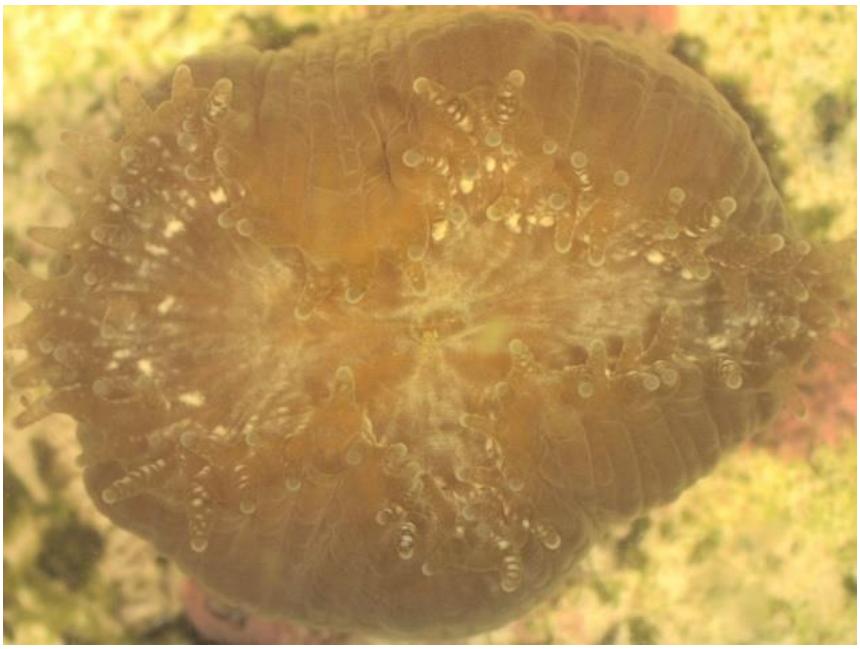
Coral Propagation: Land-based and Offshore Nursery Phase II

Final Report



Florida Department of Environmental Protection
Office of Resilience and Coastal Protection

Coral Propagation: Land-based and Offshore Nursery Phase II

Final Report

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Table of Contents

List of Figures

List of Tables

List of Acronyms

ESA	Endangered Species Act
DEP	Florida Department of Environmental Protection
FCR	Florida's Coral Reef
FWC	Florida Fish and Wildlife Conservation Commission
NOAA	National Oceanic and Atmospheric Administration
NSU	Nova Southeastern University
SE FL	Southeast Florida
SGCN	Species of Greatest Conservation Need
Coral ECA	Southeast Florida Coral Reef Ecosystem Conservation Area

1. PROJECT DESCRIPTION

Florida's Coral Reef is currently experiencing a multi-year disease-related mortality event that has resulted in massive die-offs in multiple coral species. Approximately 21 species of coral, including both Endangered Species Act-listed and the primary reef-building species, have displayed tissue loss lesions which often result in whole colony mortality. First widely observed in southeast Florida in 2014, the disease has since spread to the northernmost extent of Florida's Coral Reef, and south to the Marquesas in the Lower Florida Keys. The best available information indicates that the disease outbreak is continuing to spread southwest and throughout the Caribbean.

In the Southeast Florida Coral Reef Ecosystem Conservation Area (ECA), the stony coral tissue loss disease outbreak has reduced the abundance of corals by at least 30% and caused the loss of 60% of their live tissue (Walton et al. 2018). This is catastrophic because corals build the reefs that protect our coastlines from erosion and storm surge, and provide habitat, nursery areas, and food for over 9 million species of animals and plants, including commercial fish species. Coral populations typically recover after disturbances through sexual reproduction which results in the production of coral recruits that will replenish depleted reefs. However, because the abundance of corals on the reef is currently so low, the chance that eggs and sperm from different colonies will ever meet are severely reduced, precluding the production of offspring and the recovery of the reef through natural processes. Hence, aside from minimizing or eliminating local and global stressors to reduce coral mortality, reef recovery can be accelerated by increasing the density of corals on the reef through restoration processes. Increasing coral density on the reef can be done through asexual and sexual forms of reproduction.

Asexually reproducing corals through fragmentation has the advantage of quickly increasing coral biomass available to restoration efforts, but it does not contribute to increased genetic diversity. Fragmentation consists of breaking adult colonies into multiple smaller pieces, which are then grown in land-based and/or offshore nurseries. Smaller fragments present faster growth rates than larger colonies. This has been hypothesized to be because smaller corals allocate more energy towards growth and away from reproduction, or simply because the perimeter to area ratio is more advantageous for the growth of modular organisms. Fragmentation of branching, fast-growing corals like *Acropora* has been successfully and widely applied. More recently, boulder corals began being microfragmented, i.e., cut down into smaller pieces down to one polyp size and grown at faster rates (Forsman et al. 2015, Page et al. 2018). When grown in proximity, smaller pieces of the same initial colony can eventually fuse and form a larger/reproductive-size colony in a much shorter period (micro-colony fusion). Microfragmentation of reef-building species impacted by the SCTLD outbreak needs to be optimized and intensified in land-based and offshore nurseries to significantly enhance their density on the reef, enhance fertilization success, and ultimately promote recruitment success.

The assisted sexual reproduction of corals can massively increase the number of genetically unique corals available for restoration and increase the genetic diversity of existing populations. This entails collecting coral gametes, performing fertilization, rearing embryos to the larval stage for settlement, and growing the recruits until they reach a size suitable for outplanting. Gametes can be collected in the field during the annual coral spawning event and/or in land-based nurseries by bringing in sexually mature colonies to outdoor tanks right before spawning is projected to occur. Coral gametes can also be acquired by maintaining corals year-round in outdoors tanks exposed to natural moon and light cues under a natural annual temperature cycle, or even by replicating those same conditions in indoor aquaria (Craggs et al. 2017).

This project aims to assist the asexual and sexual propagation of coral species affected by the stony coral tissue loss disease in the ECA by increasing reef-building coral biomass available for restoration and producing corals with high genetic diversity that are better adapted to local and global stressors.

The purpose of this project is to propagate the coral species that were most impacted by the disease outbreak, *Montastraea cavernosa, Orbicella faveolata, Pseudodiploria clivosa, P. strigosa,* and *Colpophyllia natans* using sexual and asexual reproduction modes. This will allow us to increase the live coral biomass available for future restoration projects, preserve the genetic diversity of the corals that survived the disease (potentially more disease resistant), and increase the genetic diversity of the populations on the reef (new genotypes are formed through genetic recombination). Ultimately, we aim to contribute to accelerating the breeding of hardy genotypes, re-establish or enhance the natural sexual reproduction on the reef, and hence reef resilience.

We propose to use asexual and sexual reproduction techniques to propagate colonies/genotypes of reef-building coral species from the coral ECA that are susceptible to disease but were not impacted and rear them until they reached a size suitable for outplanting. These species included: *Montastraea cavernosa, Orbicella faveolata, Pseudodiploria clivosa, P. strigosa,* and *Colpophyllia natans*. Combined, these techniques will allow managers to preserve and increase the genetic diversity of these species, and more rapidly produce a greater quantity of mature-size reef-building corals to outplant. To do so, we conducted histological studies to determine the fecundity of wild colonies and colonies maintained in the land-based nursery, continued to induce gonad maturation and spawning of corals in captivity, collected gametes of wild colonies in the field and in the land-based nursery, assisted gamete fertilization, reared larvae, settled them and reared juveniles, and propagated corals through microfragmentation. We proposed to do sexual and asexual propagation, with the understanding that spawning is not always reliable, i.e. sexual reproduction (spawning, larval rearing, and early grow-out of sexual recruits) was our main goal and we allocated as many resources as necessary to those tasks, however because the sexual reproduction was only partly successful, we to still produced the same amount of coral biomass using asexual reproduction (microfragmentation techniques) and reared larvae donated by the Florida Aquarium

which parental colonies were from the Florida Keys region. These activities preserved the most resilient genotypes of the most disease-impacted reef-building corals from the ECA, propagatde them to facilitate population recovery in future restoration projects. The outcomes of this project provided an extensive number of colonies of the most resilient corals in the region and preserved their genetic information in *ex situ* tanks.

The outcomes of this project will be incorporated into an on-going coral disease response effort which seeks to improve understanding about the scale and severity of the Florida's Coral Reef coral disease outbreak, identify primary and secondary causes, identify management actions to remediate disease impacts, restore affected resources and, ultimately, prevent future outbreaks.

To ensure alignment of needs and avoid duplication of efforts, this project primarily focused on corals from the Southeast Florida Coral Reef ECA (Coral ECA), where remaining corals have survived the disease outbreak, however, larvae from the Florida Keys were also incorporated. Throughout the project, we continued to collaborate and coordinate activities with partners in other locations, sharing the best existent knowledge and practices to maximize the success for all.

This report summarizes the field and lab efforts conducted to fulfill PO B77DF8 deliverables through June 30, 2021.

2. METHODOLOGY

This work was conducted under the State of Florida Special Activity License SAL-20-2238B-SCRP.

2.1. Task 1: Assessment of coral fecundity (Renegar)

Large previously treated *Orbicella faveolata* in the field. Six large *Orbicella faveolata* colonies in the field which have been previously diseased, treated for disease, or isolates from colonies which have remained healthy throughout the past years of monitoring, ideally from a range of sizes to detect any possible senescence, were sampled (core size 2.5 x 2.5 cm) using a diamond core bit and pneumatic drill attached to a scuba tank to determine if those colonies were fecund. After sampling, large *Orbicella faveolata* colonies in the field were monitored monthly, and if needed, treated with antibiotics during monthly monitoring (separately funded project by DEP, P.I.: Dr. Brian Walker). Collected coral samples were immediately placed in a 20% Z-fix® and seawater solution and fixed for 24-48 hours. Post-fixation, samples will be decalcified in 5% HCl/ethylenediamine tetraacetic acid (EDTA) seawater solution, dehydrated in a graded series of ethanols and xylene, and embedded in paraffin wax (Paraplast Plus). Longitudinal and transverse sections at appropriate depths (4 um in thickness) were

mounted on slides, cleared in xylene, stained with Heidenhain's azocarmine-aniline blue (HAB), and analyzed for gamete content to determine fecundity via light microscopy.

Land-based nursery. A total of 6 *Montastraea cavernosa* colonies and 3 large *Orbicella faveolata* colonies have been maintained since September or October 2019 in the land-based nursery's indoor gonad maturation induction system were sampled (core size 2.5 x 2.5 cm) using a diamond core bit and pneumatic drill attached to a scuba tank. Collected coral samples were immediately placed in a 20% Z-fix® and seawater solution and fixed for 24-48 hours. Post-fixation, samples were decalcified in 5% HCl/ethylenediamine tetraacetic acid (EDTA) seawater solution, dehydrated in a graded series of ethanols and xylene, and embedded in paraffin wax (Paraplast Plus). Longitudinal and transverse sections at appropriate depths (4 um in thickness) were mounted on slides, cleared in xylene, stained with Heidenhain's azocarmine-aniline blue (HAB), and analyzed for gamete content to determine fecundity via light microscopy.

Spawning hub. Six *Pseudodiploria clivosa* samples were collected from the offshore spawning hub for histological analysis on 09/03/20 and fixed in zinc-buffered formalin (Z-fix) for at least 24 hours. Samples were decalcified in 5% HCl/ethylenediamine tetraacetic acid (EDTA) seawater solution, dehydrated in a graded series of ethanols and xylene, and embedded in paraffin wax (Paraplast Plus). Longitudinal and transverse sections (4 mm) were mounted on slides. Sections were cleared in xylene and stained with modified Heidenhain's aniline blue. Stained slides were viewed in an Olympus BX 43 light microscope at magnifications ranging from 4X to 60X and photographed with an Olympus DP21 digital camera. Histological sections were assessed for the presence or absence of male and female gonads and the reproductive stage of gametes.

2.2. Task 2: Coral spawning and gamete collection in the field and at spawning hub (Gilliam and Walker)

In Broward County, spawning of *Orbicella faveolata* and *Pseudodiploria clivosa* typically occurs from sunset to midnight 5-9 days after the full moon in August or September. Colonies of these species were found to be fecund and confirmed to be expected to spawn in September (determined based on the developmental stage of the egg, Task 1). One site in Broward County with multiple very large/old *Orbicella faveolata* colonies and the pilot spawning hub with *Pseudodiploria clivosa* were monitored every night for 5 nights from sunset to midnight during the expected window for spawning in August or September. Since these species are hermaphroditic (i.e. each colony releases both eggs and sperm in gamete bundles), colonies would be tented to collect the gametes. Tents are made of plankton mesh and have attached buoys to ensure they float above the colony. Each tent funnels gamete bundles into a collection container. Once gamete bundles from multiple colonies of the same species are collected, they would be pooled for fertilization, keeping sperm concentration at approximately 10^5 - 10^6

mL^{-1}, on the boat and brought back to the Nova Southeastern University's Guy Harvey Oceanographic Center.

2.3 Task 3: Coral spawning and gamete collection at land-based nursery (Figueiredo and Renegar)

Beginning on the full moon of August, and then in September, for 10 days every night after sunset, the pumps of the indoor and outdoor recirculating systems were shut down and colonies of *Montastraea cavernosa, Orbicella faveolata, Pseudodiploria clivosa, P. strigosa,* and *Colpophyllia natans* (Figure 1) were observed until midnight for spawning. For each species, the number and ID of colonies that spawn each night was recorded. For gonochoric species, the eggs were collected by skimming the surface with a cup, while sperm will be immediately collected with large (23mL) plastic pipettes to avoid dilution. For hermaphrodite species, egg and sperm bundles would be collected by skimming the surface with a cup.

2.4 Task 4: Fertilization, larval culture and settlement (Figueiredo)

At the land-based nursery, eggs and sperm from colonies that spawned (Task 3) were combined, keeping sperm concentration at 10^5 - 10^6 mL^{-1} to maximize fertilization. After one hour of eggs being pooled with the sperm, the eggs were washed through a series of dilutions using gravy separators to remove the sperm and avoid polyspermy. The embryos were then reared in the mass-scale larval culture system until larvae reached competency. This recirculation system is equipped with mechanical, biological and chemical filtration, UV sterilizer, heaters and chiller, and has eight 95L conical tanks allowing us to rear large quantities of larvae of several species or spawning days simultaneously. The temperature, pH, and salinity levels mimic historical conditions on the reef during the summer (not necessarily current conditions) to maximize survival.

Once the larvae became competent, they were moved to an existing indoor recirculating system holding adult corals and placed inside a mesh basket covered with settlement tiles conditioned for at least one month on the indoor tanks and sprinkled with crustose coralline algae to induce larval settlement and metamorphosis and acquisition of algal symbionts. Twenty-four hours after the competent larvae were exposed to the conditioned tile, the tiles were scored for metamorphosis. Larvae which remained swimming were provided with new settlement tiles. This process was repeated daily for one week, or until all the larvae either died or settled. Some tiles with newly settled corals were photographed with an Olympus LC20 digital camera attached to an Olympus SZ61 dissecting microscope to measure the initial surface area of the coral polyp using CellSens®.

We fertilized gametes and reared and settled the larvae of *Montastraea cavernosa* (offspring from the colonies held in the indoor gonad maturation and spawning induction system for a year). Because the colonies of *Montastraea cavernosa, Orbicella faveolata, Pseudodiploria clivosa, P. strigosa, and Colpophyllia natans* that had just been collected in September 2020 did not spawn (Task 3), and colonies of *P. clivosa* and *O. faveolata*

were not observed spawning in the field (Task 2), Task 4 had less effort than previously predicted. This effort was however compensated with additional settlement and rearing of *Dendrogyra cylindrus* (one parent from the ECA), and *C. natans, Diploria labyrinthiformis*, and *P. strigosa* larvae (parental colonies from the Florida Keys) donated to Nova Southeastern University by the Florida Aquarium (parental colonies induced to spawn in captivity).

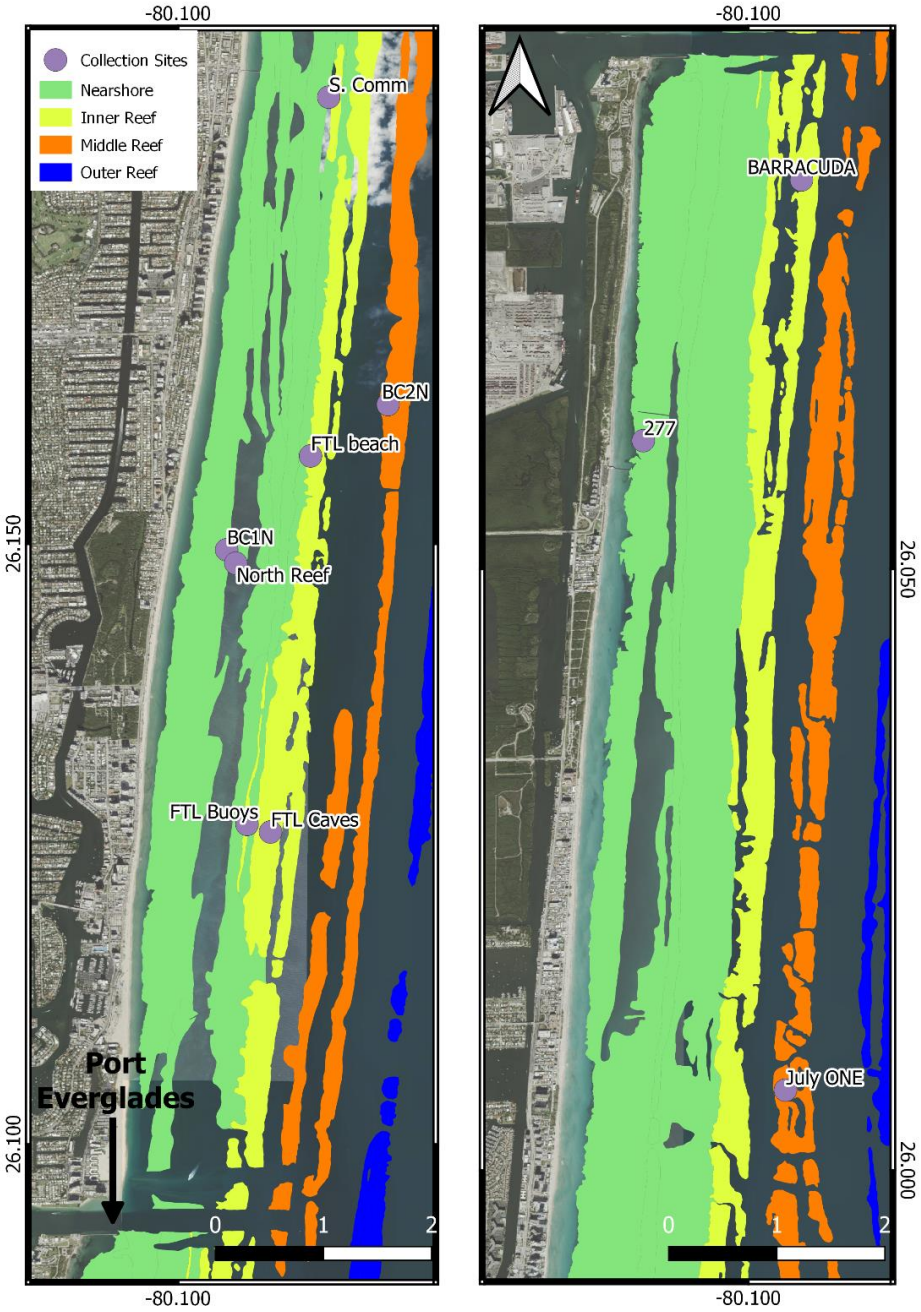

Figure 1: Map of sites of origin of the Montastraea cavernosa, Orbicella faveolata, Pseudodiploria clivosa, P. strigosa, *and* Colpophyllia natans *colonies.*

2.5 Task 5: Grow-out of sexual recruits in land-based nursery (Figueiredo)

We reared newly settled recruits of *Montastraea cavernosa* (offspring from the colonies held in the indoor gonad maturation and spawning induction system for a year). Because the colonies of *Montastraea cavernosa, Orbicella faveolata, Pseudodiploria clivosa, P. strigosa, and Colpophyllia natans* that had just been collected in September 2020 did not spawn (Task 3), and colonies of *P. clivosa* and *O. faveolata* were not observed spawning in the field (Task 2), Task 5 also had less effort than previously predicted. Similarly to Task 4, this effort was however compensated with additional rearing of *Dendrogyra cylindrus, C. natans, Diploria labyrinthiformis,* and *P. strigosa* sexual recruits derived for larvae donated to Nova Southeastern University by the Florida Aquarium.

Newly-settled corals were reared in an existing 1500L indoor recirculating system composed of 2 raceways (each 2.5 x 0.6 x 0.3 m) with a flow rate of 350L/h and shared sump equipped with a biological and chemical filtration, UV sterilizers, heaters and chillers, calcium reactors, and Radion LED lights. Newly settled corals have a very small tolerance range to environmental conditions, thus is extremely important to maintain optimal and stable environmental conditions, high water quality and provide varied food *ad libitum*. A modified annual temperature cycle was used to fluctuate temperature throughout the year, but it never goes below 23.3°C or above 28°C, as this typically impairs growth. Temperature was measured daily with a YSI® Pro20 temperature probe to ensure accuracy. The lights followed a natural photoperiod with light irradiance increasing from sunrise until solar noon (when maximum irradiance is reached) and decreasing after that until a sunset. Newly settled corals are very sensitive to high light irradiance levels and grow faster under lower irradiance levels; however once coral can feed heterotrophically and symbiosis is fully established, they require higher light levels to growth and survive. Previous experiments (McMahon 2018, Kreh 2019) have identified the optimal light levels for *Montastraea cavernosa* and *Orbicella faveolata* to be 10 µmol photons.cm^{-2} sec^{-1} during Weeks 1-4, 40 µmol photons.cm^{-2} sec^{-1} during Weeks 5-8, 60 µmol photons.cm^{-2} sec^{-1} during Week 9, 80 µmol photons.cm^{-2} sec^{-1} during Week 10, 120 µmol photons.cm^{-2} sec^{-1} during Week 11, 140 µmol photons.cm^{-2} sec^{-1} during Week 12, and 180 µmol photons.cm^{-2} sec^{-1} onwards. The salinity maintained at 35 ppt. Reverse osmosis water was added daily to the sump to replace evaporated water and maintain salinity. Water quality tests were performed weekly to determine alkalinity, ammonia, nitrite, nitrate, and phosphate concentrations. If necessary, partial water changes were performed to guarantee the water did not contain ammonia nor nitrites, and nitrates were at 0.05-0.2 ppm, and phosphate at 0.02-0.03 ppm. Adult corals of several species were placed in the tank to release algal symbionts and facilitate symbiont acquisition by the coral recruits. Corals were hand-fed daily *Nannochloropsis* and Rotigrow Plus-enriched rotifers and *Artemia*, RN Oyster Feast, RN ROE, Reef-Roids (PolypLab), and Colors (PolyLab) *ad libitum* to promote growth and enhance survival rates. Overgrowth by algae was controlled with the help of small herbivorous snails, and manually removed to minimize coral mortality and promote growth. Coral juveniles were reared in this system until they had reached a size suitable to be moved to the offshore nursery or outplanted on the reef.

Juvenile corals were examined under the Olympus stereo-dissecting microscope to score survival and photographed to measure surface area using the software CellSens to monitor growth.

2.6 Task 6: Microfragmentation and grow-out of microfragments in land-based nursery (Renegar and Figueiredo)

Corals collected for research purposes in previous years (SAL-18-2021-SCRP and SAL-19-1902-SRP) and corals of opportunity (SAL-20-2238B-SCRP) which had been collected and brought to NSU, of species susceptible to STCLD were microfragmented and grown in the land-based nursery.

Renegar's lab: Four corals of opportunity (three colonies of *Siderastrea siderea* and one colony of *Montastraea cavernosa*) were cut into ~1cm^2 pieces (32-36 fragments per colony, for a total of 132 coral microfragments) using a Gryphon Aquasaw XL Bandsaw. Once cut, fragments were attached in single-colony groups of four to tiles using cyanoacrylate gel glue (Figure 2A). When firmly attached each tile and set of fragments were then treated in a 15-minute Lugol's Iodine dip at a concentration of 2ml/L. The remaining parent colony was also dipped in the same manner and adhered to a tile to monitor recovery (Figure 2B). All tiles were placed in the onshore (building) nursery. Microfragments were reared outdoors in 400-gallon (8' x 4' x 2') fiberglass raceways utilizing artificial seawater. Temperature was maintained with a heat exchanger and process chilled water. Filtration consisted of a protein skimmer with ozonation, a UV sterilizer, and a phosphate reactor. A calcium reactor was used to maintain calcium levels and facilitate coral growth. In tank circulation was provided by submersible powerheads and wave timers. Wastewater was stored in an effluent detention tank before disposal or discharge. Corals were hand-fed daily *Nannochloropsis* and Rotigrow Plus-enriched rotifers and *Artemia*, RN Oyster Feast, RN ROE, Reef-Roids (PolypLab), and Color (PolyLab) *ad libitum* to promote growth and enhance survival rates. Tiles were cleaned weekly to minimize algal overgrowth. Water quality was monitored weekly or more frequently if needed. To measure size and growth of the fragments, scale referenced photographs were processed using Image J's polygon selection and measurement features, using the metric ruler in each image for scale. The difference in amount of living tissue on each fragment was used to calculate growth rate.

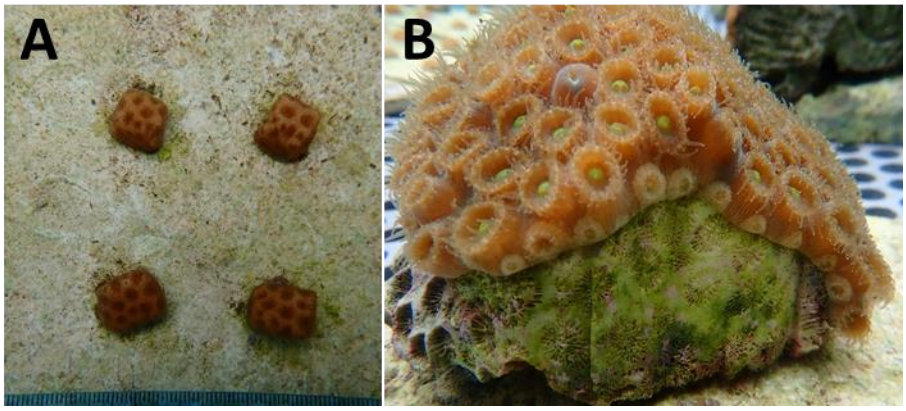

Figure 2: Coral microfragmentation. A) Microfragments of Siderastrea siderea*, NSU-SID1, plate 9, 06/14/2021; B)* Montastraea cavernosa *fragment donor colony, showing tissue regrowth over cut margin.*

Figueiredo's lab: Three colonies of *Diploria labyrinthiformis*, *Orbicella faveolata*, *Siderastrea siderea*, and *Pseudodiploria clivosa* were cut each into three large fragments (approx. 3 cm^2) and three small fragments (approx. 1 cm^2), totaling 72 microfragments. Colonies were microfragmented using a Gryphon Aquasaw XL Bandsaw with a diamond tipped blade and QEP 650XT. Fragments 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, allowing overhead pictures to capture surface. These microfragments were grown over 6 months in an 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 Coral ECA 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-2s-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).

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 over 6 months, 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. After 6 months (March 25, 2021), some of these microfragments, specifically 13 *Diploria labyrinthiformis*, 3 *Orbicella faveolata*, and 4 *Siderastrea siderea*, were further microfragmented a Gryphon Aquasaw XL Bandsaw with a diamond tipped blade and QEP 650XT creating 90 microfragments. These new microfragments were epoxied onto settlement tiles and held *in situ* for seven days before being moved to the offshore coral nursery (Task 7).

2.7 Task 7: Grow-out of newly settled corals and microfragments at the offshore nursery (Gilliam)

Sexual recruits

Juvenile *Colpophyllia natans* (n = 133), *Diploria labyrinthiformis* (n = 59), *Montastraea cavernosa* (n = 232), and *Pseudodiploria strigosa* (n = 19) on settlement tiles were transferred to an *in situ* nursery on April 1, 2021. The settlement tiles the juvenile corals grew on were strung on monofilament with ~2.5 cm long spacers in between each tile creating a 'kebab' that was hung from coral trees at 6 m depth (Figure 3). Before being moved to the *in situ* nursery, the juvenile corals on each settlement tile were counted and mapped. After six weeks at the *in situ* nursery, the kebabs were collected on May 24, 2021, and each settlement tile was analyzed for juvenile survival. They were returned to the *in situ* nursery on May 27, 2021.

Figure 3. Two kebabs hanging from a coral tree at the in situ nursery. Settlement tiles with juvenile corals were strong on monofilament with spacers in between each tile.

Microfragments

Ninety microfragments of 13 colonies of *Diploria labyrinthiformis* 3 colonies of *Orbicella faveolata*, and 4 colonies of *Siderastrea siderea* were moved to an *in situ* coral nursery and placed on a coral table on April 1, 2021 (Figure 4a). Monitoring photos were taken with a ruler on April 1, April 14, April 30, and May 14, 2021 (Figure 4b); survival and condition data were collected from the monitoring photos. Each photo was scaled and each microfragment was traced using ImageJ to determine surface area.

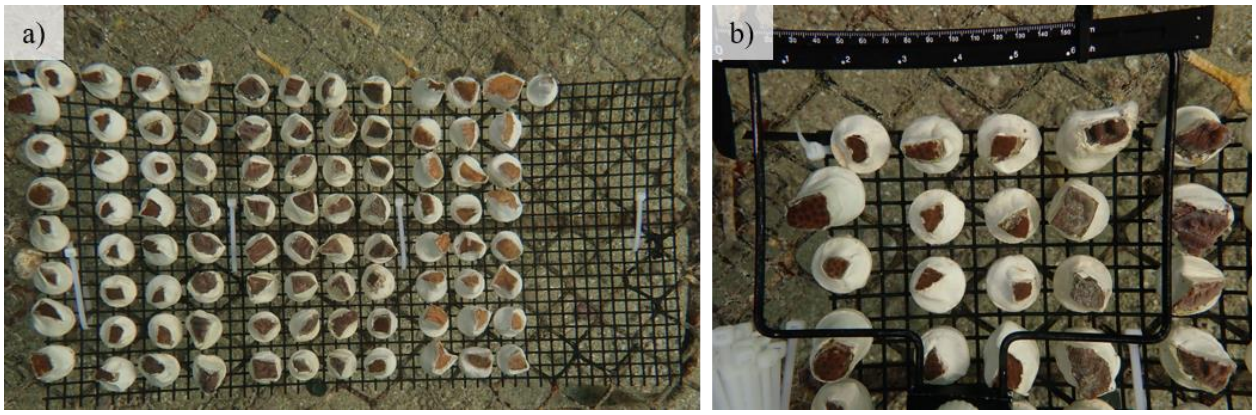

Figure 4. a) Overhead photo of microfragments taken after they were attached to the coral table on April 1, 2021; b) monitoring photo with scale bar used to trace microfragments and for survival and condition data collection.

2.8 Task 8: Preserve coral genotypes of disease impacted species and induce gonad maturation and spawning in captivity (Figueiredo)

At the beginning of this project, 159 coral colonies (of which 84 were part of the coral 'rescue' program) from the Coral ECA were being held at NSU's land-based nursery at the beginning of the Phase II of this project, specifically 55 *Montastraea cavernosa,* 31 *Orbicella faveolata*, 29 *Pseudodiploria clivosa,* 24 *P. strigosa,* 4 *Diploria labyrinthiformis* 14 *Siderastrea siderea,* 5 *Agaricia agaricites* and 4 *Colpophyllia natans* mature-size colonies of unique genotypes. A few other corals of opportunity were added later on to this collection (from collections on the Port Everglades ahead of dredging). These colonies represent a significant part of the genetic diversity of the populations of these species on the Coral ECA. Importantly, these genotypes survived the disease event and thus possibly are more disease-resistant than genotypes from other areas along Florida's Coral Reef not yet ravaged by the SCTLD. These colonies were maintained in 12 outdoor and 2 indoor recirculating tanks to preserve their genotypes *ex situ* (genotype banking/caching), and induce their gonads to mature and spawning to occur synchronously, which will allow us to produce offspring that perpetuates but also recombines their genes into new genotypes. The indoor systems were designed to replicate historical temperature, photoperiod, and solar/lunar irradiance on the reef. The indoor system is fit with a web-based microprocessor (Neptune Systems, Apex) attached to the tank and Radion LED lights. Using the edit seasonal table on the Apex classic dashboard, the target seasonal temperature, photoperiod, and lunar cycle data has been programmed. Annual variation in sea temperature on the reef was based on HOBO temperature loggers (HOBO Pro V2) data collected at the Southeast Florida Coral Reef Evaluation and Monitoring Project (SECREMP) sites between February 2007 and June 2016. After removing data points for periods with cold snaps and/or bleaching events, the data was used to create an average profile of annual temperature cycle in the region. Sunrise, sunset, moonrise, and moonset times were downloaded from www.timeanddate.com. To simulate annual variation in photon intensity, irradiance averages recorded by NASA Surface Meteorology and Solar Energy for the reefs off Fort Lauderdale were averaged and converted into data for LED programming. The outdoor tanks are exposed to natural sun and moon cycles, and temperature mimicking natural. Both indoor and outdoor aquarium systems are equipped with biological and chemical filtration, UV sterilizers, heaters and chillers, and calcium reactors or alkalinity and magnesium automatic dosers. Corals were hand-fed daily *Nannochloropsis* and Rotigrow Plus-enriched rotifers and *Artemia*, RN Oyster Feast, RN ROE, Reef-Roids (PolypLab), and Color (PolyLab) *ad libitum* to promote growth and enhance survival rates. Water quality was monitored weekly or more frequently if needed. Their health was monitored daily, and when needed the colonies were treated with antibiotics and/or probiotics. The health of the 89 colonies (84 from the initial 'rescue' project and other 5 colonies added later on) was recorded monthly.

2.9 Task 9: Cryopreservation (Figueiredo)

A technique that helps preserve the genotypes of corals is cryopreservation. Coral sperm may be frozen and stored, remaining viable for hundreds of years. This frozen sperm can be then thawed as needed to sexually cross corals from different locations, or that spawn in different days. This cryopreserved genetic material may therefore be used to strengthen small populations by adding genetic diversity. The goal of this task was to acquire the necessary equipment and supplies to cryopreserve the sperm of corals at Nova Southeastern University in following years.

3. RESULTS

3.1. Task 1: Assessment of coral fecundity (Renegar)

Large treated *Orbicella faveolata* in the field. The results for this task relative to *Orbicella faveolata* have been reported by Walker et al. 2020 "Field Operations for large Orbicella Corals Spawning: Year 2020". In sum, all colonies sampled were found to be fecund early in September.

Indoor and outdoor land-based nursery. The *Montastraea cavernosa* colonies held in indoor tanks at the land-based nursery since September 2019 colonies were all fecund. Specifically, four of the six colonies sampled were females containing oocytes in stages III & IV on July 25, 2020. The other two colonies had no visible oocytes and thus were assumed to be males (which was later confirmed during the spawning window, Task 3). The *Orbicella faveolata* held in indoor tanks at NSU since September or October 2019 and sampled on the same date contained oocytes but they appeared degraded or in earlier stages of development (Table 1).

Table 1: Fecundity status of corals induced to mature their gonads in captivity.

Species	Colony	Fecundity status
Montastraea cavernosa	1	Female: stage 3-4 oocytes
	2	No oocytes, potentially a male
	3	Female: stage 4 oocytes
	4	No oocytes, potentially a male
	5	Female: stage 3 oocytes (very few visible)
	6	Female: stage 3 oocytes (very few visible)
Orbicella faveolata	1	Oocytes visible but they appear degraded (or in earlier stage of development)
	2	Oocytes visible but they appear degraded (or in earlier stage of development)
	3	Oocytes visible but they appear degraded (or in earlier stage of development)

Spawning hub. Four out of the six colonies (67%) of *Pseudodiploria clivosa* in the spawning hubs were fecund (visible oocytes) one week prior to the expected September spawning window. Four of the six colonies sampled on 9/3/20 contained male and/or female gametes. All female gametes observed were Stage IV (Figure 5A and Figure 5B) and observed spermaries were Stage V (Figure 5B and Figure 5C) (Table 2). The low numbers of ova per polyp, appearance of spermaries and the presence of free spermatozoa in the mesenteries (Figure 5D) indicated that spawning or partial spawning had likely already occurred by the sampling date of 09/03/20.

The sampling of colonies in the field required a SAL permit. The permit SAL-20-2238B-SCRP was issued 9/2/2020.

Table 2: Fecundity status of Pseudodiploria clivosa *corals in the spawning hubs*

Spawning hub	**Colony**	**Stage**
North	1	Stage IV eggs
	2	Not fecund
	3	Stage IV eggs and Stage V sperm
South	1	Not fecund
	2	Stage IV eggs and Stage V sperm
	3	Stage IV eggs

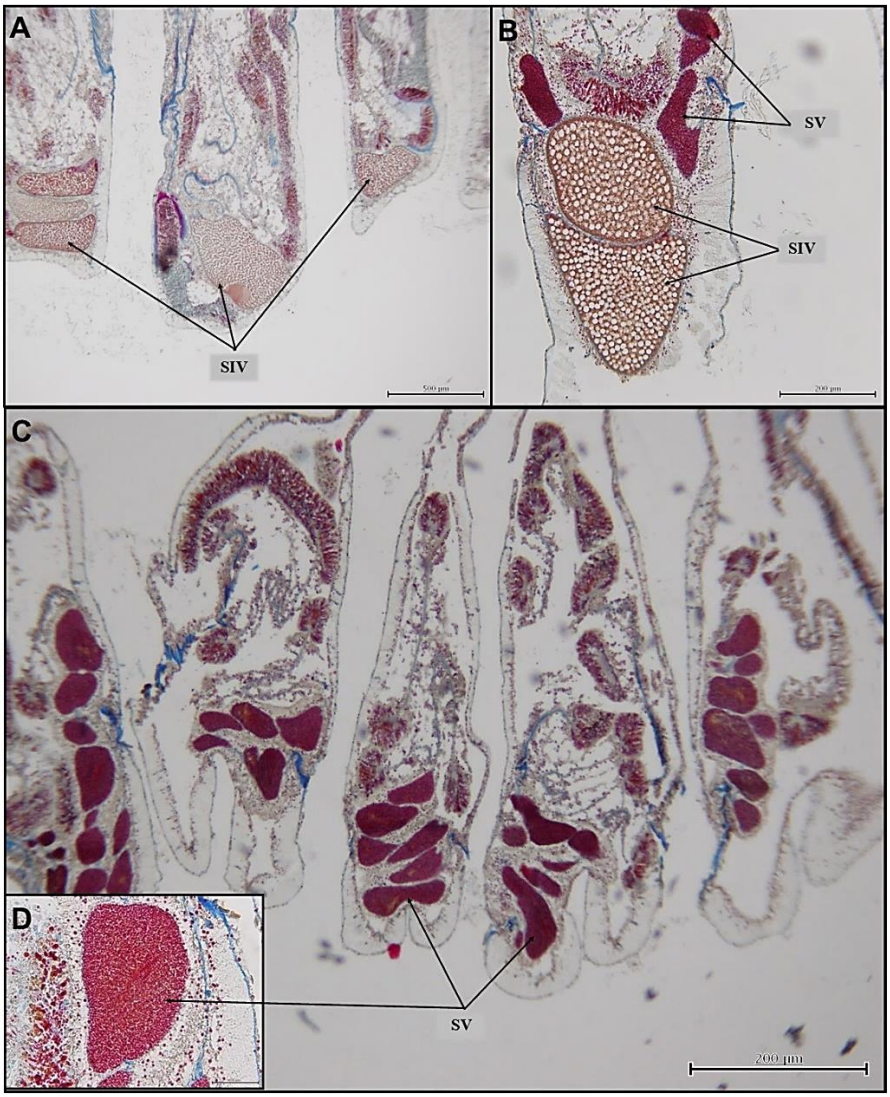

Figure 5. Pseudodiploria clivosa. *A) Stage IV oocytes/ova in mesenteries, B) Stage IV oocytes/ova and Stage V spermaries, C) Stage V spermaries, and D) Stage V spermary surrounded by free spermatozoa.*

3.2. Task 2: Coral spawning and gamete collection in the field and at spawning hub (Gilliam and Walker)

The results for this task relative to *Orbicella faveolata* have been reported by Walker et al. 2020 "Field Operations for large *Orbicella* Corals Spawning: Year 2020". In sum, colonies were found to be fecund early in September.

The colonies of *P. clivosa* in the northern spawning hub were not observed spawning during the expected spawning time window in September (despite having been found fecund a week prior, Task 1) throughout the allotted monitoring period. Colonies were

monitored every night by graduate research assistants in the NSU CRAM lab (Table 3). No issues were encountered.

SAL permits were issued to collect gametes of *Pseudodiploria clivosa* at the spawning hub and *Orbicella faveolata* at the LC site (SAL-20-2251-SCRP and SAL-20-2238B-SCRP).

Table 3: Dive logs for the dives performed at the North Hub. All dives were performed at 25ft deep and lasted 105 minutes.

Date	Divers	Time of Entrance	Time of Exit
9/6/2020	Nick Jones, Grace Hanson	8:30 PM	10:15 PM
9/6/2020	Cassie VanWynen, Daniel Perez	10:15 PM	12:00 AM
9/7/2020	Nick Jones, Grace Hanson	8:30 PM	10:15 PM
9/7/2020	Cassie VanWynen, John Alfirevich	10:15 PM	12:00 AM
9/8/2020	Nick Jones, Grace Hanson	8:30 PM	10:15 PM
9/8/2020	Nicole Hayes, Morgan Hightshoe	10:15 PM	12:00 AM
9/9/2020	Nicole Hayes, Morgan Hightshoe	8:30 PM	10:15 PM
9/9/2020	Shane Wever, Hope Hefley	10:15 PM	12:00 AM
9/10/2020	Nicole Hayes, Morgan Hightshoe	8:30 PM	10:15 PM
9/10/2020	Shane Wever, Daniel Perez	10:15 PM	12:00 AM

3.3. Task 3: Coral spawning and gamete collection at land-based nursery (Figueiredo)

Colonies of *Montastraea cavernosa* held at the land-based nursery since September 2019 were monitored daily for spawning at night during the expected spawning window of August and September. Most colonies (13) spawned in August. Spawning occurred August 8-12, and colonies started spawning from 8:28 pm to 10:31 pm. In September, colonies spawned Sep 6-8 starting at 8:30 pm to 9:54 pm (Table 4). This consisted mostly of colonies that had already spawned in August, and one that only spawned in September. The spawning in August was more synchronous and more colonies participated in the event. Fourteen of the 23 colonies were observed spawning heavily within the monitoring period (Figures 6 and 7); it is still possible that others may have spawned too, but due to the lower amount of gametes produced, synchronous spawning with other colonies which produced more gametes (Figure 8) or spawning outside monitoring period, these were not observed. Combining the results from histology and spawning observations, we conclude that at least 20 of the 23 colonies had mature gonads. Sex ratio approached 1M:1F.

Table 4: Day and time of spawning of colonies of Montastraea cavernosa induced to spawn in captivity

Date	Number of colonies spawning (sex ratio M:F)	Spawning start time	Spawning end time	Fertilization rate
8/08/20	8 (5M:3F)	21:05	22:28	>95%
8/09/20	5 (3M:2F)	20:46	22:45	>95%
8/10/20	0	-	-	-
8/11/20	5 (3M: 2F)	21:11	23:22	>95%
8/12/20	10 (6M: 4F)	20:28	22:25	>95%
8/13/20	0	-	-	-
9/04/20	0	-	-	-
9/05/20	0	-	-	-
9/06/20	1 (0 M: 1 F)	20:35	20:50	-
9/07/20	2 (1 M: 1 F)	20:30	21:54	25-30%
9/08/20	1 (1 M: 0F)	20:48	21:30	-
9/09/20	0	-	-	-

Figure 6: Male Montastraea cavernosa *spawning in the induction system in the lab.*

Figure 7: Female Montastraea cavernosa *spawning in the induction system in the lab.*

Spawning videos are publicly available at:

https://www.youtube.com/channel/UCgSHPoL07O31cM2AyW-Pu3g

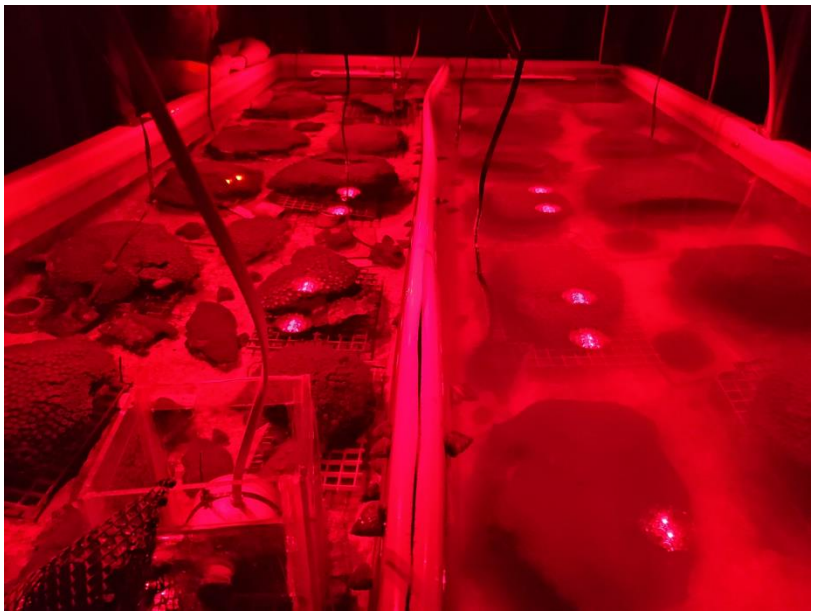

Figure 8: Highly synchronous spawning may prevent detection of spawning of colonies releasing less gametes.

Corals of *Montastraea cavernosa, Orbicella faveolata, Pseudodiploria clivosa, P. strigosa,* and *Colpophyllia natans* collected right before the expected spawning window in September were not observed spawning synchronously in the land-based nurseries.

3.4. Task 4: Fertilization, larval culture, and settlement (Figueiredo)

Montastraea cavernosa colonies held in the lab-induced spawning system (from 2019) produced very large amounts of gametes (Figure 9). Fertilization was very high (estimated at 95-100%, Figure 10, Table 4) in the two highly synchronous spawning nights in August (many females and males spawning). In September, fertilization in the one synchronous spawn (one male and one female) was estimated at 25-30% (Table 4).

Figure 9: Detail of a small portion of the pooled gametes of Montastraea cavernosa *in fertilization tub.*

Figure 10: Embryo cleavage 1h30 (left) and 12h (right) after fertilization.

Larvae were successfully cultured to competency in the mass-scale culture system (see video at the lab's YouTube channel listed above). Settlement rates of *Montastraea cavernosa* were very high. The very large amount of gametes produced (Figure 5) and resulting new settlers made it impossible to count and photograph all tiles within the day, but all tiles were censused for corals and some were photographed to determine average initial surface area (0.269 ± 0.015 mm^2). In total, 154 tiles with newly settled corals of *Montastraea cavernosa* were produced, with each tile containing 1-111 corals. New settlers appeared healthy and some already contained symbionts at the time tiles were censused (Figure 11).

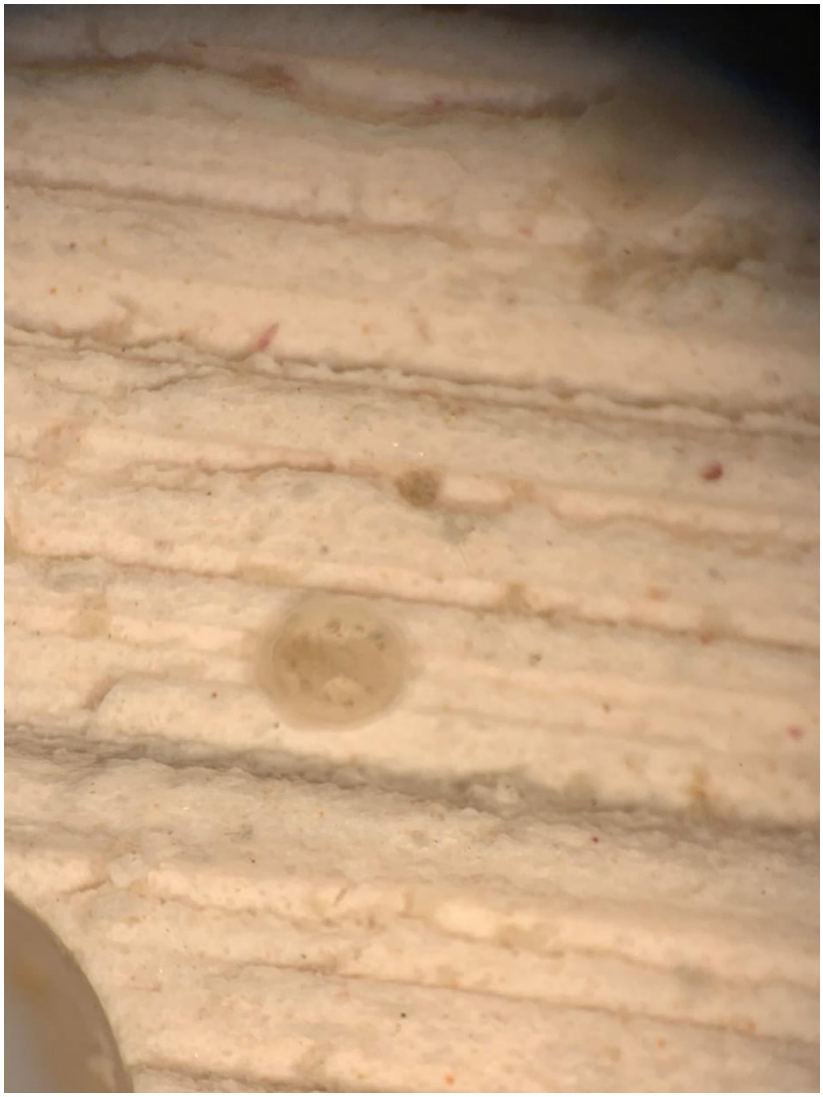

Figure 11: New settlers of Montastraea cavernosa *(one in the center, two in the top right)*

NOTE: Because the wild *Montastraea cavernosa, Orbicella faveolata, Pseudodiploria clivosa, P. strigosa,* and *Colpophyllia natans* colonies did not spawn (Task 3), and colonies of *P. clivosa* and *O. faveolata* were not observed spawning in the field (Task 2), Task 4 had less effort than previously predicted. However, as mentioned earlier, larvae from other disease susceptible species that were induced to spawn in captivity were donated by the Florida Aquarium on May, August and September 2020. Therefore, Tasks 4 and 5 included additional settlement and rearing of *Dendrogyra cylindrus* (with one parent known to be from the Coral ECA), *C. natans, Diploria labyrinthiformis,* and *P. strigosa* (parent colonies known to be from the Florida Keys). Additionally, there was a greater emphasis on the asexual propagation through microfragmentation and grow-out of corals of opportunity from the ECA (Task 6). We microfragmented corals of opportunity of SCTLD-affected species. Some of these corals have been found dislodged on the reef, while others have been taken from the Port Everglades ahead of construction.

3.5. Task 5: Grow-out of sexual recruits in land-based nursery (Figueiredo)

Approximately six months after settlement (March 4, 2021), the number of sexual recruits successfully reared was counted. The land-based coral nursery successfully reared 2844 six month old sexual recruits. Specifically, 792 *Colpophyllia natans*, 135 *Diploria labyrinthiformis* 1817 *Montastraea cavernosa*, and 100 *Pseudodiploria strigosa* (counting fused corals, i.e. chimeras, as a single individual).

On April 1, 2021, 426 sexual recruits that had been reared at the land-based nursery, specifically, 19 *Pseudodiploria strigosa*, 132 *Colpophyllia natans*, 226 *Montastraea cavernosa*, and 59 *Diploria labyrinthiformis*, were moved to the offshore nursery for grow-out (Task 7).

On June 14, 2021 the land-based nursery held a total of 978 sexual recruits (counting fused corals, i.e. chimeras, as a single individual), specifically, 57 *Colpophyllia natans*, 19 *Pseudodiploria strigosa*, 867 *Montastraea cavernosa* , and 35 *Diploria labyrinthifomis*. These numbers reflect the reduction due to deployment to offshore nursery, but also the formation of chimeras (fusion of corals in tiles where settlement rates were very high) and some mortality, mostly caused by algal overgrowth.

Over the months, sexual recruits experienced significant growth at the land-based nursery (Figure 12 and see *Diploria labyrinthiformis* growth over time on Figures 13-16). On June 14, 2021 their average surface area (± S.E.) was:

Diploria labyrinthiformis: 65.86 ± 9.68 mm^2 (Figure 17)
Montastraea cavernosa (from August): 18.28 ± 1.23 mm^2 (Figure 18)
Montastraea cavernosa (from September): 2.95 ± 1.27 mm^2
Colpophyllia natans: 16.63 ± 2.72 mm^2 (Figure 19)
Pseudodiploria strigosa: 5.29 ± 0.68 mm^2 (Figure 20)

Diploria labyrinthiformis measured on average 0.51 mm^2 when settled, thus increased 129 times in size, corresponding to a 5.03 mm^2/month. *Montastraea cavernosa* from August measured on average 0.12mm^2 when settled, thus increased 152 times in size, corresponding to a 1.65mm^2/month. Assuming the *Montastraea cavernosa* from September had a similar size to the ones from August (they were not measured but appeared similar, and this size is similar to the one registered in previous years), increased only 25 times, corresponding to a growth rate of 0.28mm^2/month.

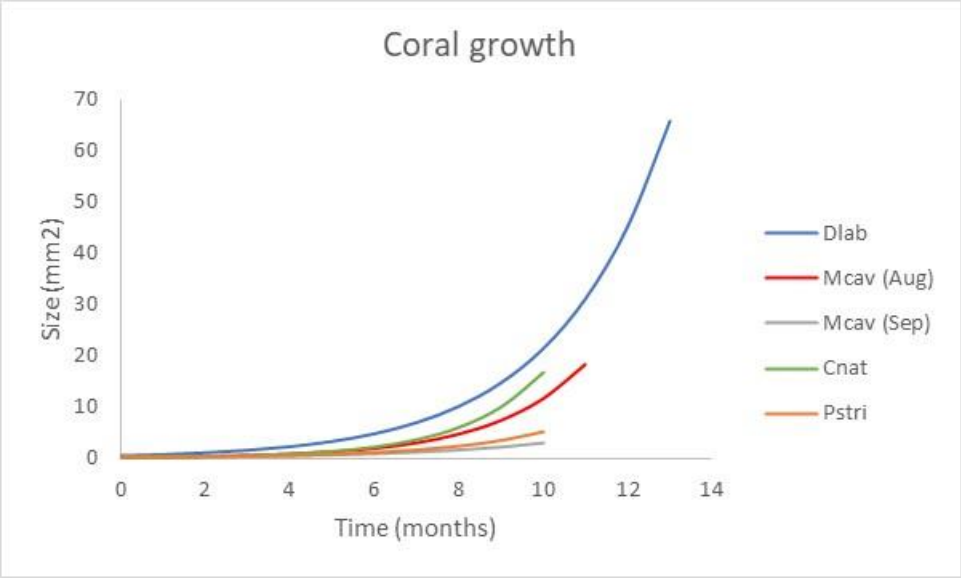

Figure 12: Growth of the newly settled corals of each species (Dlab – Diploria labyrinthiformis, *Mcav* – Montastraea cavernosa, *Cnat* – Colpophylia natans, *Pstri*- Pseudodiploria strigosa*).*

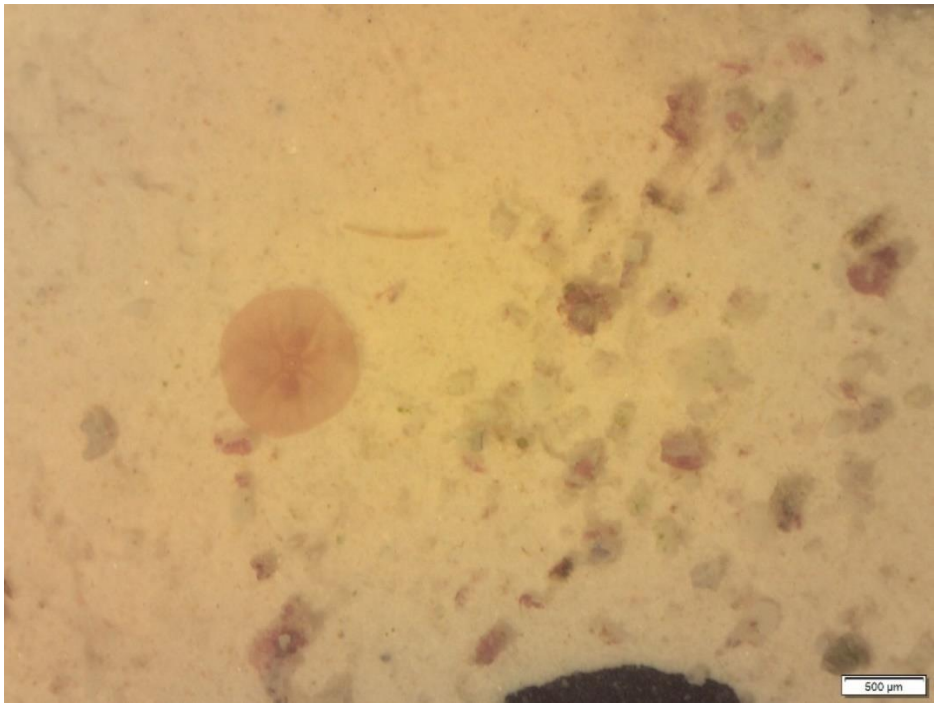

Figure 13: 5 days old Diploria labyrinthiformis.

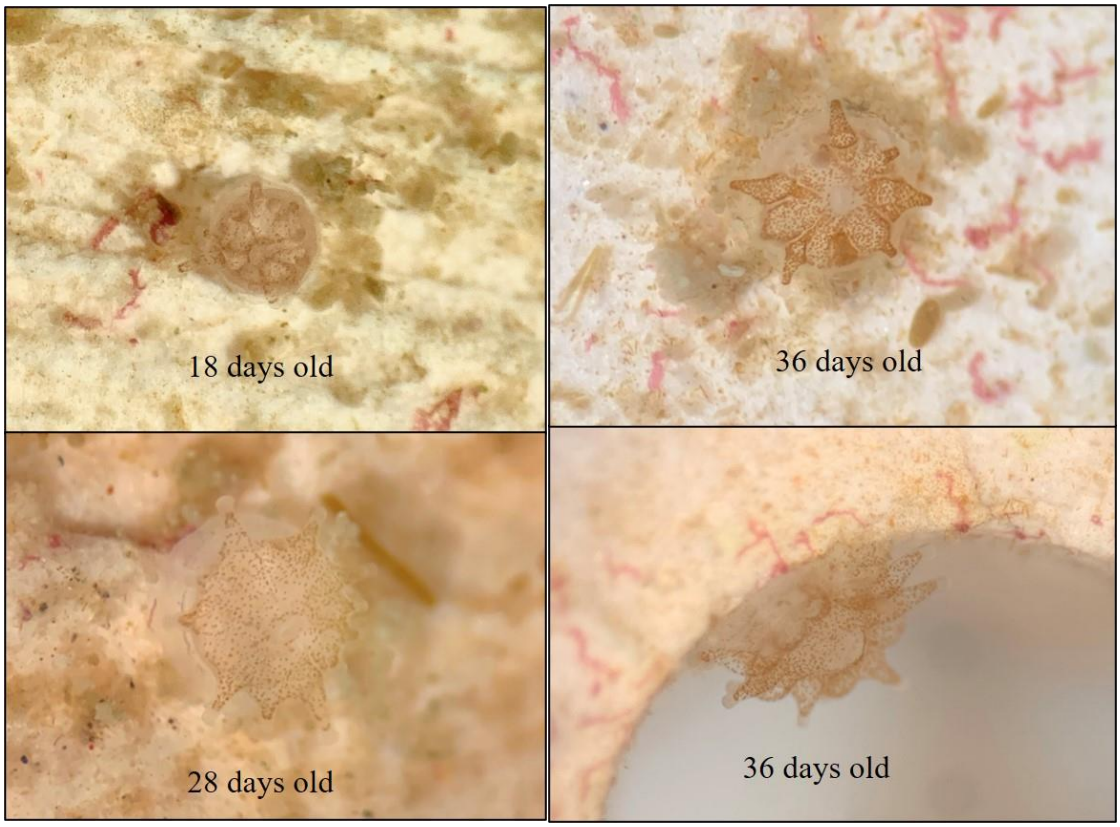

Figure 14: 18, 28 and 36 day old Diploria labyrinthiformis.

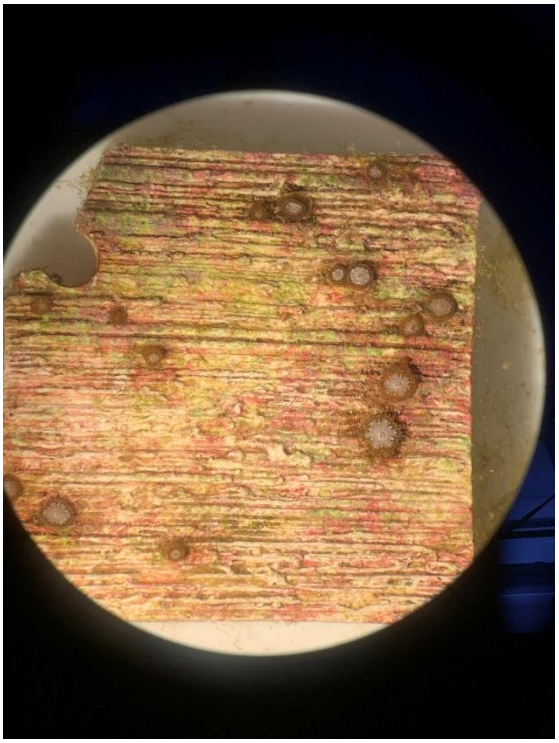
Figure 15: Four and half months Diploria labyrinthiformis.

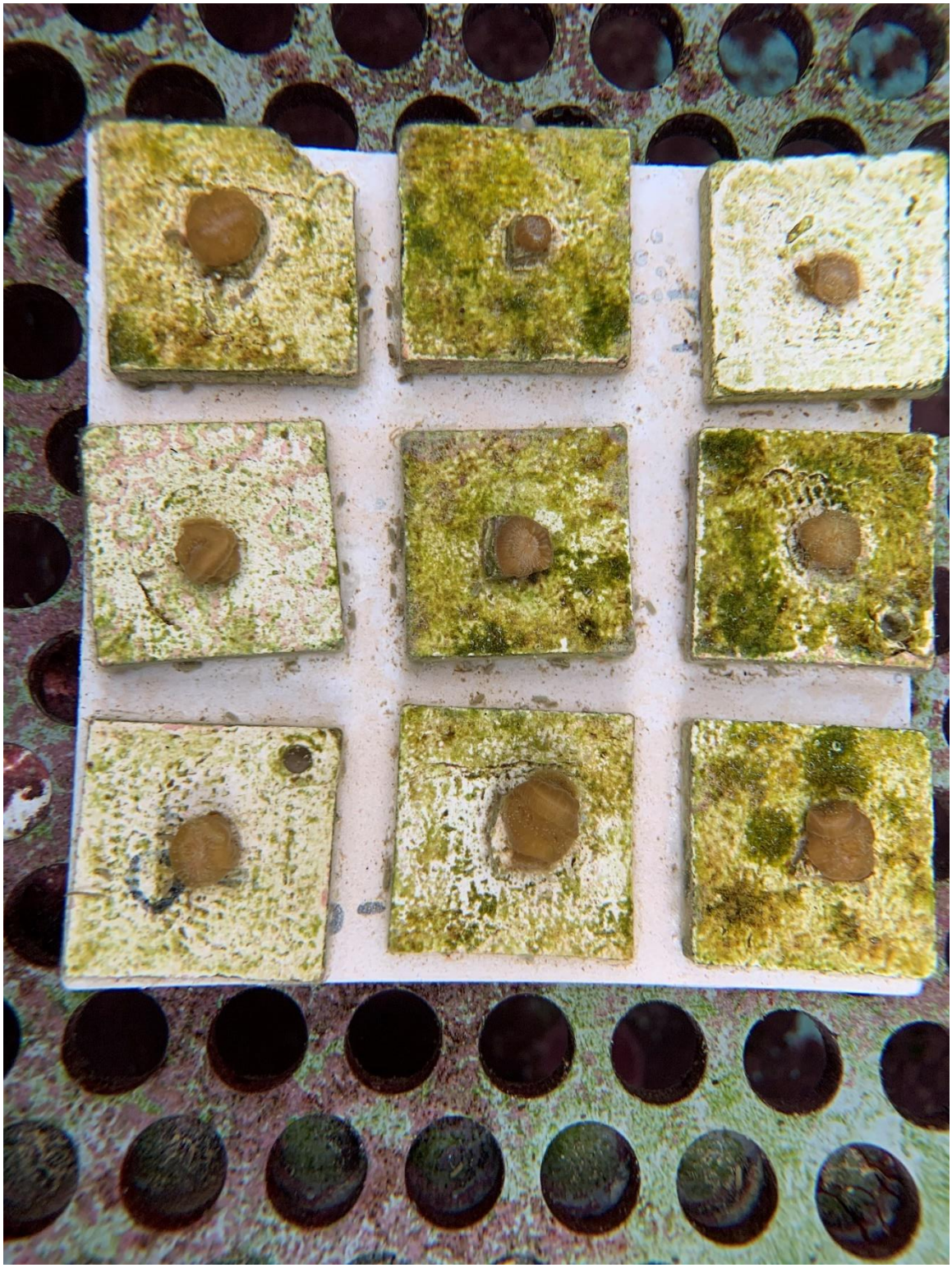

Figure 16: 1 year old Diploria labyrinthiformis *sexual recruits*

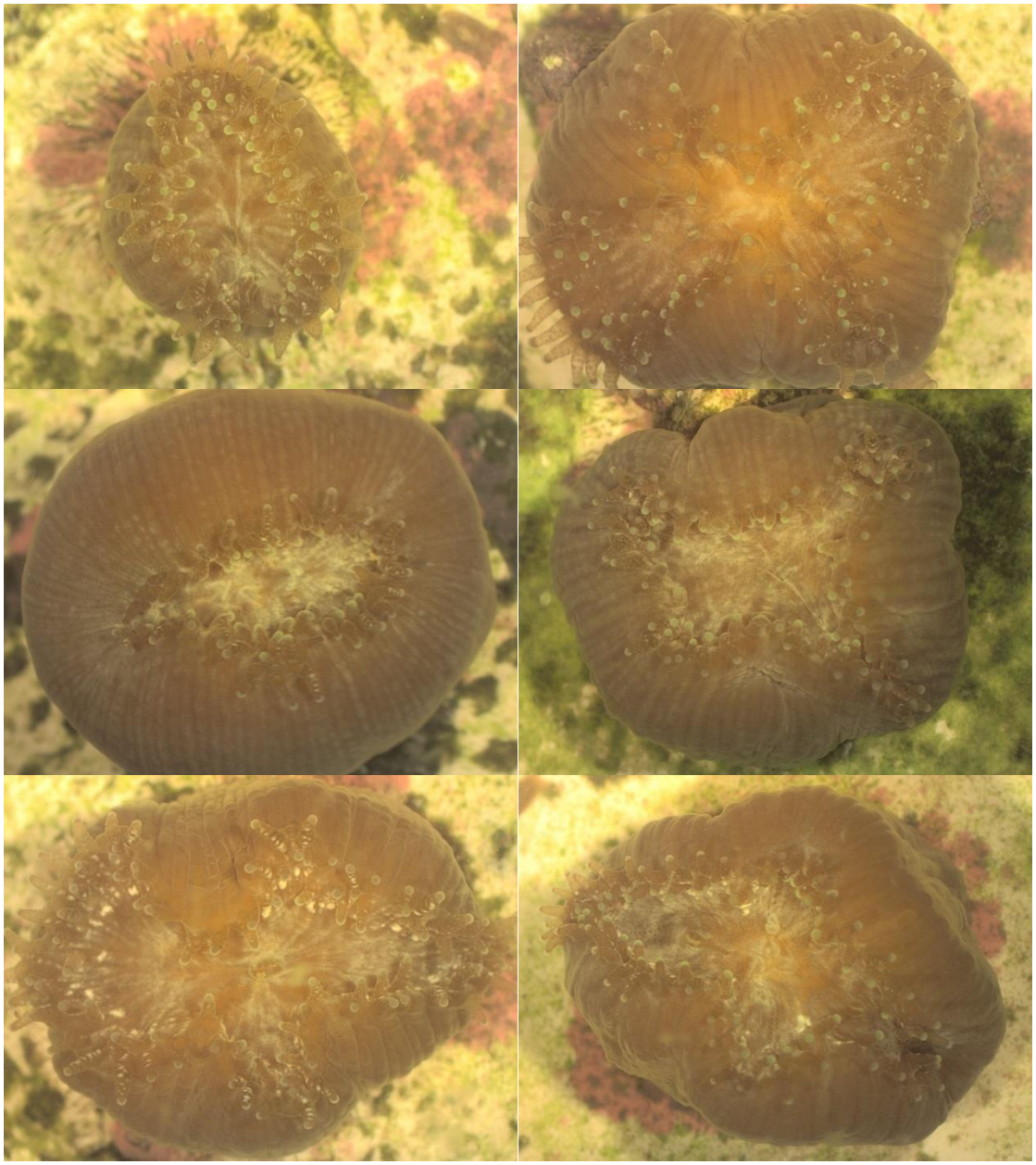

Figure 17: Diploria labyrinthiformis *sexual recruits on June 14, 2021 (13 months old).*

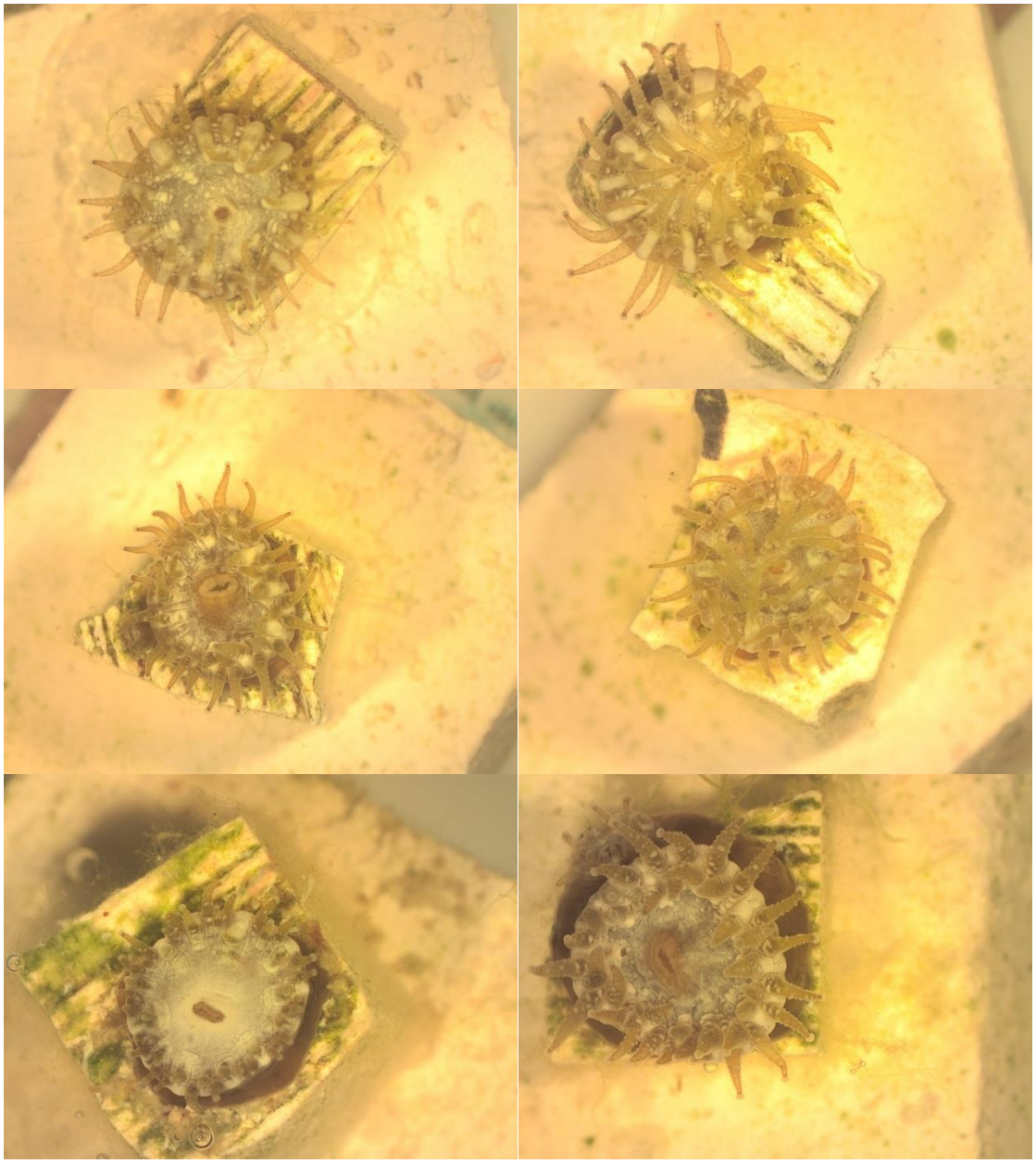

Figure 18: Montastraea cavernosa *sexual recruits on June 14, 2021 (11 months old).*

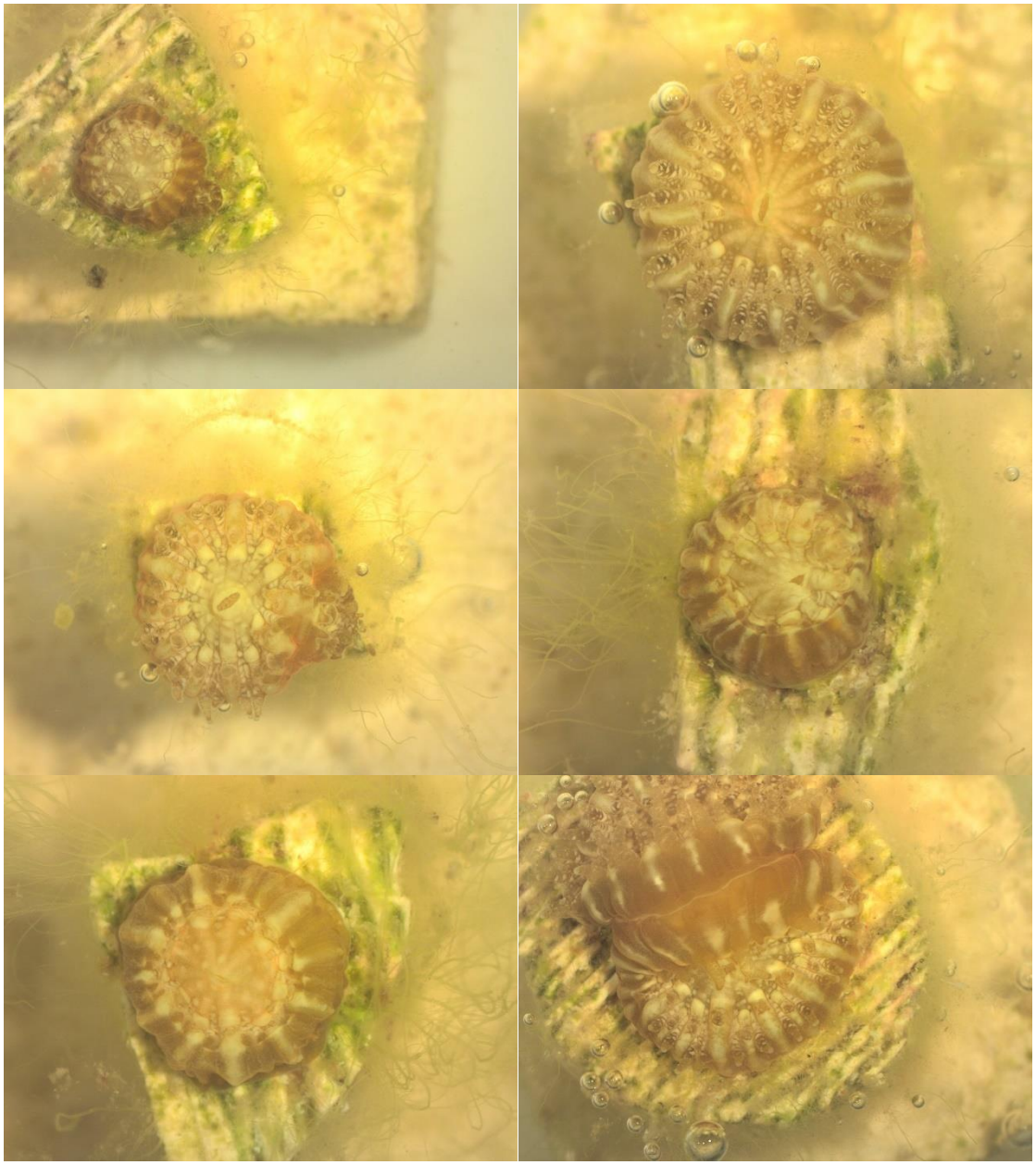

Figure 19: Colpophyllia natans *sexual recruits on June 14, 2021 (10 months old) (note that the one on the bottom right is a chimera).*

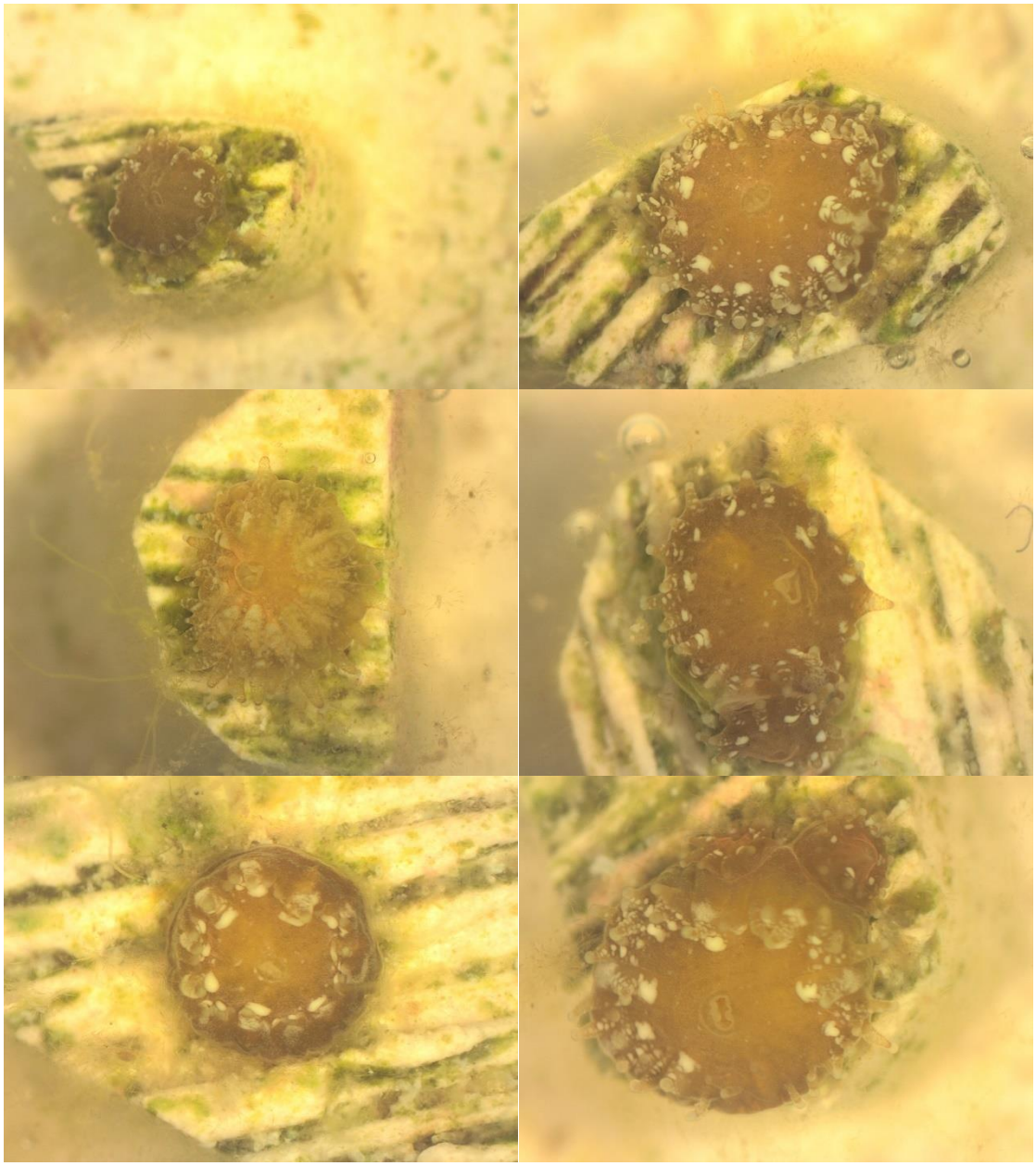

Figure 20: Pseudodiploria strigosa *sexual recruits on June 14, 2021 (10 months old).*

In addition, we had initially settled and reared *Dendrogyra cylindrus* sexual recruits, which remained healthy and growing until the Winter, but suffered a mass mortality from unknown causes. They were kept in the same tank as the sexual recruits from all other species, and these were not affected, so we do not think it was a water quality issue. One potential cause may be the lack of their species-specific symbionts *Breviolum dendrogyrus*. The *D. cylindrus* sexual recruits had acquired algal symbionts (Figure 21) but these are not the species these corals have in the wild as that was not present in our tanks.

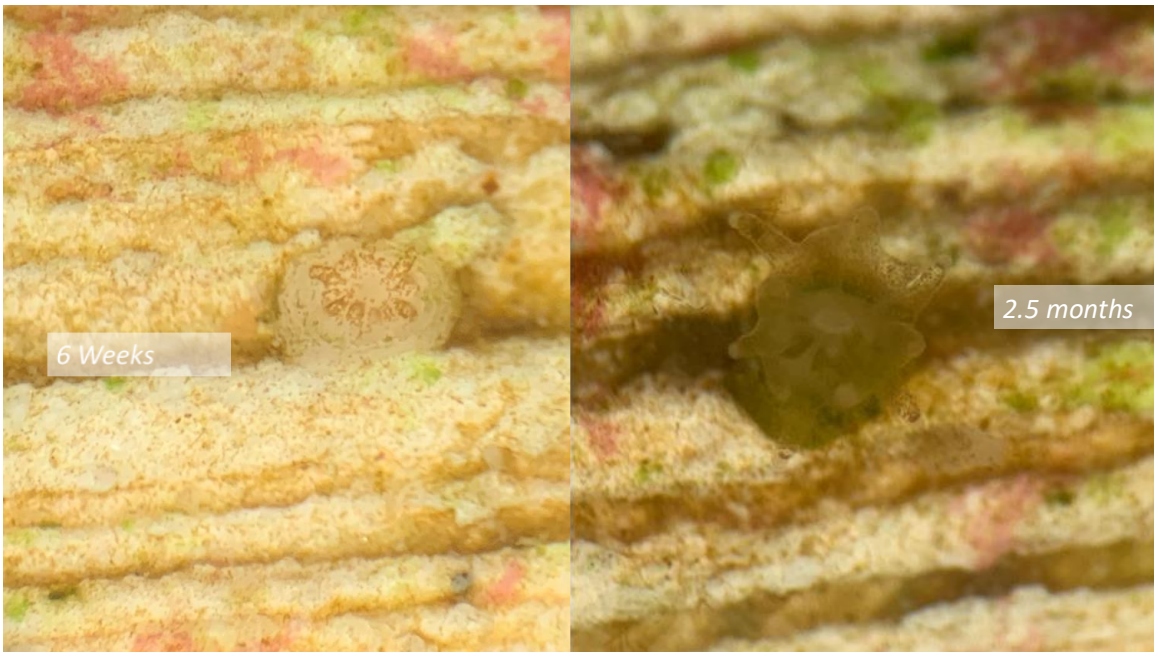

Figure 21: Dendrogyra cylindrus *sexual recruits (6 weeks and 2.5 months old).*

3.6. Task 6: Microfragmentation and grow-out of microfragments in land-based nursery (Renegar and Figueiredo)

Renegar's lab:

Fragment survivorship. All three colonies of *S. siderea* responded well to microfragmentation, with an overall 95% survivorship (Figure 22A). For colony 1, all fragments survived (100% survivorship), with limited (11%) tissue loss in only one fragment (Figure 22B). Four fragments from colony 2 did not survive (87.5% survivorship), and 2 of surviving fragments had minimal tissue loss (6.5-7.9%). One fragment from colony 3 did not survive (96.9% survivorship), and 4 fragments had some tissue loss (5.3-24.7%). In contrast, 40.6% survivorship was observed for microfragments of *M. cavernosa*, with 100% mortality in 19 fragments (Figure 22A). Eight of the surviving fragments had variable levels (5.7-76.2%) of tissue loss.

Growth rate. Mean growth rate of the surviving microfragments for both species is shown in Figure 23. Overall growth rate was higher for *S. siderea* compared to *M. cavernosa* (Figure 23A), and substantial variability was observed in growth rate of microfragments from different *S. siderea* colonies (Figure 23B).

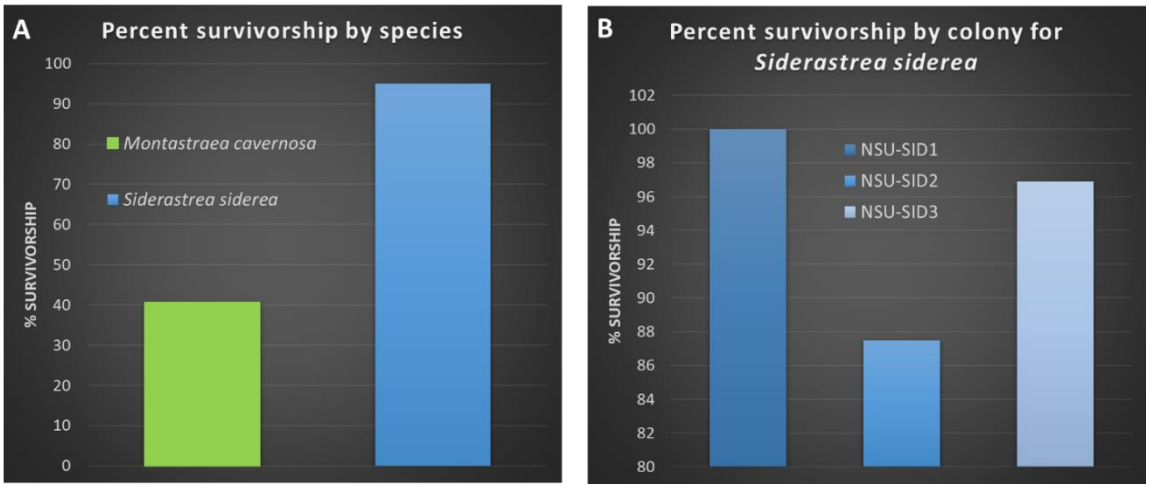

Figure 22. A) Overall % survivorship for microfragments of Montastraea cavernosa *and* Siderastrea siderea*; B) % survivorship by colony for* Siderastrea siderea *microfragments.*

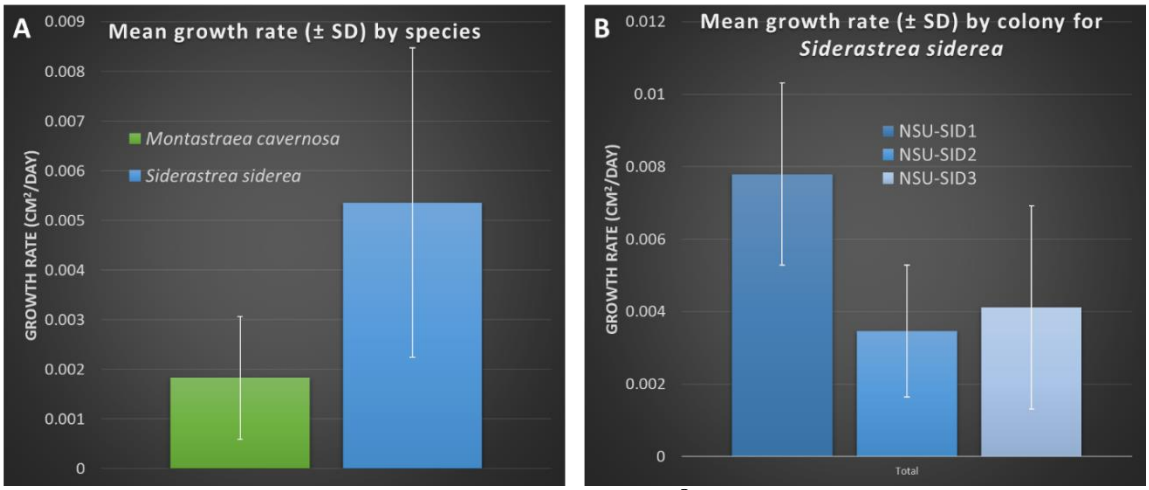

Figure 23. A) Overall mean growth rate (± SD, in cm^2/day) for microfragments of Montastraea cavernosa *and* Siderastrea siderea*; B) mean growth rate (± SD, in cm^2/day) by colony for* Siderastrea siderea *microfragments.*

Figueiredo's lab:

Microfragment survival and growth

Corals grown in the land-based nursery had 98.6% survivorship after 6 months. There was only one mortality 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. Their growth rates are summarized on Table 5 and Figure 24.

Table 5: Average growth rates of coral microframents of different initial size classes

Species	Class size	Average growth rate ($cm^2 month^{-1}$) ± S.E.
O. faveolata	Large	0.162 ± 0.109
	Small	0.070 ± 0.054
S. siderea	Large	0.409 ± 0.054
	Small	0.182 ± 0.022
P. clivosa	Large	1.341 ± 0.196
	Small	1.032 ± 0.185
D. labyrinthiformis	Large	0.936 ± 0.082
	Small	0.732 ± 0.060

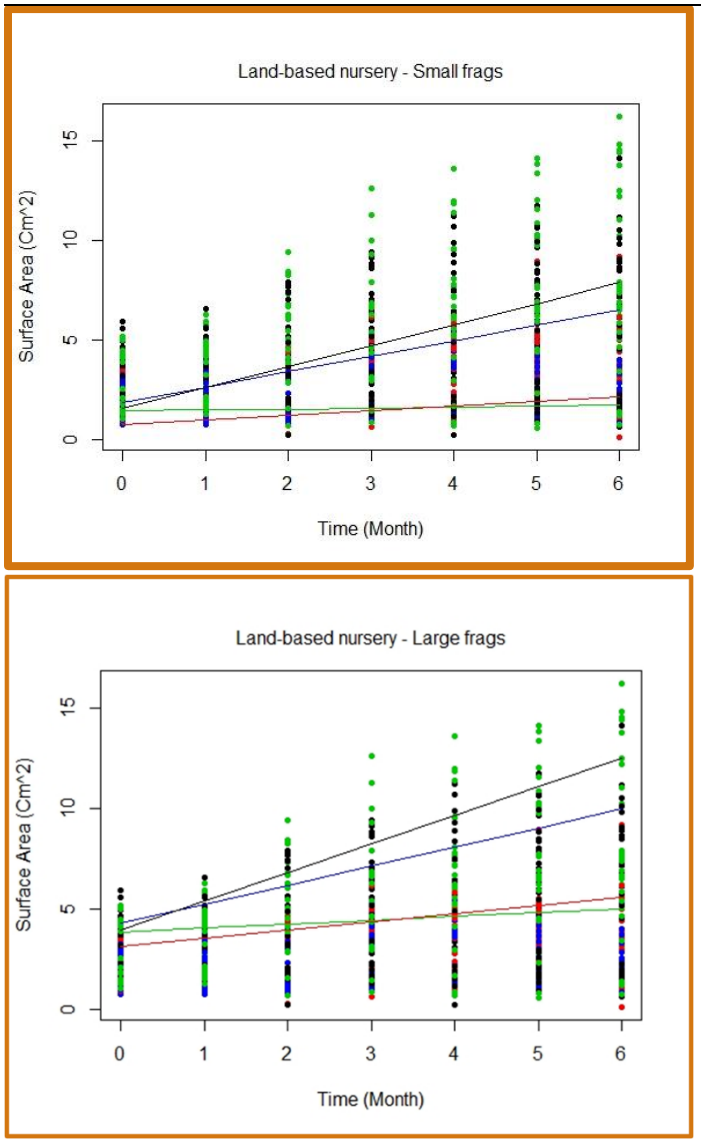

Figure 24. Average growth of the coral fragments over time, by initial size class (green - Orbicella faveolata*, red -* Siderastrea siderea*, grey-* Pseudodiploria clivosa*, blue -* Diploria labyrinthiformis).

3.7. Task 7: Grow-out of newly settled corals and microfragments at the offshore nursery (Gilliam)

Sexual recruits

After seven weeks at the *in situ* nursery, the kebabs were collected on May 24, 2021, and each settlement tile was analyzed for juvenile survival (Figure 25).

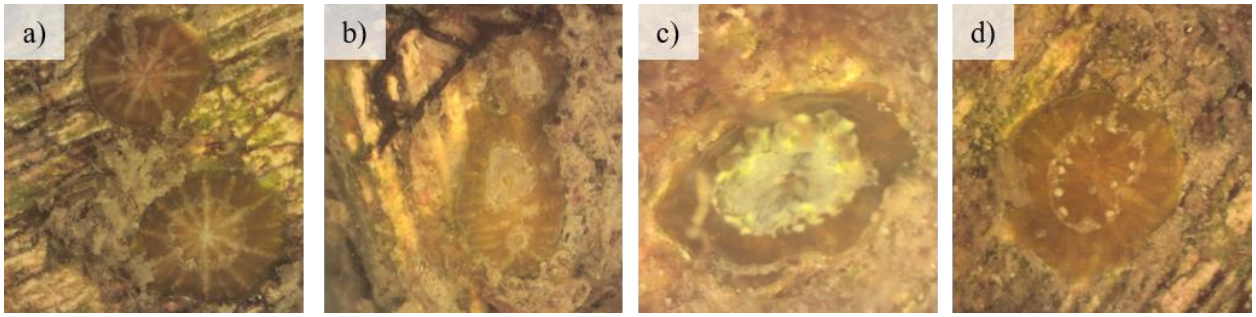

Figure 35. Photos of juvenile corals after seven weeks in situ, a) C. natans, *b)* D. labyrinthiformis, *c)* M. cavernosa, *and d)* P. strigosa.

Pooled survival by species was 83% for *C. natans*, 49% for *D. labyrinthiformis*, 55% for *M. cavernosa*, and 74% for *P. strigosa.* A survival analysis with tile, species, and kebab as factors found which kebab the juveniles were placed on was the only factor that significantly impacted survival (Survival Analysis Cox model, p = 0.0005). Mean survival ± SD per kebab for each species was 83 ± 4% for *C. natans*, 50 ± 50% for *D. labyrinthiformis*, 56 ± 26% for *M. cavernosa*, and 74 ± 20% for *P. strigosa*. The survival for each kebab is shown in Figure 25. They were returned to the *in situ* nursery on May 27, 2021.

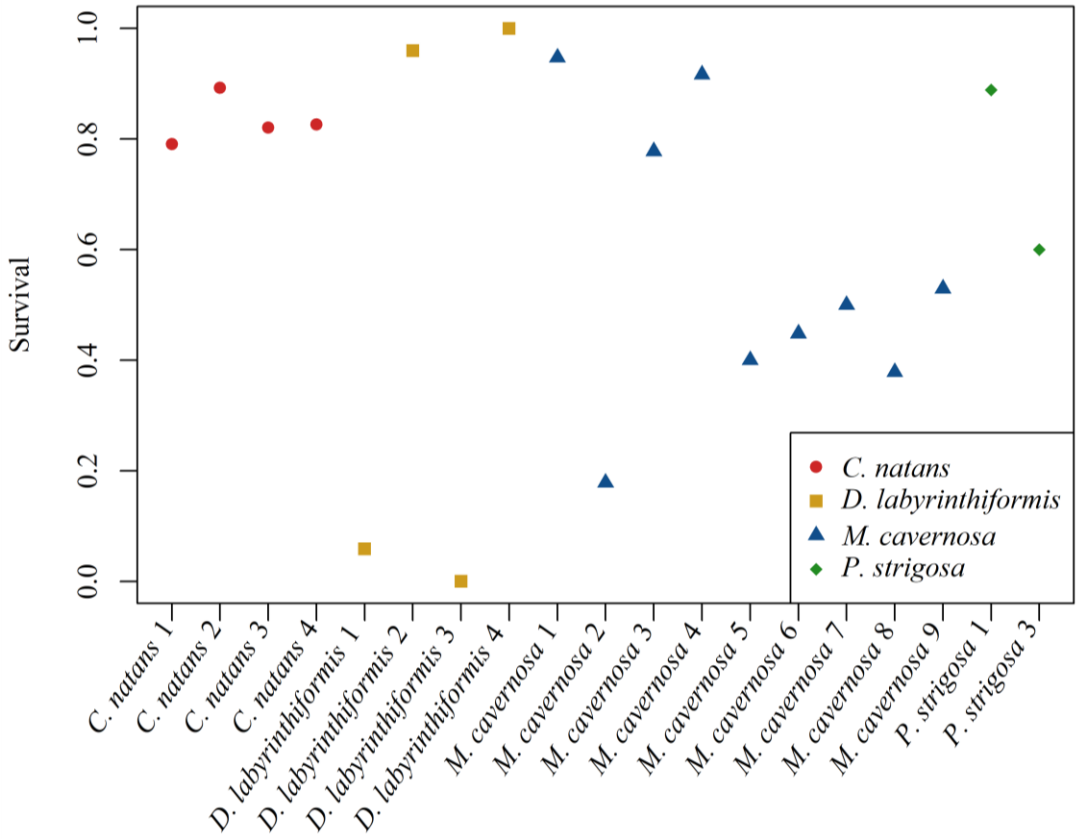

Figure 26. Percent survival of each kebab (represented by the number after the species name).

The significance of individual kebab is likely due to the differences in overgrowth communities observed on the tiles between each kebab. Tiles on the same kebab were observed to have similar overgrowth communities but between kebabs these communities differed. Kebab *D. labyrinthiformis* 3 had 0% survival and Figure 27a shows a representative photo of the overgrowth community of the tiles on the kebab. The overgrowth community on kebab *D. labyrinthiformis* 3 was observed to be more filamentous and may have shaded or abraded the juvenile corals on the kebab causing the complete mortality seen. Figure 27b is a representative photo of the overgrowth community on kebab *D. labyrinthiformis* 2, exposed tile can be seen around the juvenile *D. labyrinthiformis* in the photo. Figure 27c is a photo of two juvenile *M. cavernosa* on kebab *M. cavernosa* 7. These two corals were partially covered by the overgrowth on the tile but were still alive. The different overgrowth communities between may have caused differential ability to outcompete the juvenile corals. This may explain why the kebab was the factor that significantly affected juvenile coral survival.

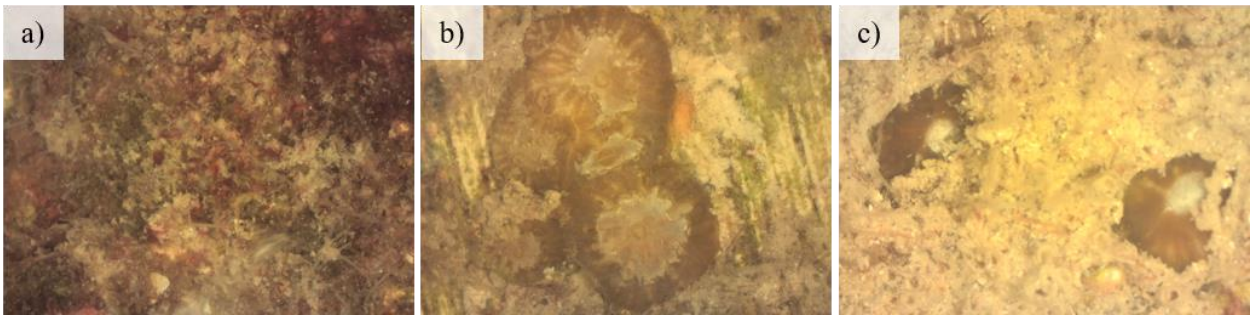

Figure 27. a) Representative photo of overgrowth community on kebab D. labyrinthiformis *3; b) representative photos of overgrowth community on kebab* D. labyrinthiformis *2; and c) representative photos of overgrowth on kebab* M. cavernosa *7.*

Microfragments

After six weeks on the coral table, six microfragments (out of 90) died and four went missing. One microfragment was lost during transport to the nursery, the remaining three went missing during the following six weeks. Pooled survival excluding the missing microfragments was 93%. All six of the dead microfragments were *D. labyrinthiformis* spread across five genotypes (Tables 6 and 7).

Table 6. List of sample size by species with percent survival, missing corals were excluded from percent survival calculation.

Species	**n**	**Percent Survival**
D. labyrinthiformis	62	90%
O. faveolata	4	100%
S. siderea	23	100%

The mean ± SD initial size for each of the three species was 1.97 ± 0.72 cm^2 for *D. labyrinthiformis*, 1.65 ± 0.79 cm^2 for *O. faveolata*, and 1.09 ± 0.49 cm^2 for *S. siderea.* Microfragment size initially decreased for all three species during the week two monitoring likely due to tissue loss cause by the stress of being moved *in situ* (Figure 28). After six weeks *D. labyrinthiformis* was the only species that saw an increase in mean size. *Siderastrea siderea* mean size showed negligible increases from weeks two to six. *Orbicella faveolata* mean size was on an increasing trend and may continue to increase beyond this study period.

Table 7. List of sample size by genotype with percent survival, missing corals were excluded from percent survival calculation.

Species	Genotype	n	Percent Survival
D. labyrinthiformis	DLAB1	5	80%
D. labyrinthiformis	DLAB2	7	86%
D. labyrinthiformis	DLAB3	6	83%
D. labyrinthiformis	DLAB4	6	100%
D. labyrinthiformis	DLAB5	4	50%
D. labyrinthiformis	DLAB6	2	100%
D. labyrinthiformis	DLAB7	3	100%
D. labyrinthiformis	DLAB8	2	100%
D. labyrinthiformis	DLAB9	3	100%
D. labyrinthiformis	DLAB10	5	100%
D. labyrinthiformis	DLAB11	5	80%
D. labyrinthiformis	DLAB12	7	100%
D. labyrinthiformis	DLAB13	7	100%
O. faveolata	OFAV1	1	100%
O. faveolata	OFAV2	1	100%
O. faveolata	OFAV3	2	100%
S. siderea	SSID1	4	100%
S. siderea	SSID2	4	100%
S. siderea	SSID3	6	100%
S. siderea	SSID4	1	100%
S. siderea	SSID5	1	100%
S. siderea	SSID6	1	100%
S. siderea	SSID7	3	100%
S. siderea	SSID8	1	100%
S. siderea	SSID9	2	100%
Total		**89**	**93%**

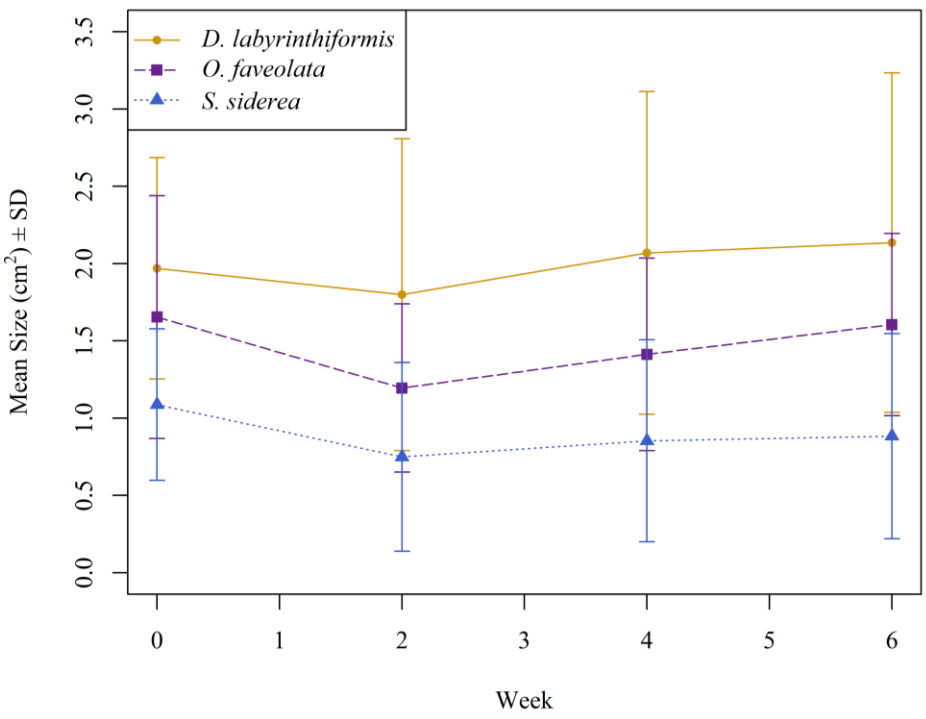

Figure 28. Mean size (cm^2) ± SD of all microfragments over time pooled by species.

Unknown tissue loss was recorded on both *D. labyrinthiformis* and *O. faveolata* (Figure 29). It was defined as tissue loss without any observable cause. This tissue loss may have been induced by the stress caused from moving the microfragments to the coral table. Paling and partial bleaching were also conditions on all three species and unknown tissue loss may have been the result of tissue loss associated with those conditions. Paling and partial bleaching were the most prevalent conditions for all species and two causes seem most likely. The first is the microfragments may have paled or partially bleached in response to the increased *in situ* light levels. The *ex situ* conditions the microfragments were reared were unable to fully replicate the light the microfragments would experience *in situ*. When the microfragments were then placed *in situ* they may have paled or partially bleached either as a stress response or to adjust the abundance of zooxanthellae in their tissues to more appropriate amounts. The second hypothesis is the microfragments may be in the process of changing the clade of their zooxanthellae from one that was more appropriate for their *ex situ* conditions to one that is more appropriate for their *in situ* conditions. This process can take time and involves the expulsion of current zooxanthellae so another clade can be taken in by the tissues. The expulsion of zooxanthellae would be demonstrated by a loss of color such as paling or partial bleaching. Given the low prevalence of complete bleaching and the low mortality, it seems possible the microfragments may recover from their sublethal paling and partial bleaching as time passes. Finally, fish bites were the only predation observed on the microfragments *in situ.* All three species were predated with *S. siderea* predated the least. Fish bite prevalence decreased over time though it is not clear why.

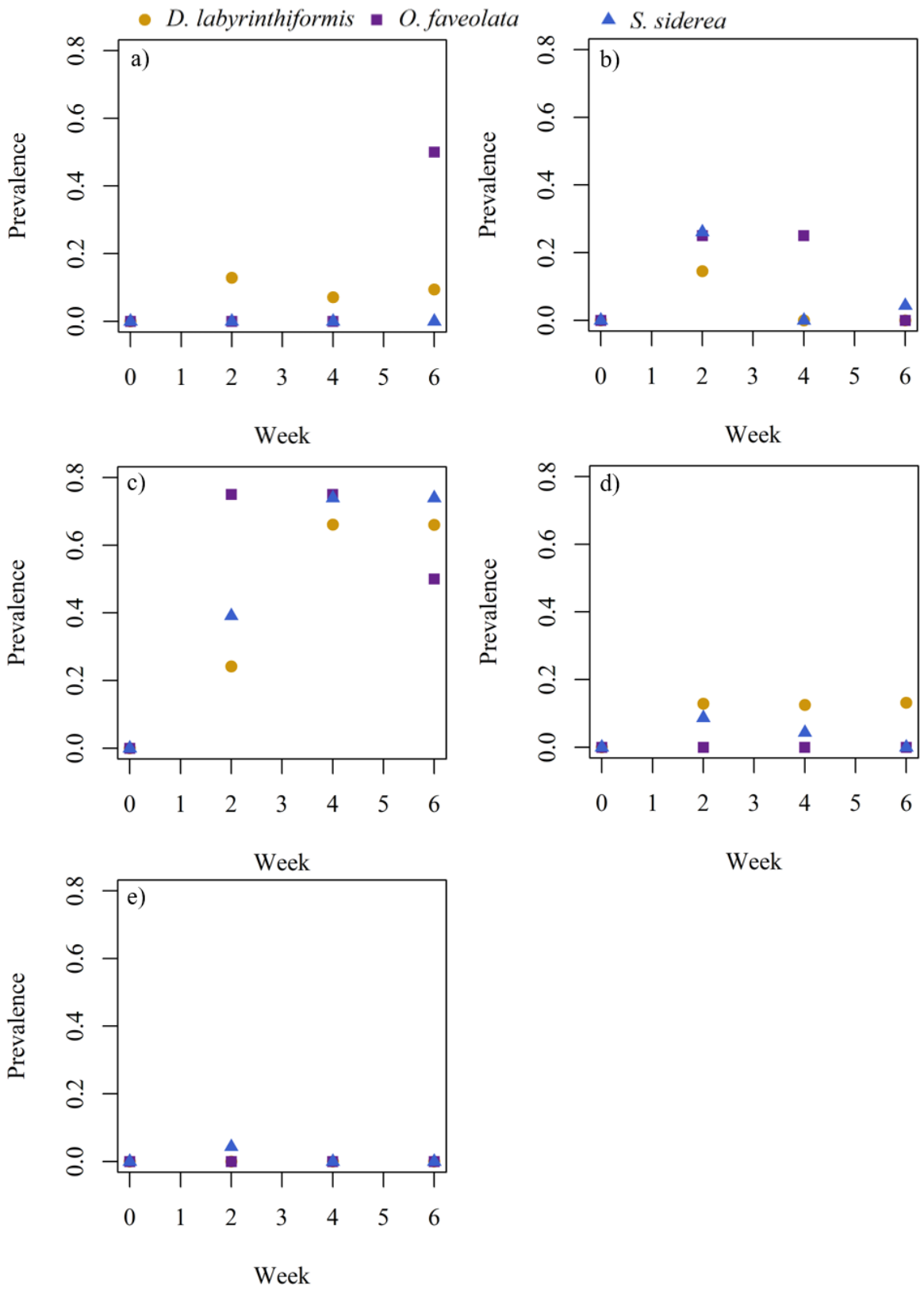

Figure 29. Prevalence by species over time of a) unknow tissue loss, b) fish bites, c) paling, d) partial bleaching, and e) bleaching.

3.8. Task 8: Preserve coral genotypes of disease impacted species and induce gonad maturation and spawning in captivity (Figueiredo)

Corals in the land-based nursery largely remained healthy throughout the year, but some losses were observed. The most significant mortality occurred right after collection (2 *Orbicella faveolata* and 5 *P. clivosa*), and then in the Winter (December-March) (multiple *Montastraea cavernosa*).

At the end of this project (June 2021), the NSU land based nursery is holding a total of 140 (larger than 10cm diameter) colonies (rescue and non-recue), specifically 6 *Colpophyllia natans* (all healthy), 40 *Montastraea cavernosa* (39 healthy, and one that is continuously improving after tissue loss this winter but still has not grown back tentacles), 30 *Orbicella faveolata* (25 healthy, 1 with discoloration, 3 with minor tissue loss this month, 1 was pale but regaining color), 20 *Pseudodiploria clivosa* (17 healthy, 2 with minor tissue loss this month, 1 was paling but is regaining color), 25 *Pseudodiploria strigosa* (24 healthy, 1 has minor tissue loss), 5 *Diploria labyrinthiformis* (all healthy) and 14 *Siderastrea siderea* (all healthy).

Seventy-seven out of the initial 89 ‘rescue’ colonies remained alive throughout the year (87% survival, Appendix Table A1). The species experiencing the greatest mortality, associated with an unknown disease, was *Montastraea cavernosa*. After an initial mass mortality in December and January, which the use of antibiotics was not able to contain, we recurred to the experimental use of probiotics developed by Dr. Blake Ushijima. The treatment of *Montastrea cavernosa* with probiotics developed for this species showed to be very effective when the colonies were just beginning to get sick, moderately effective when disease was moderately spread, and inefficient when very diseased.

3.9. Task 9: Cryopreservation (Figueiredo)

The materials and supplies to cryopreserve coral sperm were ordered as described in Table 8.

Table 8: Materials and supplies for coral sperm cryopreservation

Item	Cost per unit ($)	Number	Total ($)
liquid nitrogen	171	1	171
transfer dewer	1384	1	1384
storage dewer	2056	1	2056
canes	8	20	160
50 ml falcon tube	190	1	190
15 ml falcon tube	136	1	136
cell strainers	112	1	112
cryo marker	48	1	48
100-1000 ul pipette	881	1	881
1- 10 ul pipette	881	1	881
1000 ul pipette tips	92	1	92
100 ul pipette tips	111	1	111
racks for holding cryo vials	24	1	24
hemocytometer	183	1	183
cryo gloves (for DMSO)	60	1	60
cryo gloves (for liquid nitrogen)	242	1	242
DMSO	40	1	40
temperature probe	64	1	64
temperature reader	190	1	190
large forceps (straight)	68	2	136
cryo rack (custom make?)	150	2	300
hot water bath	50	1	50
low liquid nitrogen level alarm	895	1	895
Metal Yard Stick	23	1	23

4. DISCUSSION

Fecundity: The assessment of coral fecundity through histology revealed that most corals in field in Broward County are reproductive. About 67% of the *P. clivosa* and nearly all *Orbicella faveolata* were fecund. The exact time they spawn, however, is not clear, but most likely most spawn before or during the expected September spawning window, with some potentially spawning in August. The induction of gonad maturation of *Montastraea cavernosa* in indoor recirculating systems is highly successful, with 22 out of 24 corals having developed eggs or sperm.

Spawning: *Montastraea cavernosa* colonies held in an indoor recirculating system for the past year spawned synchronously when their temperature, and (moon and sun) light cycles were manipulated, with spawning occurring mostly in August 2020. Colonies collected from the wild or monitored in the field did not spawn within the expected September window, suggesting they may have spawned sometime before the expected window, as it had been observed in *Acropora cervicornis* in this area.

Fertilization, Larval rearing, and settlement: The techniques to fertilize coral gametes are well established, with success being higher (>95% fertilization) when a larger number of colonies spawns in the same night. The mass-scale larval rearing system proved to be an efficient and practical way to rear large numbers of coral larvae with minimum handling. Larvae of all coral species successfully settled on tiles conditioned for two months in the indoor recirculating system, and sprinkled with (crushed) crustose coralline algae.

Grow-out of recruits in land-based nursery: The techniques to rear sexual recruits in captivity keeps being improved, with survival and growth rates increasing from year to year. The training of staff, optimization of light levels and improved food led to higher survival and growth rates than observed in previous years at this facility. The only exception was *Dendrogyra cylindrus* sexual recruits, which after an initial high survival and growth, died in mass for unclear reasons. Algal overgrowth seems to be the highest cause of mortality in all species.

Survival of sexual recruits at offshore nursery: Survival of the 6 months sexual recruits was relatively moderate (83% for *C. natans*, 49% for *D. labyrinthiformis*, 55% for *M. cavernosa*, and 74% for *P. strigosa*) six weeks after deployment. This survival was, as expected, lower than in the land-based nursery; however predation did not appear to be very high. The kebabs seem to be an effective technique to prevent fish to bite the tiles. The major cause of mortality was overgrowth by algae or other benthic animals that colonize the tiles where the little recruits were growing.

Microfragmentation and grow-out of corals in land-based nursery: Mass scale coral propagation through microfragmentation at *ex situ* nurseries is viable as each colony can be cut to generate large numbers of microfragments, and these present very high survival and high growth rates. The low survivorship observed in the *M. cavernosa* microfragments is likely attributed to the small size of the microfragment ($1cm^2$) relative to their large polyp size. In sum, the optimal size of microfragments differs between species, likely related to their polyp size, with $3cm^2$ appearing to be good for most species.

Grow-out of microfragments in offshore nursery: Our results suggest that while the grow-out of massive corals through processes of microfragmentation in offshore nurseries is in its infancy, it appears to be very successful. Six weeks after deployment at the offshore nursery, the microfragments of *O. faveolata* and *S. siderea* experienced no mortality (100% survival). Six microfragments of *D. labyrinthiformis* died (90% survival). Growth was minimal likely because the slow growth rates of these species, predation (fish bites), and initial adaptation to the new environment.

Preservation of corals in captivity: *Ex situ* nurseries will play a fundamental role in the preservation and propagation of existing genotypes for future restoration. These nurseries will also be key to induce and assist the sexual reproduction of these disease-impacted species in captivity, forming new genotypes and producing a high quantity of new individuals to use in future restoration efforts. The preservation/banking of corals

representing the genotypic diversity of these species in captivity is feasible, likely due to the ability to provide the colonies with good water quality and abundance of food. Most corals remained healthy at the land-based nursery (87%). Mortality occurred right after collection (7 colonies). Colonies of *Montastraea cavernosa* experienced a disease (not SCTLD), which was not treatable with antibiotics alone, but was treatable with probiotics if treatment occurred early on. *Orbicella faveolata* seemed more vulnerable than other species to alkalinity swings, experiencing some paling or tissue loss, but most recovered after treated.

Cryopreservation: equipment was ordered and staff were trained in cryopreservation techniques.

5. RECCOMMENDATIONS FOR THE FUTURE

Fecundity: Since corals were found to be fecund, but spawning was not observed within the expected time window, we recommend the use of permanent underwater cameras to document the day and time these species spawn in Broward County. Observations of spawning at the land-based nursery, particularly the outdoor ones, this coming August and September may also provide some clues.

Spawning: Since *Montastraea cavernosa* colonies held in an indoor recirculating system for the past year spawned synchronously, thus this technique should be expanded to the other species held in captivity. It remains unclear if the synchrony of spawning can also be achieved in the temperature-regulated outdoor tanks which experience natural light cycles, although are likely exposed to light pollution. This will be tested in the following year.

Fertilization, Larval rearing, and settlement: As fertilization is typically very high, and mass-larval rearing is also successful, we recommend the development of mass-settlement system in which larvae would be transported from the mass-scale larval rearing tanks by gravity to settlement tray, minimizing handling and thus allowing to upscale ex situ coral rearing for restoration. Funds to develop such a system have been allocated.

Grow-out of recruits in land-based nursery: The techniques to rear sexual recruits in captivity keeps being improved, with survival and growth rates increasing from year to year. The training of staff, optimization of light levels and improved food led to higher survival and growth rates than observed in previous years at this facility. While we cannot assure it, we believe the quality of the gametes and larvae produced at ex situ nurseries is also superior, leading to higher survival and growth. The major constraint to the grow-out of juveniles *ex situ* is algal overgrowth. In tanks with adult corals or microfragments, algal overgrowth is typically controlled by herbivores such as *Litopoma* snails and sea urchins. However, this cannot be used during the grow-out of newly settled corals as they often accidently graze them. The use of smaller snails has been found to be

insufficient. Co-cultures with newly settled sea urchins and/or snails have been found to be a promising way to overcome algal overgrowth during the grow-out of new settled corals. Dr. Figueiredo's lab is beginning to test culturing herbivores. If successful, co-culture may be tested in following years. Another constrain to the culture of sexual recruits is the absence of the algal symbionts species more commonly found in the adult corals. When the facility has adult corals of the same species, we recommend the placement of a few adult colonies by the inlet of the tank; however, care should be taken so that adult corals experience high light levels while sexual recruits remain under a much lower light regime. In the absence of the adult corals of the same species, as it happened with *D. cylindrus*, failure is likely guaranteed. Unfortunately, the culture methodology of most algal species that commonly occur and dominate corals is not developed or is very unreliable (low quantities that crash frequently, Dr. John Parkinson, personal communication).

Survival of sexual recruits at offshore nursery: Survival of sexual recruits at the offshore nursery was not as high as desired (i.e., was lower than at the land-based nursery), but this was mostly due to overgrowth. The use of kebabs to reduce predation by fish was effective. We recommend the use of different material to settle corals. Specifically, we recommend the use/development of much smaller tiles or structures (e.g., small ceramic buttons) that minimize the free space (hard substrate) that could be occupied by other species such as barnacles or algae, but that could be also strung in a 'kebab' fashion.

Microfragmentation and grow-out of corals in land-based nursery: The low survivorship observed in the *M. cavernosa* microfragments is likely attributed to their small size; it is therefore recommended that fragments of this species be at least 2-3 times larger in size and consist of 3-4 polyps to enhance survivorship and growth.
We do not recommend the use of very small microfragments (1 cm^2), at least not for species with large polyp sizes as *M. cavernosa*. We recommend the use of 3 cm^2 microfragments as this displayed high survival and growth for most species. Several additional corals of opportunity have been collected and added to the coral nursery stock in the time since this initial group of fragments was produced. We will be using these corals to expand the microfragment program by 5 to 10 fold, and increase the number of species represented.

Grow-out of microfragments in offshore nursery: We recommend the continuation of the grow-out of microfragments of massive corals in the offshore nursery as these displayed high survival and are less laborious, less time-consuming and cheaper. However, we also recommend the microfragments to be epoxied to structures with little free hard substrate to prevent overgrowth. A technique that would hang the coral directly (without a tile), for example drilling a hole in the microfragment and hanging using a monofilament so that it dangles from a tree, should be experimented.

Preservation of corals in captivity: The use of probiotics to cure diseased *M. cavernosa* is highly recommended to be applied, and more so right at the beginning of the disease.

We recommend the development of probiotics for other species, or at least testing the use of the ideal probiotics for M. cavernosa in other species from the same region.

Cryopreservation: we recommend the continuous investment on cryopreservation and staff training as ways to increase chances of fertilization (when colonies spawn in different days). However, the lower fertilization achieved with cryopreservation when compared to natural methods, is an indicative that while necessary to safeguard species that are nearly locally extinct or to "inject" new genetic diversity in low diversity and declining population, it may not be the best use of time and funds for species where broodstock is still abundant.

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