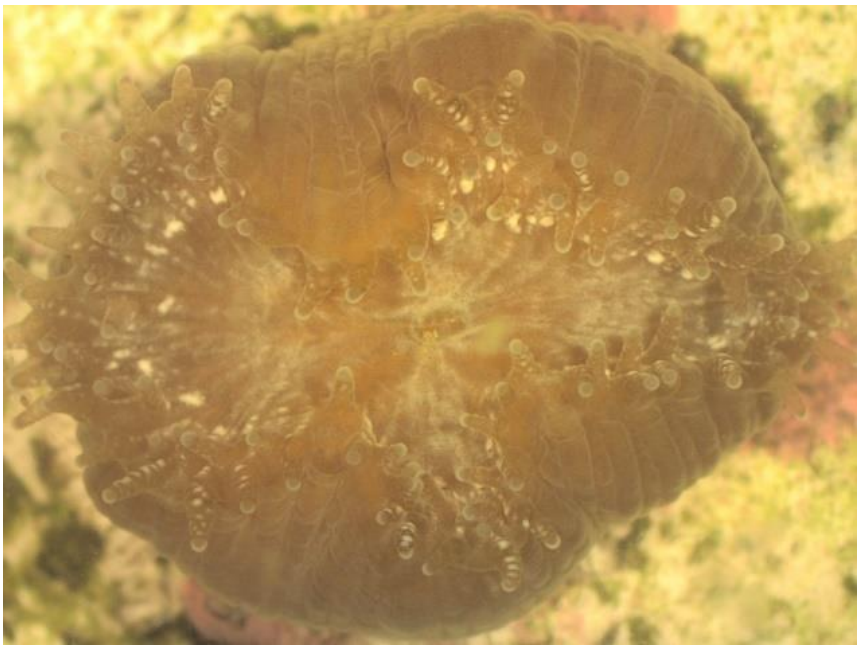
Coral Propagation: Land-based and Offshore Nursery Phase III

Final Report



Coral Propagation: Land-based and Offshore Nursery Phase III

Final Report

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January 30, 2022

Completed in Partial Fulfillment of PO B97E48 for

Florida Department of Environmental Protection
Coral Protection and Restoration Program
1277 N.E. 79th Street Causeway
Miami, FL 33138

This report should be cited as follows:

Figueiredo, J. 2022. Coral Propagation: Land-based and Offshore Nursery (Phase III). Final Report. Florida DEP. Miami, FL. Pp 38

This report was prepared for the Florida Department of Environmental Protection, Office of Resilience and Coastal Protection by Nova Southeastern University. Funding was provided by the Florida Department of Environmental Protection (Award No. B97E48) and the National Oceanic and Atmospheric Administration (NOAA) Coral Reef Conservation Program. The views, statements, findings, conclusions and recommendations expressed herein are those of the authors and do not necessarily reflect the views of the State of Florida, NOAA or any of its sub-agencies.

Acknowledgements

Thank you to the Florida Fish and Wildlife Conservation Commission State Wildlife Grant Program and the Florida Department of Environmental Protection Office of Resilience and Coastal Protection for supporting these efforts. We thank the Florida Coral Disease Advisory Committee for the large number of volunteers assisting in the meeting and planning of coral disease efforts. A big thank you to the Miami-Dade County Department of Regulatory and Economic Resources Environmental Resources Management Restoration & Enhancement Section for their help with the field efforts. We thank Lisa Gregg for assisting with permitting. We thank the many Nova Southeastern University Oceanographic Center (NSUOC) student volunteers for diving and lab assistance. Thanks to the DEP CPR staff including Kristi Kerrigan for contract and report-review coordination.

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List of Acronyms

ESA	Endangered Species Act
DEP	Florida Department of Environmental Protection
FCR	Florida's Coral Reef
NOAA	National Oceanic and Atmospheric Administration
NSU	Nova Southeastern University
SCTLD	Stony coral tissue loss disease

Executive Summary for Managers

The culture of corals at Nova Southeastern University's ex situ and in situ nurseries has progressively become more successful, but we are still encountering issues that require further optimization. One hundred and fifty-eight adult corals were kept in captivity at Nova Southeastern University's ex situ nursery, with minimal cases of disease and even less of death. Corals in both indoors and outdoor systems were induced to mature their gonads, however synchronous spawning was only successful in *M. cavernosa* kept indoors. Indoors, the lack of synchronous spawning was caused an inaccurate program that the Apex controller uses for moon light; we have not shifted to using Mobius to control the moon lights. Outdoors, light pollution enables corals to discern the moon light cycle, and thus may require to be placed under blackout curtain and artificial lighting during the month prior to spawning. Fertilization, larval rearing and settlement were very successful. Five hundred and fifty larvae were produced at NSU, of which nearly 100,000 larvae were donated to other institutions. Despite a large die-off on one of the systems (not caused by equipment failure nor water quality issues, but likely a microbial community shift), 2190 sexual recruits were successfully reared (9 months) from the larvae produced at NSU and donated by other institutions. The grow-out of microfragments and sexual recruits outdoors in tables seems to be mostly hampered by predation and may require refinement to prevent it. The co-culture of herbivores is promising to control algal overgrowth but requires testing at older life stages when light levels are higher, and algae grows more readily.

1. PROJECT DESCRIPTION

Florida's coral reefs are 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 the Florida's Coral Reef, and south to Dry Tortugas 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 Kristin Jacobs Coral Reef Ecosystem Conservation Area (ECA), the 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 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. The collection of gametes can be made 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 indoors 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.

In Phase I of this project (FY2020), we increased the capacity of NSU's land-based nursery by building 1 indoor and 8 outdoor recirculating aquarium systems to induce sexual maturation and spawning of coral species native to Florida, and collected corals of seven disease-impacted species to preserve their genetic diversity. This allowed NSU's land-based nursery infrastructure to increase and encompass 2 indoor and 12 outdoor independent aquarium systems to preserve existing genotypes and induce gonad maturation and spawning in captivity, 1 indoor mass-scale larval system composed of 8 tanks, 1 indoor independent recirculating aquaria system dedicated to early grow-out and 8 outdoor grow-out inter-connected tanks dedicated to grow-out. At the end of Phase II of

this project, the land-based nursery was holding 41 *Montastraea cavernosa*, 30 *Orbicella faveolata,* 18 *Pseudodiploria clivosa*, 25 *P. strigosa*, 4 *Colpophyllia natans,* 6 *Diploria labyrinthiformis*, and 14 *Siderastrea siderea* mature-size colonies of unique genotypes, all from the ECA. NSU also manages an offshore nursery where *Acropora cervicornis* is being grown extensively for restoration purposes, and *Orbicella faveolata, Siderastrea siderea, Porites astreoides, Agaricia agaricites, Pseudodiploria clivosa, Diploria labyrinthiformis* and *Montastraea cavernosa* are being grown on an experimental scale to optimize grow-out protocols of massive reef-building species in offshore nurseries.

In Phase II of this project (FY2021), we monitored for spawning and maintained colonies and reef-building coral species impacted by the disease, *Montastraea cavernosa, Orbicella faveolata, Pseudodiploria clivosa, P. strigosa,* and *Colpophyllia natans*, from the ECA which resisted the disease under conditions that intend to induce gonad maturation and synchronous spawning in captivity. We reared coral larvae and grew sexually-produced corals, and microfragmented and grew-out corals of opportunity in the land-based nursery and offshore nursery. This allowed 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.

For the Phase III of this project (FY2022), we proposed to use asexual and sexual reproduction techniques to propagate colonies/genotypes of reef-building coral species impacted by the disease, *Montastraea cavernosa, Orbicella faveolata, Pseudodiploria clivosa, P. strigosa, Diploria labyrinthiformis, Siderastrea siderea,* and *Colpophyllia natans,* from the ECA which resisted the disease, and to rear them until they reach a size suitable for outplanting. 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 will continue to induce gonad maturation and spawning of corals in captivity, collect gametes of colonies in the land-based nursery, assist gamete fertilization, rear larvae, settle them and rear juveniles, and propagate corals through microfragmentation. Corals will be grown in the land-based and offshore nurseries. These activities will preserve the most resilient genotypes of the most disease-impacted reef-building corals, from the ECA, propagate them to facilitate population recovery in future restoration projects. The outcomes of this project will provide an extensive number of colonies of the most resilient corals in the region and preserve 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 not to duplicate efforts, this project is targeting corals from the Southeast Florida ECA, where remaining corals have survived the disease outbreak. Since broadcast spawning coral species are known to be connected throughout the FCR, if the corals from the Southeast Florida ECA do not spawn (as in the previous fiscal year), we would fulfill the goals of this project using gametes or larvae collected from FCR corals outside this region, collected by other institutions. We continue to collaborate and coordinate activities with partners in other locations, sharing the best existent knowledge and practices to maximize the success for all.

2. METHODOLOGY

This work is being conducted under the State of Florida Special Activity License SAL-21-2238-SCRP.

2.1. Task 1: Coral spawning and gamete collection at land-based nursery (Figueiredo)

Beginning on the full moon of August 2021, 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, Colpophyllia natans, Diploria labyrinthiformis*, and *Siderastrea siderea* were observed until midnight for spawning. Note that these are the colonies that were collected in Broward County in the previous year, and that are being maintained in the tanks at NSU to preserve their genetic diversity (Task 5). The number of colonies that spawned each night were recorded. For gonochoric species, the eggs were collected by skimming the surface with a cup, while sperm was immediately collected with large (23mL) plastic pipettes to avoid dilution. For hermaphrodite species, egg and sperm bundles were collected by skimming the surface with a cup.

2.2. Task 2: Fertilization, larval culture, and settlement (Figueiredo)

At the land-based nursery, eggs, and sperm from colonies from each species that spawned in the land-based nursery were combined, keeping sperm concentration at 10^5 - 10^6 mL^{-1} to maximize fertilization. For both gametes collected in the field and in the land-based nursery, 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 will mimic historical conditions on the reef during the Summer (not necessarily current conditions) to maximize survival; water changes will be performed as needed to avoid toxic components, such as ammonia, to accumulate and harm the larvae.

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 a month on the indoor tanks and sprinkled with crustose coralline algae to induce larval settlement and metamorphosis and acquisition of algal symbionts. Twenty-four to 48 hours after the competent larvae were exposed to the conditioned tile, the tiles were observed under the scope 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.

Since not all species spawned, we received larvae from corals that spawned in Biscayne Bay and at the Florida Aquarium, and these were reared as described above to fulfill the goals of this and Task 3. Because broadcast spawning corals have been found to be well connected throughout FCR, these corals can in the future be outplanted within the ECA to restore this region.

2.3. Task 3: Grow-out of sexual recruits in land-based nursery (Figueiredo)

Newly-settled corals were initially 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 went 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 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 was maintained at 34-35 ppt. Reverse osmosis water was added daily to the sump to replace evaporated water and maintain salinity. Water quality tests were be 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 the same species were placed in the tank to release algal symbionts and facilitate symbiont acquisition by the

coral recruits. For the same effect, we cultured and seeded the tanks with algal symbionts from multiple species. 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 manually removed and, after corals reached 0.5 cm, with the help of small herbivorous snails to minimize coral mortality and promote growth.

Coral juveniles were reared at the land-based nursery until they reached a size suitable to be moved to the offshore nursery (Task 4).

2.4. Task 4: Grow-out of newly settled corals and microfragments at the offshore nursery (Gilliam)

Up to 600 newly settled corals and/or microfragments of stony coral tissue loss disease susceptible species were moved to the offshore nursery in fall 2021 or early 2022 for grow-out. These newly settled corals and/or microfragments were moved to the offshore nursery and deployed on suspended tree structures, wire platforms, and/or modules. Planar images were taken of all newly settled corals and/or microfragments at the time of deployment to the offshore nursery. For microfragments deployed to the nursery prior to the end of 2021, colony survival and condition were monitored approximately 2 weeks, 1-month, and 6-months after deployment. During each monitoring event, coral colony condition was recorded for all corals, and planar images were taken of at least 25% of the corals. For newly settled corals deployed in early 2022, the deployed settlement tile/plug were examined for colony survival in May 2022.

Up to 200 newly settled corals and/or microfragments moved to the nursery in 2021 were outplanted in fall 2021. The exact number of outplants was dependent upon survival and condition of the newly settled corals and/or microfragments prior to outplanting. Outplant colony survival and condition was monitored approximately 2 weeks, 1-month, and 6-months after deployment. During each monitoring event, coral colony condition was recorded for all corals, and planar images were taken of at least 25% of the corals.

2.5. Task 5: Preserve coral genotypes of disease impacted species and induce gonad maturation and spawning in captivity (Figueiredo)

NSU's land-based nursery has been holding an extensive number of colonies of the reef-building coral species from the Kristin Jacob's Coral Reef Ecosystem Conservation Area (ECA), specifically 41 *Montastraea cavernosa,* 30 *Orbicella faveolata*, 18 *Pseudodiploria clivosa,* 25 *P. strigosa,* 4 *Colpophyllia natans,* 6 *Diploria labyrinthiformis*, and 14 *Siderastrea siderea* colonies (totaling 138 colonies), all from the ECA. These colonies represent a significant part of the genetic diversity of the populations of these species on the ECA. Importantly, these genotypes survived the

disease event and thus possibly are more disease-resistant than genotypes from other areas along the Florida's Coral Reef not yet ravaged by SCTLD.

These colonies were maintained in 12 outdoor independent recirculating tanks and 2 indoor independent recirculating tanks (on room 242 of the Guy Harvey Oceanographic Center Building) to preserve their genotypes *ex situ* (genotype banking/caching), and induce their gonads to mature and spawning to occur synchronously (see Task 1 for the collection of gametes from these colonies), which was expected to allow us to produce offspring (described in Task 2 and 3) 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 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. 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 has been 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. 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.

2.6. Task 6: See Appendix from Reef Institute Sub-Contract

2.7. Task 7: Co-culture of coral recruits with herbivorous invertebrates (Figueiredo)

Adult colonies of *Porites astreoides* were collected in Broward County in early April 2022. Colonies were placed in a 1500L recirculating outdoor tank containing a larval release system comprised of an irrigation system leading into individual spouted bowls, that outflow into 105-micron mesh filters. Once larvae of *P. astreoides* were released, they were settled according to methods from Task 2. After larval release, all colonies were returned to their site of collection.

Three gravid female *Mithrax spinosissimus* were collected in Key West via snorkeling. Larvae were reared until metamorphosing to the juvenile stage following A. J. Spadaro's culture methodology. Crab juvenile grow-out tanks contained a rigid airline, continuous

water flow, and a 105-micron mesh filter cover on the outflow of the tank to prevent escape. The juvenile crabs were fed a diet of turf algae.

Four experimental treatments were created to test the effectiveness of juvenile *M. spinossimus* versus manual algal removal. The first treatment, algae was allowed to grow without removal. In the second treatment, algae were removed manually once per week. The third and fourth treatments consisted of low (2 crab/L) and high (4 crabs/L) densities of Caribbean King crabs ranging from 2-6 weeks of age. Tiles in the third and fourth treatments did not receive manual algal removal for the entirety of the experiment. In the third and fourth treatments, crabs were also fed turf algae.

For each treatment, twelve newly settled corals, on at least five tiles, were randomly placed into each treatment. There were two replicates created for each treatment, for a total of 96 corals represented. Experimental treatment tanks consisted of plastic 2.5L baskets suspended within an indoor recirculating system. Each basket had four holes covered in 105µm mesh to allow water flow into the basket, while not allowing juvenile crabs to escape. Corals in all treatments were fed a mixture of 1.23mL Polyp Lab ® Reef-Roids, 5mL Reef Nutrition ® Oyster Feast, live rotifers (26,400 rotifers/L) and 5mL Brightwell Aquatics ® Coral Aminos four days a week.

3. RESULTS

3.1. Task 1: Coral spawning and gamete collection at land-based nursery (Figueiredo)

We collected the spawn of three species, *Montastraea cavernosa*, *Orbicella faveolata* and *Pseudodiploria strigosa* (Table 1), but only *Montastraea cavernosa* had multiple colonies spawning heavily over several nights in July and August (Table 1, Figures 1 and 2). The others had only one or two colonies spawn or did not spawn at all. After discussing the subject with the teams from the Florida Aquarium (FLAQ) and University of North Carolina Wilmington (UNCW), we realized that the Apex system that controls the moon lights in our systems (also used by FLAQ and UNCW) does not imitate an accurate representation of the moonlight cycle, it uses an average daily change on the time of moonrise, when in reality this changes over time too. In some years, the difference from the actual cycle is minimum, but this year it had a much greater difference (up to 1.5 hrs), which may have derailed the spawning synchrony of most species. The team at FLAQ indicated that to follow the actual moon cycle they had to manually turn on and off the moon lights. It is possible that the species that did not spawn or that only had a few colonies spawn are more sensitive to the difference between sunset and moonrise than *M. cavernosa*. According to the most recent literature, the difference between sunset and moonrise seems to be essential for cuing spawning, thus we are currently researching the possibility of changing the control of moonlights to the Mobius system.

Coral colonies in the outdoor recirculating tanks were found to have eggs when histology was performed but were not observed spawning. The annual temperature cycle they were exposed to induced their gonads to mature, but the light pollution outdoors (mostly from

the Coast Guard property adjacent to NSU) interfered with the sun and moonlight exposure, preventing synchronous spawning. We are currently researching how this issue could be resolved.

We have also collected gametes of *Acropora cervicornis* from fragments of wild Broward colonies (Table 2, Figures 3 and 4)).

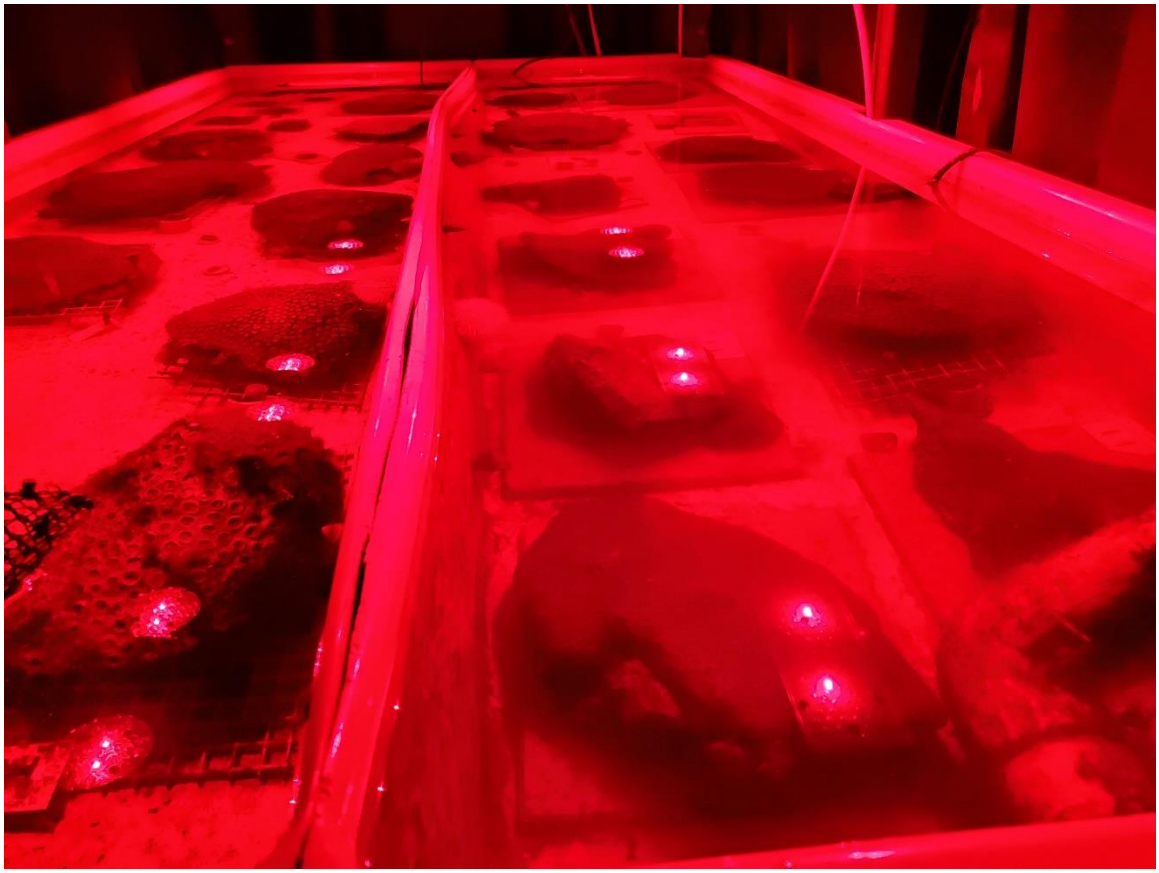


Figure 1: Spawning of *Montastraea cavernosa* in the indoors tanks (07/30/2021)

Figure 2: Gametes of *Montastraea cavernosa* pooled from multiple colonies (07/30/2021)

Figure 3: *Acropora cervicornis* setting (08/06/2021)

Figure 4: Gametes of *Acropora cervicornis* collected (08/06/2021)

*Table 1: Day and time of spawning of coral colonies induced to mature gonads and spawn in captivity (Species: MCAV – Montastraea cavernosa, OFAV – Orbicella faveolata, PSTR – Pseudodiploria strigosa; Colony sex: F – female, M – male, H – hermaphrodite, * – was of the opposite sex in 2020; UNK – unknown, N/A – not applicable as only one sex spawned, SF – self-fertilization, < before, > after)*

Date	Species	Colony ID	Colony sex	Spawning start time	Spawning end time	Spawn quantity	Fertilization	Number of larvae produced	Larval fate
7/29/21	MCAV	MC19_32 (Xmas Tree)	M	UNK	+ 20min	High	~90%	~170,000	~70,000 NSU ~100,000 lost (overflow)
		MC19_23 (Hulk)	M	22:21	22:21	Medium			
		MC19_04 (fake Mama)	M	22:49	22:49	Low			
		UNK (indoor tank Ryder)	M	UNK	UNK	Low			
		UNK (indoor tank Ryder)	F	<21:00	< 23:00	Low			
		UNK (indoor tank Ryder)	F	<21:00	< 23:00	Low			
		UNK (indoor tank Ryder)	F	UNK	< 23:00	Low			
		BRWDBC1_MCAV_01 (Oz)	F	UNK	UNK	Low			
7/30/21	MCAV	BRWDBC1_MCAV_01 (Oz)	F	22:39	22:39	Low	~90%	~220,049	~135,000 NSU ~20,000 Mote ~40,000 FLAQ ~5,000 UNCW ~20,049 UM
		MC19_23 (Hulk)	M	21:57	22:04	Medium			
		BRWDCOM_MCAV_01	M	22:01	22:10	Medium			
		MC19_04 (fake Mama)	M	22:06	22:15	Medium			
		MC19_13 (Shaggy)	F*	22:25	22:25	High			
		MC19_10	F	22:26	22:26	High			
		MC19_01	F	22:40	22:40	Low			
7/31/21	MCAV	MC19_10	F	22:15	22:15	Low	0% (very low sperm concentration)	0	N/A
		UNK (indoor tank Ryder)	M	22:00	22:00	Low			

	OFAV	BRWDBAR_OFAV_04	H	22:39	22:41	Medium	0% SF	0	N/A
8/1/21	OFAV	BRWDBAR_OFAV_04	H	22:46	22:46	Low	0% SF	0	N/A
8/2/21	MCAV	MC19_07	F	20:15	21:37	Low	N/A	N/A	N/A
	MCAV	MC19_13 (Shaggy)	F*	22:25	23:08	Low			
8/3/21	MCAV	MC19_13 (Shaggy)	F*	21:41	23:25	Low	N/A	N/A	N/A
8/26/21	MCAV	BRWDJUL_MCAV_06	F	UNK	UNK	Low	N/A	N/A	N/A
8/27/21	MCAV	MC19_20 (Mama)	F	19:40	22:00	Medium	N/A	N/A	N/A
		MC19_13 (Shaggy)	F	21:42	21:42	Low			
		MC19_34	F	21:58	21:59	Low			
8/28/21	MCAV	MC19_20 (Mama)	F	19:27	21:00	Medium	N/A	N/A	N/A
8/29/21	MCAV	MC19_20 (Mama)	F	19:58	20:20	High	~50% (low sperm concentration)	~10,000	~10,000 NSU
		MC19_32 (Christmas Tree)	M	20:57	21:25	Medium			
		MC19_07	F	21:00	21:00	Medium			
8/30/21	MCAV	MC19_20 (Mama)	F	20:12	20:12	Low	~90%	~100	~100 NSU
		MC19_32 (Christmas Tree)	M	21:18	21:18	Medium			
8/31/21	MCAV	UNK (outdoor tank Charlie)	F	>22:30	< 22:45	Low	N/A	N/A	N/A
9/3/21	PSTR	PS20_02 (Mr. Wilson)	H	23:16	00:00	Low	0% SF	0	N/A
9/21/21	PSTR	BRWDBAR_PSTR_10	H	19:46	19:58	Low	0% SF	0	N/A
9/28/21	MCAV	MC19_20 (Mama)	F	20:15	20:15	Low	N/A	N/A	N/A

Table 2: Day and time of spawning of wild Acropora cervicornis colonies induced to spawn in outdoor tanks (SF – self-fertilization, < before, > after)

Date	Site of origin of genets	Number of genets	Setting time	Spawning start time	Spawning end time	Fertilization	Number of larvae produced	Larval fate
8/5/21	Core 2	3	<22:00	22:56	23:21	~90%	~ 100,000	~ 100,000 NSU
	DC6	2	<22:00	22:56	23:21			
8/14/21	Core 2	1	<22:00	22:55	23:00	0% SF	0	N/A
8/15/21	Core 3	2	<21:45	22:45	23:00	~90%	~50,000	~35,000 NSU ~10,000 FLAQ ~5,000 UNCW
	BCA	1	<21:45	22:34	23:00			
	Scooter	3	<21:45	22:35	23:00			

Spawning videos are publicly available at:
https://www.youtube.com/channel/UCgSHPoL07O31cM2AyW-Pu3g

3.2. Task 2: Fertilization, larval culture, and settlement (Figueiredo)

In the nights where multiple colonies of the *M. cavernosa* spawned, eggs were fertilized with >90% success. The other species only had one colony spawning in a night; since they are hermaphrodite, we still assessed self-fertilization, but found 0% success, as expected. The fertilization of the eggs of *Acropora cervicornis* was also > 90% successful.

Larvae of both species were cultured in the conical tanks with large success (see number of larvae produced in Tables 1 and 2). Some of the larvae of both species were settled at NSU with 10-20% settlement success which resulted in large number of settlers, but a large number was also donated to other institutions for rearing (Tables 1 and 2).

We also received larvae of *Orbicella faveolata, Pseudodiploria clivosa, P. strigosa* and *Colpophyllia natans* from FLAQ and NOAA (Dana Williams). These larvae were settled at NSU with ca. 10% settlement success.

Species	Number of settlers	Number of Tiles
D. labyrinthiformis	15,984	864
M. cavernosa	17,917	874
A. cervicornis	2,553	138
O. faveolata	276	92
P. strigosa	2,024	184
P. clivosa	322	92
C. natans	3,542	322
TOTAL	42,618	2,566

Table 3: Number of tiles with newly settled corals for all species.

Up to the time of settlement, there were no further problems encountered.

Figure 5: Newly settled *Acropora cervicornis*

Figure 6: Newly settled *P. clivosa*

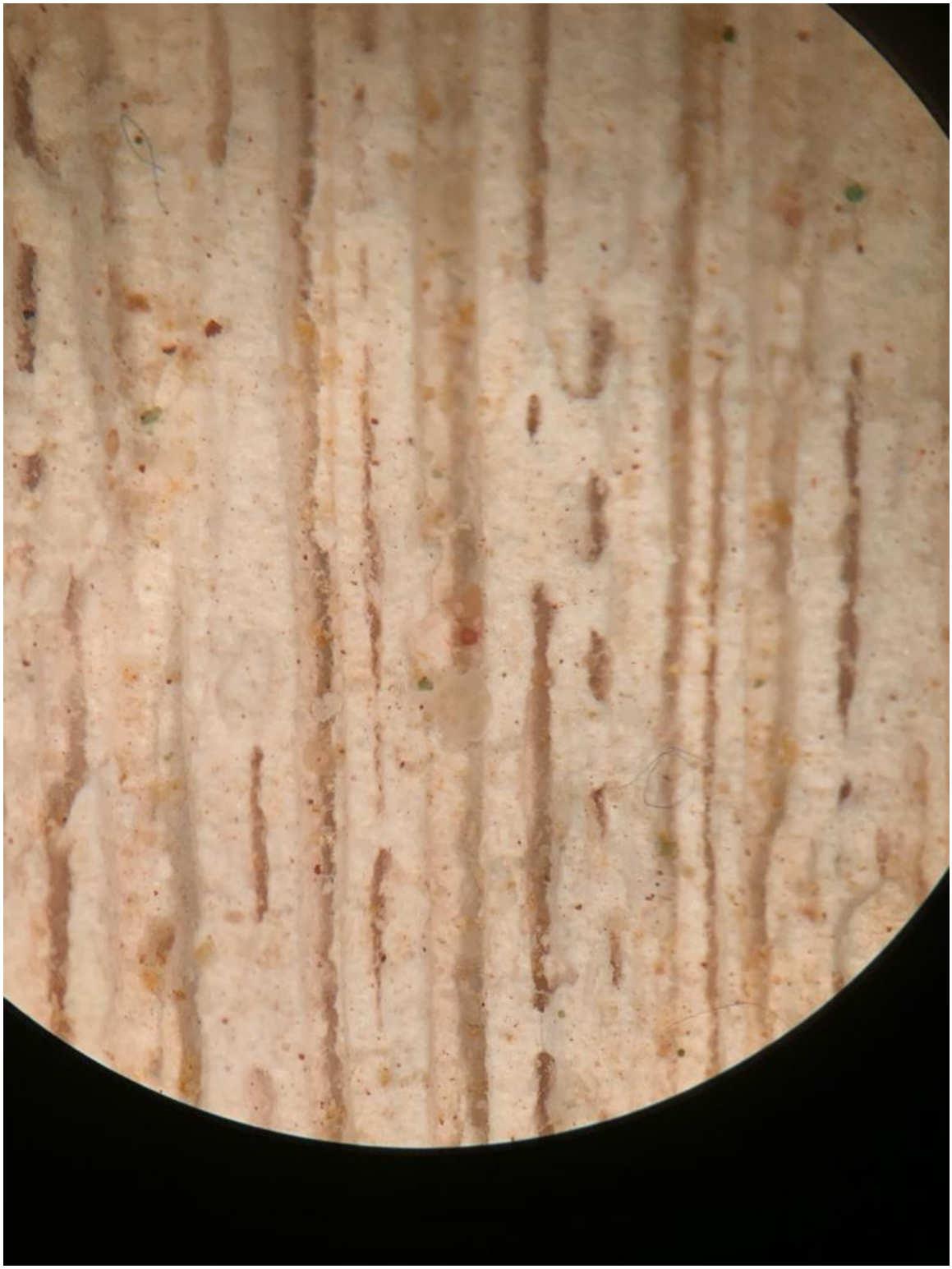


Figure 7: New settled C. natans

Videos of larvae swimming are publicly available at:
https://www.youtube.com/channel/UCgSHPoL07O31cM2AyW-Pu3g

3.3. Task 3: Grow-out of sexual recruits in land-based nursery (Figueiredo)

Newly settled recruits of all species grew and acquired symbionts. They remained healthy for 8 weeks, but after this there was a mass die-off in the system holding *M. cavernosa*, *O. faveolata*, *C. natans* and *P. strigosa*. Water quality parameters remained optimal during this period, all biosecurity measures were followed, and we did not find any (at least visible) pest consuming the recruits, thus, after consulting with other partners that have observed similarly unexplained mass die-offs, we suspect there might have been a shift in microbial community, the occurrence of a disease that quickly spread through all species, or even some other undetectable allelopathic chemical that accumulated in the tank. The corals that remained healthy were moved to a quarantine tank and treated with lugol and Restor disps which allowed some to be saved, but the total numbers of recruits of these species decreased significantly. The system containing the *Diploria labyrinthiformis* recruits was not affected. All surviving corals continued to be grown and remained healthy at the land-based coral nursery. Some recruits were moved to the offshore nursery and then outplanted on the reef or were directly outplanted on the reef. Specifically, on August 11, 2021, 1 *P. strigosa*, 1 *C. natans* and 29 *M. cavernosa* were transferred to the Layer Cakes offshore nursery. These were on plugs and placed in the nursery on a rebar table. On November 4, 2021, 50 *M. cavernosa* were moved to the Layer Cakes offshore nursery on plugs and placed on rebar tables. Approximately 3 months later, these 50 corals, plus another 100 that were kept at the nursery were outplanted at a reef. On January 2022, 2 *P. strigosa* and 126 *M. cavernosa* recruits were moved to the offshore nursery (Task 4).

The total number of coral recruits produced this spawning cycle that remained alive until the end of the project is summarized in Table 4.

Species	**Total = land-based nursery (LBN) + moved to offshore nursery or outplanted on reef (ON)**
D. labyrinthiformis	1,661 recruits = 1,661 LBN
M. cavernosa	365 recruits = 60 LBN + 305 ON
C. natans	83 recruits = 82 LBN + 1 ON
P. strigosa	51 recruits = 50 LBN + 3 ON
O. faveolata	30 recruits = 30 LBN
Total	2,190 recruits = 1,883 LBN + 309 ON

Table 4: Number of coral recruits produced during the spawning of 2021 that were successfully reared at the land-based nursery until June 2022

Future recommendations: The rapid massive die-off in one of tanks dedicated to the culture of coral recruits was a major blow on our aim to produce an even larger number of coral recruits. More importantly, we were unable to identify what caused it (as water quality was ruled out), but discussions with partners suggest it may have been caused by a shift in microbial community in the system. Further research on the dynamics of microbial communities' dynamics in tanks is required (our team at Nova Southeastern University, the University of North Carolina at Wilmington, Florida Aquarium and Mote

Marine Lab, have recently been awarded a NOAA Ruth Gates grant that will start addressing this issue in the coming 3 years). To minimize the impact events like these have on the overall production at coral nurseries, we suggest spreading the coral recruits of each species by as many systems as the facility has available. Our facilities only have 2 indoors recirculating systems (one with 2 raceways, the other just with one raceway) dedicated to the early grow-out of juveniles (the other indoors tanks are dedicated to the induction of gonad maturation and spawning of adult corals; and the outdoors tanks are not adequate to early grow-out due to having too elevated light irradiance), thus in the next year, we will split the recruits by the 2 indoors systems.

One important success we would like to report was the finding that once coral recruits reach around 12-20 weeks old (i.e. when symbiosis is fully established and tentacles are well developed), they can already handle the light levels experienced at the outdoor tanks and thus can be cultured outdoors and be spread by a greater number of tanks (minimizing risk of potential die-offs). Importantly also, we found corals to grow faster and exhibited better general health (fleshier) since relocating outdoors (compared to corals kept being reared under artificial lights) which we hypothesize may be related to being exposed to a light spectrum that is more favorable.

3.4. Task 4: Grow-out of newly settled corals and microfragments at the offshore nursery (Gilliam)

After six months of monitoring, overall outplant survival was 50%. *Colpophyllia natans* had the highest survival rate (100% n=2), while *Orbicella faveolata* had the lowest survival rate (0%, n=2). The four other outplanted species had similar survival rates (*Diploria labyrithinformis* 42%, *Montastrea cavernosa* 47%, *Pseudodiploria strigosa* 50% and *Siderastrea siderea* 59%). Of the outplants, 14 were sexual recruits while 69 were microfragments; where the microfragments had higher survival rates (52%) compared with the sexual recruits (36%). During the monitoring periods, predation was the only condition recorded on the outplants and was recorded during the 2-week monitoring and 1-month monitoring. Only two colonies were recorded with predation, 1 *M. cavernosa* and 1 *C. natans*. No disease or bleaching was observed. As the sexual recruits tended to be smaller than the microfragments, size may have been a contributing factor to survival, in addition to species. Growing sexual recruits out to a larger size before outplanting may help increase survival for some species.
Colonies placed in the nursery and monitored for six months had better overall survival of 89%, when compared to the outplants (50%). Survival by species ranged from 85% for *M. cavernosa* (n=303) to 100% for *P. strigosa* (n=2). Microfragments in the nursery also had greater survival (95%) compared to sexual recruits (66%). *Montastrea cavernosa* had 177 microfragments in the nursery and 126 sexual recruits. After six months, the *M. cavernosa* microfragments had 100% survival while the sexual recruits had 65% survival. Higher predation instances were observed in the nursery when compared to the outplants. Predation was highest on the recruits, where 59% experienced predation at the 2-week monitoring compared to only 1% of the microfragments. Disease was recorded during the 2-week monitoring and 6-month monitoring but only 12 total colonies were affected. Overgrowth interactions were also recorded and had the highest instance during the 6-

month monitoring period where 18 colonies had tissue loss due to overgrowth. After this study, we recommend testing out new nursery structures other than coral tables to house microfragments and sexual recruits to try and minimize predation, as that seemed to be the greatest cause of mortality in nursery corals.

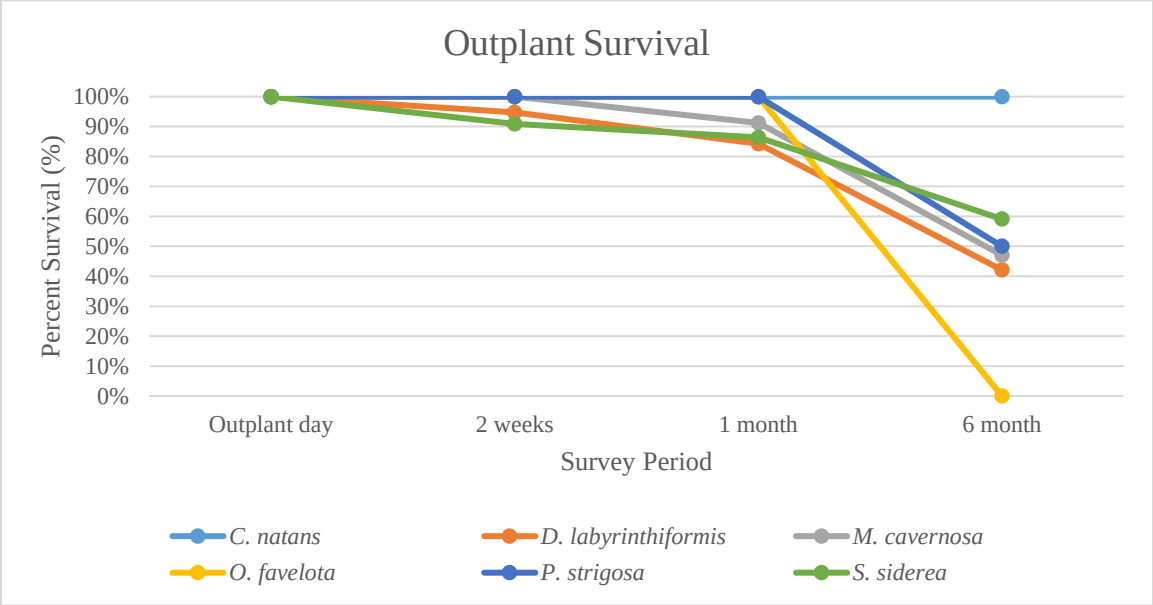


Figure 8: Survival of outplanted corals by species

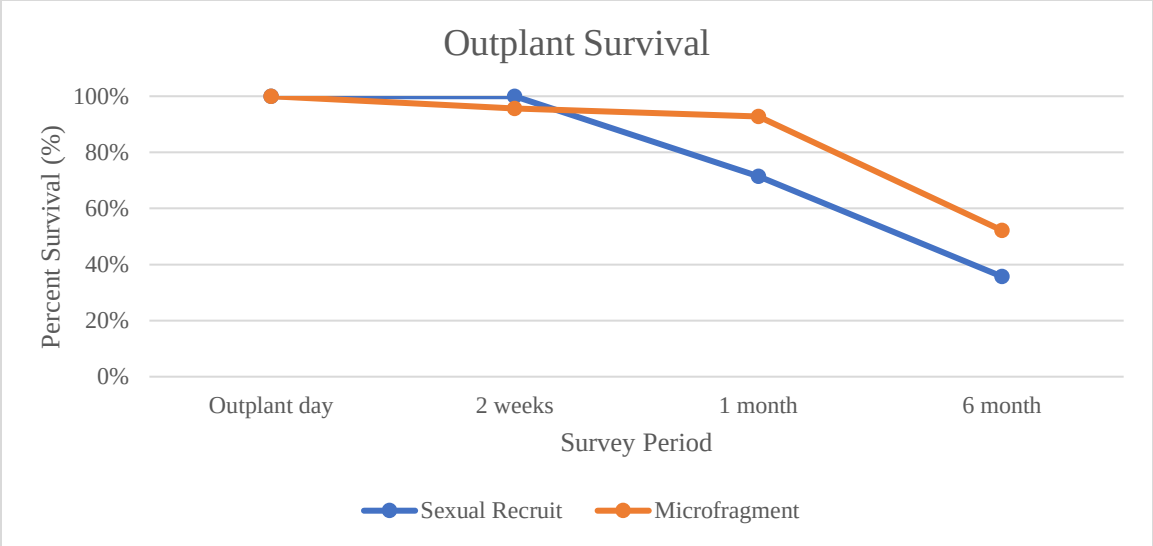


Figure 9: Survival of outplanted corals by life stage

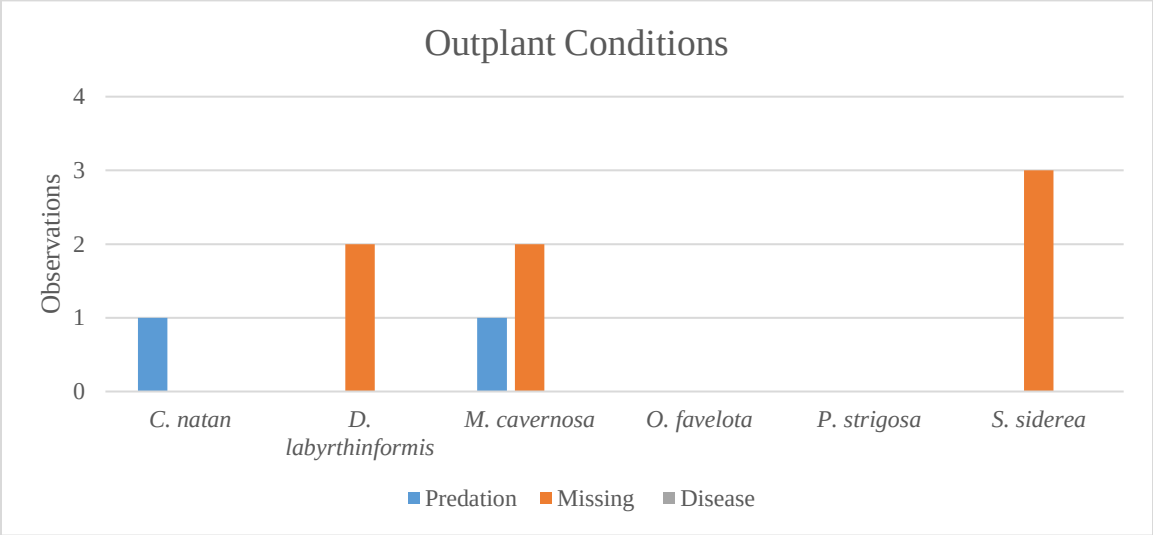


Figure 10: Causes of mortality detected through observation

3.5. Task 5: Preserve coral genotypes of disease impacted species and induce gonad maturation and spawning in captivity (Figueiredo)

One hundred and fifty-eight coral colonies, specifically 9 *C. natans*, 25 *P. strigosa*, 54 *M. cavernosa*, 20 *P. clivosa*, 6 *D. labyrinthiformis*, 14 *S. siderea* and 30 *O. faveolata*, were maintained at the land-based nursery, because it received 20 new colonies. Seven of the colonies held which were not part of the rescue program were moved (whole or microfragmented) to the offshore nursery or outplanted to the reef for other research projects and/or restoration efforts. There was some tissue loss in a few colonies, mostly *O. faveolata* (several were unhealthy at the time of collection) and *P. strigosa* (in the Winter months) (see Table 5). Unhealthy colonies were treated alternating dips of Iodine and Restor amino acids which in almost all cases slowed or stalled tissue loss. Nine colonies died which corresponds to 5.6% of the colonies held. Three *D. labyrinthiformis* died due to the malfunction of a heater, 1 *P. clivosa* died but was already unhealthy at the time of arrival to the nursery, and 5 *P. strigosa* died of disease during the winter/cooler months. We hypothesize *P. strigosa* is more prone to disease under lower temperatures, and thus suggest trying not to lower the temperature in the Winter as much. *Orbicella faveolata* is known to be very sensitive to any swings in environmental conditions, particularly alkalinity, which is possible to have happened during transportation and/or acclimation to the lab conditions and caused the instances of ‘unhealthy’.

Species	Tag #	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
C. natans	1160_CNAT_010									H	H	H
C. natans	1160_CNAT_011									H	H	H
C. natans	BM1083_CNAT_009									H	H	H
C. natans	BRWDBAR_CNAT_002	H	H	H	H	H	H	H	H	H	H	H
C. natans	BRWDBAR_CNAT_003	H	H	H	H	H	H	H	H	H	H	H
C. natans	BRWDBAR_CNAT_004	H	H	H	H	H	H	H	H	H	H	H
C. natans	BRWDJUL_CNAT_001	H	H	H	H	H	H	H	H	H	H	H
C. natans	CN21_01	H	H	H	H	H	H	H	H	H	H	H
C. natans	CN21_02	H	H	H	H	H	H	H	H	H	H	H
D. labyrinthiformis	T1085_DLAB_002									H	H	D
D. labyrinthiformis	T1085_DLAB_005									H	D	D
D. labyrinthiformis	V1237_DLAB_003									H	D	D
D. labyrinthiformis	DLX_001	H	H	H	H	H	H	H	H	H	H	H
D. labyrinthiformis	DLX_002	H	H	H	H	H	H	H	H	H	H	H
D. labyrinthiformis	DLX_003	H	O									
M. cavernosa	BRWD277_MCAV_001	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWD277_MCAV_002	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDBAR_MCAV_001	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDBAR_MCAV_002	H	H	H	H	H	H	H	H	U	H	U
M. cavernosa	BRWDBAR_MCAV_003	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDBC1_MCAV_001	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDBC2_MCAV_002	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDCAVE_MCAV_002	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDCAVE_MCAV_003	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDCAVE_MCAV_004	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDCOM_MCAV_001	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDJUL_MCAV_001	H	H	H	H	H	H	H	H	H	H	H

M. cavernosa	BRWDJUL_MCAV_002	H	H	U	U	U	H	H	H	H	H	H
M. cavernosa	BRWDJUL_MCAV_004A	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDJUL_MCAV_004B	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDJUL_MCAV_004C	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDJUL_MCAV_006	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MCX_02	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_03	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_23a	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_30	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_22	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC_NSH_3						H	H	H	H	H	H
M. cavernosa	MC19_10	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_20	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_01	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_04	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_34	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC_30_02						H	H	H	H	H	H
M. cavernosa	MC_299_3						H	H	H	H	H	H
M. cavernosa	MC19_18a	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_23b	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MCX_03	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC_NSH_1						H	H	H	H	H	H
M. cavernosa	MC19_32	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC_NSH_2						H	H	H	H	H	H
M. cavernosa	MC19_07	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_18b	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC_209_2						H	H	H	H	H	H
M. cavernosa	MC19_33	H	H	H	H	H	H	H	H	H	H	H

M. cavernosa	MC19_31a	H	H	H	H	U	H	H	H	H	H	H
M. cavernosa	MC19_31b	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_05	H	H	H	U	H	H	H	H	H	H	H
M. cavernosa	MC19_29	U	U	U	U	U	H	H	H	H	H	H
M. cavernosa	MC19_19	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_16	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_21	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_06	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC20_01	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC20_02	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC20_03	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC20_04	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC20_05	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC20_06	H	H	H	U	U	H	H	H	H	H	H
O. faveolata	BRWDBAR_OFAV_001	U	H	U	U	H	H	H	H	H	H	H
O. faveolata	BRWDBAR_OFAV_002	U	H	H	H	H	H	H	H	H	H	H
O. faveolata	BRWDBAR_OFAV_003	H	H	H	H	H	H	H	H	H	H	H
O. faveolata	BRWDBAR_OFAV_004	H	H	U	H	H	H	H	H	H	H	H
O. faveolata	BRWDBAR_OFAV_005	U	H	U	U	H	H	H	H	H	H	H
O. faveolata	BRWDBC1_OFAV_001	H	H	H	H	H	H	H	H	H	H	H
O. faveolata	BRWDBC1_OFAV_003a	H	H	H	H	H	H	H	H	H	H	H
O. faveolata	BRWDBC1_OFAV_003b	H	H	H	H	H	H	H	H	H	H	H
O. faveolata	BRWDBC1_OFAV_004	H	H	H	H	H	H	H	H	H	H	H
O. faveolata	BRWDBC1_OFAV_005	H	H	H	H	H	H	H	H	H	U	U
O. faveolata	BRWDBC2_OFAV_001	H	H	H	H	U	H	H	H	H	H	H
O. faveolata	BRWDBOU_OFAV_001	U	U	U	H	U	H	H	H	H	H	H
O. faveolata	BRWDBOU_OFAV_002	H	H	H	H	H	H	H	H	H	H	H
O. faveolata	BRWDBOU_OFAV_003	H	H	H	H	H	H	H	H	H	H	H

O. faveolata	BRWDCOM_OFAV_001	U	H	H	H	H	H	H	H	H	H	H
O. faveolata	BRWDJUL_OFAV_001	H	U	H	H	U	H	H	H	H	H	H
O. faveolata	BRWDJUL_OFAV_003	H	H	U	U	H	H	H	H	H	H	H
O. faveolata	BRWDJUL_OFAV_004	H	H	H	H	H	H	H	H	H	H	H
O. faveolata	OF19_06	H	H	H	H	H	H	H	H	H	H	H
O. faveolata	OF21_01				H	H	H	H	H	H	H	H
O. faveolata	OF19_01	H	H	U	U	U	H	H	H	H	H	H
O. faveolata	OF19_02	H	H	H	H	U	H	H	H	H	U	U
O. faveolata	OF19_03	H	H	U	U	H	H	H	H	H	H	H
O. faveolata	OF19_04	H	H	U	U	H	H	H	H	H	H	H
O. faveolata	OF19_05	H	H	U	U	U	H	H	H	H	H	H
O. faveolata	OF19_07	H	H	H	H	U	H	H	H	H	H	H
O. faveolata	OF19_08	H	H	H	H	U	H	H	H	H	H	H
O. faveolata	OF19_09	H	H	H	H	H	H	H	H	H	H	H
O. faveolata	OF19_10	H	O									
O. faveolata	OF19_11	H	O									
P. clivosa	BRWD277_PCLI_002	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_004	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_005	H	U	U	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_006A	H	H	H	H	H	H	H	H	U	H	H
P. clivosa	BRWD277_PCLI_006B	U	H	D	D	D	D	D	D	D	D	D
P. clivosa	BRWD277_PCLI_007	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_008	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_010	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_011	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_012a	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_012b	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_012c	H	H	H	H	H	H	H	H	H	H	H

P. clivosa	BRWD277_PCLI_014	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_015	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWDBAR_PCLI_002	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWDCOM_PCLI_001	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	PEJ_PCLI_005	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	PEJ_PCLI_011A	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	PEJ_PCLI_11B	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	PC20_01	H	H	H	H	H	H	H	H	H	H	H
P. strigosa	1160_PSTR_001									H	H	H
P. strigosa	BRWDBAR_PSTR_001	H	H	H	H	H	H	U	U	D	D	D
P. strigosa	BRWDBAR_PSTR_002A	H	H	H	H	U	U	U	H	H	H	H
P. strigosa	BRWDBAR_PSTR_003	H	H	H	H	H	H	H	H	H	H	H
P. strigosa	BRWDBAR_PSTR_006	H	H	H	H	H	H	H	H	H	H	H
P. strigosa	BRWDBAR_PSTR_007	H	H	H	U	U	U	H	H	H	H	H
P. strigosa	BRWDBAR_PSTR_008	H	H	H	H	H	H	H	H	H	H	H
P. strigosa	BRWDBAR_PSTR_009	H	U	U	H	U	H	H	H	H	H	H
P. strigosa	BRWDBAR_PSTR_010	H	H	H	U	U	U	D	D	D	D	D
P. strigosa	BRWDBAR_PSTR_011	H	H	U	U	U	U	U	U	U	H	H
P. strigosa	BRWDBAR_PSTR_012	H	H	H	H	H	H	H	H	H	H	H
P. strigosa	BRWDBAR_PSTR_013	H	H	H	H	H	H	H	H	H	H	H
P. strigosa	BRWDBC2_PSTR_001	H	H	H	H	H	H	H	U	U	H	H
P. strigosa	BRWDBC2_PSTR_002	H	H	H	H	H	H	H	H	H	H	H
P. strigosa	BRWDCAVE_PSTR_001	H	H	H	H	H	H	H	H	H	H	H
P. strigosa	BRWDFTB_PSTR_001	H	H	H	H	H	H	H	H	U	H	H
P. strigosa	BRWDJUL_PSTR_001	H	H	H	H	H	H	H	H	H	H	H
P. strigosa	PEJ_PSTR_053	H	H	H	H	H	H	H	H	H	H	H
P. strigosa	U3060_PSTR_004									H	H	H
P. strigosa	PS20_02	H	H	H	H	H	U	U	U	D	D	D

P. strigosa	PS20_01	H	H	H	H	U	U	H	H	H	H	H
P. strigosa	PS20_03	H	H	U	U	D	D	D	D	D	D	D
P. strigosa	PS20_04	U	D	D	D	D	D	D	D	D	D	D
P. strigosa	PS20_05	H	O									
P. strigosa	PS20_06	H	O									
S. siderea	SSX_001	H	H	H	H	H	H	H	H	H	H	H
S. siderea	SSX_002	H	H	H	H	H	H	H	H	H	H	H
S. siderea	SSX_003	H	H	H	H	H	H	H	H	H	H	H
S. siderea	SSX_004	H	H	H	H	H	H	H	H	H	H	H
S. siderea	SSX_005	H	H	H	H	H	H	H	H	H	H	H
S. siderea	SSX_006	H	H	H	H	H	H	H	H	H	H	H
S. siderea	SSX_007	H	H	H	H	H	H	H	H	H	H	H
S. siderea	SSX_008	H	H	H	H	H	H	H	H	H	U	U
S. siderea	SSX_009	H	H	H	H	H	H	H	H	H	H	H
S. siderea	SSX_010	H	H	H	H	H	H	H	H	H	H	H
S. siderea	SSX_011	H	H	H	H	H	H	H	H	H	H	H
S. siderea	SSX_012	H	H	H	H	H	H	H	H	H	H	H
S. siderea	SSX_013	H	H	H	H	H	H	H	H	H	H	H
S. siderea	SSX_014	H	H	H	H	H	H	H	H	H	H	H

Table 5: Health status of all colonies maintained at the land-based nursery (H - healthy, U -unhealthy, D – deceased, O – whole colony or microfragments outplanted; lack of information for a month it means the colony was only collected afterwards)

3.6. Task 6: See Appendix from Reef Institute

3.7. Task 7: Co-culture of coral recruits with herbivorous invertebrates

The newly settled *Porites astreoides* survival and growth in the treatments where tiles were cleaned weekly and not cleaned was similar (Figures 11 and 12). This suggests that the quantity of algae developing on the tiles was still insufficient to negatively impact the corals. This is like because at this stage corals are grown under very low light irradiance levels (20-50 PAR) which do not elicit a fast algal growth.

The newly settled *Porites astreoides* kept in treatments with crabs survived and grew less than the ones kept in treatments where tiles were cleaned weekly, which suggest there was some level of predation. This could indicate that the co-culture with crabs is not adequate. However, that may not be the case because, as explained above, there was very little algae growing on the tiles thus the predation on corals by the crabs was likely due to the lack of algae on tile to graze upon.

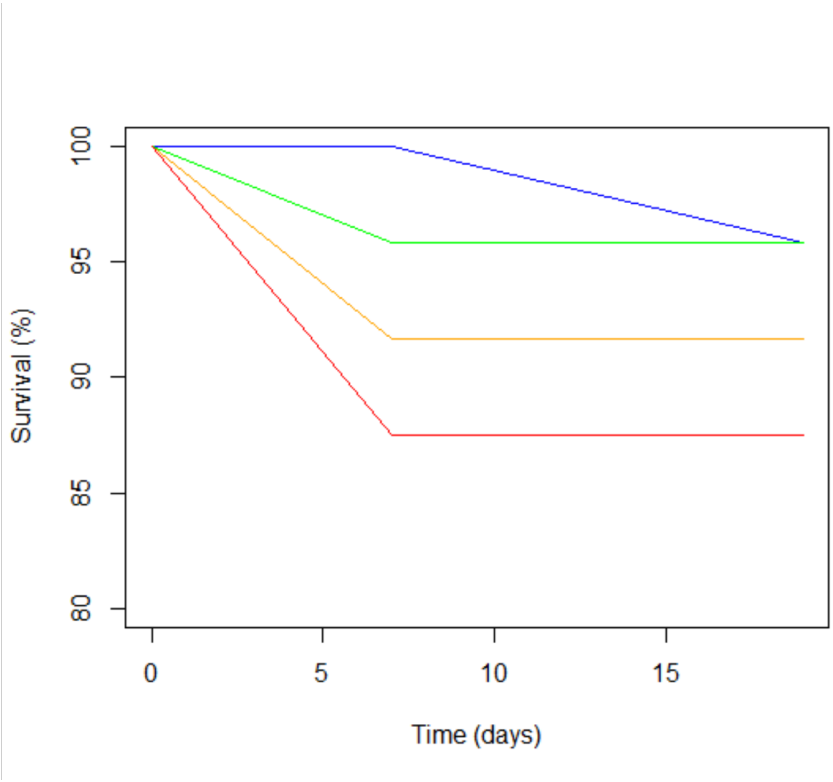


Figure 11: Survival of newly settled Porites astreoides over time in all treatments (blue – no clean, green – manual cleaning once per week, orange – low crab density, red – high crab density)

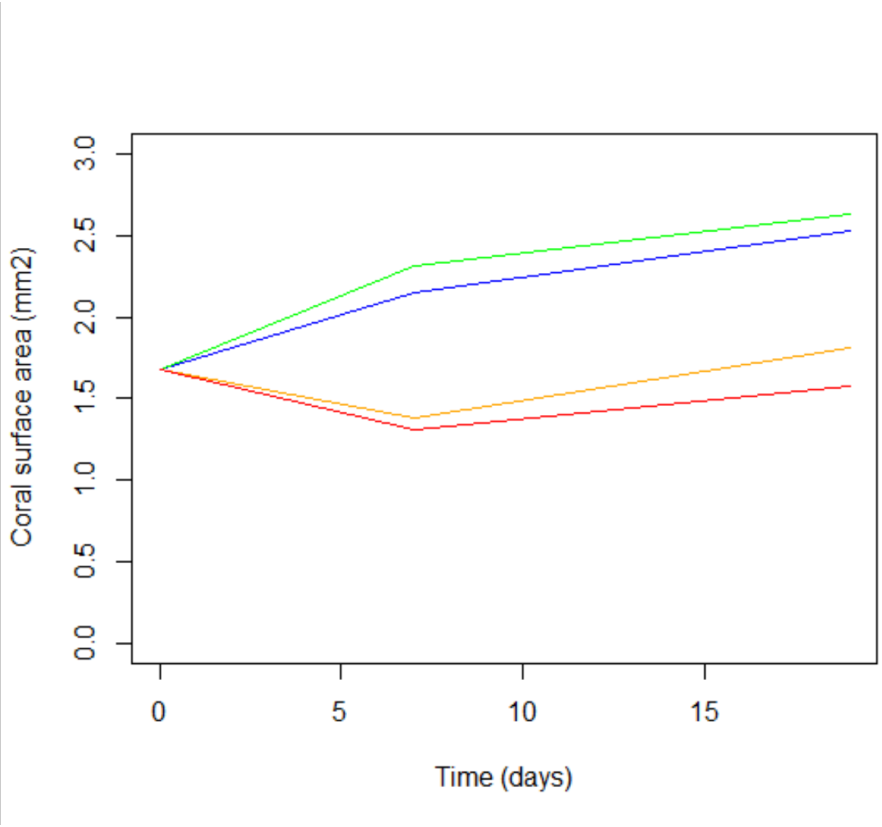


Figure 12: Growth of newly settled Porites astreoides in all treatments (blue – no clean, green – manual cleaning once per week, orange – low crab density, red – high crab density)

To more definitely decide on the effectiveness of using these crabs to control algal overgrowth and thus promote the survival and growth of coral recruits, we suggest testing the same treatments with corals over 8-12 weeks old, a time in which light irradiance is higher and thus promotes algal overgrowth.

4. CONCLUSIONS & RECOMMENDATIONS

Spawning: The Apex system that controlled the moon lights indoors does not accurately imitate the moonlight cycle; it uses an average daily change on the time of moonrise, when in reality this changes over time too. In some years, the difference from the actual cycle is minimum, but this year it had a much greater difference (up to 1.5 hrs), which may have derailed the spawning synchrony of most species. *M. cavernosa* spawned indoors very successfully maybe because is less dependent on the moon cycle. It is possible the others are more sensitive to the difference between sunset and moonrise than *M. cavernosa*. According to the most recent literature, the difference between sunset and moonrise seems to be essential for cuing spawning, thus we have now changed the control of moonlights to the Mobius system.

In the tanks outdoors, the annual temperature cycle induced gonad maturation, but the light pollution outdoors (mostly from the Coast Guard property adjacent to NSU) interfered with the moonlight exposure, preventing synchronous spawning. Next year, we will do a pilot blacking out two outdoors tanks starting a month before expected spawning and put lights inside the curtain to test synchronous spawning.

Fertilization, larval rearing and settlement: The existing technique for egg fertilization is very successful (>95%) when a larger number of colonies spawn, likely due to genotype diversity and compatibility likelihood increase as result. Larval rearing in mass scale using the conical tanks is also very successful. Larval settlement success seems to differ from species to species, parental genotype and history, but overall, it is a well-established methodology.

Grow-out *ex situ*: The rapid massive die-off in one of tanks dedicated to the culture of coral recruits was a major blow on our aim to produce an even larger number of coral recruits. More importantly, we were unable to identify what caused it (as water quality was ruled out), but discussions with partners suggest it may have been caused by a shift in microbial community in the system. Further research on the dynamics of microbial communities' dynamics in tanks is required (our team at Nova Southeastern University, the University of North Carolina at Wilmington, Florida Aquarium and Mote Marine Lab, have recently been awarded a NOAA Ruth Gates grant that will start addressing this issue in the coming 3 years). To minimize the impact events like these have on the overall production at coral nurseries, we suggest spreading the coral recruits of each species by as many systems as the facility has available. Our facilities only have 2 indoors recirculating systems (one with 2 raceways, the other just with one raceway) dedicated to the early grow-out of juveniles (the other indoors tanks are dedicated to the induction of gonad maturation and spawning of adult corals; and the outdoors tanks are not adequate to early grow-out due to having too elevated light irradiance), thus in the next year, we will split the recruits by the 2 indoors systems.

One important success we would like to report was the finding that once coral recruits reach around 12-20 weeks old (i.e. when symbiosis is fully established and tentacles are well developed), they can already handle the light levels experienced at the outdoor tanks and thus can be cultured outdoors and be spread by a greater number of tanks (minimizing risk of potential die-offs). Importantly also, we found corals to grow faster and exhibited better general health (fleshier) since relocating outdoors (compared to corals kept being reared under artificial lights) which we hypothesize may be related to being exposed to a light spectrum that is more favorable.

Grow-out in situ: Predation of fragments and sexual recruits being grown in situ remains an issue that requires further research. Research from other groups (Diego Lirman, at University of Miami) seems to suggest that temporary complete protection from predators does not prevent predation after protection is removed, but partial protection seems to elicit an anti-predatory response on the corals. Despite more research being needed, this could be useful to develop future structures to protect fragments; however,

sue to their small size, this could not be applied to sexual recruits, and thus require further research.

Coral husbandry: Largely successful, with instances of disease which mostly can be treated, and even less of death.

Co-culture of herbivores: The effectiveness of using crabs to control algal overgrowth and promote the survival and growth of coral recruits, should be tested with corals over 8-12 weeks old, a time in which light irradiance is higher and thus promotes more algal overgrowth. This work will be conducted in the next year.

5. LITERATURE CITED

Craggs et al. 2017. Inducing broadcast coral spawning ex situ: closed system mesocosm design and husbandry protocol. *Ecology and Evolution* 7:11066-11078.

Forsman et al. 2015 Growing coral larger and faster: micro-colony-fusion and a strategy for accelerating coral cover. *PeerJ* 3: e1313

Page et al. 2018. Microfragmenting for the successful restoration of slow growing massive corals. *Ecological Engineering* 123: 86-94.

Walton et al. 2018. Impacts of a regional, multi-year, multi-species coral disease outbreak in Southeast Florida. *Frontiers in Marine Science* 5: 323.

STATE OF FLORIDA
DEPARTMENT OF ENVIRONMENTAL PROTECTION
Exhibit A –
Final Report Submission Form for Scope of Work

DEP Agreement No.:	B97E48
Project Title:	Intensive Closed Land-Based Coral Systems: for research, gene banking, and amplification: Phase 3
Principle Investigator Name:	Dr. Charles Gregory DVM and David Gross
PI's Funding Manager Name:	Leneita Fix
Reporting Period:	June 1–June 30, 2021

Task 1: Coral Collection and Acquisition

Reporting for the fiscal year started in July. No rescue corals were added to the Reef Institute (TRI) in July through December 31st.

Discussions were had in July with MOTE marine labs in Summerland Key about the possibility of being given some of their fertilized unsettled juveniles. At the time, the goal was to bring larvae into the land-based systems for the purpose of settlement for growth, with a hope of using them towards restoration. Unfortunately, *Acropora **c**ervicornis* spawned early this year, and so this was unable to happen.

In November, TRI received over 300 colonies of settled juvenile *Acropora **c**ervicornis* from MOTE Marine Lab in Summerland Key. 100 of these were from spawning events in 2020 and 200 were from the 2021 spawning season. While TRI was not able to obtain the larvae for settlement, we have been able to host these juveniles for rearing. MOTE would like to see these grown and then used for out planting and restoration efforts. It is estimated that TRI will be caring for them for at least two years before they move into restoration efforts. TRI will be discussing out planting strategies and future placement of these corals with FWC and all necessary stakeholders. These corals are healthy and growing.

In August, the Florida Coral Rescue Project made the choice to move three colonies of coral, two *Orbicella faveolata* and one *Pseudodiploria clivosa* from the Reef Institute to MOTE Marine Labs in Sarasota into a spawning system in their lab. It is the understanding of TRI that corals may be moved in and out of their facility in the future to best suit the needs of the Florida Coral Rescue Project.

Florida Aquarium provided the Reef Institute with 200 juvenile colonies of (100) C*olpophyllia natans* and (100) *Diploria labyrinthiformis* that had been spawned and reared at the Florida Aquarium coral research facility at Apollo Beach. These juveniles have been at TRI since August and are growing well. TRI remains in conversation with the Florida Coral Rescue Team

as to what will be will be the future placement of these corals in the ocean. TRI continues to work with the Florida Coral Rescue Project to receive permission to see some of these colonies out planted in Palm Beach County. Currently, Palm Beach County is waiting on a dredging project to be completed through 2023 before any corals are out planted. TRI will provide corals for restoration as is most needed across Florida.

While TRI was granted permission to retrieve coral gametes from Peanut Island in the Lake Worth Lagoon, TRI did not witness any spawning during the 2021 season. TRI believes weather and artificial lighting near the area may have created differing cues for coral spawn in the area. While TRI is not convinced that corals did not spawn in the area, TRI did not witness spawning. TRI is going to visit the Peanut Island lagoon in the next month or so in the evening to look more closely at This has caused TRI to work more closely with Palm Beach County Environmental Resource Management and Parks Department about changes that can be made to protect future of spawning in this area. Additionally, TRI is currently creating an updated monitoring list of reproductively viable corals at Peanut Island. TRI is already actively planning for the 2022 spawning season in August. The hope is that there will be more clarity this year as to if coral colonies are spawning, and if they are mistaking cues from artificial lighting.

While TRI did not collect any wild larvae during spawning season, it was wise to have systems prepared to receive larvae. For this reason, in September TRI was able to receive 20,000 C*olpophyllia natans* and 15,000 *Pseudodiploria clivosa* coral larvae that were spawned at the Florida Aquarium coral research facility in Apollo Beach. The larvae were placed in 3 systems that includes 7 aquariums for settlement and settlement studies. Those systems were conditioned with live rock and coralline algae for several months in preparation for spawning season. TRI received unsettled larvae approximately 2 days after spawning. Settled coral larvae was detected under a microscope approximately 5 days after they spawned. Coral larvae settled on glass, tiles, edges of systems and a variety of other locations. These have all been moved from settlement areas onto individual tiles. The Reef Institute estimates that there are approximately 1,000 surviving juvenile corals. It has come to our attention that TRI had one of the highest success rates of settlement and growth of the various facilities who received larvae from the Florida Aquarium. Many of the other facilities have seen between 2 - 20 successful recruits

TRI is in continued conversation with MOTE Marine Lab scientists and the Florida Coral Rescue Center on ways we can collaborate for holding and acquisition in future efforts. MOTE visited TRI in November, at which time they indicated that TRI will be a site used for spawning settlement in 2022. TRI has learned much about the care and rearing of *Acropora cervicornis* over the past year. TRI's focus remains with species of coral most susceptible to Stony Coral Tissue Loss Disease. However, working with a variety of stony corals from the Caribbean ensures that biodiversity is preserved as corals are grown for out planting efforts.

TRI discovered several corals that had come in undetected on live rock over two years ago. Most notably, multiple colonies of *Porites porites* have been growing. TRI scientists made the decision to use micro fragmentation of 3 colonies splitting them into about 80 micro fragments for the purpose of research on growth rates. These have been placed on tiles to monitor growth and keep for future research needs. They continue to grow and be fragged for multiplication efforts.

Favia fragum colonies continue to spawn under the care of the Reef Institute. The first spawning event was discovered in March of 2021. Those initial colonies continue to grow. TRI estimates there to be about 120 colonies of *Favia fragum* of various ages. The Reef Institute continues to seekiadvice from the Florida Coral Rescue Team if they would like any of these juveniles to eventually move to other members of the Florida Coral Rescue Project. TRI remains the only facility currently holding, spawning, and rearing *Favia fragum.*

In January, the Florida Rescue and Propagation Project moved 30 colonies of *Dendrogyra cylindrus* to TRI from the Florida Aquarium rescue center in Apollo Beach, FL. The Reef Institute was chosen to hold this vulnerable species due to the extraordinary care that has been shown to other corals. Currently, the Florida Aquarium and TRI are the only two facilities holding *Dendrogyra cylindrus.* They are on a Florida seasonal light cycle developed by Nikki Fogarty's lab at the University of North Carolina and on seasonal temperatures developed by the Florida Aquarium. They continue to do well. It is estimated that an additional 20 colonies will be moved from Florida Aquarium to the Reef Institute in July so FLAQ can continue to have additional space for spawning and rearing of juveniles.

On March 29, the Reef Institute received 12 wild collected *Acropora palmata* for a combined study on microbiomes with Mote's International Coral Gene Bank in Sarasota, Florida. The goal is to determine bacteria (16S sequencing) and Symbiodiniaceae (ITS2 sequencing) dynamics of *Acropora palmata* genotypes used in restoration. The goals are to look at what happens when transferred from the field to two different holding facilities, if the microbiome is affected when exposed to a Lugol's Iodine dip at each facility, and if there is any difference in coral health when held in the same system as other corals at each facility. This research will help lend data to the moving of corals onto land and back into the ocean. Research is completed and these colonies are now living at the Reef Institute. The Reef Institute will follow up with MOTE to discuss the best long-term placement of these corals.

On April 27th, The Reef Institute (TRI) received a surprise phone call from NOAA. *Diploria labyrinthiformis* had spawned early in the wild. They had coral larvae that needed to be settled for future out planting efforts. On April 28th, TRI received approximately 30,000 larvae. These were placed in three systems for settlement and rearing. They continue to be monitored for health and growth.

On May 28th the Reef Institute (TRI) received information from the Florida Aquarium that their *Diploria labyrinthiformis* had spawned in their Apollo Beach facility. TRI was contacted to receive larvae for the purpose of rearing juveniles for out planting. On May 29th TRI received approximately 96,000 larvae. These were placed in four vats for settlement and rearing. They have settled and are being cared for.

The Florida Coral Rescue and Propagation project has confirmed the Reef Institute will receive all corals from two rescue projects in October. The number of corals is still to be determined, but the Florida Coral Rescue and Propagation Project assessed all space at the Reef Institute for future projects. The Reef Institute will continue with a combined effort of caring for corals in the rescue project and rearing juveniles for out planting.

As the Reef Institute has added additional space outdoors there will be continued discussions on how to best utilize that space. Currently salvage corals and juveniles that are at least six months old will move to that space. This makes room for continued space during spawning season for larval settlement, and methods for better raising juveniles for future out planting. Leaders of the Florida Coral and Rescue Project are suggesting that juveniles should be about two years old for out planting. This additional outdoor holding provides space for corals to grow in natural sunshine with controlled temperatures so they can be staged for preparation for out planting efforts.

TRI remains waiting as the Florida Coral Rescue Team makes decisions on needs and next steps in new rescues, holding, movement of corals. The Reef Institute has been talking to the Florida Coral Rescue and Propagation Project for over a year about bringing rescues into the Reef institute from Peanut Island. There have been multiple unknow delays, and TRI continues in conversations on how to best move forward with bringing some corals in from this area. TRI did experience a sump issue with their larval system, that has since been remedied. This will aid in the determination on how many *Diploria labyrinthiformis* from the April/May spawning season will grow into adulthood. At this juncture juveniles are too young to determine the survival rate.

- **Deliverables due for this task:**

  Pictures of all corals currently in holding are uploaded into both the Florida Coral Rescue Project database and shared via Google Drive with DEP and all-important parties. Photos are available for each month in the appropriately marked folders. Final photos and information are uploaded.

- **Description of how deliverables are submitted and where to find them in submission:**

  2021 Coral Photos
  2022 Coral Photos

Task 2: Maintenance of corals and water quality. Coral growth monitoring.

Starting in July, TRI began watching two *Solenastrea bournoni* colonies and approximately eight of *Favia fragum* colonies were moved into a treatment area. It was believed it could be red ciliated protozoans irritating both species. This was later confirmed through close monitoring.

By early August a third colony of *Solenastrea bournoni* was added to TRI's watch list. However, by late August all three colonies appeared to be recovering. By late September these colonies had fully recovered and the "Revive" dips were ended. These colonies began to reexhibit similar symptoms again in May, and Revive dips were reinitiated in early June. These three colonies continue to be closely monitored.

July through December TRI had ongoing issues with *Favia fragum* colonies. TRI currently has 58 colonies of *Favia fragum* in holding. As previously mentioned, eight colonies were initially

moved into a treatment area. In September, these eight remained under treatment. It was confirmed the issue was a red ciliated protozoan irritating the coral. As TRI remains the only facility holding *Favia fragum,* their care remains a learning experience. In September, TRI did lose one colony (F23). In December we lost two more colonies (F22a and F28). An algae outbreak was mitigated in our system holding *Favia fragum* in May. Hair algae was removed from tiles, and from the system so corals were not agitated by an algal overgrowth. 22 colonies *of Favia fragum* were dipped in revive for 3 minutes on both May 26, 27 and 31 to treat for opportunistic red ciliates. All corals are recovering, algal overgrowth has been mitigated.
It appears that red ciliates that have been creating stress for 051,053,059 and 037. 037 has tissue loss in four polyps and 051 appears to have had a bleaching event. All four colonies continue to have Revive dips and are being closely monitored.

We did have a few issues in addition to the red ciliates. In August, it was discovered a juvenile pistol shrimp that had presumably "hitch hiked" on some live rock. It created a hole in one polyp of a single colony of *Montastraea cavernosa.* The shrimp was discovered after dipping the coral in "Revive." The colony has since fully recovered and regrown the lost tissue.

Two tiles with multiple fragments of *Orbicella faveolata* appeared to have a small, slow-moving infection that primarily causes discoloration of polyps. This was initially discovered in August. Members of the Florida Coral Rescue Project suspected that an endolithic alga was causing issues within this species. Dr. Gregory presented the issues with the coral at the Florida Coral Holders meeting in October to receive official opinions on the cause of the infection. As these corals were rescued off a metal sea wall and have metal embedded in their skeleton, it was suspected this could also be a cause of the issues with the corals. Heavy metals are regularly removed from the water where these corals reside. After placing these colonies in a more direct water flow, the issues began to resolve. Keeping these colonies in an area with high water flow keeps the colonies healthy.

In November, the Reef Institute changed their measuring techniques for the corals. TRI had been measuring height, width, and length of each colony in the Florida Coral Rescue Project in our holding using the program "ImageJ" for accuracy. Scientists were noticing issues with how they were being measured (i.e., length and width includes dead areas or gaps and does not include geometry for calculations). After discovering a brief from Sarah Hamlyn at MOTE Marine Labs, a change was made to follow the protocols used at MOTE. This included mounting our camera on a stationary stand to create a consistency in photographing. TRI measures the area of coral, in place of height, width and length. This change was first reflected in November reporting and continues in December reporting. This methodology of measuring appears to be a more accurate way to continue to gather data on the growth of the corals in TRI's care.

The Reef Institute continues to deal with an acute disease process on about 10 random heads of Eusmilia fastigiata (EFAS). Dr. Charles Gregory presented an overview of the analysis of ongoing issues with these colonies that started in January to the official coral holders in Mid-March.

As mentioned in previous reports, the diminishing alkalinity was discovered due to a blocked line. The alkalinity bumped down to 7.0 on January 10th then bumped up to 7.75 in one day (1/11) and to 8.7(1/12) over 2 days. By early January a few irritated margins on 5 Eusmilia fastigiata colonies was limited to single or 2 heads. These were treated with revive dips to mitigate opportunistic ciliated protozoans. On January 12th, it was discovered that a few EFAS corals were showing streaks of whitening along sides of heads, some progressing up to edges of heads up to the mouth. Over the next few days, it was discovered that a significant number of red ciliates appeared when observed through a microscope. This was believed to be possibly opportunistically infecting damaged tissues. On February 7th, 9th, 11th, 23rd, and 28th antibiotic dips were performed, as well as trials with feeding corals food soaked in antibiotics. Even with subsequent rinsing of corals this seemed to prompt slight water cloudiness corresponding to a bacterial bloom. The protein skimmer on the system was tweaked to correspond with this new bloom.

Diseased heads were separated from healthy heads and remounted on new tiles. The fear was that the treatments and handling of corals could be causing ongoing stress leading to disease spread. The disease continued to manifest itself even on single healthy heads with their move onto individual tiles. The disease spread to corals that had never received treatment. During the beginning of March antibiotics were halted on diseased heads, and revive dips were used as the course of treatment.

After consultation with the coral holders, and specifically the Florida Aquarium, it was discovered this species of coral has been difficult in holding across facilities. The suggestion was made in the coral holders working group to restart antibiotics, but to keep corals exposed to medication for longer periods of time.

Ampicillin 50 mg/l per one inch of salt water held in bath for 10 days. was started on March 23rd for 4 colonies and the disease seems to be arrested but the initial conditions of many of the heads was poor. Some of the original EFAS that were moved to their own tiles and into different systems have begun to recover. However, about 6-8 heads continue to struggle to recover from the onslaught of disease. The Reef Institute continues to deal with an acute disease process on about 10 random heads of Eusmilia fastigiata (EFAS). Dr. Charles Gregory presented an overview of the analysis of ongoing issues with these colonies that started in January to the official coral holders in Mid-March.

After multiple months of struggling with an acute disease process on about 10 random heads of *Eusmilia fastigiata* (EFAS) most of the lineages in the care of the Reef Institute lingered past any point of recovery in May. The following lineages in the care of the Reef Institute have been lost:

NC0002_EFAS_004
6_EFAS_006
BW16_EFAS_004
NC0001_EFAS_001
NC0002_EFAS_015a
WS_EFAS_004

Dr. Charlie Gregory is in the process of writing up a white paper for the Florida Coral Rescue and Propagation project on processes and procedures learned from caring for the EFAS. The hold is that it could be part of a long-term husbandry and disease management compendium.

The belief is that it began with an alkalinity swing after which these corals began to struggle. The acute physiological stressor is believed to have immunocompromised the corals for the long-term leading to a variety of opportunistic problems.

Two of the colonies of *Dendrogyra cylindrus* were discovered to have worms agitating the colony and creating holes in the skeletal structure. These worms were removed and the *Dendrogyra cylindrus* appear to be healthy. They continue to be monitored closely, but no new holes have appeared.

Meandrina meandrites (WS_MMEA_004) lost multiple polyps in the center of the colony. This has been treated with alternating revive and lugol's iodine in 2 waves through May and continues to have weekly Revive dips through June. The belief is that the polyp bleached and died acutely likely from something possibly ingested. The colony continues to recover.

Atypical polyp bleaching on WS_MCAV_005 has additionally resulted in 1 polyp lost, and a slight bleaching event at the top of the colony. This continues to be monitored closely along with a slight bleaching in WS_MCAV_020. Corals continue to eat and are being cared for.

- **Deliverables due for this task:**

  Spreadsheets of water quality, treatment and health records, and growth are shared via Google Drive. All photos are shared via Google Drive.

- **Description of how deliverables are submitted and where to find them in submission:**

  DEP Reporting
  DEP Reporting 2 (2022 Data)

Task 3: Reporting

- **Progress for this reporting period:**

  There are two required calls to participate in the Florida Coral Rescue Project. The Disease Advisory Committee meets biweekly and reviews any additional information pertinent to stony coral tissue loss disease. The AZA Coral Holders Call meets weekly and reviews all guidelines and parameters in participating as a long-term holding facility. All calls are recorded. For AZA call logs and recordings for proof of attendance please contact: Beth Firchau at bfirchau@AZA.org. For DAC call logs please contact Victoria Barker at: victoria.barker@noaa.gov.

- **Identify any delays or problems encountered:** None
- **Deliverables due for this task:**

Call Log for June:

Date	Time	TRI Staff Attendance	Name of Call
6/1/22	1:30 - 3:00 PM	Dr. Charlie Gregory	DAC Call
6/2/2022	1:00 - 2:00 PM	Dr. Charlie Gregory Chad Scott	AZA-FRTRP Coral Health Management Advisory Group
6/2/2022	2:00-2:30 PM	Chad Scott	AVID RFID Testing group
6/6/22	11:00 – 12:00AM	Chad Scott Charis Peterson	Restoration Meeting
6/8/2022	1:30 - 3:00 PM	Dr. Charlie Gregory	DAC Call
6/15/2022	1:30 - 3:00 PM	Chad Scott	DAC Call
6/9/2022	1:00 - 2:00 PM	Dr. Charlie Gregory	AZA Coral Holders
6/16/2022	1:00 - 1:45 PM	Dr. Charlie Gregory/Chad Scott	AZA Coral Holders
6/23/2021	3:00 - 4:00 PM	Dr. Charlie Gregory	AZA Coral Holders
6/29/2022	1:30 - 3:00 PM	Dr. Charlie Gregory	DAC Call
6/30/2022	1:00 - 1:45 PM	Dr. Charlie Gregory/Chad Scott	AZA Coral Holders

Task 4: Expand Outdoor Holding Space

Final Report:

The 6 vats funded for this expansion have been plumbed, set up, and are ready for cycling. The hope is to move corals into these between July and August. Some products for building were delayed due to supply chain issues and shipping delays. They have been installed, and all systems are ready to prepare for coral holding and propagation. The Reef Institute is still figuring out the additional shade cloths and covers that will be needed to properly protect the system from the elements. While these were ordered and installed, the Reef Institute may add additional protection against Florida's extreme weather.

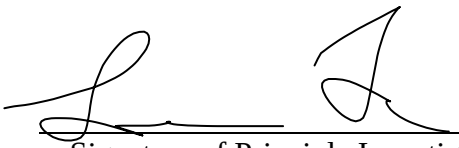
DEP Addition

DEP Receipts

All final photos, power points, and a walk-through video have been uploaded to Google Drive. This can be found here:

Final Reports: Final Reports Are Here

This report is submitted in accordance with the reporting requirements of the above DEP Agreement number and accurately reflects the activities associated with the project.



Signature of Principle Investigator or Designee

6/13/22

Date