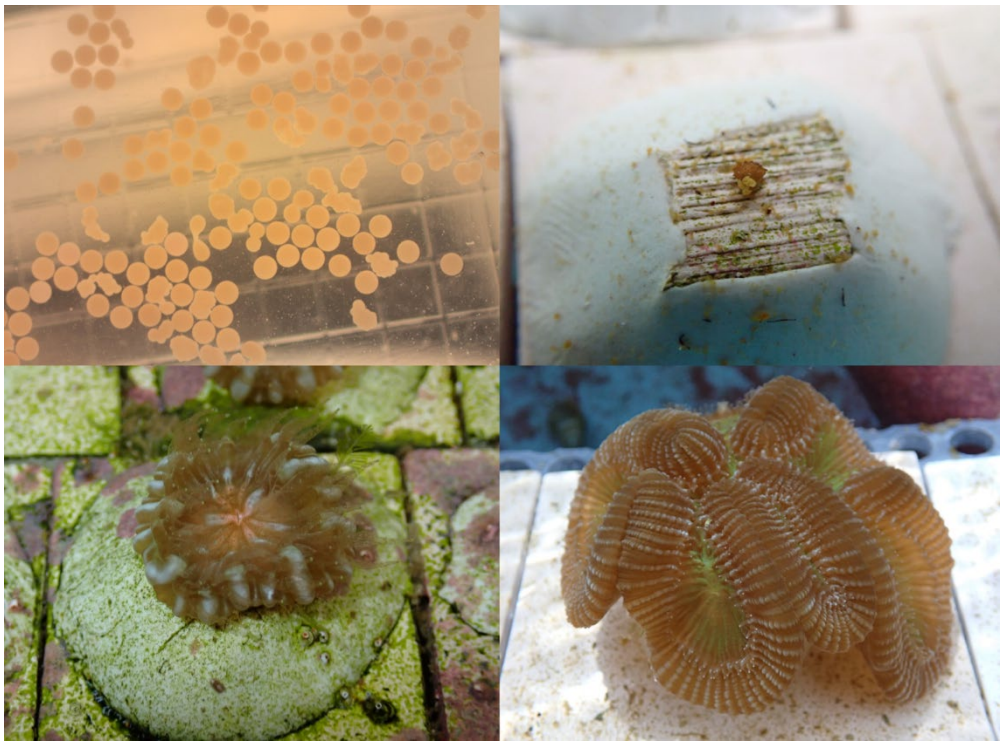
Coral Propagation: Land-based and Offshore Nursery Phase IV

Final Report



Coral Propagation: Land-based and Offshore Nursery Phase IV

Final Report

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Management Summary

The culture of corals at Nova Southeastern University's *ex situ* and *in situ* nurseries is increasingly successful. One hundred and twenty-four adult corals were kept in captivity at Nova Southeastern University's *ex situ* nursery, with a few cases of disease and death, likely caused by a dramatic shift in temperature during cold snap events which led to a shift in tank microbiome. The elimination of light pollution to the induction systems led species which had never spawned at our facilities, *Orbicella faveolata, Pseudodiploria clivosa, P. strigosa,* to do so this year. Still, we saw a reduction in fecundity of *M. cavernosa* and several colonies shifting from female to males, likely caused by a reduction in feeding. As a result, the feeding regime was increased right after spawning. This year's success in rearing coral juveniles was much higher than in previous year, with over 4.4k corals produced that have been or will soon be transferred to the offshore nursery or outplanted on the reefs of the Coral ECA. The increased production of sexually-produced corals for outplanting has been the result of research conducted in this and previous years. The larvae of seven species were also used in experiments to assess their survival over time and competency dynamics. This data was incorporated into bio-physical dispersal models to make recommendations on the best reefs to restore along Florida's Coral Reef (FCR) and within its several regions, including the Coral ECA. The grow-out of sexual recruits at the offshore nursery seems to be mostly hampered by predation and may require refinement to prevent it. The co-culture of *Mithrax* crabs to control algal overgrowth proved to be harmful to the corals, thus should not be pursued. A new induction room was planned and the equipment necessary to set it up was acquired.

Executive Summary

The culture of corals at Nova Southeastern University's *ex situ* and *in situ* nurseries has progressively become more successful. One hundred and twenty-four adult corals were kept in captivity at Nova Southeastern University's *ex situ* nursery, with a few cases of disease and death due to tank water quality fluctuations. Corals kept indoors were induced to mature their gonads. The elimination of light pollution in the lab using blackout curtains led to species which had never spawned at our facilities, *Orbicella faveolata, Pseudodiploria clivosa, P. strigosa,* to do so this year. Still, we saw a reduction in fecundity of *M. cavernosa* with several colonies shifting from female to males. We think this was caused by a reduction in feeding implemented in the previous year to decrease the concentration of nitrates and phosphates which tend to boost algal overgrowth. Reduced feeding likely reduced fecundity and led to shifts from producing energy-expensive egg to energy-cheap sperm. As a result, right after spawning, we shifted the feeding regime back to higher quantities, matching what is used in other successful coral nursery facilities. The coral larvae produced in our lab and the ones donated to us were settled and raised until they reached a size suitable to be transferred offshore. This year's success in rearing coral juveniles was much higher than in previous year, with our facilities being able to raise over 4.4k corals that have been or will be transferred to the offshore nursery or outplanted on the reefs of the Coral ECA.

The increased production of sexually-produced corals for outplanting has been the result of research conducted in this and previous years, including development of a hands-off free transfer of larvae from larval tanks to settlement trays, optimization of light regimes during early grow-out, improved techniques to accelerate acquisition of algal symbionts, use of herbivores to control algae, more frequent manual removal of algae, improvement of water quality through the use dosing and calcium reactors, separating coral juveniles that settled on the same tile into individual tiles earlier on, and moving them outdoors when they reach 3 months. The larvae of seven species were also used in experiments to assess their survival over time and competency dynamics. This data was modeled and shared with partners at the Universite Catolique de Louvain (Belgium) which have now developed connectivity models for these seven species for the entire Florida's Coral Reef. The Nature Conservancy is currently using that information and combining it with demographic data to make recommendations on the best reefs to restore along the FCR and within its several regions, including the ECA. The grow-out of sexual recruits at the offshore nursery seems to be mostly hampered by predation and may require refinement to prevent it. The co-culture of *Mithrax* crabs to control algal overgrowth proved to be harmful to the corals, thus should not be pursued. A new induction room was planned and the equipment necessary to set it up was acquired.

The findings of this project demonstrate methods to protect and produce genotypes of corals through active restoration, which directly supports a resilience based and adaptive management approach. The knowledge on reproduction of massive coral species within the Coral ECA have many unknowns. This project contributes to the increase our knowledge of the reproductive timing and modes, and improvement of culturing conditions for corals, and production of corals to restore corals in the Coral ECA.

Acknowledgements

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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
Coral ECA	Kristin Jacobs 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 through the 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 (Coral 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 Coral 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 one indoor and eight 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 will see 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, one 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 is holding 41 *Montastraea cavernosa*, 30 *Orbicella faveolata,* 18 *Pseudodiploria clivosa,* 25 *P. strigosa,* four *Colpophyllia natans,* six *Diploria labyrinthiformis, and* 14 *Siderastrea siderea* mature-size colonies of unique genotypes, all from the Coral 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 ofreef-building coral species impacted by the disease, *Montastraea cavernosa, Orbicella faveolata, Pseudodiploria clivosa, P. strigosa,* and *Colpophyllia natans*, from the Coral ECA which resisted the disease under conditions that intend to induce gonad maturation and synchronous spawning of captivity. We reared coral larvae and grew sexually-produced corals, as well as 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 aimed to contribute to accelerating the breeding of hardy genotypes, re-establish or enhance the natural sexual reproduction on the reef, and hence reef resilience. In Phase III of this project (FY2022), we continued to monitor spawning and maintain colonies of reef building coral species impacted by disease, *M. cavernosa, O. faveolata, P. strigosa, P. clivosa* and *C. natans*. We began a co-culturing project between *Mithrax*

spinossisimus crabs and *Porites astreoides* recruits, to determine the optimal methods of macroalgae removal from coral recruits. Issues of light pollution within our outdoor nursery were addressed by trialing blackout curtains on two of our outdoor recirculating systems, which we hoped would lead to an increased number of adult colonies spawning. We worked to improve our new larval rearing and settlement system, to increase the scale of work within our lab. In Phase IV, we aimed to optimize our spawning, settlement, and rearing processes to allow us to upscale the amount of coral we can produce.

For Phase IV of this project (FY2023), 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 Coral ECA which resisted the disease, and rear them until they reached a size suitable for outplanting. Combined, these techniques 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 continued 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 were grown in the land-based and offshore nurseries. 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) is our main goal and we allocated as many resources as necessary to those tasks, however if the sexual reproduction is not or only partly successful, we committed to still produce the same amount of coral biomass using asexual reproduction (microfragmentation techniques). These activities preserve the most resilient genotypes of the most disease-impacted reef-building corals, from the Coral ECA, propagate them to facilitate population recovery in future restoration projects. The outcomes of this project 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 have been incorporated into an on-going coral disease response effort which seeks to improve understanding about the scale and severity of 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 duplicate efforts, this project targeted corals from the Coral ECA, where remaining corals have survived the disease outbreak. Since broadcast spawning coral species are known to be connected throughout Florida's Coral Reef (FCR), if the corals from the Coral ECA did not spawn, we fulfilled the goals of this project using gametes or larvae collected from FCR corals outside this region, collected by other institutions. 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.

2. METHODOLOGY

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

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, Pseudodiploria. strigosa, Colpophyllia natans*, *Diploria labyrinthiformis,* and *Siderastrea siderea* were observed until midnight for spawning. Note: these colonies were collected in Broward County in 2019 and 2020 and are being maintained in the tanks at NSU to preserve their genetic diversity (Task 5). The number of colonies that spawned each night was 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

Eggs and sperm from colonies of each species that spawned in the land-based nursery from Task 1 were combined, keeping sperm concentration at 10^5 - 10^6 mL^{-1} to maximize fertilization. One hour after the eggs were 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 mimicked historical conditions on the reef during a typical summer (not necessarily current conditions) to maximize survival; water changes were performed as needed to avoid toxic components such as ammonia to accumulate and harm the larvae.

Once the larvae become 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. 24h to 48h after the competent larvae were exposed to the conditioned tile, the tiles were observed under the scope for metamorphosis. The larvae which remain swimming were provided with new settlement tiles. This process was repeated daily for one week, or until all the larvae either died or settled.

As we did not obtain a lot of gametes from some of the coral species held at NSU, we received larvae from corals from other regions within the FCR that spawned in the field and at other institutions. The Florida Aquarium and University of North Carolina Wilmington, and these were reared as described above to fulfill the goals of this and Task 3 below. Because broadcast spawning corals have been found to be well connected throughout Florida's Coral Reef (FCR), these corals can be outplanted within the Coral ECA to restore this region.

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

Newly settled corals were initially reared in an existing 1500L indoors recirculating system composed of 2 raceways (each 2.5 m x 0.6 m 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*. We used a modified annual temperature cycle that mimics the natural annual temperature cycle but never goes below 23.3°C or above 28°C, as this typically impairs growth. Temperature was measured daily with a YSI® Pro30 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 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 grow 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 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 does not contain ammonia nor nitrites, and nitrates are at 0.05-0.2 ppm, and phosphate at 0.02-0.03 ppm.

Adult corals of the several 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 seed 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 Golden Pearls (Aquatic foods) *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. At the 6 months mark, the number of corals of each species was counted.

2.4. Task 4: Grow-out of newly settled corals at the offshore nursery

Six hundred and two newly settled corals of *Diploria labyrinthiformis* were moved to the offshore nursery in late fall 2022 or early 2023 for grow-out. These newly settled corals 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 at the time of deployment to the offshore nursery. Colony survival and condition was monitored approximately 1 week, 1-month, 3-month, and will be monitored at 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 2023, the deployed settlement tile/plug will be examined for colony survival in May 2023.

2.5. Task 5: Preserve coral genotypes of disease impacted species and induce gonad maturation in spawning capacity

NSU's land-based nursery holds an extensive number of colonies of the reef-building coral species from the Coral ECA, specifically 54 *Montastraea cavernosa*, 28 *Orbicella faveolata*, 19 *Pseudodiploria clivosa*, 18 *P. strigosa*, nine *Colpophyllia natans*, two *Diploria labyrinthiformis*, and 14 *Siderastrea siderea* mature-size colonies of unique genotypes (144 colonies total). These colonies represent a significant part of the genetic diversity of the populations of these species in 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 SCTLD.

These colonies were maintained in 12 outdoor independent recirculating tanks and two indoor independent recirculating tanks (in 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 allowed 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 cycles. Both indoor and outdoor aquarium systems are equipped with biological and chemical filtration, UV sterilizers, heaters and chillers, and calcium reactors or alkalinity and calcium automatic dosers. The indoor system was fitted 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 was 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. Corals were hand-fed daily *Nannochloropsis* and Rotigrow Plus-enriched rotifers and *Artemia* nauplii, RN Oyster Feast, RN ROE, Reef-Roids (PolypLab), and Golden Pearls (Aquatic Foods) *ad libitum* to promote growth and enhance survival rates. Water quality was monitored weekly or more frequently if needed.

2.6. Task 6: Co-culture of coral recruits with herbivorous invertebrates

Three gravid female *Mithrax spinosissimus* were collected in Key West via snorkeling in the summer of 2022 and transported to Nova Southeastern University (SAL-22-2238-SCRP). Females were placed into 38L polystyrene tubs equipped with a rigid airline and continuous water flow. When larvae were released, they were gently flushed through the outflow of the tank into a 105 µm mesh filter. Larvae were transferred to a 208L conical tank for two days, after which they were transferred into 38L Nalgene tubs to metamorphose into benthic megalopae. Post-larval grow-out tanks contained a rigid airline, continuous water flow, and a 105µm mesh filter cover on the outflow of the tank to prevent escape. The juvenile crabs were fed a diet of *Menidia menidia* and turf algae.

Three experimental treatments were created to test the effectiveness of juvenile *M. spinossimus* versus manual algal removal. The first treatment, manual algal removal, was performed *ad libitum*. The second and third treatments consisted of low (one crab/ three L) and high (two crabs/ three L) densities of Caribbean King crabs ranging from two to six weeks of age. Tiles in the second and third treatments did not receive manual algal removal for the entirety of the experiment. In the second and third treatments, crabs continued to be fed *M. menidia* and turf algae. For at least two "rescue" coral species for which settlers are attained, 20 newly settled corals (on at least five tiles) (Task 2) was randomly placed into each treatment. There were two replicates created for each treatment, for a total of 80 corals represented per species. There was an equal composition of all coral species represented between tanks. Experimental treatment tanks contained the same

equipment as crab post-larval grow out tanks. 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.

A second experiment was conducted where juvenile crabs were driven to NSU's coral nursery in late January. A pilot study was first conducted to determine if the juvenile crabs have a diet preference. The algal load in the indoor aquariums is often a unique blend of microalgae (often diatoms) and macroalgae (such as *Caulerpa* spp. and *Halimeda* spp.). Identifying the types of algae juvenile crabs fed upon ensured crabs were grazing daily and can be sustained in this experiment.

The follow up experiment was conducted February 9th to February 17th, 2023. Crabs were housed with coral recruits across two indoor nursery systems, Rico or LS, in varying levels of density. New mesh baskets were made with ~400µm mesh and supplied with an aeration line. Three baskets were fitted per raceway. Two raceways were used, for a total of six baskets. Each raceway had one replicated treatment: control (manual algae removal), low density crabs (one crab per three L of tank water), and high density of crabs (two crabs per three L of tank water). Each basket received 18 *M. cavernosa* corals and 12 *P. strigosa* corals. Tiles with coral recruits were counted and photographed at the beginning and at the end of the study. Photographs of the tiles were visually assessed to determine the algal growth. Coral survivorship was collected by looking at each coral under the microscope for presence or absence at the beginning (before crabs) and after 10 days. To test the effect of treatment (i.e. low density, high density, or manual cleaning) and species (i.e. *M. cavernosa* and *P. strigosa*) on coral survivorship, a mixed effects model was run. Replicate tank was included as a random factor in the model.

2.7. Task 7: Coral larval competency and survivorship curves

Species-specific reproductive and larval traits that influence dispersal kernels include the onset and duration of larval competency, and rates of coral larval mortality; however, these are largely undescribed for the corals species of our region at the resolution necessary to simulate dispersal. We measured competency and survivorship in seven species by spawning adult colonies in the laboratory, performing cross-fertilization, and observing settlement and survivorship in indoor coral larval mesocosms. To estimate long-term survival, coral embryos/larvae were reared without settlement cues and counted daily (four rep. of 100 embryos/larval treatment, as in Figueiredo et al. 2014). To assess larval competency (acquisition and loss), every day for 10 days, and after that biweekly for two months, five replicates of 20 larvae (reared without settlement cues) were exposed to a pre-conditioned tile (colonized by coralline algae and bacterial biofilm). After 24 h, the number of larvae metamorphosing was recorded to estimate the daily proportion of competent larvae (ready to settle). This data was then used to model larval survival and competency dynamics as described in Connolly & Baird (2010), and extensions by Figueiredo et al. (2013 and 2014). These models were utilized by other research groups to empirically calibrate bio-physical models of larval dispersal of corals that assess connectivity along Florida's Coral Reef.

From May to September 2022, six coral species were studied to determine the proportion of larval survival over time. The six species studied were *D. labyrinthiformis, Acropora cervicornis, P. strigosa, C. natans, M. cavernosa,* and *O. faveolata.* After confirmation of fertilization, 400 fertilized embryos were collected and placed into four temperature-controlled jars each containing 100 fertilized embryos. Total number of viable larvae were counted daily to obtain data on the proportion of larval survival over time. Jars containing larvae receive a water change daily to maintain water quality. Once one hundred percent mortality was reached for each species, the experiment was ended.

Additionally, seven species of coral were studied to determine the proportion of competent larvae over time. In addition to the six species studied for survival, *P. clivosa* was also included in the competency trials. Once rotation and motility were observed, 3,500 larvae were collected and placed into three separate holding bowls. The seawater was changed daily. Each day, 10 larvae were randomly collected from the bowls and placed into each of the four replicate jars, each containing one ceramic tile sprinkled with crustose coralline algae (CCA), to induce settlement and metamorphosis. After 24h, these tiles were removed from the jar, censused for larvae settled and/or metamorphosed. The experiment ended when there were no more mobile larvae within the holding bowls.

Larval survival and competency dynamics for each species were modelled according to the methodology described by Figueiredo et al. (2014). Briefly, for mortality, our most general model is the "generalized Weibull". This model allows for "bathtub-shaped" mortality curves, where mortality is high initially, decreases as larvae age, and then increases again as larvae become very old. We also consider special cases of the generalized Weibull. For instance, the standard Weibull survival model, which allows monotonic increases or decreases in mortality rate over time, is the limiting case of the generalized Weibull and the exponential model, according to which mortality rate is constant over time, is the special case of the Weibull model. For each species, the survival data was fit to the three models, and the best fitting one was selected.

For the acquisition of competency, since there is a minimum period of time required for individuals to complete embryogenesis and develop the structures needed for settlement, this rate was initially considered zero and afterwards (the estimated minimum time to competency) larvae acquired competence at a constant stochastic rate. For the loss of competence, our most general model is the "generalized Weibull". This model allows for "bathtub-shaped" loss of competence curves, where the rate of loss of competence is high initially, decreases as larvae age, and then increases again as larvae become very old. However, we might expect instead that loss of competence increases over time as larvae become very old and eventually deplete their energy reserves. Therefore, we also consider special cases of the generalized Weibull. For instance, the standard Weibull model, which allows monotonic increases or decreases in competency rate over time, is the limiting case of the generalized Weibull and the exponential model, according to which rate of loss of competency is constant over time. The competency data was fit to the three models, and the best fitting one was selected.

2.8. Task 10: Infrastructure for induction of spawning indoors

Many of the adult corals maintained at our facilities are outdoors where light pollution prevents them from spawning. Since the corals kept indoors in induction systems typically spawn, we planned to expand our indoor induction facilities to house all adult corals indoors and thus induce them to mature their gonads and spawn synchronously. This requires developing a new induction room equipped with up to six new induction culture systems. These systems will in the following year(s) be set up to induce spawning three different times in the year, making more space available in the lab 242 of the Guy Harvey Oceanographic Center to rear coral larvae, newly settled corals, and early juveniles, and in outdoor tanks for the grow-out of juveniles until they reach the size suitable for outplanting. This task refers solely to the acquisition of the equipment necessary to set up six new induction tank systems. The finishing of the new room on the ground floor of the Oceanographic Center (electrical upgrade, construction of wall and drains, and preparation of the floor) where these systems will be housed will only be concluded in the following year as its construction requires city permits that would not be available in time.

3. RESULTS

3.1. Task 1: Coral Spawning and Gamete Collection at land-based nursery

Across July to September 2022, spawning activity was observed in a total of 16 *M. cavernosa* colonies, one *O. faveolata* colony, four *P. strigosa* colonies, and two *P. clivosa* colonies held within the induction systems at NSU's coral nursery (Fig. 1). This year was a milestone as the induction of gametogenesis in many bouldering and brain coral species had never been previously achieved. We believe this year's success was the result of the reduction of light pollution in the lab (the lab was blacked out and all light sources covered). Additionally, this year we manipulated the lighting regime in each induction system to entrain the corals circadian rhythm to release their egg and sperm in the daytime. The adjustment proved to be feasible and most of the spawning observed happened between the hours of 11:00 to 18:00 GMT-5. Once egg and sperm were released, gametes were collected and combined for fertilization (Fig. 2). Depending on the timing of spawning and viability of gametes, several batches were produced per species. Once eggs were fertilized, the eggs were washed two to three times, depending on the starting sperm density (Fig. 3A), and transferred into a 25-gal fiberglass conical bottom tank (Fig. 3B). Tanks were set up days prior to spawning and received aeration and water flow to ensure they are primed for embryo transfer. Once the planula stage was reached, and larvae started searching for substrate, they were transferred from the mass scale larval culture tank to the mass-scale settlement tank, through the outflow and distributed across numerous settlement trays lined with preconditioned ceramic tiles (Fig. 4D).

Figure 1 *Indoor induction system is comprised of two fiberglass raceways running parallel that are supplied from the same seawater sump. Tanks are filled with four different species targeted to reproduce in the 2022 spawning season.*

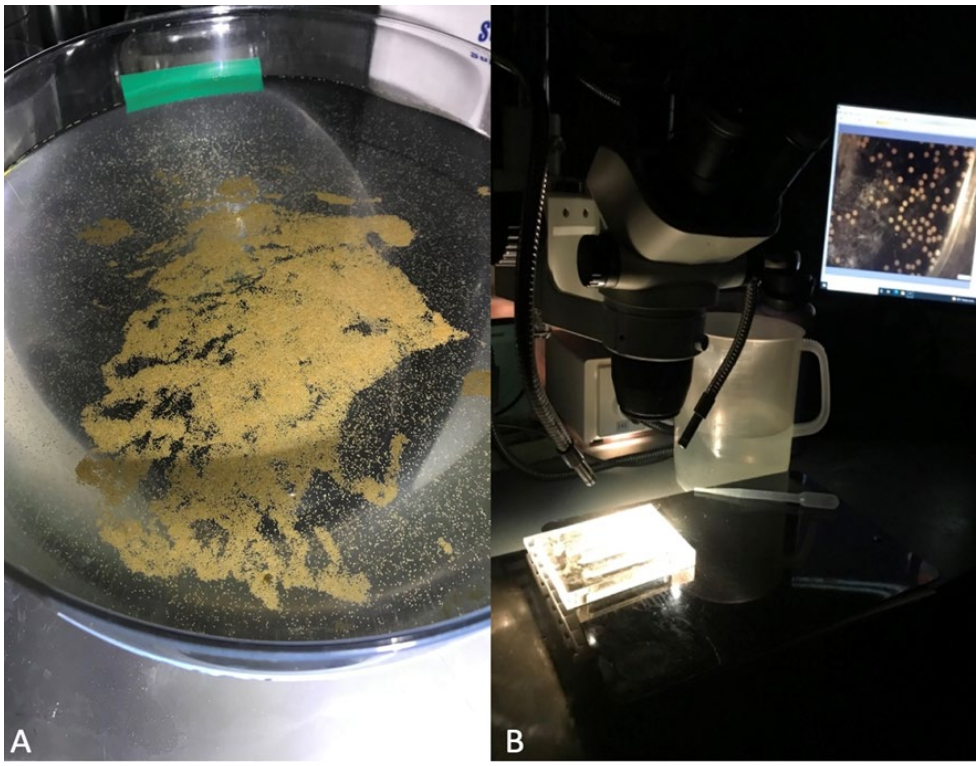


Figure 2 *(A) Floating gametes from Montastraea cavernosa colonies pooled into one container. (B) Example of checking for embryological development in fertilized eggs under the microscope.*

Figure 3 *(A) Montastraea cavernosa eggs being washed from excess sperm to prevent polyspermy in a gravy separator. (B) Transferring embryos into a 25-gal fiberglass conical bottom tank primed with new, aerated seawater where the reminder of their embryo development is spent.*

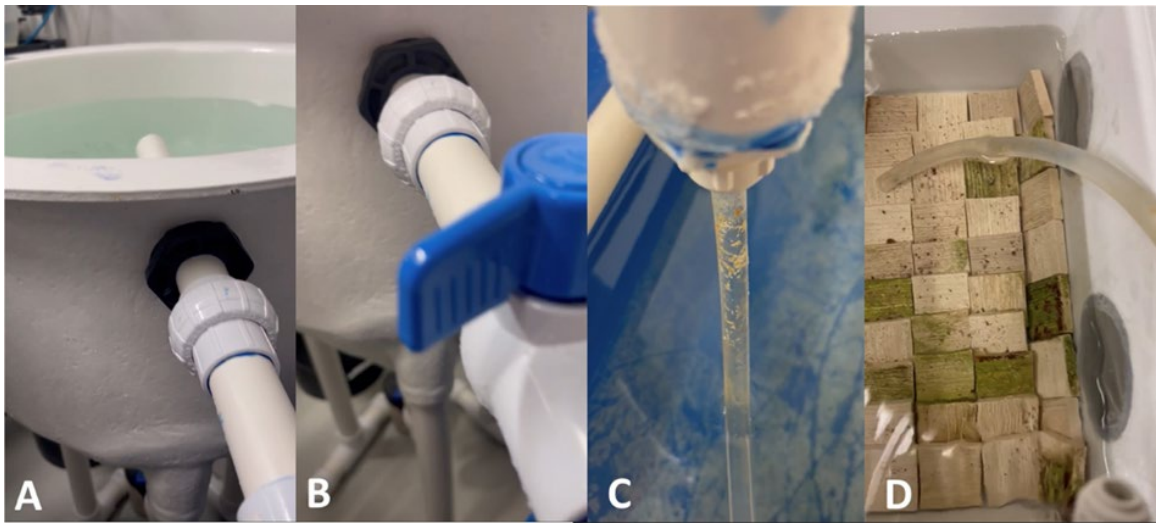


Figure 4 *Overview of water flow from the 25-gal conical bottom fiberglass tanks into a settlement bin lined with preconditioned tiles. Seawater containing larvae flows out of the tank's outflow (A) PVC pipe, controlled by a ball valve (B), which is connected to vinyl tubing (C), which delivers larvae to the settlement baskets with tiles (D).*

3.2. Montastraea cavernosa Summary

This facility has normally spawned *M. cavernosa* corals on an annual basis. Spawning activity was prolific in 2022, there were 44 individual observations of spawning activity, and the spawn came from 16 unique colonies of *M. cavernosa* (Refer to Table 1). Spawning for *M. cavernosa* was observed on four days across July 20th, 2022 to July 30th, 2022 (7 to 17 DAFM). A total of five colonies, four male and one female, released gametes in July. Only one day, July 20th, had both sexes release synchronously and fertilization did not occur as the sperm density was too low. Spawning for MCAV colonies was observed on nine days across August 10th, 2022 to August 24th, 2022 (-1 to 13 DAFM). A total of 15 *M. cavernosa* colonies, 12 male and three female, had released gametes in August; All five colonies that spawned in July also spawned in August. Near the end of the spawning window in August, when the highest frequency of male and female colonies released their spawn synchronously, we had fair amount of fertilization and production of viable larvae (Refer to Table 1). Spawning in one male and one new female *M. cavernosa* colonies was observed only on one night on September 18th (8 DAFM), where fertilization was successful, and larvae were produced.

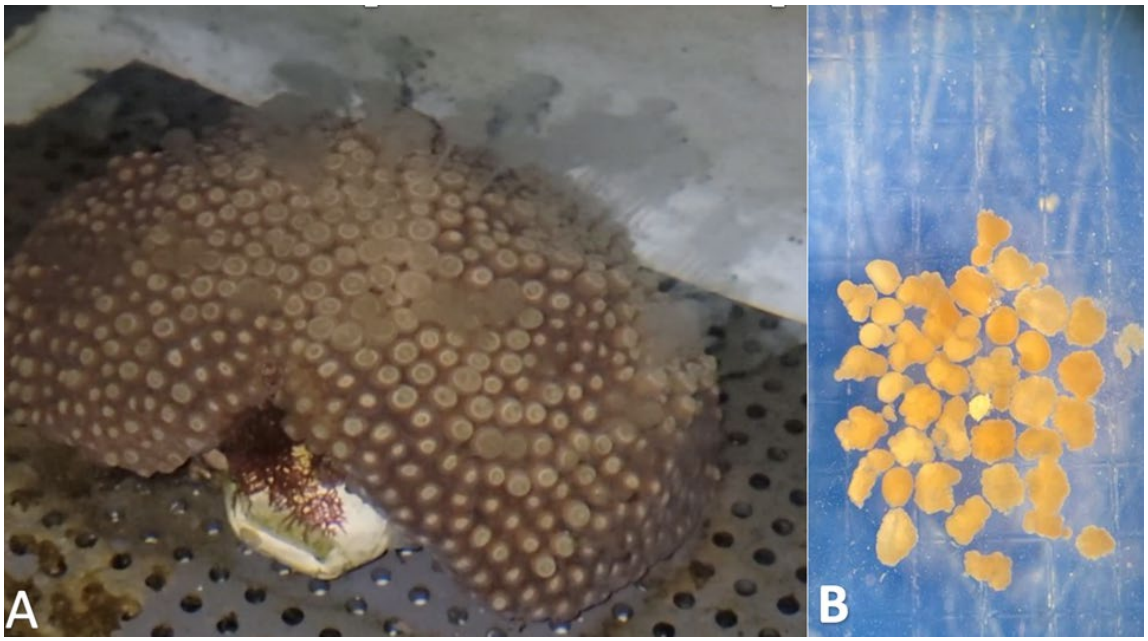


Figure 5 *(A) Montastrea cavernosa colony releasing sperm plumes across the whole colony. (B) Example of fertilized Montastrea cavernosa eggs undergoing cell division. For scale use the 1 mm square grid lines on Sedgewick rafter counting slide.*

3.3. Orbicella faveolata Summary

Spawning in the resident *O. faveolata* had only been observed once in 2020; this year we observed one colony release gametes again. Though it was not enough to make any crosses, it is still a sign that culture conditions are correct and improving. Hopefully with a few small adjustments to the general care and feeding, we will observe more spawning in years to come.

3.4. Pseudodiploria spp. Summary

For the first time in this facility, *Pseudodiploria* spp. released gametes in 2022 spawning season. Spawning for *Pseudodiploria clivosa* was limited this year, only 2 colonies released bundles on one day on September 18th (8 DAFM); Some fertilization did occur, and small number of viable larvae were produced (Refer to Table 1). Spawning for *Pseudodiploria strigosa* was observed on 2 days across September 18th and September 19th, 2022 (8 DAFM). A total of 4 unique colonies released bundles in a high spawn load and resulted in high levels of fertilization, >95%, and the production of a large amount of larvae (Table 1). Since *P. strigosa* colonies only released synchronously on one night, there was no other opportunity to work with *P. strigosa* larvae. The next day one *P. strigosa* coral colony released again but no other colony released, so no effort was made to self-fertilize these gametes (Refer to Table 1).

There are many challenges associated with the induction of brain corals: acclimation to system, nutrition, historical and current health state, and size are all playing a role in captive spawning in these species. The corals in the outdoors systems did not spawn due to light

pollution, and indoors there is little space available, so we did not have many colonies of each species in the indoors induction systems, which contributed to the lower number of colonies of *Pseudodiploria* spawning. Additionally, to help maintain water quality optimal, particularly keeping nitrates and phosphate levels low, we had reduced the feeding of adult corals. We now believe that this food reduction, while it did not affect coral health, may have reduced their fecundity; we have increased feeding again. Hopefully by maintaining high levels of general care and a nutritional feeding regime, we can see these numbers increase next spawning season.

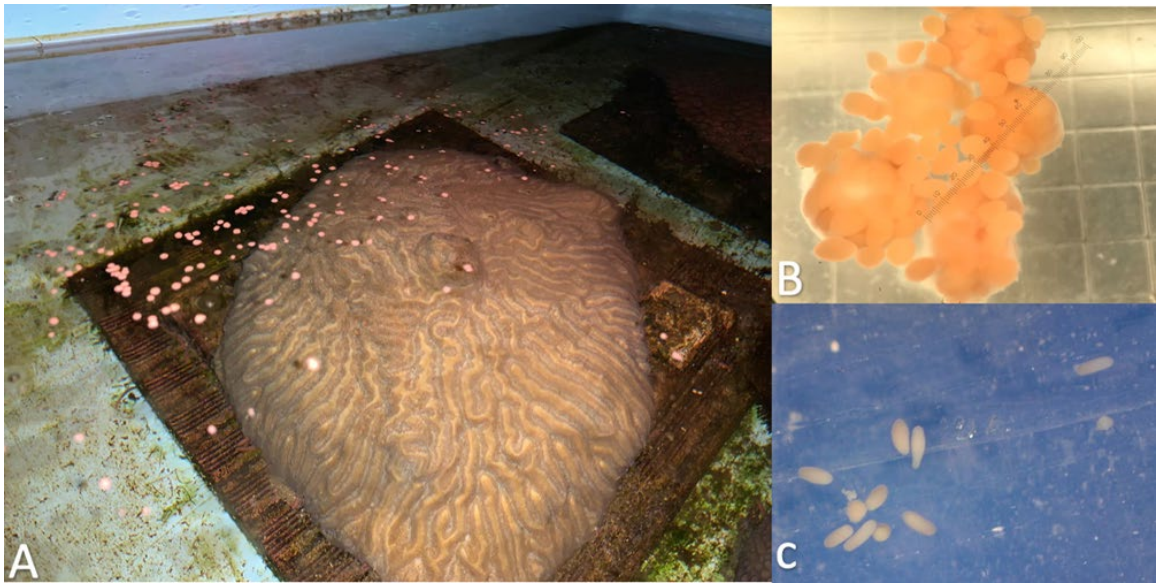


Figure 6 *(A) Pseudodiploria strigosa colony actively releasing gamete bundles with some gamete bundles floating at the surface. (B) Intact gamete bundles released from Pseudodiploria clivosa. For scale use the 1 mm square grid lines on Sedgewick rafter counting*

Table 1. Summary of Spawning observations outlining the date and time of spawning of captive coral colonies, the fertilization rates, and estimated number of larvae produced. All larvae produced remained at NSUs coral nursery. (Species: MCAV – *Montastraea cavernosa*, OFAV – *Orbicella faveolata*, PSTR – *Pseudodiploria strigosa,* PCLI – *Pseudodiploria clivosa;* Colony sex: F – female, M – male, H – hermaphrodite, N/A – not applicable)

Date	Species	Colony ID	Colony Sex	Spawning Start time	Spawning End time	Spawn quantity	Fertilization	Num of larvae produced	Larval fate
7/20/2022	MCAV	MC19_22	M	13:30	14:00	Low	0%	0	N/A
7/20/2022	MCAV	MC19_10	M	13:32	14:00	Low	0%	0	N/A
7/20/2022	MCAV	BRWDCOM_MCAV_01	F	13:34	13:46	Low	0%	0	N/A

7/21/2022	MCAV	MC19_18	M	13:21	14:00	Low	0%	0	N/A
7/21/2022	MCAV	MC19_04	M	13:25	13:40	Low	0%	0	N/A
7/24/2022	MCAV	MC19_10	M	13:25	14:00	Low	0%	0	N/A
7/30/2022	MCAV	MC19_10	M	12:47	13:10	High	0%	0	N/A
8/10/2022	MCAV	MC19_04	F	13:52	14:23	Low	0%	0	N/A
8/11/2022	MCAV	MC19_04	F	13:17	13:45	Medium	0%	0	N/A
8/12/2022	MCAV	MC19_04	F	13:17	13:45	Medium	0%	0	N/A
8/18/2022	MCAV	BRWDCAVE_MCAV_03	M	12:29	13:35	Low	0%	0	N/A
8/20/2022	MCAV	BRWDCAVE_MCAV_04	M	12:01	13:00	Medium	0%	0	NA
8/20/2022	MCAV	BRWDCAVE_MCAV_03	M	12:37	13:15	Medium	0%	0	N/A
8/20/2022	MCAV	MC19_20	M	12:50	13:20	Low	0%	0	N/A
8/20/2022	MCAV	MC19_10	M	12:54	13:30	Low	0%	0	N/A
8/20/2022	MCAV	MC19_01	M	12:50	13:35	High	0%	0	N/A
8/20/2022	MCAV	MC19_23 (right)	M	13:22	13:30	Low	0%	0	N/A
8/20/2022	MCAV	BRWDCOM_MCAV_01	F	13:45	14:40	Low	0%	0	N/A
8/21/2022	MCAV	BRWDCOM_MCAV_01	F	11:00	11:48	Medium	0%	0	N/A
8/21/2022	MCAV	MC19_34	M	11:21	11:48	Low	0%	0	N/A
8/21/2022	MCAV	MC19_18 (Left)	M	12:15	13:00	High	0%	0	N/A
8/21/2022	MCAV	MC19_10	M	12:20	13:20	Medium	0%	0	N/A
8/21/2022	MCAV	MC19_22	M	12:20	13:20	Low	0%	0	N/A

8/21/2022	MCAV	MC19_23 (right)	M	12:34	13:20	High	0%	0	N/A
8/21/2022	MCAV	BRWDCAVE_MCAV_04	M	12:35	13:00	Low	0%	0	N/A
8/21/2022	MCAV	BRWDCOM_MCAV_01	F	12:47	13:10	Low	0%	0	N/A
8/21/2022	MCAV	MC19_04	F	13:15	13:30	Medium	0%	0	N/A
8/21/2022	MCAV	MC20_03	F	13:00	13:15	Low	0%	0	N/A
8/22/2022	MCAV	MC19_18 (Left)	M	12:22	13:22	Low	0%	0	N/A
8/22/2022	MCAV	BRWDCOM_MCAV_01	F	13:03	13:13	Low	0%	0	N/A
8/22/2022	MCAV	MC19_04	F	13:13	13:23	Low	0%	0	N/A
8/23/2022	MCAV	MC19_04	F	12:07	12:50	High	5.4%	0	NSU
8/23/2022	MCAV	MC19_10	M	12:39	13:00	Low	5.4%	0	NSU
8/23/2022	MCAV	MC_NSH_3	M	12:45	13:05	Low	5.4%	0	NSU
8/23/2022	MCAV	BRWDCOM_MCAV_01	F	13:00	13:40	Low	5.4%	0	NSU
8/24/2022	MCAV	MC_NSH_3	M	12:05	12:40	Low	15.3%	~30,000	NSU
8/24/2022	MCAV	MC19_10	M	12:40	13:10	Low	15.3%	~30,000	NSU
8/24/2022	MCAV	BRWDJUL_MCAV_6	M	12:40	13:05	Medium	15.3%	~30,000	NSU
8/24/2022	MCAV	MC19_03	M	12:55	13:10	Low	15.3%	~30,000	NSU
8/24/2022	MCAV	BRWDCOM_MCAV_01	F	13:03	13:15	Low	15.3%	~30,000	NSU
8/24/2022	MCAV	MC19_04	F	13:15	13:40	Medium	15.3%	~30,000	NSU
8/24/2022	MCAV	MC19_22	M	13:20	13:36	Low	15.3%	~30,000	NSU

9/18/2022	PSTR	BRWDJUL_PSTRI_01	H	10:40	12:03	High	>95%	~7,000	NSU
9/18/2022	PSTR	BRWDBAR_PSTR_09	H	10:50	UNK	UNK	>95%	~7,000	NSU
9/18/2022	PSTR	BRWDBAR_PSTR_08	H	11:03	UNK	Low	>95%	~7,000	NSU
9/18/2022	PSTR	BRWDBAR_PSTR_013	H	11:40	UNK	Low	>95%	~7,000	NSU
9/18/2022	MCAV	BRWD277_MCAV_02	F	12:00	UNK	Medium	27%	~21,000	NSU
9/18/2022	MCAV	MC19-20	M	10:40	UNK	High	27%	~21,000	NSU
9/18/2022	OFAV	BRWDJUL_OFAV_01	H	12:15	14:45	Low	0%	0	N/A
9/18/2022	PCLI	BRWD277_PCLI_07	H	18:23	19:35	Medium	6%	~5,000	NSU
9/18/2022	PCLI	UNK	H	18:55	19:45	Low	6%	~5,000	NSU
9/19/2022	PSTR	BRWDBAR_PSTR_013	H	10:40	12:00	High	0%	0	N/A

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

3.5. Task 2: Fertilization, larval culture, and settlement

In the nights spawning was synchronous, and multiple colonies released, fertilization was normally present and produced a considerable number of larvae. On a successful spawning day when fertilization was the highest (>95%), as seen on 9/18/2022, was largely due to large spawn load and was highly synchronous among the parents (Refer to Table 1 in Task 1). Many days of poor or no fertilization also occurred, with many days in July and September having only one colony spawning, or the sperm concentrations were too low thus producing no larvae. Across many days when fertilization was very low, <5%, there was an effort to rear the larvae, but it resulted in a low yield. A few common issues encountered was that the spawning was not as tightly synchronous as we would have expected, and the viable, motile sperm concentration was often too low to fertilize successfully. Ensuring there is a fine control over culture conditions (environmental cues) and ensuring corals were close in proximity (chemical cues) can contribute to more

synchronous spawning activity. The reduction of feeding of the adults in the previous months may have also contributed to the reduced fecundity and gamete viability (low sperm mobility), impacting fertilization rates.

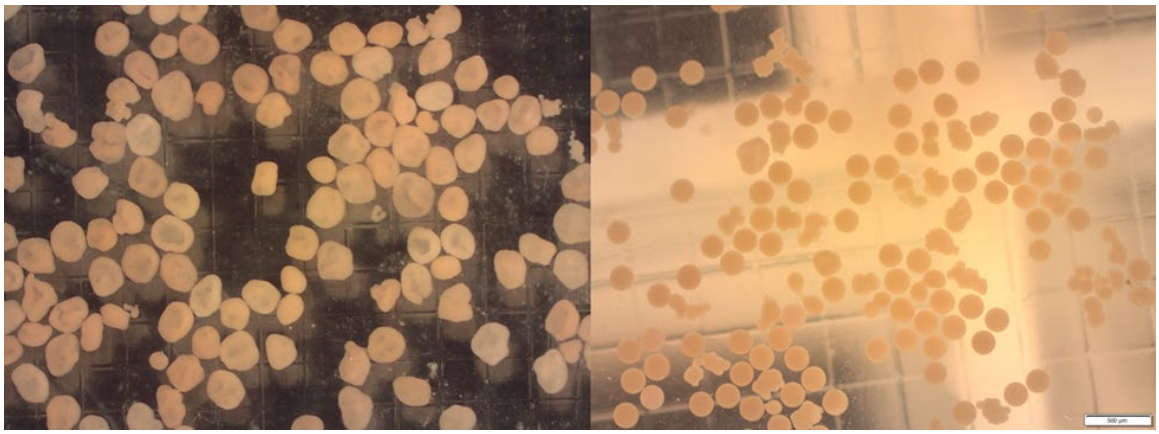


***Figure** 7 Example of high fertilization success in Acropora cervicornis (left) and moderate fertilization success in Montastraea cavernosa (right).*

Due to the effort to continue to research on reproduction and early life stages of these corals and lack of in-house spawning activity for species like *O. faveolata, P. strigosa,* and *P. clivosa* and , a donation of larvae was made by affiliates at University of North Carolina Wilmington (*P. clivosa*) and affiliates the Florida Aquarium (*P. strigosa*); This donation allowed us the opportunity to rear and settle *P. strigosa* and *P. clivosa* larvae (Refer to Table 2). Due to the lack of in-house spawning activity, a donation of *O. faveolata* larvae was made by NOAA affiliates; This donation allowed us the opportunity to rear and settle *O. faveolata* larvae (Refer to Table 2).

This year's spawning season resulted in a total of 334 *Montastraea cavernosa* tiles, 706 *P. strigosa* tiles, 181 *P. clivosa* tiles, 216 *O. faveolate* tiles, 173 *C. natans,* 1227 *D. labyrinthiformis,* and 41 *A. cervicornis* tiles (Refer to Table 3). Tiles varied with the number of individuals on them, some have a few as one and some have upward of 40+, the total number of settled corals will be monitored through time as they start to grow. After coral larvae are introduced to a suitable substrate, preconditioned ceramic tiles, they are monitored for metamorphosis into a skeleton-depositing polyp. Once coral polyps are fused to the ceramic tile, they are counted and transferred into a raceway housing adult coral colony (indoors under artificial lighting). Coral recruits are monitored for their general condition and survival over time. No issues had occurred with the settlement of coral larvae.

Table 2. Summary of larvae donated by collaborators in 2022. (Species:OFAV – *Orbicella faveolata*, PSTR – *Pseudodiploria strigosa,* PCLI – *Pseudodiploria clivosa,* ACER – *Acropora cervicornis,* DLAB – *Diploria labyrinthiformis;* Colony sex: H – hermaphrodite, DD- data deficient)

Transfer Date	Species	Donation Source	Sex	Spawn start time	Spawn End time	Spawn Quantity	Fertilization rates	Larvae produced

8/15/22	ACER	NOAA	H	22:00	23:00	DD	DD	~40,000
8/19/2022	OFAV	NOAA	H	00:10	00:40	DD	DD	~20000
8/21/2022	PSTR	FLAQ	H	19:53	21:00	DD	DD	~21000
8/21/2022	CNAT	FLAQ	H	19:04	21:00	DD	DD	~24000
9/17/2022	PCLI	UNCW	H	00:00	01:00	DD	DD	~20000

Table 3. Summary of the number of tiles with newly settled corals across each species spawned or larvae donated in August and September 2022.

	Number of tiles with Settlers per month	Number of tiles with Settlers per month	Total number of tiles with settlers
Species of interest	August	September	
Montastraea cavernosa	155	179	334
Pseudodiploria strigosa	175	531	706
Pseudodiploria clivosa	174	7	181
Orbicella faveolata	216	N/A	216
Colpophyllia natans	173	N/A	173
Acropora cervicornis	41	N/A	41

3.6. Task 3: Grow out of sexual recruits in land -based nursery

Table 4. Number of coral recruits produced during the spawning season of 2022 that were successfully reared at the land-based nursery as of June 2023.

Species	Total = Land-based (LBN) + moved to offshore nursery (ON)
Montastraea cavernosa	323 (LBN)
Pseudodiploria strigosa	1323 (LBN)
Pseudodiploria clivosa	378 (LBN)
Orbicella faveolata	176 (LBN)
Colpophyllia natans	219 (LBN)
Diploria labyrinthiformis	1305 (LBN) + 602 (ON)
Total	3,784 (LBN) + 602 (ON) = 4,386 Corals

Newly settled recruits of the species of interest, *M. cavernosa, P. strigosa, P. clivosa, O. faveolata, C. natans, D. labyrinthiformis,* and *A. cervicornis* were held across four different indoor recirculating raceways during early grow-out period. Tanks with coral recruits are named Ryder, Rico, Roomie, and LS. Once sexual recruits started to uptake their algal symbionts (Symbiodiniaceae), they received overhead lighting and routine husbandry. Overhead lighting started at a lower photosynthetically active radiation (PAR) level, ~15 µmol photons m^{-2} s^{-1} and was incrementally increased over 12 weeks until they reached higher levels ~180 µmol photons m^{-2} s^{-1}. During this time, the health and overall condition

of the recruits remained stable (Fig. 1-4). *A. cervicornis* settlers had a difficult time uptaking zooxanthellae as no adult corals of this species were held in the aquariums and none of the recruits made it past the first month. All other species of recruits successfully took up and retained algal symbionts, starting around two to three weeks of being placed near adult coral colonies of like species and the food slurry seeded with several species of Symbiodiniaceae. Survivorship of the recruits remained high across the 32-57 weeks they were held in the indoor raceways, likely due to the inoculation with algal symbionts in the early stages and optimal water quality and environmental conditions.

Corals like *D. labyrinthiformis, P. strigosa, P. clivosa,* and *C. natans* reached such large sizes it was no longer possible to capture the whole coral tissue area under the microscope, thus overhead photos were taken of the corals using the tile (1 ¼" L x 1 ¼" W) as a scale reference (Fig. 5-8). Species like *M. cavernosa* (Fig. 9) and *O. faveolata* have comparatively slower growth. During the 32–37-week time frame, *P. strigosa* and *O. faveolata* (Fig. 10) were observed to have budding polyps in some individuals. There were no issues with nuisance pests nor signs of disease in these corals.

Given all maintenance animals (i.e. urchins and snails) were removed from raceways with coral recruits, there were steadily increasing amounts of nuisance algae covering the tanks and coral tiles. While manual removal was often the best fix, algae overgrowth remains a major issue when PAR is high. Beginning at 12 weeks, manual algal removal was necessary two to three times per week to keep algae at bay. Once corals reached 0.25-0.5 cm in diameter and were clearly visible, they were moved to the outdoor raceways which are stocked with maintenance animals. Detritus on the tiles were removed by daily pipetting water on top of the recruits three to four times a week, which keep algae growth at minimum on the tiles. Manual removal of algae was done *ad libitum*. All coral recruits that were born during the August spawning were moved to the outdoor raceways around the middle of November. Corals born during the September spawning were moved to the outdoor raceways in the middle of January. Coral recruits were placed into outdoor tanks named D, F, and G. Coral recruits were reared until they reached suitable size to be relocated to the offshore nursery (Task 4).

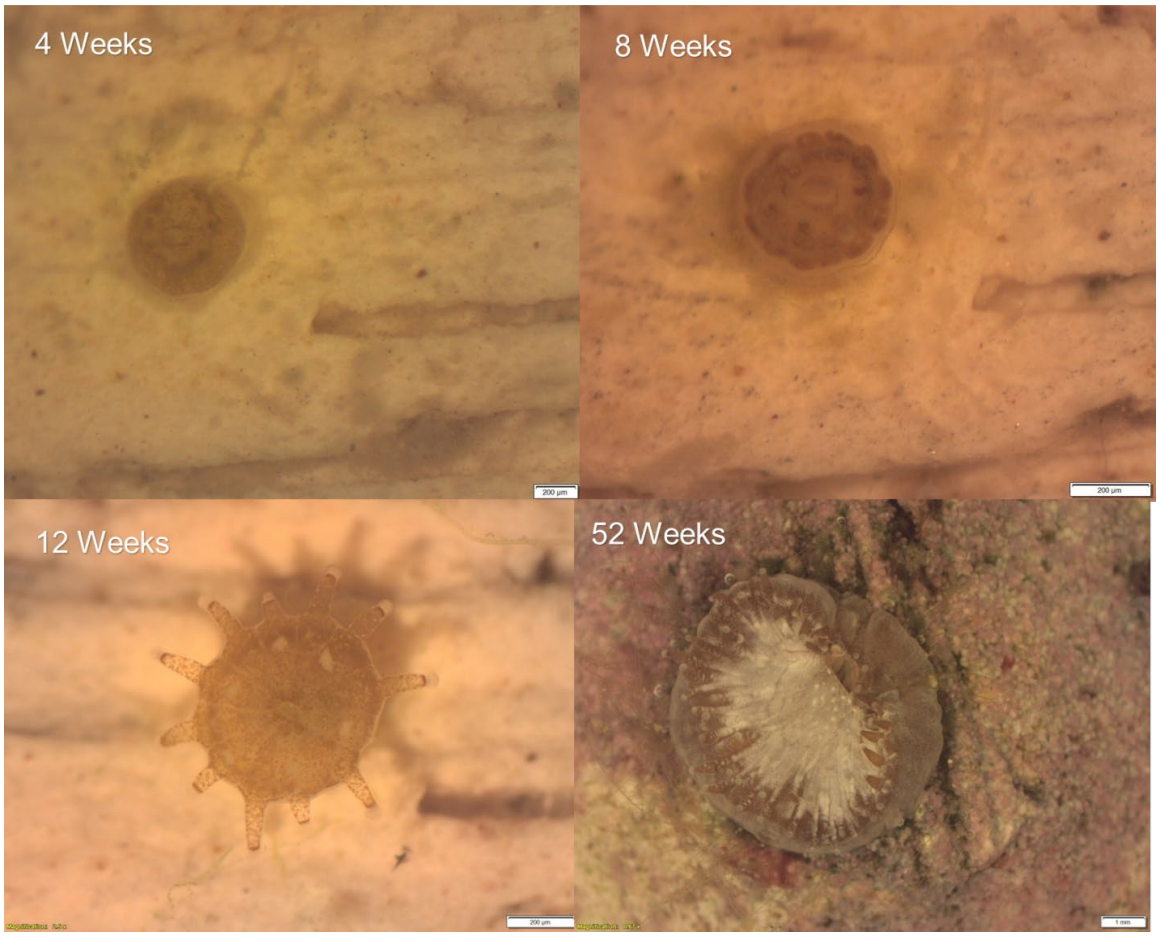


Figure 8 *Growth over time in D. labyrinthiformis sexual recruits at four weeks (top left, 200 µm scale), eight weeks (top right, 200 µm scale), 12 weeks (bottom left, 200 µm scale), 52 weeks (bottom left, 1 mm scale) of age.*

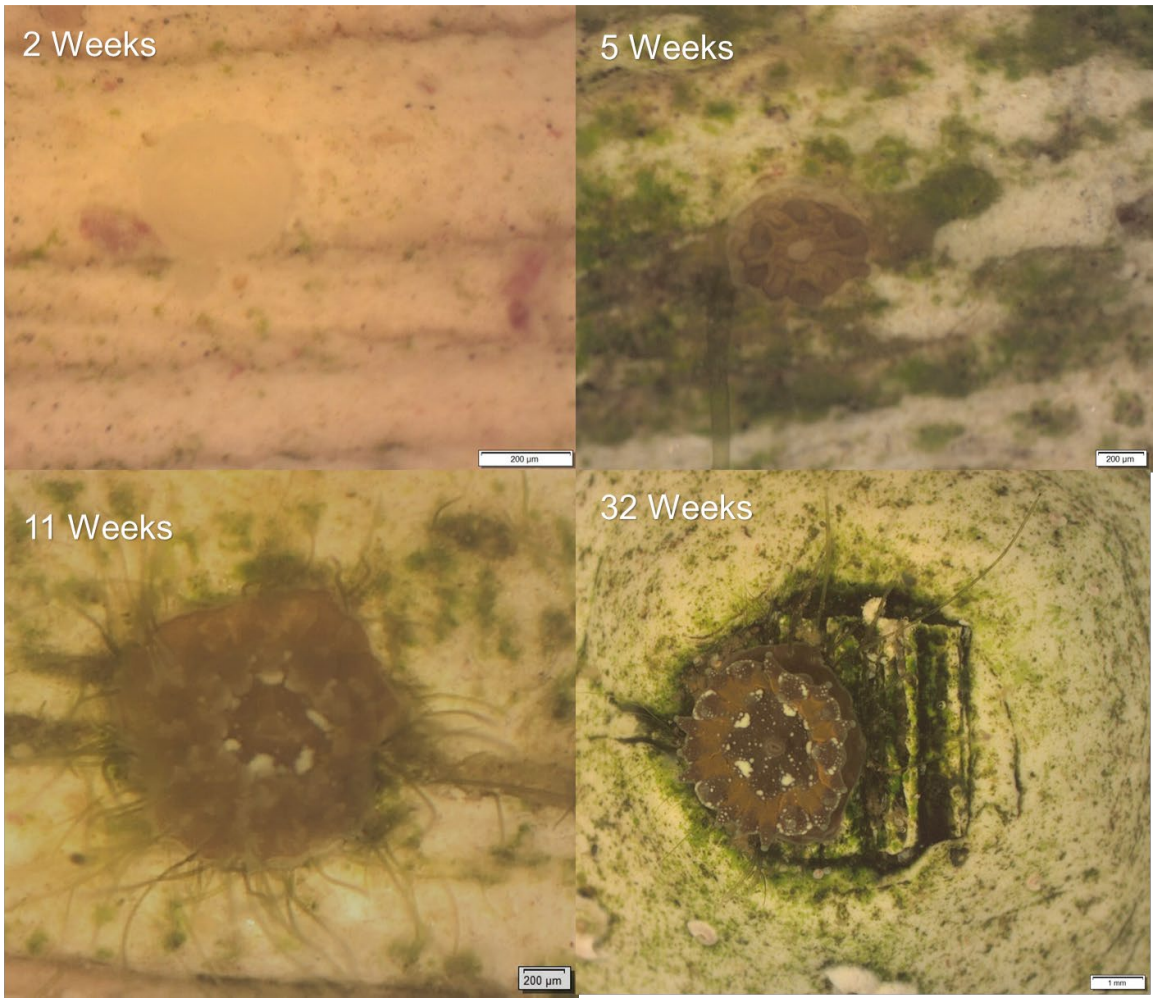


Figure 9 *Growth over time of O. faveolata sexual recruits at two weeks (top left, 200 µm scale), five weeks (top right, 200 µm scale), 11 weeks (bottom left, 200 µm scale), and 32 weeks (bottom right, 1 mm scale) of age.*

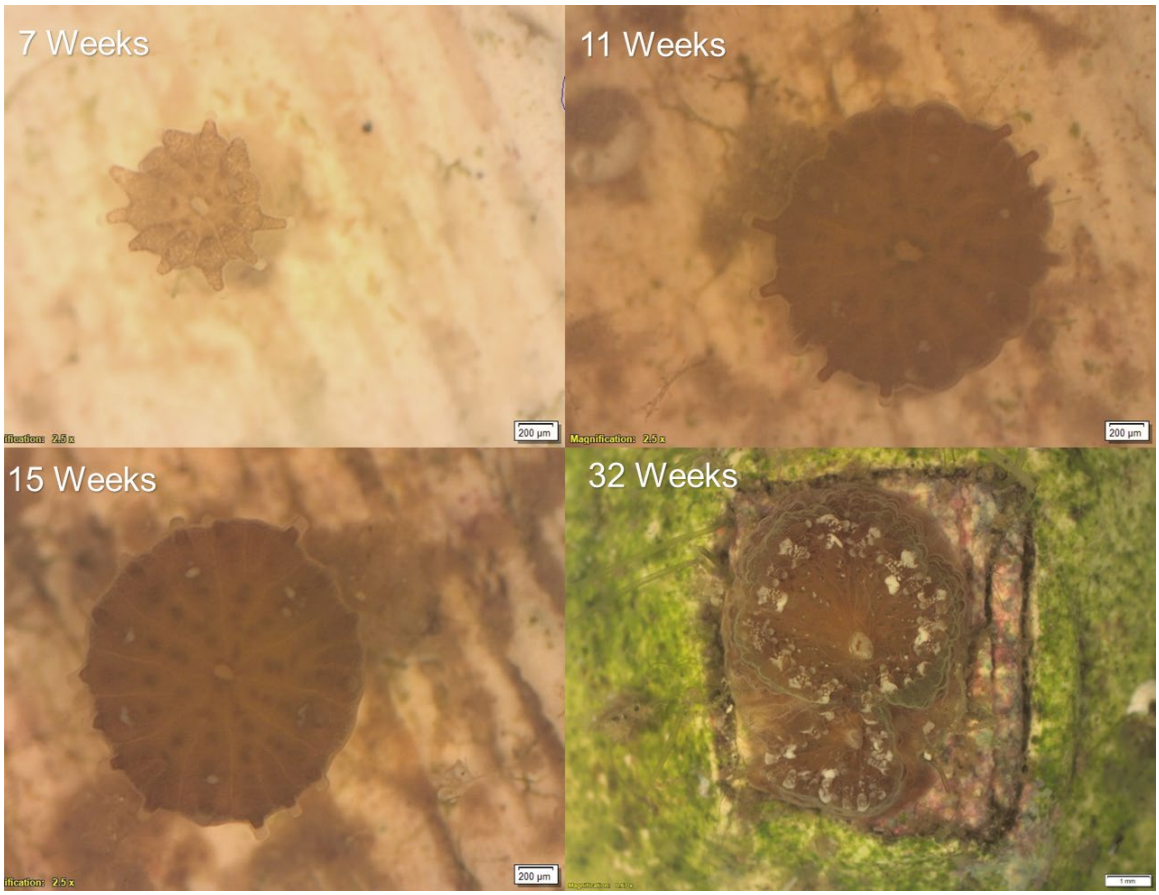


Figure 10 *Growth over time of P. strigosa sexual recruits at two weeks (top left, 200 µm scale), five weeks (top right, 200 µm scale),11 weeks (bottom left, 200 µm scale), and 32 weeks (bottom right, 1 mm scale) of age.*

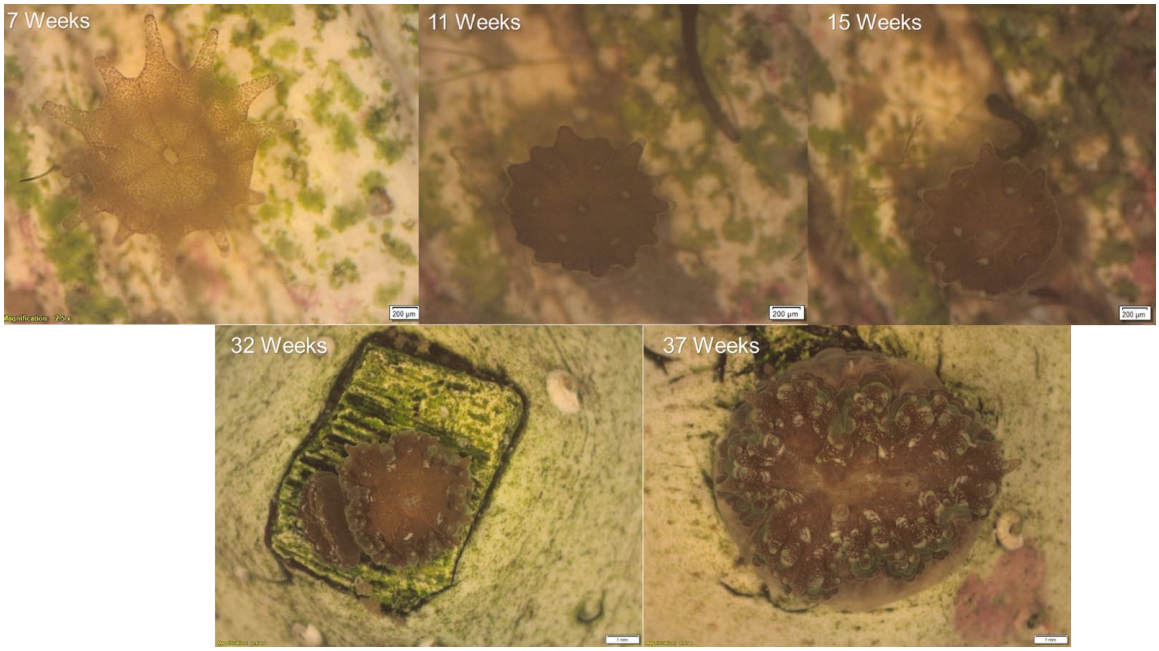


Figure 11 *Growth over time of P. clivosa sexual recruits at seven weeks (top left, 200 µm scale), 11 weeks (top middle, 200 µm scale), 15 weeks (top right, 200 µm scale), 32 weeks (bottom left, 1 mm scale), and 37 weeks (bottom right, 1 mm scale) of age.*

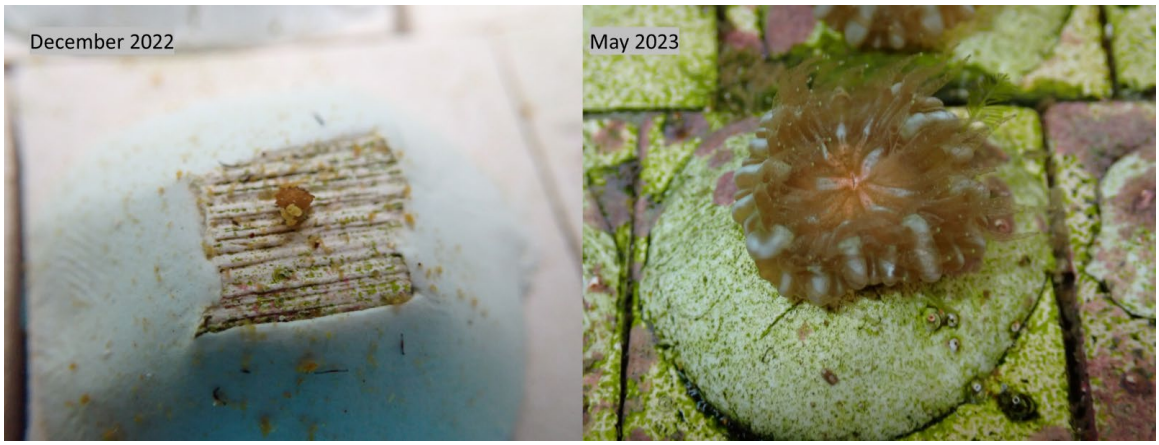


Figure 12 *Example of C. natans sexual recruits in December 2022 (left) and in May 2023 (right). Tile width is 31.75 mm for scale.*

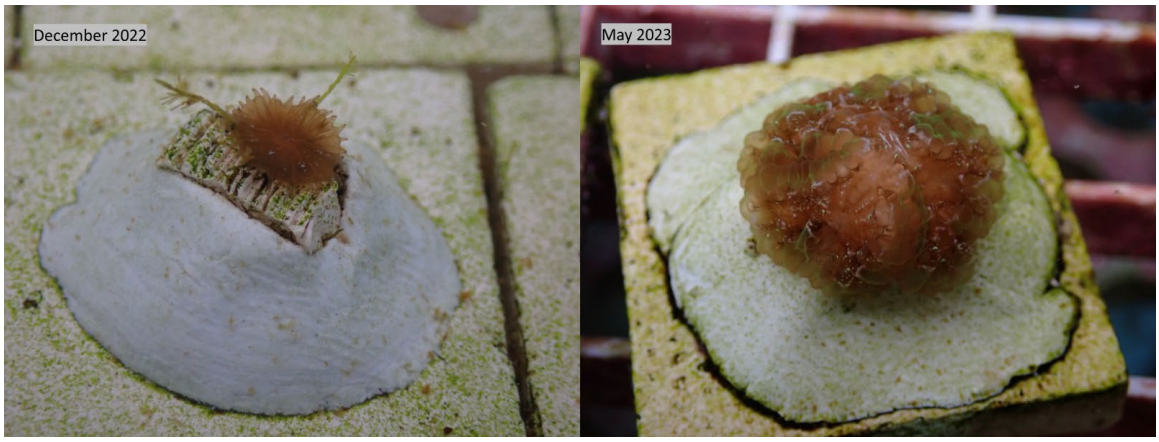


Figure 13 *Example of P. clivosa sexual recruits in December 2022 (left) and in May 2023 (right). Tile width is 31.75 mm for scale.*

Figure 14 *Example of P. strigosa sexual recruits in December 2022 (left) and in May 2023 (right). Tile width is 31.75 mm for scale.*

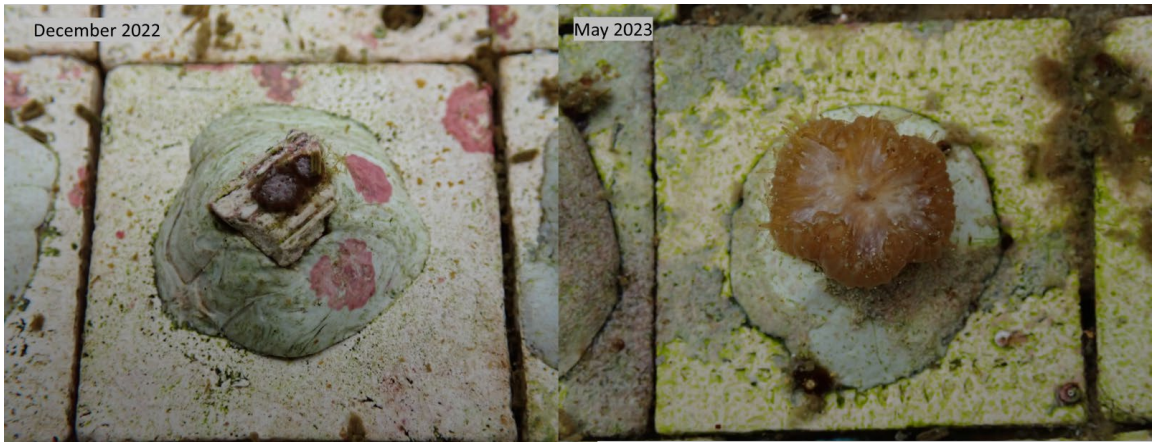


Figure 15 *Example of D. labyrinthiformis sexual recruits in December 2022 (left) and in May 2023 (right). Tile width is 31.75 mm for scale.*

Figure 16 *M. cavernosa sexually propagated coral recruit at 37 weeks of age.*

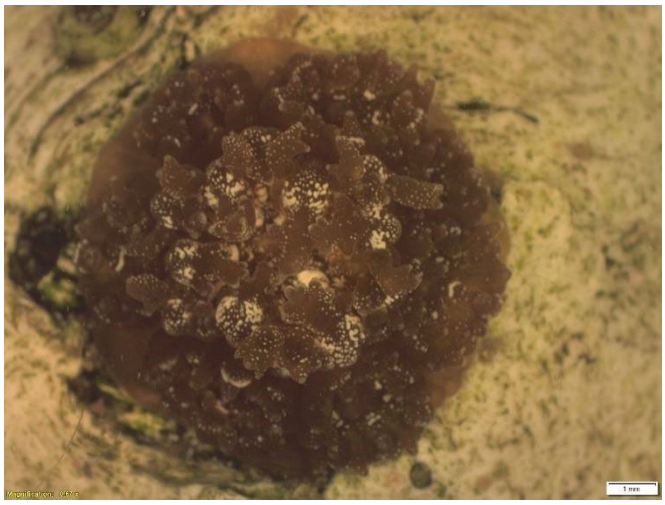


Figure 17 *Example of O. faveolata starting to bud more polyps at around 32 weeks of age.*

3.7. Task 4: Grow-out of newly settled corals and microfragments at the offshore nursery

In January 2023, 602 *D. labyrinthiformis* sexual recruits held in the *ex situ* coral nursery were designated to be moved to the offshore nursery. Out of those, 302 ranged from 0.50 to 1 cm in diameter and the remaining 300 were smaller in size, ranging from 0.1 to 0.50 cm in diameter (Fig. 18). Of those ~300 per size class, 150 recruits of each size class were put into either a stationary module platform or a suspended coral tree (Fig. 19). Corals were epoxied to the module, while corals on the tree had a screw attached to the bottom of the tile so they could be positioned and secured into the tree. Corals were vet checked and moved to the offshore nursery where photographs were taken at deployment and at the end of the monitoring period. Additionally, survivorship of corals of two size classes and in the two nursery platform types will be monitored over time.

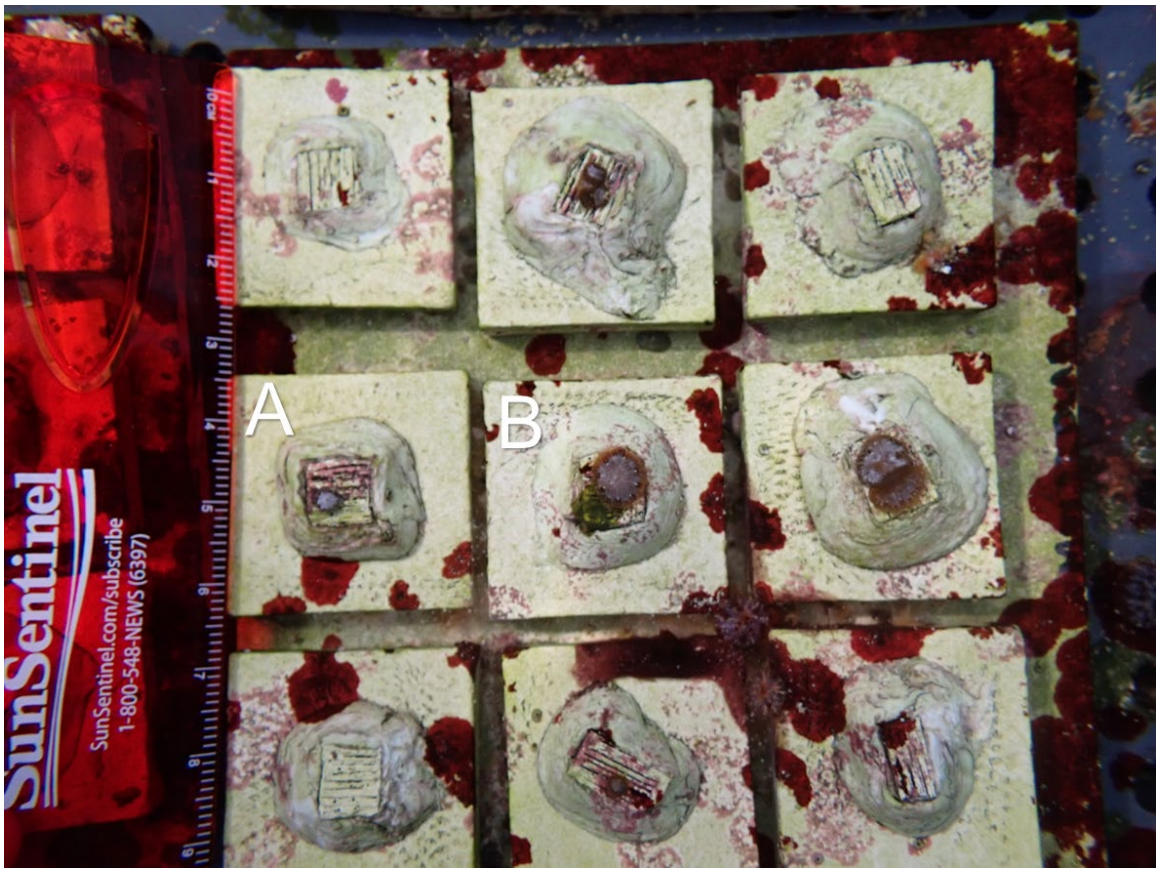


Figure 18 *Example of the two size classes of Diploria labyrinthiformis corals. A represents corals 0.1-0.5 cm in diameter and B represents the larger size, 0.5-1 cm in diameter. Ruler for scale.*

Figure 19 *Example of two attachment locations in the offshore nursery, a module (left) or a coral tree (right).*

Dr. Gilliam's CRRAM Laboratory deployed 602 grooved brain coral (*D. labyrinthiformis*) recruits in NSU's offshore nursery in February 2023. The recruits were spawned *ex situ* in Dr. Figueredo's Laboratory at NSU during the 2022 spawning season. In the nursery, recruits were separated into two size classes: small (0.1-0.5 cm) and large (>0.5 cm) and deployed on three different nursery structures: cement modules, mesh tables, and mid-water nursery trees. Divers monitored recruits at three time points: one week, one month and three months post-deployment (Fig. 20). Recruit survival three months post-deployment in the nursery was 82%, and was variable between nursery structures, with 13% mortality occurring on trees, 3% on modules, and 0.4% on tables. Survival was similar between recruit size classes, with 10% mortality occurring in small recruits and 7% in large. Across all structures, 3% of recruits showed signs of initial predation 1-week post-deployment (Fig. 21), which contributed to initial mortality but the main contributor to recruit mortality was overgrowth and competition. Even though nursery trees were cleaned before deployment, overgrowth of hydroids and fire coral were the main causes of both small and large recruit mortality on trees (Fig. 22). Mortality remains low on tables and modules; however, observations of overgrowth on tables could impact survival at future monitoring events (Fig. 23).

Overall, short-term (<6 months) *D. labyrinthiformis* recruit survival is high in the offshore nursery but using new/clean trees or regularly maintaining structures will be crucial to their

long-term success. The next step will be to outplant a subset of recruits to restoration sites and monitor survival, growth, and presence of conditions (predation, bleaching, disease, etc.). Additionally, we will measure the area of recruits using ImageJ to quantify growth in the nursery between size classes and structures.

Due to disease outbreaks and other environmental stressors, there is a low abundance of mature *D. labyrinthiformis* in southeast Florida contributing to natural spawning events on the reef. Therefore, ex situ induced spawning is important to create more genetically distinct individuals for restoration. Optimizing recruit growth and survival in an offshore nursery is the first step to restoring this reef-building species and the results from this project will inform best nursery practices when working with *D. labyrinthiformis* recruits.

Figure 20 *Diver monitoring D. labyrinthiformis recruits on a cement module with a camera and PVC framer.*

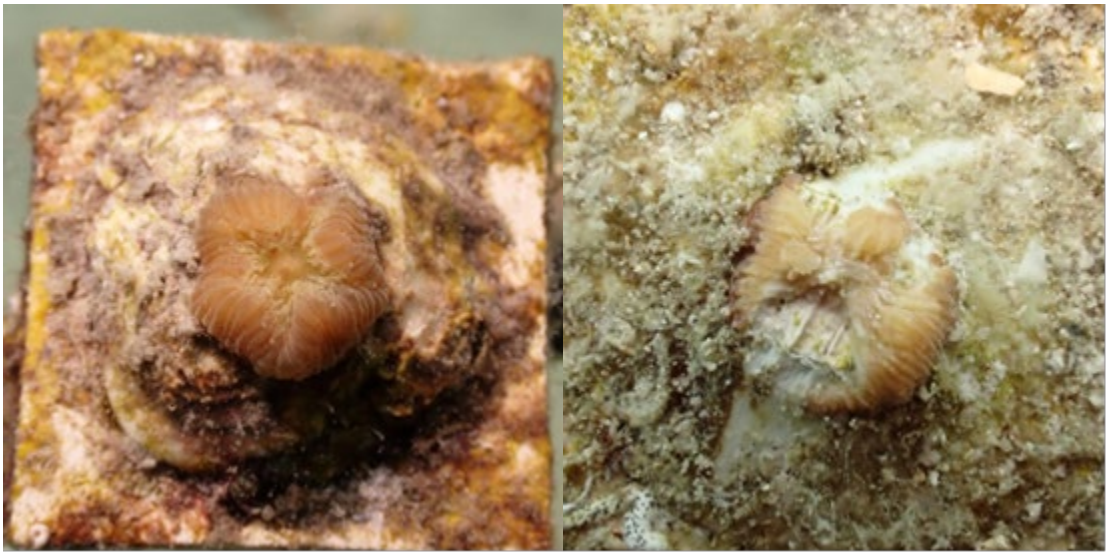


Figure 21 *Healthy D. labyrinthiformis recruit with no predation (left) and recruit with predation (right)*

Figure 22 *Settlement tiles completely covered in fire coral (left) and a recruit competing with hydroids (right).*

Figure 23 *Overgrowth of sponges on settlement tiles on a mesh table.*

3.8. Task 5: Preserve coral genotypes of disease impacted species and induce gonad maturation and spawning in captivity

147 coral colonies were held across seven indoor raceways and eight outdoor raceways. In total, there are 55 *M. cavernosa* (Fig. 25), 20 *P. strigosa,* 20 *P. clivosa* (Fig. 27), 28 *O. faveolata* (Fig. 26), nine *C. natans* (Fig. 24), two *D. labyrinthiformis,* and 13 *S. siderea* in the nursery. Indoor raceways operate in pairs (two raceways share the same sump/seawater) and receive artificial overhead lighting, whereas the outdoor raceways operate independently from one another and receive natural sunlight (reduced by shade cloth to mimic light levels at reef depth). The indoor tanks with adult corals are named Ryder, Rico, Roomie, and LS. The outdoor tanks with adult corals are named A, B, C, G, H, 1,2, and 4. Outdoor raceways do face more challenges as they are exposed to fluctuating air temperature and humidity, which can place more stress on the equipment. Water temperature and salinity were monitored daily for any deviations from target ranges. For the corals held both indoors and outdoors, the feeding regime was adjusted after spawning events, where all the corals received additional aminos and crude protein rich foods in their diet. This increase resulted in an increase in the concentration of nutrients in the water. To offset the additional input of organic material, larger water changes were performed to keep the correct ratio of nutrients in the seawater across all raceways.

Corals with a spawning history, of suitable size, and healthy status either remained or were moved to the indoor spawning systems in late 2022. A total of 35 *M. cavernosa*, six C. *natans*, seven P. *strigosa*, two D. *labyrinthiformis*, 14 P. *clivosa*, five O. *faveolata* are currently housed in the induction systems. Once corals were settled into their respective systems for long term housing, the food regime was changed with the consideration of 1) biomass within the tank, 2) the potential of becoming gravid, and 3) water quality parameters. Tanks with adults require much more food than tanks for grow out of recruits and/or juveniles. We are feeding potentially spawning adults' high protein and lipid dense foods to help supplement while they are undergoing gametogenesis. Corals were monitored weekly throughout time to check on health conditions and water was tested in house weekly to measure the nutrients over time. Systems are maintained daily to ensure that culture conditions are stable.

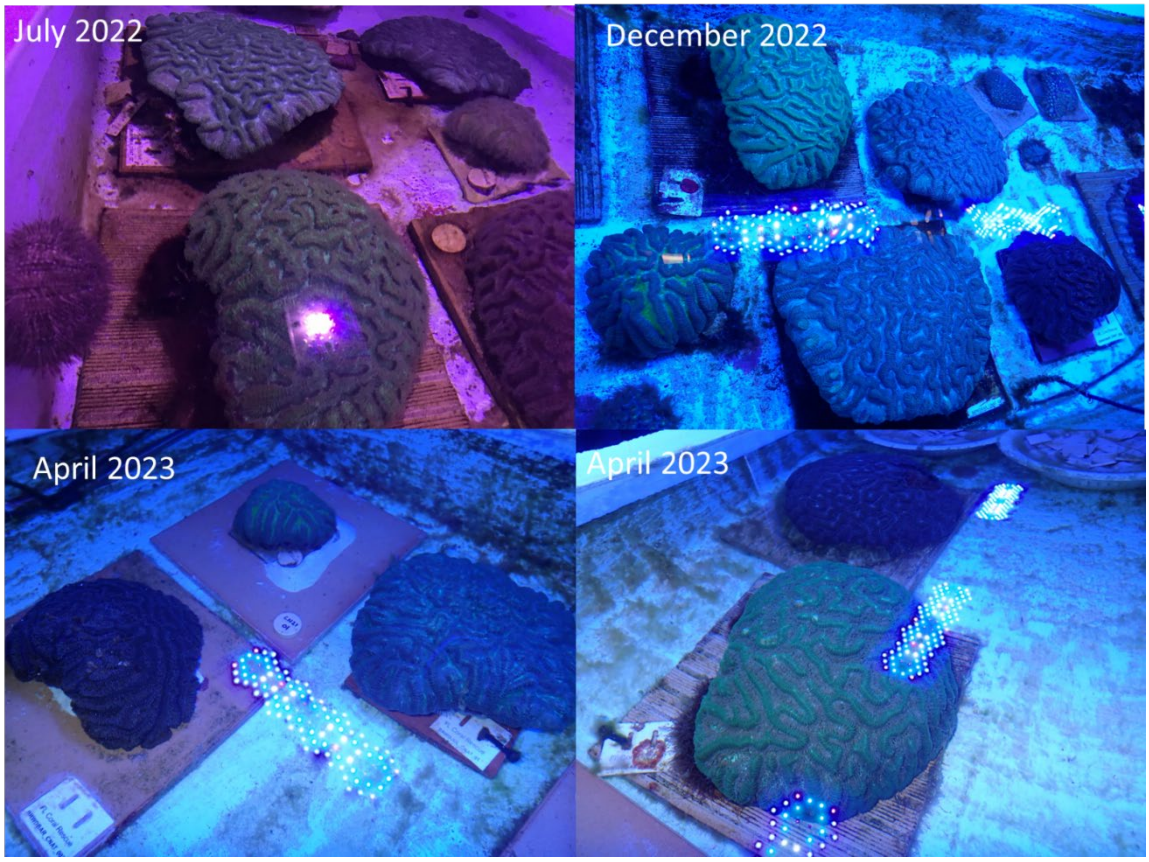


Figure 24 *Group of potential spawning Colpophyllia natans colonies in indoor induction systems in July 2022 (top left), December 2022 (top right), and April 2023 (bottom left and right).*

Figure 25 *Montastraea cavernosa colony, MC19-18a and MC19-18b, held in the indoor induction system in July (left), December 2022 (middle), and April 2023 (right).*

From July 1, 2022, to May 15, 2023, twenty-one colonies died which corresponds to 14% of the colonies held; most of this mortality occurred during the transition from Summer/Fall to Winter (Table 5). In summertime, three *C. natans* and two *O. faveolata* colonies became unhealthy, experienced tissue necrosis, and despite being treated with prophylactics, did not recover. After spawning, eight *M. cavernosa* colonies started to pale and were treated

with Restor Amino acid dips (Brightwell Aquatics); these colonies were housed with other *M. cavernosa* corals which remained healthy throughout. The unhealthy *M. cavernosa* colonies eventually recovered and became fully pigmented again. We hypothesize this was due to natural shifting in zooxanthellae communities toward the end of the summertime. In wintertime, three *O. faveolata* colonies became unhealthy and two ended up dying in December. At the same time, there was another episode of tissue loss in nine *P. strigosa* colonies held within the outdoor raceway "Barley". These colonies were treated using Lugol's Iodine dips, Restor (Brightwell Aquatics) Amino acids dips, and antimicrobial KoralMD (Brightwell Aquatics) dips over time, but those methods failed to cease the recession, and they eventually died. We hypothesize this mortality event was due to changes in seawater microbial communities, cooler temperatures, lower alkalinity levels paired with higher nitrate concentrations in that tank. *P. strigosa* held in other tanks at similar temperatures and nutrient concentrations did not experience tissue loss, so we hypothesize that the "Barley" system had bacterial changes which lead to a disease event in the very sensitive *P. strigosa* corals. As time progressed, one *C. natans*, two *P. clivosa*, and two *P. strigosa* exhibited signs of tissue necrosis, likely latent effects of lowered immunity when water quality and tank chemistry shifted. Corals were treated as described above; however, corals were lost. Mortality events like the ones described above are still hard to explain and to avoid. Since water temperature was among targeted values, we hypothesize that colonies become more vulnerable when high nutrient upload and/or lower alkalinity coincide with reduced energy storage after spawning events and/or moments of greater shift in photoperiod and natural change in temperature (from Summer to Winter), as these may be accompanied by shift to other algal and/or microbial communities present on the reef, which are not present in the lab.

Two *O. faveolata* colonies apart of the AZA-FRT Rescue Project that were held in NSU's outdoor raceways, with no prior health concerns, were relocated to another facility within the AZA-FRT Rescue Project in late December 2022.

In mid-April 2023, the Broward County region experienced unexpected flash flooding with a reported 25" of rainfall. These heavy rains had impacted the outdoor nursery area. Many tanks had rain intrusion in the sumps and raceways which affected the salinity levels, there was electrical malfunctions due to the abundance of water spray near electrical boxes and outlets which lead to several circulation pumps and reactors not working, and the grounds were flooded with leaf litter clogging drains. All were addressed throughout the flooding and anything that could not have been rectified immediately was addressed first thing in the morning when heavy rains subsided. One tank experienced salinity as low as 30 ppt, but water changes were quickly performed. All tanks' salinities returned to normal by that next morning. We did not find any immediate coral health related issues. However, we hypothesize that those events lead to a *P. clivosa* colony and *O. faveolata* colony succumbing to the shock in the tanks and experienced tissue loss in May 2023. A treatment plan of amoxicillin was prescribed to the *O. faveolata* colony for 10 days in hopes of stopping the recession. After treatment, this coral colony recession stopped and is now in a quarantine raceway outside for further recovery. Unfortunately, the *P. clivosa* lost its remaining tissues rapidly and is now deceased.

Figure 26 *Healthy adult Orbicella faveolata colony apart of the AZA-FRTRP held in NSU's outdoor raceways.*

Figure 27 *Example of a sheeting, healthy Pseudodiploria clivosa colony apart of the AZA-FRTRP held in NSU's outdoor raceways.*

Table 5. Health status of all colonies maintained at the land-based nursery across August to December 2022 (H– Healthy (green), U–Unhealthy (yellow), D – deceased (red), M – Moved to new facility (blue).

Species	Coral ID	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	April	May	June
C. natans	CN21_01	H	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	H
C. natans	1160_CNAT_010	H	H	U	D	D	D	D	D	D	D	D	D
C. natans	1160_CNAT_011	H	U	D	D	D	D	D	D	D	D	D	D
C. natans	BM1083_CNAT_009	H	H	H	H	H	H	U	D	D	D	D	D
C. natans	BRWDBAR_CNAT_02	H	H	H	H	H	H	H	H	H	H	H	H

C. natans	BRWDBAR_CNAT_03	H	H	H	H	H	U	H	H	H	H	H	H
C. natans	BRWDBAR_CNAT_04	H	H	H	H	H	H	H	H	H	H	H	H
C. natans	BRWDJUL_CNAT_01	H	H	H	H	H	H	H	H	H	H	H	H
D. labyrinthiformis	DLX-01	H	H	H	H	H	H	H	H	H	H	H	H
D. labyrinthiformis	DLX-02	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC_209_2	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC_299_3	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC_30_2	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC_30_2	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC_NSH_1	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC_NSH_2	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC_NSH_3	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MCX_01	H	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	H
M. cavernosa	MCX_03	H	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	H
M. cavernosa	MC19_03	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_04	H	H	H	U	U	H	H	H	H	H	H	H
M. cavernosa	MC19_05	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_06	H	H	H	U	H	H	H	H	H	H	H	H
M. cavernosa	MC19_07	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_10	H	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	H
M. cavernosa	MC19_18a	H	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	H
M. cavernosa	MC19_19	H	H	H	U	U	H	H	H	H	H	H	H
M. cavernosa	MC19_20	H	H	H	H	U	H	H	H	H	H	H	H
M. cavernosa	MC19_21	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_22	H	H	H	U	U	H	H	H	H	H	H	H
M. cavernosa	MC19_23a	H	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	H
M. cavernosa	MC19_29	H	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	H
M. cavernosa	MC19_31a	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_31b	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_32	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC19_33	H	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	H
M. cavernosa	MC20_01	H	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	H
M. cavernosa	MC20_03	H	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	H
M. cavernosa	MC20_05	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	MC20_06	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWD277_MCAV_01	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWD277_MCAV_02	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDBAR_MCAV_01	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDBAR_MCAV_02	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDBAR_MCAV_03	H	H	H	H	H	U	H	H	H	H	H	H

M. cavernosa	BRWDBC1_MCAV_01	H	U	U	U	U	U	H	H	H	H	H	H
M. cavernosa	BRWDBC2_MCAV_02	U	U	U	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDCAVE_MCAV_02	H	H	U	U	U	U	U	U	H	H	H	H
M. cavernosa	BRWDCAVE_MCAV_03	H	H	H	U	U	U	H	H	H	H	H	H
M. cavernosa	BRWDCAVE_MCAV_04	H	U	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDCOM_MCAV_01	H	H	H	U	U	H	H	H	H	H	H	H
M. cavernosa	BRWDJUL_MCAV_01	H	H	H	H	H	H	U	U	H	H	H	H
M. cavernosa	BRWDJUL_MCAV_02	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDJUL_MCAV_4a	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDJUL_MCAV_4b	H	H	H	H	H	H	H	H	H	H	H	H
M. cavernosa	BRWDJUL_MCAV_06	H	H	H	U	U	U	U	U	U	U	U	U
O. faveola	OF19_01	H	H	H	H	U	D	D	D	D	D	D	D
O. faveola	OF19_02	H	H	H	H	H	H	H	H	H	H	H	H
O. faveola	OF19_03	H	H	H	H	H	H	H	H	H	H	H	H
O. faveola	OF19_04	U	U	U	U	U	U	U	D	D	D	D	D
O. faveola	OF19_05	H	H	H	H	H	H	H	H	H	H	H	H
O. faveola	OF19_06	H	H	H	H	H	H	H	H	H	H	H	H
O. faveola	OF19_07	D	D	D	D	D	D	D	D	D	D	D	D
O. faveola	OF19_08	H	H	H	H	H	H	H	H	H	H	H	H
O. faveola	OF19_09	H	H	U	U	U	D	D	D	D	D	D	D
O. faveola	OF20_01	H	U	U	U	U	U	H	H	H	H	H	H
O. faveola	OF21_01	H	H	H	H	H	H	H	H	H	H	H	H
O. faveola	BRWDBAR_OFAV_01	H	H	H	H	H	H	H	H	H	H	H	H
O. faveola	BRWDBAR_OFAV_02	H	H	H	H	H	H	H	H	H	H	H	H
O. faveola	BRWDBAR_OFAV_04	H	H	H	H	H	H	H	H	H	H	H	H
O. faveola	BRWDBAR_OFAV_05	H	H	H	H	U	U	H	H	H	H	H	H
O. faveola	BRWDBC1_OFAV_01	H	H	H	H	H	H	H	H	H	U	U	U
O. faveola	BRWDBC1_OFAV_03a	H	H	H	H	H	U	H	H	H	H	H	H
O. faveola	BRWDBC1_OFAV_03b	H	H	H	H	H	H	H	H	H	H	H	H

O. faveola	BRWDBC1_OFAV_04	H	H	H	H	H	H	H	H	H	H	H	H
O. faveola	BRWDBC1_OFAV_05	U	U	U	U	U	U	H	H	H	H	H	H
O. faveola	BRWDBC2_OFAV_01	H	H	H	U	U	U	H	H	H	H	H	H
O. faveola	BRWDBOU_OFAV_01	U	U	U	U	U	U	H	H	H	H	H	H
O. faveola	BRWDBOU_OFAV_02	H	H	H	H	H	M	M	M	M	M	M	M
O. faveola	BRWDBOU_OFAV_03	H	H	H	H	H	H	H	H	H	H	H	H
O. faveola	BRWDCOM_OFAV_01	H	H	H	H	H	H	H	H	U	U	U	U
O. faveola	BRWDJUL_OFAV_01	H	H	H	H	H	H	H	H	H	U	U	U
O. faveola	BRWDJUL_OFAV_03	U	U	H	H	H	H	H	H	H	H	H	H
O. faveola	BRWDJUL_OFAV_04	H	H	H	H	H	M	M	M	M	M	M	M
P. clivosa	PC21_01a	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	PC21_01b	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	PC21_02a	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	PC21_02b	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_02	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_03	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_04	H	H	H	H	U	U	U	U	U	U	D	D
P. clivosa	BRWD277_PCLI_05	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_006a	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_07	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_08	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_10	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_11	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_12a	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_12b	H	H	H	H	H	U	U	U	U	U	U	U
P. clivosa	BRWD277_PCLI_12c	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWD277_PCLI_15	H	H	H	H	H	H	H	H	H	H	H	H

P. clivosa	BRWDBAR_PCLI_02	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	BRWDCOM_PCLI_01	H	H	H	H	H	H	H	H	H	H	H	H
P. clivosa	PEJ_PCLI_005	H	H	H	U	U	U	U	U	U	D	D	D
P. clivosa	PEJ_PCLI_11a	H	H	H	H	H	U	U	H	H	H	H	H
P. clivosa	PEJ_PCLI_11b	H	H	H	H	H	H	H	U	D	D	D	D
P. strigosa	PS20_01	H	H	H	H	H	H	H	H	H	H	H	H
P. strigosa	1160_PSTR_001	H	H	U	U	U	D	D	D	D	D	D	D
P. strigosa	BRWDBAR_PSTR_002A	H	H	H	H	H	U	D	D	D	D	D	D
P. strigosa	BRWDBAR_PSTR_002B	H	H	H	H	H	U	U	U	U	D	D	D
P. strigosa	BRWDBAR_PSTR_03	H	H	H	H	U	D	D	D	D	D	D	D
P. strigosa	BRWDBAR_PSTR_06	H	H	H	U	U	U	H	H	H	H	H	H
P. strigosa	BRWDBAR_PSTR_07	H	H	H	H	H	U	U	U	U	U	U	U
P. strigosa	BRWDBAR_PSTR_08	H	H	H	H	H	H	H	H	H	H	H	H
P. strigosa	BRWDBAR_PSTR_09	H	H	H	U	U	U	H	H	H	H	H	H
P. strigosa	BRWDBAR_PSTR_11	U	U	U	U	U	U	D	D	D	D	D	D
P. strigosa	BRWDBAR_PSTR_12	H	H	H	H	H	H	H	H	H	H	H	H
P. strigosa	BRWDBAR_PSTR_13	H	H	H	H	H	H	H	H	H	U	U	U
P. strigosa	BRWDBC2_PSTR_01	H	U	U	U	U	D	D	D	D	D	D	D
P. strigosa	BRWDBC2_PSTR_02	H	H	H	H	U	D	D	D	D	D	D	D
P. strigosa	BRWDCAVE_PSTR_01	H	H	H	H	H	H	H	H	H	H	H	H
P. strigosa	BRWSFTB_PSTR_01	H	H	U	U	U	U	U	D	D	D	D	D
P. strigosa	BRWDJUL_PSTR_01	H	H	H	H	H	H	H	H	H	H	H	H
P. strigosa	PEJ_PSTR_051	H	U	U	U	U	U	D	D	D	D	D	D
P. strigosa	PEJ_PSTR_053	U	U	U	U	U	D	D	D	D	D	D	D
P. strigosa	U3060_PSTR_004	H	H	U	U	U	U	D	D	D	D	D	D
S. siderea	SSX_001	H	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	H
S. siderea	SSX_003	H	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	H
S. siderea	SSX_005	H	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	H
S. siderea	SSX_008	H	H	H	H	H	H	H	H	H	H	H	H
S. siderea	SSX_009	H	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	H
S. siderea	SSX_011	H	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	H
S. siderea	SSX_013	H	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	H

3.9. Task 6: Co-culture of coral recruits with herbivorous invertebrates

Adult crabs were brought to the indoor nursery in the summer of 2022 and placed in aquaria until they released larvae (Fig. 28). Crabs were checked to see if they were gravid by carefully checking under their abdomen (Fig. 28). The released larvae were placed in larval culture tanks but did not complete development (likely due to food unsuitability), they never metamorphosed, so these could not be used for the experiment. In an effort to move forward with the study, Mote Marine Laboratory donated early-stage crabs (i.e. juvenile stage) to complete the experiment. Juvenile crabs, *Porites astreoides,* and *Agaricia agaricites* coral recruits were moved into respective, replicated baskets held within an indoor raceway. Unfortunately, a few weeks into the experiment both the coral recruits and juvenile crabs had a sweep of mortality. We hypothesize that the mesh used proved to be too fine and did not allow for enough water exchange. The restricted water flow led to poor water quality within the baskets resulting in inhospitable conditions. The experiment was ultimately finished at that time as there was no alternative cohort of corals or crabs to use to replicate the study. This study was restarted early in 2023.

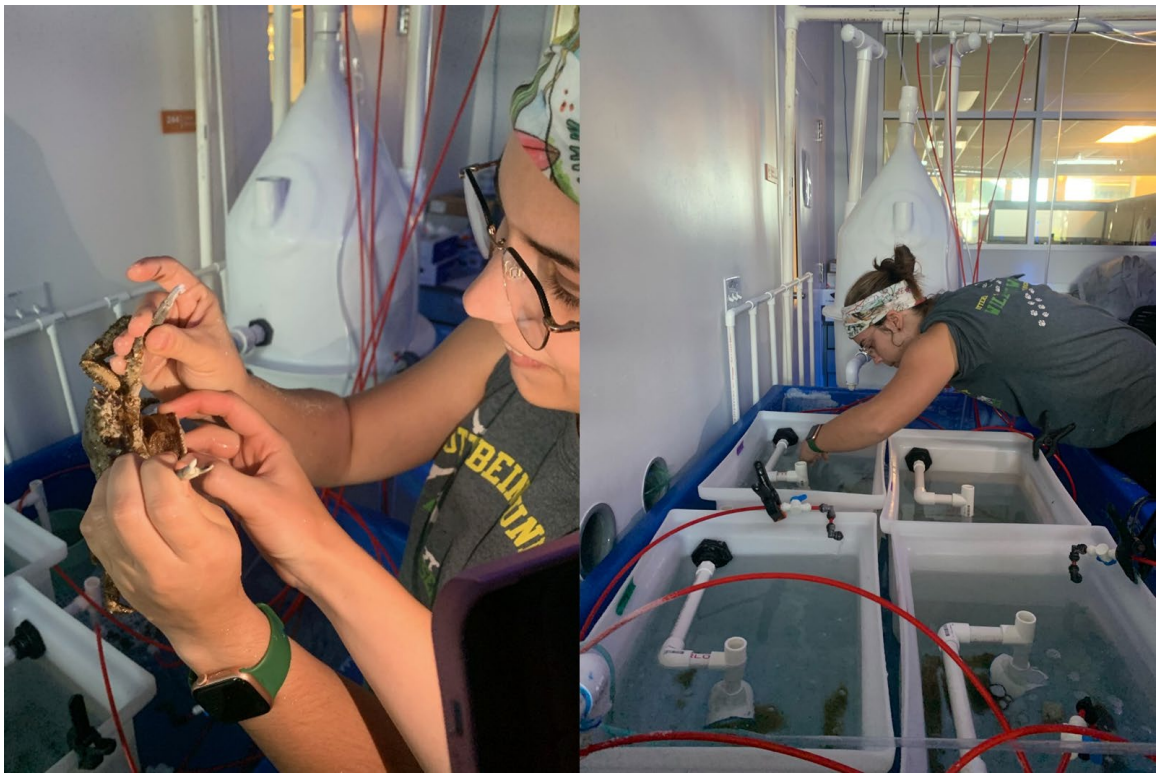


Figure 28 *Example of checking adult female king crabs (Mithrax spinosissimus) to see if they are gravid (left) and monitoring for the release of larvae (right).*

As algal overgrowth jeopardizes the survival of early life stage corals we were testing if using juvenile crabs could reduce the algal load on tiles with coral recruits, this would help maintain algal loads in grow out tanks with corals which would help up-scaling production of corals. We found high grazing activity on all types of algae provided. Since grazing of tank algae occurred, the experiment was resumed as proposed. Overhead photos of the crabs placed into the treatments were taken to measure the carapace width of crabs (Table 6).

Table 6. Summary table showing the mean and standard deviation of the carapace width of the crabs within each treatment and replicated tank. LD: lower density treatment, HD: High density treatment.

Tank	Treatment	AVG	SD
Rico	LD	1.58	0.25
Rico	HD	1.11	0.35
LS	LD	1.41	0.26
LS	HD	1.13	0.27

It is apparent from visual assessments that the algal growth in the high-density crab treatment and the manual removal treatment was similar; algae appeared in higher

quantities in the low-density crab treatment algae, thus was not as effective (Fig. 29). When many crabs died, the experiments were stopped on February 17th in the afternoon; it is unknown whether the culture conditions harmed the crabs, as water quality was stable, but it is possible that reduced water flow inside cages may have negatively impacted the crabs.

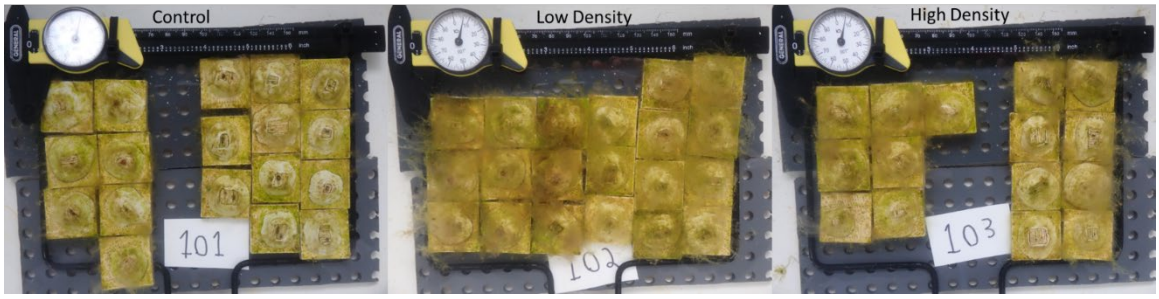


Figure 29 *Algal load on tiles cleaned manually (left) compared with tiles with low density crabs (middle), and high-density crabs (right) after 10 days of trials.*

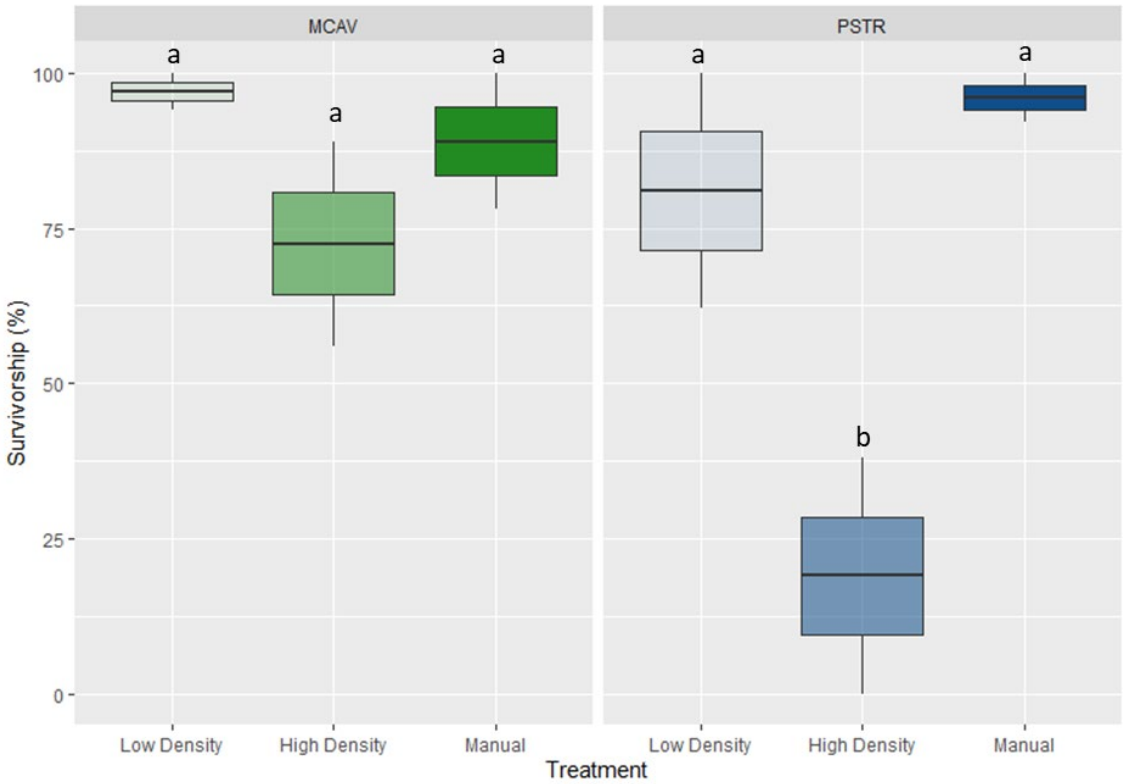


Figure 30 *Boxplots comparing survivorship of M. cavernosa (MCAV) and P. strigosa (PSTR) recruits in two different co-culture treatments, low density (one crab/ three L) or high density (one crabs/ three L), and the tiles that received manual cleaning (control group herbivore). The solid line in the box represents the median; the dotted line represents the average. The bottom and top bars in the boxes represent the 25th and 75th percentiles; whiskers above and below the box are the 10th and 90th percentiles, respectively.*

The survivorship in the control groups for *M. cavernosa* and *P. strigosa* remained high at 89% and 96%, respectively (Fig. 30). Across the remaining treatments, some mortality was observed; *P. strigosa* had the lowest survivorship in the high crab density treatment with only 19% of the treatment group corals making it to the end of the trials (Fig. 30). A mixed effect model showed there was a significant difference in survivorship across the species ($F=12.32$, $p=0.02$). A post hoc test showed there was no effect of treatment on the survivorship across treatments for *M. cavernosa* corals, whereas there was an effect of treatment on the survivorship of *P. strigosa*. Specifically, there was a significant difference in survivorship between the high-density treatment compared to the Manual treatment ($p=0.044$) and low-density treatment ($p=0.012$). It is hypothesized that the corals were predated within the high density of herbivores as there was a large competition for food, leaving the corals vulnerable once other food sources became scarce. Looking at the coral tiles under the microscope, we found no skeleton present, indicating the whole individual was removed. While it was hypothesized that some mortality could have been attributed to the stress of being moved to the respective experimental tanks, a one-way ANOVA showed there were no significant differences in survivorship between Rico and LS tanks ($F=1.921$, $p=0.19$).

Starting and continuing king crab cultures in much larger mesocosms with coastal derived seawater had shown great successes. In this study we were addressing how the crabs fared in smaller, coral grow-out tanks with artificial seawater. This research shows it may be feasible to co-culture crabs with early life stage corals, as demonstrated in the low-density culture treatment where the corals had equal survivorship to the manual cleaning. However, the fact that corals in the high crab density treatment were attacked may suggest that even a lower density of king crabs may not be safe in the long term. Additional trials should use a low density of king crabs (i.e. “1 crab/ 3 L” or lower) monitoring grazing activity over time compared to algal growth to determine a baseline and then adjust crab density depending on culture conditions. While the low-density crab treatment had the most algae cover after 10 days, it is preferred to start with a low density versus starting with too high density and risking corals being predated upon. Low density of crabs and continuing some manual algae removal would be necessary in the early stages until the optimal crab density is reached. The size of the king crab also plays a large role in the optimal density to use. Anecdotally, as the crabs became larger (1-2 cm in carapace width) they were more noticeably territorial when food is limited. This suggests it may be better to start with a larger cohort of smaller crabs (< 1 cm carapace width). As time went on, we found when the crabs become 3-4 cm in carapace, the fewer crabs in the tank the better, it is suggested to test using one to two individuals (with > 3 cm carapace width) per every 350 L or lower.

3.10. Task 7: Coral Larval Competency and Survivorship Curves

Figure 31 *Example of experimental set up. Jars containing larvae for survival trails (left) and holding bowls containing motile larvae for competency trials (right).*

Interspecific variation in the proportion of larvae alive and proportion of competent larvae over time were observed, as expected. In the absence of settlement cues, coral larvae remained swimming until exhausting their energy reserves. Hence, larval survival decreased over time for all species, but the mortality rates differed between species (Fig. 32). The larvae of each species completed larval development and acquired competency at different times; the rate of loss of competency also differed between species (Fig. 33). The larval survival and competency dynamics of seven Caribbean coral species were described and modeled. These larval dynamics models are being incorporated into bio-physical dispersal models by Prof. Emmanuel Hanert (Universite Catolique Louvain, Belgium) to estimate the connectivity for each coral species along the Florida's Coral Reefs and aid in determining optimal locations to protect and restore (King et al. 2023).

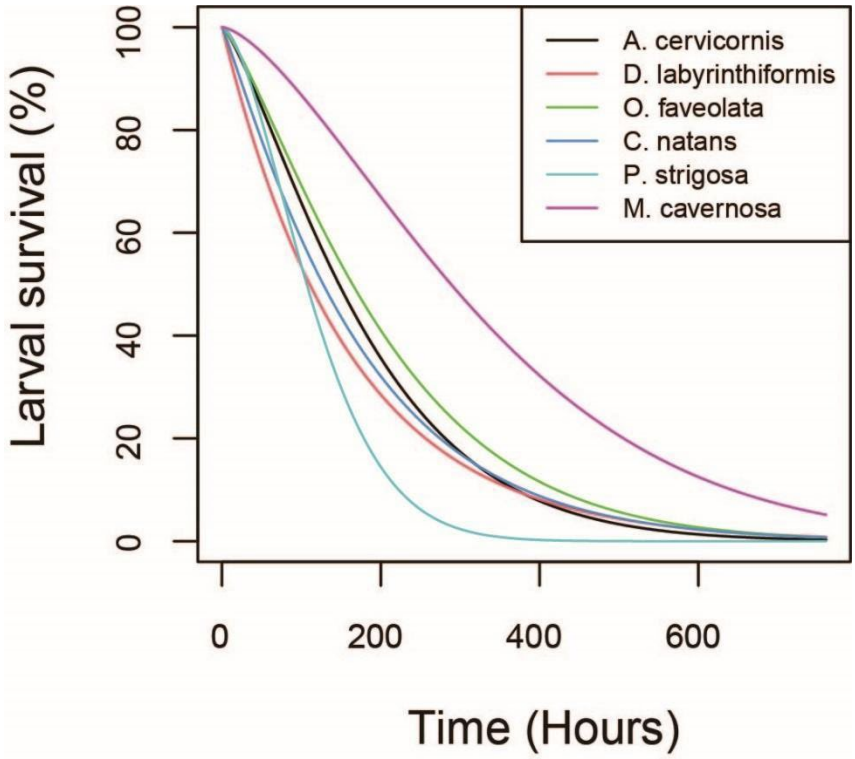


Figure 32 *Percent larvae alive over time for the six Caribbean coral species studied. A. cervicornis (black), C. natans (blue), D. labyrinthiformis (orange), M. cavernosa (magenta), O. faveolata (green), and P. strigosa (light blue). The model of best fit for sha pe of mortality of all species was the standard Weibull model except for, D. labyrinthiformis, whose model of best fit was the exponential model.*

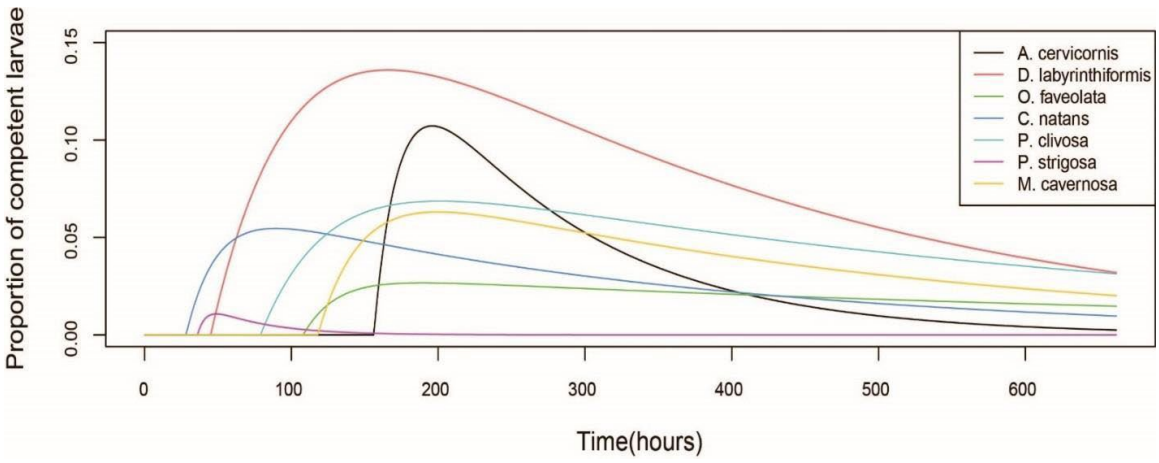


Figure 33 *Proportion of competent larvae over time for the seven species studied. A. cervicornis (black), C. natans (blue), D. labyrinthiformis (orange), M. cavernosa (yellow), O. faveolata (green), P. strigosa (magenta), and P. clivosa (light blue). The model of best fit for shape of loss of competency of all species was the exponential model.*

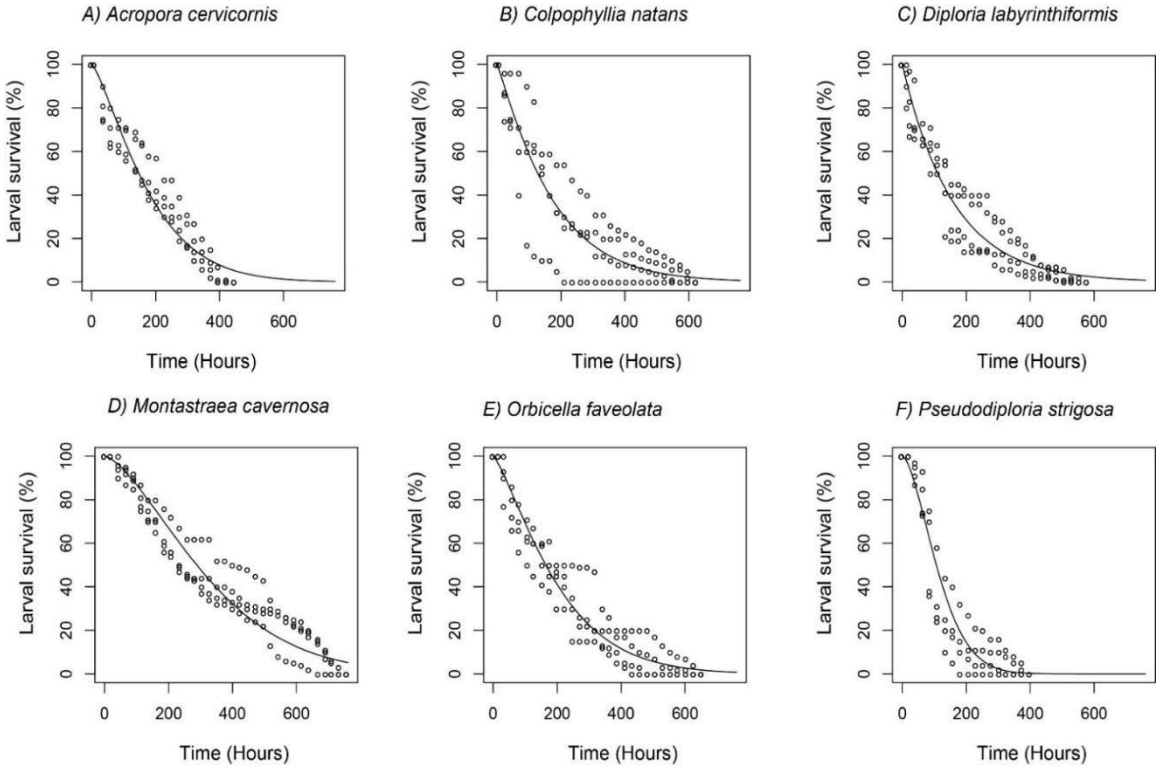
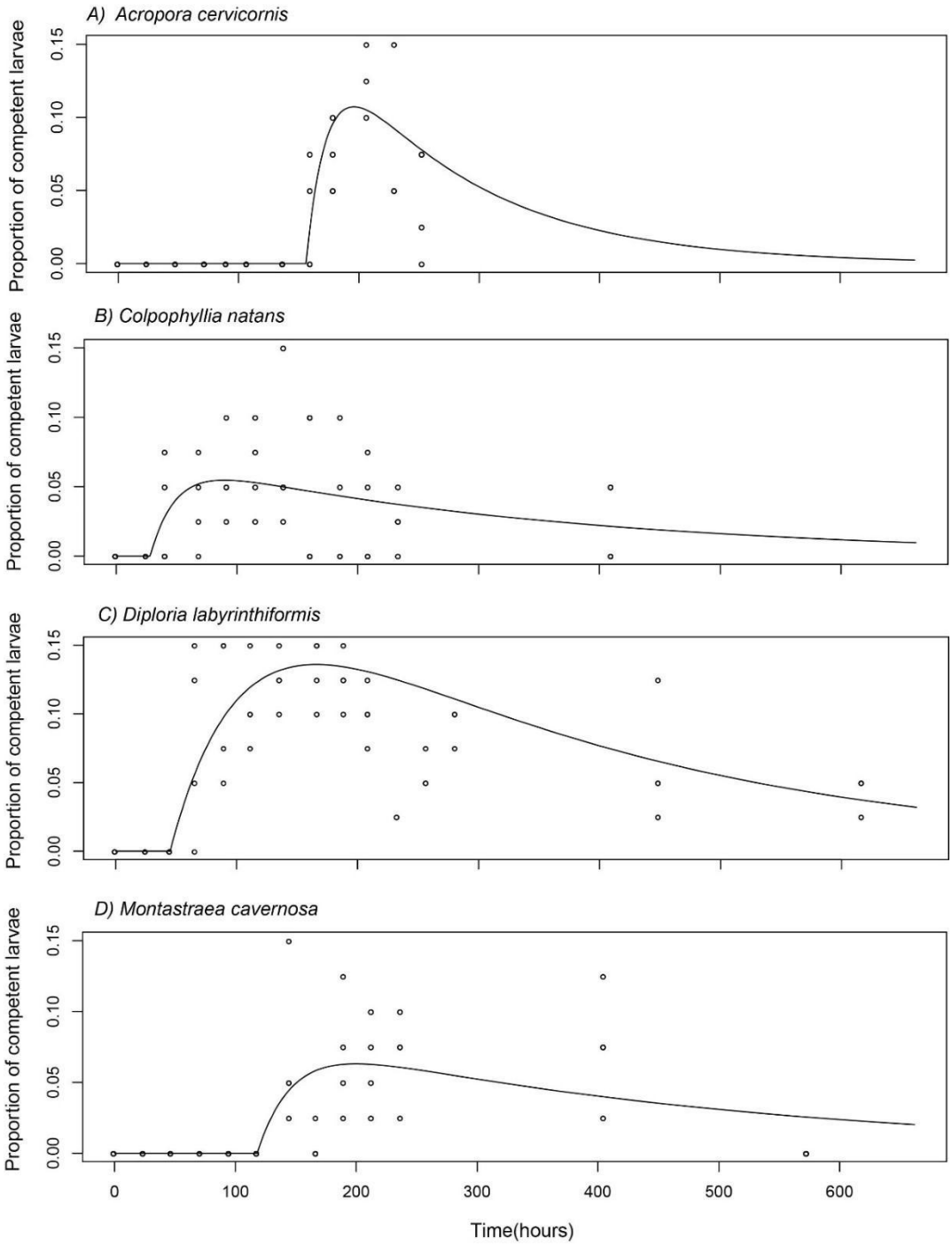


Figure 34 *Percent larvae alive over time for the six Caribbean coral species studied. A) A. cervicornis, B) C. natans, C) D. labyrinthiformis, D) M. cavernosa, E) O. faveolata, and F) P. strigosa. The model of best fit for shape of mortality of all species was the standard Weibull model except for, C) D. labyrinthiformis, whose model of best fit was the exponential model.*


A) Acropora cervicornis
B) Colpophyllia natans
C) Diploria labyrinthiformis
D) Montastraea cavernosa
Proportion of competent larvae
0.00
0.05
0.10
0.15
0
100
200
300
400
500
600
Time(hours)

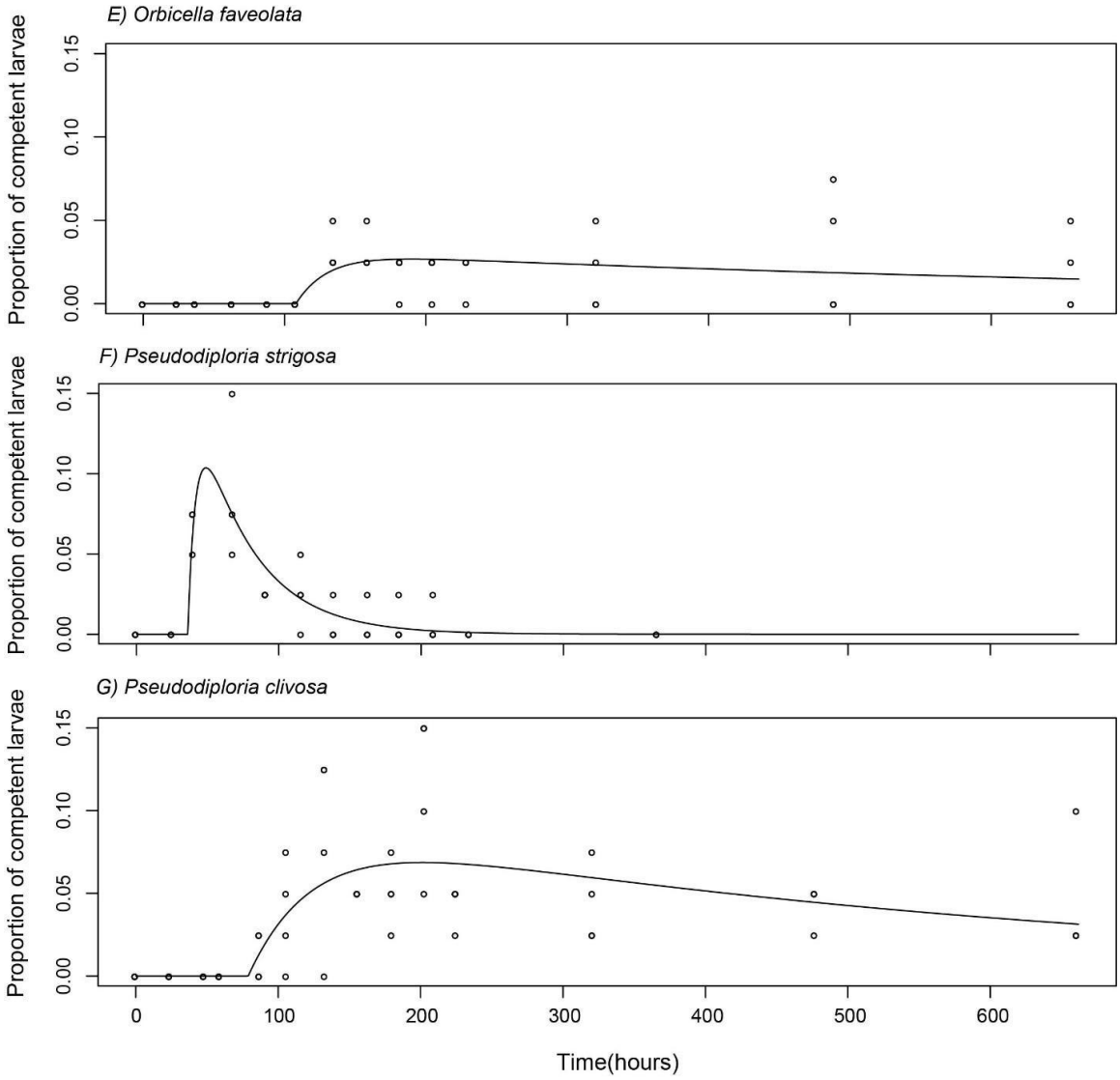


Figure 35 *Proportion of competent larvae over time for the seven species studied. A) A. cervicornis, B) C. natans, C) D. labyrinthiformis, D) M. cavernosa, E) O. faveolata, F) P. strigosa, and G) P. clivosa. The model of best fit for shape of loss of competency of all species was the exponential model.*

1.1. Task 10: Infrastructure for induction of spawning indoors

Nova Southeastern University's facility team worked closely with architects to develop blueprints for the new induction room. These blueprints will be used to work with a construction team to build the physical room at NSU's Guy Harvey Oceanographic Center pump room. Equipment such as raceways (8' x 4' x 16") have been molded and made through Hydro Composites. Due to their process (e.g. making the mold, making the raceway, trimming the edges, and applying the protective gel coat on interior and exterior) the lead time was 10 weeks. Tanks were delivered on June 20th, 2023. The inductions systems frames will continue to be worked on. Other equipment such as UV sterilizers,

protein skimmers, calcium reactors, aquarium controllers, chillers, etc. were also ordered and delivered. All equipment will remain in storage until the room is ready for build-out.

2. CONCLUSIONS & RECOMMENDATIONS

2.1. Gonad maturation and Spawning

Spawning corals *ex situ* requires precisely mimicking annual temperature, sun and moon light cycles, as well as maintaining good water quality and providing adequate quantity of nutritious food. For the first time we had colonies of four species spawn at Nova Southeastern University's indoor induction rooms. This success is due to maintaining good water quality, specifically a better handle on keeping alkalinity levels high through a combination of the use of calcium-reactors and dosing, and the reduction of light pollution in the lab, which was achieved by covering the tanks with black out curtains.

Contrarily to previous years, we saw a reduction in fecundity in the *M. cavernosa* colonies, with many switching from females to males. We suspect the feeding regime played a large role in this, as corals were not fed as much in the year prior to spawning and thus may have shifted away from developing energy-costly eggs to produce energetically cheap sperm. The heavily male-biased male:female ratio of spawning colonies reduced the chances of obtaining large quantities of larvae. The low fertilization rates obtained in some days may be due to genetic incompatibilities (high relatedness between parental colonies), but probably even more likely due to lower sperm motility, a common observation when corals have reduced energy reserves to allocate to reproduction. The feeding regime was increased immediately after spawning and thus we expect to see a large improvement in fecundity in the colonies of *M. cavernosa* and in all other coral species held. We recommend food to be supplied at a quantity proportional to the biomass within a tank more times a week. Specifically, we recommend feeding one tsp of Reef Roid (PolyLabs), one tsp of Golden Pearls (respective size based on age of coral), one mL of Selcon (American Marine Inc.), and two mL of Coral Aminos (Brightwell Aquatics) four times a week.

2.2. Fertilization, larval rearing, and settlement

Our historical data and existing literature show that fertilization rates are typically very high (>95%) when many colonies (of both sexes) spawn and do so synchronously. This happens because it is more likely there will be compatible genotypes, sperm motility is highest, and there are plenty of eggs to be fertilized. Due to the reduced feeding period explained above, many nights, few colonies released gametes so not enough embryos were produced.

When fertilization rates were high, larval culture was extremely successful as unfertilized eggs did not release lipids to the water that deteriorate the water quality and/or trap healthy embryos.

Larval settlement was estimated between 10-20% which, considering historical records in this and other facilities, is relatively high. The hands-off approach of transfer of the larvae from the conical tanks to the settlement trays (slow drain) has not only reduced the labor involved at this stage, but it has also reduced the number of larvae that used to get injured in filters and thus increased overall settlement.

2.3. Grow-out *ex situ*

Newly settled corals require low water flow and low light levels in their first months. As they become better attached to the substate and acquire algal symbionts, they start requiring higher light levels. High light is essential for the corals to survive and grow faster. However, if corals have yet to take up symbionts when they are introduced to light (around one month old), they typically experience higher mortality rates and slower growth than corals which have a high density of symbionts. Thus, we found it to be extremely important to infect newly settled corals with symbionts as soon as possible. Our recommendation is to place adults of the same species in the tank, provide mucus of adult colonies (that include shed symbionts) and/or add symbionts cultured in lab along with the feed.

Post-settlement mortality rates tend to decrease sharply over time. This is likely due to higher levels of sensitivity to fluctuation of water quality parameters, lower ability to capture food and (lower) extent of acquisition of symbionts.

After three months old, corals were moved from the indoor systems to outdoors systems. This transfer often resulted in a visible increase in growth. Coral individuals became visible by the naked eye within two to three months of culturing and many colonies across many species reached close to 20 mm in diameter after 40 weeks.

We plan to continue to assess (and optimize) the effect of light conditions, specifically light spectrum over time, during the grow-out of coral recruits, and the dynamics of microbial communities in tanks through a research project funded by with a NOAA's Ruth Gates grant.

2.4. Grow-out *in situ*

One major reason corals face mortality in the offshore nursery is predation and competition. While practitioners routinely clean nursery structures, it is impossible to eliminate those pressures. While research on anti-fouling structures or applications is being conducted, currently the best approach to reduce the deleterious effects of predation and competition is to only transfer larger coral recruits to the offshore nurseries when they have reached a larger size: greater than dime (17.91 mm diameter), but ideally the size of a quarter (24.26 mm diameter). Cleaning tiles with coral recruits at the offshore nursery is more laborious compared to cleaning larger coral colonies as there is more exposed tile surface on the tiles that algae, sponges, hydroids, and others can colonize and overgrow the coral and/or compete for space. We also recommend distributing them amongst many types of structures to test if one produces better results.

2.5. Coral husbandry

Raising corals long term requires consistent and close care, as corals are very sensitive to fluctuations in water flow, light, water quality, and food. In an established tank system, we see little to no issues with disease or health concerns. Most issues occur when a dramatic event, such as weather (cold spell) or equipment malfunctions, occur. We recommend always checking weather forecast and use preventative equipment to minimize any potential impacts, like extra heaters when temperatures are expected to be abnormally lower or lowering plastic shades to prevent rainwater to enter the tank when high winds and rain are forecasted. Additionally, having several contingency plans for equipment failures is critical in operating a nursery, such as having alternative systems to use or back up parts on hand. While malfunctions are often unforeseen, many steps can be taken in advance to ensure aquaria can function properly and provide stable conditions for corals to remain healthy even if a piece of equipment fails. Having back-ups for all essential equipment, supplies and SOP explaining how each equipment is exchanged to hasten repairs and equipment replacement is also essential. Conducting daily health checks to detect the onset of any disease, as well as having a detailed SOP for the diagnosis and respective recommended treatment of diseases is essential to ensure the treatment is quickly provided and colonies are more likely saved.

2.6. Co-culture of herbivores

Co-culturing corals with herbivores would be ideal to prevent algae to overgrow coral juveniles. Unfortunately, we are not yet convinced the crab species tested would be ideal for this purpose. *Mithrax* crabs do control the algae, but corals also died, likely eaten by the crabs. For crabs to survive, algae need to be abundant. The risk of using too many large crabs is that corals become prey, but not using enough crabs will not mitigate algae effectively. The possibility of using these crabs is not yet fully excluded, but an optimal crab density would have to be found.

2.7. Larval survival and competency

Measuring larval competency in laboratory proved successful and provided valuable data to develop bio-physical models of coral larval dispersal. The Nature Conservancy is currently combining the connectivity estimates obtained from these models with coral demographic data along FCR to make recommendations on the best reefs to restore along FCR and within its several regions, including the Coral ECA.

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