Assisted Sexual Reproduction of Corals and Grow-out of Coral Recruits: Spawning Year 2019
Final Report

Florida Department of Environmental Protection
Coral Reef Conservation Program

Assisted Sexual Reproduction of Corals and Grow-out of Coral Recruits: Spawning Year 2019 Final Report

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Acknowledgements

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List of Acronyms

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

1. PROJECT DESCRIPTION

The purpose of this project was to assist the reproduction and propagation of previously known, large (≥ 2 m diameter), species of greatest conservation need (SGCN) corals of the species *Orbicella faveolata* and *Montastraea cavernosa* colonies in Southeast Florida (SE FL). This included collecting gametes during spawning, fertilizing eggs, rearing larvae and growing recruits into colonies that can be used for future restoration, using the large colonies of corals of opportunity to induce gonad maturation and spawning in captivity in the next spawning season, and propagating smaller pieces of coral of opportunity via microfragmentation. These activities will preserve the genotypes of the largest, oldest, and most resilient corals in SE FL and the Keys and facilitate population recovery after the disease event. The outcomes of this project will provide numerous colonies composed of the largest, most resilient corals in the region and save their genetic information in *ex situ* tanks.

This project is 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.

This report summarizes the field and lab efforts conducted to fulfill PO B54DE4 deliverables through June 30, 2020.

2. METHODOLOGY

This work was conducted under the State of Florida Special Activity Licenses SAL-19-1902-SRP and SAL-19-2022A-SRP which authorized the deployment of settlement tiles and gamete collection of ESA-threatened *Orbicella* and *Montastraea* corals for the intent of assisting in sexual reproduction and the collection of corals of opportunity.

2.1. Task 1: Coral Spawning and Gamete Collection

Orbicella faveolata is a broadcast spawner with an annual reproduction cycle. In Broward County, Florida, spawning typically occurs 5 – 8 days after the full moon in August and September between 21:30-22:30 (pers. obs.). Because these species are hermaphroditic (contain both eggs and sperm in gamete bundles), we endeavored to tent ten large colonies (over 2m in diameter) each night coinciding with the 3rd to 9th day after the full moon of August (8/19 – 8/23/2019). Tents were made of No-See-Um Mosquito Netting weighted at the bottom and buoyant at the collection tube to ensure they float above the colony. Each tent facilitates the upward funneling of gamete bundles into a collection container with the intent that once gamete bundles were collected, they would be pooled in buckets for fertilization on the boat and brought immediately back to the Nova Southeastern University's Guy Harvey Oceanographic Center.

Dana Williams (NOAA) successfully collected gametes from *Orbicella faveolata* at Horseshoe Reef Upper Florida Keys on August 22nd, 2019 at 23:30. A portion of the resulting larvae were donated to NSU for settlement and rearing.

Montastraea cavernosa is also a broadcast spawner with an annual reproduction cycle. In Broward County, Florida, spawning typically occurs 5 – 8 days after the full moon in late August/early September between 21:30-22:30. Twenty-eight colonies of *Montastraea cavernosa* were collected on September 16th by Dr. Figueiredo's graduate students (Samantha King, Morgan Short, and Elizabeth McDonald) at three sites in Broward County (26° 9.420'N, -80° 5.309'W; 26° 9.120'N, -80° 5.340'W; and 26° 8.735'N, -80° 5.782'W), brought to NSU Oceanographic Center, and monitored every night for spawning from 1900 to 2330. This species is gonochoric. Eggs were collected with a cup and sperm was collected with a turkey baster.

2.2. Task 2: Fertilization, Larval rearing and Settlement

For *Montastraea cavernosa*, eggs and sperm were pooled, brought to the laboratory and after sperm concentration was adjusted to $1x10^{-6}$ sperm cells mL^{-1}. After one hour, the sperm was removed by a series of dilutions. The embryos were then randomly divided into bowls at a density of < 1 embryo mL^{-1}, and reared at the temperature, pH and salinity levels mimic historical conditions on the reef during the Summer (not necessarily current conditions) to maximize survival. Water was changed daily to avoid toxic components such as ammonia to accumulate and harm larvae.

For both *Orbicella faveolata* and *Montastraea cavernosa,* one day prior to the expected acquisition of competency (~3 days), larvae were transferred to larger bowl with a flat bottom covered with settlement tiles pre-conditioned (allowed to be colonized by naturally occurring bacteria and coralline, which are settlement cues for coral larvae; Ritson-Williams et al. 2009) for one month at the NSU offshore coral nursery to induce larval settlement and metamorphosis. The settlement tiles will be deployed on the reef 1.5 months before spawning to become conditioned, i.e. these tiles will be collected during coral spawning and maintained in the recirculating tanks near the adult corals until used for larval settlement to preserve their cues. Forty-eight hours after the larvae were exposed to the conditioned tile, the tiles were scored for metamorphosis. Larvae which remain swimming were provided with new settlement tiles. This process was repeated daily until all the larvae either died or settled. One to 3 days after settlement, tiles with newly settled corals were photographed with an Olympus LC20 digital camera attached to an Olympus SZ61 dissecting microscope. The pictures allow to later identify each individual by its position within the tile and measure the initial surface area of the coral polyp using CellSens®.

2.3 Task 3: Genotype banking, Gonad maturation, and Microfragmentation

Since we did not observe coral spawning offshore in Miami-Dade or Broward counties, we fulfilled the overall objective of the project by propagating colonies that can be used for future restoration. Specifically, we banked the genotypes of large *Orbicella faveolata*

colonies found injured on the reefs, used the smallest pieces for propagation through microfragmentation techniques, and the largest pieces to try to induce gonad maturation and synchronized spawning *ex situ*. *Montastraea cavernosa* colonies previously collected for spawning in Broward County's reefs were also be maintained in recirculating systems to induce gonad maturation and spawning *ex situ*. These activities help us preserve the genetic diversity and hopefully assisting the reproduction of these very large/old Endangered-Species-Act (ESA)-threatened-corals of the species which have been most affected by disease. This also allows us to learn if these corals can reproduce and if environmental stressors are inhibiting sexual reproduction. We currently hold a permit to collect other corals of opportunity (loose or injured colonies) that may be found on the reef which may allow us to opportunistically increase the genetic diversity banked. This proposed Change of Scope was approved on Dec 12, 2019.

Genotype banking

Corals of opportunity have been transported to NSU and maintained at indoor and outdoor recirculating systems equipped with biological filtration, protein skimmer, calcium/carbon reactor and UV sterilizer. Corals have been handfed daily (pipette food particles to the coral tentacles), at least 1h/day, *Nannochloropsis*-enriched rotifers and Reef-Roids (PolypLab) *ad libitum* to promote growth. The salinity has been maintained at 35. Reverse osmosis water has been added daily to the sump to replace evaporated water and maintain salinity. An EHEIM Jager submersible heater and a chiller have been used to maintain the desired temperature. Temperature has been measured daily with an YSI® Pro20 temperature probe to ensure accuracy. In the indoor systems, LED lights are being used to mimic a natural diel cycle with light irradiance increasing from sunrise until solar noon (when maximum irradiance is reached), and decreasing after that until a sunset. Water quality tests have been performed weekly to monitor alkalinity, ammonia, nitrite, nitrate, and phosphate concentrations. When necessary, partial water changes were performed to guarantee the water does not contain ammonia nor nitrites, and nitrates are at 0.05-1ppm, and phosphate at 0.02-0.03ppm. Algal growth has been controlled using herbivorous snails and by manual removal weekly to minimize coral mortality and promote growth.

Gonad maturation

Using sexual reproduction to propagate corals allows increasing the genetic diversity of the corals available for future restoration efforts (new genotypes are formed through genetic recombination). Gonad maturation and annual spawning events are synchronized by a series of environmental cues, such as annual temperature, sun, and moon cycles. The larger fragments of the injured colonies have been selected for gonad maturation induction as they are more likely to be sexually mature. These fragments are being housed in outdoor and indoor recirculating systems designed to replicate historical annual cycles of temperature, photoperiod, and solar/lunar irradiance on the reef that are known to induce gonad maturation, and synchronize release of sperm and eggs in captivity (Craggs et al. 2017). The indoor system has been fit with a web-based microprocessor (Neptune Systems, Apex) attached to the tank and Radion LED lights. Using the edit seasonal table on the Apex classic dashboard, the target seasonal temperature, photoperiod, and lunar cycle data has been programmed. Annual variation in sea

temperature on the reef has been based on HOBO temperature loggers (HOBO Pro V2) data collected at the Southeast Florida Coral Reef Evaluation and Monitoring Project (SECREMP) sites between February 2007 and June 2016. After removing data points for periods with cold snaps and/or bleaching events, the data was used to create an average profile of annual temperature cycle in the region. Sunrise, sunset, moonrise and moonset times were downloaded from www.timeanddate.com. To simulate annual variation in photon intensity, irradiance averages recorded by NASA Surface Meteorology and Solar Energy for the reefs off Fort Lauderdale were averaged and converted into data for LED programming.

Microfragmentation
The smaller pieces of the originally large injured colonies were propagated through microfragmentation techniques (Forsman et al. 2015). These pieces were cut into 1-10 polyps fragments, epoxied to a title, and grown in the above described recirculating tanks using a modified annual temperature cycle that never goes below 24°C, as this typically impairs growth. All fragments were monitored and measured to assess growth. The provenience of each piece will be recorded to allow using micro-fusion techniques and produce larger size colonies to use in future restoration efforts.

3. RESULTS

3.1. Task 1: Field operations

The 2019 field operations to observe spawning and collect gametes occurred from August 19 to August 23 and from September 19 to September 22 at one site in Broward County and one site in Miami-Dade County.

The Broward County site, LC-003, was located in 20 ft on the nearshore ridge complex in Fort Lauderdale, FL, near Hugh Taylor Birch State Park. This location was chosen because it contains sixteen documented live *Orbicella* colonies greater than 2 m in diameter within a 20 x 20 m area (*Figure 1*). The Miami-Dade County site, LC-062, was located in 20 ft on the nearshore ridge complex in Key Biscayne, FL, near Crandon Park. This location was chosen because it contains 3 documented live *Orbicella* colonies greater than 2 m in diameter and many smaller colonies within a 40 x 20 m area (Figure *2*).

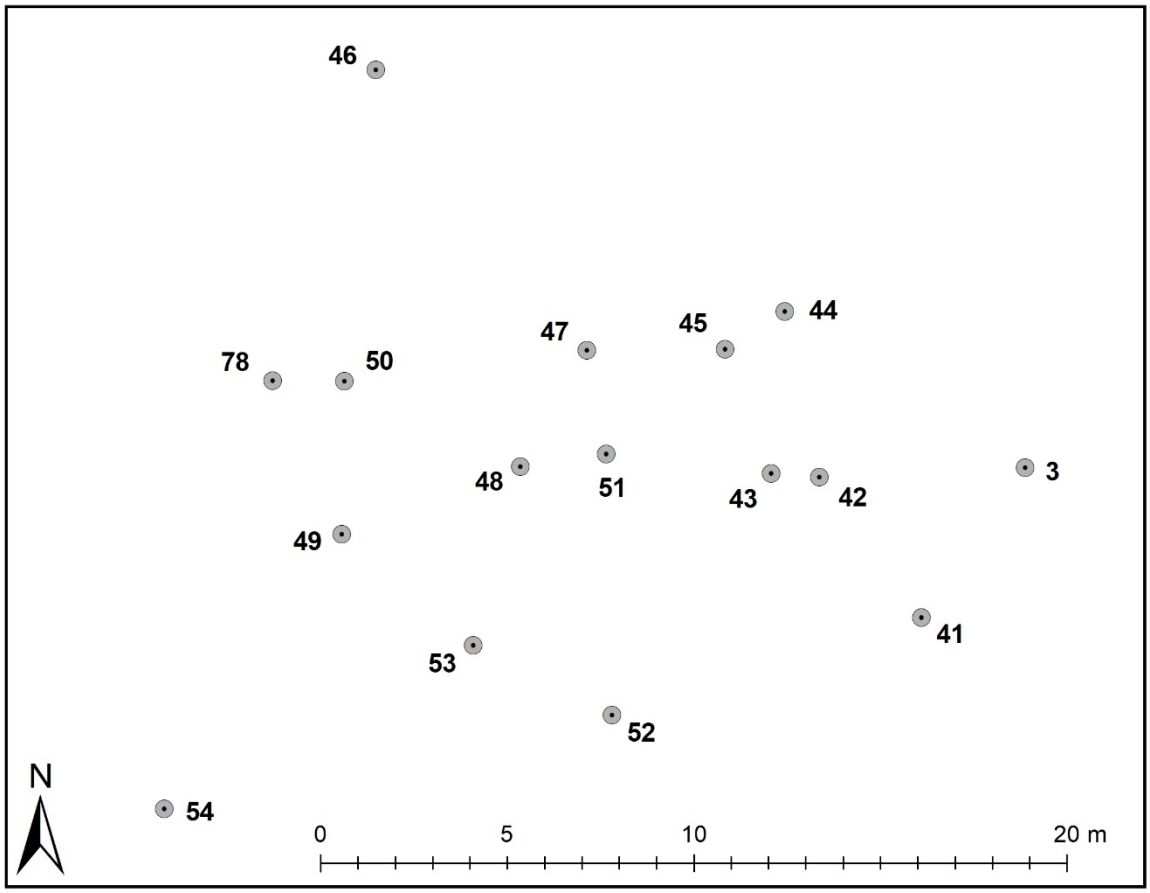

Figure 1. The location of the spawning dives referred to as site LC-003 (far right). Each dot represents the documented location of a live large *Orbicella* greater than two meters in diameter.

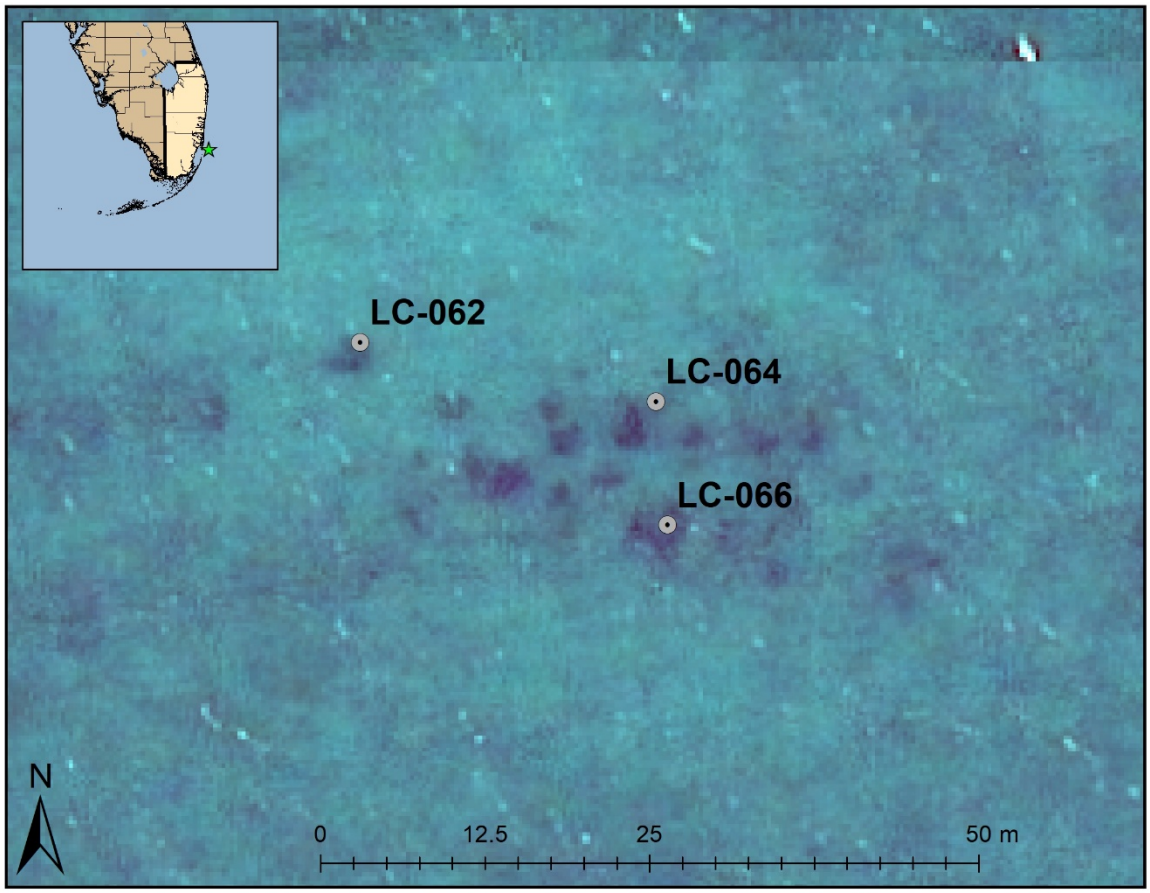

Figure 2. The location of the spawning dives referred to as site LC-062 (far left). Each dot represents the documented location of a live large *Orbicella* greater than two meters in diameter. Other dark spots are large colonies.

Daily operations involved a field and lab team in place working late hours to ensure all tasks could be accomplished if spawning was observed and gametes collected on any given sampling day. *Table 1* shows the field personnel and their roles during the August spawning operations. In Broward, there were seven personnel the first night and six each thereafter. In Miami-Dade, there were six people the first and last night and seven people the remaining nights. The weather was conducive to diving all nights, however conditions were more favorable August 21-23.

Nightly field operations on each boat were similar. A vessel with 12 tanks and at least five divers departed from NSU's marina and Crandon Park Marina at 8 pm. Once on site, a dive flag was deployed on the coordinates and the first dive team went in to set up the site with line and glow sticks and for diver navigation to and from the boat in the dark. The boat anchored and the first dive team dove until around 10 pm to observe corals and confirm no spawning was occurring. At around 10:15, all divers outfitted red flashlights and nets and extra Falcon tubes dove and went to their assigned corals. Divers monitored several nearby corals by periodically checking for setting bundles every couple of minutes with the red lights (*Figure 3*). No spawning was observed in August, thus the teams surfaced by 11:45 pm and returned to the dock.

Table 1. Personnel and roles during August spawning field operations. X marks participating personnel. Light grey indicates completed work.

Broward Site

				August 2019				
				Mon 8/19	Tue 8/20	Wed 8/21	Thu 8/22	Fri 8/23
Name	Role	Location	Organization	7:00 - 11:30 PM	8:00 - 11:45 PM	8:00 - 11:45 PM	8:00 - 11:45 PM	8:00 - 11:45 PM
Landis Bullock	Captain	Broward	NSU	X	X	X	X	X
Murphy MacDonald	Fertilizer	Broward	NSU	X	X	X	X	
Kelly Pitts	Diver	Broward	NSU	X	X	X	X	X
Sammi Buckley	Diver	Broward	NSU	X	X	X	X	X
Morgan Short	Diver	Broward	NSU	X	X	X	X	X
Scott Jones	Diver	Broward	Smithsonian	X	X	X	X	
Brian Walker	Diver	Broward	NSU	X				X
Kristi Kerrigan	Diver	Broward	FDEP					X

Miami-Dade Site

				August 2019				
				Mon 8/19	Tue 8/20	Wed 8/21	Thu 8/22	Fri 8/23
Name	Role	Location	Organization	7:00 - 11:30 PM	8:00 - 11:45 PM	8:00 - 11:45 PM	8:00 - 11:45 PM	8:00 - 11:45 PM
Rebecca Ross	Captain	Miami-Dade	MDRER	X	X	X	X	X
Sam King	Fertilizer	Miami-Dade	NSU	X	X	X	X	X
Liz Fromuth	Diver	Miami-Dade	NSU	X	X	X	X	X
Alysha Brunelle	Diver	Miami-Dade	NSU	X	X	X	X	X
Jenna Soulliere	Diver	Miami-Dade	MDRER	X	X	X	X	X
Michael Greenemeier	Diver	Miami-Dade	MDRER	X	X	X	X	X
Brian Walker	Diver	Miami-Dade	NSU		X	X	X	

Table 2. Personnel and roles during September spawning field operations. X marks participating personnel. Light grey indicates completed work.

Broward Site

				September 2019				
				Wed 9/18	Thu 9/19	Fri 9/20	Sat 9/21	Sun 9/22
Name	Role	Location	Organization	7:00 PM - 12 AM	7:00 PM - 12 AM	8:00 PM - 12 AM	8:00 PM - 12 AM	8:00 PM - 12 AM
Landis Bullock	Captain	Broward	NSU	X	X	X	X	X
Murphy MacDonald	Fertilizer	Broward	NSU	X	X	X	X	X
Sammi Buckley	Diver	Broward	NSU	X	X	X	With Miami-Dade	With Miami-Dade
Morgan Short	Diver	Broward	NSU	X	X		X	X
Emily Nixon	Diver	Broward	Smithsonian		X	X	X	
Kelly Pitts	Diver	Broward	Smithsonian		X	X	X	X
Ana Zangroniz	Diver	Broward	Sea Grant/UF		X		X	X
Kurtis Gregg	Diver	Broward	NOAA			X	X	X
Nicole D'Antonio	Diver	Broward	FDEP	X				X
Nick Turner	Diver	Broward	NSU	X				

Miami-Dade Site

				September 2019				
				Wed 9/18	Thu 9/19	Fri 9/20	Sat 9/21	Sun 9/22
Name	Role	Location	Organization	7:00 PM - 12 AM	8:00 PM - 12 AM	8:00 PM - 12 AM	8:00 PM - 12 AM	8:00 PM - 12 AM
Rebecca Ross	Captain	Miami-Dade	MDRER	X	X	X	Back up	X
Jenna Soulliere	Diver	Miami-Dade	MDRER	X	X	X	X	X
Michael Greenemeier	Diver	Miami-Dade	MDRER	X	X	X		
Sara Thanner	Diver	Miami-Dade	MDRER				X	
Sam King	Fertilizer	Miami-Dade	NSU	X	X	X	X	X
Liz Fromuth	Diver	Miami-Dade	NSU	X	X	X	X	X
Alysha Brunelle	Diver	Miami-Dade	NSU	X	X	X	X	X
Sammi Buckley	Diver	Miami-Dade	NSU	*With Broward	*With Broward	*With Broward	X	X

Figure 3. A diver monitoring a coral by periodically checking for setting bundles every couple of minutes with the red lights.

In September, the weather forecast was very poor for diving operations. On September 18, the National Weather Service predicted 4 to 6 ft seas with occasional 8 ft and 6 to 8 ft seas with occasional 10 ft for September 19 – 20. The planned dive schedule was left in place and divers stayed on call each day to see if the weather changed. Each day the conditions were evaluated for potential dive operations. On September 18, conditions were not as bad as predicted, so dive operations occurred as planned. However, no spawning was observed. Dive operations were cancelled on the remaining days in September due to deteriorated, unsafe conditions (F*igure 4*).

Figure 4. September 20, 2019 ocean condition at Dr. Von D. Mizell-Eula Johnson State Park.

3.2. Task 2: Fertilization, larval rearing and settlement

The larvae of *Orbicella faveolata* donated by Dana Williams (NOAA) were received at NSU on August 24th. Larvae were fully developed and ready to settle, so when provided with tiles pre-conditioned at the NSU offshore coral nursery, they settled in high numbers and several acquired symbionts; quick check on 26 (out of close to 200 tiles) (~13%) randomly selected tiles, identified 43 settlers. This equates to about 330 settled larvae. Unfortunately, one to 2 days afterwards, while we were still undergoing scoring (we had 198 recruits scored and photographed at that point) we found that most of, and eventually all, of the settlers had died.

We suspect the total mortality of settled larvae was due to an issue with the conditioned tiles brought in from the field nursery. There was no indication of a system issue. All physical parameters in tank and water quality had remained at optimal levels and all the other corals kept in the same tank, including very sensitive ones such as juveniles of *Acropora palmata,* remained visibly healthy. Concomitant to the *Orbicella* larvae settlement, we had *Acropora cervicornis* larvae settle in tanks with non-conditioned tiles and tanks with the same conditioned batch as the *Orbicella*. While more *A. cervicornis* larvae settled on the conditioned tiles than on the non-conditioned tiles, all but one of the settlers on conditioned tiles died, whereas most of the larvae on the non-conditioned tiles survived. This suggests that there was something harmful on the conditioned tiles from the field; likely a harmful bacteria or virus since we could not find any predator.

In total, only 3 of the 21 (14%) *Montastraea cavernosa* colonies collected were observed spawning in September. This was a much lower percentage than previous years where 30-50% colonies collected spawned in the laboratory. The spawning colonies did so synchronously on nights 7-9 after the full moon between 9:07 and 9:20 pm and finished around 9:45 pm at the latest (**Error! Reference source not found.**). These were composed of one male that spawned over 3 nights (dribbles on the first 2 nights and large spawn on the 3rd night), and two females, one of which just dribbled on the first day,

while the second dribbled for 2 days, and then partially spawned on the third day. The eggs and sperm of all colonies that spawned were pooled for fertilization.

Table 3. Number of females and males of *Montastraea cavernosa* that spawned over the monitoring time and fertilization rates obtained (DBFM- days before full moon, DAFM- days after the full moon).

Date	Number of females spawned	Number of Males spawned	Fertilization rate
Sep 16 (3 DAFM)	0	0	-
Sep 17 (4 DAFM)	0	0	-
Sep 18 (5 DAFM)	0	0	-
Sep 19 (6 DAFM)	0	0	-
Sep 20 (7 DAFM)	2 (dribble)	1 (dribble)	<0.5%
Sep 21 (8 DAFM)	1 (dribble)	1 (dribble)	<0.5%
Sep 22 (9 DAFM)	1 (partial spawn)	1 (spawn)	<0.5%
Sep 23 (10 DAFM)	0	0	-

After three days in culture bowls, five *Montastraea cavernosa* embryos developed into larvae and successfully settled on tiles. None of these settlers completed metamorphosis and all ultimately died.

Our fertilization rates were way below the rates obtained by other researchers this year and by our lab in previous years (10 – 20%). We initially hypothesized that the low fertilization rates were due to the very low sperm density since only a few polyps of a colony released sperm the first two nights. However, on the third night, we obtained a high/optimal sperm concentration and still had extremely low fertilization success. Perhaps the low fertilization was due to low sperm motility or the colonies that spawned are closely related. We did not investigate the sperm motility during spawning and further testing would be necessary to determine any genetic linkages.

The issues experienced during the 2019 spawning and fertilization projects are disheartening and perhaps point to bigger reason why we are not seeing high natural recruitment on the reefs. There no existing records of observations of *Orbicella* spawning in the field in SE FL. Granted, this was mostly due to bad weather keeping us on shore during the optimal spawning nights, however we still do not know if they are reproductive. We witnessed very low *Montastraea cavernosa* fecundity and larval survival from colonies collected in the field and brought back to the lab. And we had almost complete mortality of all of our larva that settled on the tiles conditioned in the field. These results may be indicative of a very stressed reef system that is unable or unlikely to recover by natural reproduction. This is all the more reason to further investigate coral reproduction in SE FL. We need to confirm if the large *Orbicella* are fecund and the timing of reproduction. If they aren't fecund, can we initiate spawning by removing the stressors? What is deterring survival of settled larvae on conditioned tiles?

Perhaps this is affecting the survival to the naturally recruiting larvae on the reef as well. Answers to these questions may help the goal of restoring the natural processes of reproduction and recruitment at a relevant scale.

3.3. Task 3: Genotype banking, Gonad maturation induction, and Microfragmentation

Genotype banking

Twenty-one mature-size colonies of *Montastraea cavernosa* were collected in Broward County prior to spawning, and multiple fragments of originally 3 large *Orbicella faveolata* colonies which were found injured on the reef were also collected. Specifically, in August two *O. faveolata* corals were found injured by recent previous boat anchoring. LC-062 was fractured in several places and it appeared that the colony was lifted and slightly moved (Figure 5). At site LC-003, a one-meter diameter colony was found loose with several scrapes and gouges with loose live fragments laying nearby (Figure 6). Fragments were cached at both sites and left *in situ* until the permit could be amended to include their collection. On August 23rd, 27 coral fragments were transported to NSU and maintained in recirculating systems.

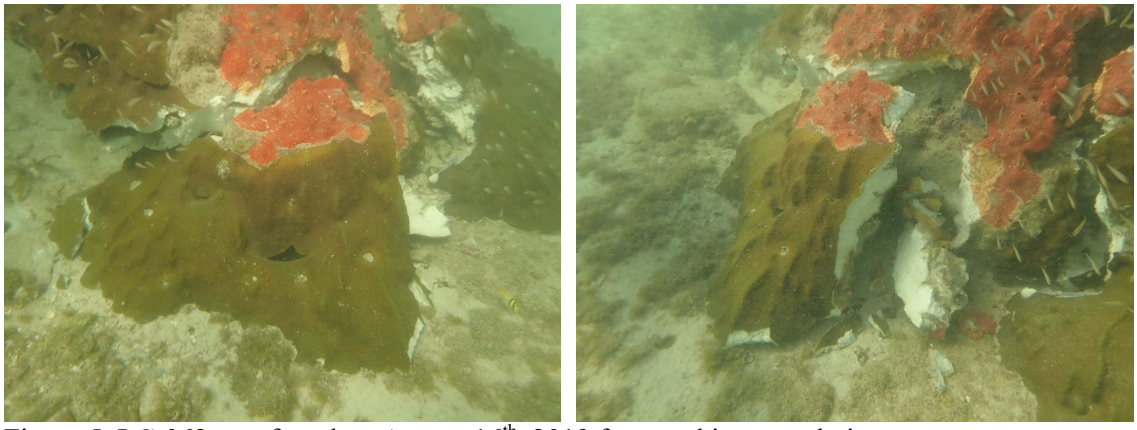

Figure 5. LC-062 was found on August 16th, 2019 fractured in several pieces.

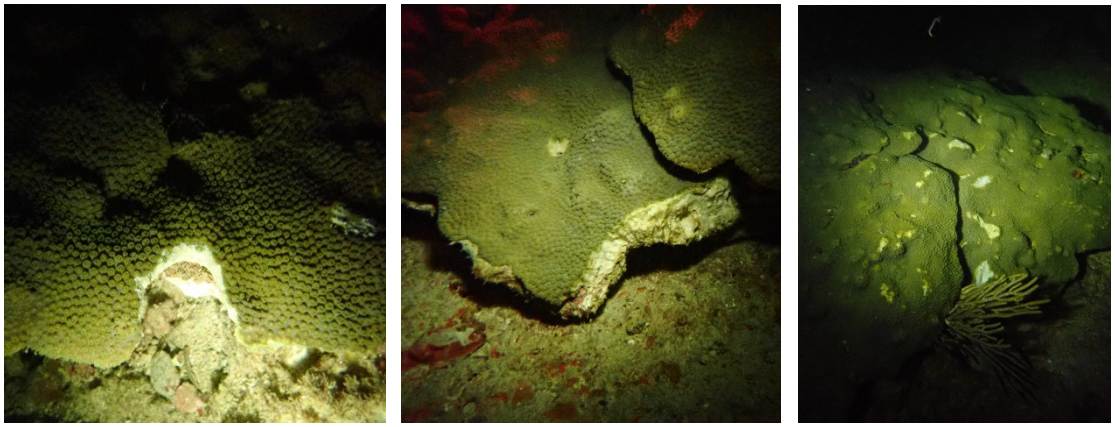

Figure 6. A fragmented, loose ~1m diameter *Orbicella* at site LC-003.

Fragments of a third *O. faveolata* colony were found injured on a reef off Broward County (LC-003) on October 15th, and brought to NSU on October 22nd. These fragments appeared to have been injured for a while, several them being bleached for being upside down for a prolonged period of time, and containing scrapes (Figure 7).

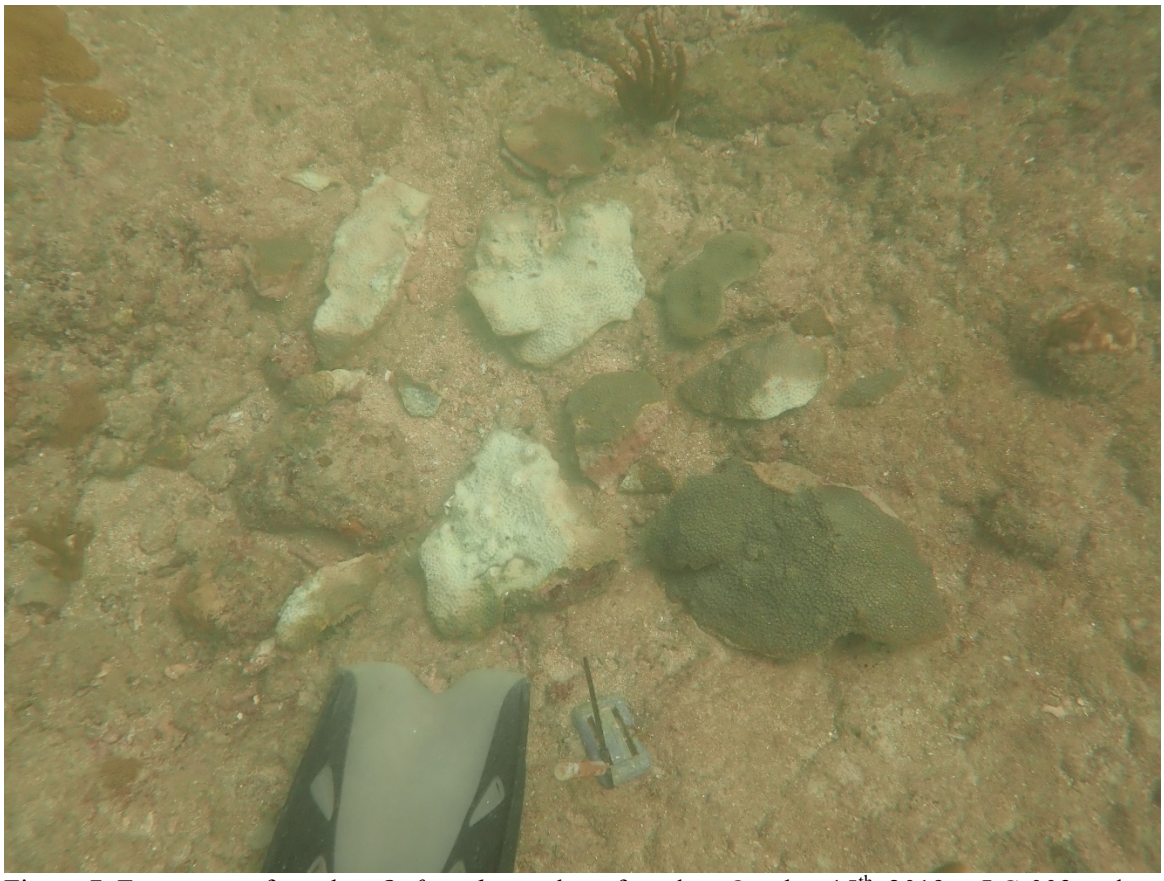

Figure 7. Fragments of another *O. faveolata* colony found on October 15th, 2019 at LC-003 and brought to NSU on October 22nd, 2019

These fragments came bleached and/or injured to the lab. During the first 3 months of holding at NSU, their condition improved considerably, healing lesions and regaining pigmentation (Figure 8a-e), and they grew considerably (approximately 1 cm along the margins, ca. 2-3 polyp rows of growth from the fragmentation edge, in just 4 months, Figure 9). All fragments of the original 3 large *O. faveolata* colonies were monitored daily and presented good health prior to microfragmentation (Figure 10a-c).

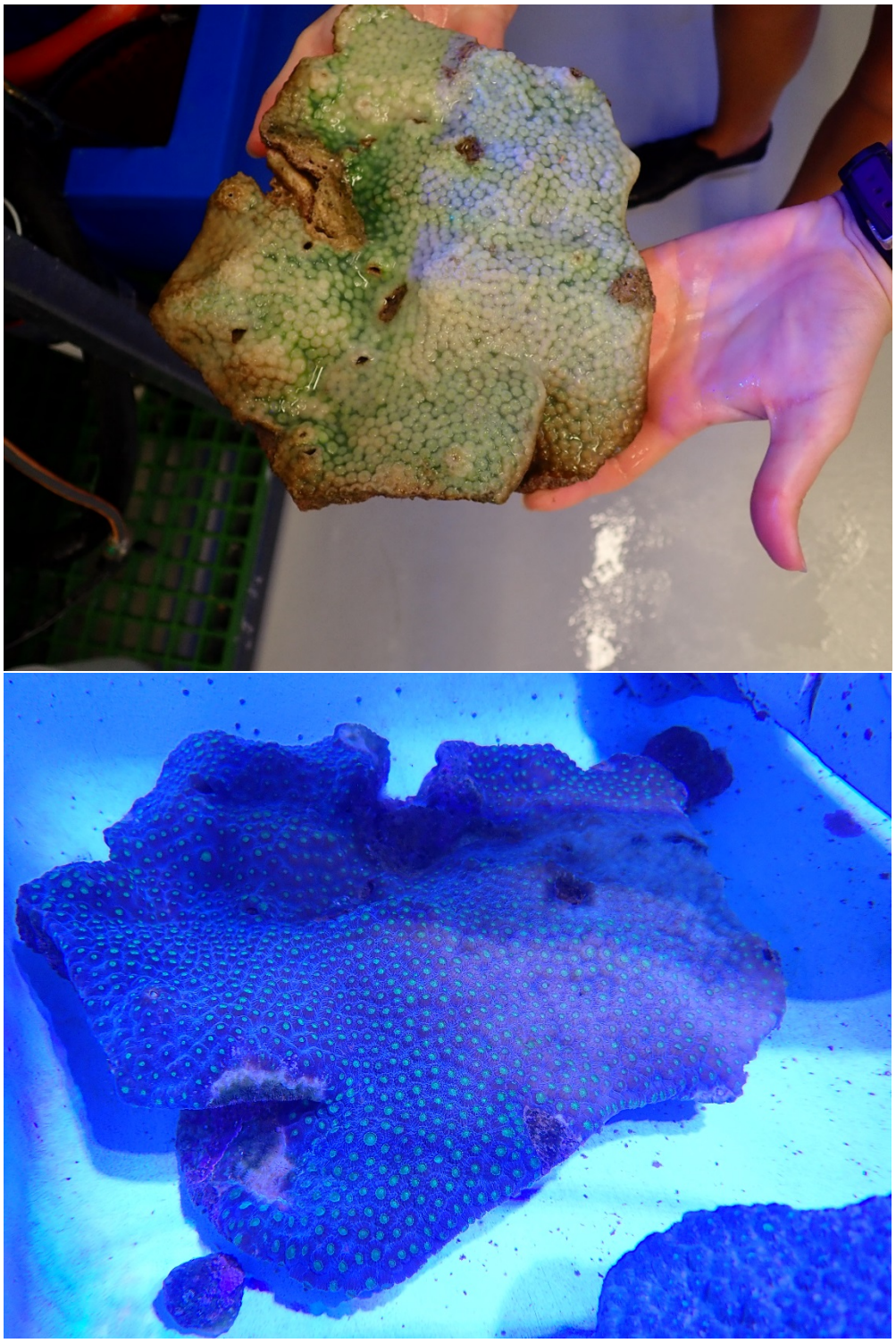

Figure 8a. Fragment of *O. faveolata* colony found at the time of collection (October 22nd, 2019, top) and after two and half months at NSU (January 8th, 2020, bottom)

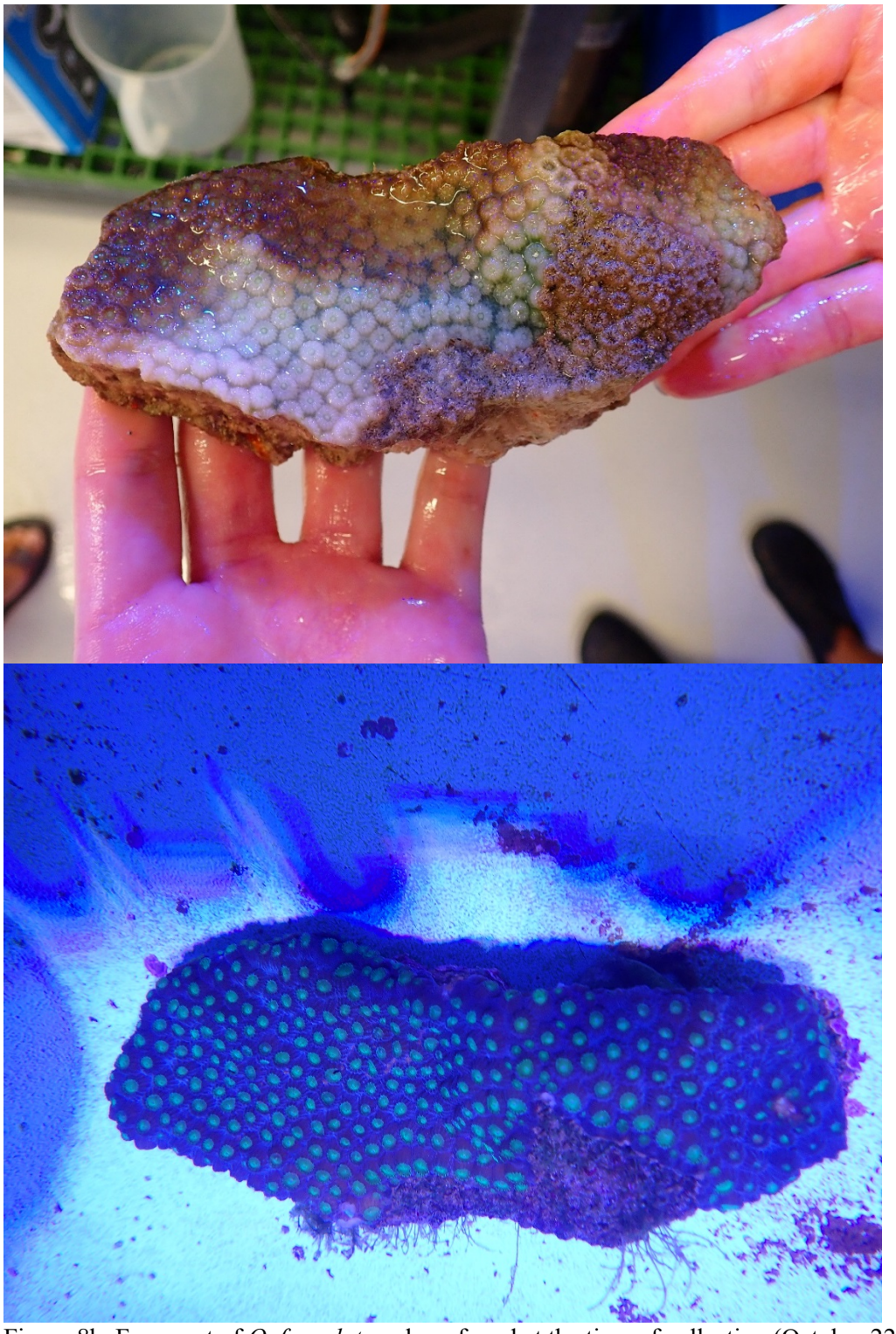

Figure 8b. Fragment of *O. faveolata* colony found at the time of collection (October 22nd, 2019, top) and after two and half months at NSU (January 8th, 2020, bottom)

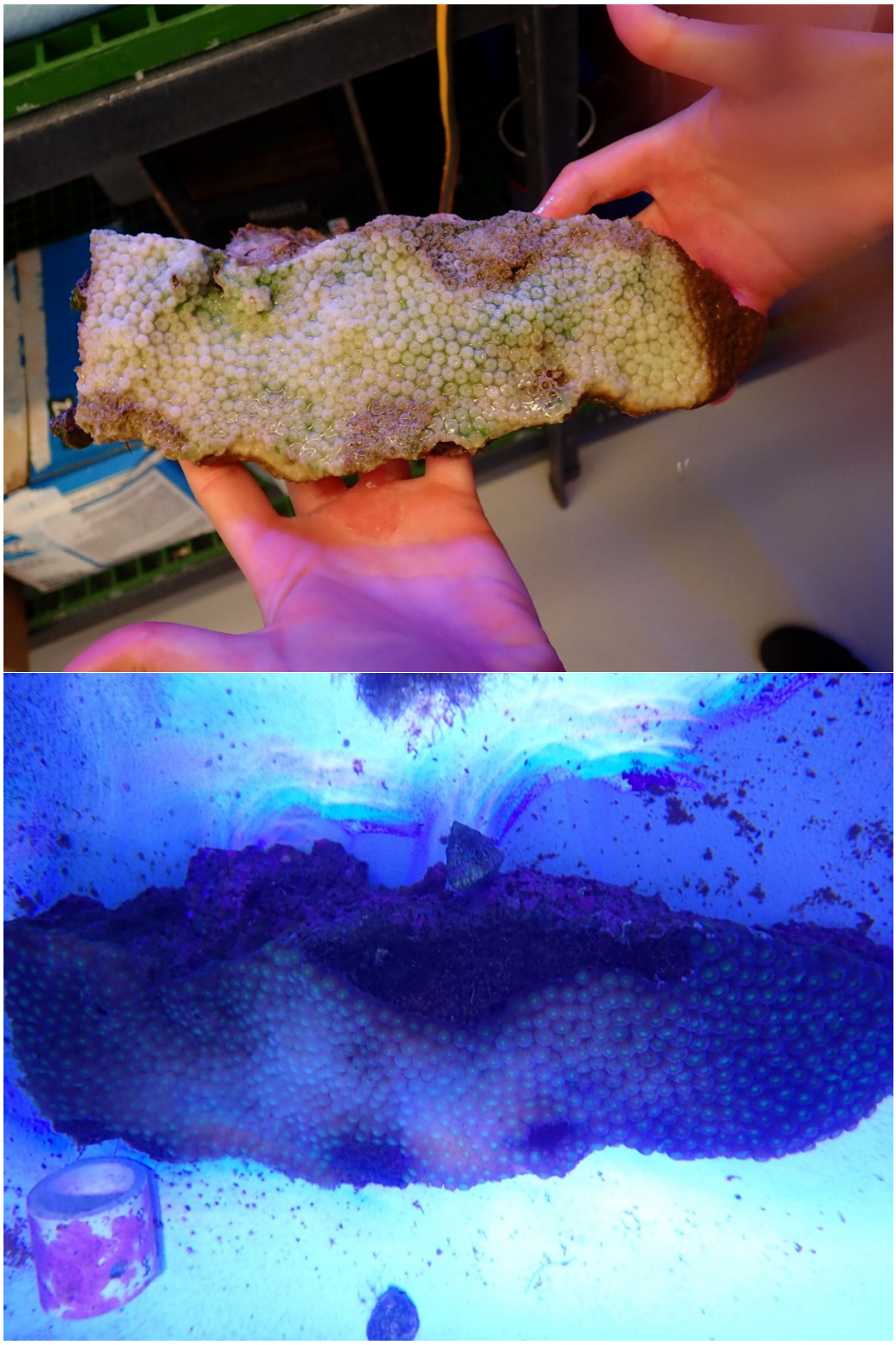

Figure 8c. Fragment of *O. faveolata* colony found at the time of collection (October 22nd, 2019, top) and after two and half months at NSU (January 8th, 2020, bottom)

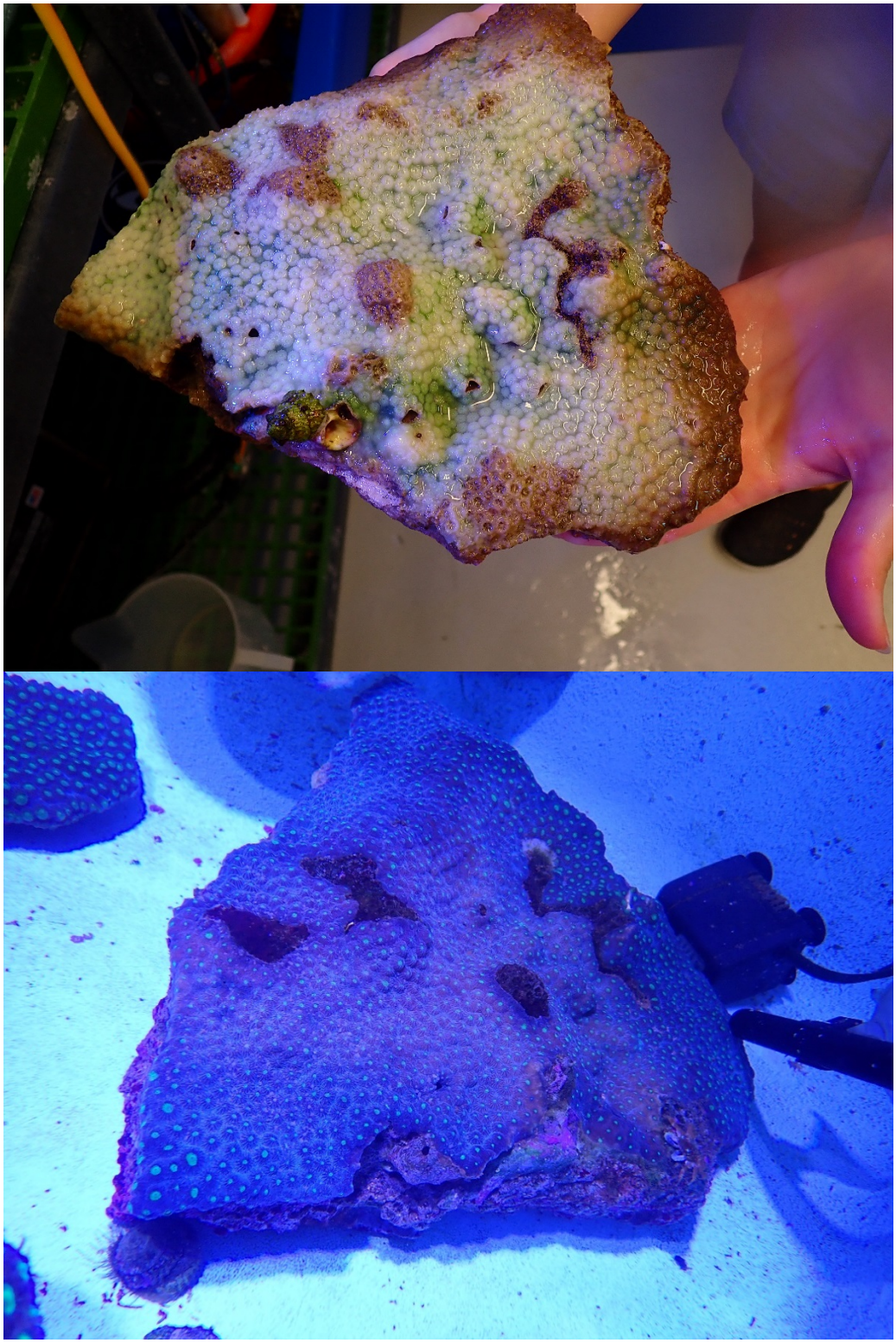

Figure 8d. Fragment of *O. faveolata* colony found at the time of collection (October 22nd, 2019, top) and after two and half months at NSU (January 8th, 2020, bottom)

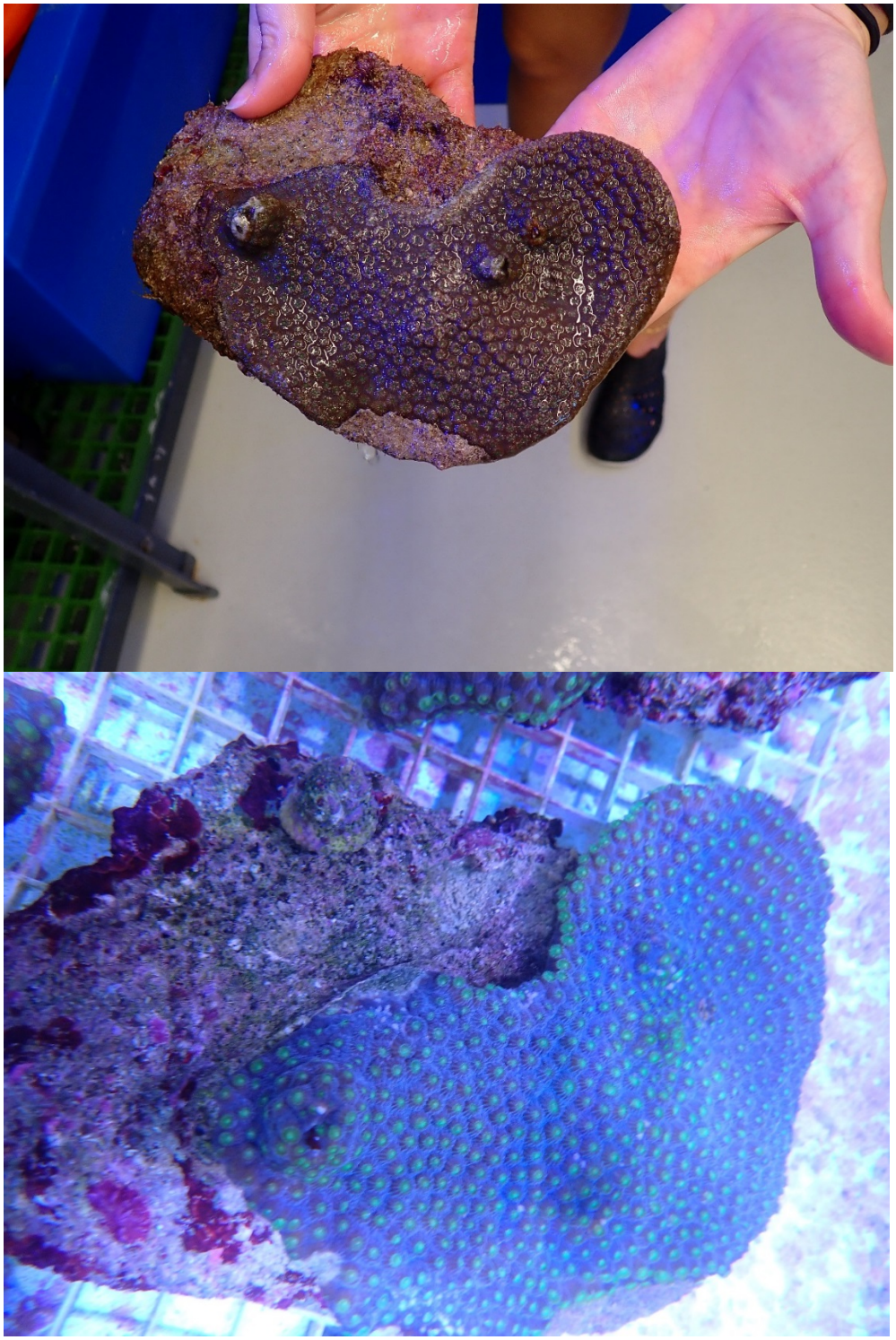

Figure 8e. Fragment of *O. faveolata* colony found at the time of collection (October 22nd, 2019, top) and after two and half months at NSU (January 8th, 2020, bottom)

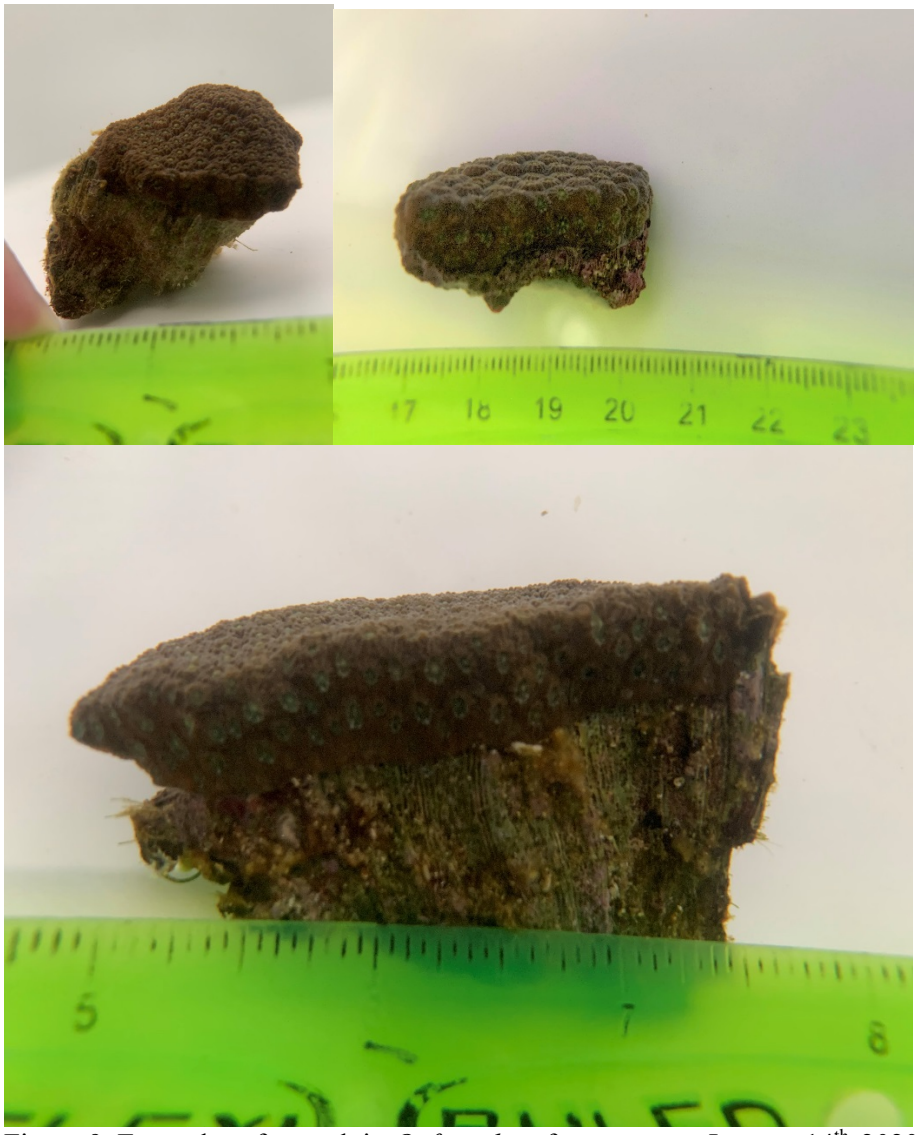

Figure 9. Examples of growth in *O. faveolata* fragments on January 14th, 2020 (approximately 1 cm, 2-3 polyp rows of growth from fragmentation edge)

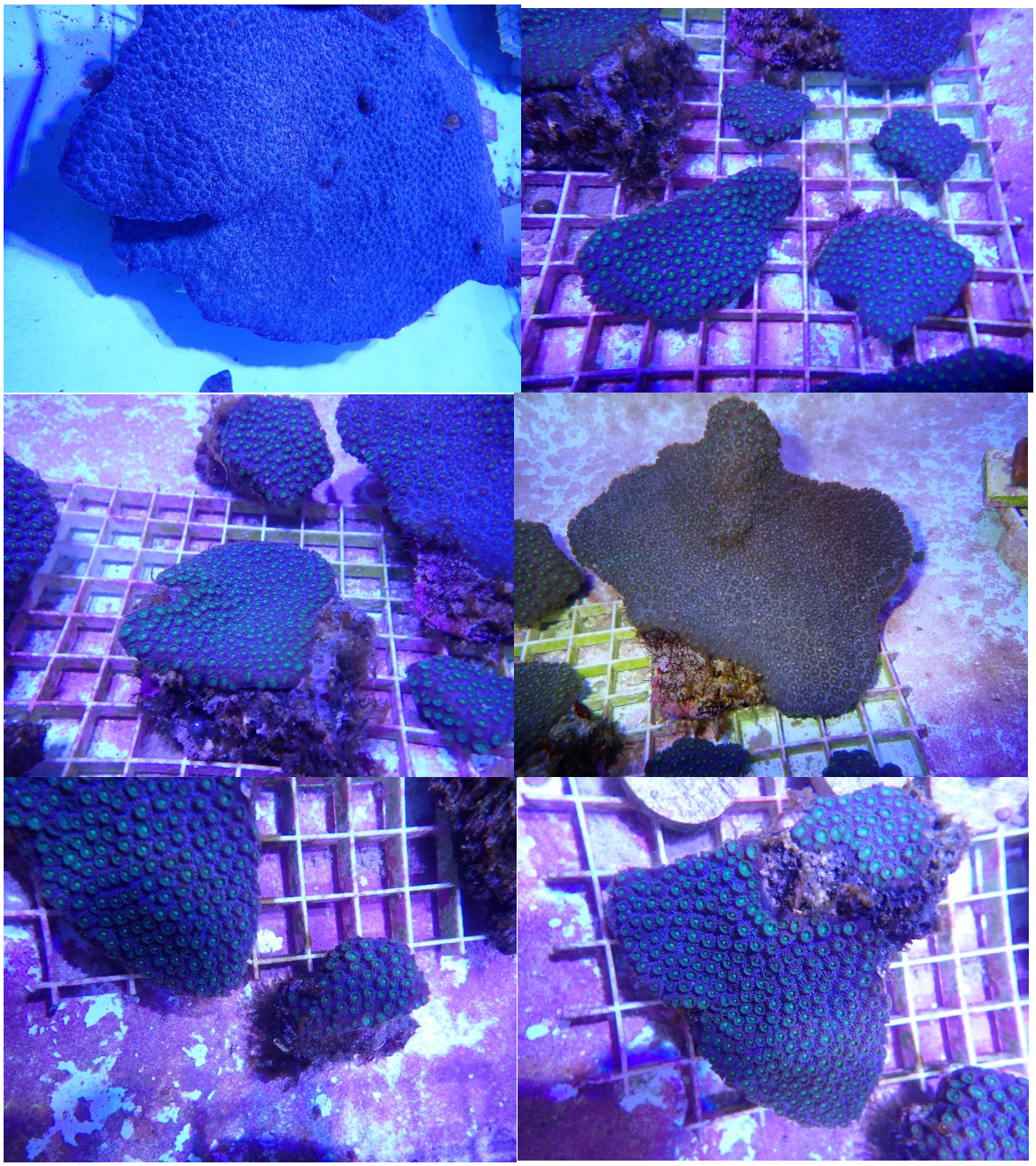

Figure 10a. Fragments of *O. faveolata* kept in the lab for 2.5-4 months

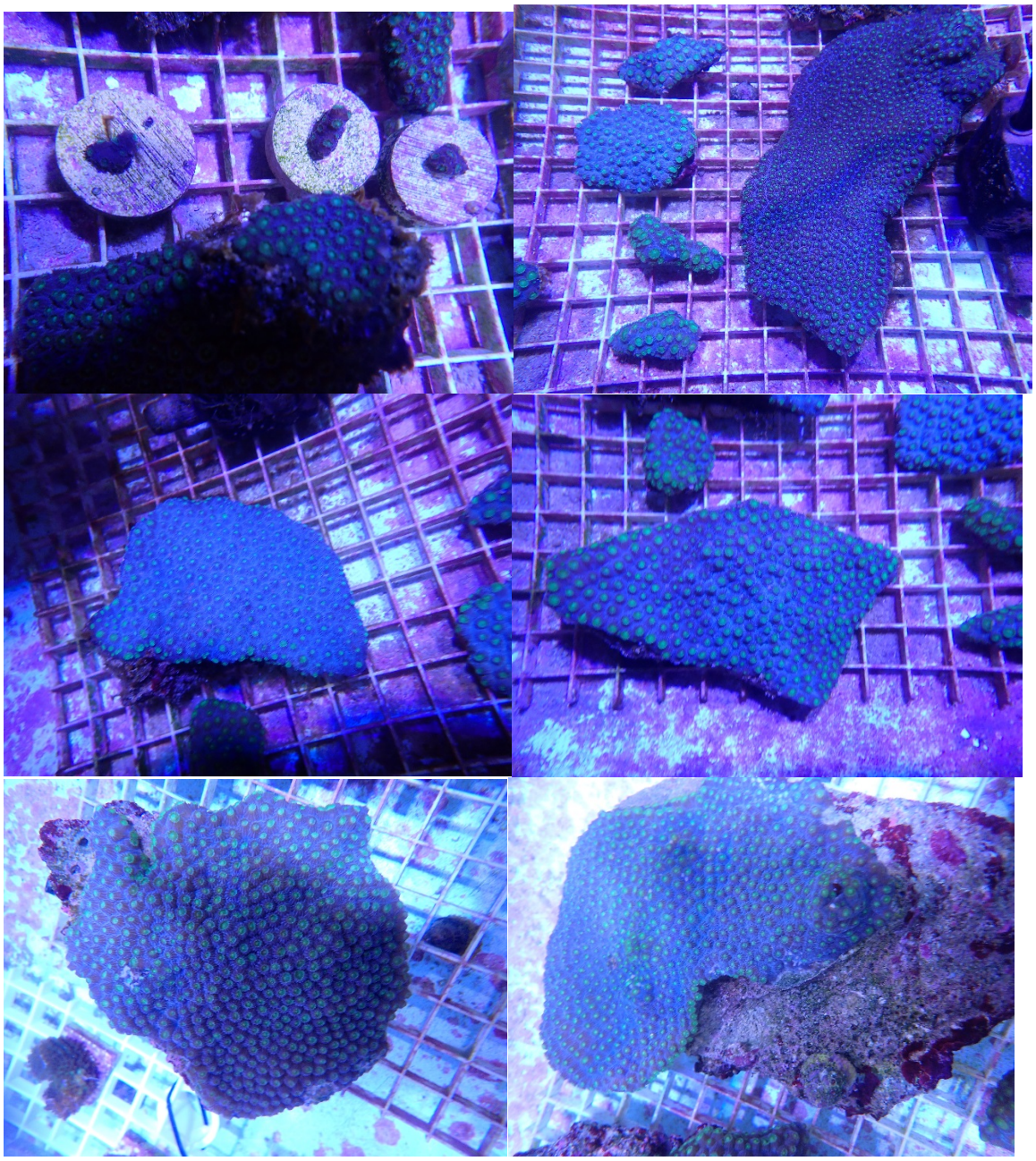

Figure 10b. Fragments of *O. faveolata* kept in the lab for 2.5-4 months

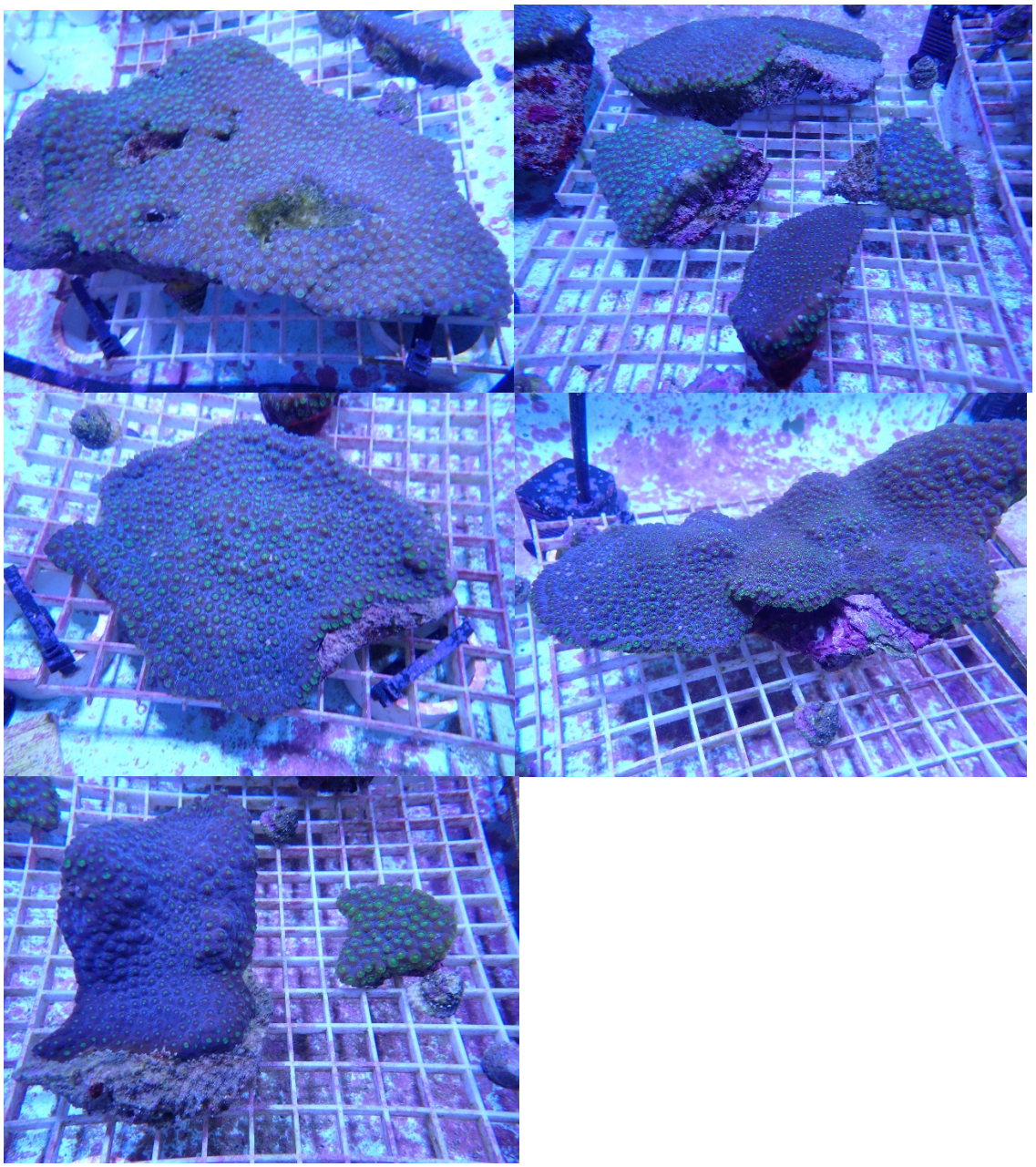
Figure 10c. Fragments of *O. faveolata* kept in the lab for 2.5-4 months

Gonad maturation induction

Seven colony fragments of *O. faveolata* are being maintained in the indoor recirculating system, and three colony fragments are being maintained in the outdoor recirculating system under conditions to induce gonad maturation and spawning in captivity (as described in Methods). Twenty two *Montastraea cavernosa* colonies are being maintained in the indoor recirculating system, and four large colonies are being maintained in the outdoor recirculating system under conditions to induce gonad maturation and spawning in captivity (as described in Methods). These *O. faveolata* and *M. cavernosa* colonies have remained healthy (Figures 11a-b and 12a-f, respectively) and

showed fast growth rates (approximately 1.5-2 cm, 3-5 polyp rows of growth from fragmentation edge, Figure 13), and will be maintained for future natural induction during the 2020 spawning.

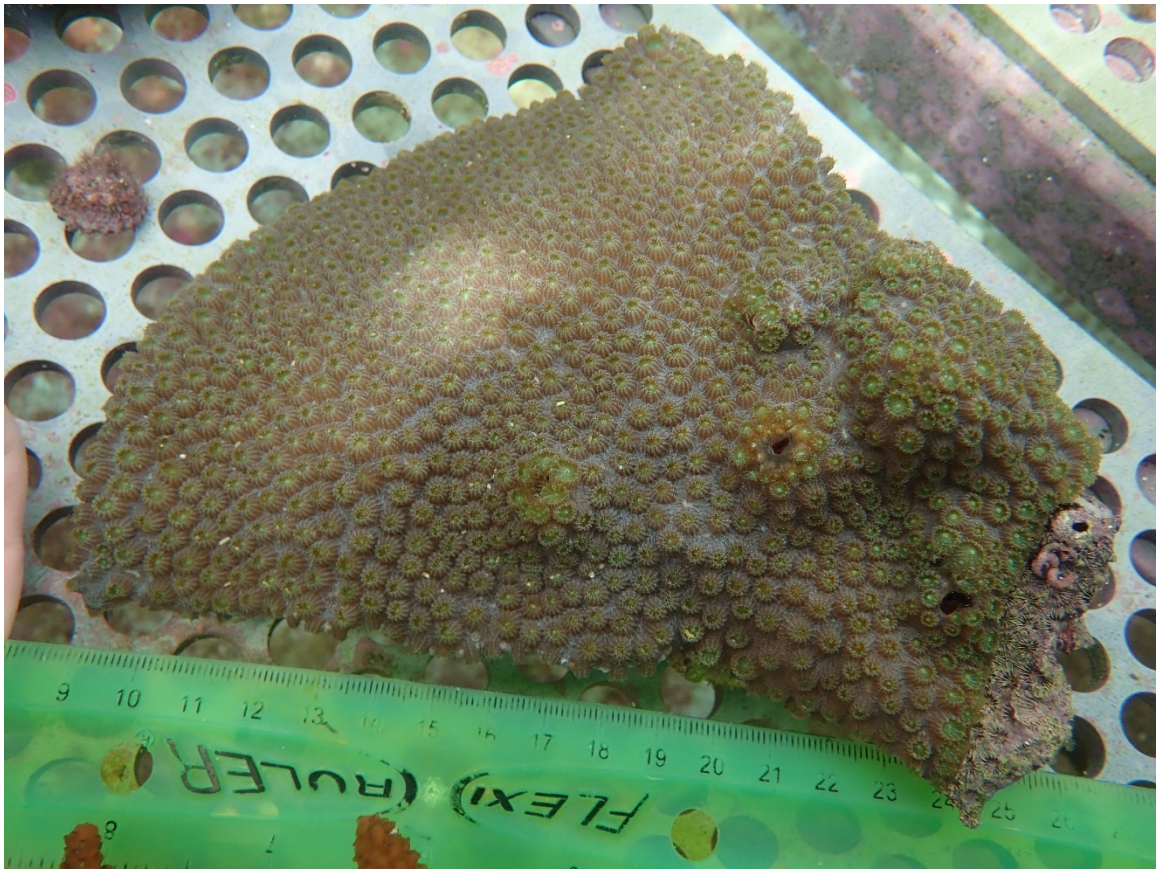

Figure11a: *Orbicella faveolata* colony maintained in the outdoor system to induce gonad maturation and spawning in captivity (March 27, 2020)

Figure11b: *Orbicella faveolata* colonies maintained in the indoor system to induce gonad maturation and spawning in captivity (March 27, 2020)

Figure12a: *Montastraea cavernosa* colonies maintained in the outdoor system to induce gonad maturation and spawning in captivity (March 27, 2020)

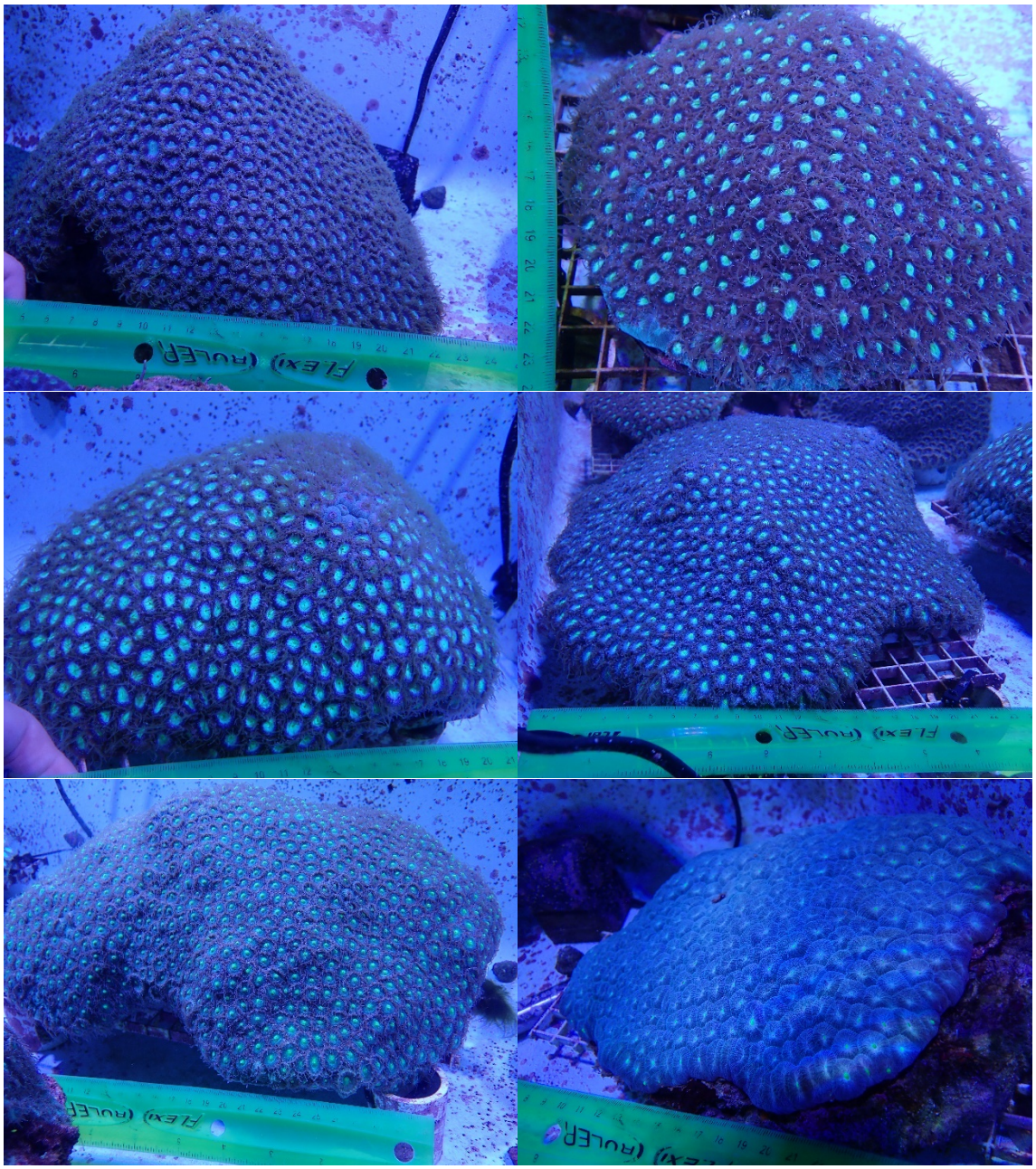

Figure12b: *Montastraea cavernosa* colonies maintained in the indoor system to induce gonad maturation and spawning in captivity (March 27, 2020)

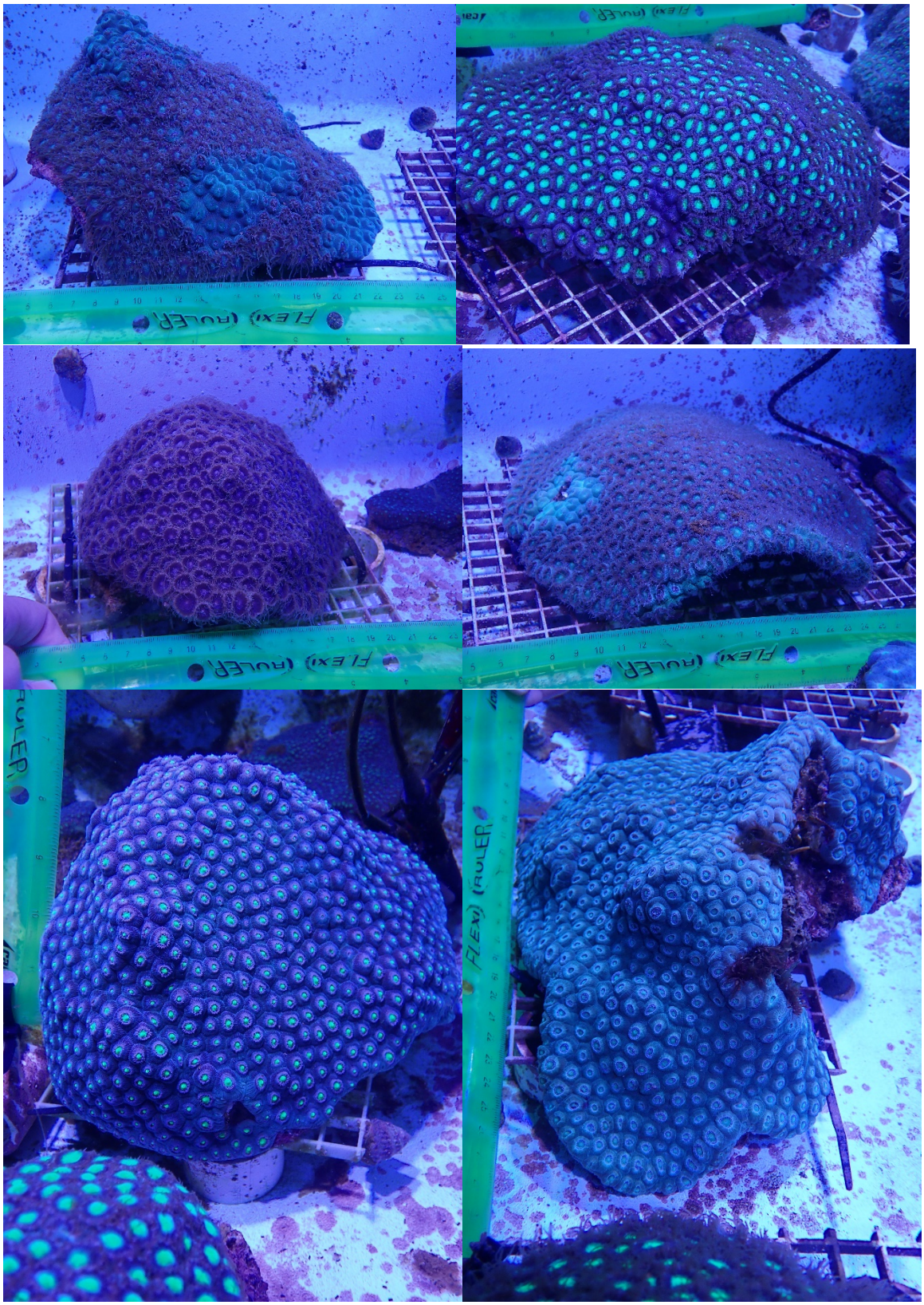

Figure12c: *Montastraea cavernosa* colonies maintained in the indoor system to induce gonad maturation and spawning in captivity (March 27, 2020)

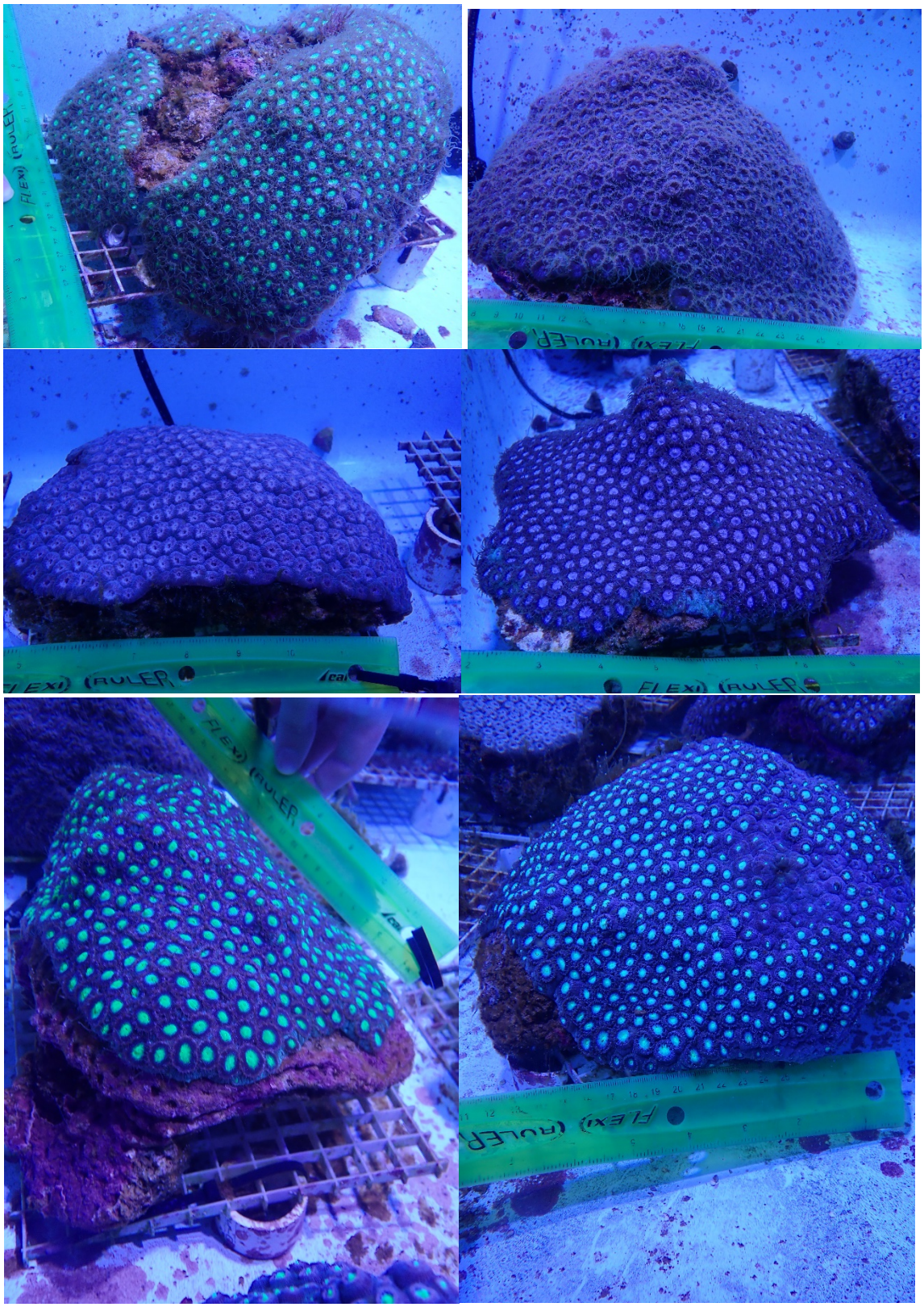

Figure12d: *Montastraea cavernosa* colonies maintained in the indoor system to induce gonad maturation and spawning in captivity (March 27, 2020)

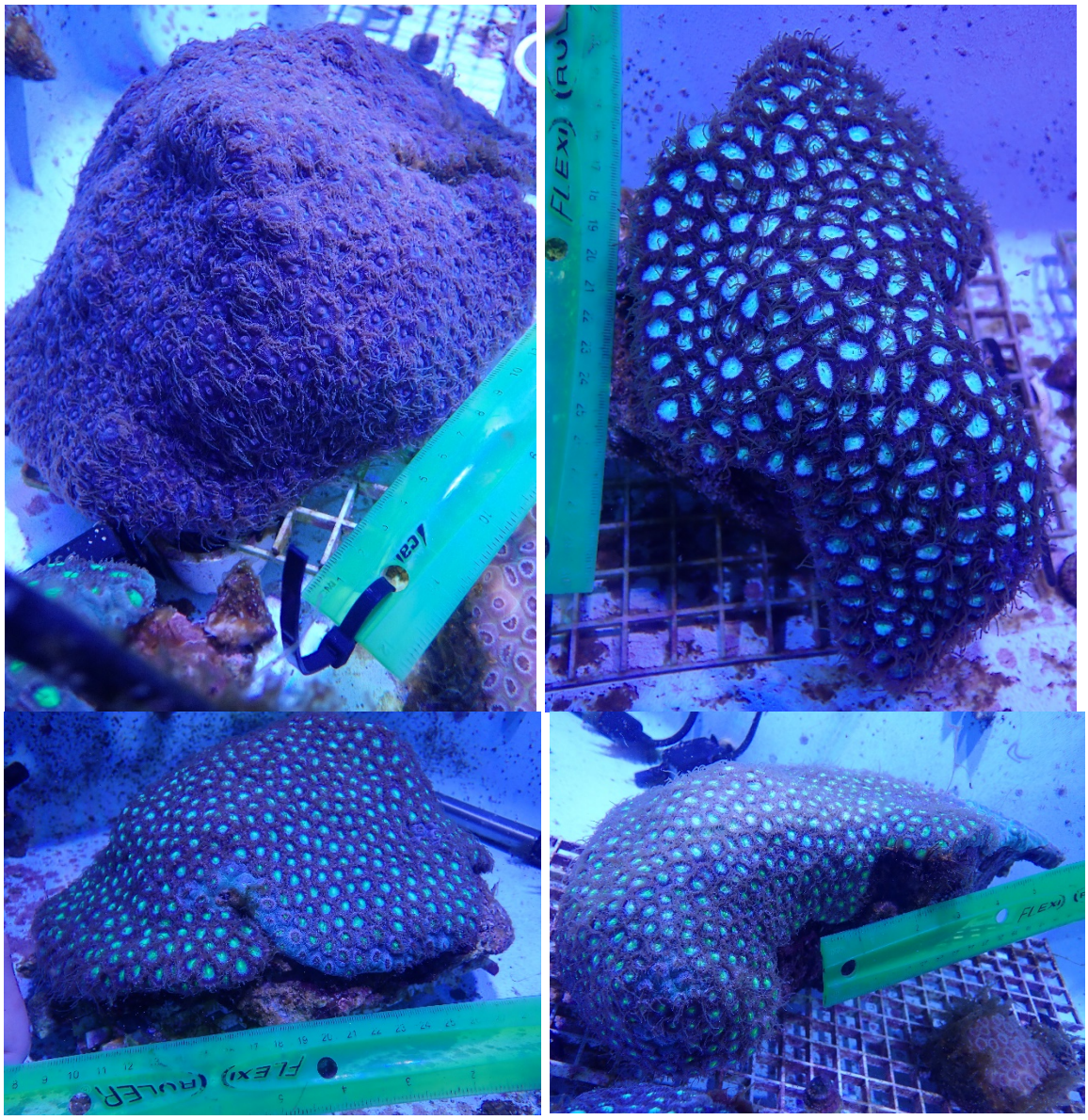

Figure12e: *Montastraea cavernosa* colonies maintained in the indoor system to induce gonad maturation and spawning in captivity (March 27, 2020)

Figure12f: *Montastraea cavernosa* colonies maintained in the indoor system to induce gonad maturation and spawning in captivity (March 27, 2020)

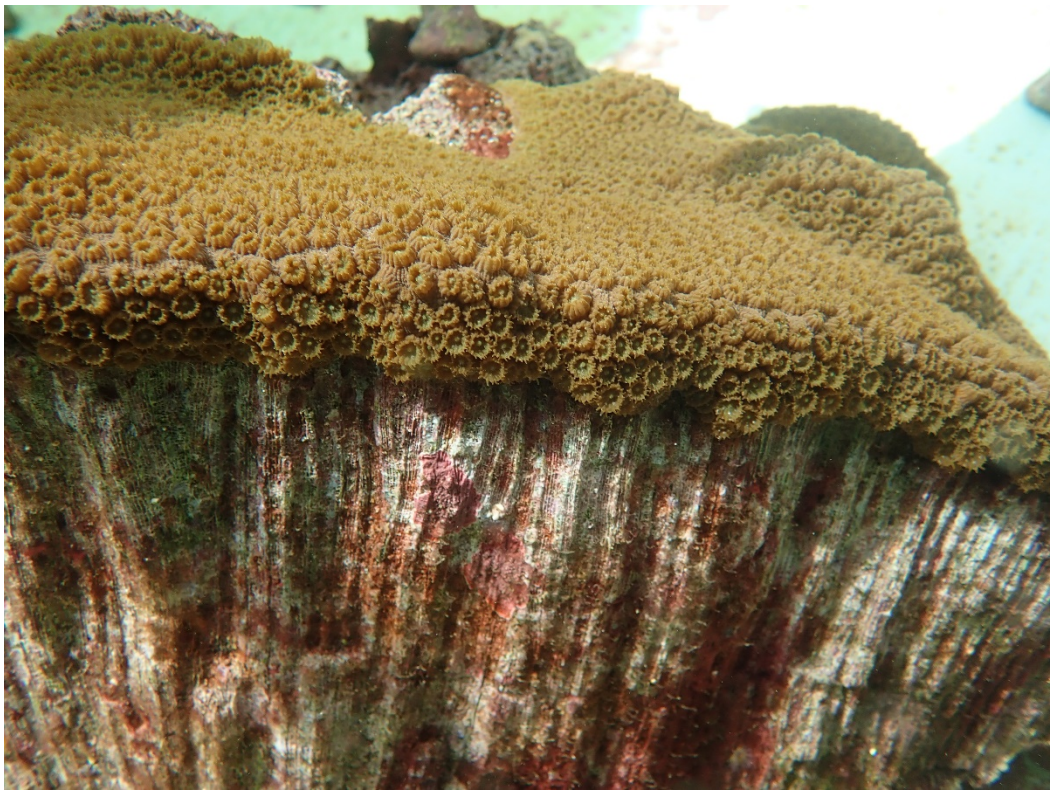

Figure 13: Example of growth in large *O. faveolata* fragment on March 27, 2020 (approximately 1.5-2 cm, 3-5 polyp rows of growth from fragmentation edge)

Microfragmentation

Four hundred and eighty microfragments of *Orbicella faveolata* (average 1.97±0.03 cm^2) from 3 genotypes of very large >2m colonies (two from Broward and one from Miami-Dade Counties) totaling ca. 950cm^2 of live tissue have been maintained in aquaria. Four to six microfragments from the same original fragment of the same genotype were glued to the same tile to allow for future micro-colony fusion (Figure 14).

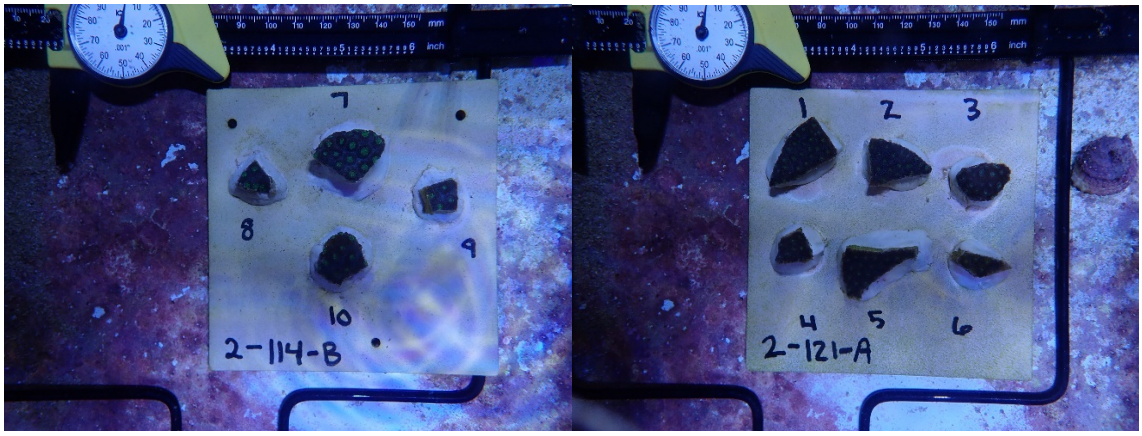

Figure 14. Some of the microfragments of *O. faveolata* at NSU, right after cutting

After 3 months, fragments had on average a 27% larger surface area, ca. 0.89 cm^2 more tissue, meaning they grew at a rate of 0.29 cm^2 per month (Figure 15), which is very good considering it included an initial phase of lesion repair.

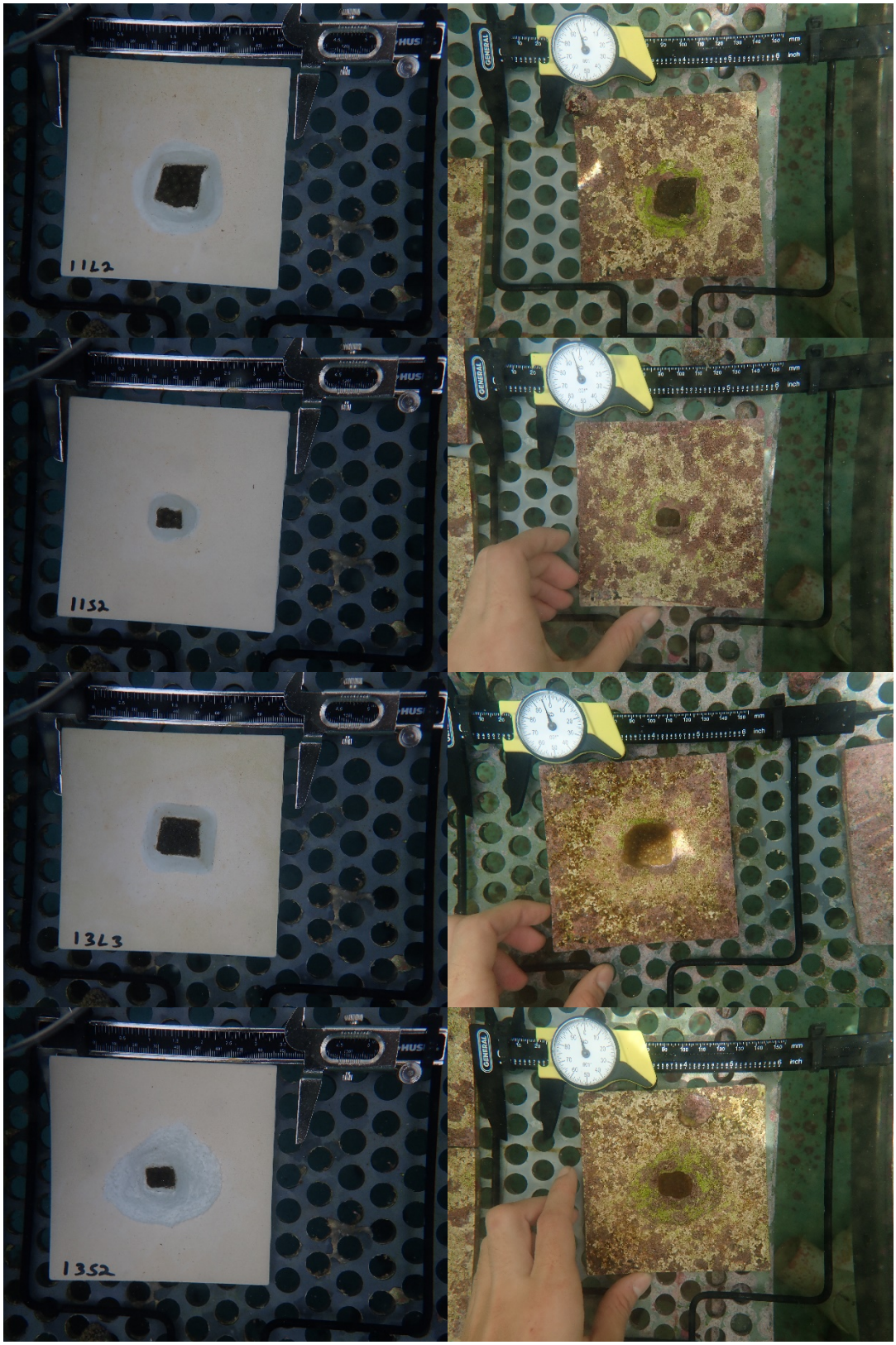

Figure 15. Microfragments of *O. faveolata* right after microfragmentation (left) and three months later showing substantial growth (right), respectively

4. DISCUSSION

Since the large *Orbicella faveolata* was not observed to spawn in Miami-Dade or Broward counties in August, we suggest that these colonies either likely spawned in September (as in most of the Caribbean) or are not sexually reproductive. If not sexually reproductive, these could be due to poor environmental conditions or senescence. Along with observed low fecundity of *Montastraea cavernosa* colonies (14%), these results may be indicative of a very stressed reef system that is unable or unlikely to recover by natural reproduction, therefore urging us to further investigate coral reproduction in SE FL. Future studies should include assessing the fecundity of colonies of these species on the reef prior to the expected spawning through histological analysis. If corals are not fecund, the ability to sexually reproduce them in captivity by removing the stressors needs to be fully assessed.

The high post-settlement mortality of *O. faveolata* from the Keys which were settled in tiles conditioned in Broward suggests the presence of some deleterious agent (chemical or biological) on the reef that may deter natural recruitment. The agent deterring survival of settled larvae on conditioned tiles should be studied as it is possibly affecting the survival to the naturally recruiting larvae on the reef as well. Answers to these questions may help the goal of restoring the natural processes of reproduction and recruitment at a relevant scale. To avoid this issue, in future spawning seasons we will follow the protocol used by other research institutions which consists of conditioning the tiles in the tanks along with adult corals for 1-2 months, and then sprinkle them with crushed pieces of crustose coralline algae for settlement induction before exposing them to the larvae.

The preservation/banking of corals representing the genotypic diversity of these species in captivity is feasible, likely due to the ability to provide the colonies with good water quality and abundance of food. We recommend these colonies to be genotyped to assess their genetic diversity and thus determine if they constitute a good representation of local genetic diversity.

Mass scale coral propagation through microfragmentation at *ex situ* nurseries is viable as these present high growth rates (0.29 cm^2 per fragment per month), and each colony can be cut to generate large numbers of microfragments. In this project, 3 very large colonies/genotypes of opportunity generated 10 colony fragments for gonad maturation and spawning induction ex situ, and 480 microfragments.

5. CONCLUSIONS AND RECOMMENDATIONS FOR THE FUTURE

The fecundity of corals and the ability of larvae to settle and recruits to survive on the reef needs to be assessed.

Ex situ nurseries will play a fundamental role in the preservation and propagation of existing genotypes for future restoration. These nurseries will also be key to induce and assist the sexual reproduction of these disease-impacted species in captivity, forming new

genotypes and producing a high quantity of new individuals to use in future restoration efforts.

Recommendations for future efforts:

1. **Collect histological samples to determine**:
    a. **Reproductive state of colonies**.
    Colonies monitored or collected for spawning should be sampled prior to the predicted spawning period to confirm if they are reproductive using histological analysis;
    b. **Spawning month**
    Samples collected from colonies on the months prior to spawning would also allow us to determine the exact month of spawning for this species (typically consistent for each colony), which allow us to concentrate the field effort exclusively on one month. Note that while some colonies may spawn in different months (one month earlier or later), there is usually a month in which a greater percentage of the colonies of a population of each species spawns;
2. **Assess the effect of environmental conditions on reproductive capacity**
    To determine if reproductive capacity is being impaired by poor environmental conditions, some (fragments or) colonies of these species should be kept year-round in systems that mimic natural environmental cues for spawning (sun and moon light, and temperature) and optimal water quality and food availability, and observed for spawning. To determine if environmental conditions in the field are impairing the sexual reproduction of corals we can compare each colony's fecundity when brought to the lab right before their expected spawning window (i.e. completed gametogenesis offshore) with their fecundity after remaining one year in captivity (i.e. gametogenesis completed in the land-based nursery). This direct within-colony comparison method will allow us to control for genotype effects;
3. **Maximize post-settlement survival**
    In future settlement, use tiles that were conditioned in tanks (optimal water quality) sprinkled with very small pieces of crustose coralline algae.

6. LITERATURE CITED

Craggs, J., J. R. Guest, M. Davis, J. Simmons, E. Dashti, and M. Sweet. 2017. Inducing broadcast coral spawning ex situ: Closed system mesocosm design and husbandry protocol. Ecology and Evolution 7:11066-11078.

Forsman, Z. H., C. A. Page, R. J. Toonen, and D. Vaughan. 2015. Growing coral larger and faster: micro-colony-fusion as a strategy for accelerating coral cover. PeerJ 3:e1313.