# Large *Orbicella* Assisted-Reproduction Report

**Florida Department of Environmental Protection**
**Coral Reef Conservation Program**

# Large *Orbicella* Assisted-Reproduction Report

Final Report

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# List of Acronyms

| | |
|---|---|
| ESA | Endangered Species Act |
| DEP | Florida Department of Environmental Protection |
| FRT | Florida Coral Reef Tract |
| FWC | Florida Fish and Wildlife Conservation Commission |
| NOAA CRCP | National Oceanic and Atmospheric Administration Coral Reef Conservation Program |
| NSU | Nova Southeastern University |
| SE FL | Southeast Florida |

## 1. PROJECT DESCRIPTION

The purpose of this project was to assist the reproduction and propagation of previously-identified, large (≥ 2 m diameter), Endangered-Species-Act (ESA)-threatened-corals of the species *Orbicella faveolata* from SE FL and the Keys as well as *Montasraea cavernosa* colonies in SE FL, both species most impacted by the coral disease outbreak. This includes collecting gametes during spawning, fertilizing eggs, rearing larvae and growing recruits into colonies that can be used for restoration. Field collections of SE FL large colonies were unsuccessful however the other project objectives were still met by raising fertilized *Orbicella* spat from the Florida Keys and using them in light experiments. The *O. faveolata* gametes were used to run light experiments to optimize grow-out and the *M. cavernosa* gametes were used to rear larvae and grow recruits into colonies that can be used for restoration. The outcomes of this project provided numerous colonies of reef-building species and saved their genetic diversity *in ex* situ tanks.

This report summarizes the field and lab efforts conducted to fulfill PO B39AA7 Tasks 1, 2 and 3 deliverables.

## 2. METHODOLOGY

This work was conducted under the State of Florida Special Activity Licenses SAL-18-2021-SRP and SAL-18-1902-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.

### 2.1. Task 1: Field Operations 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 3$^{rd}$ to 9$^{th}$ day after the full moon of August (8/29 – 9/4/2018). 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.

Larvae from *Orbicella faveolata* from Horseshoe Reef Upper Florida Keys were donated by Dana Williams (NOAA). These larvae were obtained from gametes released after the full moon of July.

*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 (pers. obs.). As part of a separate

supplemental research project, twenty-eight colonies of *Montastraea cavernosa* were collected on August 24th by Dr. Figueiredo's graduate students (Samantha King, Allan Anderson, Morgan Stephenson, Nicholas McMahon, 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

Once brought to the laboratory, gametes were pooled and after one hour and a half, the sperm were removed by a series of dilutions. The eggs/embryos were observed under the microscope every 30 minutes afterwards to detect cleavage, to score fertilization. The embryos were then randomly divided into polystyrene containers at a density of < 1 embryo $mL^{-1}$, and were reared until larvae become competent (~3 days for both *Orbicella faveolata* and *Montastraea cavernosa, pers. obs.*); water was changed daily. One day prior to the expected acquisition of competency, the bottoms of the polystyrene containers were covered with pre-conditioned settlement tiles to induce larval settlement and metamorphosis. The settlement tiles were previously conditioned on the reef for 1 month so that coralline algae and bacteria, settlement cues for coral larvae, colonize the tile (Ritson-Williams et al. 2009). Twenty-four hours after the competent larvae were exposed to the pre-conditioned tile, the tiles were scored for metamorphosis. Swimming larvae were provided with new settlement tiles. This process was repeated daily until all the larvae either died or settled. Each day, tiles with newly settled corals were photographed with an Olympus LC20 digital camera attached to an Olympus SZ61 dissecting microscope. The pictures facilitated later identification of each individual by its position within the tile and the measurement of the initial surface area of the coral polyp using CellSens®.

### 2.3. Task 3a: Mass-scale Juvenile grow-out

Juveniles were reared in an indoor 1500L recirculating system composed of two raceways (2.5 X 0.6 X 0.3 m) with a flow rate of 350L/h and shared sump. This recirculating culture system was equipped with biological filtration, protein skimmer, carbon reactor and UV sterilizer. An EHEIM® Jager submersible heater and a chiller was used to maintain temperature. A modified annual temperature cycle was used to fluctuate temperature throughout the year, never going below 24°C, as this typically impairs growth (personal observation). Temperature was measured daily with an YSI® Pro20 temperature probe to ensure accuracy. Aquaillumination® Sol LED lights were used to produce a light irradiance regime adequate to the newly settled corals. The lights followed the photoperiod for the Caribbean with light irradiance increasing from sunrise at 06:00 until solar noon (when maximum irradiance is reached) and decreasing after that until a sunset. The salinity was maintained at 35±1 psu. Reverse osmosis water was added daily to the sump to replace evaporated water and maintain salinity. Water quality tests were performed weekly to determine ammonia, nitrite, nitrate and phosphate concentrations. If necessary, partial water changes were performed. Every week from the

settlement date, each tile was examined under the Olympus stereo-dissecting microscope to score survival, and photographed to measure surface area using the software CellSens.

### 2.4 Task 3b: Experiment to optimize light irradiance

*One of the greatest constraints for culturing sexually-produced corals is high mortality in the the first months after settlement. Coral recruits appear vulnerable to high light irradiance, likely likely due to the incomplete establishment of symbiosis. We conducted an experiment to optimize optimize the grow-out of sexually-produced coral recruits of O. faveolata from the Upper Florida Florida Keys. Specifically, we determined the optimal light irradiance after settlement and the and the optimal rate of increase in light irradiance during the grow-out phase (first 4 months months post- settlement). To do so, once larval development was completed, coral larvae were were settled on pre-conditioned tiles (as described in 2.3). Tiles with newly settled recruits were recruits were placed in the recirculating raceway system composed of two fiberglass raceways raceways 240 cm x 60 cm x 30 cm connected to a common sump, filtration and sterilization). sterilization). Newly settled juveniles were exposed to 10µmol photons $m^{-2}$ $s^{-1}$ until they were 5 were 5 weeks old and then progressively moved to higher irradiance levels at different rates and rates and times, based on the weekly performance of the recruits, to maximize the number of of recruits alive at the end of the experiment (Figure 1,*

Table 1). Each raceway was lit by Aqua Illumination Hydra 26 HD LEDs lights. The light spread of each of these LEDs is approximately 60 x 60 cm, so each raceway was divided into 6 sections of this size (12 sections total), using black plastic bags to eliminate the possibility of light scattering between sections. One section was used to house adult corals to shed symbionts to the recruits and each of the remaining sections was assigned to one treatment, with each treatment being replicated twice in the system (one per raceway). We used at least 15 recruits per replicate (30 replicates per treatment).

*Table 1. Lighting levels (µmol photons $m^{-2}$ $s^{-1}$) in the five light treatments.*

| **Treatment** | **Time (weeks)** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| **1** | 10 | 10 | 10 | 10 | 60 | 60 | 60 | 60 | 60 | 80 | 100 | 120 | 140 | 140 | 180 | 180 |
| **2** | 10 | 10 | 10 | 10 | 60 | 80 | 80 | 80 | 80 | 100 | 120 | 140 | 160 | 160 | 180 | 180 |
| **3** | 10 | 10 | 10 | 10 | 40 | 40 | 40 | 40 | 60 | 80 | 120 | 140 | 180 | 180 | 180 | 180 |
| **4** | 10 | 10 | 10 | 10 | 40 | 40 | 60 | 60 | 60 | 100 | 120 | 160 | 160 | 160 | 180 | 180 |
| **5** | 10 | 10 | 10 | 10 | 20 | 20 | 40 | 40 | 40 | 60 | 80 | 120 | 140 | 140 | 180 | 180 |

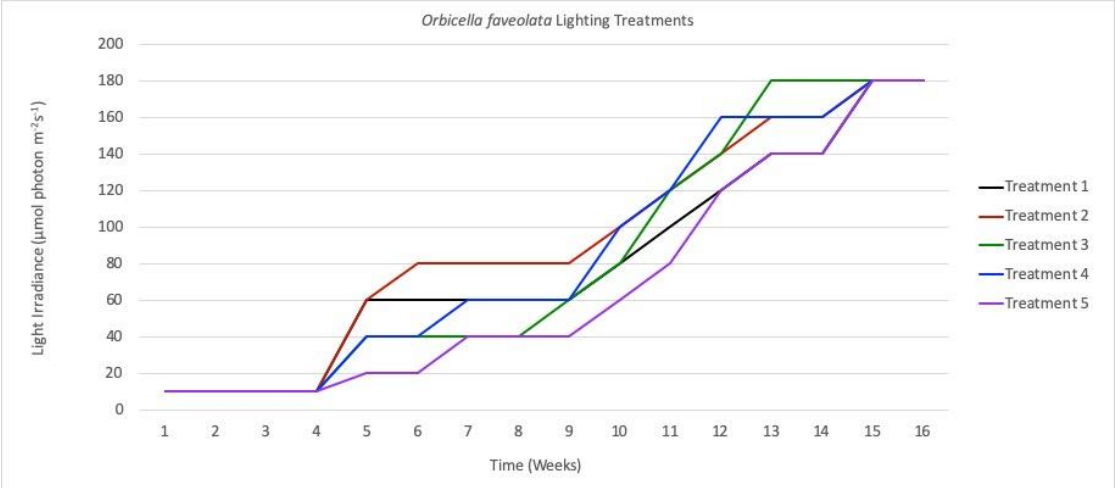


*Figure 1. Light levels (µmol photons $m^{-2}$ $s^{-1}$) in the five light treatments.*

The survival, growth and pigmentation of recruits was examined weekly under an Olympus LC20 digital camera attached to an Olympus SZ61 dissecting stereoscope to measure surface area of the polyps with the software cellSens®. Corals were scored for survival (0 – alive, 1 – dead) and pigmentation (scale 1-6, 1-pale, 6-dark, based on the Coral Watch's "Coral Color Reference Card").

## 3. REPORT

The purpose of field efforts reported herein was to assist the reproduction and propagation of previously-identified, large (≥ 2 m diameter), Endangered-Species-Act (ESA)-threatened-corals of the species *Orbicella faveolata* from SE FL. This includes collecting gametes during spawning, fertilizing eggs, rearing larvae and growing recruits into colonies that can be used for restoration. These activities would preserve the genotypes of the largest, oldest, and most resilient corals in SE FL 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. Unfortunately, this objective was not achieved.

### 3.1. Task 1: Field operations

Field operations to observe spawning and collect gametes occurred between August 27 and September 4, 2018 in Fort Lauderdale, FL, near LC-003. 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 (**Error! Reference source not found.**).

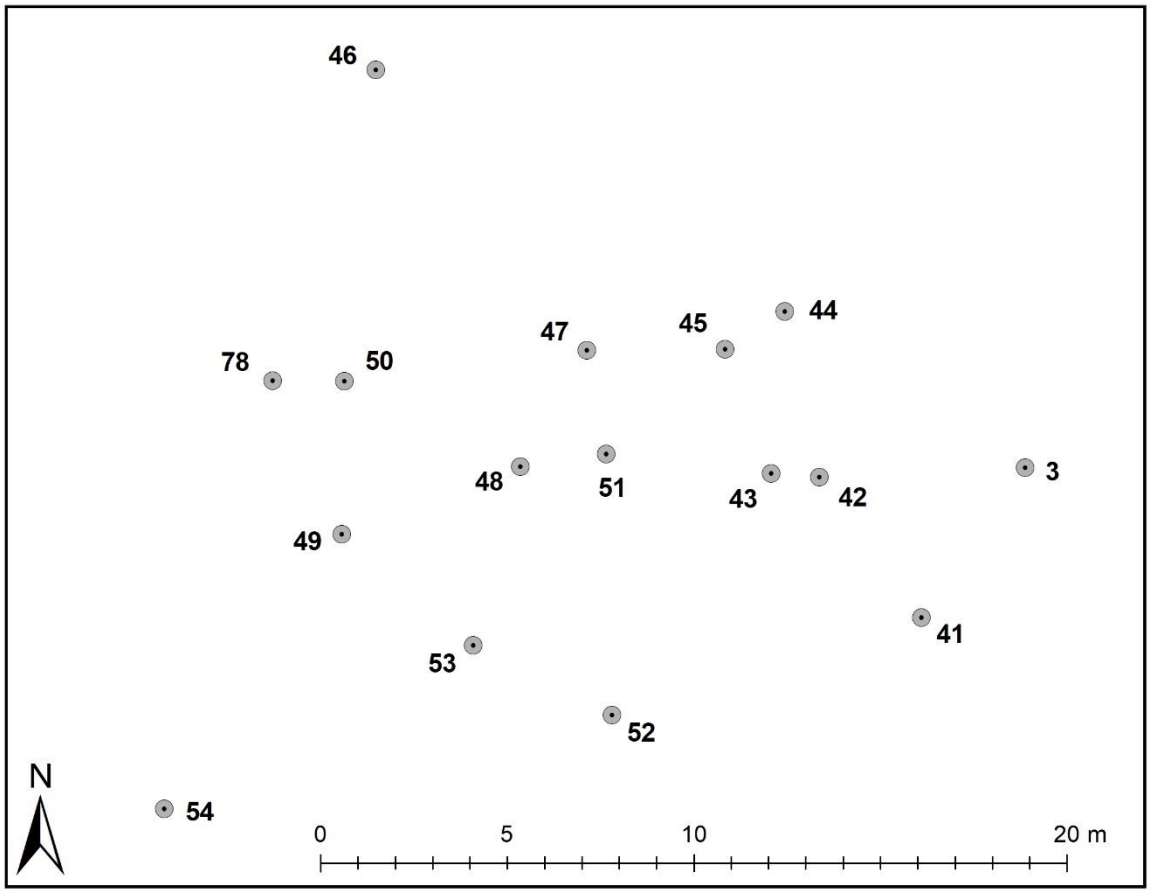


*Figure 2. The location of the spawning dives referred to as site LC-003 (far right). Each dot represents the documented location of a live large Orbicella greater than two meters in diameter.*

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 2 shows the personnel and their roles during the project. For spawning there were eight vessel personnel and between three and four lab personnel standing by. The first four nights were attempted using an NSU vessel (Table 3). The last four nights were attempted using the American Dream II. This was due to the high winds and rough sea state during the formation and passing of Tropical Storm Gordon.

**August 27, 2018 – Site setup**

On Monday, August 27, 2018 a team of seven visited the site to become acquainted with the area during daylight hours, label the LC corals with survey tape, and devise a plan for night operations. The corals were labeled using reference photos printed on underwater paper. The conceived plan involved staging three teams of two divers about five meters apart in a line extending westward from LC-003 where each diver would monitor two large corals. The site was marked by a removable weighted line and glow sticks that were attached each night to outline the three survey locations within the site and the beginning and end of the line.

**August 29, 2018**

A team of two divers was deployed on site at 1700 to set up the transect and glow sticks during daylight hours. This team returned to dock at 1900. At 2000, a 30-minute pre-dive meeting was held by the PI and Co-PI to go over protocols, spawning video examples, and last-minute details. At 2100 a team of eight departed NSU and traversed to the site. Once on site, two divers were deployed to place a lighted dive buoy on LC-003 for reference when anchoring. Those divers were retrieved and the vessel was anchored to the East of LC-003, laying back over the site. At 2200, two divers descended to observe corals at the site. At 2230 four more divers descended and took their stations along the transect to observe their assigned large corals. No spawning was observed. At 2345, divers were retrieved and the team headed home. Seas were quite rough on the return trip and the vessel could only safely travel approximately 5 knots. Therefore, we returned to dock at 0030 on August 30.

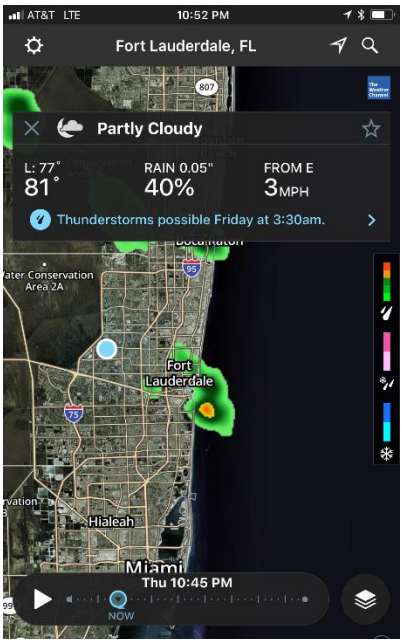


*Figure 3. Screenshot of the weather radar showing a thunderstorm on site at the time of the planned spawning dive.*

**August 30, 2018**

The marine forecast was questionable for the evening with seas at the limit of normal daytime operations. At 2030, a team of eight divers loaded the boat and headed out towards the spawning site. There was a thunderstorm to the East headed directly to the dive site which was expected to increase the wave energy (Figure 3). Once outside of the Port Everglades inlet, the seas were deemed too rough for safe operations as the boat and crew were being tossed around in the dark. The dive operation was abandoned and the team returned to the dock around 2100.

*Table 2. Personnel and roles during spawning field operations. Shading indicates aborted operations.*

| Location | Monday (8/27) | Wednesday (8/29) | Thursday (8/30) | Friday (8/31) |
|---|---|---|---|---|
| BOAT | Brian Walker (boat captain) | Brian Walker (boat captain) | Brian Walker (boat captain) | Brian Walker (boat captain) |
| BOAT | Sam King (diver) | Sam King (boat fertilization) | Sam King (boat fertilization) | Sam King (boat fertilization) |
| BOAT | Shelby Eagan (diver) | Shelby Eagan (diver) | Shelby Eagan (diver) | Shelby Eagan (diver) |
| BOAT | Alysha Brunelle (diver) | Alysha Brunelle (diver) | Alysha Brunelle (diver) | Liz Fromuth (diver) |
| BOAT | Liz Fromuth (diver) | Liz Fromuth (diver) | Liz Fromuth (diver) | Allan Anderson (diver) |
| BOAT | Murphy McDonald (diver) | Allan Anderson (diver) | Allan Anderson (diver) | Murphy McDonald (diver) |
| BOAT | Sarah Koerner (diver) | Murphy McDonald (diver) | Grace Hanson (diver) | Sarah Koerner (diver) |
| BOAT | | Sarah Koerner (diver) | Nicole Hayes (diver) | Nick McMahon (diver) |
| | | | | |
| LAB | | Joana Figueiredo (lab) | Joana Figueiredo (lab) | Joana Figueiredo (lab) |
| LAB | | Morgan Stephenson (lab) | Morgan Stephenson (lab) | Morgan Stephenson (lab) |
| LAB | | Helena (lab) | Helena (lab) | Helena (lab) |
| LAB | | | Murphy McDonald (lab) | |

| Location | Saturday (9/1) | Sunday (9/2) | Monday (9/3) | Tuesday (9/4) |
|---|---|---|---|---|
| BOAT | Brian Walker (photo/video) | Brian Walker (photo/video) | Brian Walker (photo/video) | Brian Walker (topside assistant) |
| BOAT | Sam King (boat fertilization) | Sam King (boat fertilization) | Sam King (boat fertilization) | Sam King (boat fertilization) |
| BOAT | Shelby Eagan (diver) | Shelby Eagan (diver) | Shelby Eagan (diver) | Grace Hanson (diver) |
| BOAT | Alysha Brunelle (diver) | Alysha Brunelle (diver) | Alysha Brunelle (diver) | Alysha Brunelle (diver) |
| BOAT | Liz Fromuth (diver) | Liz Fromuth (diver) | Liz Fromuth (diver) | Liz Fromuth (diver) |
| BOAT | Allan Anderson (diver) | Allan Anderson (diver) | Allan Anderson (diver) | Allan Anderson (diver) |
| BOAT | Murphy McDonald (diver) | Murphy McDonald (diver) | Murphy McDonald (diver) | Nicole Hayes (diver) |
| BOAT | Nick McMahon (diver) | Nick McMahon (diver) | Nick McMahon (diver) | Nick McMahon (diver) |
| | | | | |
| LAB | Joana Figueiredo (lab) | Joana Figueiredo (lab) | Joana Figueiredo (lab) | Joana Figueiredo (lab) |
| LAB | Morgan Stephenson (lab) | Helena (lab) | Morgan Stephenson (lab) | Morgan Stephenson (lab) |
| LAB | Helena (lab) | Sarah Koerner (lab) | Helena (lab) | Helena (lab) |
| LAB | Sarah Koerner (lab) | | Sarah Koerner (lab) | Sarah Koerner (lab) |

*Table 3. Boat logs indicating the date, personnel, site, and brief operational notes. Shading indicates aborted operations.*

| Date | Vessel | Personnel | Site | Notes |
|---|---|---|---|---|
| 8/27/2018 | Navigator | Brunelle, Walker, Eagan, Fromuth, Koerner, McDonald, King | LC-003 | Site setup, coral identification, and tagging. |
| 8/29/2018 | Twin Vee | Brunelle, Walker, Eagan, Fromuth, McDonald, Anderson, Koerner, King | LC-003 | Spawning dives conducted. None observed. |
| 8/30/2018 | Twin Vee (no charge) | Brunelle, Walker, Eagan, Fromuth, Hayes, Anderson, Hanson, King | LC-003 | Conditions too rough for vessel size. Operations aborted. |
| 8/31/2018 | Twin Vee (no charge) | Walker, Eagan, Fromuth, McDonald, Anderson, Koerner, King, McMahon | LC-003 | Conditions too rough for vessel size. Operations aborted. |
| 9/1/2018 | American Dream II | Brunelle, Walker, Eagan, Fromuth, McDonald, Anderson, King, McMahon | LC-003 | Spawning dives conducted. None observed. |
| 9/2/2018 | American Dream II (no charge) | Brunelle, Walker, Eagan, Fromuth, McDonald, Anderson, King, McMahon | LC-003 | Conditions too rough for safe operations. Operations aborted. |
| 9/3/2018 | American Dream II (no charge) | Brunelle, Walker, Eagan, Fromuth, McDonald, Anderson, King, McMahon | LC-003 | Conditions too rough for safe operations. Operations aborted. |
| 9/4/2018 | American Dream II | Brunelle, Walker, Hanson, Fromuth, Hayes, Anderson, King, McMahon | LC-003 | Spawning dives conducted. None observed. |

**August 31, 2018**

The marine forecast was questionable for the evening with seas at the limit of normal daytime operations. However, the NSU teams that had been out during the day were able to safely complete their tasks. Thus at 2030, a team of eight divers loaded the boat and headed out towards the spawning site. On the way out of Port Everglades inlet, at least five waves crested over the bow of the vessel. The conditions were again deemed too rough for safe operations as the boat and crew were being tossed around in the dark. The dive operation was abandoned and the team returned to the dock around 2100.

**September 1, 2018**

The seas were above the limit of normal daytime operations on NSU vessels, therefore we hired the American Dream II dive operation to transport divers to the spawning site. At 2000, a team of eight divers met at the American Dream II and loaded the vessel (Figure 4). Scott Sheckman, from Friends of Our Florida Reefs, met the team on site and took photographs and conducted short video interviews about the project. At 2100 the team departed and traversed to the site. Seas were 3 to 5 feet, but the 46-foot vessel handled it without issue. Operations commenced similarly to the August 29 operations as planned. Conditions underwater were challenging with low visibility and high surge. Divers found it difficult to stay in place to observe their corals and found getting back on the boat challenging. No spawning was observed. Operations ended at 2345 and the team returned to the dock. Equipment and personnel were unloaded during a heavy rainstorm with lightening around 0030.

*Figure 4. Dive team preparing to depart the dock on the American Dream II to collect spawn. Photo by Scott Sheckman.*

**September 2, 2018**

The seas were above the limit of normal daytime operations on NSU vessels (Figure 5). The entire team met at the American Dream II dock to discuss the possibility of diving. The captain had been out earlier in the day and did not recommend attempting the dive. The dive was cancelled without leaving the dock to avoid any vessel fees and to ensure divers were not put in unsafe conditions. Operations were rescheduled for September 4.

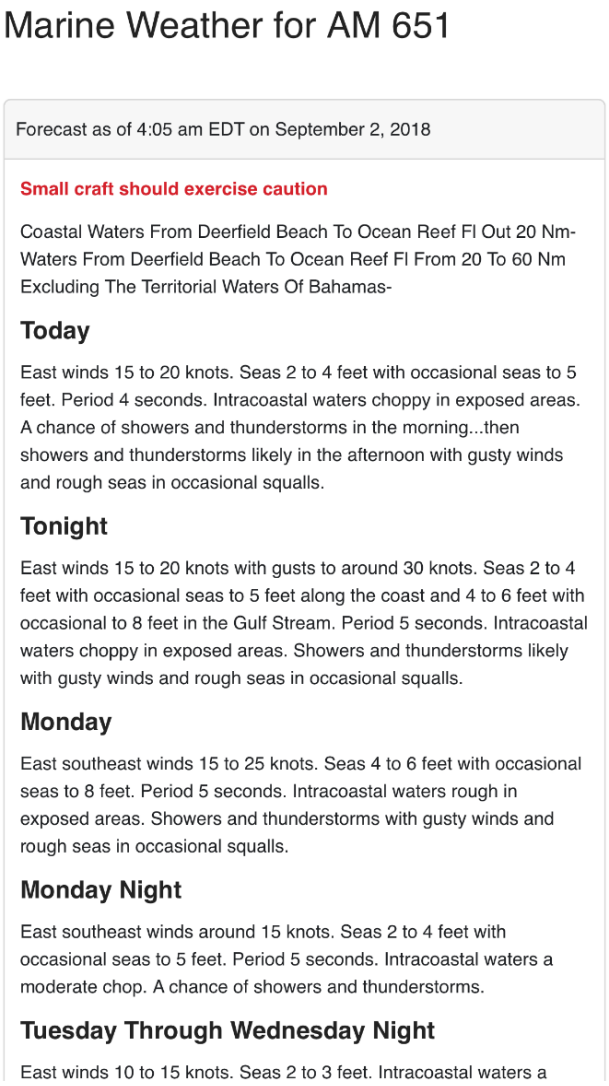


*Figure 5. Cell phone screenshot of the Weather Underground marine forecast for September 2, 3, and 4.*

**September 3, 2018**

The seas were above the limit of safe diving operations. Operations were cancelled earlier in the day and no attempt was made to dive.

**September 4, 2018**

The seas were above the limit of normal daytime operations on NSU vessels, therefore we hired the American Dream II dive operation to transport divers to the spawning site. At 2000, a team of eight divers met at the dock and loaded the vessel. Conditions were similar to the September 1 dive. Operations commenced as planned. Divers noted a very turbid layer of water on top of the site (the first 10 ft down) and low visibility below with high surge. No spawning was observed. Operations ended at 2345 and the team returned to the dock.

### 3.2. Task 2: Fertilization, larval rearing and settlement

In total, 13 of the 28 colonies of *Montastraea cavernosa* were observed spawning on nights 3-7 after the full moon (Table 4). The only nights where both male and female colonies spawned was 5, 6 and 7 days after the full moon. The eggs and sperm of all colonies that spawned were pooled for fertilization.

*Table 4. 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 |
|---|---|---|---|
| Aug 24 (2 DBFM) | 0 | 0 | - |
| Aug 25 (1 DBFM) | 0 | 0 | - |
| Aug 26 (Full Moon) | 0 | 0 | - |
| Aug 27 (1 DAFM) | 0 | 0 | - |
| Aug 28 (2 DAFM) | 0 | 0 | - |
| Aug 29 (3 DAFM) | 0 | 1 | - |
| Aug 30 (4 DAFM) | 0 | 1 | - |
| Aug 31 (5 DAFM) | 1 | 1 | 97% |
| Sep 1 (6 DAFM) | 2 | 2 | 78% |
| Sep 2 (7 DAFM) | 2 | 3 | 83% |
| Sep 3 (8 DAFM) | 0 | 0 | - |
| Sep 4 (9 DAFM) | 0 | 0 | - |
| Sep 5 (10 DAFM) | 0 | 0 | - |
| Sep 6 (11 DAFM) | 0 | 0 | - |

Fertilization rates were generally high (Table 4), which is indicative that the parental genotypes were genetically different and likely non-related.

After 3 days in culture bowls, we obtained 1,171 larvae of *Montastraea cavernosa* (Figure 6 andFigure 7). Of these, 171 settled and metamorphosed in 117 settlement tiles, thus settlement success was estimated at 14.6%. This success rate is within the rates obtained by other researchers and our lab in previous years (10 – 20%). The newly settled recruits had on average (± SE) 0.568 ± 0.017 mm diameter and 0.269 ± 0.015 $mm^2$ of surface area (see actual size measurements "Week 1 size" under the folder "Mcav grow out 2018" in the removal hard drive submitted with this report).

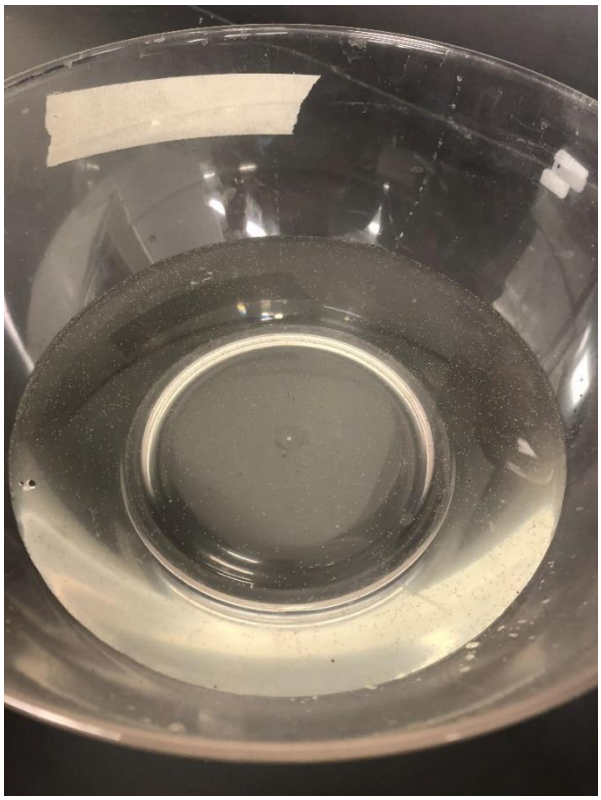

*Figure 6. Fertilization bowl where sperm and eggs of* Montastraea cavernosa *were pooled.*

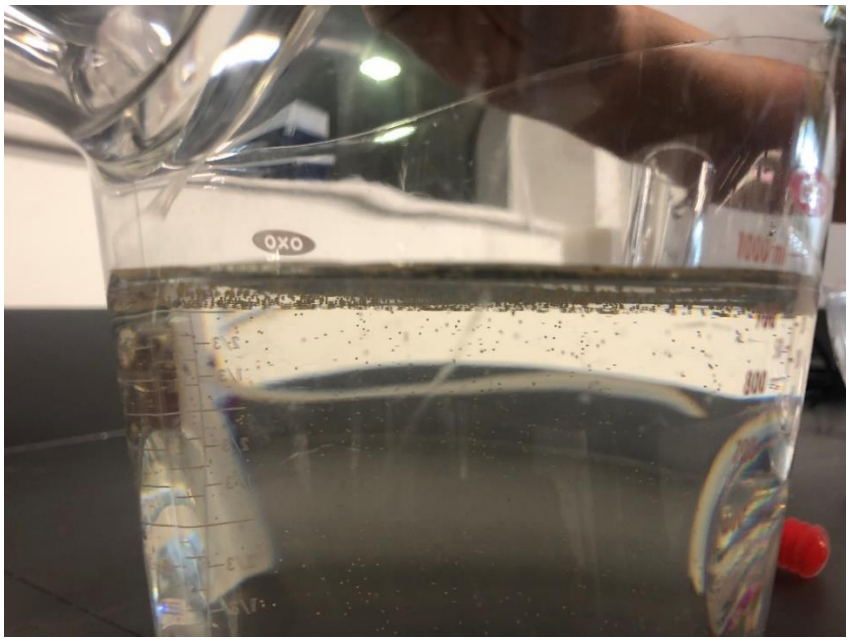


*Figure 7. Fat separator containing the coral gametes. This is used after fertilization occurs to remove the sperm from the solution containing the eggs to avoid polyspermy.*

The larvae of *Orbicella faveolata* donated by Dana Williams (NOAA) spawned at 2330 on August 2nd and were received at NSU on August 4th. According to Dana Williams, these larvae were obtained from gametes of 14 colonies from Horseshoe Reef in Key Largo (an estimated 25% of each colony released gametes on that day). An estimated 8,750 received larvae were fully developed and ready to settle thus they were distributed by 15 polystyrene containers with settlement tiles. A total of 849 coral recruits settled, which leads to a 10.3% settlement success rate (Figure 8). Newly settled corals measured on average (± SE) 0.1374 ± 0.0068 $mm^2$. Some coral settlers ended up not completing metamorphosis (~34%), thus ultimately we obtained 196 coral recruits (newly settled and metamorphosed corals) to conduct the light experiment (Task 3b) and 212 recruits to grow in mass scale (Task 3a).

*Figure 8. Newly settled* Orbicella faveolata *corals.*

### 3.3. Task 3a: Mass-scale Juvenile grow-out

*Orbicella faveolata*

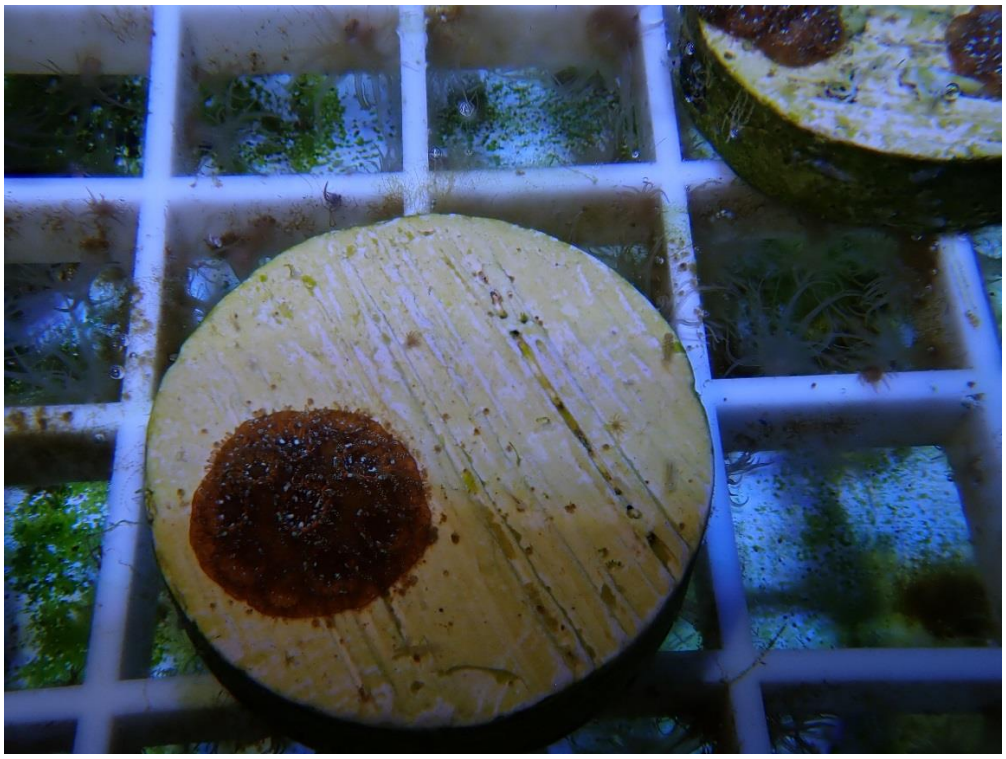

*Figure 9. Six months old* Orbicella faveolata *(tile has 2.5 cm diameter).*

Post-settlement mortality rates were initially high but decreased significantly over time as expected (Figure 10). Note that the light regime had not been optimized for this species yet, so we conservatively maintained the corals at lower light levels for a longer period time that it would be desired, which by weeks 12-17 proved to be deleterious. The optimal light levels are assessed on Task 3b. Still ca. 45% of the corals remained alive at week 8 and 20% by week 17, which is considerably better than the expected 5% in the field. After lights levels were increased, mortality stabilized at week 17. Unfortunately, in January we had an *Aiptasia* outbreak in the tanks that resulted in a significant mortality. The anemone *Aiptasia* was inadvertently introduced in the tanks by the rock around and below the adult coral colonies placed in the tanks to shed algal symbionts and facilitate symbiont acquisition by the young corals. We routinely eliminated all anemones growing near the recruits and added cleaner shrimp *Lysmata wurdemanni* to control the outbreak. *Aiptasia* anemone sting the corals, which eventually die (see mortality of 23-25 weeks old corals). To avoid further mortality, we moved the coral recruits to another culture raceway tank that had been recently set up which allowed us to eliminate the *Aiptasia* threat. However, this new tank still had high levels of phosphate contributing to fast-growing turf algae that, despite the use of a phosphate reactor and herbivorous snails, contributed to a low, but steady mortality rate on our cultures (Figure 10). The *O.*

*faveolata* recruits increased their surface area by nearly 473x, with the surviving corals having a median size of ca. 65 $mm^2$ by week 40 (Figure 11). This is a considerably fast growth, however if we examine the growth curve (Figure 11), we can see that several recruits did not grow during the *Aiptasia* outbreak (see weeks 22-24). Further, the recruits did grow as much after being moved to the new tank (after week 29, Figure 11), which was likely due to competition with the turf algae. See the photos in the hard drive attached to this report pictures of the recruits over time under the folder "Ofav growth out 2018".

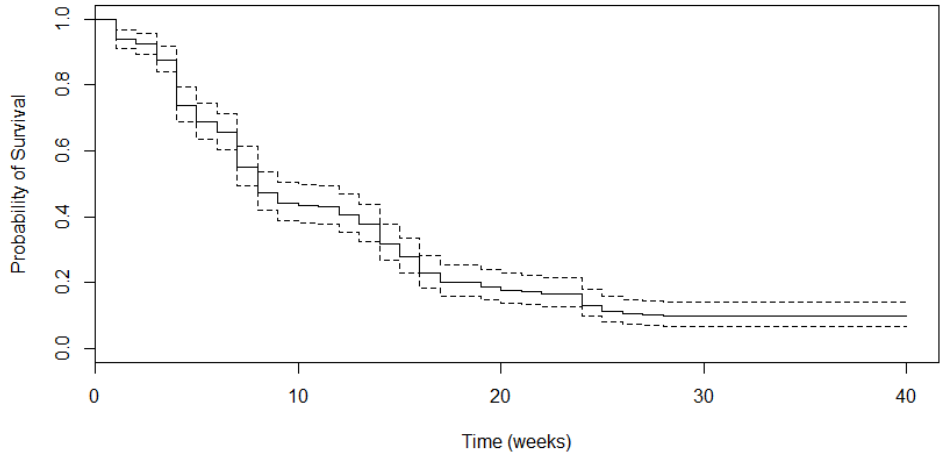


*Figure 10. Survival of* O. faveolata *recruits over time.*

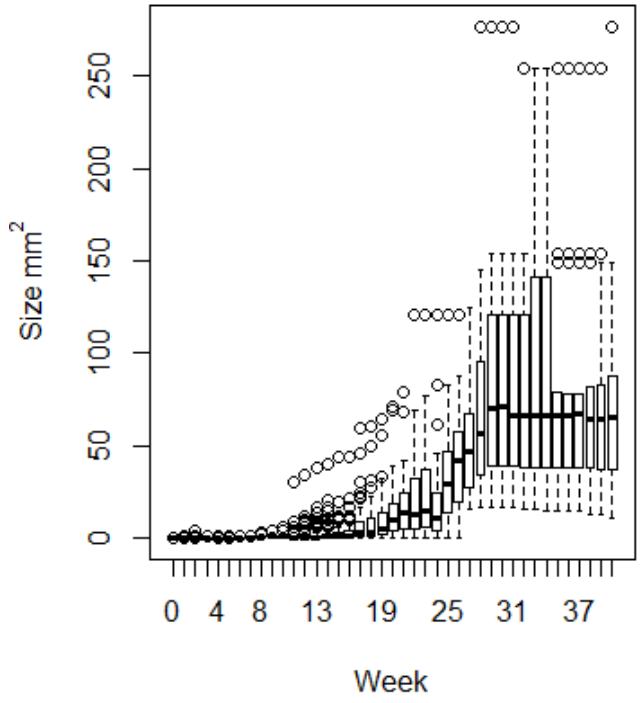


*Figure 11. Growth (surface area) of* O. faveolata *recruits over time.*

*Montastraea cavernosa*

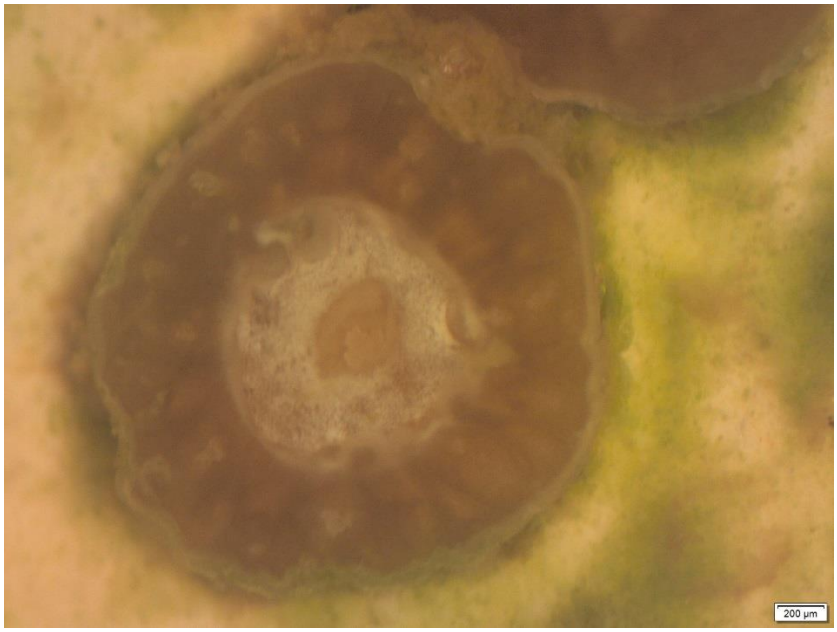


*Figure 12. Seventeen weeks old* Monstastraea cavernosa *recruit.*

Post-settlement mortality rates were initially high, but decreased significantly over time as expected, particularly after week 10, where we had about 30% of the initial colonies remaining (Figure 13). This a good result considering the typical mortality rates of newly settled recruits on the reef are estimated to be above 95%. Also, note that because this species spawned on month later than *Orbicella faveolata* we used results of Task 3b to maximize their survival. Unfortunately, the Aiptasia outbreak (see above) also affected this species, and the recruits experienced a high mortality event when they were 18-20 weeks old (Figure 13) and lost some tissue (Figure 14). After that, in the new tank (which had high levels of phosphate), there was still some mortality, albeit lower, due to the competition with the fast-growing turf algae (Figure 13).

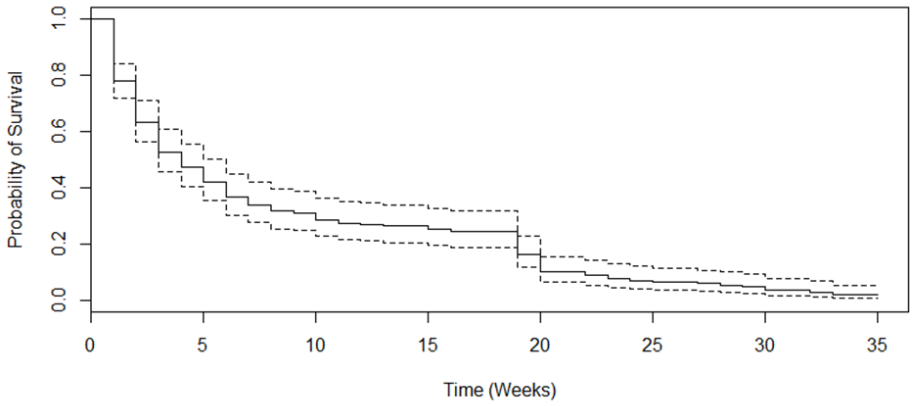


*Figure 13. Survival of the* Monstastraea cavernosa *recruits.*

The *Monstastraea cavernosa* recruits increased their surface area by nearly 15x, with the surviving corals having a median size of 4 $mm^2$ by week 35 (Figure 14). While this growth seems relatively high, this is considerably less than what had been attained in previous years. If we examine the growth curve (Figure 14), we can see that several recruits lost tissue during the *Aiptasia* outbreak and did not grow as fast once moved to the other tank, which may necessitate the allocation of energy to tissue repair, instead of towards growth, due to competition with the turf algae. See in the hard drive attached to this report pictures of the recruits over time under the folder "Mcav growth out 2018".

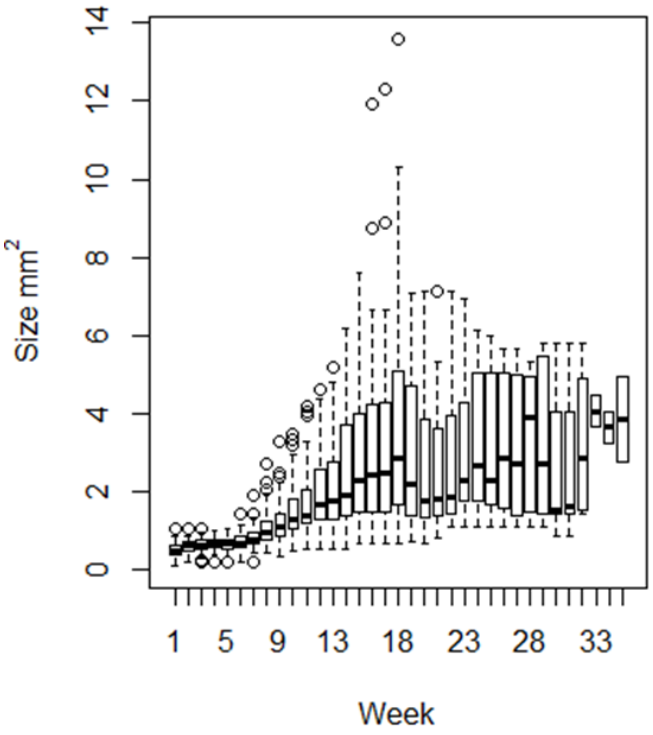


*Figure 14. Growth (surface area) of* Montastraea cavernosa *recruits over time.*

The Marine Larval Ecology and Recruitment Lab at NSU, led by Dr. Figueiredo has been rearing *Montastraea cavernosa* corals from gametes from Broward County colonies since 2016. We currently hold 31 *Monstastraea cavernosa* corals that were reared from gametes released by colonies from Broward County in the Fall 2016. These colonies were grown directly beside the corals recruits from this project, but did not experience any mortality due to the *Aiptasia* nor the turf algae, and continued to grow, albeit not as much in the new tank. This is because once they reach a certain size, they become less sensitive to environmental changes and can better compete against algae. These (almost 3 years old) corals are now on average 49.6±5.26 $cm^2$ (see Figure 15, and photos and measurements of the 31 corals in the hard drive attached to this report in the folder "Mcav growth out 2016").

*Figure 15. Two years and eight months old* Montastraea cavernosa *coral reared in the lab from gametes released by colonies from Broward County in the Fall 2016.*

### 3.4. Task 3b: Experiment to optimize light irradiance - *Orbicella faveolata*

Light treatment had significant effect on the survival, growth and coloration of *O. faveolata* recruits ($p= 0.00824$ and $p= 2.68x10^{-14}$, respectively). The light regime that led to the highest survival was Treatment 3 (Figure 16). Treatment 2 initially had higher mortality than Treatment 3, but after week 8 had similar survival rates to Treatment 3, promoted faster growth (Figure 17), and had higher coloration (Figure 18). Additionally, all treatments displayed greater mortality when exposed to light levels above 120 µmol photons $m^{-2}s^{-1}$, thus it is uncertain if higher light levels should be used to rear these recruits, at least within this initial four-month period.

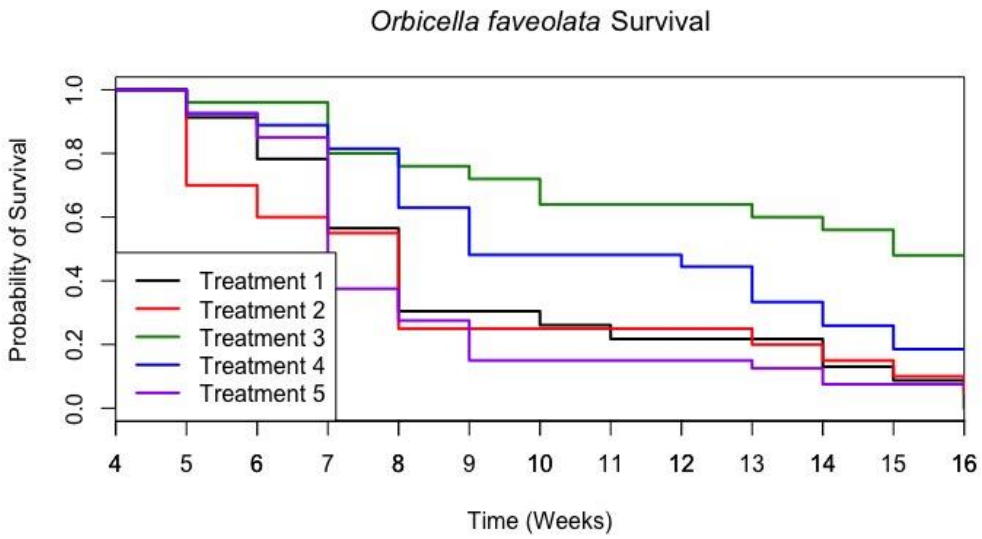


*Figure 16. Survival curve of* Orbicella faveolata *recruits in the five light treatments.*

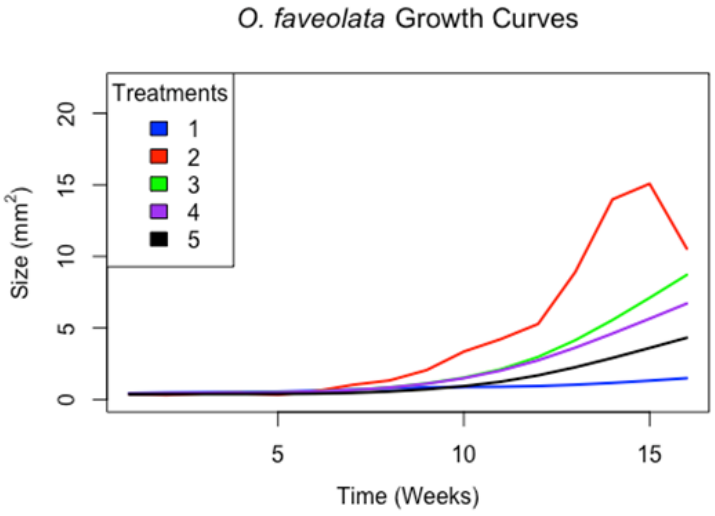


*Figure 17. Growth models (surface area over time) for* O. faveolata *under the five light treatments. (Note the drop in surface area on week 15 in Treatment 2 is due to mortality of a few very large recruits, lowering the average size).*

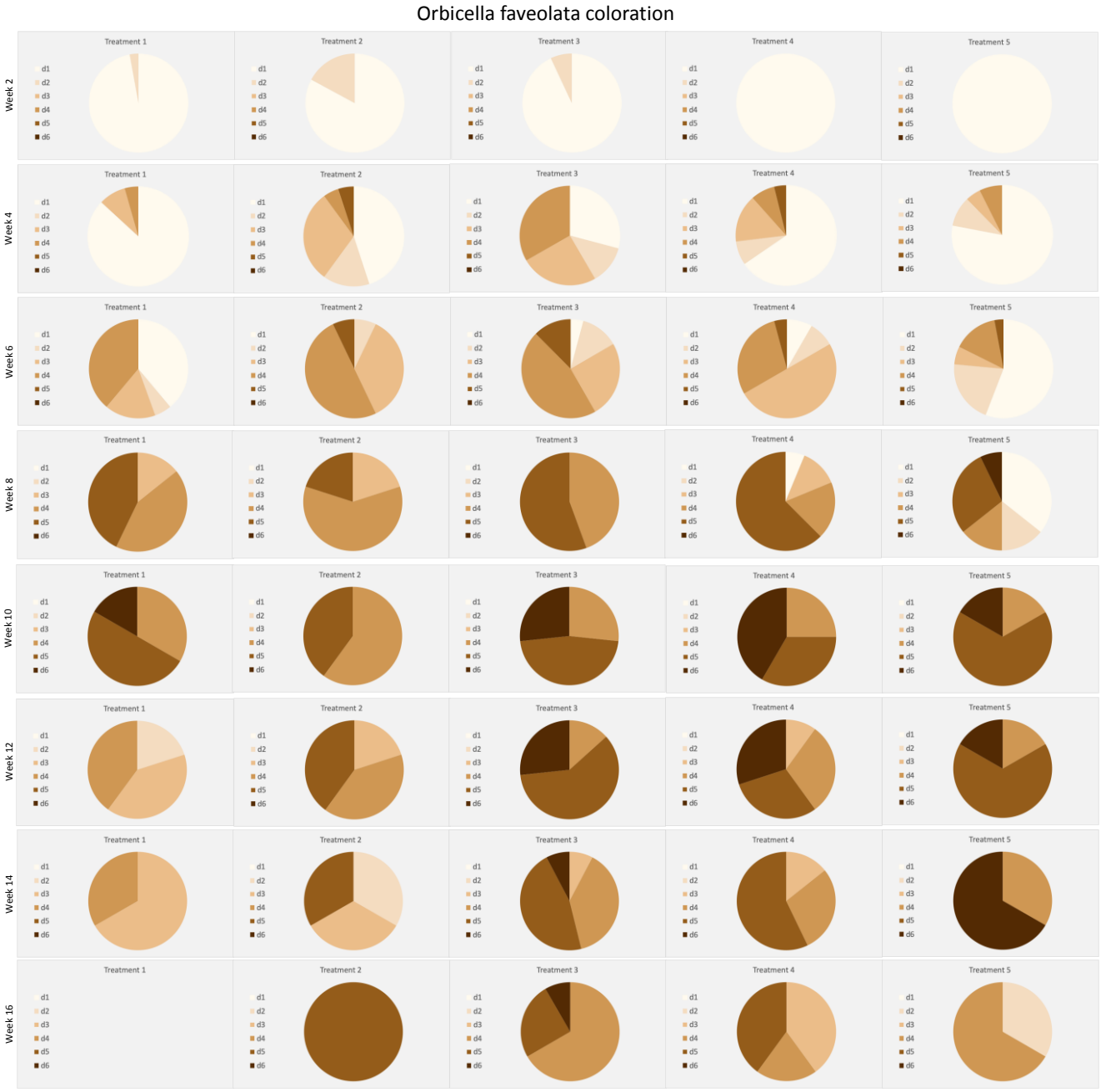


*Figure 18. Pigmentation of* O. faveolata *recruits over time under the five light treatments. Pie charts represent the proportion of individuals in each treatment with the coloration scored using the Coral Watch's Coral Color Reference Card.*

These results allowed us to determine that the light regime that promotes higher survival, faster growth and good coloration (an indication of good health) of *O. faveolata* recruits is: weeks 0-4: 10 µmol photons $m^{-2}s^{-1}$, weeks 5-7: 40 µmol photons $m^{-2}s^{-1}$, weeks 8-10: 80 µmol photons $m^{-2}s^{-1}$, and weeks 11 onwards: 120 µmol photons $m^{-2}s^{-1}$).

## 4. RECOMMENDATIONS

The lessons we have learned are:

1) Coral colonies collected from the reef a couple of days before spawning successfully spawn in the lab with synchrony similar to what happens on the reef (corals spawn on the same days and at the same time), which allow us to guarantee successful fertilization of gametes from a very diverse group of corals, thus enhancing genetic mixing and diversity.
2) For gonochoric species (species with separate sexes like *Montastraea cavernosa*), it is essential to collect a relatively high number of corals (ca. 30 colonies) to guarantee that we are working with a broodstock that has enough males and females (note: colony gender is not obvious unless polyps are dissected).
3) While colonies release gametes when they are still small (e.g. colonies of *Montastraea cavernosa* typically can release gametes at sizes 15 - 20 cm diameter), fecundity is exponentially higher in larger colonies (e.g. for *Montastraea cavernosa*, colonies with >30 cm diameter), and thus this should be prioritized for gamete collection.
4) Fertilization rates are optimized (>80%) when sperm concentration is within $10^5$-$10^6$ sperm cells.mL$^{-1}$, and gametes originate from different colonies.
5) Larval rearing is optimized when larvae are kept in lower densities, water quality is good, and temperature, pH, and salinity levels mimic historical conditions on the reef during the Summer (not necessarily current conditions offshore).
6) Larval settlement and symbiont acquisition are maximized if tiles are kept for 1.5-2 months on the reef prior to spawning. While larvae can settle when tiles are conditioned for a shorter period of time (ca. 2 weeks) and sprinkled with crustose coralline algae powder, symbiont acquisition can be much reduced.
7) Symbiont acquisition and selection is enhanced by placing adult corals of the same species (ideally mixed with other coral species) in the tank.
8) Newly settled corals typically experience high levels of mortality during the first weeks, but mortality rates tend to decrease considerably over time (or as their size increases). Newly settled recruits have a very small tolerance range to environmental conditions, thus is extremely important to maintain high water quality, some water flow but not very strong, and provide varied food daily.
9) Newly settled corals are very sensitive to high light irradiance levels and grow faster under lower irradiance levels; however once symbiosis is fully established, they require higher light levels to grow and survive. Our 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.

10) Feeding coral recruits is essential to promote growth and enhance survival rates. A varied feeding regime should be provided and include *Nannochloropsis* enriched rotifers, newly hatched *Artemia* nauplii, and Reef-Roids (PolypLab). Coral recruits should be fed daily, at least 1h/day, by hand-feeding (pipette food particles to the coral tentacles) to maximize uptake.
11) Water should contain no ammonia nor nitrites, nitrates should be 0.05-1ppm, and phosphate should be 0.02-0.03ppm. Very low levels of nitrates and phosphates prevent growth as these are required by the algal symbionts to conduct photosynthesis. Very high levels of nitrates and phosphates are harmful and often result in turf algae outbreaks.
12) Temperatures that mimic late Spring / early Summer promote the fastest growth. Temperatures typical of the Fall and Winter temperatures reduce or may even cease growth.
13) Sudden changes in salinity, particularly levels below 34 ppt and above 37 ppt are deadly, killing juvenile corals in less than a day.
14) For both *M. cavernosa* and *O. faveolata*, the period up to 10 weeks seems to be the most sensitive, and where the greatest mortality occurs. From that point forward mortality rates can be very low, provided water quality conditions are optimal and stable, and no harmful pests such as *Aiptasia* or turf algae outbreaks occur. However, this can be controlled.
15) Outbreaks of the anemone *Aiptasia* are highly deleterious to juvenile corals. *Aiptasia* sea anemones sprout out of the rock/base of adult corals placed in tanks to release algal symbionts to facilitate acquisition of symbionts by the coral juveniles. *Aiptasia* numbers explode rapidly in coral culture tanks because they feed on the same food as the corals, benefit from the light (they share the same algal symbionts as the corals), and reproduce asexually by fragmentation, i.e. each piece can grow another anemone. *Aiptasia* anemones sting juvenile corals, leaving them vulnerable and often leading to death. These *Aiptasia* oubreaks can be controlled and eventually eliminated by placing cleaning shrimps *Lysmata wurdemanni* in the tanks. These shrimps eat the *Aiptasia* and are not harmful to the corals.
16) Control of algal growth is essential to minimize mortality and promote growth. Turf algae commonly outbreak on recently established culture tanks. They benefit from light and higher levels of phosphate and nitrates. They not only shade coral recruits and they seem to produce a substance that is toxic/deleterious the young corals. They can be controlled using herbivorous snails, and by reducing phosphate levels (which are typically high on well water). Using phosphate reactors is often insufficient to lower phosphate levels to the levels required to control algal growth. Phosphate levels were only sufficiently reduced by using ultra-activated phosphate-adsorption media (such as Brightwell Xport-PO4 - 1/2" cubes).

## 5. SUMMARY

In summary, no *in situ* spawning was observed in SE FL of *Orbicella faveolata* over the three successful dive nights and the spawning event in SE FL was not captured due to conditions brought on by TS Gordon. However, we obtained a similar amount of *O. faveolata* gametes that were collected in the Upper Keys by Dana Williams (NOAA). In addition, we obtained a similar amount of *Montastraea cavernosa* gametes in SE FL from previously collected colonies, one of the species most impacted by disease. The Florida Reef Tract (FRT) *O. faveolata* gametes were used to fulfil the objective of assisting the reproduction of Endangered-Species-Act (ESA)-threatened-corals of the species which have been most affected by disease.

We have established a successful methodology to assist the sexual reproduction of *Montastraea cavernosa* and *Orbivella faveolata*, and to produce recruits which can be grown into colonies to be used for restoration. The fertilization rates of *Montastraea cavernosa* were very high (78-97%) and larvae developed well. Settlement rates were estimated at 14.6% for *M. cavernosa* and 10.3% for *O. faveolata*. After settlement and metamorphosis, both species readily acquired symbionts in our tanks. Post-settlement mortality rates were initially high, but abated considerably 10 weeks after settlement, with 30% of the initial corals remaining. The light experiments with *O. faveolata* allowed us to determine an optimal light regime (Weeks 0-4: 10 µmol photons $m^{-2}s^{-1}$, weeks 5-7: 40 µmol photons $m^{-2}s^{-1}$, weeks 8-10: 80 µmol photons $m^{-2}s^{-1}$, and weeks 11 onwards: 120 µmol photons $m^{-2}s^{-1}$). This is expected to further increase survival above 50% after 16 weeks, and promote considerably faster growth. After 10 weeks the levels of mortality remained extremely low until in the middle of January, when the culture tanks experienced a major outbreak of anemone *Aiptasia* (that was inadvertently introduced in the tanks by the rock around and below the adult coral colonies placed in the tanks to shed algal symbionts and facilitate symbiont acquisition by the young corals). The *Aiptasia* anemones sting corals and led to a high mortality event of 22-24 weeks old *Orbicella faveolata* and 18-20 weeks old *Montastraea cavernosa* recruits. We routinely eliminated all anemones growing near the recruits and added cleaner shrimp to control the outbreak, but ultimately had to move the coral recruits to a new culture tank that had been recently set up which allowed us to completely eliminate the *Aiptasia*. However, this new tank still had high levels of phosphate contributing to fast-growing turf algae that, despite the use of a phosphate reactor and herbivorous snails, contributed to a low, but steady mortality rate on our cultures. The optimal conditions to assist the sexual reproduction and grow these species in land-based nurseries, and recommendations to control harmful pests are listed on section 4. Once corals reach a certain size, they become more tolerant to a wider range of environmental conditions and are able to withstand stressors, including pests. For example, the 31 *Montastraea cavernosa* corals reared from gametes released by colonies from Broward County in the Fall 2016 (now with an average 49.6±5.26 $cm^2$) were grown right next to the corals recruits from this project, but did not experience any mortality due to the *Aiptasia* nor the turf algae, and continued to growth. This work provides valuable information for future sexual reproduction and grow-out restoration activities, which should facilitate population recovery after the disease event.

We are currently holding 33 *Montastraea cavernosa* (multiple of which result from the fusion of 2 or 3 sexually-produced recruits) from SE FL, and 1 colony of *Orbicella faveolata* (that resulted from the fusion of 3 sexually-produced recruits) from the Upper Keys. These colonies will be maintained in captivity until reaching a size suitable for microfragmentation, and potential outplanting trials. To prepare the tanks to grow newly settled recruits starting in August 2019, we are currently trialing the switch to using man-made seawater in our systems, rather than well water, as the high levels of ammonia and phosphate in well-water are not as suitable for corals and favor the growth of harmful macroalgae.

Using assisted sexual reproduction and grow-out in land-based nurseries of corals to assist reef recovery will require a trained and well-staffed land-based nursery. Based on our growth observations, colonies of *M. cavernosa* and *O. faveolata* may be suitable for outplanting within 2-3 years after spawning, however ideal age/size for outplanting needs to be assessed by trialing the outplanting of different aged/sized individuals and monitoring their relative survival to determine the optimal season and size/age for outplanting. Each culture system (~6 $m^2$) can hold ca. 3000 newly-settled coral recruits, which can be scaled up, depending on the number of species and number of recruits/species aimed to rear. Culture tanks should be well-established (filtration system can take 2-3 months to mature) before the introduction of coral recruits (see section 4 for more detailed recommendations). When the collection of wild adult colonies and transfer to captivity for spawning is not possible due to colony size and species endangered-status, gametes should be collected in the wild during the annual spawning event, weather-permitting. Whenever possible, we recommend the collection of adult colonies (which have matured in the wild) right before they are expected to spawn in nature (around the full moon of August or September) for collection of gametes in captivity; these adult colonies may be returned to the reef after spawning or maintained in captivity to induce gonad maturation. Mature adult coral colonies may be kept in captivity year-round to induce spawning through manipulation of light and temperature and provision of a good diet (Craggs et al. 2017); eggs typically take 9-10 months to develop and mature. This latter methodology is more expensive and laborious than collection of gametes from colonies matured on the reef as it requires an indoor laboratory and more advanced technology to mimic moon and sun light annual cycles, but it will likely be essential for the recovery of species with a very low density on the reef, particularly after the mass mortality caused by disease in SE FL.