Quicklook Report

Coral Spawning 2023: Activities and Observations

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Summary

Spawning observations and gamete collections in 2023 were made for the coral species *Diploria labyrinthiformis* (DLAB) in April and May, *Acropora palmata* (APAL), *A. cervicornis* (ACER), and *Pseudodiploria strigosa* (PSTR) in August, and *Orbicella faveolata* (OFAV) and *Montastraea cavernosa* (MCAV) in August and September. During the summer months of July and August, seawater temperatures in the Florida Keys reached record highs resulting in widespread coral bleaching and mortality by the end of the summer season. During the spawning months of August and September, bleaching varied widely among the monitored colonies and coral spawning was observed in normal-colored (i.e., no visual sign of bleaching), pale, and bleached colonies. In general, spawning activity was lower than normal and included several uncharacteristic observations (e.g., smaller and less buoyant gamete bundles), which may be in part due to the timing or interannual variability, but may also be due to thermal stress. However, from colonies that did spawn, we observed low fertilization rates across the majority of gamete-mixed batch batches, suggesting that the viability of the gametes released by these colonies may have been compromised by the thermal stress event. Larvae produced from these collections were settled and reared for all species except PSTR in which spawning was not observed. Additional larvae were supplied from research and restoration partners including the University of Miami, The Florida Aquarium, Biscayne National Park, and the University of North Carolina at Wilmington. All larvae were reared and settled at the Coral Research and Assessment Lab (CoRAL) wet lab facility in Miami, Florida. A portion of the larvae were used in experiments examining the effect of sedimentation on coral recruitment and post-settlement survivorship. The remaining larvae were settled onto artificial substrates where they are being raised for future research and restoration projects. Spawning observations and gamete collections were conducted in collaboration with the Reef Futures Lab at the University of Miami Rosenstiel School of Marine, Atmospheric & Earth Science, SECORE International, and the Coral Health and Disease Program at the Hollings Marine Lab (NOAA NCCOS; Dr. Cheryl Woodley) where gametes were used for various projects led by each group.

Introduction

The Coral Research and Assessment Lab (CoRAL) at NOAA's Southeast Fisheries Science Center has monitored spawning patterns and collected gametes from APAL and OFAV in the upper Florida Keys since 2000. This year marks the third year utilizing our new and recently expanded CoRAL wet lab facility located at the University of Miami's Experimental Hatchery. This expansion allows us to bring gametes collected from Key Largo, Florida back to our Miami-based wet lab to complete the gamete fertilization process, rear larvae, settle recruits, and continue the recruit grow-out for future research and restoration projects. The CoRAL wet lab includes an experimental tank system consisting of 30 independent, temperature-controlled aquaria fed by mechanically filtered (5 micron) and UV-sterilized flow-through seawater. The experimental tank system provides an opportunity to conduct experiments capable of highly replicated treatments at any of the larval rearing, settlement, and grow-out phases. Using this experimental system in the fall of 2023, we tested the effect of sedimentation on the survivorship of recruits of numerous species at the ages of 1-month, 3-months, and 6-months. Additionally, we tested the effect of sedimentation on settlement rates of six species (ACER, APAL, OFAV, CNAT, PSTR, PCLI) using replicated settlement chambers in temperature-controlled seawater baths in our lab space at NOAA's Southeast Fisheries Science Center.

During the summer of 2023, record high seawater temperatures were observed on coral reefs throughout south Florida and the Florida Keys resulting in a widespread coral bleaching event. The heat wave started six weeks prior to the first fall-spawning window which occurred in August. During the spawning months of August and September, a range of bleaching conditions were observed including non-bleached colonies, pale colonies, partially bleached colonies, and fully bleached colonies. Bleaching severity varied between sites, coral species, and spawning windows. Evidence suggests that some coral's reproductive capacities can be impacted following thermally-induced bleaching (Johnston et al. 2020), but it was unclear if the 2023 fall spawning event would be affected by the 2023 summer heat stress. In fact, all species monitored in the Florida Keys during August and September showed spawning activity, except for PSTR in which only one colony was monitored and did not spawn. Spawning activity was observed across the range of colony conditions, from colonies that were non-bleached to colonies that were bleached. Although colonies spawned, divers from our team observed uncharacteristically smaller sized gamete bundles that were less buoyant when released than typically observed in previous years. These observations suggest that gamete development may have been compromised by the 2023 marine heat wave, though additional research is required to establish a causative link between thermal stress and decreased reproductive success. However, low fertilization rates of the majority of two-parent crosses and batches made from these gametes lends additional support for the hypothesis that the reproductive potential of corals was compromised by the 2023 marine heat wave. As a consequence of low fertilization rates, we also observed uncharacteristically low larval settlement rates from batches produced by gametes collected from the field.

Methods

In 2023, the CoRAL team monitored six coral species for spawning activity: DLAB, APAL, ACER, OFAV, PSTR, and MCAV. All species monitored, except for MCAV, are hermaphroditic broadcast spawning corals that release bundles containing both eggs and sperm. Gamete collection for all hermaphroditic broadcast spawning corals was accomplished by placing a spawning net on the colony and accumulating gamete bundles in a collection tube attached at the top of the net. Gamete collection for MCAV, a gonochoric species in which individual colonies release either eggs or sperm, was

accomplished by siphoning either eggs or sperm released from the colony into a large syringe. For all species, once gametes were collected, the gamete fertilization and larval rearing process is similar. Prior to spawning observations and collections, a setup dive was conducted to facilitate locating and identifying colonies during spawning dives.

Species specific site preparation

Diploria labyrinthiformis (DLAB)

Prior to monitoring DLAB for spawning, tags were attached to the substrate next to monitored colonies for re-identification and future genotyping, and stakes were hammered into the reef to attach a transect tape during spawning dives to facilitate coral relocation. At the beginning of each spawning dive, we placed collection nets on a subset (approximated 35) of tagged corals located along the transect lines. Bundle setting in DLAB occurs so rapidly that we have yet to witness it in the field, despite three years of spawning observations and collections. Consequently, collection nets are placed on DLAB colonies ahead of the predicted spawning window to avoid missing the release of gametes and subsequent collections.

Montastraea cavernosa (MCAV)

Prior to monitoring MCAV for spawning, colonies were located and marked with a buoyant plastic chain marker with a unique number placed next to each colony to facilitate locating colonies during the spawning dive. Due to the high abundance of MCAV colonies at North North Dry Rocks Reef, only a subset of colonies at this site were marked with floating chain markers. Since this species is gonochoric, and thus individual colonies only release either sperm or eggs (however, we observed one colony to release both eggs and sperm in 2022 and 2023) that quickly dissipate in the water column, the gamete collection method for MCAV differs from hermaphroditic broadcast spawning species that release buoyant bundles easily collected in a net. Briefly, for MCAV gamete collections divers swim from colony to colony looking for sperm or eggs being released from the polyps. Once spawning activity is observed, the diver places a large (500ml) catheter tipped syringe near the polyp mouths and pulls the plunger to siphon the gametes into the syringe. Once the syringe is full or the colony is no longer releasing gametes, the syringe is capped with a cinched piece of airline tubing to prevent the gametes from escaping. The syringes with gametes are then brought to the boat where the gametes are mixed and the fertilization process begins.

Acropora palmata (APAL) and *A. cervicornis* (ACER)

Prior to monitoring APAL and ACER for spawning, a set-up dive was conducted at each reef where divers identified the location of individual genotypes for all wild APAL colonies, and marked a subset of outplanted colonies using small floats that raised a genotype-tag approximately two feet off the benthos to facilitate colony identification at night (Figure 1). During spawning dives, if a colony was observed with gamete bundles setting in the polyps, a collection net was placed over part or all of the colony. APAL and ACER typically display bundle setting in advance of bundle release (~15 min), allowing time to retrieve nets and place them on a colony once setting in the polyp mouths is observed.

Orbicella faveolata (OFAV)

The CoRAL team has monitored the population of OFAV at Horseshoe Reef since 2018 for spawning activity and have tagged specific parent colonies used for annual spawning observations. Prior to the spawning dive, tags for each colony were found and uncovered so the tag ID was visible. Buoyant plastic chain markers were then strategically placed near a subset of colonies to make the area easier to navigate and find tagged colonies during the spawning dive. Similar to APAL and ACER, when a colony was observed with gamete bundles setting in the polyp mouths, divers expeditiously placed a collection net on part of the colony or the entire colony.

Pseudodiploria strigosa (PSTR)

A single colony was observed for spawning during the MCAV/OFAV spawning dives. No net was deployed on this colony as no spawning was observed.

Gamete collection, fertilization and larval rearing

For all hermaphroditic broadcast spawning species monitored (APAL, ACER, DLAB, OFAV, and PSTR), gamete bundles are collected via a net placed over the colony. The top of the net consists of a plastic transmission funnel, the mouth of which leads to a 50ml centrifuge tube screwed onto a permanently attached cap at the top of the net (Figure 2). The cap has a hole drilled in the center that is placed over the narrow end of the funnel allowing gamete bundles to pass through but retains the threaded sides for attaching and swapping centrifuge tubes. Because gamete bundles are buoyant, when they are released during spawning the bundles float towards the top of the net where they are concentrated and collected in the tube. Once the collection tube at the top of the net is partially filled with gamete bundles (~5-10ml of gamete bundles), the tube is removed, capped, and labeled to identify the genotype or colony ID from which the gamete bundles originated. Collection tubes with gametes are then brought to the boat where gametes from different parents are mixed to create either two-parent or multi-parent crosses. Gametes of all species collected are mixed using this process to allow for fertilization to occur. The resulting batches are then transported to the CoRAL wet lab in Miami, where they are immediately decanted and diluted with seawater to reduce the sperm concentration (hereafter, referred to as decanted) and left overnight in table top bins to continue the fertilization process. Because OFAV spawns on the same night as MCAV but approximately 2-3 hours later, MCAV gametes are mixed immediately but remain concentrated in closed containers stored in coolers on the boat for several hours longer than the other species before they are decanted and diluted back at the CoRAL wet lab. The morning after gamete collection, fertilization rates are estimated and the batches are distributed to larval rearing kreisels where the larvae remain for the duration of the settlement phase. During the settlement phase, pre-conditioned ceramic tiles and/or plugs are placed in the kreisels to provide settlement substrates. Once larvae have recruited to the substrates and are fully attached without risk of dislodgement, the substrates are removed from the kreisel and placed in a rearing tank for use in further experiments or for grow-out.

Spawning observations

Previous spawning observations made by our lab and other research organizations informed predicted peak-spawning windows in the Florida Keys region. For each species, predicted peak-spawning windows and actual days and times monitored in 2023 are listed in Table 1. Dates for each

site and species monitored and the number of larvae produced from each spawning event are listed in Table 2.

Florida Keys observations

Diploria labyrinthiformis (DLAB)

DLAB spawning observations and collections are conducted at an unnamed patch reef in the upper Florida Keys within the boundary of John Pennekamp State Park. DLAB spawning observations began in 2020, although no spawning was observed that year. 2023 marks the third year our team has observed wild colonies of DLAB spawn in the upper Florida Keys. Prior to our observations of colonies spawning in 2021, there were no reports of spawning activity in wild Florida DLAB populations, and, thus, a predicted spawning window (Table 1) is based on the two preceding years of observations at this single patch reef.

April 2023 monitoring of DLAB for spawning activity was conducted on April 16 and 17 (10 and 11 days after the full moon [AFM], respectively). Spawning was observed on April 16, and no colonies were observed spawning on April 17. On April 16, five colonies released a small number of bundles that were collected. The first observation of gamete bundle release occurred at approximately 18:15. Once spawning colonies were no longer observed releasing bundles, all collection tubes with bundles were brought back to the boat where they were mixed to create five batches, four separate 2-parent crosses and one 5-parent mix, at approximately 19:00. These batches were then transported to the CoRAL wet lab in Miami where the batches were diluted and the fertilization process continued overnight. The next morning (06:00), estimated fertilization rates ranged from 94 to 100% among the five batches, at which point the embryos were placed into larval rearing kreisels for the duration of the larval phase, yielding an estimated 37,500 DLAB larvae. Fourteen hours after larvae were placed in the kreisels, preconditioned settlement substrates were introduced into the kreisels to promote larval settlement. Once recruits were attached without risk of dislodgement, substrates were removed from the kreisels and transferred to tanks for grow-out at the CoRAL wet lab.

May 2023 monitoring of DLAB spawning was conducted on May 15-17 (10, 11 and 12 days AFM). A small amount of spawning was observed on May 15 and no colonies were observed to spawn on May 16-17. On May 15, two colonies released a very small amount of spawn at approximately 18:25. Once spawning colonies were no longer observed releasing bundles, collection tubes were brought to the boat and mixed into a single batch. The resulting batch was transported to the CoRAL wet lab in Miami where the batches were diluted and the fertilization process continued overnight. The next morning (0600), the fertilization rate was estimated at 100%, and the embryos were placed in a single kreisel for the larval phase producing approximately 2,000 larvae. These larvae were then settled onto substrates, once firmly attached the substrates were removed and placed in tanks for the grow-out phase.

Acropora palmata (APAL) and *A. cervicornis* (ACER)

August 2023 spawning observations were made at three different sites in the upper Florida Keys for both wild and outplanted APAL genotypes, while ACER outplants were only observed at one of the sites. Both APAL and ACER were monitored at North Dry Rocks on August 2-3 (nights 1 and 2 AFM),

only APAL was monitored at Sand Island Reef on August 4 (night 3 AFM), and only APAL was monitored at Elbow Reef on August 5 (night 4 AFM).

North Dry Rocks has a sparse population of naturally occurring (wild) APAL consisting of four genotypes. Beginning in 2012, the Coral Restoration Foundation (CRF) outplanted hundreds of ACER colonies at this site. In 2015, in partnership with CRF, we outplanted four genotypes of APAL along experimental transects at North Dry Rocks Reef. In 2018, approximately 1,000 APAL and 1,000 ACER colonies were outplanted through a cooperative agreement between the NOAA Restoration Center and CRF. In 2019, we began monitoring the wild and 2015 APAL outplants along with the 2012 ACER outplants. In 2020, we first observed spawning among the 2015 APAL and 2012 ACER outplants, and in 2021, we first observed spawning among the wild APAL. In 2022, we began monitoring the 2018 outplants and observed spawning. During the 2023 spawning window, APAL colony condition at this site included normal coloration, partial bleaching, fully bleached, and some colonies exhibited tissue loss. North Dry Rocks is a shallow reef that is inshore of the main reef tract and experienced high-water temperatures beginning in early July (Figure 3).

In 2016, again in partnership with CRF, we outplanted 288 fragments of APAL consisting of eight different genotypes within ~3x2m plots (n = 24 fragments per plot) on the northern side of Sand Island Reef where no live naturally occurring (wild) APAL was remaining. Each plot represented a replicated treatment testing the effect of clumped vs mixed genotypes and the effect of high (n = 8 genotypes) vs low (n = 2 genotypes) genotypic diversity within a plot. These outplants at Sand Island Reef had not been monitored for spawning prior to the 2023 fall spawning season. During the 2023 spawning window, APAL colonies at this site ranged from normal in color to partly bleached and some colonies were exhibiting tissue loss. Sand Island is on the main reef tract and experienced moderately elevated water temperatures beginning in early July (Figure 3).

Elbow Reef has a sparse to moderate abundance of wild APAL with documented spawning observations since 2005. In 2016, 288 fragments of APAL were also outplanted at Elbow Reef as described above for Sand Island Reef. These outplants were first monitored for spawning in 2021, where we observed outplanted colonies of numerous genotypes spawning synchronously with wild colonies at Elbow Reef (Williams et al. 2023). During the 2023 spawning window, APAL colonies at this site were slightly pale to normal in color because water temperatures were cooler at this site relative to other sites monitored for spawning (Figure 3).

On August 2-3 (nights 1 and 2 AFM) at North Dry Rocks, our team, along with divers from SECORE and the University of Miami, observed spawning for both wild and outplanted APAL (2015 and 2018 outplants) and outplanted ACER (2012 and 2018). All outplant cohorts were observed to spawn. During both nights, nine APAL genotypes and at least three ACER genotypes spawned, and colonies were observed to start releasing bundles at approximately 22:15. Collection tubes containing bundles were brought to the boat for mixing. On night 1 AFM, seven APAL batches (six 2-parent and one 5-parent) and one 4-parent ACER batch were mixed at approximately 23:45. On night 2 AFM, five APAL batches (four 2-parent and one 4-parent) and two ACER batches (one 3-parent and one 4-parent) were mixed at approximately 23:30. After batches were mixed, gametes were transported to the CoRAL wet lab where they were diluted and the fertilization process continued. The following morning (0800), night 1 AFM fertilization rates among batches were uncharacteristically low, ranging from 60% to 96% for APAL and 15% to 73% for ACER. Fertilization rates for night 2 AFM batches were low as well, ranging from 60% to 83% for APAL and 32% to 86% for ACER. Based on these fertilization rates, an estimated

total of 57,500 APAL larvae and 14,000 ACER larvae were produced from night 1 AFM, and 21,500 APAL larvae and 4,300 ACER larvae were produced from night 2 AFM. Typically, fertilization rates less than 80% would be discarded as there is high likelihood for the batch to crash, particularly if batches are in confined tanks with little or no water turnover. However, our flow through kreisel systems along with regular cleaning of the surface of the water with cling wrap allowed us to save many of the larvae without crashing the entire batch.

On August 4 (night 3 AFM) at Sand Island Reef, all seven of the remaining live genotypes of the 2016 APAL outplants were monitored for spawning. Because this is not a site we anticipated monitoring for spawning, colonies were not pre-labeled and thus their identity was unknown. However, based on the experimental design under which the colonies were outplanted, we are confident that at least three genotypes contributed to the spawning event. Colonies were observed to start releasing bundles at approximately 2130. Once tubes were partially filled with bundles, they were removed from collector nets and brought to the boat for mixing into a single batch. Gametes were transported to the CoRAL wet lab where batches were rinsed and fertilization continued overnight. The following morning (0800), fertilization rates were uncharacteristically low, ranging from 38% to 69%. The two batches from Sand Island Reef with the highest fertilization rates (64% and 69%) were added to larval kreisels producing an estimated 10,000 larvae.

On August 5 (night 4 AFM) at Elbow Reef, light spawning was observed among wild colonies only. Although water temperatures were lower (Figure 3) and colonies were normal to mildly bleached in color, we still observed bundles that were smaller in size and less buoyant than seen in previous years. Gamete bundles were collected from two wild genotypes, and brought to the boat and mixed into a single batch given the low volume of gametes collected. The gametes were transported to the CoRAL wet lab where they were diluted, split into three containers and the fertilization process continued overnight. The following morning (0800), fertilization rates were low, averaging 59% among the three containers. The batch with the highest fertilization rate (64%) was added to a larval kreisel producing an estimated 3,200 larvae.

Montastraea cavernosa (MCAV)

On August 7-8 (nights 6 and 7 AFM), we monitored approximately 10 colonies of MCAV around the north mooring of Horseshoe Reef for spawning activity. During these observations, most colonies were pale but not fully bleached. We began monitoring colonies at 20:45, and first observed spawning activity at approximately 21:15. Eggs were collected from one colony and sperm was collected from three colonies, and both eggs and sperm were released and collected from an additional colony. This phenomenon of a single MCAV colony releasing both eggs and sperm was also observed at this site in 2022; however, we are uncertain that the colony that released both eggs and sperm in 2023 was the same colony that released both gamete types in 2022. At approximately 21:40 when colonies were no longer releasing gametes, the syringes filled with gametes were brought to the boat for mixing. A single batch was mixed at 21:35 and transported back to the CoRAL wet lab where the batch was decanted and allowed to fertilize overnight. The following morning, the fertilization rate was 100%, and the embryos were added to larval kreisels producing approximately 3,000 larvae.

On September 5 (night 6 AFM), we monitored at least 30 colonies of MCAV at the south mooring of North North Dry Rocks Reef. On this night, some colonies were normal in color while the majority of colonies were pale. Colonies were monitored from 20:45 - 21:45, but none were observed to spawn.

Orbicella faveolata (OFAV)

On August 7-8 (nights 6 and 7 AFM), our team, along with divers from SECORE and the University of Miami, monitored at least 12 colonies of OFAV around the north mooring of Horseshoe Reef for spawning activity. During these nights, colonies ranged in bleaching severity from pale to partially bleached. We began monitoring colonies at 22:45, and at approximately 23:15 colonies were observed with gamete bundles setting in their polyps. At least five colonies were observed to spawn; however, our team obtained gamete bundles from two colonies. Collection tubes for these two colonies were brought to the boat making a two-parent batch at 00:10. The batch was brought back to the CoRAL wet lab and rinsed with seawater and allowed to continue fertilizing overnight. The following morning (0800), fertilization rates were estimated at 0% suggesting that the two colonies we collected gametes from were clones (i.e. the same genotype), and the batch was discarded.

On September 5 (night 6 AFM), our team monitored 17 colonies of OFAV at the south mooring at North North Dry Rocks Reef. On this night, colonies ranged in bleaching severity from non-bleached to partially bleached with gamete bundles observed setting in areas with fully bleached tissue and non-bleached tissue. We began monitoring colonies at 22:45, and at approximately 23:20 two colonies were observed with gamete bundles setting in their polyps. Bundles were collected from the two colonies, and collection tubes were brought to the boat and mixed in a single 2-parent batch at 00:05. The batch was transported to the CoRAL wet lab, decanted, and allowed to continue fertilizing overnight. The following morning (0800), fertilization rates were estimated at 99%, and the embryos were added to larval rearing kreisels producing approximately 53,460 larvae.

Miami 'urban coral' spawning observations

In 2023, we partnered with the Coral Program at NOAA's Atlantic and Oceanographic & Meteorological Laboratory to conduct spawning observations of populations of PSTR, OFAV, and CNAT located near the Port of Miami, FL. Colonies of these populations have been a focus of demographic monitoring by AOML's Coral Program for several years as they persist in sub-optimal conditions (e.g. high temperatures, large thermal variability). However, this was the first year that these colonies were monitored for spawning activity to document if they are reproductively active. During the spawning monitoring window, colonies at this site ranged from pale to fully bleached, as well as visibly recovered from bleaching during the second spawning window (Figure 4).

On August 7-9 (nights 6-8 AFM) and September 5-7 (nights 6-8 AFM), a CoRAL team member joined the AOML Coral Program to monitor colonies of PSTR, OFAV, and CNAT at MacArthur Causeway North (25.77293, -80.15263). Each night, we began monitoring 12 colonies of CNAT at 19:00 and continued until 21:00. The same night, we monitored 10 colonies of PSTR and 2 colonies of OFAV at 22:10 and continued until 23:30. No colonies were observed to spawn during any of these observation windows. In addition to spawning observations, small tissue samples were collected pre-spawn on 08/07/2023 and post spawn on 09/11/2023 for histology analyses that are currently being processed by collaborators at Louisiana State University.

Larvae acquired from other research partners

As a result of low fertilization rates for numerous batches, our larval supply was limited in 2023. To increase our larval numbers necessary for planned experiments and grow-out stock, we received larvae

from other organizations that collected spawn during the August and September spawning season. The Reef Futures Lab at the University of Miami Rosenstiel School of Marine, Atmospheric & Earth Science supplied 47,250 ACER larvae produced from spawn collected at their *in situ* coral nursery in August 2023. The Florida Aquarium supplied larvae from multiple species that spawned in their land-based nursery in August and September 2023: 10,000 ACER larvae (August), 51,800 CNAT (September), 54,500 PSTR (September) and 38,583 PCLI (September). Biscayne National Park supplied approximately 40,000 OFAV and 10,000 *Orbicella annularis* (OANN) larvae produced from spawn collected from wild colonies on an unnamed patch reef at the south end of Biscayne National Park. The University of North Carolina at Wilmington supplied approximately 50,000 *Pseudodiploria clivosa* (PCLI) larvae produced from *ex situ* spawning collections in September from colonies originating from reefs offshore of Ft. Lauderdale, FL.

Effects of sedimentation on coral settlement and recruit survivorship

Larvae produced from our 2023 spawning collections and partners were leveraged to facilitate a series of lab-based experiments (FDEP Award #BA6281 to Dr. Dana Williams) aimed at understanding the effects of sedimentation on coral settlement and the young (i.e., 1-month, 3-month, and 6-month) coral recruits. Larval settlement assays testing the effect of different sediment depths and the presence of sediment were conducted for APAL, ACER, OFAV, PSTR, PCLI, and CNAT, and sediment exposure trials on recruits were conducted using CNAT, PSTR, OFAV, ACER, and DLAB.

Field and lab support

Field and lab support from the SEFSC CoRAL Team includes Allan Bright, Kat Grazioso, Mark Ladd, Dana Williams, Sophia Ippolito, and Dylan Orcutt. Additional field support from collaborating organizations are as follows: AOML (Michael Studivan, Taylor Gill, Graham Kolodziej, Allyson DeMerlis, Patrick Kiel), UM Coral Futures Lab, NOAA NCCOS Hollings Lab, The Florida Aquarium, and SECORE. Histology and fecundity analyses on urban coral samples are being completed by Louisiana State University (Daniel Holstein, Ashley Rossin, Gillian Coleman, Morgan Coleman). The urban coral spawning observations and histological analyses were supported by SERO HCD FY23 funding to Michael Studivan, Ian Enochs, and Mark Ladd, with collections permitted under FWC SAL SAL-22-2116B-SCRP. Spawning monitoring and collection activities in the FKNMS were permitted by FKNMS-2018-163-A1 and funded by CRCP projects 1091 and 321367.

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Table 1. 2023 spawning observations in Key Largo, FL, and subsequent larval production.

Date	dAFM	Site	Wild/ Outplant	Species Monitored	Spawning		# Genets		Larvae Produced
					Observed	Collected	Monitored	Spawned	
4/16/2023	10	DLAB Patch	Wild	*D. labyrinthiformis*	Yes	Yes	≥68	5	37,500
4/17/2023	11	DLAB Patch	Wild	*D. labyrinthiformis*	No	No	≥68	0	---
5/15/2023	10	DLAB Patch	Wild	*D. labyrinthiformis*	Yes	Yes	≥68	2	2,000
5/16/2023	11	DLAB Patch	Wild	*D. labyrinthiformis*	No	No	≥68	0	---
5/17/2023	12	DLAB Patch	Wild	*D. labyrinthiformis*	No	No	≥68	0	---
8/2/2023	1	North Dry Rocks	wild	*A. palmata*	Yes	Yes	4	1	57,500
			outplant		Yes	Yes	25-30	≥7	
			outplant	*A. cervicornis*	Yes	Yes	5-10	≥4	13,900
8/3/2023	2	North Dry Rocks	wild	*A. palmata*	Yes	Yes	4	1	21,400
			outplant		Yes	Yes	25-30	≥5	
			outplant	*A. cervicornis*	Yes	Yes	5-10	≥2	4,300
8/4/2023	3	Sand Island	outplant	*A. palmata*	Yes	Yes	≥7	≥3	9,850
8/5/2023	4	Elbow	wild	*A. palmata*	Yes	Yes	10	2	3,200
			outplant		No	No	6	0	
8/7/2023	6	Horseshoe	wild	*O. faveolata*	Yes	Yes	14	≥3	---
				M. cavernosa	Yes	Yes	≥10	4	3,000
				P. Strigosa	No	No	1	0	---
8/8/2023	7	Horseshoe	wild	*O. faveolata*	Yes	Yes	14	≥2	---
				M. cavernosa	No	No	≥10	0	---
9/5/2023	6	North North Dry Rocks	wild	*O. faveolata*	Yes	Yes	17	2	53,460
				M. cavernosa	No	No	≥30	0	---

*dAFM is days after the full moon

Table 2. Predicted and monitored spawning time windows for target coral species, as well as observed spawning times by species in Key Largo, FL.

	Predicted Window			Monitored Window			Spawning Times	
Species	Months	Night AFM	Time	Dates	dAFM	Time	Night AFM	Time
D. labyrinthiformis	April, May, June	10	18:00 - 19:00	April 16 - 17	10, 11	17:50 - 19:00	10	18:15
				May 15 - 17	10, 11, 12	17:30 - 19:00	10	18:25
A. palmata	July, August	1 - 6	22:00 - 23:00	August 2 - 5	1, 2, 3, 4	21:45 - 23:00	1, 2, 3, 4	~22:25
A. cervicornis	July, August	1 - 6	22:00 - 23:00	August 2 - 3	1, 2	21:45 - 23:00	1, 2	~22:25
M. cavernosa	August, September	6, 7	20:00 - 22:45	August 7 - 8	6, 7	20:45 - 21:45	6	21:15
				September 5	6	20:45 - 21:45	---	---
O. faveolata	August, September	6 - 8	23:00 - 0000	August 7 - 8	6, 7	22:45 - 0005	6, 7	23:20
				September 5	6	22:45 - 0005	6	23:20
P. strigosa	August, September	6 - 8	21:40 - 0000	August 7	6	20:45 - 21:45	---	---
						22:45 - 0005		

*dAFM is days after the full moon

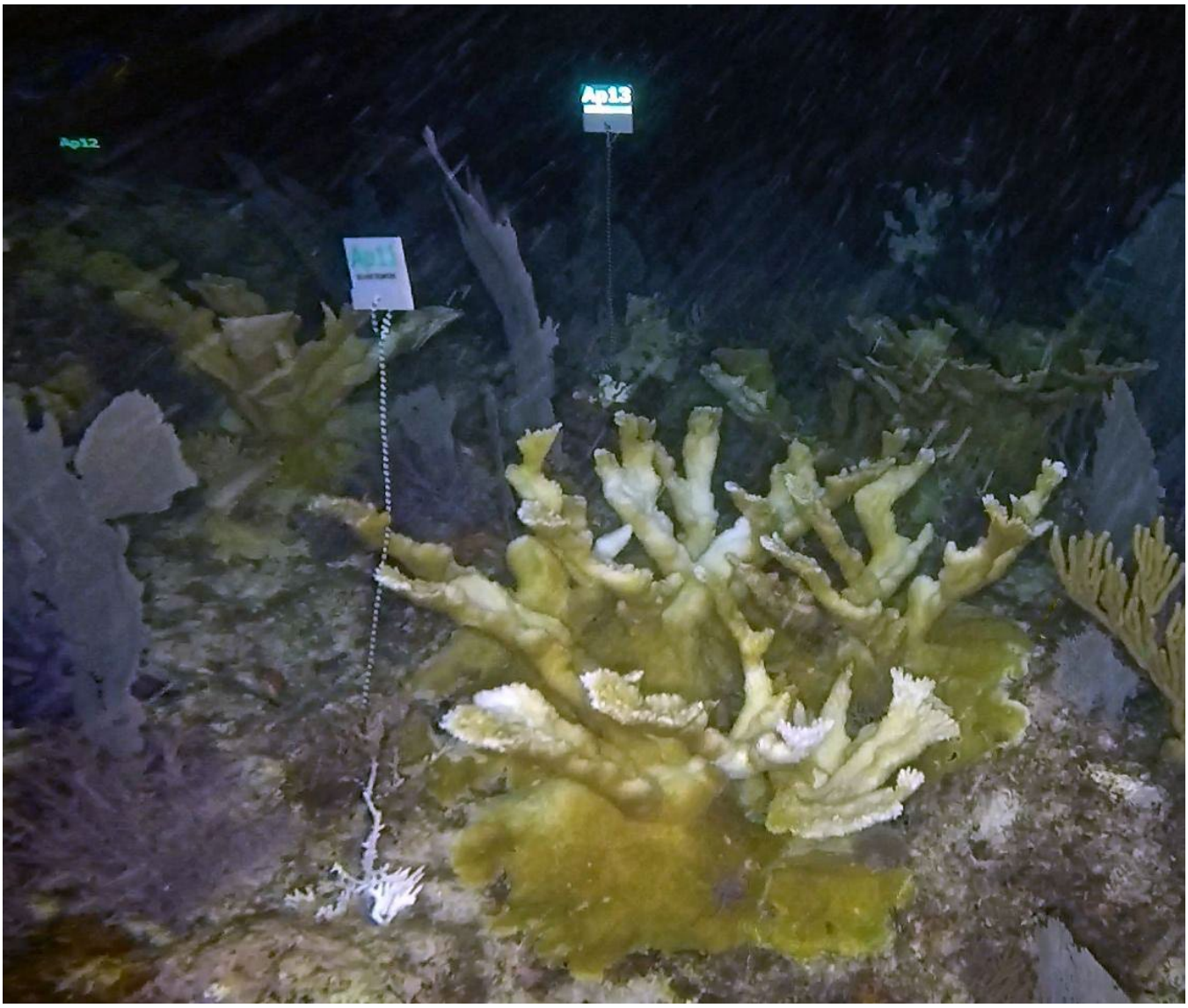

Figure 1. *Acropora palmata* colonies marked with small, buoyant genotype-tags that are raised approximately two feet off the benthos to facilitate colony identification at night. Photo depicts partially bleached APAL colonies at North Dry Rocks Reef, Key Largo, FL on August 3, 2023.

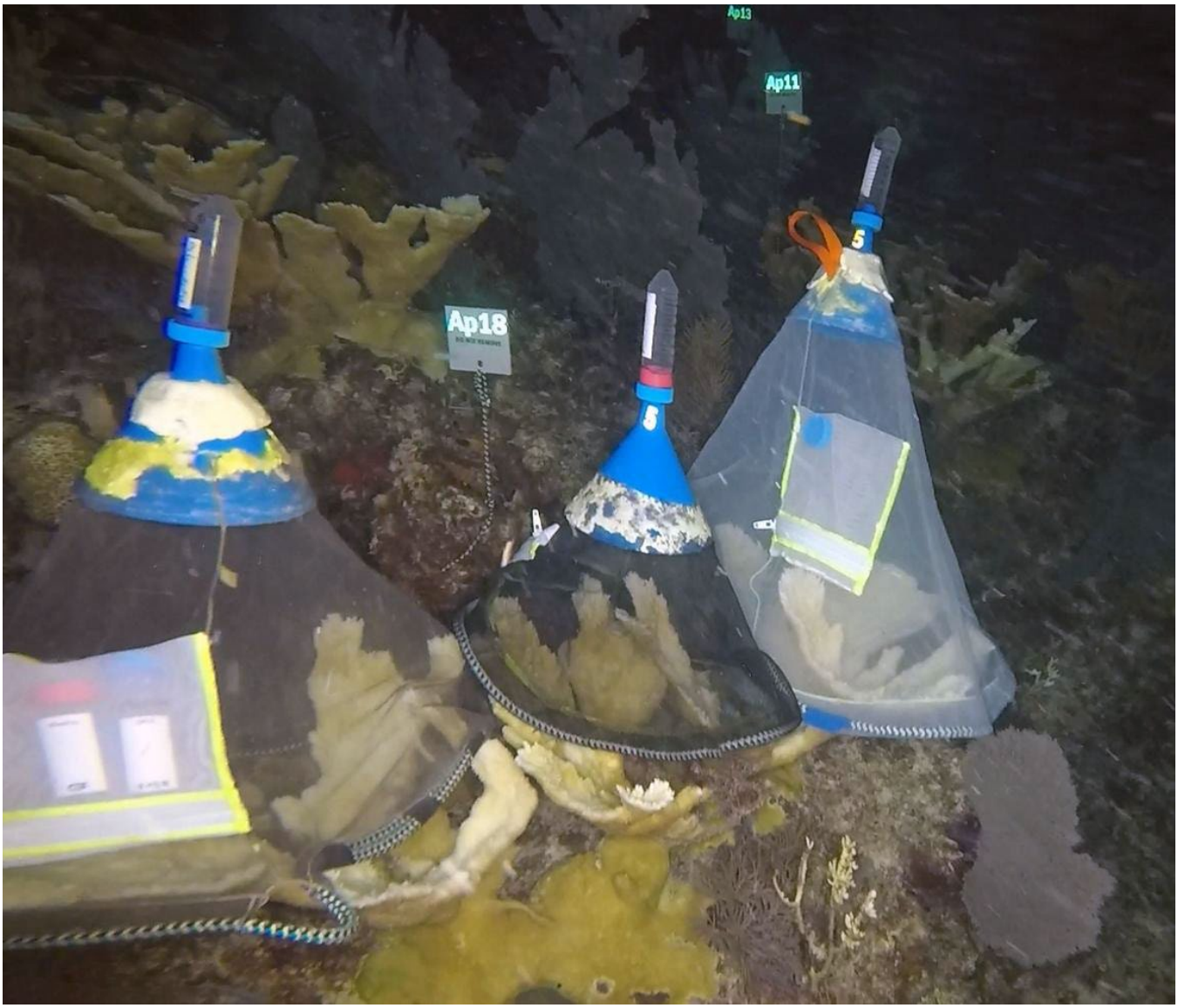

Figure 2. Spawning nets with collection tubes on *Acropora palmata*. Photos depict partially bleached *A. palmata* at North Dry Rocks Reef, Key Largo, FL on August 3, 2023.

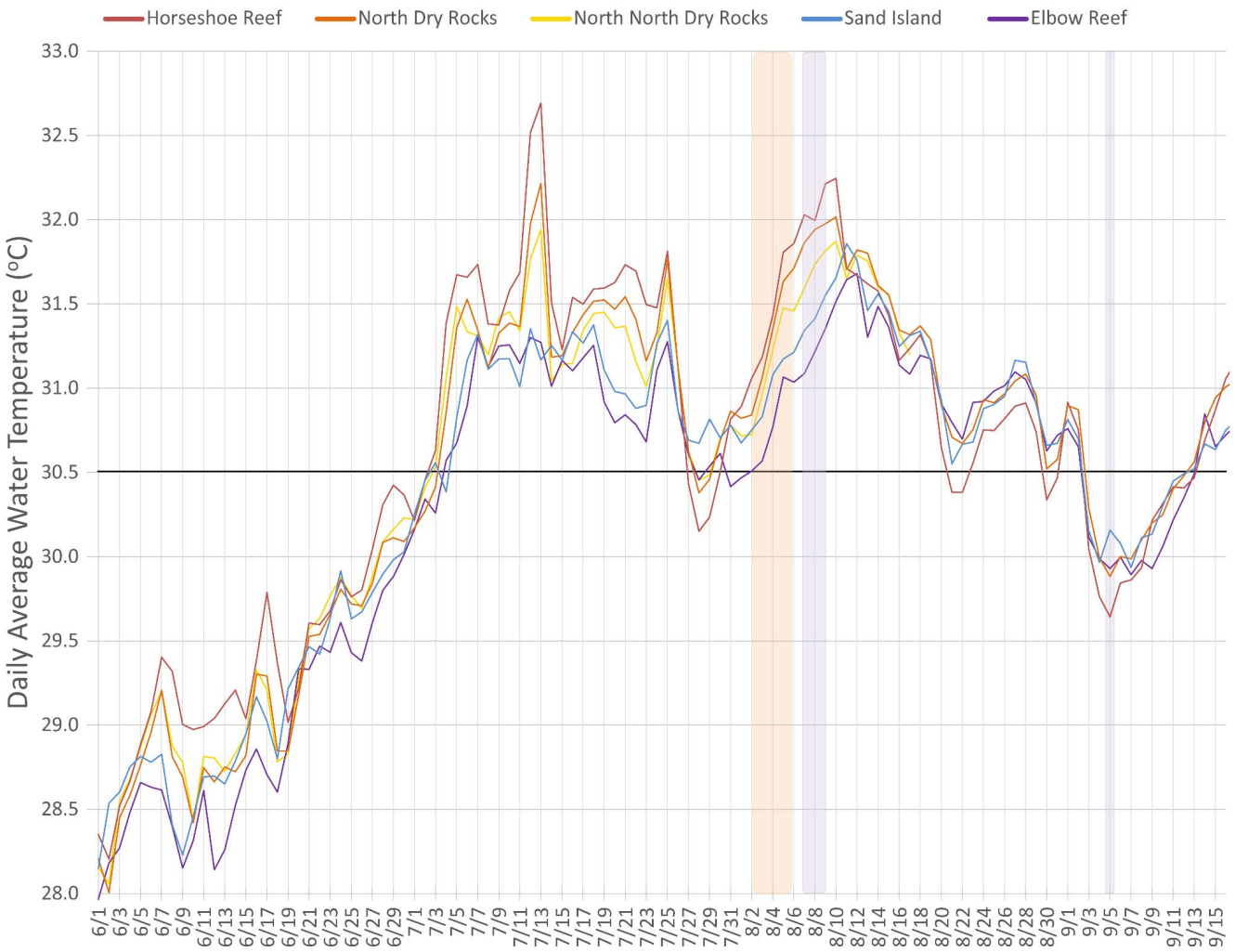

Figure 3. Daily average water temperatures measured at spawning sites from Onset HOBO MX2203 loggers deployed on the reef. The *Acropora* spp. August spawning window is shown in orange and the *Montastraea cavernosa* and *Orbicella faveolata* August and September windows in purple. The line at 30.5 °C shows the temperature above which heat stress accumulates in most coral species.

Figure 4. A *Colpophyllia natans* colony monitored for spawning activity at the MacArthur Causeway North 'urban coral' site in the Port of Miami. Top: The colony showing signs of paling/bleaching on August 7, 2023 during the first spawning observation window, with a recent core sample for pre-spawning histology. Bottom: Apparent recovery of the colony from bleaching by September 5, 2023 during the second spawning observation window.