

Quicklook Report

Coral Spawning 2024: Activities and Observations

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Overview

The Coral Research and Assessment Lab (CoRAL) at NOAA's Southeast Fisheries Science Center has monitored coral spawning patterns and collected gametes from APAL and OFAV in the upper Florida Keys since 2000. This year marks the fourth year utilizing our new and recently expanded CoRAL wet lab facility located at the University of Miami's Experimental Hatchery allowing 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.

2024 spawning observations and gamete collections were made for the coral species *Diploria labyrinthiformis* (DLAB) in April and May, *Acropora palmata* (APAL) in July and August, and *Acropora cervicornis* (ACER), *Orbicella faveolata* (OFAV), *Montastraea cavernosa* (MCAV), *Pseudodiploria strigosa* (PSTR) and *Eusmilia fastigiata* (EFAS) in August.

Due to the 2023 marine heatwave, observations for APAL are now limited to Elbow Reef as it is the only known site in the upper Florida Keys where there are enough mature APAL colonies remaining that belong to multiple genets to make spawning observations and collections (Williams et al. 2024). In previous years, we were able to make ACER spawning observations on outplanted colonies at North Dry Rocks Reef; however, none of the acroporid corals at North North Dry Rocks Reef survived 2023, and the most accessible mature ACER colonies in summer 2024 were colonies in the Reef Renewal USA's Tavernier nursery. Other monitored species (DLAB, OFAV and PSTR) did not experience notable changes in abundance between 2023 and 2024 at the sites we use for spawning collections so we were able to monitor the same populations as in recent years.

Record high seawater temperatures that were observed in 2023 throughout south Florida and the Florida Keys resulted in widespread coral bleaching where a range of bleaching conditions were

observed including non-bleached, pale, partially bleached and fully bleached colonies. Evidence suggests that some coral's reproductive capacities can be impacted following thermally-induced bleaching (Johnston et al. 2020), and it was apparent that some reproductive faculties may have been affected during the August and September spawning windows in 2023. All species monitored in the Florida Keys during August and September 2023 did in fact show spawning activity except for PSTR in which only one colony was monitored and did not spawn. However, divers from our team observed gamete bundles that appeared uncharacteristically smaller in size and less buoyant. Additionally, fertilization rates and settlement rates of APAL two-parent crosses were lower in 2023 than typical, suggesting some impact on reproductive success from the thermal stress event.

In 2024, reproductive capabilities did not appear to be inhibited by the 2023 thermal stress event. Monitored colonies of APAL, DLAB, and OFAV spawned and produced viable gametes leading to relatively high fertilization rates as expected based on recent years. Observations for ACER, MCAV, PSTR, and EFAS were limited to a single day in August and did not yield enough gametes to attempt fertilization (except for MCAV, see below, and EFAS which is a brooding coral species), precluding any conclusions regarding the potential impact of the 2023 heat wave on the reproductive capacity of these species. These species were monitored for a single day in August observing approximately 10% of MCAV colonies spawn, one ACER colony spawn, one EFAS colony spawn and no colonies of PSTR spawn. The small amount of MCAV gametes collected were mixed producing a small batch of fertilized embryos. However, the embryos were kept in suboptimal conditions resulting in no surviving larvae for settlement. Based on the spawning observations made in 2024, we can surmise that reproductive capabilities of the surviving population in 2024 were not inhibited by the 2023 thermal stress event as the majority of species monitored were observed to spawn producing viable gametes that led to apparently healthy coral larvae.

All coral larvae produced from gamete collections in the field were reared and settled at the Coral Research and Assessment Lab (CoRAL) wet lab facility in Miami, Florida. Larvae produced from spawn collections were settled on substrates in lab-based experiments evaluating the effect of different grazer assemblages on recruitment success and settlement substrate burial on coral recruitment success. Two additional coral larval species (*Pseudodiploria clivosa* [PCLI] and *Colpophyllia natans* [CNAT]) were acquired from The Florida Aquarium for the substrate burial experiment. The remaining larvae not used for experiments were settled under normal conditions and will be grown out in the CoRAL wet lab facility to be used in future experiments and restoration projects. Spawning observations and gamete collections were conducted in collaboration with the Coral Program at NOAA's Atlantic and Oceanographic & Meteorological Laboratory (AOML), the Reef Futures Lab at the University of Miami Rosenstiel School of Marine, Atmospheric & Earth Science (UM RSMAES), and Florida International University (FIU) where gametes were used for various projects led by each group.

Methods

In 2024, the CoRAL team monitored seven coral species for spawning activity: DLAB, APAL, ACER, OFAV, PSTR, EFAS and MCAV. Formal genotyping has only been done for the monitored APAL and ACER. For other monitored species, individual colonies are assumed to be distinct genotypes. Clonality is common in OFAV (and EFAS) so this assumption certainly leads to collection of observations and gametes from fewer genets than estimated. Clonality is less likely among DLAB and PSTR so the assumed number of genets is likely to match the number of colonies.

All species monitored, except for MCAV and EFAS, 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. 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 1](#)). 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 while retaining 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 ([Figure 2](#)). 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.

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. EFAS is a brooding coral species releasing fertilized eggs (i.e. zygotes) which can be collected using the same technique for MCAV spawn.

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) - unnamed sites**

Spawning observations and collections for DLAB were conducted at two unnamed inshore patch reefs in the upper Florida Keys within the boundary of John Pennekamp State Park during predicted spawning windows in April and May 2024. One patch reef (25.13401°, -80.3292°) has been monitored for DLAB spawning since 2020. A second neighboring patch reef (25.1253°, -80.3342°) was monitored for the first time this year (2024). 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 site navigation. At the beginning of each spawning dive, collection nets were placed on a subset of tagged corals (approximately 35) located along the transect lines. Bundle setting and release in DLAB occurs so rapidly that we have yet to witness it in the field, despite four 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) - North North Dry Rocks Reef**

Spawning observations and collections for MCAV were conducted at North North Dry Rocks Reef (25.13642°, -80.2894°), a spur-and-groove habitat on the Florida Reef Tract off-shore of Key Largo, during the predicted spawning window in August 2024. Prior to monitoring MCAV for spawning, colonies were located and temporarily marked with buoyant plastic chain markers containing a unique ID tag and attached glow stick 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 spawning-sized colonies present 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) - Elbow Reef**

Spawning observations and collections for APAL were conducted at Elbow Reef (25.142899°, -80.258221°), a spur-and-groove habitat on the Florida Reef Tract off-shore of Key Largo, during predicted spawning windows in July and August 2024. Elbow Reef has sparsely distributed wild APAL with documented spawning observations since 2005. In 2016, in partnership with CRF, we outplanted 288 fragments of APAL consisting of eight different genotypes within ~3x2m plots (n = 24 fragments per plot) near our long-term wild APAL monitoring plots. These outplants were first monitored for spawning in 2021, where we observed outplanted colonies of multiple genotypes spawning synchronously with wild colonies at Elbow Reef (Williams et al. 2023).

Prior to monitoring APAL for spawning at Elbow Reef, a set-up dive was conducted where divers identified and marked the location of known distinct genotypes for all wild and outplanted APAL colonies using buoyant plastic chains with floats and genotype ID tags to facilitate colony identification at night. 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 typically displays bundle setting at least 15 minutes before bundle release, allowing time to retrieve nets and place them on a colony once setting is observed in the polyp mouths.

***Acropora palmata* (APAL) and *Acropora cervicornis* (ACER) - Reef Renewal Nursery**

APAL and ACER spawning activity was also monitored at Reef Renewal's field coral nursery (24.982°, -80.438°) for one night in August. Prior to monitoring at Reef Renewal's coral nursery, a dive was conducted during daylight hours to familiarize divers with the location of APAL and ACER nursery trees and to clean genotype tags on the trees for genotypic identification during the spawning dive. The spawning dive was conducted as described for Elbow Reef.

***Orbicella faveolata* (OFAV) - North North Dry Rocks Reef**

Spawning observations and collections for OFAV were conducted at North North Dry Rocks Reef (25.13642°, -80.2894°) during the predicted spawning window in August 2024. Prior to the spawning dive, buoyant plastic chain markers with a unique ID tag and attached glow stick were placed near colonies for ease of location at night. At the time of the spawn window, divers swam from colony to colony looking for gamete bundles setting in the polyp mouths. Similar to APAL, when a colony was observed with gamete bundles setting, divers expeditiously placed a collection net on part of the colony or the entire colony.

***Pseudodiploria strigosa* (PSTR) - North North Dry Rocks Reef**

A single PSTR colony was identified and monitored during the MCAV and OFAV spawning dives at North North Dry Rocks Reef (25.13642°, -80.2894°) in August 2024. As the duration of bundle set time for this species is uncertain, a collection net was placed over this colony during the first dive of the night

and was removed at the end of the last dive of the night. Divers monitored the collection tube for gamete bundles throughout all dives.

***Eusmilia fastigiata* (EFAS) - North North Dry Rocks Reef**

A single EFAS colony was identified and monitored during the OFAV spawning dive at North North Dry Rocks Reef (25.13642°, -80.2894°) in August 2024. Brooded fertilized embryos were observed in polyp tentacles which were slowly being released, but collection was not attempted as divers did not have the appropriate collection tools (i.e., syringes used for MCAV gamete collection).

Spawning observations, collections and larval rearing

Peak spawning windows in the Florida Keys region are predicted based on information from previous spawning observations made by our lab, as well as those from other research organizations. For each species, predicted peak spawning windows and actual days and times monitored in 2024 are listed in [Table 1](#). Dates for each site, species monitored, and the number of larvae produced from each spawning event, are listed in [Table 2](#).

During spawning observations, collections are made as described above. Once collection tubes or syringes are capped with gametes, they are 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 diluted with 20-micron filtered seawater to reduce the sperm concentration. Embryos are then ladled from the surface of the water and distributed among table top bins filled with 20-micron filtered seawater ([Figure 3](#)) and left overnight to continue the fertilization process (for all species except DLAB which are directly added to larval rearing kreisels). 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 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 ([Figure 4](#)). 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.

***Diploria labyrinthiformis* (DLAB)**

April 2024 monitoring of DLAB for spawning activity was conducted at two inshore patch reefs on April 4 and 5 (10 and 11 days after the full moon [AFM], respectively). During both dates, the CoRAL team monitored one patch reef and the UM Reef Futures Lab monitored a second. No spawning was observed at either patch reef during these dates.

May 2024 monitoring of DLAB spawning was conducted at two inshore patch reefs on May 2 and 3 (9 and 10 days AFM) by the same teams as in April, and one patch reef was monitored on May 4 (11 days AFM) by the UM Coral Futures Lab. Spawning was observed at both patch reefs on May 3 only. Gamete bundle release occurred between 18:10-18:30 at both sites. At the DLAB patch observed by the CoRAL team, when spawning colonies were no longer releasing bundles, all collection tubes with bundles were brought back to the boat where they were mixed to create four batches, three separate 2-

parent crosses and one 6-parent mix, at approximately 18:55. The fertilization process began in the concentrated mixed batches while the gametes were transported to the CoRAL wet lab in Miami. Gametes collected from the 2nd patch reef were handled by the Coral Futures Lab. Once in the CoRAL wet lab, batches were transferred to larval rearing kreisels at approximately 21:45 in effect diluting the sperm concentration. All water flow was turned off in the kreisels which allowed the fertilization process to continue overnight. The next morning (09:30), estimated fertilization rates were 100% for all batches yielding approximately 35,000 DLAB larvae.

These larvae were used to set up two lab-based experiments. The remaining larvae were left in kreisels for continued rearing. Larvae were first observed swimming approximately 19 hours after spawning. Precondition settlement substrates were introduced into the kreisels to promote settlement approximately 41 hours after spawning. 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.

***Acropora palmata* (APAL)**

July 2024 monitoring of APAL spawning activity was conducted by the CoRAL team at Elbow Reef during nights of July 22 and 25-27 (nights 1 & 4–6 AFM). Spawning was observed on July 26 and 27 for both outplanted and wild colonies at approximately 22:20. On July 26, three of the six wild genotypes that were monitored spawned and five of the six outplanted genotypes spawned. Once collection tubes were partially filled with bundles, they were removed from the collector nets and brought to the boat where they were mixed creating six 2-parent crosses and one 4-parent mix. Gametes were mixed on the boat at approximately 23:30. Gametes were then transported to the CoRAL wet lab where batches were diluted with filtered seawater and transferred to table-top bins allowing fertilization to continue overnight. The following morning (08:20), the fertilization rates among batches were estimated to be an average of 82%, and batches were transferred to larval kreisels producing an estimated 36,500 larvae. On July 27, one wild genotype and one outplanted genotype spawned a small amount. Gametes were brought to the boat and mixed at 23:17 producing a single 2-parent batch. Gametes were transported to the CoRAL wet lab, diluted with filtered seawater and transferred to table-top bins where fertilization continued overnight. The following morning (07:45), fertilization was estimated to be 86% producing approximately 8,200 larvae, and larvae were transferred to kreisels for rearing.

For APAL larvae made from July 26 spawn, larvae were first observed to start swimming 3 days after spawning (dAS). For larvae made from July 27 spawn, swimming larvae were first observed 2 dAS. At 5 dAS a portion of July 26 larvae were removed from kreisels and used in lab-based experiments. The remaining larvae were reared in the kreisels for settlement and grow out. Once larvae were removed for experiments, pre-conditioned substrates were added to kreisels for settlement. Recruit settlement was first observed 5 dAS.

August 2024 monitoring of APAL spawning activity was conducted by the CoRAL team at Reef Renewal's field coral nursery on the night of August 21 (night 2 AFM) and Elbow Reef on nights August 22 and 24 (nights 3 and 5 AFM). No APAL colonies were observed spawning during any of these nights.

***Acropora cervicornis* (ACER)**

August 2024 monitoring of ACER spawning activity was conducted by the CoRAL team at Reef Renewal's field coral nursery on the night of August 21 (night 2 AFM). Small patches of bundles were observed setting on one colony around 21:00, ~80 minutes before bundles are typically released for

this species. Spawning occurred at approximately 22:25. Since only one ACER colony spawned, collected gametes were released at the site.

***Montastraea cavernosa* (MCAV)**

August 2024 monitoring of MCAV spawning activity was conducted by the CoRAL team, along with divers from FIU, at North North Dry Rocks Reef on August 25 (night 6 AFM). Divers began monitoring approximately 30 MCAV colonies at 20:30. The first colony observed releasing gametes was at approximately 21:30. A total of four colonies were observed to spawn, three female colonies and one male colony. Once colonies were no longer observed releasing gametes, divers returned to the boat with the collected gametes at 21:55. On the boat, the gametes were mixed at 22:20 producing a single 4-parent batch. The concentrated batch of gametes were stored in a cell culture flask while divers conducted the next dive monitoring OFAV for spawning activity. Once spawning dives were completed the gametes were transported back to the CoRAL wet lab where the batch was diluted with filtered seawater and transferred to table-top bins allowing fertilization to continue overnight. When the batch arrived at the wet lab (~03:00), the culture was not healthy. Analysis under the microscope revealed cellular pieces likely from broken fertilized embryos. Few intact embryos were observed; yet, all were fertilized suggesting that fertilization was high, but the extended wait-time on the boat and transit back to the wet lab resulted in degradation of the culture. The following morning (09:00), very few embryos were remaining in the culture. All developing larvae eventually died before settlement could be achieved.

***Orbicella faveolata* (OFAV)**

August 2024 monitoring of OFAV spawning activity was conducted by the CoRAL team, along with divers from FIU, at North North Dry Rocks Reef on August 25 (night 6 AFM). Divers began monitoring approximately 17 OFAV colonies at 22:45, and at approximately 23:00 colonies were observed with gamete bundles setting in their polyps. At least 10 colonies were observed to spawn, and collection nets were placed on all colonies with bundles setting. Once collection tubes were partially filled with gametes, they were removed and brought to the boat where gametes were mixed making one multi-parent batch at 00:15. The batch was transported to the CoRAL wet lab where it was diluted with filtered seawater and distributed among table top bins and allowed to continue fertilization overnight. The following morning (08:00), fertilization rates were estimated at 89% producing an estimated 302,700 larvae, and were transferred to larval rearing kreisels.

Larvae were first observed swimming at 2 dAS. Pre-conditioned substrates were added to some kreisels at 3 dAS, while other kreisels had none added as larvae from those kreisels were to be used for experiments. Settlement was first observed 5 dAS. At 6 dAS, a portion of larvae were removed from kreisels without substrates and used in lab-based experiments. The remaining larvae were reared in the kreisels for settlement and grow out.

***Psuedodiploria strigosa* (PSTR)**

During the MCAV and OFAV spawning dives on August 25 (night 6 AFM) at North North Dry Rocks Reef, a single PSTR colony was identified and monitored for spawning activity. Spawning was not observed.

***Eusmilia fastigiata* (EFAS)**

During the OFAV spawning dive on August 25 (night 6 AFM) at North North Dry Rocks Reef, a single EFAS colony was identified and monitored for spawning activity. At approximately 23:40, the brooded fertilized embryos were observed in polyp tentacles and were slowly being released. No embryos were collected as divers did not have syringes to capture the embryos. When divers ended the dive at midnight, the colony was still releasing embryos while some were still visible in the polyp tentacle.

Miami ‘urban coral’ spawning observations

In 2023, the CoRAL team partnered with the Coral Program at NOAA’s AOML to conduct spawning observations of populations of PSTR, OFAV, and CNAT located near the Port of Miami, Florida (25.77293, -80.15263). 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 and large thermal variability). This partnership continued into 2024 with the additional monitoring of corals of opportunity (COOs). Twenty-four COOs (11 CNAT, 6 PSTR, 4 OFAV, 2 DLAB, and 1 PCLI) from the MacArthur Causeway North were collected and brought to the University of Miami’s land-based nursery in July, August, and October 2024 to monitor for ex-situ spawning.

On August 25-27 (nights 6-8 AFM) and September 23-25 (nights 6-8 AFM), a CoRAL team member joined the AOML Coral Program to monitor 6 PSTR, 2 OFAV, and 11 CNAT colonies at MacArthur Causeway North and the COOs being held at the University of Miami’s land-based nursery. Monitoring at both sites began at 19:00 for CNAT and continued until 21:00. The OFAV and PSTR colonies were also monitored from 22:10 to 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, 4/3/2024, 7/27/24, and 8/23/2024 and post spawn on 09/11/2023, 6/3/2024, 9/26/2024, and 9/27/2024 for histology analyses that are currently being processed by collaborators at Louisiana State University.

Larvae acquired from other research partners

To supplement our supply of larvae for use in lab-based experiments, we acquired additional larvae from Biscayne National Park (BNP) and The Florida Aquarium in May and August, respectively. Biscayne National Park supplied 25,000 DLAB larvae produced from spawn collected at a reef site within the park on May 3 (night 10 AFM). The Florida Aquarium supplied an estimated 10,000 PCLI larvae produced from spawn collected on August 26 (night 7AFM) and 10,000 CNAT larvae produced from spawn collected on August 27 (night 8 AFM). Larvae from both species were produced from spawn collected in The Florida Aquarium’s land-based nursery.

Understanding the effects of sedimentation on coral settlement and recruit survivorship

Larvae produced from our 2024 spawning collections and partners were leveraged to facilitate a series of lab-based experiments (FDEP Award #C3FC5D to Drs. Victor Rodriguez-Ruano and Dana Williams), aimed at understanding the effects of sedimentation on coral settlement. Larval settlement

assays to test the effect of different sediment depths and sediment grain sizes were conducted for DLAB, APAL, ACER, OFAV, PCLI, and CNAT. An additional set of tank-based larval settlement experiments to test the effect of different grazer assemblages on field conditioned substrates was conducted using DLAB and APAL larvae produced from our 2024 spawning collections.

Field and lab support

Field and lab support from the SEFSC CoRAL team includes Mark Ladd, Dana Williams, Allan Bright, Kathryn Grazioso, Sophia Ippolito, Colin Murphy, Victor Rodriguez-Ruano, Eliana Galindo, and Alex Howard. Additional field support from collaborating organizations are as follows: AOML (Michael Studivan, Ashley Rossin, Emilia Silverberg, and Ashley Stevens), FIU (Alain Duran, Silvana Guzman, and William Barriera), and UM Coral Futures Lab. Histology and fecundity analyses on urban coral samples are being completed by Louisiana State University (Daniel Holstein, Ashley Rossin, Gillian Coleman, and 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-SCRIP. Spawning monitoring and collection activities in the FKNMS were permitted by FKNMS-2024-178 and funded by CRCP projects 1091 and 31416. Observations and collections from DLAB took place on patch reefs within the John Pennekamp State Park within the FKNMS and were permitted by FDEP-03042415 and FKNMS-2024-178.

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Table 1. Predicted and monitored spawning time windows for target coral species, as well as observed spawning times by species in Key Largo, FL.

Species	Predicted Window			Monitored Window			Observed Spawning Times	
	Months	Night AFM	Time (EDT)	Dates	Night AFM	Time (EDT)	Night AFM	Time (EDT)
DLAB	Apr, May	9-11	18:00 - 19:00	Apr 4 - 5	10, 11	17:50 - 19:00	10	~18:15
				May 2 - 4	9, 10, 11	17:30 - 19:00	10	~18:25
APAL	Jul, Aug	1 - 6	22:00 - 23:00	Jul 22, 25 - 27	1, 4, 5, 6	21:45 - 23:00	1, 2, 3, 4	~22:25
				Aug 21, 22, 24	2, 3, 5	21:45 - 23:00	--	--
ACER	July, Aug	1 - 6	22:00 - 23:00	Aug 21	2	21:45 - 23:00	1, 2	~22:25
MCAV	Aug, Sep	6 - 7	20:00 - 22:45	Aug 25	6	20:45 - 21:45	6	~21:15
OFAV	Aug, Sep	6 - 8	23:00 - 00:00	Aug 25	6	22:45 - 00:05	6, 7	~23:20
PSTR	Aug, Sep	6 - 8	21:40 - 00:00	Aug 25	6	20:45 - 21:45	---	---
EFAS	---	---	---	Aug 25	6	22:45 - 00:05	---	---

Table 2. 2024 spawning observations in Key Largo, FL, and subsequent larval production.

Date	dAFM	Site	Colony Origin	Species Monitored	Spawning		# Genets*		Larvae Produced
					Observed	Collected	Monitored	Spawmed	
4/4/2024	10	DLAB Patch	wild	DLAB	No	No	30	0	--
		Brain Town			No	No	20	0	--
4/5/2024	11	DLAB Patch	wild	DLAB	No	No	30	0	--
		Brain Town			No	No	20	0	--
5/2/2024	9	DLAB Patch	wild	DLAB	No	No	30	0	--
		Brain Town			No	No	20	0	--
5/3/2024	10	DLAB Patch	wild	DLAB	Yes	Yes	30	~6	35000
		Brain Town			Yes	No	22	4	**
5/4/2024	11	Brain Town	wild	DLAB	No	No	20	0	--
7/22/2024	1	Elbow	wild	APAL	No	No	6	0	--
			outplant		Yes	Yes	6	1	--
7/25/2024	4	Elbow	wild	APAL	No	No	6	0	--
			outplant		No	No	6	0	--
7/26/2024	5	Elbow	wild	APAL	Yes	Yes	6	3	36500
			outplant		Yes	Yes	6	5	
7/27/2024	6	Elbow	wild	APAL	Yes	Yes	6	1	8200
			outplant		Yes	Yes	6	1	
8/21/2024	2	Reef Renewal	nursery	APAL	No	No	3	0	--
				ACER	Yes	No	12	1	
8/22/2024	3	Elbow	wild	APAL	No	No	6	0	--
			outplant		No	No	6	0	
8/24/2024	5	Elbow	wild	APAL	No	No	6	0	--
			outplant		No	No	6	0	
8/25/2024	6	North North Dry Rocks	wild	OFAV	Yes	Yes	~17	≥10	302700
				MCAV	Yes	Yes	~30	4	<100
				PSTR	No	No	1	0	--
				EFAS	Yes	No	1	1	--

*Formal genotyping has only been done for APAL and ACER. For other species individual colonies are assumed to be distinct genotypes. This assumption is known to be unreliable for OFAV, but for the other species it is a reasonably likely assumption.

**Brain Town DLAB gamete collection, larval production, and rearing were conducted by the UM Reef Futures Lab.



Figure 1. Spawning net with collection tube on outplanted *Acropora palmata* at North Dry Rocks Reef, Key Largo, FL on August 2, 2023. *Photo credit: O Williamson*



Figure 2. Collection tube with *Diploria labyrinthiformis* gamete bundles at an unnamed patch reef in Key Largo, FL, on May 3, 2024.



Figure 3. Table-top bins with *Acropora palmata* gametes at the CoRAL wet lab in Miami, FL, on July 26, 2024.

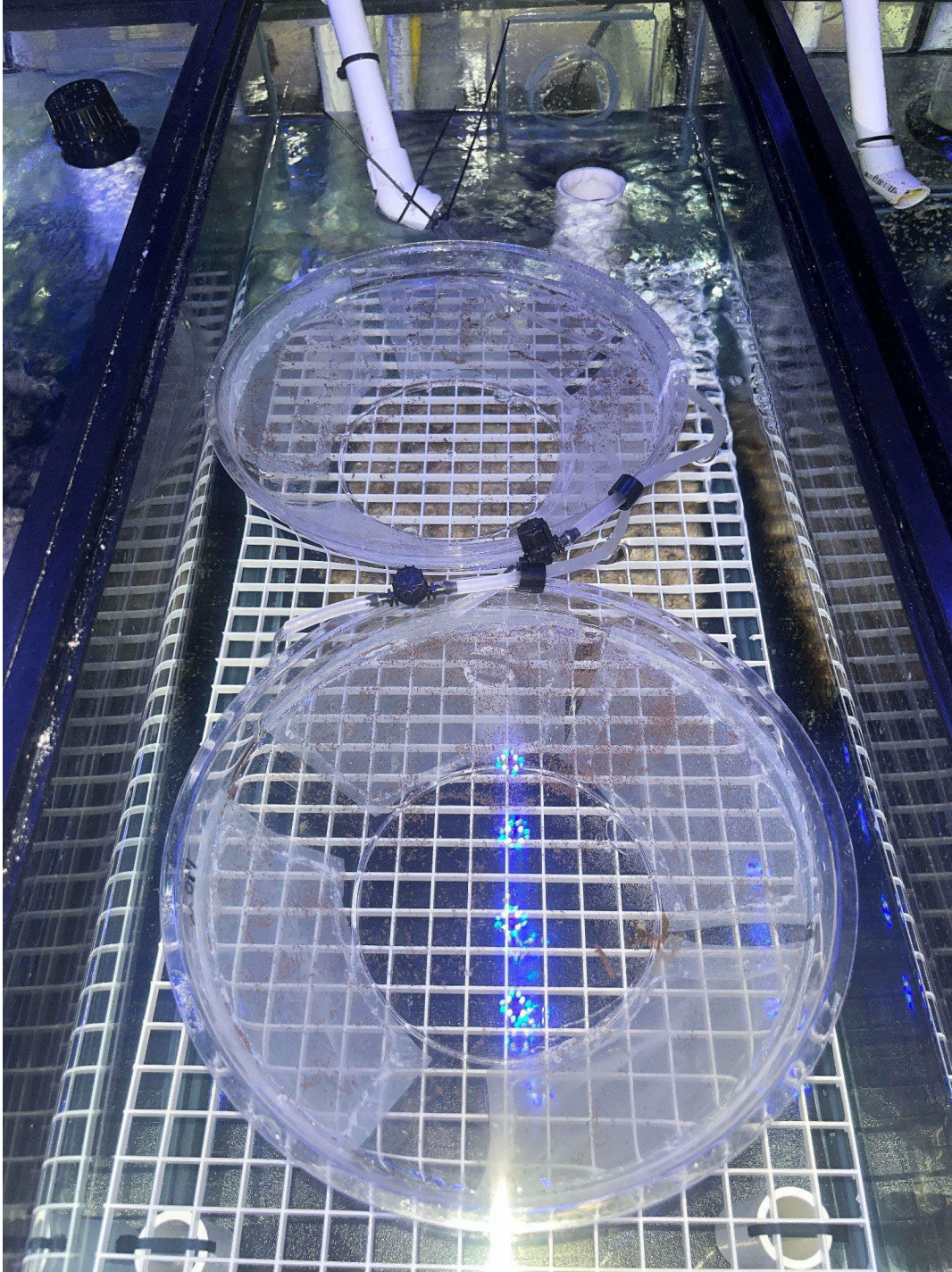


Figure 4. Larval kreisels with *Diploria labyrinthiformis* larvae at the CoRAL wet lab in Miami, FL, on May 4, 2024.