Quicklook Report

Coral Spawning 2022: Activities and Observations

NOAA Fisheries

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Summary

Spawning observations and gamete collections were made for the coral species *Diploria labyrinthiformis* (DLAB) in April, *Acropora palmata* (APAL) and *A. cervicornis* (ACER) in July and August, and *Orbicella faveolata* (OFAV), *Montastraea cavernosa* (MCAV), and *Pseudodiploria strigosa* (PSTR) in August and September of 2022. Larvae were cultured for all species except *P. strigosa* in which spawning was not observed. Larvae were reared at the Coral Research and Assessment Lab (CoRAL) Nursery in Miami, Florida, and settled onto artificial substrates where they are being raised for future research and restoration projects. Additionally, in partnership with the University of Miami Rosenstiel School of Marine, Atmospheric & Earth Science Coral Futures Lab, sperm was cryopreserved from APAL, ACER, and OFAV. We partnered with three additional research groups; the Coral Health & Disease Program at Hollings Marine Lab (Cheryl Woodley), Florida Aquarium (Keri O'Neil), and SECORE (Margaret Miller), to collect gametes for various projects led by each group. Additionally, larvae from our batch cultures were provided to other research and restoration partners including UM RSMAS Coral Futures Lab, SECORE, Florida Aquarium, Florida International University (FIU) and the Reef Institute. In Partnership with SECORE we conducted an experiment evaluating *Millepora complanata* as a settlement cue for APAL and OFAV.

Figure 1 Collection of gamete bundles from *Acropora palmata* outplants at North Dry Rocks in the upper Florida Keys in August 2022. Photo Credit: Liv Williamson.

Introduction

The Coral Research and Assessment Lab (CoRAL) at the Southeast Fisheries Science Center has observed spawning patterns and collected gametes from APAL (**Fig. 1**) and OFAV in the upper Florida Keys since 2000. This year marks the second year utilizing our new and recently expanded CoRAL wet lab (**Fig. 2**). This new facility allowed us to bring gametes collected from Key Largo 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. In 2022, we completed the addition of an experimental system to the CoRAL wet lab consisting of 24 independent, temperature-controlled aquaria fed by filtered (5 micron) and UV-sterilized flow-through seawater. This experimental tank system allows us 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 2022, we tested the effect of the presence of *Millepora complanata* (MCOM) on settlement success of APAL and OFAV larvae.

Figure 2. Raceways and experimental aquaria holding recruitment substrates with recently settled larvae in the new SEFSC experimental system.

Species Observed:

*Diploria labyrinthiformis (*DLAB*)*

We began monitoring DLAB colonies for spawning activity on an unnamed patch reef in the upper Florida Keys in 2020. In 2020, spawning was not observed during the consecutive five months in which observations were made (May - September). On the first night that observations were made in 2021 (May 6), a large spawning event was observed among DLAB colonies at that site. In 2022, due to the timing of the full moon, we began observations on April 26. Spawning nets were placed on 12 colonies ranging in size from 4 cm to 50 cm with the goal of identifying a minimum colony size threshold for reproductive activity in DLAB. Spawn was collected from 8 of those colonies on April 26, 2022. Colonies that released gametes ranged in size from 10 cm to 50 cm. Spawning occurred at approximately 18:20, which is the early end of the predicted window. Inclement weather precluded our team from making observations on the following day (April 27, 2022). However, because all of the colonies did not release gametes on April 26, we suspect that more spawning activity occurred on April 27 or during the May spawning window.

Gamete bundles were collected in falcon tubes and immediately transported to a CoRAL team member on the surface. A batch culture of gametes from the 14 parents was mixed on the boat at approximately 18:30. Shortly after mixing gametes, the bundles broke up allowing fertilization to begin while we were still on site. Aliquots of eggs and sperm were placed in flasks of seawater for transport to Miami. The remainder of the gametes were poured back into the water immediately before leaving the site (approximately 19:30). Because fertilization likely occurred on the boat, releasing the embryos may have resulted in a greater number of larvae being

produced than would have occurred naturally at that site. The embryos transported to the lab in Miami were estimated to have ~90% fertilization. The embryos were placed into kreisels in our seawater system for the larval phase (9 days). Larvae began probing settlement substrates placed on the bottom of the kreisels two days after spawning (dAS). Attached recruits were first observed 4 dAS. Settlement substrates remained in the kreisels until 10 dAS to ensure recruits were fully attached, after which they were removed from the kreisels and transferred to tanks for ongoing growout in our seawater system.

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

Spawning observations were made at North Dry Rocks in the upper Florida Keys among wild and outplanted APAL genotypes and outplanted ACER. North Dry Rocks has a sparse population of four genotypes of naturally occurring (wild) APAL. Beginning in 2012 the Coral Restoration Foundation (CRF) planted hundreds of ACER at this site. In 2015, in partnership with CRF, we planted four genotypes of APAL along experimental transects on the reef. In 2018, ~1,000 APAL and ~1,000 ACER were planted 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. In 2020 and 2021, we observed spawning in the 2015 APAL and 2012 ACER. We did not monitor the 2018 outplants in previous years because they were not large enough to spawn.

On nights 2 (July 15) and 3 (July 16) after the July full moon (Table 1), we monitored the 2015 APAL and the 2012 ACER and no spawning activity was observed. Due to the limited number of divers available, we did not include the 2018 APAL in our July monitoring.

After the August full moon, a second boat chartered by the Woodley Lab (NOAA Coral Health & Disease Program at Hollings Marine Lab) enabled us to recruit additional divers from Florida Aquarium, SECORE, and the University of Miami (UM) to make observations on a larger number of corals including the APAL and ACER outplanted to North Dry Rocks in 2018. In total, 32 APAL genotypes (3 wild, 29 outplanted) and an unknown number (>7) of ACER genotypes were monitored by a team of six divers over 2,400m^2 of reef area on August 13 and 14. Of the monitored genotypes, 1 wild and 17 outplanted genotypes were observed to spawn (Table 2; **Fig. 1**), 13 genets spawned both nights and 4 of the genets were observed to only spawn on one of the two nights. Gametes collected for batch cultures were mixed on the R/V Palmata II while on site. Gametes collected for parent crosses were brought to the chartered vessel and returned to shore to conduct the reciprocal crosses. The Reef Futures Lab at UM cryopreserved sperm from 9 individual APAL genets as well as a batch pooled from 11 genotypes.

On August 13, gametes from 6 APAL parent genotypes were mixed and returned to the CoRAL wet lab to allow time for fertilization. The next morning, fertilization was found to be ~90% and the embryos were placed into kreisels (**Fig. 2**). On August 14, gametes from APAL were used to make two batches- one with six genotypes and one with five genotypes. Fertilization was >95% for both batches.

Because spawning activity was very high on August 13 (night 2AFM) and 14 (night 3AFM) and we did not have the capacity to raise more larvae, we did not go out to make observations for the remainder of the *Acropora* window (August 15 to August 16). Surplus larvae were distributed to several research and restoration partners as listed in the 'Collaborations' section below.

*Montastraea cavernosa (*MCAV*)*

On August 17 (night 6AFM) we monitored colonies of MCAV at the north mooring of Horseshoe Reef for spawning activity. In past years, we have observed several colonies release sperm and one colony release eggs,

but we have never successfully collected gametes from MCAV colonies at Horseshoe Reef or any other site prior to 2022. We identified the locations of >20 colonies and began monitoring for spawning activity at 20:15. Sperm was collected from at least four different MCAV colonies and eggs were collected from three colonies. The majority of gamete release was observed between 21:05 and 21:30, though eggs were observed being released before this window and sperm was observed afterwards. Typically, a small portion of a colony was observed releasing sperm or eggs at any one point. However, this year included two notable observations. First, we observed an entire male colony release sperm at the same time when it was hit by a small (<10 cm) nocturnal fish attracted to our dive lights (colloquially known as 'slappy-fish'), potentially suggesting that the physical stimulus of impact induced the release of sperm. Secondly, we observed a single colony release both sperm (first; from the base of the colony) and eggs (after the release of sperm; from the middle and top portions of the colony), indicative of a hermaphroditic MCAV colony.

Gametes were combined on the boat at 21:30 and allowed to fertilize on the boat while divers began monitoring OFAV for spawning. Once the culture arrived back at the lab, it had been 6 hours after the gametes were combined. Fertilization could not be determined as the culture condition was poor and contained higher concentrations of cell fragments than visibly-viable embryos. Considering the extended duration of time before the gametes arrived at the lab in Miami, the culture would have likely been in better condition if it had been diluted further and transferred to an airless container following the OFAV collection dive. Upon arriving at the Miami wet lab, the culture was cleaned, diluted and left to continue the fertilization process overnight. Approximately 12 hours after spawning, embryos were individually hand-picked from the culture and placed in a kreisel for larval development and settlement. Despite the poor conditions during the fertilization process, viable larvae continued through the developmental stage and successfully settled.

*Orbicella faveolata (*OFAV*)*

On August 17 (night 6AFM) we monitored OFAV at the north mooring of Horseshoe Reef for spawning activity. We have monitored this patch of OFAV since 2018 and it reliably spawns and the resulting cultures reliably have high fertilization, although we have not genotyped the colonies so we do not know how many parents there are. This year our team of three divers tagged 14 colonies and began monitoring for spawning at 22:40 alongside two divers from SECORE and UM from a second vessel. Bundles from one colony released around 23:25, before bundles were even set in the other colonies. The remainder of the colonies' bundles set between 23:50 and 00:10 and released bundles from 00:05 to 00:40 (O. Williamson pers comm). The timing of bundle set and release of the first colony was consistent with previous observations of the colonies in this patch but the timing of the remaining colonies was substantially later and less synchronous than observed among this patch of colonies in the past.

Divers collected gametes from 9 colonies and tubes were shuttled to each boat. Gametes taken to the R/V Palmata II were mixed on the boat at 00:45, transported to the lab in Miami, and allowed to fertilize overnight. Fertilization was ~ 90%, and the embryos were placed in kreisels the following morning. Gametes taken to the second vessel were used for reciprocal crosses and other research by the Woodley Lab as well as for batch cultures for research by UM and SECORE. Additional larvae from our batch culture were shared with other research and restoration groups as listed in the Collaborations section below. Additionally, the remaining larvae (~200K) were liberated on the reef the following day.

Orbicella annularis

Personnel at Biscayne National Park (A. Bourque) collected and cultured *Orbicella annularis* (OANN) from Alina's Reef on August 17 of 2022. On August 20, 1000 larvae were transferred to our lab in Miami. The culture contained a lot of debris and cell fragments. Consequently, the culture was poured through a sieve, successfully cleaning the culture of most of the debris and cell fragments, but resulted in a culture of smaller-sized larvae as it's likely any viable, larger larvae were retained in the sieve. The larvae were transferred to a kreisel where the remaining decomposing debris was regularly cleaned by "sweeping" the water's surface with saran wrap. This culture resulted in the recruitment of OANN larvae on 28 settlement substrates.

*September Boulder Corals (*OFAV*,* PSTR*, and* MCAV*)*

On nights 6 and 7 after the September full moon, we partnered with SECORE and the UM Reef Futures Lab to monitor several boulder coral species at the unnamed patch reef where we have collected DLAB in the past. Divers set up haphazard transects and placed temporary markers by ~12 OFAV, ~4 *Pseudodiploria strigosa* (PSTR) colonies to aid in monitoring. Numerous MCAV along the transects were also monitored. Four divers monitored these colonies from 19:00 to 23:00 on both nights and no spawning was observed.

Millepora complanata as a settlement cue for APAL and OFAV

Methods: In August of 2022, we conducted aquaria-based experiment to assess the effect of (1) the presence, and, (2) distance from, *Millepora complanata* (MCOM) on the settlement of APAL and OFAV larvae generated from our 2022 spawning collections. To do so, we established eight experimental aquaria (n = 4 per species). Each aquarium consisted of a glass 20-gallon tank connected to a 20-gallon sump fed with a slow trickle of filtered (5µm) and UV-sterilized seawater such that water within each tank was turned over ~6x per day. Water temperatures were kept at 28.5℃ ±0.3 for the duration of the experiment via a Neptune Apex® Controller System that monitored and regulated temperature via a temperature probe, heater, and cold seawater line for each tank. Each experimental aquarium contained two kreisels. Within each kreisel, we lined the bottom with unseasoned ceramic tiles soaked in seawater for 48 hours

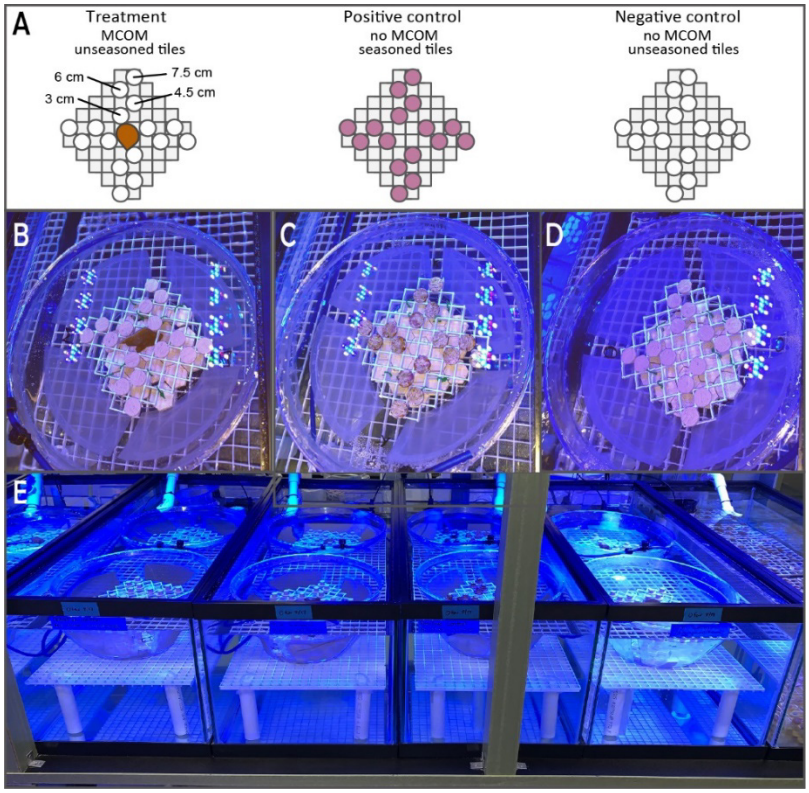

Figure 3 (A) Schematic of one complete replicate for the settlement experiment. (B) Kreisels with larvae for one of the *M. complanata* (MCOM) replicates containing a MCOM fragment and unseasoned settlement substrates. (C) Kreisels with larvae for one of the positive control replicates containing no MCOM and seasoned settlement substrates. (D) Kreisels with larvae for one of the negative control replicates containing no MCOM and unseasoned settlement substrates. (E) The experimental tank system with treatments established in each independently controlled tank.

prior to the experiment. We then placed a single diamond-shape piece of egg crate made out of PVC into each kreisel on top of these tiles to elevate experimental plugs off the substrate (**Fig. 3**). Sixteen experimental plugs were placed on top of each piece of egg crate at a distance of 3, 4.5, 6, or 7.5 cm from the center (n = 4 plugs per distance). Plugs for OFAV larvae were placed upside down based on previous observations of preference to settle on the undersides of plugs for this species. Unseasoned plugs soaked in seawater for 48 hours prior to the experiment were used for the Negative Control treatment. Unseasoned plugs soaked in seawater for 48 hours prior to the experiment were also used for the MCOM treatment, which also received a single blade ~5 x 3 cm (L x W) of MCOM that was placed in the center of the egg crate. To create Positive Control treatments, we used seasoned plugs that had been deployed to a reef site in the Upper Florida Keys (Sand Island) on May 17 of 2022 and were left for three months (May to August). These plugs were retrieved in mid-August, lightly scrubbed with a brush, and placed in a 20-gallon tank overnight with ~15 juvenile *Diadema antillarum* that grazed the plugs and removed the majority of upright macroalgae. Because of a limited number of tanks (n = 8 total), we established n = 1 for Negative Control and Positive Control treatments, and n = 2 +MCOM treatments for each species.

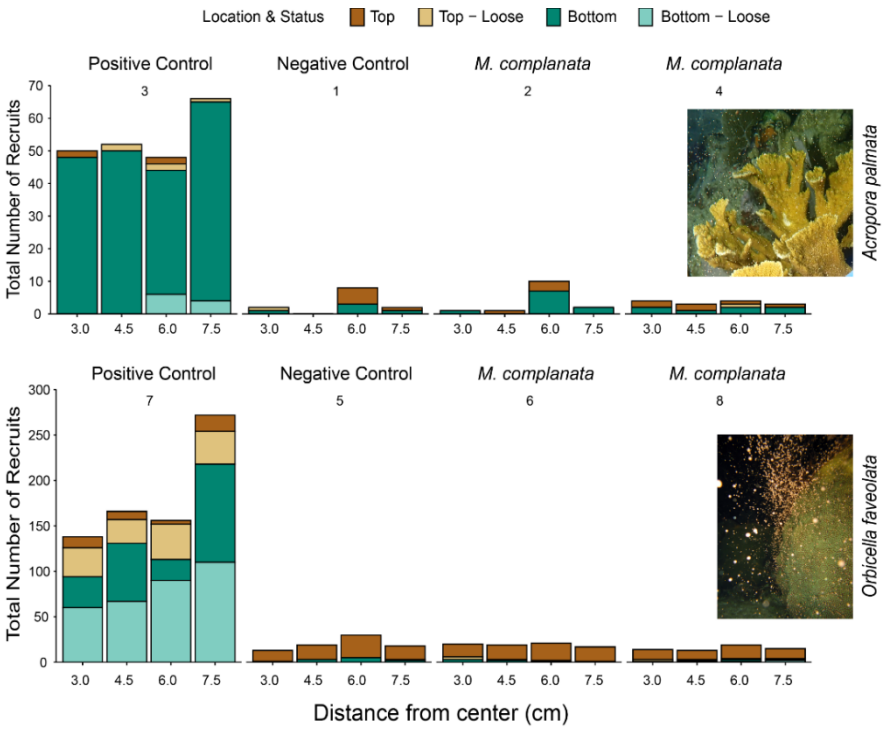

Figure 4 Total number of *Acropora palmata* (top) and *Orbicella faveolata* (bottom) recruits settled on substrates under different treatments in the summer of 2022. Different colors represent the location (brown shades = top of plug; green shades = bottom of plug) and if a recruit was firmly attached to the substrate (darker shades) or loosely attached (lighter shades). Labels above each panel indicate treatment (Positive Control, Negative Control, or *M. complanata*) and the individual tank number for that replicate (range: 1-8).

Fertilized embryos of APAL were added to kreisels on August 14 2022, and fertilized embryos of OFAV were added to kreisels on August 18 2022, approximately 8 hours after gametes were mixed for both species. Settlement substrates and MCOM fragments were added to their respective kreisels on August 21, 8 dAS for APAL and 4 dAS for OFAV. Kreisels were monitored daily for the accumulation of debris on the surface that results from the breakdown of embryos and were cleaned as needed using sheets of saran wrap that effectively trap lipids and other debris from the surface but not larvae. On August 25 (OFAV) and August 27 (APAL) all experimental plugs were surveyed under a dissecting microscope to quantify the number of recruits and their location. For each plug we surveyed all surfaces. For each recruit observed, we recorded if it was settled on the top (i.e. surface facing upwards) or bottom (i.e. surface facing downward) of the plug, and also recorded if it was "loosely" attached to the plug, a common phenomenon at this stage whereby the larvae has not completely secured itself to the substrate. The distance from the center of the kreisel (Positive and Negative Controls) or from the MCOM fragments (+MCOM treatment) was recorded for each plug to assess any spatial patterns in settlement.

Settlement rates for both species were substantially higher on seasoned tiles in the Positive Control treatments compared to the Negative Control and MCOM treatments (**Fig. 4**). The addition of a single blade of MCOM to kreisels had no effect on the number of settlers or their spatial distribution for both APAL and OFAV. The consistent lack of a settlement response to the presence of MCOM suggests that the presence of this species did not induce settlement in the larvae of APAL or OFAV.

Collaborations

Our collections were shared with several other research and restorations groups listed below.

UM RSMAS (Reef Futures Lab; A. Baker):
- 40,000 ACER larvae
- 400 APAL larvae

SECORE (M. Miller):
- 185,000 APAL larvae

Nova SE (J. Figueiredo):
- 110,000 ACER larvae
- 100,000 OFAV larvae

Florida Aquarium (K. O'Neil):
- 275,000 APAL larvae

The Reef Institute (L. Fix):
- 50,000 APAL larvae
- 50,000 OFAV larvae

NOAA-NCCOS (Hollings Marine Lab; C. Woodley):
- 10,000 APAL larvae
- 10,000 OFAV larvae

Field Support (Divers, topside, shore coordination)

NOAA SEFSC CoRAL Team: Allan Bright, Kat Grazioso, Mark Ladd, and Dana Williams.
UM Coral Futures Lab: Katherine Hardy and Liv Williamson
NOAA-NCCOS Hollings Marine Lab Coral Health & Disease Program: Murphy Macdonald, Lisa May, Zach Moffit, Cheryl Woodley
Florida Aquarium: Keri O'Neil, Emily Williams
SECORE: Hannah Ditzler and Margaret Miller

Permits & Funding

Collections made for the reported work were permitted by Florida Keys National Marine Sanctuary (FKNMS-2018-163) and Florida Department of Environmental Protection (04222125K). This work was funded by NOAA's Coral Reef Conservation Program.

Table 1. Spawning observations for 2022 at North Dry Rocks (NDR), Horseshoe Reef and an unnamed patch reef ("DLAB Patch") in the upper Florida Keys.

Date	dAFM	Site	Wild/ Outplant	Species Monitored	Spawn Observed	Spawn Collected	# Genets Monitored	# Genets Spawned	Larvae produced
4/26/2022	10	DLAB Patch	Wild	*D. labyrinthyformis*	Yes	Yes	12	8	250,000
7/15/2022	2	NDR	Wild	*A. palmata*	No	---	3	0	----
7/15/2022	2	NDR	Outplant	*A. palmata*	No	---	4	0	----
7/15/2022	2	NDR	Outplant	*A. cervicornis*	No	---	Unkn	0	----
7/16/2022	3	NDR	Wild	*A. palmata*	No	---	3	0	----
7/16/2022	3	NDR	Outplant	*A. palmata*	No	---	4	0	----
7/16/2022	3	NDR	Outplant	*A. cervicornis*	No	---	Unkn	0	----
8/13/2022	2	NDR	Wild	*A. palmata*	Yes	Yes	3	1	335,000**
8/13/2022	2	NDR	Outplant	*A. palmata*	Yes	Yes	29	15	335,000**
8/13/2022	2	NDR	Outplant	*A. cervicornis*	Yes	Yes	8	≥3	240,000
8/14/2022	3	NDR	Wild	*A. palmata*	Yes	Yes	3	1	165,000**
8/14/2022	3	NDR	Outplant	*A. palmata*	Yes	Yes	29	15	165,000**
8/14/2022	3	NDR	Outplant	*A. cervicornis*	Yes	Yes	Unkn	≥7	240,000
8/17/2022	6	Horseshoe	Wild	*O. faveolata*	Yes	Yes	~8*	8	710,000
8/17/2022	6	Horseshoe	Wild	*M. cavernosa*	Yes	Yes	~15*	8	10,000
9/16/2022	6	DLAB Patch	Wild	*O. faveolata*	No	---	Unkn	0	----
9/16/2022	6	DLAB Patch	Wild	*M. cavernosa*	No	---	Unkn	0	----
9/16/2022	6	DLAB Patch	Wild	*P. strigosa*	No	---	Unkn	0	----
9/17/2022	7	DLAB Patch	Wild	*O. faveolata*	No	---	Unkn	0	----
9/17/2022	7	DLAB Patch	Wild	*M. cavernosa*	No	---	Unkn	0	----
9/17/2022	7	DLAB Patch	Wild	*P. strigosa*	No	---	Unkn	0	----

** dAFM is days after the full moon*

*** total from wild and outplanted mixed culture*

Table 2. *Acropora palmata* spawning observations among outplanted and wild genotypes at North Dry Rocks Reef in August 2022.

Original Nursery Name	Current Nursery Name	Genet Origin: Keys Region	Genet Origin: Reef Name	8/13	8/14	Cryo-preserved
TR1	Apal-057	Upper	Turtle Rocks	---	---	---
CF2	Apal-062	Upper	South Carysfort	---	---	---
CF4	Apal-061	Upper	South Carysfort	---	---	---
AA3	Apal-068	Upper	Triple A	X	X	---
Ap2	Apal-002	Upper	U91 Patch	---	---	---
Ap4	Apal-004	Upper	Elbow	---	---	---
HS1	Apal-066	Upper	Horseshoe	X	X	---
Ap1	Apal-001*	Upper	North Dry Rocks	X	X	X
Ap35	Apal-035*	Upper	North Dry Rocks	---	---	---
None	None*	Upper	North Dry Rocks	---	---	---
GR1	Apal-055	Upper	Grecian Rocks	---	---	---
FR1	Apal-053	Upper	French	---	---	---
Ap16	Apal-016	Upper	Molasses	X	X	---
Ap17	Apal-017	Upper	Molasses	X	X	X
ML16	Apal-069	Upper	Molasses	X	X	---
ML2	Apal-050	Upper	Molasses	X	X	---
ML3	Apal-051	Upper	Molasses	---	---	---
Phil	Apal-070	Upper	Phils	X	X	X
PK2	Apal-034	Upper	Pickles	---	---	---
PK4	Apal-067	Upper	Pickles	---	X	---
PK10	Apal-078	Upper	Pickles	---	---	---
SN1	Apal-010	Upper	Snapper Ledge	---	---	---
CN1	Apal-030	Middle	Conch	X	X	X
CN2/3	Apal-071	Middle	Conch	X	X	---
Ap9	Apal-009	Middle	Delta Shoal	---	X	X
Ap11	Apal-011	Middle	Sombrero	X	---	X
Ap12	Apal-012	Middle	Sombrero	X	X	X
Ap13	Apal-013	Middle	Sombrero	---	---	---
Ap14	Apal-014	Middle	Sombrero	---	---	---
Ap24	Apal-024	Lower	Looe Key	X	---	---
Ap18	Apal-018	Lower	Sand Key	X	X	X
Ap28	Apal-028	Lower	Man Key	X	X	X

** Wild genotype naturally present at North Dry Rocks; Apal-001 is present both as a wild colony and as outplanted colonies.*