Quick Look Report: *Dendrogyra cylindrus* spawning– August 2019

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Fish and Wildlife Research Institute – South Florida Regional Lab Restoration Ecology Team (FWC)
Florida Keys National Marine Sanctuary (FKNMS)
Coral Restoration Foundation (CRF)
Keys Marine Laboratory (KML)
Volunteers

Abstract:

In August 2019, Nova Southeastern University led a multi-agency effort to observe and collect gametes from spawning pillar coral (*Dendrogyra cylindrus*) colonies in the Western Dry Rocks region of the Lower Florida Keys. For two nights, four boats and 29 personnel representing five agencies/institutions recorded data and collected eggs and sperm for propagation of the species. Field spawning closely matched predicted dates and times from previous years. Larvae were transported to Florida Aquarium's Center for Conservation for settlement. Development could not be observed several hours after spawning, but the following morning, numerous larvae were observed. Many of these were greatly diminished in size from the normal larvae and may be a result of damaged eggs or embryos disturbed during early development. The impact of Stony Coral Tissue Loss Disease on the parent colonies may also have reduced developmental success, and the impact of this disease on *D. cylindrus* populations has reduced wild colony numbers to a point where future wild spawning collections are most likely unobtainable. However, in addition to the juveniles produced in the past and the larvae produced from 2019 collections, the long-term knowledge gained from wild spawning observations and collections has built the knowledge that now allows facilities to spawn, collect, settle, and rear juveniles of this species into the future.

Background:

The pillar coral *Dendrogyra cylindrus* is a rare but conspicuous coral on Caribbean reefs. It is unique in its taxonomy as the only species within its genus, and it is unique in its structure as the only columnar coral in the Caribbean. It has been listed as "threatened" under the US Endangered Species Act, "threatened" by the State of Florida, and "vulnerable" by the IUCN.

Baseline surveys of the population on the Florida Reef Tract (FRT) from Dry Tortugas to West Palm Beach since 2013 describe a population of approximately 789 live *D. cylindrus* colonies representing approximately 185 genotypes. Back-to-back bleaching events in 2014 and 2015 and chronic white plague led to mortality of some

individuals near the time of the baseline surveys. The progression of Stony Coral Tissue Loss Disease (SCTLD) through the Florida Reef Tract since 2014 subsequently led to widespread mass mortality of the species (Fig 1). Surveys through August 2019 document only 159 colonies (80% loss) and 86 genotypes (54% loss) remaining. The majority of the extant colonies are currently affected with SCTLD and are not expected to survive the year.

D. cylindrus is generally gonochoric, meaning that individuals are either male or female (but see results). Gametes (sperm and eggs) must encounter each other to produce fertilized eggs that develop into swimming planula larvae that must then find a place to successfully settle and grow into new colonies. Because most remaining adult genotypes are widely dispersed, the likelihood of *in situ* gamete encounters is extremely low. Juveniles of this species have not been documented in Florida surveys, and it is suspected that this species is reproductively extinct in the region.

Since 2012, annual observations of male and female *D. cylindrus* spawning on the FRT have allowed for accurate predictions of spawning times. Spawning in the population occurs 2-4 nights after the full moon of August, and 55-115 minutes after sunset. Males generally spawn a few minutes before females. Identifying the sex of a colony is not visually possible until the moment of spawning.

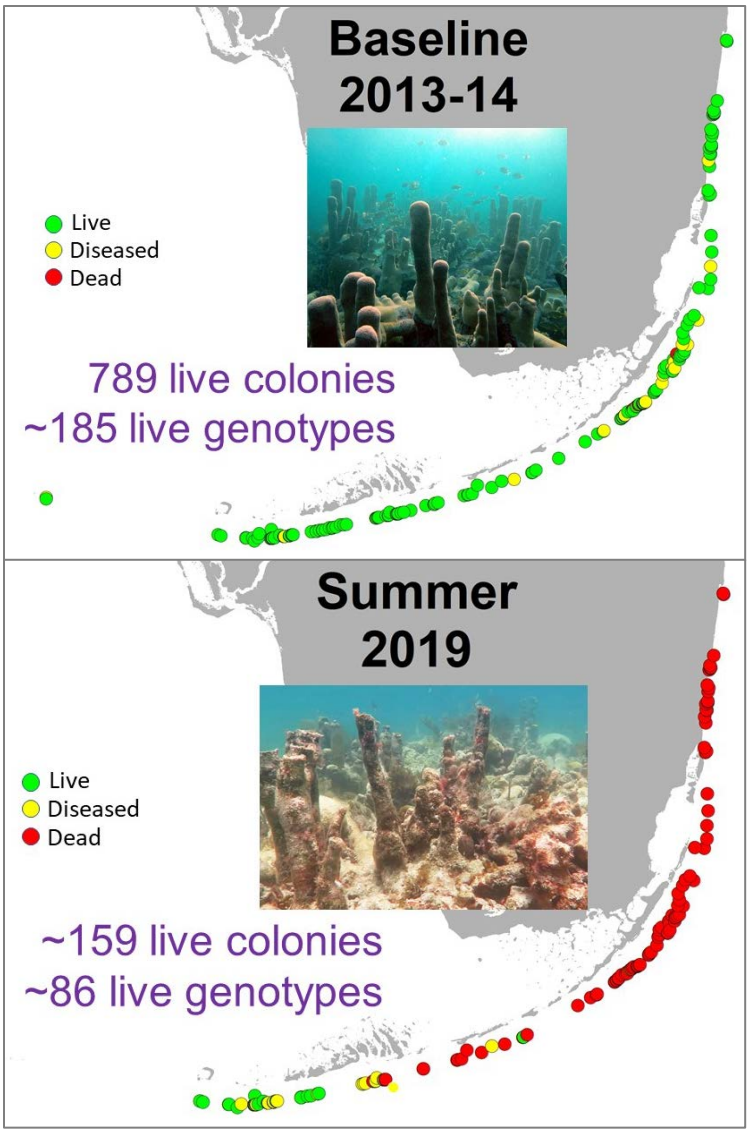

Fig 1. Distribution and status of *D. cylindrus* colonies on the Florida Reef Tract. Baseline 2013-2014 surveys show a background level of disease. Surveys five years later document almost complete mortality from the Middle Keys through Southeast Florida, with disease progressing through the Lower Keys. Photos are taken from the same location at a site in the Upper Keys.

Since November 2015, a genetic rescue project has collected and housed fragments of *D. cylindrus* genotypes in onshore aquarium facilities. In 2016, 2017, and 2018, fragments at Mote, KML, and FLAQ that were exposed to natural light cycles were observed spawning, though timing was asynchronous to wild colonies. Beginning in 2019, a subset of these fragments at FLAQ were also placed in indoor aquaria where temperature and light were controlled to replicate average reef conditions during gametogenesis and spawning.

Successful settlement of wild-collected of *D. cylindrus* larvae was first accomplished in Florida in 2016. From that settlement event, three individual genetically distinct juveniles have since survived to 3 years of age. *Ex situ* fertilization and settlement was first successfully accomplished in 2018 when colonies held at KML spawned and

juveniles were settled at FLAQ. Ten genetically distinct individuals from that event are still alive and growing. Bottlenecks identified for ongoing efforts include: inconsistent fertilization rates and low settlement rates.

Goals for the 2019 field spawning event were as follows:

1. Record date and time of gamete release in the field to add to and confirm predictive models of spawning.
2. Record gender of observed wild colonies for further information on gender ratios and hermaphroditism.
3. Attempt fertilization, larval rearing, and settlement of *D. cylindrus* juveniles.

Additional efforts, headed by FLAQ, included 1) observation of *ex situ* colonies exposed to natural light and observation and 2) collection and rearing of gametes from colonies placed in controlled light/temperature regimes. These are reported upon elsewhere as they are outside the scope of this work.

2019 Protocols

The Western Dry Rocks region was targeted for observations and collections due to the quantity of genetically distinct colonies as well as a progressing disease boundary that resulted in this location being the only remaining site to house large amounts of live *D. cylindrus*. The site contained 17 colonies representing between 8 and 12 distinct genotypes (while some colonies were genotyped in 2016, others were after genotyping was conducted and have not yet been sampled for genetic analysis). All known colonies within this region were tended by dive teams during spawning windows (Figure 2).

Field surveys were conducted on August 17 and August 18. Teams of SCUBA divers were deployed on each colony from 8:45 – 9:50 pm. Each team recorded the timing of spawning, proportion of colony spawning, and gender. They also collected gametes using large plastic bags weighted with rigid rims (Figure 3). In addition to bag collections, small amounts of sperm were also collected with 60 cc syringes (silicone rather than rubber rings) to provide concentrated samples. All gametes were returned to boats immediately after spawning. On

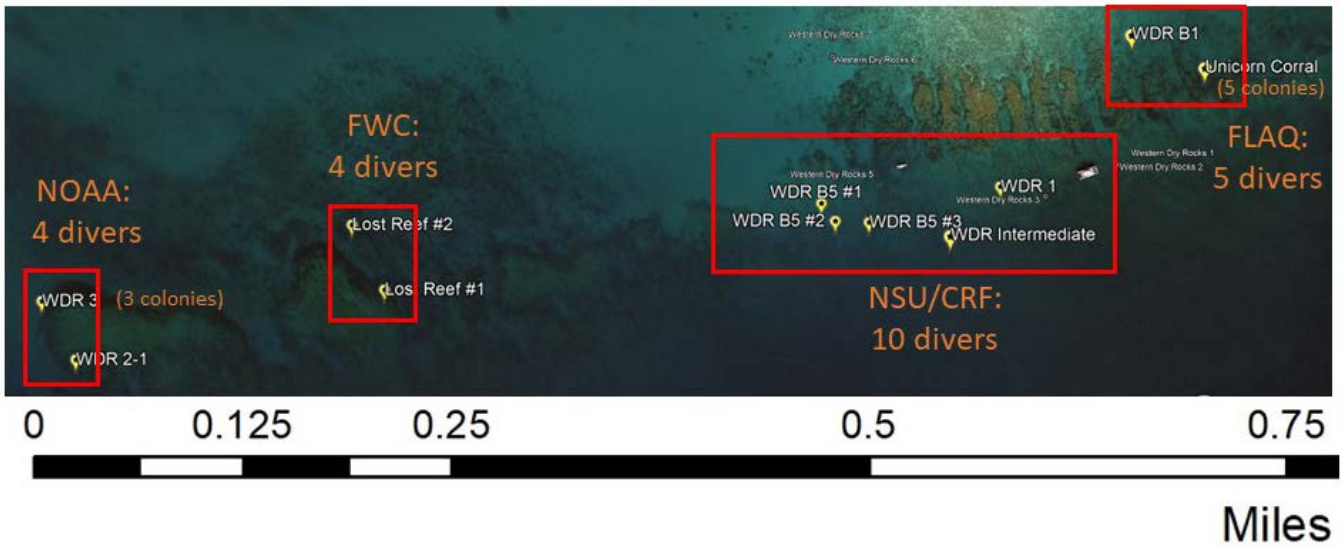

Fig 2. Location of *D. cylindrus* colonies in the Western Dry Rocks region. Boxes and text represent the colonies and teams deployed for observation during spawning.

Aug 17, bags of sperm were poured through a 600 micron sieve to remove zooplankton. Eggs were siphoned into a 75 micron filter submerged within seawater to concentrate them before sperm was added (Figure 3). Sperm from each male was combined into each egg-containing bucket to increase genetic recombination potential. On Aug 18, all sperm was collected in syringes (smaller releases), and these were added directly to concentrated eggs. Samples were transported in buckets back to shore.

Fig 3. Coral gamete collection device made of weighted hose and large plastic bag (left). In-water sieve used to gently concentrate eggs (right. Photo by CRF).

2019 Field Results

Field spawning was observed on 10 of 17 (59%) field colonies on August 17 and 6 of 16 field colonies (38%) on August 18. These nights were selected based on observations at five Florida sites over seven years that show peak spawning on nights 3 and 4 after the full moon (Figure 4). In 2019, both nights resulted in gametes from male and female colonies. Males spawned between 21:20-21:40 (80-100 minutes after sunset) and females between 20:55-21:43 (55-103 minutes after sunset).

2012	Aug 1 23:27	2	3	4 PCF	5 PCF	6
2013	Aug 20 21:45	21	22	23 PCF	24 PCF	25
2014	Aug 10 14:10	11	12 PCF DRTO	13 PCF DRTO	14 DRTO	15
2015	July 30	31 06:45	Aug 1	2 DRTO	3 Coffins	4
2016	Aug 17	18 05:29	19	20 Coffins Sombrero	21 Sombrero	22
2017	Aug 7 14:12	8	9 Looe	10 Looe	11	12
2018	Jul 27 16:22	28	29 Looe	30 Looe	31	Aug 1
2019	Aug 14	15 08:31	16	17 WDR	18 WDR	19

Fig 4. Observations of *D. cylindrus* spawning nights within the Florida Reef Tract from 2012-2019. Yellow circles and times represent the date and local time of the full moon. Blue clouds indicate the presence of spawning, with size indicative of the intensity. Grey crosses are nights when colonies were observed but did not spawn. Text notes the site(s) of the observations.

These dates and times are synchronous with observations of *D. cylindrus* spawning in Florida in previous years (Figure 5). In 2019, males spawned on average 92 minutes after sunset (SE: 2 minutes) and females on average 93 minutes after sunset (SE: 8 minutes). One small (50x48x58 cm) female spawned notably early (55 minutes after sunset). Average timing of egg release was not significantly different from previous years (ANOVA). Average timing of sperm release was not significantly different from previous years with the exception of 2018. The timing of male spawning in 2018 was significantly lower than gamete relase in any other year (ANOVA. $p < 0.001$). One hypothesis for this early release was the infection of 2018 observed corals with Stony Coral Tissue Loss Disease. However, 10 of the 17 colonies observed in 2019 were also affected with SCTLD, and all released gametes in accordance with predictions from non-2018 years. We propose that early sperm release is triggered by the occurrence of the full moon earlier in the gametogenesis cycle. The 2018 spawning full moon was July 27; the 2015 moon was July 30. Spawning for all other years in which spawning was timed was August 7-17. It is possible that

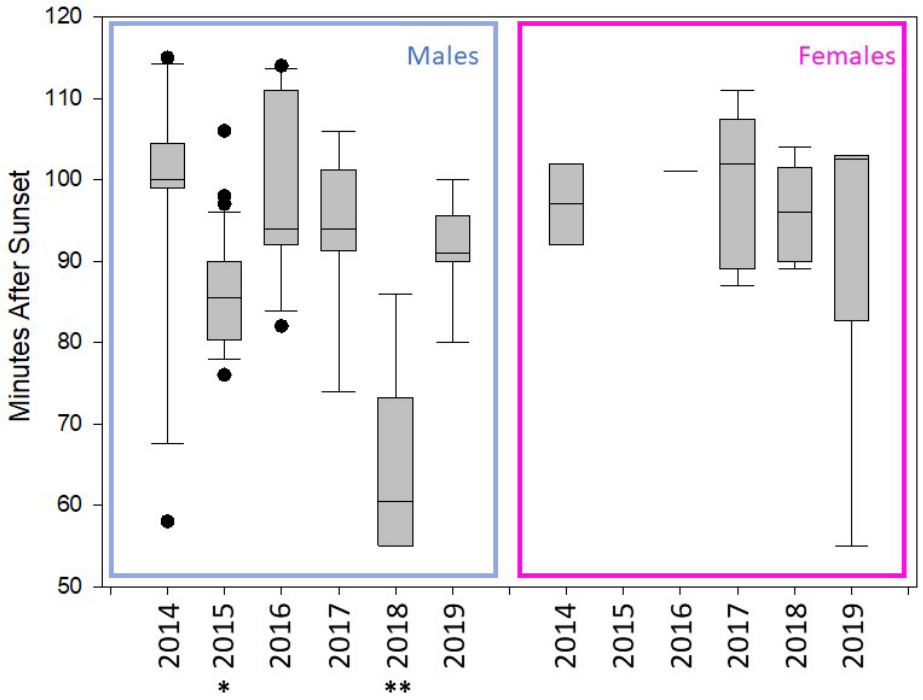

Fig 5. Spawning times of wild colonies across all observation sites by year. Box plots show each median value boxed by 25-75% of observations. Whiskers encompass 10-90% of observations, with dots showing outliers. A one-way ANOVA showed no difference in annual spawning times among females, but did identify 2018 (**) males as spawning significantly earlier than in all other years ($p < 0.001$) and 2015 males (*) as spawning earlier than 2014 and 2016 males.

Colony	Genotype	Diseased?	8/17/2019	8/18/2019
WDR 3-1	D1411	yes	80%	10%
WDR 3-2	prob D1411	no		
WDR 3-3		no	1%	
WDR 2	D1194	yes	30%	
Lost Reef 1	unknown	yes	90%	5%
Lost Reef 2	unknown	yes		
WDR B5-#1	unknown	no	100%	50%
WDR B5-#2	unknown	yes	100%	
WDR B5-#3	unknown	yes	20%	
WDR Intermediate	unknown	yes		
WDR 1	D1246	yes	40%	
WDR B1	unknown	no	50%	not observed
Unicorn Corral #1	prob clonal	yes		80%
Unicorn Corral #2/3		no	40%	
Unicorn Corral #4		no		75%
Unicorn Corral #5		yes		45%
Unicorn Corral #6		no		

Fig 6. Spawning data for each colony observed in 2019. Information includes genotype (conducted by Baums lab, 2016), presence/absence of recent mortality as a result of SCTLD, percentage of colony spawning on each night, and gender (pink = eggs, blue = sperm).

an atypically early moon could result in physiological differences in the sperm or differences in environmental cues that would result in early release.

Of the thirteen colonies that spawned in 2019, seven spawned only on Aug 17, three spawned only on Aug 18, and three spawned on both nights (Figure 6). The colonies that spawned both nights were of the same gender each night. One likely case of hermaphroditism between ramets was observed. Though not yet genotyped, the close proximity of the colonies at the Unicorn Corral site (1.3 to 4.8 meters) suggests they are clonal. Two of the spawning colonies at the site released as males (one on Aug 17 and one on Aug 18), and two released as females (Aug 18).

D. cylindrus has traditionally been considered a gonochoric species. However, observations at a clonal site in the Upper Keys between 2012-2014 documented males and females on genetically identical colonies, sex switching between years, and even production of eggs and sperm within a single colony (Neely et al. 2018). It was suspected that this hermaphroditism was rare, as three other large clonal sites have been gonochoric throughout colonies and over multiple years. However, assessments of 29 known genotypes that were observed over multiple ramets, nights, years (including 2019), or locations (lab and field) indicated 11 of them to be hermaphroditic in some form. This suggests that hermaphroditism is not particularly rare within Florida Reef Tract *D. cylindrus*, and cannot be used to identify non-clonal individuals.

2019 Fertilization and Larval Development

Mixing of gametes occurred between 22:15-22:40 on August 17, and from 21:50-22:20 on August 18. Eggs were thus exposed to sperm between 35-80 minutes after gamete release on Aug 17 and 13-49 minutes after release on Aug 18. The difference is accounted for by slightly different methodologies. In other coral species, concentration of gametes and time from spawning to fertilization are both important factors in coral fertilization rates. Thus, the first night's process was seeking to minimize plankton and concentrate the eggs. The second night's process was focused on more rapid fertilization with the tradeoffs of less-concentrated eggs and increased zooplankton

Florida Aquarium protocols and results (details provided by Rachel Serafin & Keri O'Neil):

Buckets of larvae from Aug 17 spawning were delivered to FLAQ staff at Keys Marine Lab at 1:06 am. Fertilization was checked; cell division was not observed, but gametes were treated as fertilized. All eggs/embryos were washed and consolidated. Sperm was present, but concentration seemed dilute in comparison to work with *Acropora cervicornis*. To remove zooplankton, most eggs/embryos were gently cupped while some were either pipetted or slowly siphoned through a sieve submerged in water. Gametes were then transferred into fat separators, rinsed with fresh filtered saltwater, and put into polystyrene pans around 3:45 am. To avoid further disruption, no post-rinsing counts were conducted.

Gametes were checked the following morning (8:00 am). Most (estimated 98%) eggs had lysed and were decomposing, and surface waters were mopped multiple times throughout the day. At 2:00 pm, approximately 150 larvae of normal appearance larvae were observed swimming. In addition, an estimated 300 swimming larvae of significantly smaller size were observed. It is hypothesized that these smaller larvae are a result of disruptions to the gametes during cell division. This has been found in some other coral species to occur via perturbation during early cell division and leads to smaller but still viable larvae (Heyward and Negri 2012,

Chamberland et al. 2017). All larvae were separated by size class and put into 1 liter bottles for transport to the Center for Conservation the night of Aug 18
Larvae from Aug 18 spawning were transported by bucket directly to Florida Aquarium's Center for Conservation. They arrived at 6:30 am on Aug 19 and were sorted by pipetting potential larvae into sterile seawater containers.

The evening of Aug 19, all larvae from both nights were placed into a plastic wash basin with 55 micron mesh on the sides. The basin was placed in a recirculating quarantine system containing sand and crushed CCA retrieved from tanks with adult *D. cylindrus*. It is hoped that free-living zooxanthellae were present within the substrate and available to settlers. Within the settlement basins were settlement tiles of various shapes and sizes. Bins will remain untouched for several weeks, as previous settlement events have shown early settlers to be poorly attached until skeletal structures are deposited.

General notes on fertilization/rearing:

Despite improved on-water protocols to concentrate eggs and minimize zooplankton by sieving, 2019 fertilization rates as measured 3.5 hours after gametes were mixed indicated zero fertilization. However, the presence of larvae the following day indicates that fertilization did occur. Though not directly comparable due to time differences in observations between years, it is likely that fertilization rates were lower than normal in 2019. Apparent fertilization rates have varied over field spawning years. In 2016 and 2017, fertilization was greater than 90%. In 2018, fertilization was less than 10%. This was attributed to the early release of male gametes during that spawning season, but 2019 observations suggest alternate hypotheses:

- Delayed fertilization. Though spawning of males and females was relatively synchronous in 2019 compared to 2018, the methods for concentration of eggs did delay gamete mixing. However, timing of fertilization during the second night in particular was similar to 2016 and 2017, yet fertilization rates remained low. Minimizing the time between spawning and fertilization continues to be a variable worth considering; sperm motility is a known factor affecting fertilization rates in other species.
- Egg and larval damage. The presence of small swimming larvae suggests that at least some developing embryos underwent disruptive cleavage. Two of the egg collections from Aug 17 were dumped directly into bins rather than undergoing gentle siphoning which may have caused damage; however, the others were not disturbed. Post-fertilization transport in buckets took notably longer in 2019 than in previous years; it is possible that this led to diminished fertilization rates.
- Though low fertilization in 2018 was attributed to early sperm release, low rates in 2018 and 2019 could in fact be the result of the condition of the parent corals. In both years, most individuals were affected with Stony Coral Tissue Loss Disease. Though releases were still large, the condition of the gametes themselves may be poorer and thus result in low fertilization/development rates.

Future Efforts

The larvae settling at Florida Aquarium's Center for Conservation will be checked in September to determine whether settlers are present. Field observations from 2012-2019 are in preparation for a manuscript to be submitted September 2019.

It is likely that no future field spawning collections will be possible for *D. cylindrus* in Florida. Western Dry Rocks represents the westernmost location of multiple adjacent genotypes and was targeted in 2019 as the last

surviving individuals of the species on the FRT. Over 50% of the colonies were infected at the time of 2019 spawning and are not expected to survive until August 2020.

Nevertheless, the many years of field observations and rearing of larvae in laboratory settings have provided the information needed to predict and even replicate *D. cylindrus* spawning in the lab and to better understand and improve development and settlement. Efforts by the Florida Aquarium to artificially control spawning within this species promise opportunities to initiate multiple spawning events a year, and to have immediate capability to fertilize and provide a calm and safe haven for development and settlement of this species.

Acknowledgements:

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