Nursery management and treatment of disease-ravaged pillar coral (*Dendrogyra cylindrus*) on the Florida Reef Tract

Final Monitoring Report

Florida Department of Environmental Protection
Coral Reef Conservation Program

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1.0 Project Background

The Atlantic Pillar Coral, *Dendrogyra cylindrus*, has suffered catastrophic losses along the Florida Reef Tract in the past three years due primarily to disease. Beginning in January 2016, the Pillar Coral Genetic Rescue Project is a targeted effort between multiple partner agencies to bring in fragments of distressed genotypes for long-term care, growth, and emergency treatment. The Florida Aquarium joined this effort in August 2016, and has housed pillar coral colonies at the Center for Conservation (CFC) in Apollo Beach, Florida since this time.

The Florida Aquarium has worked to optimize ex-situ culture conditions for pillar coral, including modifying aquaria systems to increase growth rates and testing disease treatment methods in order to better manage the ex-situ population and develop treatments that may be applicable in-situ. Monthly health assessment data is collected on all colonies in the collection per permit requirements. In addition, as a certified member of the Association of Zoos and Aquariums (AZA), The Florida Aquarium maintains an updated inventory and medical records of all animals at the facility. Historical health assessment, inventory, and medical treatment data was compiled for this project report.

Throughout the genetic rescue project, fragments have also been transported to and held at the National Oceanographic and Atmospheric Administration – National Ocean Service laboratory in Charleston, SC (NOS), The Elizabeth Moore International Center for Coral Reef Research & Restoration in Summerland Key, FL (Mote), and the Keys Marine Laboratory in Long Key, FL (KML). A summary spreadsheet showing the current disposition of all fragments, collection sites, genotype, and the status of the wild colonies is provided in the **Data Deliverables – Task 1_DCYL Frags in Nurseries.xlsx.** As of June 2018, the pillar coral genetic archive at CFC consists of 106 colonies from up to 33 unique genotypes, 15 of which are extinct in the wild and 5 of which are currently diseased in the wild (**Table 1**).

2.0 Ex-situ Growth, Bleaching and Long-term Health Observations (Task 2)

Several key modifications were made to aquarium systems at the CFC in order to maximize coral health and growth. These modifications included upgrades to the chilling capacity of aquaria, increased water circulation, adjusting light levels to stimulate calcification rates, and reducing dissolved organic carbon by utilizing specialized filter media. All pillar coral colonies are now maintained at 400 – 600 µmol photons/m^2/sec of photosynthetically active radiation (PAR), a key factor to increasing growth. Several genotypes show a high growth rate in the archive, visible by encrusting growth over the epoxy attachment point and concrete base that each colony is mounted on (**Photos 1 and 2**).

In order to determine which genotypes show good growth characteristics, a collection of photographs taken in April/May 2017 were compared to those taken in April/May 2018 for each colony. Based on the review of these photographs and visual inspection in the nursery, each genotype was given a growth rate ranking of 1 (fast, visibly noticeable coverage over attachment point and base), 2 (noticeable growth but not fully covering attachment point or onto base) or 3 (minimal growth).

In three cases, partial generalized bleaching or paling has occurred within the archive that is unrelated to disease and does not occur with any signs of tissue recession. These cases have occurred either in late summer, when water temperatures are warmer, or in spring, when light intensity is increasing in the nursery. In every case, these fragments have fully recovered and resumed normal coloration **(Photo 3).**

In order to summarize disease occurrence/susceptibility, treatments, growth, and bleaching occurrence for each genotype currently held in the CFC nursery, historical data was compiled for each fragment of each genotype, and consolidated into a single spreadsheet by genotype and/or collection location. This summary table is provided in the **Data Deliverables – Documents – Task 2_Genotype Characteristics.xlsx**.

3.0 Disease Treatment Trials and Progression (Task 3)

Fragments Brought to CFC in 2016 - Prior to Antibiotics

In August and September 2016, the first 22 *D. cylindrus* fragments were brought to the CFC from Keys Marine Laboratory and Mote Marine Laboratory. Shortly after arrival, six individuals showed tissue recession that began either at the base of the fragment or at a single focal point on colony and progressed rapidly (**Photo 4**). The receding line of tissue was often preceded by a 1-2 cm area of pale tissue with poor polyp extension, similar to white plague signs in the wild. Potentially diseased corals were separated into smaller medical aquaria. The diseased area was amputated and samples were preserved. Corals were given a 15-minute dip in a Lugol's iodide solution at a concentration of 0.5 ml Lugol's per liter of seawater. This treatment is traditionally used in the aquarium industry as both a prophylactic dip and medical treatment for corals.

Medicated Lugol's dips and amputations were repeated as necessary, and corals were maintained in the cleanest possible aquarium conditions including low total organic carbon (TOC), low nutrients and cool water temperatures (less than 24ºC). Out of the six original corals, 13 total fragments resulted after amputations. Two were lost completely (15.4%), seven were stabilized after repeated medicated dips and amputations, and four had re-occurring symptoms into 2017 (**Table 2**). The four fragments with re-occurring symptoms were eventually treated with antibiotic dental paste in 2017 and were stable after treatment. Overall, treatment with Lugol's dips and amputations alone were successful in 53.4% of cases where corals showed symptoms indicative of disease. However, repeated amputations often resulted in very small fragments.

In three other individuals, a generalized patchy loss of coenosarc tissue was observed that progressed at a slow rate, and was not consistent with disease symptoms mentioned above (**Photo 5**). In addition, one individual showed signs of "brown jelly" syndrome, a condition associated with ciliate infection in aquarium corals (colony 111b). Relocation to medical aquaria, amputation of the "brown jelly" area, and a single medicated dip in Lugol's iodide was 100% effective in these four cases. Biologists and veterinary staff at the CFC believe that these cases were not due to the "tissue loss disease" as seen in wild corals, however no microbial analysis was done.

In order to investigate the potential difference in aquarium conditions between holding raceways and medical aquaria, water quality samples were sent to an analytical laboratory to be tested for major ion constituents, trace metals, nutrients, and total organic carbon. Results showed that the medical aquaria had lower concentrations of total organic carbon (< 2.0 mg/l), while all other elements were comparable. The Florida Aquarium then implemented measures to control dissolved organic carbon in all raceways/aquaria by the addition of media reactors containing activated carbon and Polyfilter (Poly-Bio-Marine, Inc.), both known to absorb organic material.

Time series photographs of all corals that arrived in 2016 that were treated for signs of tissue recession and/or disease are provided in the **Data Deliverables – Photographs – 2016 Collection Treatments.**

Fragments Brought to CFC in 2017 – Treatment with Antibiotic Paste

In February 2017, 23 pillar coral colonies were brought to the CFC that were collected from sites with active disease, but were collected from visually healthy colonies ('Category 2' fragments). Beginning approximately one month after arrival, several corals from diseased sites began to show signs of disease (**Photo 6**). Primary symptoms included rapid tissue recession from the base of the colony or a single focal point, often preceded by a pale area with poor polyp extension. Early warning signs were seen as a single pale spot with poor polyp extension (**Photo 7**). Although fragments were collected from different colonies or from different ramets of the same colony, signs of disease manifested at similar times in corals collected from the same sites. All fragments that were collected from Conch Reef and Staghorn Acres were affected.

A total of twelve potentially diseased fragments were amputated as necessary and this resulted in 20 total pieces of coral that needed treatment. Initially, fragments were amputated and dipped in Lugol's iodide or Revive (Two Little Fishes, Inc.). Shortly prior to this disease outbreak, an antibiotic laden dental paste mixture had been developed and used successfully by Dr. Cheryl Woodley at NOAA-NOS in Charleston, SC. In order to attempt to arrest the ongoing disease outbreak at the CFC, The Florida Aquarium received the dental paste mixture in April 2017. Amoxicillin at a ratio of 250 mg amoxicillin/4g of dental paste was mixed together immediately prior to treatment. The dental paste mixture is applied to the coral out of the water and is applied onto the skeleton of the coral along the receding margin (**Photo 8**).

A summary of what treatments were provided to which fragments is shown in **Table 3**. Of the 20 fragments, two were lost before antibiotic treatment was available, five recovered with dips and amputation (25%) and the remainder (13 fragments) were treated with amoxicillin dental paste. Of the 13 fragments treated with antibiotics, 12 recovered and are currently alive at the CFC (92.3% survival).

Time series photographs of all corals that arrived in 2017 that were treated for signs of tissue recession and/or disease are provided in the **Data Deliverables – Photographs – 2017 Collection Treatments.**

Drug Delivery Formulation with CoreRx

In order to further investigate the antibiotic dissolution properties of the amoxicillin/dental paste matrix used in disease treatment at the CFC, and gather data to support the use of the compound in the wild, The Florida Aquarium contacted a local pharmaceutical formulation and manufacturing company, CoreRx (Clearwater, FL). CoreRx conducted dissolution trials on the amoxicillin/dental paste formula, and found that the antibiotic released into solution in 16 hours (**Figure 1**). Real-time dissolution testing is conducted in artificial seawater in replicated dissolution vessels using a fiber optic UV monitoring system (Pion, Inc.). The Florida Aquarium continued to consult with CoreRx formulators to develop a drug delivery system that would release the antibiotic over a longer period of time, and also could be applied underwater with good adhesion to the coral skeleton even in high water flow.

For each formula, the drug dissolution profile was first tested by CoreRx and targeted for a 2-3 day release of antibiotic. Promising formulas were then tested at the CFC for adhesion to coral skeleton. Initial trials consisted of a white petrolatum base with various excipients (inactive ingredients) to improve drug retention and potential delivery into coral mucus and tissue. However, consistent issues with adhesion and stability of the product resulted in abandoning the initial white petrolatum base formula in May 2018. A new drug delivery formula was developed and tested in May/June 2018 for this project based on a silicone adhesive/sealant with additional excipients to promote delivery to the coral and to slow the release profile of the drug. A drug development and dissolution testing summary, including a list of ingredients, is provided in the **Data Deliverables – Documents – Coral Adhesive Dissolution Report.docx**.

The most recent iteration of this formula was tested at the CFC beginning on June 3, 2018. This formula showed the ability to be applied underwater in water flow and good adhesion onto coral skeleton as long as there was not a heavy biofilm on the skeleton (**Photos 9 and 10**). Removing any biofilm with a wire brush prior to application increased adhesion. The tested formula showed a 36-hour antibiotic dissolution profile. Samples of this formula were provided to Dr. Karen Neely for laboratory testing on diseased corals at Keys Marine Laboratory beginning on June 5, 2018.

Fragments Brought to CFC in 2018 and Current Treatments

In April 2018, an additional 21 fragments were brought to the CFC from both wild collection and transfer from Keys Marine Laboratory. On May 21, 2018, slight tissue recession was observed along the margin of one colony (275). This colony was treated with amoxicillin dental paste on May 24, 2018 and has been monitored daily for recovery (**Photo 11**). Recession appears to have stopped, despite the treatment falling off of the coral within the first 24 hours.

In addition, fragment 197-F01 has shown re-occurring symptoms of disease, characterized by a distinct pale margin and tissue recession beginning on one corner. This fragment has undergone previous antibiotic treatment, and occasionally shows re-occurrence of symptoms. The fragment was treated on May 24, 2018 and is being monitored daily for recovery. Recession appears to have stopped, however the tissue at one end remains pale.

Time series photographs of all corals that are currently undergoing treatment at the CFC are provided in the **Data Deliverables – Photographs – Current Treatments.**

4.0 Summary, Current Status, and Recommendations (Task 4)

A total of 82 fragments of *D. cylindrus* have been brought to the CFC nursery, either directly from wild collection or from other ex-situ facilities. Through fragmentation, mostly to control disease spread, these original 82 corals have now been divided into 106 pieces. Currently, two corals are undergoing antibiotic treatment and recovery (197-F01 and 275). All other corals in the collection are healthy. Photographs of all corals in the CFC pillar coral archive from May 2018 are provided in the **Data Deliverables – Photographs – Inventory 052818** and current health assessment data is provided in **Data Deliverables – Documents-Task 4_May Health Assessment.xlsx.**

Overall, some fragments were able to be stabilized through amputation, medicated baths, and consistent good husbandry practices including minimal organic content in the water. In corals that arrived in 2016, prior to the availability of antibiotic treatment, 53.4% of the corals that broke with disease after arrival were successfully treated in this manner. In corals that arrived in 2017, only 25% were stabilized using only amputation and medicated baths. In total, amoxicillin dental paste has been used on 18 coral fragments, and 15 of these corals survived. The three individuals that did not survive were very small fragments that had been repeatedly amputated and treated with medicated dips. If corals were treated early, the treatment has been 100% effective. For ex-situ population management, amputation and treatment with antibiotic ointment as soon as symptoms appear is the recommended course of action.

The partnership with CoreRx has allowed us to understand more fully the antibiotic release characteristics of various treatment options. Looking at the current coral disease problem from a medical/veterinary perspective, it is sensible to consider developing a medical treatment for the disease and pursuing the possibility of treating corals in the wild. Although the reality of treating corals on a large scale may not be feasible or is environmentally risky, these techniques can be targeted to highly reduced populations such as *D. cylindrus*, or high value target reefs.

Further studies into the microbial community before and after antibiotic treatment would help to clarify the effects of the antibiotic treatment and possible reasons for recovery. In addition, further testing into drug delivery methods may allow the development of a compound that is targeted for delivery of the antibiotic into coral mucus and/or tissue cell membranes, but not release the compound into the water column. In addition, other non-antibiotic active ingredients can be investigated and delivery systems developed for those compounds if shown to be effective against the disease. Partnering with companies that conduct this sort of work for human and animal pharmaceuticals may lead to new innovations in ways to respond to coral disease outbreaks.

Table 1. Genotype ID, collection site, and number of fragments of *D. cylindrus* in each size class currently held at the Florida Aquarium Center for Conservation ex-situ nursery. XS: Less than postage stamp, S: Less than slice of bread, M: Less than notebook paper, L: Larger than notebook paper

Genotype	Tag IDs	Site Collected	Size Class				Total
			XS	S	M	L	
D1042	145, 191, 192, 194-197, 199, 200	Conch3, Conch 4	1	12			13
D1055	255	Sombrero SPA 2-2			1		1
D1059	062	DAP 07		2			2
D1066	065	Sombrero SPA 1		2			2
D1076	204, 252	LKL 1			2		2
D1092	025, 13	Marker 32-1		4			4
D1103	187, 201	Crocker 3			1	1	2
D1105	211, 212, 213, 214	Critter Ridge		12	1		13
D1109	023, 288, 289	Marker 32-3, Col 2,3		3			3
D1128	0025	Bahia Honda 4		4			4
D1131	205, 206, 207, 253	Coffins		10	2		12
D1170	022	Marker 32-3, Col 1,7		1			1
D1240	066	Sombrero Out		2			2
D1367	080, 153, 154, 155	Carysfort 8		1	3		4
D1369	087, 089	Elbow 5		3	1		4
D1371	100	French 2		1			1
D1375	129, 189, 190	Conch 1		2	1		3
D1376	111b	Crocker 4		1			1
D1377	203	Crocker 4			1		1
D1378	254	Long Key Bridge (Ari's)	1		2		3
D1380	208, 209, 210	Stag Acres		3			3
D1392	276, 277	Looe West 3		2			2
D1393	278	Looe West 5		1			1
D1395	279, 280	Pelican 2, Col 1		2			2
D1397	122	Molasses 10		3			3
D1398	131	Pickles 8			1		1
D1402	024	Marker 32-2, Col 2-2		1			1
D1404	026, 054, 055	Middle Sambo		5			5
D1410	10	Western Dry Rocks 2, Col 2		1			1
Unk1	281, 282, 283, 284, 285	Lonny's Pillars		1	4		5
Unk2	275	Mote SWG				1	1
Unk3	286, 287	Western Sambos		1	1		2
Unk4	056	DAP 33		1			1
		Grand Total	**2**	**81**	**21**	**2**	**106**

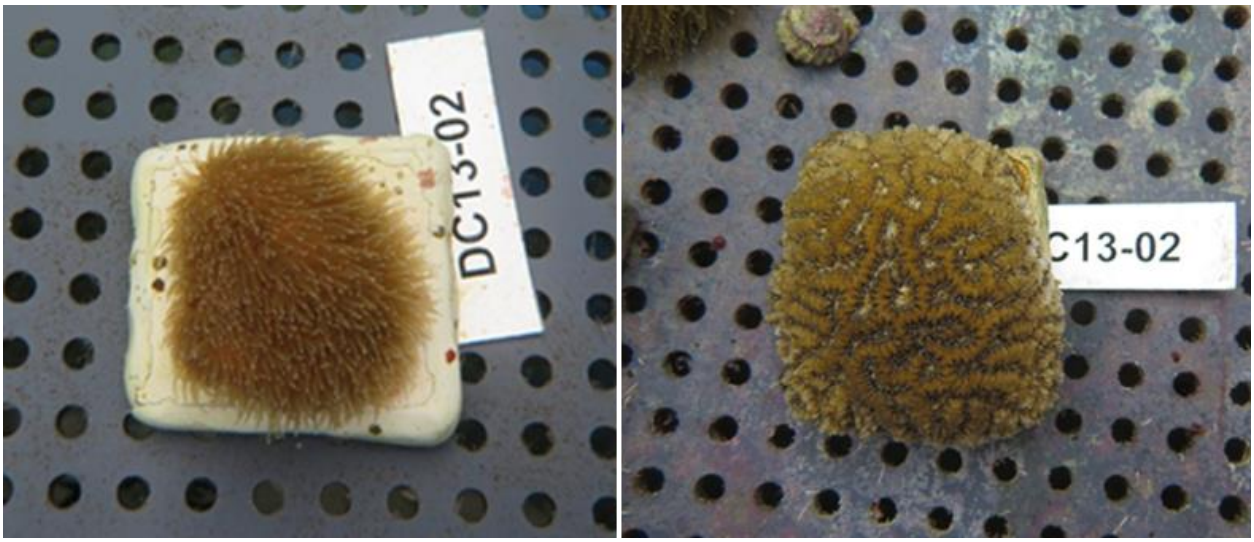

Photo 1. *D. cylindrus* fragment 13-02 on May 25, 2017 (Left) and May 28, 2018 (Right). Genotype shows good growth over attachment point and base.

Photo 2. *D. cylindrus* fragment 208 shortly after amputation and amoxicillin paste treatment on April 28, 2017 (left) and in the CFC nursery on June 28, 2018 (right).

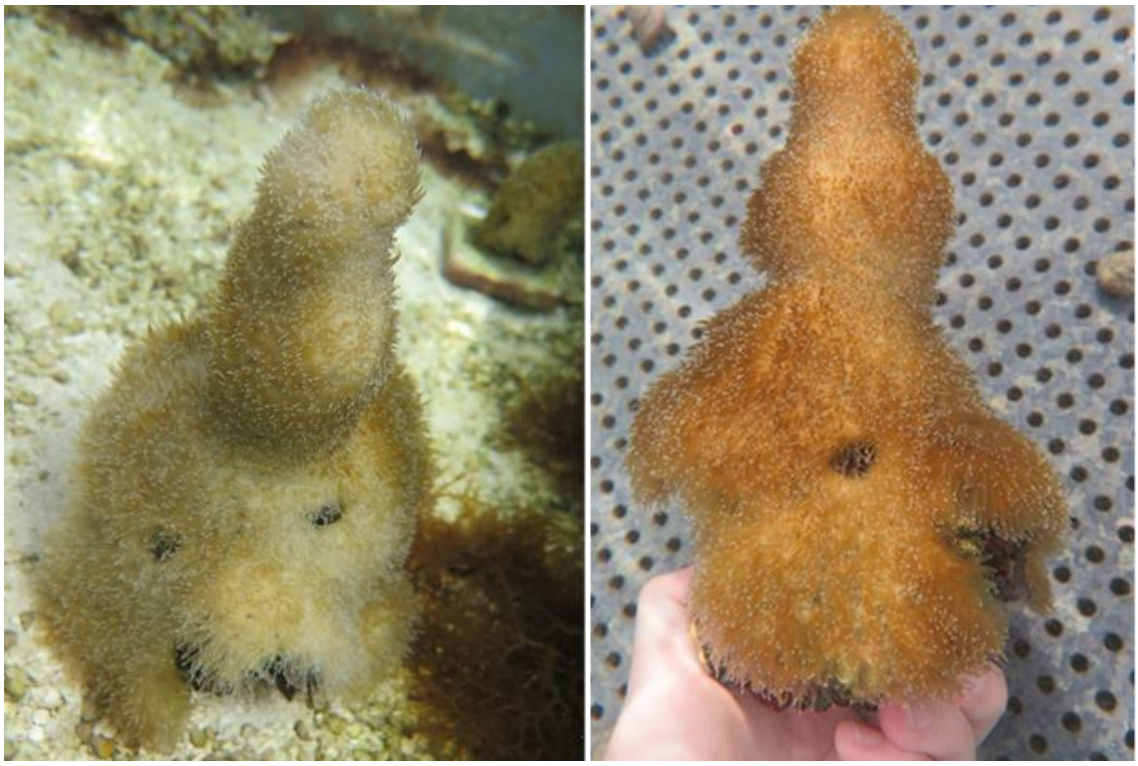

Photo 3. *D. cylindrus* colony 131 showing partial bleaching on September 26, 2017 (left) and recovering color on November 25, 2017 (right).

Table 2. Tag ID numbers, treatments and outcome for corals brought to the CFC in 2016. F-numbers in TagID indicate a fragment of the original coral. *Dip/Amp*: medicated dips in Lugol's Iodide and amputation of diseased tissue. *Amox Paste*: Treated with amoxicillin dental paste mixture.

YearIn	TAGID	Dip/Amp	Amox Paste	Alive?
2016	102	Y	N	N
2016	122	Y	Y	Y
2016	122-F01	Y	N	Y
2016	122-F02	Y	Y	N
2016	127	Y	N	N
2016	065	Y	N	Y
2016	065-F01	Y	N	Y
2016	066	Y	N	Y
2016	066-F01	Y	N	Y
2016	089	Y	N	Y
2016	089-F01	Y	Y	Y
2016	089-F02	Y	Y	N
2016	089-F03	Y	Y	Y

Photo 4. Examples of disease symptoms observed in the CFC nursery in 2016. Fragment Tag 102 (left) and 066 (right).

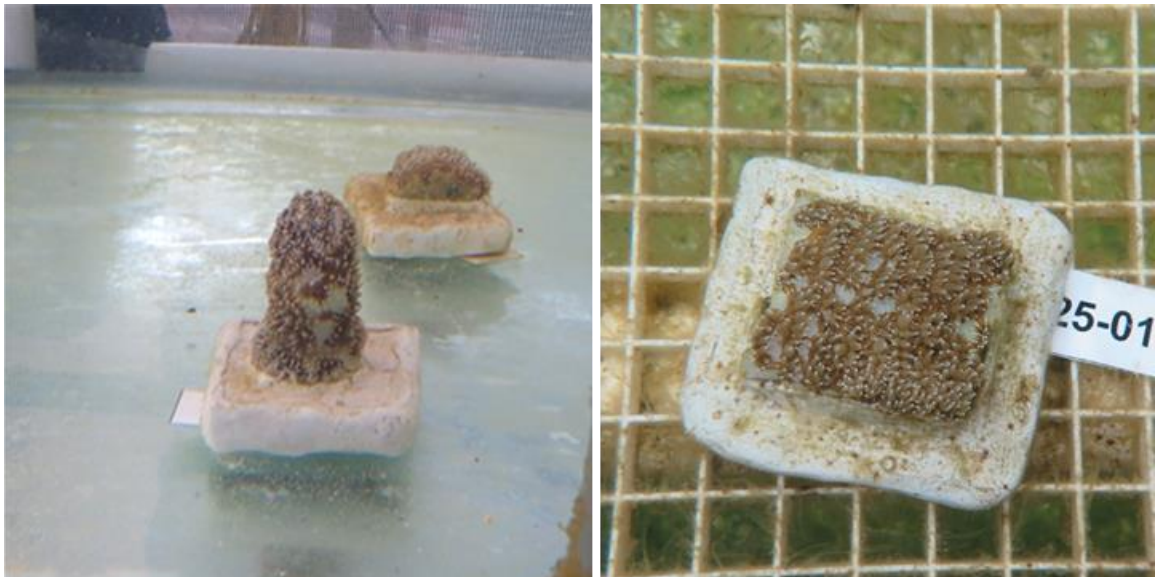

Photo 5. Examples of slow, generalized coenosarc recession observed in some fragments. Fragment 10-01 (left) and fragment 0025-01 (right).

Photo 6. *D. cylindrus* fragments 195 (left) and 192 (right) showing pale tissue, poor polyp extension, and tissue recession on March 21, 2017. Fragment on the right is shown in Lugol's Iodide dip. Both fragments were collected at Conch Reef on February 17, 2017.

Photo 7. Early sign of disease on fragment 208 seen as pale spot of tissue with poor polyp extension.

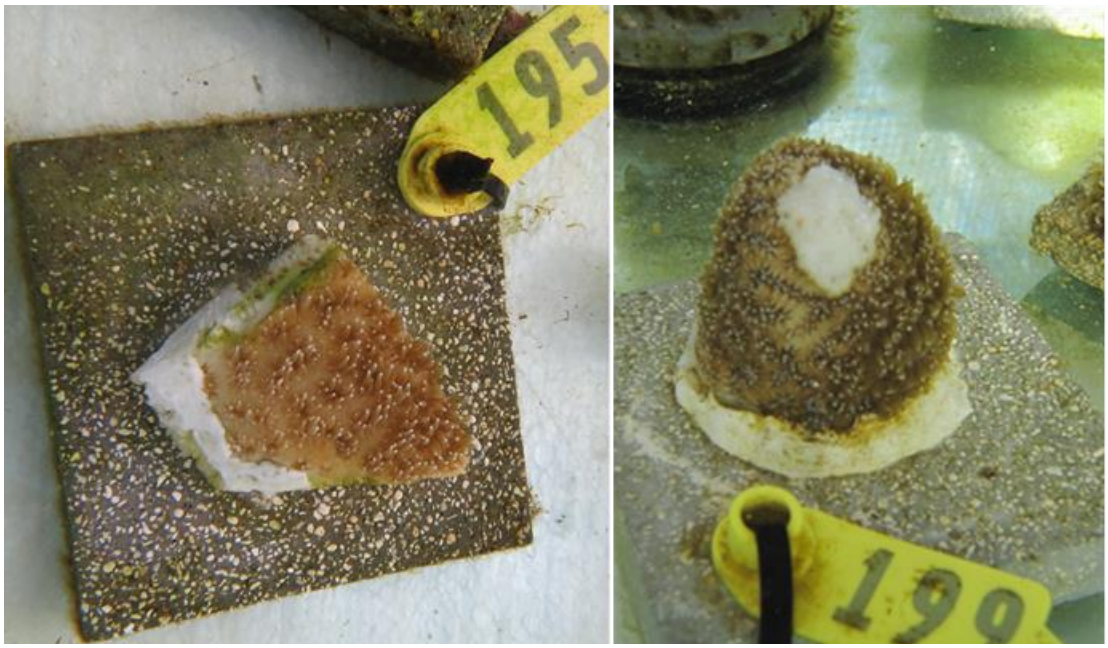

Photo 8. Fragments treated with amoxicillin laden dental paste mixture

Table 3. Tag ID numbers, treatments and outcome for corals brought to the CFC in 2017. F-numbers in TagID indicate a fragment of the original coral. *Dip/Amp*: medicated dips in Lugol's Iodide or Revive and amputation of diseased tissue. *Amox Paste*: Treated with amoxicillin dental paste mixture.

YearIn	TAGID	Dip/Amp	Amox Paste	Alive?
2017	191	Y	Y	N
2017	191-F01	Y	N	N
2017	192	Y	N	N
2017	194	Y	N	Y
2017	195	Y	Y	Y
2017	195-F01	Y	Y	Y
2017	195-F02	Y	Y	Y
2017	196	Y	Y	Y
2017	197	Y	N	Y
2017	197-F01	Y	Y	Y
2017	197-F02	Y	Y	Y
2017	197-F03	Y	N	Y
2017	199	Y	Y	Y
2017	199-F01	Y	N	Y
2017	200	Y	N	Y
2017	208	N	Y	Y
2017	209	N	Y	Y
2017	210	N	Y	Y
2017	207	N	Y	Y
2017	207-F02	N	Y	Y

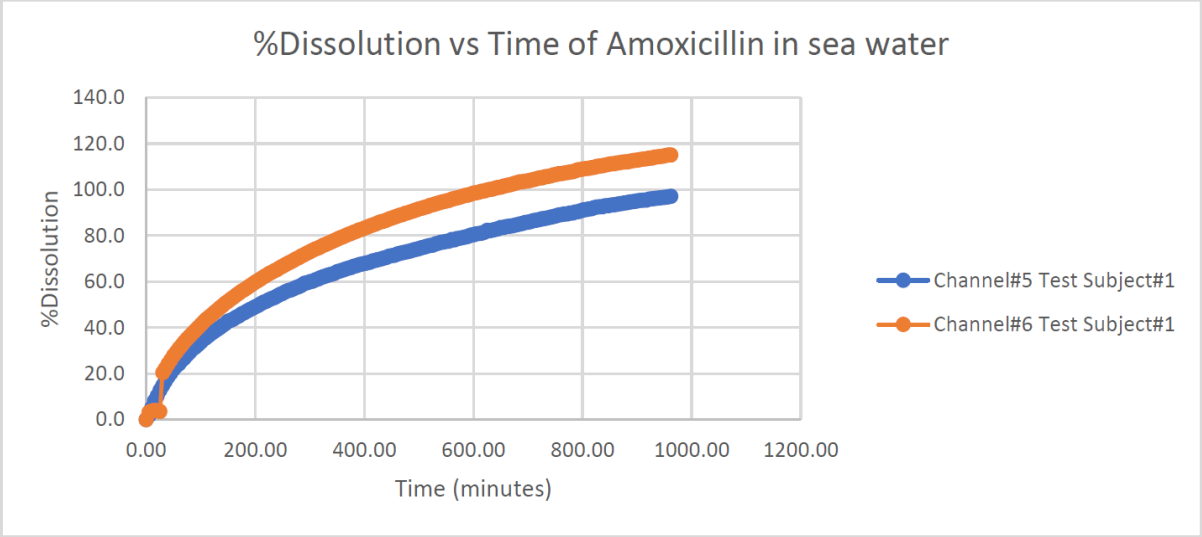

Figure 1. Dissolution of amoxicillin from the dental paste formula used to treat diseased pillar coral colonies ex-situ. Results from CoreRx.

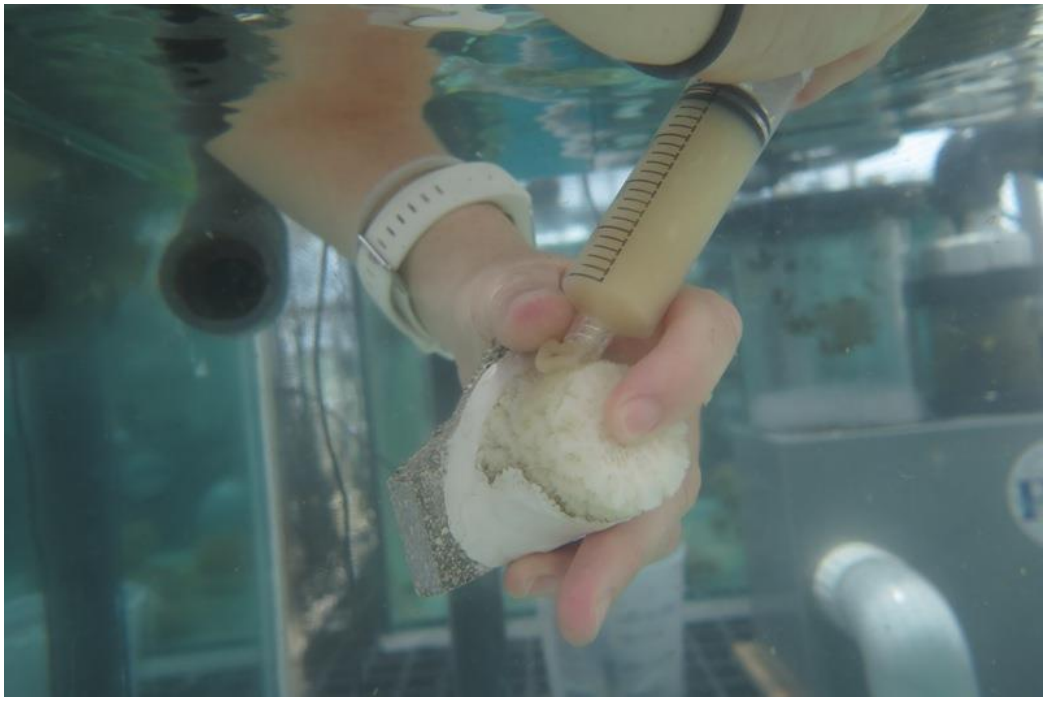

Photo 9. Testing application of CoreRx drug delivery formula on pillar coral skeleton in high water flow and underwater on June 3, 2018.

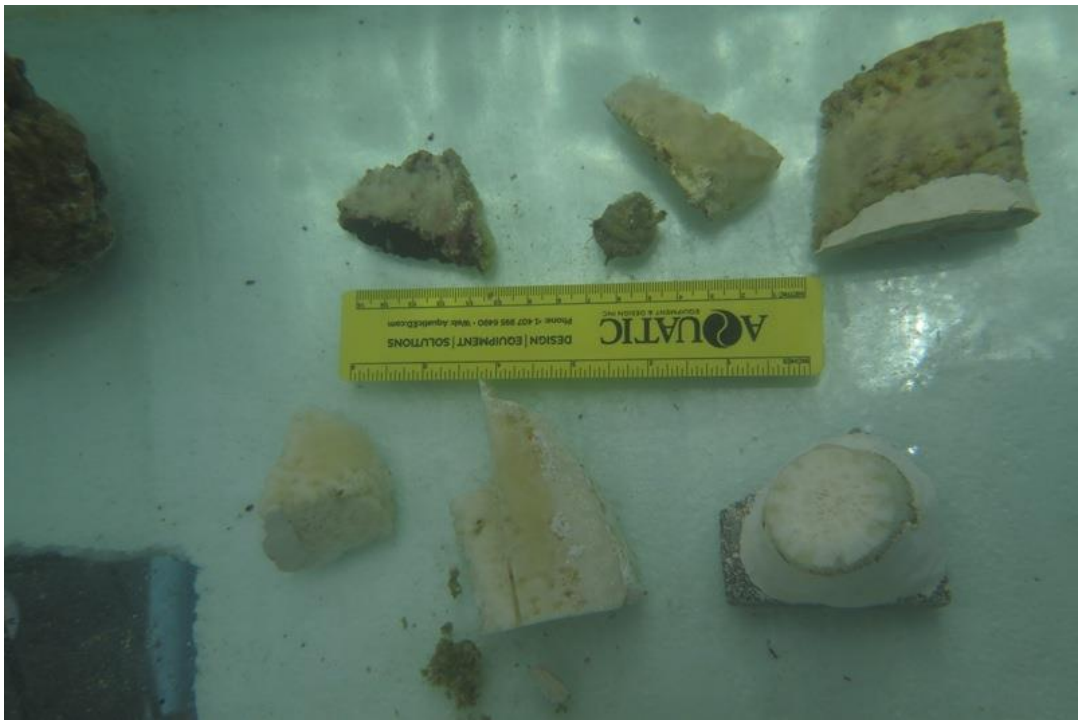

Photo 10. Testing adhesion of CoreRx drug delivery formula on coral skeletons with varying levels of biofilms.

Photo 11. *D. cylindrus* colony 275 collected from the wild in April 2018 immediately after amoxicillin dental paste application on May 24, 2018.