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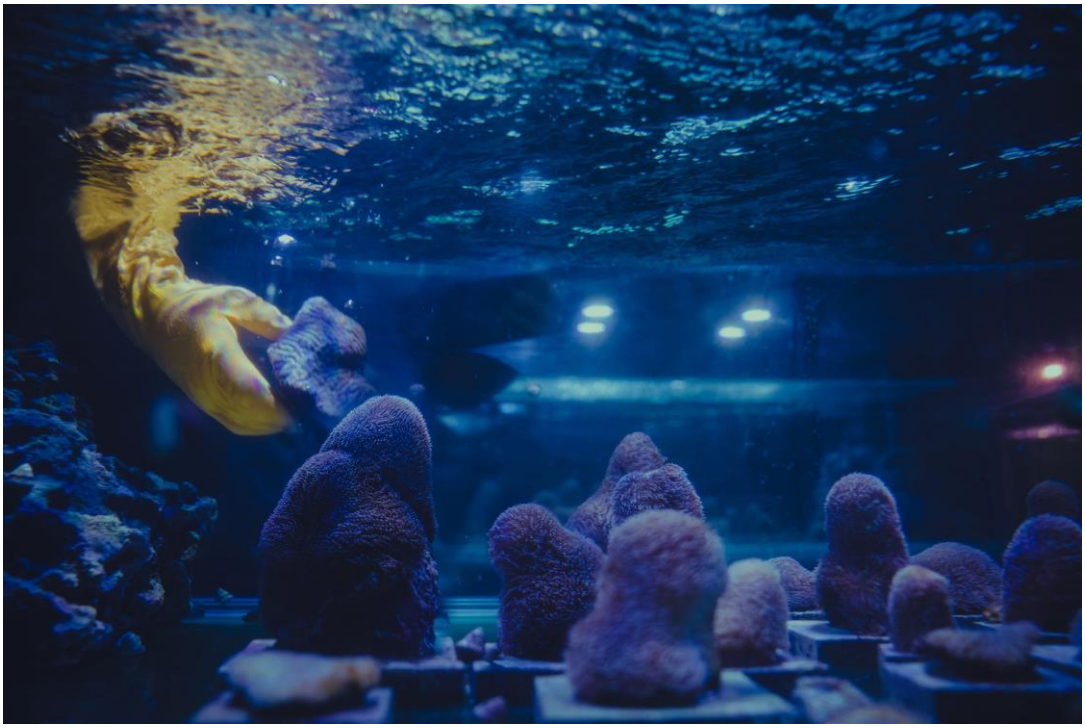
**NOAA Technical Memorandum NOS NCCOS 245**

**NOAA Technical Memorandum Coral Reef Conservation Program (CRCP) 37**

# *Exploratory Treatments for Stony Coral Tissue Loss Disease: Pillar Coral (Dendrogyra cylindrus)*



Collection of *Dendrogyra cylindrus* fragments from Florida reefs rescued and rehabilitated at NOAA NOS NCCOS in Charleston, SC. (*Photo credit: Paul Chelmis*)

NOAA National Centers for Coastal Ocean Science
Stressor Detection and Impacts Division
Key Species and Bioinformatics Branch
Charleston, SC

**November 2020**

| United States Department of Commerce | National Oceanic and Atmospheric Administration | National Ocean Service |
|---|---|---|
| Wilbur L. Ross, Jr.<br>Secretary | Neil A. Jacobs, Ph.D.<br>Assistant Secretary of Commerce,<br>Acting NOAA Administrator | Nicole R. LeBoeuf<br>Acting Assistant Administrator |

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# *Exploratory Treatments for Stony Coral Tissue Loss Disease: Pillar Coral (Dendrogyra cylindrus)*


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## About this document

This report represents a chronology over four years (2016-2019) of seven collection and rescue events, experimental treatments, and rehabilitation of *Dendrogyra cylindrus* genotypes afflicted with stony coral tissue loss disease (SCTLD) from Florida reefs. The laboratory work described here was conducted by NOAA's National Centers for Coastal Ocean Science (NCCOS), Coral Health and Disease Program. Our work was part of a larger effort to preserve as many genotypes of this species as possible from extinction, driven by a deadly disease outbreak. Using a pragmatic approach to the exploratory research reported here, we dealt with the sickest of the sick specimens that were collected to remove as many representatives of this species as possible from harm's way. With each new group of diseased *D. cylindrus* coming in for treatment, our knowledge, skill and success in rehabilitation increased. These combined efforts resulted in our facility receiving 208 fragments; 176 specimens of the original fragments were successfully treated with no further sign of disease and represented at least 76 distinct genotypes. These small remnants of once tall stately pillars now reside in *ex situ* facilities awaiting a time when they can contribute to restoring this species to the wild, through growth and fragmentation and/or ex situ sexual reproduction.

NCCOS and NOAA's Coral Reef Conservation Program (CRCP) provided funding for this project. NCCOS scientists led the efforts described here in collaboration with partners from Biscayne National Park, Southeast Regional Office of NOAA Fisheries, Nova Southeastern University, Keys Marine Laboratory, Florida Aquarium, Mote Marine Laboratory, Frost Museum and Florida Keys National Marine Sanctuary who made this work possible.

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# Executive Summary

Pillar coral (*Dendrogyra cylindrus*) is a rare Caribbean coral with only one species in its genus. Because of population declines, the species was listed as threatened under the U.S. Endangered Species Act in 2014 (Federal Register 2014). By 2020, the species had further catastrophic population declines of an estimated 70 % along the Florida Reef Tract from back-to-back bleaching events, white plague, and a spreading disease now referred to as stony coral tissue loss disease (SCTLD) (Precht 2016; Neely *et al.* 2017; FKNMS 2018). The estimated number of surviving genotypes within the Florida population has declined from 181 to 51 (Neely personal communication March 2020). The remaining population is assumed to be reproductively extinct and at high risk for regional extinction (Neely *et al.* 2017). At least 40 of the remaining genotypes are heavily diseased or have less than 5% tissue remaining (Neely *et al.* 2020).

In 2016, a multi-institutional collaboration was initiated to rescue remaining genotypes from the wild and place them into *ex situ* and *in situ* nurseries. As part of this effort, the NOAA NOS NCCOS Coral Health and Disease Program participated by conducting exploratory experimentation to treat, recover and rehabilitate diseased *D. cylindrus* genotypes. These efforts culminated in the following conclusions:

- Antibiotic treatments (amoxicillin and ampicillin) were successful in arresting the tissue loss and further disease progression of most diseased *D. cylindrus* fragments treated in the laboratory.
- A customized drug delivery vehicle, coral dental paste (CDP), was found effective in providing targeted drug delivery and controlled release of the therapeutic agents.
- Based on this work the most effective treatment is a timely regimen of:
    - Resecting the diseased portion of the fragment up to 1 cm ahead of the disease margin
    - Immersing the fragment in 0.5 mL/L Lugol's solution (10 % potassium iodide, 5 % iodine) for 15 min followed by an artificial seawater rinse
    - Applying amoxicillin in CDP (50 mg/mL) to the freshly resected tissue margin
        - In some cases 7 days of ampicillin at 100 mg/L in artificial seawater with daily 100% treatment renewal may be indicated
    - Performing daily artificial seawater changes (100 %) for two weeks post treatment to ensure the coral remains asymptomatic
- Early intervention combined with spot-treatment of diseased areas after initial treatment reduced coral fragment mortality to nearly zero. After best practices were implemented, a 97 % success rate was achieved.

Seven collection efforts (2016-2019) combined with acquisitions transferred from two other laboratories resulted in our facility receiving 208 fragments (an additional 29 subfragments were created during triage); 176 specimens of the original fragments were successfully treated and show no further sign of disease. The original fragments were collected from 83 different colonies representing at least 76 distinct genotypes.

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# List of Acronyms and Abbreviations

| | |
|---|---|
| BIP | 2,2’-bipyridil |
| BISC | Biscayne National Park |
| BJS | brown jelly syndrome |
| CDP | coral dental paste |
| CRF | Coral Restoration Foundation |
| DCYL | *Dendrogyra cylindrus* |
| DP | Dottie’s Pharmacy |
| DRTO | Dry Tortugas |
| E7 | Elbow Reef site 7 |
| ESA | Endangered Species Act |
| FKNMS | Florida Keys National Marine Sanctuary |
| FLAQ | Florida Aquarium |
| frag | coral fragment |
| FWC | Florida Fish and Wildlife Conservation Commission |
| FWRI | Fish and Wildlife Research Institute |
| h | hour |
| KML | Keys Marine Laboratory |
| L | liter |
| LKLD | Long Key Ledge reef sites |
| lb | pound(s) |
| min | minute(s) |
| mg | milligram |
| mL | milliliter |
| Mote | Mote Marine Laboratory |
| NCCOS | National Centers for Coastal Ocean Science |
| NMFS | National Marine Fisheries Service |
| SERO | Southeast Regional Office |
| NOAA | National Oceanic and Atmospheric Administration |
| NOS | National Ocean Service |
| PAR | photosynthetically active radiation |
| PCCA | Professional Compounding Centers of America |
| PCF | Pillar Coral Forest |
| PP | Plantation Pharmacy |
| ppt | parts per thousand |
| spp. | species |
| SCTLD | stony coral tissue loss disease |
| TMASW | Tropic Marin artificial seawater |
| TR2 | Turtle Rocks reef site 2 |
| U | units |
| W | watts |

# Introduction

Pillar coral (*Dendrogyra cylindrus*) is a rare Caribbean coral with only one species in its genus and hosts a highly specific algal symbiont, *Breviolum dendrogyrum* (Lewis *et al.* 2019). It is a stony coral with colonies able to reach 3 meters in height. Colonies emerge as cylindrical spires that grow vertically from an encrusting base and can extend more than 10 cm in diameter (Humann & Deloach 2013). Because of population declines from a combination of threats including ocean warming, ocean acidification, and disease, *D. cylindrus* was listed as threatened under the U.S. Endangered Species Act (ESA) in 2014 (Federal Register 2014). By 2020, the species had further catastrophic population declines of an estimated 70 % along the Florida Reef Tract from back-to-back bleaching events, white plague, and a spreading disease now referred to as **stony coral tissue loss disease** (SCTLD) (Precht 2016; Neely *et al.* 2017; FKNMS 2018). The estimated number of surviving genotypes within the Florida population has declined from 181 to 51 (Neely personal communication March 2020). The remaining population is assumed to be reproductively extinct and at high risk of regional extinction (Neely *et al.* 2017). At least 40 of the remaining genotypes in the wild are heavily diseased or have less than 5 % tissue that remains (Neely *et al.* 2020).

In 2015, a severe mortality event affecting the entire Florida population of pillar coral and at least 12 other coral species was reported (Precht 2016). The first SCTLD observation was near Virginia Key, Miami-Dade County, Florida. By January 2018, the disease had spread north to St. Lucie Inlet in Martin County and south to Marathon in the Florida Keys. As of March 2020, the disease continued to spread southward throughout the Florida Keys, affecting over 20 coral species. The disease signs associated with SCTLD are focal or multi-focal coalescing, sharply demarcated acute to subacute areas of tissue loss that can begin at the base of the colony and spread apically or appear randomly distributed. In some individuals, the tissue loss area may be bordered by a narrow band of bleached tissue at the tissue-loss margin, though this is not common in D. cylindrus. The etiology of this disease is currently unknown.

The first set of diseased pillar coral fragments was transferred from Biscayne National Park to the NOAA NCCOS Charleston Coral Laboratory in South Carolina for experimental treatment in May 2016. The continuing dramatic decline of the Florida *D. cylindrus* populations prompted a multi-agency coordinated effort in July 2016 to “rescue” fragments of as many extant genotypes as possible to provide a genetic tissue bank, as well as investigate viable treatment and restoration techniques (Moore *et al.* 2017). Fragments collected from the July 2016 rescue event were held in three additional land-based culture facilities including Mote Marine Laboratory (Mote; Summerland Key, FL), Keys Marine Laboratory (KML; Layton, FL), Florida Aquarium (FLAQ; Apollo Beach, FL), and two offshore nurseries (Coral Restoration Foundation (CRF) and Mote). As part of this rescue effort, 20 diseased *D. cylindrus* fragments were collected for treatment testing at the NOAA NCCOS coral facility.

A second multi-agency rescue was conducted in February 2017 to collect remaining genotypes; the most severely diseased fragments were transferred to the NOAA NCCOS coral facility. As the SCTLD outbreak progressed south along the Florida Reef Tract, four additional rescues occurred between April 2018 and October 2019 to acquire new genotypes previously unaffected by disease. The NOAA NCCOS coral facility in Charleston, SC, received a total of 208 pillar coral fragments from the seven collections and acquisitions from two other laboratories representing at least 76 putative genotypes. Partnerships between federal, state, academic, and non-profit agencies have allowed for the rapid response and care for this species to save what remains of the population for future restoration (Neely *et al*. 2017; O'Neil *et al.* 2018).

# Chapter 1. Biscayne National Park *Dendrogyra cylindrus* Rescue

On May 13, 2016, the NOAA Fisheries Office of Protected Resources informed the NOAA NCCOS Coral Health and Disease Program of an ongoing and severe mortality event affecting *Dendrogyra cylindrus* throughout the Florida Reef Tract. Available monitoring data (May 2016) showed that the situation was critical in the Southeast Florida region (~96 % tissue loss and ~85 % total colony mortality) and severe throughout the Florida Keys (~67 % tissue loss, ~7 % total colony mortality). Earlier in the year, Keys Marine Lab (KML), Florida Fish and Wildlife Conservation Commission's (FWC) Fish and Wildlife Research Institute (FWRI) and Mote Marine Laboratory (Mote) launched the project "Pillar coral (*D. cylindrus*) fragment rescue and relocation for preservation of genetic diversity in the Florida Keys Reef Tract" (Jennifer Moore, personal communication). As part of this program, the NOAA NCCOS Coral Health and Disease Program initially received five small diseased fragments (Table 1, Table 2) of *D. cylindrus* on 5/16/2016 from Biscayne National Park for treatment.

## Methods

### *Coral collection and transport*

Five pillar coral fragments from three different colonies (comprising one genotype) were collected from Biscayne National Park (BISC) on May 16, 2016 (Figure 1; Table 1) by park staff. Samples were obtained from colonies 1, 5, and 12 (colony numbers assigned in Neely database, K. Neely personal communication). The colony 12 sample was small with tissue only at the base. A hammer and chisel method was used for sampling with a new chisel used for each colony. The specimens from colonies 1 and 5 were placed in a cooler of ambient seawater while the fragment from colony 12 was held in a separate bucket. Coral fragments were cushioned with Ziploc® bags during transport to shore. Once on shore, coolers with specimens were placed in the shade with aeration for approximately four hours during which time the plastic was removed from the fragments and the transport seawater was replaced with fresh natural seawater. Due to logistical constraints, the BISC specimens were transported to Key Largo, FL, and held for 1.5 days before transport to the NOAA NCCOS Charleston coral facility for treatment. The newly collected specimens were held in 10-gallon coolers filled with approximately 5 gallons of 80 % source water and 20 % artificial seawater (Tropic Marin®, 35 ppt, TMASW, prepared using deionized water). The seawater temperature was regulated with a series of small chiller coils attached to a programmable circulating bath (PolyScience, Niles, IL). Water circulation and aeration were provided by an Aqua-Supreme® AP-8 air pump (Danner, Islandia, NY) and a small airstone in each cooler. During the day, open coolers were placed under shade trees to provide filtered sunlight, and then were brought inside at night for continued monitoring. Daily water changes (~50 %) with TMASW were performed. Corals were transported from Florida to the NOAA NCCOS Charleston coral facility in coolers with constant temperature regulation via van A/C calibrated to 24 °C using a Thermapen® digital thermometer (ThermoWorks, American Fork, UT) and circulation/aeration via pump and airstone.

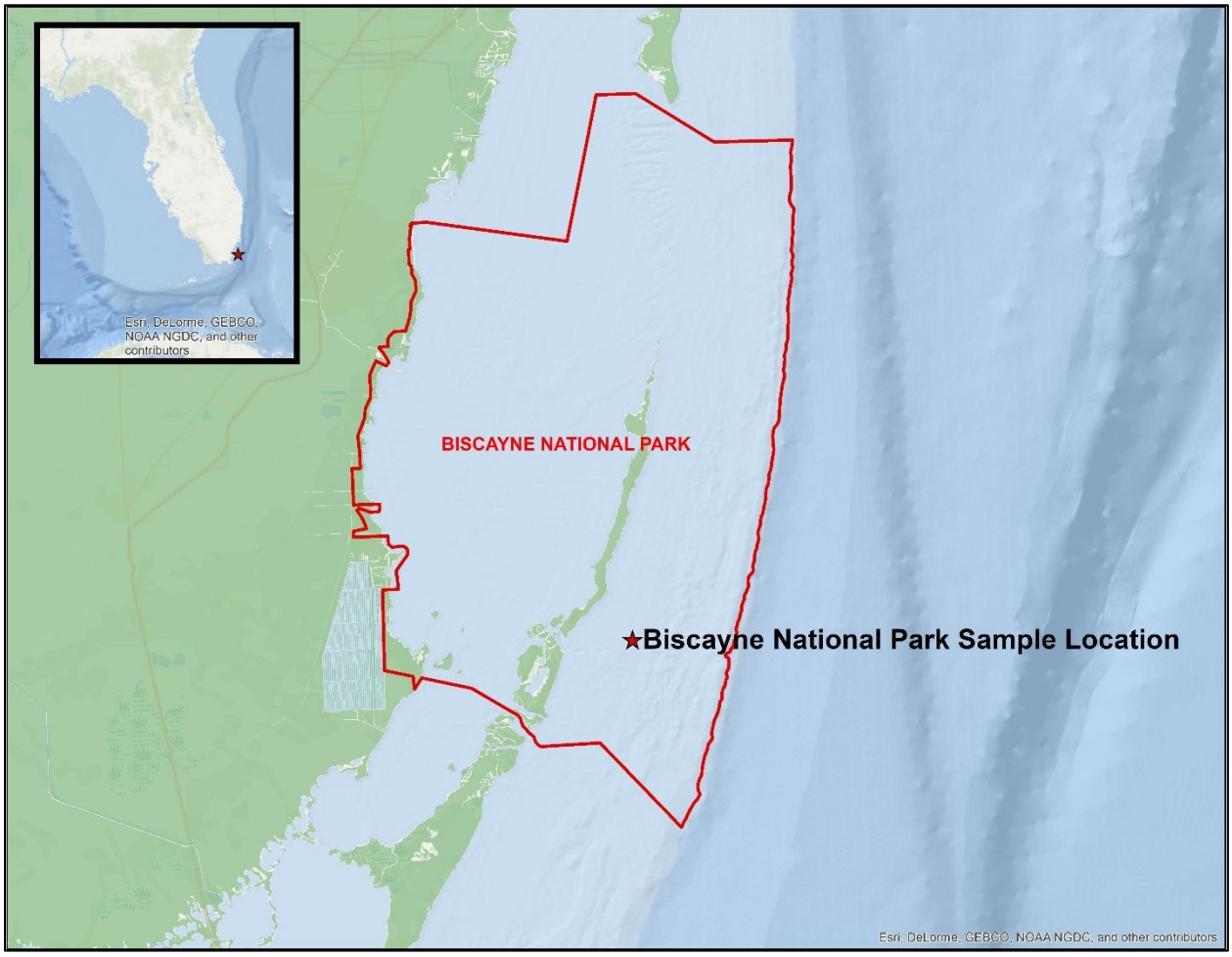


**Figure 1. Location of *Dendrogyra cylindrus* fragments collected from Biscayne National Park.**

**Table 1. GPS coordinates of Biscayne National Park *Dendrogyra cylindrus* collections.**

| Collection Site | Latitude | Longitude |
|---|---|---|
| Biscayne National Park | 25.389 | -80.189 |

## Observations and treatment regimes

On arrival in Charleston on May 18, 2016, *D. cylindrus* fragments were placed in three 5-gallon aquaria containing 100 % TMASW, each equipped with a Marineland® 50 W heater (Blacksburg, VA) set to 26 °C and an airstone to provide circulation/aeration. A light fixture containing two 54 W T-5 light bulbs (Giesemann Aquablue+, Nettetal, Germany) and emitting 72-83 µmol/$m^2$/s photosynthetically active radiation (PAR) was placed above all three tanks with a reduced (6 h on/18 h off) light cycle. Coral fragments were designated with location of parent colony, colony number, and unique identifier.

Corals and assigned treatments are listed in Table 2. Two broad-spectrum antibiotics (ampicillin and gentamicin) with differing modes of action were tested for efficacy against the tissue loss disease. Ampicillin is a beta-lactam derivative, which inhibits the enzyme transpeptidase required for bacterial cell wall synthesis (Harwick *et al.* 1973). Gentamicin binds to the bacterial 30S ribosomal subunit, inhibiting protein synthesis (Weinstein *et al.* 1967). Concentrated antibiotic stock solutions were made at 1000X in Type 1 water, filter sterilized (0.2 µm) and stored at 4 °C in the dark until use.

**Table 2. Treatments for *Dendrogyra cylindrus* fragments from Biscayne National Park.**

| Fragment | Collection Site | Colony # | Genotype | Treatment | Treatment Time (d) |
|---|---|---|---|---|---|
| BISC-01-001 | Biscayne NP | 1 | D1279 | 100 mg/L ampicillin | 11 |
| BISC-01-002 | Biscayne NP | 1 | D1279 | 25 mg/L gentamicin* | 7 |
| BISC-05-003 | Biscayne NP | 5 | D1279 | 25 mg/L gentamicin* | 7 |
| BISC-05-004 | Biscayne NP | 5 | D1279 | 100 mg/L ampicillin | 11 |
| BISC-12-005 | Biscayne NP | 12 | D1279 | 100 mg/L ampicillin | 11 |

*The gentamicin treatment did not arrest disease progression. Fragments were subjected to two additional antibiotic regimens: 100 mg/L ampicillin (concurrently with gentamicin) followed by 100 mg/L paromomycin.

The five *D. cylindrus* fragments were under observation for an initial 12 h period. Colony replicates (BISC-01 and BISC-05 fragments) were placed in two separate aquaria. The small colony 12 fragment was placed in a third aquarium. Continued tissue sloughing was observed after 12 h for all fragments, so two treatment regimens were initiated: 1) 100 mg/L ampicillin in TMASW with 100 % daily water changes and 2) 25 mg/L gentamicin in TMASW with 100 % daily water changes. One representative fragment from each of the three coral colonies sampled (BISC-01-001, BISC-05-004 and BISC-12-005) was designated for ampicillin treatment in two separate 5-gallon treatment tanks while the replicate fragments from colonies 1 and 5 (BISC-01-002 and BISC-05-003) were designated for the gentamicin treatment. Excess skeleton was trimmed from fragments before being placed in the appropriate treatment solution. Corals were fed a sterile food source daily consisting of Bio-Pure® frozen rotifers, *Artemia* nauplii, and cyclopods (Hikari, Hayward, CA).

### *Ampicillin treatment*

Ampicillin is a broad-spectrum antibiotic, but is more effective against Gram-positive bacteria such as enterococci strains (Harwick *et al.* 1973). The three coral fragments were treated with ampicillin (Fisher Scientific, Hampton, NH; catalog number AAJ1125922) for 11 days based on a commonly used antibiotic regimen. Following treatment termination, corals were placed in TMASW (no antibiotic) with 100 % seawater changes for 3 days to evaluate treatment effectiveness. After no disease

progression was observed, these fragments were moved to a larger (20-gallon) system which included live rock harvested from the Florida Keys (KP Aquatics, LLC, Tavernier, FL), a Koralia® 750 circulation pump (Hydor, Sacramento, CA), Remora® protein skimmer (AquaC, San Diego, CA), 100 W heater (Marineland, Blacksburg, VA), and a custom-made calcium reactor for water chemistry stability. Lighting over the tank was increased over time from two to four 54 W T-5 light bulbs (ATI Coral Plus®, ATI North America) which yielded approximately 230-250 µmol/$m^2$/s PAR and the light cycle was gradually increased from 6 h on/18 h off to 10 h on/14 h off. The corals appeared stable (no tissue sloughing observed) after 1 month (as of 6/15/2016) (Figure 2) after which water changes were performed on an as needed basis and feeding was reduced to three times per week (Monday, Wednesday, Friday). Fragments have continued to thrive and two of the three fragments have been placed in other facilities (Frost Museum, Miami FL; FLAQ).

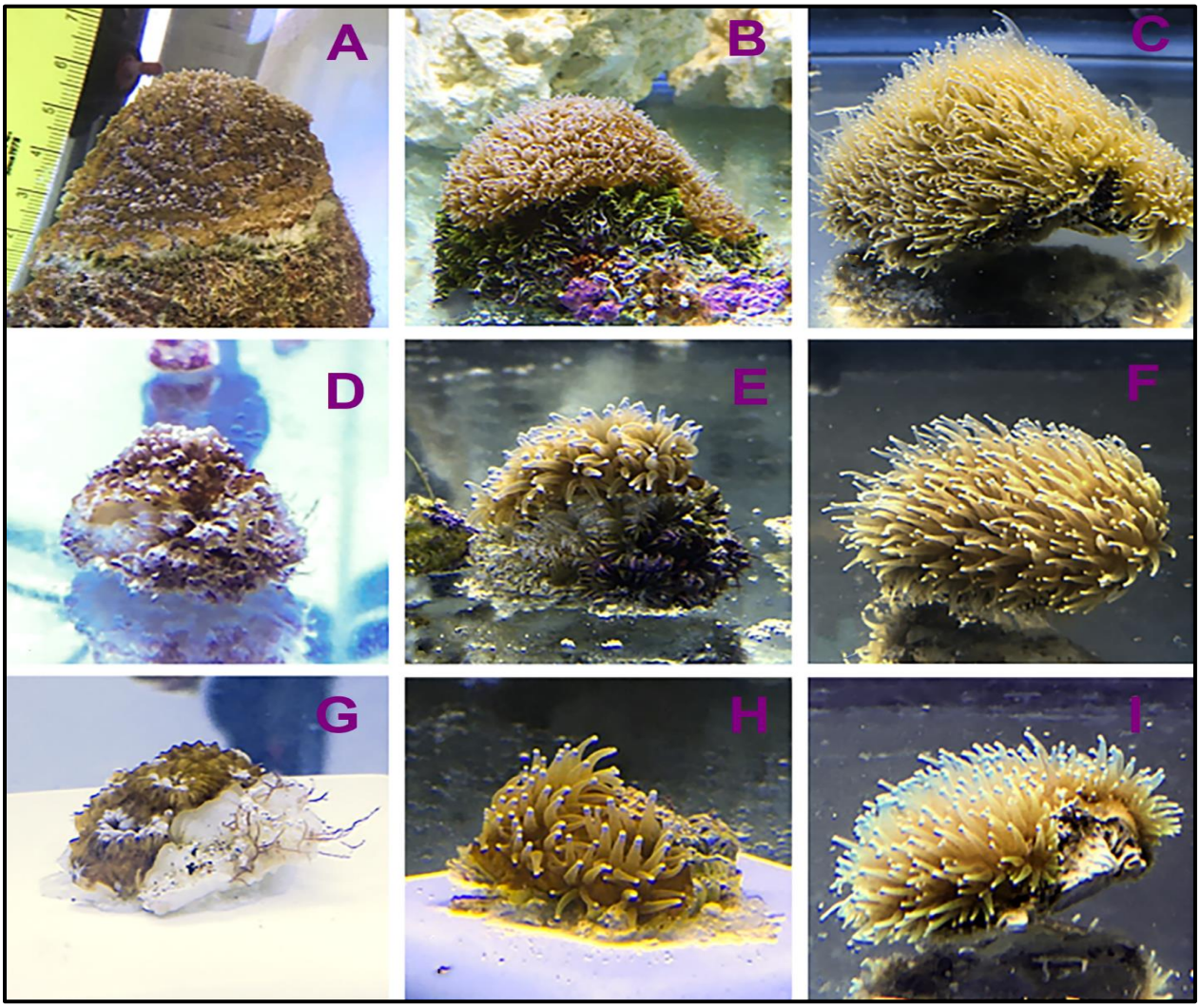


**Figure 2. Recovery of Biscayne National Park coral fragments. Fragments BISC-01-001 (panels A-C), BISC-05-004 (panels D-F) and BISC-12-005 (panels G-I) before and after treatment regimen of 100 mg/L ampicillin for 11 days. Panels A, D and G images taken prior to treatment (5/20/2016). Panels B, E and H imaged on 6/13/2016, following treatment and after transfer to larger tank. Panels C, F and I are images from 1/11/2018. Note tissue growth between 6/13/2016-1/11/2018.**

### *Gentamicin treatment*

Gentamicin is a broad-spectrum antibiotic that is most effective against Gram-negative organisms such as *Pseudomonas*, *E. coli*, *Serratia* and *Klebsiella*. It also shows efficacy in treating *Staphylococcus*, a Gram-positive bacterium. In human medicine, effective doses of gentamicin are much lower as compared to ampicillin (Weinstein *et al.* 1967). Our laboratory previously had tested gentamicin effects on cultured dinoflagellates up to concentrations of 25 mg/L with no adverse effect to the symbiont, thus we decided to use this concentration as a second treatment for the diseased *D. cylindrus*. Pillar coral fragments BISC-01-002 and BISC-05-003 (one fragment from colony 1 and one fragment from colony 5) were actively sloughing tissue upon arrival. After the initial 12 h observation period, both fragments were placed on a 25 mg/L gentamicin (PhytoTechnology Laboratories, Lenexa, KS; catalog number G570) regimen with daily 100 % water changes. Temperature and lighting were the same as for the ampicillin-treated fragments. Overnight, significant tissue loss was observed and as the gentamicin appeared to be ineffective, a concurrent ampicillin treatment (100 mg/L) was started with 100 % water changes daily. Excess skeleton was trimmed from each fragment on 5/20/2016. Gentamicin treatment was continued for a total of 7 days and ampicillin treatment for 11 days. Corals were placed into a clean 5-gallon tank and were antibiotic-free for two days (6/1/16 to 6/3/16), however, tissue sloughing continued at which time a paromomycin treatment (100 mg/L) was initiated.

### *Paromomycin treatment*

Paromomycin is a broad-spectrum antibacterial agent, but has no activity against anaerobes. It also is an effective anti-protozoal treatment. As an aminoglycoside, it inhibits protein synthesis by binding to the 16S rRNA within the 30S ribosomal subunit and seems to act synergistically with β-lactams and other cell wall-active agents (Durante-Mangoni *et al.* 2009). The paromomycin (PhytoTechnology Laboratories, Lenexa, KS; catalog number P710) treatment was continued for 10 days with daily 100% water changes (Figure 3). Corals had some tissue sloughing during treatment, and polyps were retracted. Following paromomycin treatment, coral fragments were moved to a clean 5-gallon tank with no antibiotic and daily 100 % water changes. Corals were fed the same daily diet as described previously. Tissue sloughing continued for both fragments until all tissue was gone (BISC-01-002 on 6/20/2016 and BISC-05-003 on 6/21/2016). The last section of tissue that sloughed from BISC-05-003 was archived immediately in histological fixative for future analysis.

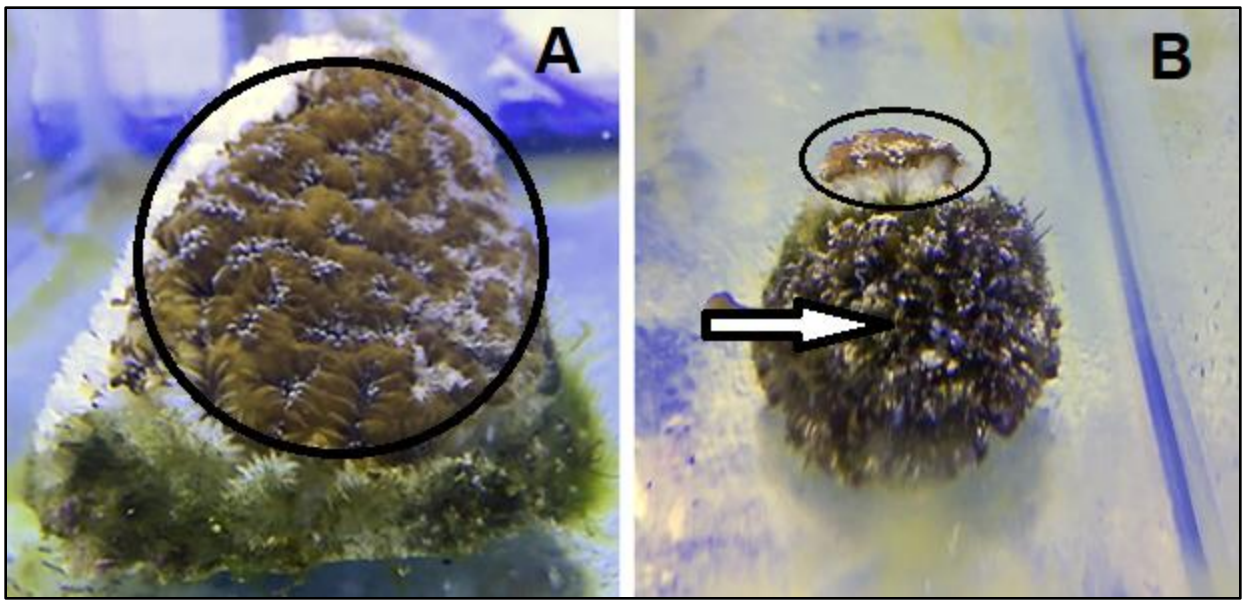


**Figure 3. *Dendrogyra cylindrus* fragments showing continued tissue loss during treatment. Panel A (BISC-01-002) and panel B (BISC-05-003) shown on 6/13/17. Circled areas = remaining live tissue. BISC-01-002 was completely devoid of tissue on the back side (panel A). Arrow in panel B indicates center of large algal overgrowth area. The multiple-antibiotic treatment regimen was not successful, and may have contributed to the complete tissue loss of the fragments. Both were dead by 6/21/2016.**

## Conclusions

- Three of the five *D. cylindrus* fragments (BISC-01-001, BISC-05-004, and BISC-12-005) that received a daily 100 mg/L dose of ampicillin for 11 days (with 100 % daily treatment renewal) stopped sloughing tissue and eventually made full recoveries.

- All three *D. cylindrus* fragments from Biscayne National Park were placed together in one 20-gallon aquarium (described above) after no disease signs were observed for several months. As of July 2020, these fragments remain disease free and are growing. BISC-12-005 remains at the NOAA Charleston coral culture facility, while BISC-01-001 was moved to FLAQ facilities and BISC-05-004 was moved to the Frost Museum.

- The two fragments (BISC-01-002 and BISC-05-003) initially treated with 25 mg/L gentamicin never recovered after treatments with multiple antibiotics. These two fragments appeared to be in the poorest state of health when received, but it is unclear if their demise was due to a more advanced state of disease progression, a more aggressive disease form, the added stress of multiple antibiotic treatments applied concurrently, or a combination of these factors.

# Chapter 2. Upper Keys *Dendrogyra cylindrus* Rescue I

During July 2016, a multi-organization rescue effort was conducted to collect as many genotypes of *Dendrogyra cylindrus* as possible and relocate these voucher specimens to shore-based laboratories or offshore coral nursery sites. The decision to pursue genotype rescue was prompted by forecasts of pending severe bleaching conditions that would possibly kill all remaining colonies in the wild. Collaborating in this effort were NOAA NMFS SERO, Keys Marine Laboratory (KML), Florida Aquarium (FLAQ), Coral Restoration Foundation (CRF), Mote Marine Laboratory (Mote), University of Miami, Nova Southeastern University, University of Florida, Florida Fish and Wildlife Conservation Commission (FWC), U.S. National Park Service (NPS), and the NOAA NCCOS Charleston Laboratory. The primary target of the rescue was to secure apparently healthy specimens for relocation. However, based on earlier success in treating *D. cylindrus* from Biscayne National Park (BISC), the role of the NOAA NCCOS Charleston Laboratory was to obtain diseased specimens to conduct treatment trials with various therapeutics. Thus, 20 pillar coral fragments manifesting signs of SCTLD were collected and transferred to the NOAA NCCOS Charleston coral facility for experimental treatment.

## Methods

### *Coral collection and transport*

Coral fragments (n=20) were collected from four distinct sites along the Florida Reef Tract on 7/26/2016 (Figure 4; Table 3). Five fragments were collected from Pillar Coral Forest (PCF), seven from Turtle Rocks site 2 (TR2), seven from Elbow Reef site 7 (E7) and one from Long Key Ledge site 1 (LKLD1). The fragment from LKLD1 and two fragments from Elbow Reef (E7-91 and E7-92) were originally deemed healthy and held at KML, but later manifested signs of disease. Colonies were transported by van in 10-gallon coolers (one cooler per collection site) as described in Chapter 1. Biscayne National Park *Dendrogyra cylindrus* Rescue. An abbreviation for each site name was combined with original fragment ID numbers to create individual coral identifiers (Table 4) and labels were glued (Seachem® Reef Glue, Madison, GA) to areas of bare skeleton on each colony.

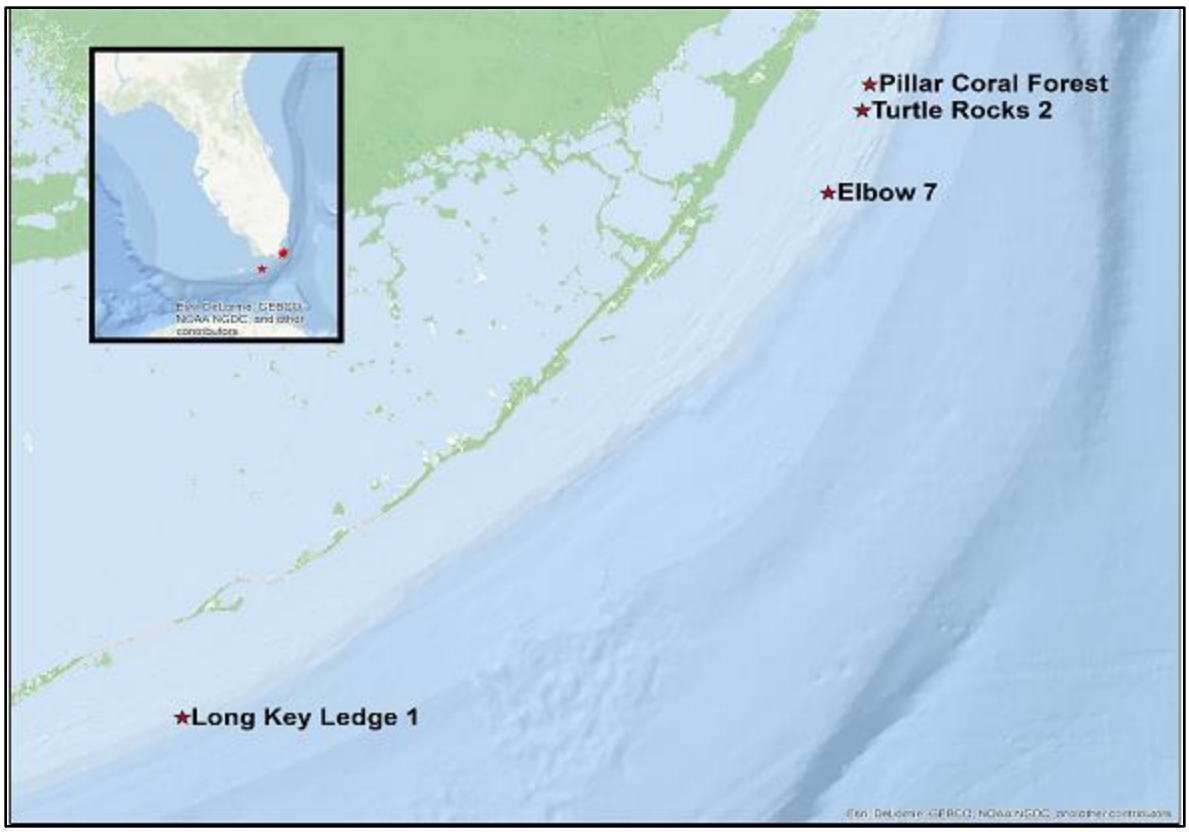


**Figure 4. Upper Keys Rescue I: *Dendrogyra cylindrus* collection sites along the Florida Reef Tract.**

**Table 3. : Rescue I: GPS coordinates of diseased *Dendrogyra cylindrus* collections.**

| Collection Site | Latitude | Longitude |
|---|---|---|
| Pillar Coral Forest | 25.274 | -80.211 |
| Turtle Rocks 2 | 25.251 | -80.218 |
| Elbow Reef 7 | 25.177 | -80.251 |
| Long Key Ledge 1 | 24.7178 | -80.845 |

**Table 4. Diseased *Dendrogyra cylindrus* fragments collected July 2016 for experimental treatments.**

| Coral ID | Collection Site | Genotype | # of Subfragments |
|---|---|---|---|
| **PCF-33** | Pillar Coral Forest | D1198 | 1 |
| **PCF-37** | Pillar Coral Forest | D1198 | 3 |
| **PCF-38** | Pillar Coral Forest | D1198 | 3 |
| **PCF-39** | Pillar Coral Forest | D1198 | 0 |
| **PCF-40** | Pillar Coral Forest | D1198 | 1 |
| **TR2-25** | Turtle Rocks 2 | D1365 | 4 |

| Coral ID | Collection Site | Genotype | # of Subfragments |
|---|---|---|---|
| **TR2-32** | Turtle Rocks 2 | D1365 | 0 |
| **TR2-34** | Turtle Rocks 2 | D1365 | 3 |
| **TR2-35** | Turtle Rocks 2 | D1365 | 0 |
| **TR2-55** | Turtle Rocks 2 | D1365 | 0 |
| **TR2-56** | Turtle Rocks 2 | D1365 | 0 |
| **TR2-65** | Turtle Rocks 2 | D1365 | 0 |
| **E7-41** | Elbow 7 | unknown | 0 |
| **E7-42** | Elbow 7 | unknown | 0 |
| **E7-43** | Elbow 7 | unknown | 0 |
| **E7-53** | Elbow 7 | unknown | 0 |
| **E7-60** | Elbow 7 | unknown | 0 |
| **E7-91** | Elbow 7 | unknown | 0 |
| **E7-92** | Elbow 7 | unknown | 0 |
| **LKLD1-73** | Long Key Ledge 1 (Middle Keys) | D1076 | 2 |

### *Fragment processing*

Upon arrival at the Charleston coral facility (7/27/2016), excess dead skeleton was trimmed from each fragment using a rotary tool (Dremel®, Mount Prospect, IL) with an EZ-Lock 1-1/2" diamond-wheel attachment. At this time, a strong sulfur smell originating from within the freshly cut skeleton of several colonies was detected. During trimming, eight smaller subfragments from four different colonies (TR 25-A, TR 25-B, TR 25-C, PCF 33-A, PCF 38-A, PCF 38-B, PCF 38-C, PCF 40-A) were generated due to uneven disease progression and/or because colonies were too large to fit into a 2.5-gallon treatment tank. The eight subfragments were held in separate small aquaria according to parent colony identifier, along with nine additional subfragments (TR 25-2A, TR 34-A, TR 34-B, TR 34-C, PCF 37-A, PCF 37-B, PCF 37-C, LKLD1 73-A, LKLD1 73-B) created at later dates.

### *Treatment system specifications*

The treatment system (Figure 5A) consisted of two custom-built 24" x 56" x 6" tall glass water baths, each with a Maxi-Jet® 1200 circulation pump (Marineland, Blacksburg, VA) and a HPS-200® 200 W digital heater (Finnex, Chicago, IL) set to 26 °C. Four 54 W T-5 Coral Plus light bulbs (ATI, ATI-North America) provided 110-150 µmol/$m^2$/s photons depending on placement under the light. Each water bath held ten 2.5-gallon glass treatment tanks each with a custom 1/8" thick clear annealed glass lid (Charleston Glass, Charleston, SC) and polypropylene siphon tube installed in one corner for water changes. A customized 20-valve air manifold fitted with silicone airline tubing and 20 individual airstones driven by an AP-60 air pump (Danner, Islandia, NY) provided aeration and water circulation for each treatment tank. A shop vacuum powerhead (Wet/Dry Shop Vacuum Powerhead model BH0100) was fitted to a wastewater

container (Figure 5B.a) and the vacuum hose graduated to fit snugly over each tank's siphon tube in order to aspirate all old treatment water and prevent cross contamination. Fresh TMASW was pumped into each treatment tank from a large 55-gallon polypropylene cylindrical mixing reservoir (Nalgene®, Rochester, NY; Figure 5B.b).

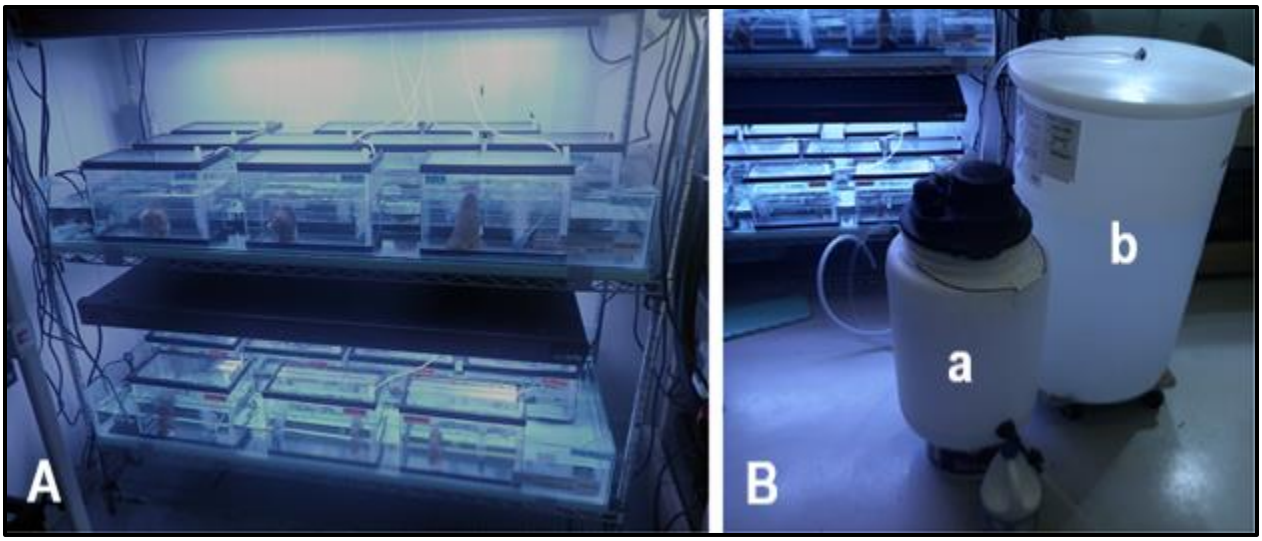


**Figure 5. *Dendrogyra cylindrus* treatment system. Panel A: Treatment system with 20 *D. cylindrus* fragments. Panel B: Modified shop vacuum (a) and saltwater mixing reservoir (b) for water changes.**

### *Experimental treatment regimens*

Four treatment regimens were designed to address the unknown etiology of the disease using antibiotic-based and non-antibiotic approaches to identify an effective treatment. The first treatment was a water change (100 %) every 24 h for 10 days with TMASW (prepared to 35 ppt in deionized water, at 26 ˚C) to reduce microbial and nutrient load. A second treatment incorporated two 15-min immersions in 0.5 mL/L Lugol's stock solution (10 % potassium iodide, 5 % iodine) in TMASW at experiment initiation and another after 24 h, followed by daily 100 % water changes with freshly made TMASW for an additional nine days. Lugol's solution was used because of its antiseptic properties. Two remaining treatments included two antibiotics, ampicillin or paromomycin, with daily treatment renewal (100 mg/L in TMASW, 100 %) for 10 days. Both antimicrobial compounds are broad-spectrum agents; however, ampicillin is more effective against Gram-positive bacteria, while paromomycin targets bacterial and some protozoal infections (Kim *et al.* 2016). Both have been used successfully to treat white band disease in *Acropora cervicornis* without negative effects (Sweet *et al.* 2014). Each of the 20 fragments was assigned randomly to one of the four treatment groups. At least one individual from each collection site was represented in each treatment, except Long Key Ledge site 1 from which there was only one fragment.

All treatment tanks were filled with 5 L of treatment solution, except tanks for fragments PCF-25 and E7-91, which were filled with 7 L due to large (tall) fragment size. Water baths around the tanks were maintained at 26.0±0.5 °C. Daily 5 mL feedings of a

solution containing Bio-pure *Artemia* nauplii, rotifers, and cyclopods (Hikari, Hayward, CA) were administered at least one hour before water changes. Lighting was reduced to seven hours (light cycle from 9:30 am – 4:30 pm). After two days with no antibiotic, the next course of action was determined by visual health assessments. Treatment follow-up options were: 1) to continue current treatment, 2) to initiate new treatments, or 3) to place the apparently healthy fragment in a permanent holding system. Permanent systems consisted of 40-gallon tanks (35 ppt TMASW) each equipped with a Remora protein skimmer, a customized glass calcium reactor (Greatglas, Wilmington, DE), a Koralia 1100 circulation pump and a HPS-200 200 W digital heater set to 26 °C. Lighting was provided over each system with four 54 W T-5 Coral Plus light bulbs (PAR = 130-165 µmol/$m^2$/s) and approximately 20 lb of live rock was added to each for bio-filtration. Live rock was seeded from KP Aquatics live rock (Tavernier, FL) that was collected prior to disease outbreak.

## Results

### *Experimental treatments for SCTLD*

Of the 20 test fragments, four did not survive the initial treatment regimen and included one from each test group: PCF-33 (Lugol's), E7-41 (water change only), TR2-65 (paromomycin) and PCF-40 (ampicillin). The 16 remaining fragments were still losing tissue at day 12 of the experiment, but at different rates (Figure 6; Table 5) and were transferred to four independent permanent systems (one per treatment type) for rehabilitation.

Three of the fragments from the Lugol's treatment (TR2-35, E7-42, LKLD1-73) continued to exhibit slow tissue recession but were not actively sloughing tissue after placement in the permanent system. The remaining fragment from this treatment, E7-92, was visibly diseased compared to other fragments and thus was kept in the original treatment tank for an extended period to be treated with CVS Health® Triple Antibiotic Ointment (400 units bacitracin, 3.5 mg neomycin and 5000 units polymyxin B) around the tissue margin. Fragment E7-92 died on 8/19/2016.

Fragment conditions after antibiotic treatments varied. Paromomycin-treated fragments (PCF-37, TR2-34, E7-60, E7-91) were sloughing tissue following treatment but at a slower rate than during treatment. Pillar coral fragments treated with ampicillin (TR2-32, TR2-55, E7-53, PCF-38) experienced varying levels of recovery after being transferred to permanent systems. Fragment TR2-32 continued to slough tissue ≥ the rate observed while undergoing treatment and died on 8/24/2016. Fragments TR2-55 and E7-53 continued to slough tissue but at a slower rate than during treatment. Fragment PCF-38, which arrived in a partially bleached state, ceased sloughing tissue, showed thickened and darkened tissue and increased polyp extension.

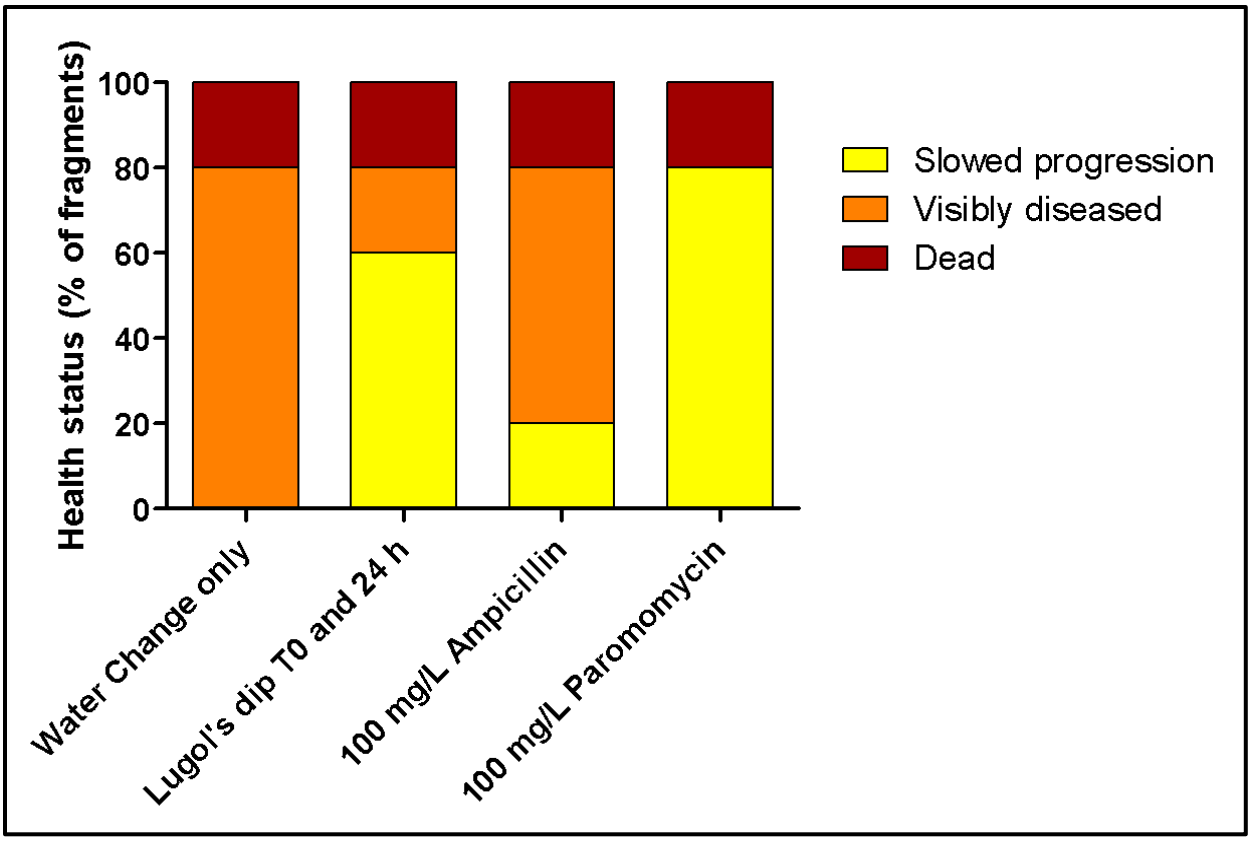


**Figure 6. Health status of *Dendrogyra cylindrus* fragments following initial 10-day treatments (n=5). Twenty fragments with SCTLD were subjected to one of four treatment regimens: no antibiotic with 100 % water changes (fresh artificial seawater), Lugol's immersion and 100 % water changes with fresh artificial seawater, 100 % water changes with 100 mg/L ampicillin in artificial seawater and 100 % water changes with 100 mg/L paromomycin in artificial seawater. Fragments were scored as either dead, visibly diseased (active tissue sloughing) or slowed disease progression (tissue sloughing but at a relatively slower rate). One fragment in each treatment died during the exposure period. All fragments still exhibited disease signs after treatment termination.**

Of the five fragments (TR2-25, TR2-56, PCF-39, E7-43, E7-41) undergoing the water-change-only treatment, fragment E7-41 sloughed all tissue four days after treatment initiation. The remaining four fragments still appeared visibly diseased and tissue recession remained constant for 10 days; they were subjected to additional treatments in an effort to save the remaining tissue. Fragment TR2-25 was placed on a 10-day regimen of ampicillin (100 mg/L, refreshed daily). This fragment was ultimately deemed recovered. Fragment E7-43 was similarly treated with ampicillin but did not respond. PCF-39 was treated with one liberal application of CVS Health Triple Antibiotic Ointment (400 units bacitracin, 3.5 mg neomycin and 5000 units polymyxin B) around the tissue margin with daily (100 %) water changes. Eight days after the ointment application, PCF-39 was removed from the treatment, rinsed with TMASW, and placed in a TMASW-only treatment tank with daily water changes (100 %). PCF-39 continued to experience slow tissue recession until all tissue was gone (8/24/2016, 18 days post-treatment). The same antibiotic ointment was applied twice to TR2-56 but each time

detached from the coral skeleton. Since TR2-56 and E7-43 did not respond to secondary treatments, tertiary therapies using different dosages of amoxicillin were administered.

Amoxicillin is a moderate-spectrum, β-lactam antibiotic (derivative of penicillin) used to treat infections primarily from *Streptococcus*, *Bacillus*, *Enterococcus*, *Haemophilus*, *Helicobacter,* and *Moraxella* bacterial genera (Neu 1974). It has an advantage over similar antibiotics as it diffuses into tissues more easily; however, this characteristic also makes it more difficult to incorporate into an aqueous solution. A treatment of 65 mg of amoxicillin (PhytoTechnology Laboratories, Lenexa, KS; catalog number A122) mixed into 1.5 mL (43 mg/mL) of a dental compounding agent (CDP) with a timed-release feature was chosen (see Chapter 3. Testing of Drug Delivery Vehicles). This mixture was applied to the tissue margin of fragment TR2-56. This tertiary treatment of TR2-56 was successful. Fragment E7-43 received a tertiary treatment on 9/27/2016 with amoxicillin mixed into CDP (40 mg/mL) applied around the tissue margin and then placed into a permanent aquarium. This fragment fully recovered by 10/24/2016 (27 days post-treatment with amoxicillin).

### *Secondary infection: 'Brown Jelly Syndrome'*

After being treated for SCTLD, fragments in three of the four permanent treatment tanks (for treatments by Lugol's, ampicillin, and paromomycin) contracted a second disease that resembled what has been termed 'brown jelly syndrome' (BJS) in the reef aquarium industry (Sweet 2013). Ten of the twelve fragments in these tanks (TR2-35, E7-42, LKLD1-73, PCF-38, TR2-55, E7-53, PCF-37, TR2-34, E7-60, E7-91) were infected with BJS between 8/14/2016 and 8/24/2016. This disease may be related to brown band disease in the wild according to Sweet *et al.* (2013). As a colony became infected, the tissue margin would appear blistered, become gelatinous and affected tissues would rapidly disintegrate (liquefy) when disturbed (Figure 7). The advancement of disease signs was very rapid and was accompanied by what appeared to be an outbreak of ciliates ingesting the affected tissue. Localized treatments of 60 mg/mL of ampicillin, amoxicillin or paromomycin in CDP, and treating with 50 mL/L of Bayer® Advanced Complete Insect Killer (active ingredients: imidacloprid - 0.15 % beta-cyfluthrin - 0.05 %) in TMASW for 15 min were not effective in stopping the advancement of this disease (Table 5). One fragment (E7-91) did not survive these treatments. The nine remaining fragments still showing BJS signs underwent an additional treatment:

1. All dead skeleton was removed and corals were amputated just ahead of the diseased tissue margin into apparently healthy tissue.
2. Coral fragments were dosed once with 1 mL/L Lugol's solution in 35 ppt TMASW for 15 min and rinsed well with fresh TMASW.
3. Coral fragments then were put on a 7-day, 100 mg/L daily dose of ampicillin in TMASW with 100 % daily treatment changes.

Following amputation of the diseased tissue margin and immersion in Lugol's and ampicillin treatment, seven fragments with BJS fully recovered (no signs of tissue recession was observed, tissue regenerated along the margin, tissue thickened and

darkened, and polyps were fully extended). These seven fragments were placed in newly established permanent systems along with other fragments and subfragments according to collection site. Two of the nine fragments with BJS (E7-53 and E7-60) were in a state of recovery but died on 10/9/2016 and 10/13/2016, respectively, likely due to a three-day power loss in the culture facility during Hurricane Matthew. It is unclear whether BJS returned to these two fragments before they died or they succumbed due to the adverse culture conditions. Final status of all 20 fragments is detailed in Table 5.

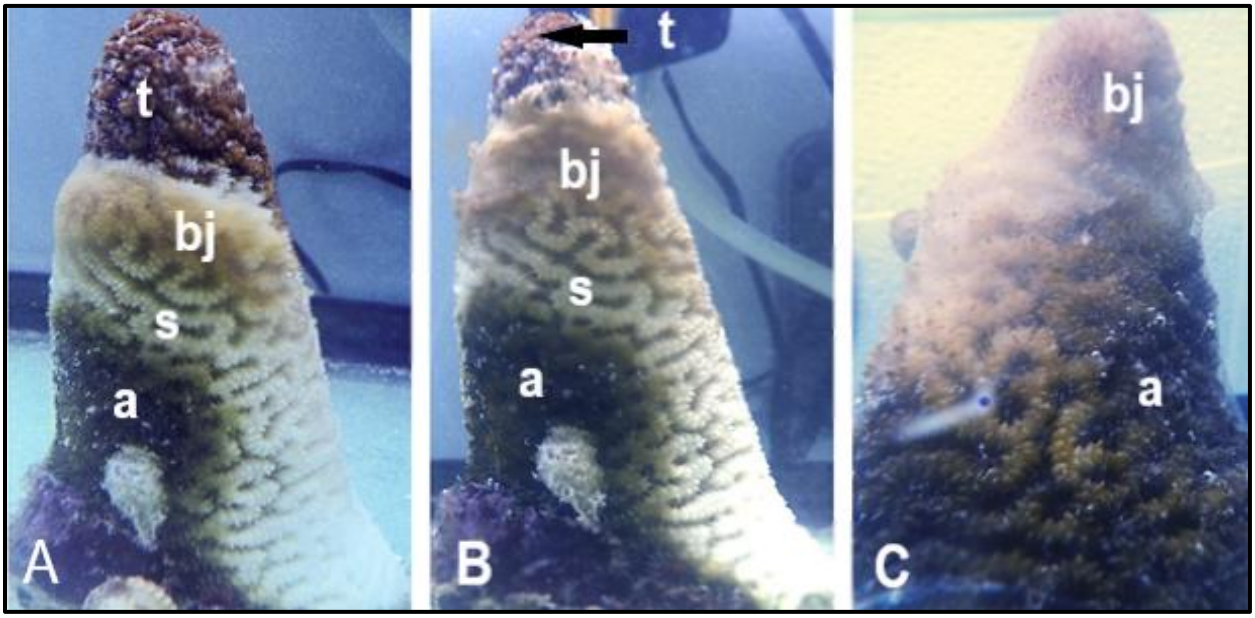


**Figure 7. Fragment E7-91 showing rapid onset of 'brown jelly syndrome'. Panel A – 8/14/16; panel B – 8/15/16; panel – C 8/19/16. A localized amoxicillin treatment in CDP applied on 8/18/216 was ineffective. Shown are coral tissue (t), gelatinous brown jelly tissue (bj), bare coral skeleton (s), and skeleton with algal overgrowth (a) over time.**

### *Specialized subfragment treatments*

Upon arrival in Charleston, SC (7/27/2016), eight small subfragments were created while amputating excess skeleton and diseased tissue. These included one subfragment each from PCF-33 and PCF-40, and three subfragments each from PCF-38 and TR2-25. These small subfragments were used to test the effectiveness of several different drug delivery vehicles, which is outlined in Chapter 3. Testing of Drug Delivery Vehicles. Due to the success of one of the drug delivery trials, untreated subfragments or those not responding to other treatments were treated with approximately 60 mg of amoxicillin mixed into 1 mL CDP (Table 6). Treatment was applied to a fragment after its removal from the tank and excess water dried at the tissue-skeleton interface. Artificial seawater (35 ppt) was then dripped over the area to activate the CDP to promote adherence to the skeleton. Subfragments were housed in our main culture room in (600 mL) TMASW in 1000 mL glass beakers with aeration. Room temperature was set to 23.5 °C and lighting was provided by indirect exposure to four metal halide lights (1000 W; 14,000 °K). Of the six subfragments that received this treatment, only one (PCF-33) which was already noted to be in a very poor condition, died. The remaining five had fully recovered by 9/16/2016 and were placed in permanent culture systems according to collection site.

An additional eight subfragments (LKLD1-73 (n=2); TR2-34 (n=3); PCF-37 (n=3)) were created between 8/14/2016 and 9/6/2016 from attempts to treat BJS (Table 6). The amoxicillin/CDP treatment was applied to four of the eight subfragments (Table 6) when other treatments were ineffective. A second treatment of a daily 200 mg/L ampicillin dose for 7 days was also tested on two subfragments (73-B and 34-B, Table 6).

Note that all subfragments from PCF-37, TR2-34, and LKLD1-73 were previously treated while attached to their respective parent colonies when they started to display signs of BJS. Before subfragments were created, PCF-37 and PCF-38 were treated with amoxicillin (60 mg/mL in CDP) for two days, followed by paromomycin (60 mg/mL in CDP) for two days and then immersed in 250 mL/L Bayer Advanced Complete Insect Killer for 15 min. Fragment LKLD1-73 was treated with amoxicillin (60 mg/mL in CDP) for two days and then immersed in 250 mL/L Bayer Advanced Complete Insect Killer for 15 min. When none of these treatments proved to be effective against BJS, diseased tissue was cut away resulting in the subfragments.

**Table 5. Primary and secondary treatments for *Dendrogyra cylindrus* fragments exhibiting signs of tissue loss disease.**

| Treatment Condition | Coral ID | Condition after Initial Treatment 8/12/2016 | Contracted "Brown Jelly" | Additional Treatment* 8/15-9/27/2016 | Condition on 8/24/2016 | Condition on 10/25/2016 |
|---|---|---|---|---|---|---|
| 100% Water change only | PCF-39 | visibly diseased | no | 3 | DEAD | DEAD |
| | TR2-25 | visibly diseased | no | 2 | recovering | **Recovered** |
| | TR2-56 | visibly diseased | no | 3,1 | recovering | **Recovered** |
| | E7-41 | DEAD | DEAD | DEAD | DEAD | DEAD |
| | E7-43 | visibly diseased | no | 2,6 | visibly diseased | **Recovered** |
| Lugol's immersion T0 and T24 h | PCF-33 | DEAD | DEAD | DEAD | DEAD | DEAD |
| | TR2-35 | slowed disease | yes | 1,5,4 | recovering | **Recovered** |
| | E7-42 | slowed disease | yes | 1,5,4 | recovering | **Recovered** |
| | LKLD1-73 | slowed disease | yes | 1,5,4 | recovering | **Recovered** |
| | E7-92 | visibly diseased | no | 3 | DEAD | DEAD |
| 100 mg/L Ampicillin | PCF-40 | DEAD | DEAD | DEAD | DEAD | DEAD** |
| | PCF-38 | slowed disease | yes | 4 | recovering | **Recovered** |
| | TR2-32 | visibly diseased | no | DEAD | DEAD | DEAD |
| | TR2-55 | visibly diseased | yes | 4 | recovering | **Recovered** |
| | E7-53 | visibly diseased | yes | 1,4,6 | recovering | DEAD (storm) |
| 100 mg/L Paromomycin | PCF-37 | slowed disease | yes | 1,7,5,4 | visibly diseased | **Recovered** |
| | TR2-34 | slowed disease | yes | 1,7,5,4 | recovering | **Recovered** |
| | TR2-65 | DEAD | DEAD | DEAD | DEAD | DEAD |
| | E7-60 | slowed disease | yes | 1,7,5,4 | recovering | DEAD (storm) |
| | E7-91 | slowed disease | yes | 1 | DEAD | DEAD |

*Additional Treatment Key: Additional treatments are listed chronologically.
[1]Amoxicillin (60-65 mg/mL in CDP) was applied at the disease margin
[2]Ampicillin (100 mg/L in artificial seawater) for 10 days
[3]CVS Triple antibiotic ointment - disease margin (400 U bacitracin, 3.5 mg neomycin, 5000 U polymyxin B)
[4]Fragmented coral ahead of disease margin, 15 min Lugol's immersion then 100 mg/L ampicillin for 7 days
[5]Bayer Advanced Complete Insect Killer (50 mg/L) for 15 min, rinsed and placed in clean tank with ASW
[6]Amoxicillin (0.4 mL of 40 mg/mL in CDP) applied to disease margin
[7]Paromomycin (65 mg in 1.5 mL CDP) applied to disease margin
**PCF-40 had a subfragment from original fragment that was treated with amoxicillin/CDP and survived.

**Table 6. Complete listing of *Dendrogyra cylindrus* subfragments, treatments, and condition.**

| Sub-fragment ID | Parent ID | Fragment Date | Initial Treatment 7/27-8/15 | Final Treatment | Condition as of 10/25/2016 |
|---|---|---|---|---|---|
| 25-2A | TR2-25 | 8/15/2016 | Ampicillin 10 day @ 100 mg/L | NONE | **Recovered** |
| 25-A | TR2-25 | 7/27/2016 | NONE | Amoxicillin 60 mg/mL CDP | **Recovered** |
| 25-B | TR2-25 | 7/27/2016 | NONE | NONE | DEAD |
| 25-C | TR2-25 | 7/27/2016 | NONE | NONE** | **Recovered** |
| 33-A* | PCF-33 | 7/27/2016 | Amoxicillin 60 mg/mL Plasticiser | Amoxicillin 60 mg/mL CDP | DEAD |
| 34-A | TR2-34 | 8/24/2016 | Paromomycin 10 day 100 mg/L | Amoxicillin 60 mg/mL CDP | **Recovered** |
| 34-B | TR2-34 | 8/24/2016 | Paromomycin 10 day 100 mg/L | 7 day 200 mg/L Ampicillin | **Recovered** |
| 34-C | TR2-34 | 8/24/2016 | Paromomycin 10 day 100 mg/L | NONE | DEAD |
| 37-A* | PCF-37 | 9/6/2016 | Paromomycin 10 day 100 mg/L | Amoxicillin 60 mg/mL CDP | DEAD |
| 37-B* | PCF-37 | 9/6/2016 | Paromomycin 10 day 100 mg/L | Amoxicillin 60 mg/mL CDP | DEAD |
| 37-C* | PCF-37 | 9/6/2016 | Paromomycin 10 day 100 mg/L | NONE | DEAD |
| 38-A | PCF-38 | 7/27/2016 | Pracasil Applied on tissue margin | Amoxicillin 60 mg/mL CDP | **Recovered** |
| 38-B | PCF-38 | 7/27/2016 | Amoxicillin 56 mg/mL Plasticiser | Amoxicillin 60 mg/mL CDP | **Recovered** |
| 38-C | PCF-38 | 7/27/2016 | NONE | Amoxicillin 60 mg/mL CDP | **Recovered** |
| 40-A* | PCF-40 | 7/27/2016 | Ampicillin (57 mg)/ mL Mucolox | Amoxicillin 60mg/mL CDP | **Recovered** |
| 73-A | LKLD1-73 | 8/24/2016 | Lugol's immersion 15 min @ 0.5 mL/L | Amoxicillin 60 mg/mL CDP | **Recovered** |
| 73-B | LKLD1-73 | 8/24/2016 | Lugol's immersion 15 min @ 0.5 mL/L | Ampicillin -7 day 200 mg/L CDP | **Recovered** |

*Subfragments that were in extremely poor condition before their final treatment.
**A treatment of 60 mg amoxicillin in a thicker dental paste was attempted on subfragment 25-C but would not come out of the syringe. A very small amount of liquid (<0.1 mL) was squeezed out onto the skeleton/tissue margin before it was placed back into its treatment tank so it may have received a very small dose of amoxicillin.
NONE = 100 % water change only

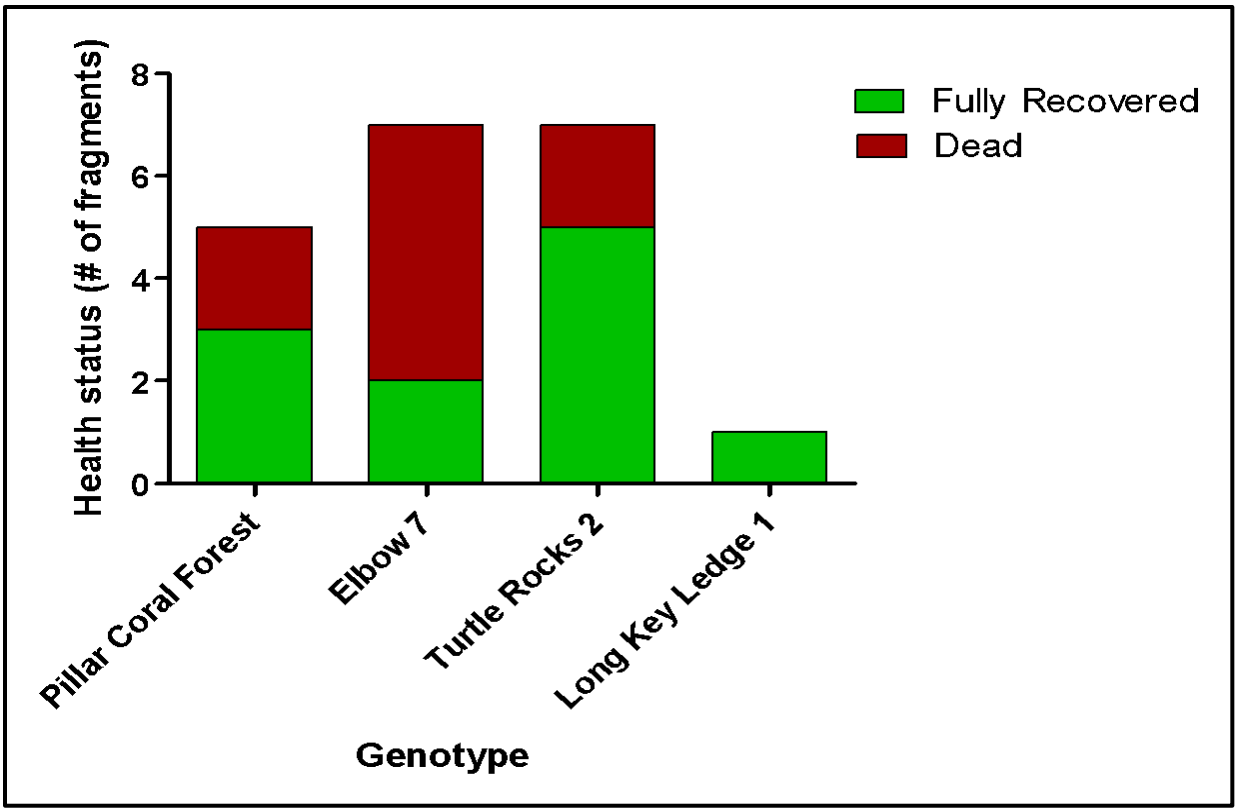


**Figure 8. Results of disease treatments for 20 *Dendrogyra cylindrus* fragments.**

## Conclusions

- A total of 20 fragments and 17 subfragments from four collection sites underwent various treatments. Ten fragments and 11 subfragments fully recovered for a survival rate of 50 % and 65 %, respectively. Although PCF-40 did not survive initial treatment, a subfragment (40-A) did, increasing survival rate of the original fragments to 55 % (Figure 8).

- Two fragments in a state of recovery (E7-60 and E7-53) died. This was believed to be due to a 3-day power failure during Hurricane Matthew (2016).

- A secondary disease (BJS) spread throughout the permanent aquaria systems soon after fragments finished their initial treatment regimens, making it difficult to evaluate the effectiveness of individual treatments for SCTLD. It is clear that 100 % daily water changes alone are ineffective. While Lugol's immersion (0.5 mL/L) at T0 and T24 h initially seemed to slow disease progression, it did not halt it; hence, Lugol’s alone proved to be an insufficient treatment.

- Dosing with ampicillin (100 mg/L daily for 7-10 days or 200 mg/L daily for 7 days) proved to be partially effective on its own or in combination with the amoxicillin-CDP treatment. Determining the efficacy of this treatment was confounded by the outbreak of BJS.

- After other treatments appeared unsuccessful, treating original fragments and additional subfragments with a localized application of amoxicillin in CDP resulted in the recovery of 17 / 22 fragments (77 %).

- The effectiveness of the paromomycin treatment was inconclusive due to subsequent BJS treatments.

- The fragments undergoing BJS treatment lost significant amounts of tissue. Vigorous rinses with TMASW to remove infected tissues, resection of the diseased tissue and immersion in Lugol's solution (1 mL/L) for 15 min, followed by a week of ampicillin appeared successful for mitigating BJS (7 of 9 BJS infections cured). It is unclear which component(s) of the treatment were crucial to success.

- The most successful SCTLD treatments included:

    1. Removing excess skeleton and diseased tissue margin, as fragment size dictates, and rinsing fragment with TMASW.
    2. Placing fragment into an antiseptic solution (0.5 mL/L of Lugol's in TMASW) for 15 min and subsequently rinsing fragment with TMASW.
    3. Applying one of the antibiotic treatments:
        a. Ampicillin 100 mg/L in TMASW (treatments are renewed 100 % daily for at least 7 days)
        b. Amoxicillin 40-60 mg/mL in CDP applied to tissue margin with 100 % daily water changes

# Chapter 3. Testing of Drug Delivery Vehicles

Curing diseased corals with presumed infectious pathogens has been limited to treating small fragments in laboratory aquaria over extended periods of time (e.g., 10-day regimen). This approach requires significant amounts of therapeutic compounds, is limited to compounds with high solubility in seawater, and is not amenable to field situations. Taking advantage of drug delivery technologies engineered for dentistry and medicine, specifically pharmacy compounding, provided an opportunity for targeted delivery and controlled release of therapeutic agents to diseased corals.

Pharmacy compounding is a practice used to prepare customized medications. Many independent compounding pharmacies are members of the Professional Compounding Centers of America (PCCA) organization, which provides to members pharmaceutical grade chemicals, base formulas, and nutraceuticals as well as access to proprietary formulas and consultation on preparing novel compounds. Two local PCCA-member compounding pharmacies were contacted for assistance in developing a drug delivery vehicle that would allow direct application to coral, withstand immersion in seawater and water currents created by aeration from airstones, and provide timed-release of the medication. Premade base compounds were purchased from one pharmacy (Dottie's Pharmacy (DP), Charleston, SC) for testing. Several of these base compounds claimed to have antimicrobial properties alone. Four of the available base compounds were tested (Table 7) for their efficacy alone or with amoxicillin added. Procedures are detailed in Regimens 1 & 2 that follow.

Consultations with a second pharmacy (Plantation Pharmacy (PP), Charleston, SC) resulted in a collaboration with PCCA to formulate several customized base compounds modified from a formulation used as a drug vehicle in dentistry. Different formulations were tested to determine an optimal formulation that would adhere to coral skeleton underwater for an extended time in small aquaria (1-5 L) with vigorous aeration. The formulation with optimal performance was termed 'coral dental paste' or CDP, and was used as a base material to deliver amoxicillin at a concentration of 50 mg/g (mL). The procedures used for testing are detailed in Regimen 3 that follows.

## Treatment Regimen 1

Six premade base compounds were recommended by DP and purchased on 7/28/2016 as possible vehicles for drug delivery or for use as antimicrobial agents alone (Table 7). Three of these were tested (due to limited availability of diseased fragments). On 7/30/16 an "extender" (with timed-release properties) to prolong the antibiotic delivery for the PCCA Plasticized-base was recommended by DP and added to subsequent DP formulations.

**Table 7. List of pre-made base compounds for use in wet environments.**

| Product | Lot Number | Chemical ID | Description / Application | Tested | Results |
|---|---|---|---|---|---|
| PCCA Mucolox | Lot # 7135339, exp. 11/30/17 | 14177 | Binds to mucus membranes | yes | Mucolox with ampicillin initial treatment **NOT EFFECTIVE** Switch to Amoxillin |
| PCCA Base, PRACASIL-Plus | Lot # 6474815 exp. 10/11/17 | 14121 | Composed of silicone with antibiotic and antifungal properties | yes | Used alone not effective, water heavily fouled **NOT EFFECTIVE** |
| PCCA Plasticized-base | Lot # 6584484, exp. 10/6/17 | 10158 | None provided | yes | Used with amoxicillin **BEST RESPONSE of pre-made bases**, but came off skeleton quickly |
| PCCA Methocel E4M Premium CR (Hydroxypropyl) methyl cellulose USP | Lot # C175834, exp. 8/11/2020 | 13815 CAS: 9004-65-3 | Timed-release compound for addition to PCCA plasticized-base at 40% wt/vol | yes | Addition with plasticized base and amoxicillin showed efficacy, however base did not adhere very well to coral skeleton |
| PCCA VERSA base Gel | Lot# 6766207 | 13870 | None provided | no | |
| Zinc oxide ointment | Lot# FE6369 | 14698 | Manufactured by Fougera | no | |
| White petrolatum ointment | Lot#FG6566, exp. 6/30/18 | 14680 | Manufactured by Fougera | no | |

Of these six initial compounding bases suggested to perform well in wet environments, three treatments were initiated on 7/28/2016 (Table 8). Prior to application, excess water from each fragment was removed by blotting with a laboratory wipe.

**A-1 PCCA Mucolox** (0.5 mL) was mixed with 56.7 mg ampicillin and applied to the tissue/skeleton margin of 40-A.

**B-1 PCCA Plasticized-base** (1 mL) was mixed with 55.7 mg amoxicillin and applied to the tissue/skeleton margin of 38-B.

**C-1 PCCA base, PRACASIL-Plus** (1 mL) was applied to fragment 38-A with no additional drug added.

A diseased fragment with a particular treatment application was placed into a 1 L clean glass beaker containing 600 mL of TMASW (35 ppt). Each beaker was aerated by a

single airstone attached to silicon tubing and the bubble rate was adjusted to a slow stream (1-2 bubbles/s). Beakers were held at ambient temperature (23.3 °C) with indirect metal halide lighting (1000 W, 14,000 °K; Hamilton Technologies, Los Angeles, CA).

Corals were examined on 7/29/2016 after 11 h of incubation in the dark (10:30 pm – 9:30 am). A 100 % water change was conducted for each beaker, and fragments were photographed. Prior to the water change, the following observations were made:

- Treatment A-1: water remained clear overnight.
- Treatment B-1: water remained clear overnight; however, the plasticized base was coming off the fragment.
- Treatment C-1: water was heavily fouled overnight.

At 24 h after initial treatment (9:30 pm, 7/29/2016) a second complete water change was performed on each treatment beaker. Prior to the water change, the following observations were made:

- Treatment A-1: showed slight fouling of the water. Tissue loss continued, though some of the Mucolox remained attached to the coral. It was decided to add 50 mg amoxicillin to 600 mL of TMASW for in-water treatment in an effort to save the quickly dying fragment (rescue mode).
- Treatment B-1: performed the best of the three treatments, although the plasticized base was mostly released from the skeleton. Though tissue loss seemed to slow, it was decided to add 50 mg amoxicillin to 600 mL of TMASW for in-water treatment (rescue mode).
- Treatment C-1: was ineffective with additional coral tissue loss over the previous 12 h and water was heavily fouled. A complete TMASW change was made and 50 mg amoxicillin/600 mL was added to the treatment vessel (rescue mode).

**Table 8. Treatment timeline for drug delivery testing of *Dendrogyra cylindrus* fragments from Pillar Coral Forest and Turtle Rocks.**

| Frag ID | 25-A | 33-A | 38-A | 38-B | 38-C | 40-A |
|---|---|---|---|---|---|---|
| July 27 2016 | **Tank 1** TMASW | **Tank 2** TMASW | **Tank 1** TMASW | **Tank 1** TMASW | **Tank 1** TMASW | **Tank 2** TMASW |
| July 28 2016 | 100 % TMASW change | 100 % TMASW change | **Treatment C-1 Pracasil Alone – no added drugs** | **Treatment B-1 Plasticized /amoxicillin (56 mg/mL base)** | 100% TMASW change | **Treatment A-1 Mucolox+ ampicillin (57 mg/mL base)** |
| July 29 2016 | 100 % TMASW change | 100 % TMASW change | **Water fouled – not effective** changed 100 % TMASW+ 50 mg amoxicillin/600 mL | **Best response, little fouling but Plasticizer was coming off skeleton;** 100 % TMASW + 50 mg amoxicillin/600 mL | 100 % TMASW change | **Continued tissue loss** changed TMASW + 50 mg amoxicillin/ 600 mL TMASW |

| Frag ID | 25-A | 33-A | 38-A | 38-B | 38-C | 40-A |
|---|---|---|---|---|---|---|
| **July 30 2016** | 100 % TMASW change + rotifer food | 100 % TMASW change + rotifer food; Treatment B-2 Plasticizer amoxicillin (60 mg/mL base) | 100 % TMASW change + Treatment A-2 Plasticizer base + 40 mg Methocel+ 60 mg amoxicillin/ mL base | 100 % TMASW change + rotifer food; Treatment B-2 Plasticizer amoxicillin (60 mg/mL base) | 100 % TMASW change + rotifer food; Treatment B-2 Plasticizer amoxicillin (60 mg/mL base) | 100 % TMASW change + Treatment A-2 Plasticizer base + 40 mg Methocel+ 60 mg amoxicillin/ mL base |
| **July 31 2016** | 100 % TMASW change + rotifer food | 100 % TMASW change + rotifer food | 100 % TMASW change + rotifer food | 100 % TMASW change + rotifer food | 100 % TMASW change + rotifer food | 100 % TMASW change + rotifer food |
| **Aug 1 2016** | 100 % TMASW change + rotifer food | **Test frag for CDP from PP; mixed with 60 mg amoxicillin** | 100 % TMASW change + rotifer food | 100 % TMASW change + rotifer food | 100 % TMASW change + rotifer food | 100 % TMASW change + rotifer food |
| **Aug 2 2016** | 100 % TMASW change + rotifer food | 100 % TMASW change + rotifer food | 10 0% TMASW change + rotifer food | 100 % TMASW change + rotifer food | 100 % TMASW change + rotifer food | 100 % TMASW change + rotifer food |
| **Aug 3 2016** | Amoxicillin (60 mg) in CDP; 100 % TMASW change + rotifer food, 24 h *Artemia* and cyclopod food | replace amoxicillin (60 mg) in 1mL CDP | replace amoxicillin (60 mg) in 1mL CDP | replace amoxicillin (60 mg) in 1mL CDP | replace amoxicillin (60 mg) in 1mL CDP | replace amoxicillin (60 mg) in 1 mL CDP |
| **Aug 4 2016** | | **Dead** | | | | |
| **Aug 9 2016** | Moved into treatment system, treatment remains the same | **Dead** | Moved into treatment system with fragment 40-A, treatment remains the same | Moved into treatment system with fragment 38-C, treatment remains the same | Moved into treatment system with fragment 38-B, treatment remains the same | Moved into treatment system with fragment 38-A, treatment remains the same |

## Treatment Regimen 2

After the initial 36 h (7/30/2016) treatment incubations, coral fragments were fed (Hikari Bio-Pure rotifers) and a 100 % water change was performed. Tissue loss had slowed but the pre-made compounding-base drug delivery vehicles did not adhere well to the skeleton, resulting in fouled treatment waters. In order to rescue the test fragments,

amoxicillin was added to the treatment vessels. Since the Plasticized-base performed the best in the initial trial, DP suggested the addition of a timed-released material, Methocel E4M Premium CR (Hypromellose USP). The mixture ratio is 40 % Methocel to 60 % Plasticized base. On 7/30/2016, two new treatments were prepared for specimen application to previously treated fragments (Table 8).

- Treatment A-2: 60 mg amoxicillin with 40 mg Methocel mixed into 1 mL Plasticized-base and applied to the tissue/skeleton margin of fragments
  - Fragment 40-A (Treatment A-1)
  - Fragment 38-A (Treatment C-1)
- Treatment B-2: 60 mg amoxicillin only, mixed with 1 mL Plasticized-base for application
  - Fragment 38-B (Treatment B-1)
  - Fragment 38-C (No previous treatment)
  - Fragment 33-A (No previous treatment, but initially in very poor condition)

All corals were fed (Hikari Bio-Pure rotifers) prior to receiving the daily 100 % water change with TMASW.

Though tissue loss appeared to be slowing, our goal was to find a drug delivery vehicle that would adhere to the skeleton and last for 5-7 days, slowly releasing the antibiotic treatment over time. This was not accomplished with the pre-made base drug-delivery pastes tested.

## Treatment Regimen 3

On 8/1/2016, a custom formulation of a paste used for drug delivery in dentistry was designed for better underwater endurance and prepared by PP. The resulting proprietary formulation, referred to as coral dental paste (CDP), was prepared in two steps, requiring 48 h with these components: Methocel K100M (hypromellose USP), sorbitan monooleate NF (SPAN 80) and shea butter/medium chain triglyceride BAS/L.

Approximately 1 mL of the CDP was mixed with 60 mg of amoxicillin, resulting in a creamy consistency. Note the amoxicillin does not fully dissolve but forms a suspension in the CDP. The treatment paste was applied to the tissue margin after blotting excess water from the skeleton with a laboratory wipe. This, along with slowly introducing the fragment to TMASW, helps set the CDP and improves adherence. Fragment 33-A from Treatment B-2 was used as a test fragment to determine the adherence properties and duration of the application.

After 48 h, almost all the CDP remained attached to the skeleton. Due to its advanced deterioration at the start of the treatment regimens, this fragment did not survive and was removed from the experiment on 8/3/2016. However, the performance of this drug delivery vehicle appeared to be superior to the others previously tested (Treatment Regimens 1 & 2), so it was used for treatments of additional fragments that had

undergone the in-water antibiotic treatments but were not fully recovered (Chapter 2: Tables 5, 6).

Several pre-made drug delivery compounds (Table 7, Table 8) were tested on five subfragments (33-A, 38-A, 38-B, 38-C, 40-A) but were unsuccessful. Those five plus one additional subfragment (25-A) were treated with approximately 60 mg amoxicillin per mL CDP applied around the entirety of the coral tissue margin (see example in Figure 9). Subfragments were incubated as described above for treatment regimen 1. Of the six subfragments that received this treatment, only one (33-A), which was already observed to be in a very poor state of health, died. The remaining five had fully recovered by 9/6/2016 and were placed in permanent culture systems according to collection site.

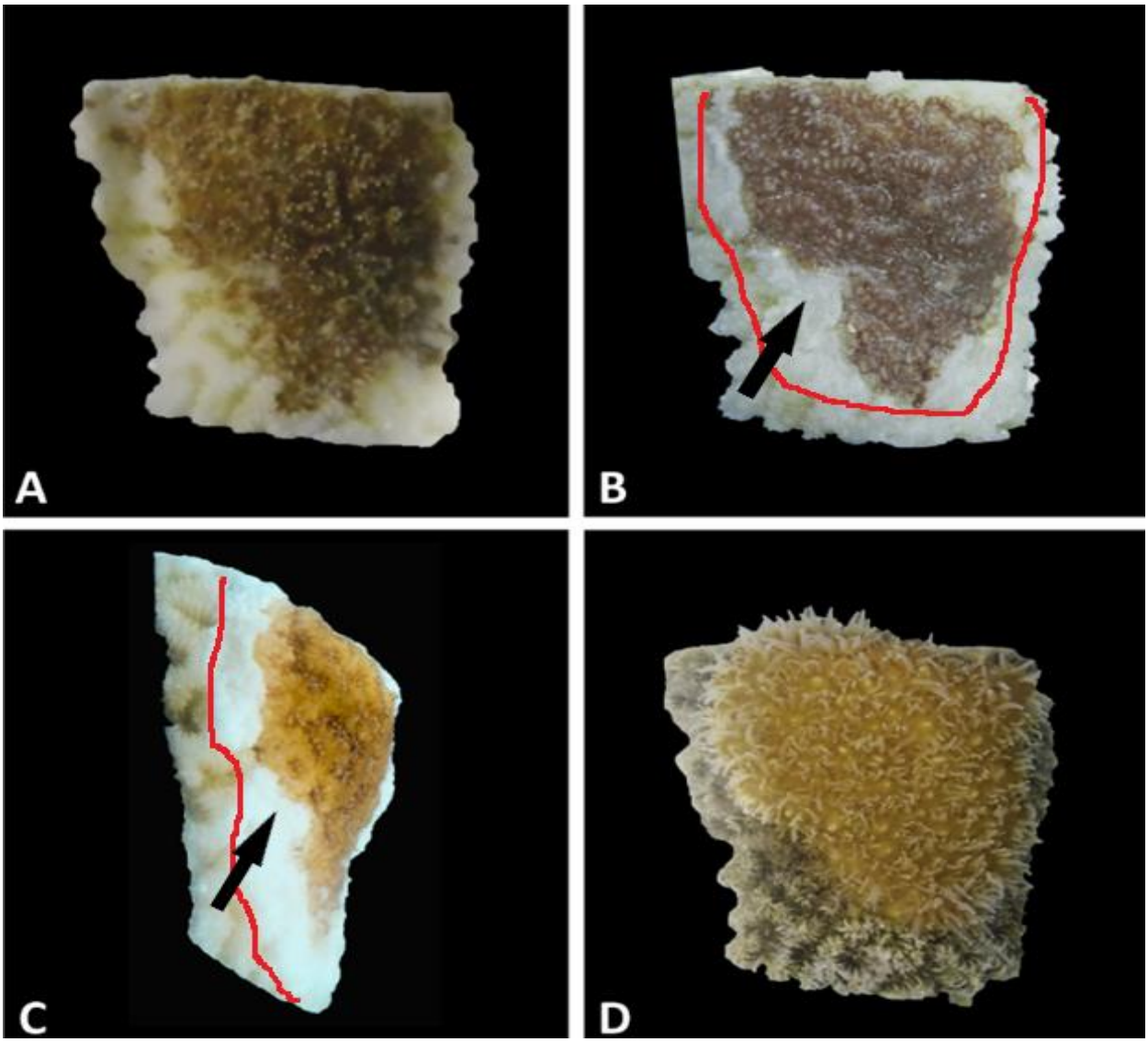


**Figure 9. *Dendrogyra cylindrus* recovery. Subfragment PCF 40-A showing tissue loss in its original condition (panel A); immediately following amoxicillin/CDP application (panel B); side view, 12 days after treatment with amoxicillin/CDP (panel C); and showing full recovery (panel D).**

## Conclusions

- None of the three pre-made base compound agents that were tested (PCCA Mucolox, PCCA Plasticized-base, PCCA Pracasil-Plus) had sufficient integrity and adherence properties to be used as an underwater targeted drug delivery vehicle.

- A custom-made formulation based on a PCCA dental paste formula with a timed-release agent (referred to as coral dental paste, CDP) adhered to coral skeleton. Blotting excess water from the skeleton prior to CDP application improved adherence. Subsequently, it maintained integrity underwater (i.e., did not dissolve) for at least 5 days.

- The application of the CDP containing 60 mg/mL amoxicillin was highly effective in arresting tissue-loss affecting *D. cylindrus*. Although the test consisted of a very small sample size, the only fragment lost was one that had only a few polyps and was in poor condition at the onset of the test.

- A recent dissolution-rate test by a pharmaceutical laboratory, CoreRX (Tampa, FL) suggested a 100 % release of the amoxicillin from the CDP into the diluent media within 16 h.

# Chapter 4. Upper Keys *Dendrogyra cylindrus* Rescue II

In January 2017, planning for the second Florida Keys rescue of *Dendrogyra cylindrus* began with a call to action by Dr. Karen Neely (NSU) because many sites had not been visited during the first rescue (July 2016), and new site assessments had identified undocumented populations. In addition, sites that had previously shown no signs of disease were reevaluated. The objective of this rescue was to collect *D. cylindrus* ahead of the disease front for the preservation of genotypes in either *in situ* or *ex situ* nurseries. When diseased sites were encountered, fragments were collected for experimental treatment at the NOAA NOS NCCOS Charleston Laboratory. The second rescue and assessment was conducted in February 2017, led by Dr. Neely with support from the Florida Aquarium (FLAQ), Keys Marine Laboratory (KML), Mote Marine Laboratory (Mote), and the Florida Fish and Wildlife Conservation Commission (FWC). Site assessments and collections were conducted according to the "DCYL Frag Protocol" (Neely, personal communication) to comply with the FKNMS permitting requirements and support continued monitoring of wild populations.

## Methods

### *Coral collection, pretreatment and transport*

*Dendrogyra cylindrus* fragments were collected from colonies at 11 sites near Key Largo, FL, between 2/14/2017 and 2/17/2017 (Figure 10; Table 9). Fragments were designated as Category 1 (apparently healthy, no disease observed at collection site), Category 2 (apparently healthy, disease observed at collection site) or Category 3 (visibly diseased colony). Diseased specimens, grouped by site, were maintained in separate flow-through systems at KML for up to 3 days. On 2/17/2017, all diseased corals were fragmented with a tile saw or Dremel tool to remove as much dead skeleton as possible. Interior skeleton was resected from fragments when a sulfur odor was detected. Mucus swabs were collected from an apparently healthy area and a diseased tissue margin on each fragment (Table A20). These samples were immediately frozen in a liquid nitrogen vapor shipper and archived (-80 °C) upon arrival at the NOAA NOS NCCOS Charleston Laboratory. If sufficient diseased tissue was available, samples were preserved in a zinc-formalin fixative (5X concentrated; Z-fix®, Anatech, Battle Creek, MI) diluted to 1X (3.7 % formalin) in TMASW (36 ppt) for histological analysis, or quickly frozen in a liquid nitrogen vapor shipper for future molecular work. Once all fragments were processed, they collectively underwent immersion in 0.5 mL/L Lugol's solution (10 % potassium iodide, 5 % iodine stock solution) in TMASW (36 ppt) for 15 min followed by a rinse in TMASW. Diseased fragments were placed into 10-gallon coolers (grouped by collection site) filled with approximately 5 gallons of TMASW (36 ppt) for transport. On arrival from the field (2/17/19), four seemingly healthy fragments (Category 1 and Category 2) were placed directly into transport coolers half filled with TMASW. This entire process yielded 38 individual fragments (Table 10).

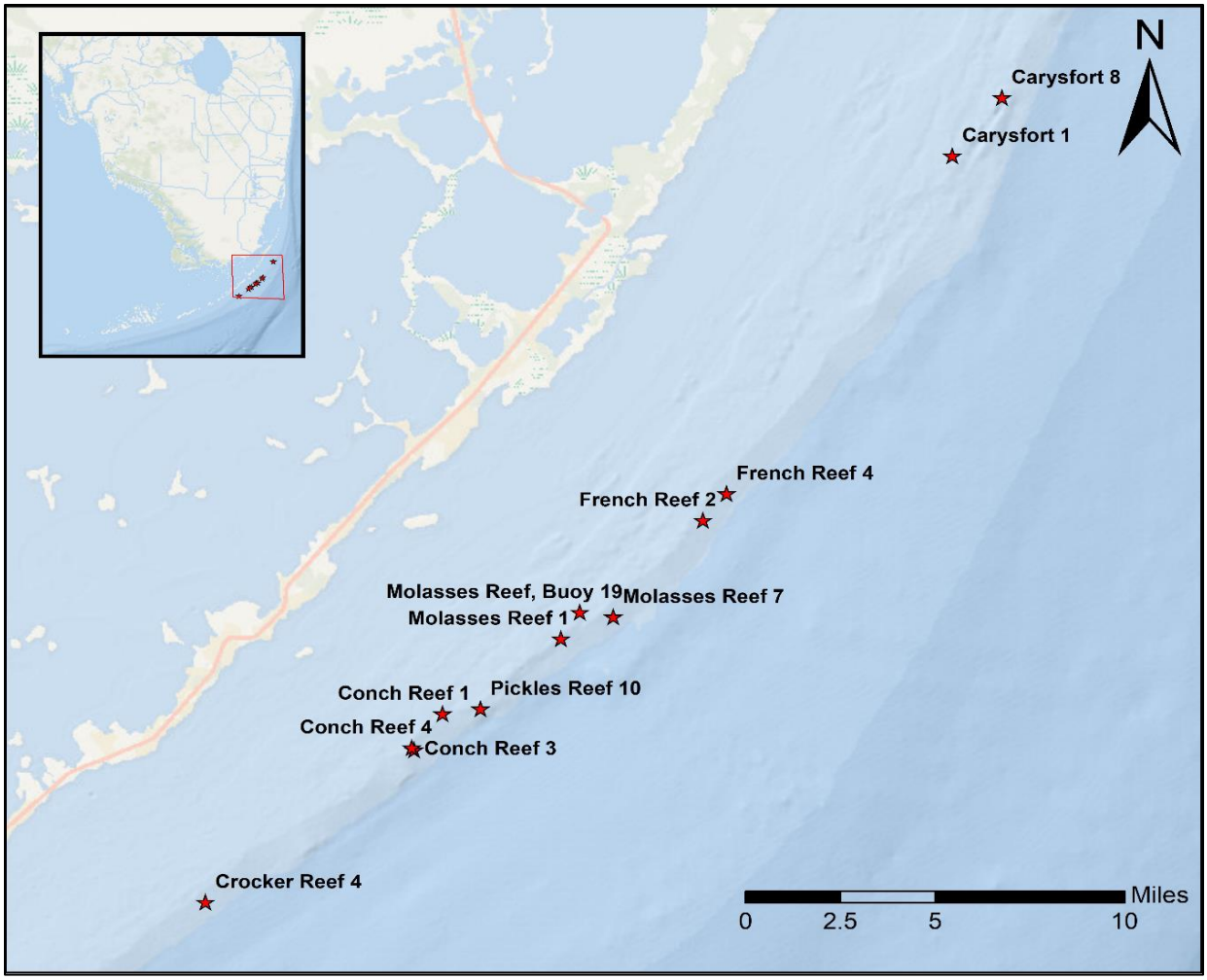


**Figure 10. Upper Keys Rescue II: *Dendrogyra cylindrus* collection sites along the Florida Reef Tract.**

**Table 9. Rescue II: GPS coordinates of diseased *Dendrogyra cylindrus* collections.**

| Collection Site | Latitude | Longitude |
|---|---|---|
| Carysfort Reef 8 | 25.226 | -80.208 |
| Carysfort Reef 1 | 25.202 | -80.229 |
| French Reef 4 | 25.062 | -80.325 |
| French Reef 2 | 25.051 | -80.335 |
| Molasses Reef, Buoy 19 | 25.013 | -80.387 |
| Molasses Reef 7 | 25.011 | -80.373 |
| Molasses Reef 1 | 25.002 | -80.395 |
| Pickles Reef 10 | 24.973 | -80.429 |
| Conch Reef 1 | 24.971 | -80.445 |
| Conch Reef 4 | 24.956 | -80.457 |
| Conch Reef 3 | 24.957 | -80.458 |
| Crocker Reef 4 | 24.893 | -80.545 |

Pillar coral fragments were transported by van in coolers with airstones driven by a Danner® AP-20 air pump (Islandia, NY) and a custom manifold. The van with coolers was parked in an enclosed garage overnight (temperature was maintained at 23-26 °C). On 2/19/2017, all fragments were transported under temperature-controlled conditions (24 °C) with constant aeration to the NOAA NCCOS Charleston coral facility.

### *Coral antibiotic treatment*

Upon arrival in Charleston, SC, fragments were placed into 20 clean treatment tanks as described in Chapter 2 (see *Treatment System Specifications*). Some smaller fragments from a single site were grouped together due to lack of additional individual treatment tanks. Treatments were assigned:

1. Ampicillin (25 mg/L TMASW) for 10 days (4 tanks, 11 fragments including three designated Category 1 and one designated Category 2)
2. Ampicillin (50 mg/L TMASW) for 10 days (4 tanks, 7 fragments)
3. Paromomycin (100 mg/L TMASW) for 10 days (4 tanks, 5 fragments)
4. Amoxicillin (50 mg/mL in coral dental paste, CDP) applied at the disease margins, the amount of CDP used was dependent on length of coral tissue margin (8 tanks, 15 fragments)

Reduced dosages of ampicillin (relative to Chapter 2: 100 mg/L for Treatments 1 & 2) were tested to determine if a lower dose was effective. Treatment 4 received 100 % daily seawater changes (TMASW, 36 ppt) following a single application of amoxicillin in CDP while the remaining treatments received 100 % daily treatment water changes (TMASW, 36 ppt, with respective antibiotic). All fragments were fed a solution containing Hikari Bio-Pure *Artemia* nauplii, rotifers, and cyclopods at least one hour before each water change.

Category 1 and Category 2 fragments (no signs of disease) continued to receive 25 mg/L ampicillin for the entire 10-day treatment. Many Category 3 fragments in the ampicillin and paromomycin treatments continued to lose tissue after 48 h, but at different rates; therefore, treatments were discontinued for the fragments with the most severe tissue loss. The paromomycin treatment (Treatment 3) was replaced with 50 mg/mL amoxicillin in CDP (Treatment 4). All Category 3 fragments receiving Treatment 1 or Treatment 2 were eventually switched to Treatment 4 between 2-6 days into their original treatments.

**Table 10. Rescue II: *Dendrogyra cylindrus* fragments with collection data and treatment.**

| Frag # | Parent Colony Tag # | Date Collected | Site Name | Geno-type | Health Cate-gory | Initial Treat-ment | Secondary Treatment Date | Brown Jelly | Recovery Status |
|---|---|---|---|---|---|---|---|---|---|
| **DC-156a** | 3 | 2/14/17 | CARY-1 | D1368 | 3 | 2 | 2/24/17 | no | **Recovered** |
| **DC-156b** | 3 | 2/14/17 | CARY-1 | D1368 | 3 | 2 | 2/24/17 | no | **Recovered** |
| **DC-157** | 1 | 2/16/17 | FRENCH-2 | D1371 | 3 | 4 | NA | yes | **Recovered** |

| Frag # | Parent Colony Tag # | Date Collected | Site Name | Geno-type | Health Cate-gory | Initial Treat-ment | Secondary Treatment Date | Brown Jelly | Recovery Status |
|---|---|---|---|---|---|---|---|---|---|
| **DC-158** | 1 | 2/16/17 | FRENCH -2 | D137 1 | 3 | 2 | 2/24/17 | yes | Dead 5/2/17 |
| **DC-159** | 1 | 2/16/17 | FRENCH -2 | D137 1 | 3 | 3 | 2/24/17 | yes | Dead 4/1/17 |
| **DC-160** | 1 | 2/16/17 | FRENCH -2 | D137 1 | 3 | 4 | NA | yes | **Recovered** |
| **DC-161** | 1 | 2/16/17 | FRENCH -2 | D137 1 | 3 | 1 | 2/24/17 | yes | Dead 3/28/17 |
| **DC-162** | 1 | 2/16/17 | FRENCH -2 | D137 1 | 3 | 2 | 2/24/17 | yes | Dead 4/1/17 |
| **DC-163** | 1 | 2/16/17 | FRENCH -2 | D137 1 | 3 | 1 | 2/24/17 | yes | **Recovered** |
| **DC-164** | 1 | 2/16/17 | FRENCH -2 | D137 1 | 3 | 2 | 2/24/17 | no | Dead 3/27/17 |
| **DC-165** | 1 | 2/16/17 | FRENCH -2 | D137 1 | 3 | 4 | NA | yes | **Recovered** |
| **DC-166** | 1 | 2/16/17 | FRENCH -2 | D137 1 | 3 | 2 | 2/24/17 | yes | Dead 4/19/17 |
| **DC-167** | 1 | 2/16/17 | FRENCH -2 | D137 1 | 3 | 4 | NA | yes | **Recovered** |
| **DC-168** | 1 | 2/16/17 | FRENCH -2 | D137 1 | 3 | 1 | 2/24/17 | yes | Dead 3/21/17 |
| **DC-169** | 1 | 2/16/17 | FRENCH -4 | D137 0 | 3 | 4 | NA | yes | Dead 3/30/17 |
| **DC-170** | 1 | 2/16/17 | FRENCH -4 | D137 0 | 3 | 1 | 2/24/17 | yes | **Recovered** |
| **DC-171** | 1 | 2/16/17 | FRENCH -4 | D137 0 | 3 | 4 | NA | yes | Dead 3/30/17 |
| **DC-172** | 1 | 2/16/17 | FRENCH -4 | D137 0 | 3 | 4 | NA | yes | **Recovered** |
| **DC-173a** | 4 | 2/16/17 | BUOY-19 | D137 3 | 3 | 1 | none | no | Dead 2/21/17 |
| **DC-173b** | 4 | 2/16/17 | BUOY-19 | D137 3 | 3 | 1 | none | no | Dead 2/21/17 |
| **DC-174b** | 1 | 2/16/17 | BUOY-19 | D137 3 | 3 | 3 | 2/20/17 | no | **Recovered** |
| **DC-174c** | 1 | 2/16/17 | BUOY-19 | D137 3 | 3 | 4 | NA | no | **Recovered** |
| **DC-175** | 1 | 2/16/17 | BUOY-19 | D137 3 | 3 | 4 | NA | no | Dead 2/21/17 |
| **DC-176** | 1 | 2/16/17 | BUOY-19 | D137 3 | 3 | 4 | NA | no | Dead 2/21/17 |
| **DC-177** | 1 | 2/16/17 | BUOY-19 | D137 3 | 3 | 1 | 2/24/17 | no | Dead 4/3/17 |
| **DC-178** | 1 | 2/16/17 | MOL-1 | D137 4 | 3 | 3 | 2/24/17 | no | **Recovered** |
| **DC-179** | 1 | 2/16/17 | MOL-1 | D137 4 | 3 | 4 | NA | no | **Recovered** |
| **DC-180** | 1 | 2/16/17 | MOL-7 | D137 2 | 3 | 2 | 2/24/17 | no | **Recovered** |
| **DC-181a** | 1 | 2/16/17 | MOL-7 | D137 2 | 3 | 4 | NA | no | **Recovered** |
| **DC-182** | 1 | 2/16/17 | MOL-7 | D137 2 | 3 | 3 | 2/21/17 | no | **Recovered** |
| **DC-183** | 1 | 2/16/17 | MOL-1 | D137 4 | 3 | 3 | 2/24/17 | no | **Recovered** |
| **DC-184** | 1 | 2/16/17 | MOL-1 | D137 4 | 3 | 4 | NA | no | **Recovered** |

| Frag # | Parent Colony Tag # | Date Collected | Site Name | Geno -type | Health Cate- gory | Initial Treat- ment | Secondary Treatment Date | Brown Jelly | Recovery Status |
|---|---|---|---|---|---|---|---|---|---|
| **DC-185** | 1 | 2/16/17 | MOL-1 | D137 4 | 3 | 4 | NA | no | Dead 2/20/17 |
| **DC-186** | 1 | 2/17/17 | PICK-10 | D128 6 | 3 | 4 | NA | no | **Recovered** |
| **DC-188** | 2 | 2/17/17 | CONCH-1 | D137 5 | 1 | 1 | none | no | **Recovered** |
| **DC-193** | 3 | 2/17/17 | CONCH-3 | D104 2 | 2 | 1 | none | no | **Recovered** |
| **DC-198** | 1 | 2/17/17 | CONCH-4 | D104 2 | 1 | 1 | none | no | **Recovered** |
| **DC-202** | 3 | 2/17/17 | CROCK-4 | D137 6 | 1 | 1 | none | no | **Recovered** |

Initial treatment 1. Ampicillin (25 mg/L TMASW) for 10 days
Initial treatment 2. Ampicillin (50 mg/L TMASW) for 10 days
Initial treatment 3. Paromomycin (100 mg/L TMASW) for 10 days
Initial treatment 4. Amoxicillin (50 mg/mL in CDP) applied at the disease margins
* Secondary treatment: Amoxicillin (50 mg/mL in CDP) applied at the disease margins

## Results

The survival of *D. cylindrus* was 61 % (23/38 fragments). Mortality due to SCTLD was 18 % (7/38 fragments) and due to BJS was 21 % (8/38 fragments). Representative specimens from each site recovered (Table 10; Figure 11).

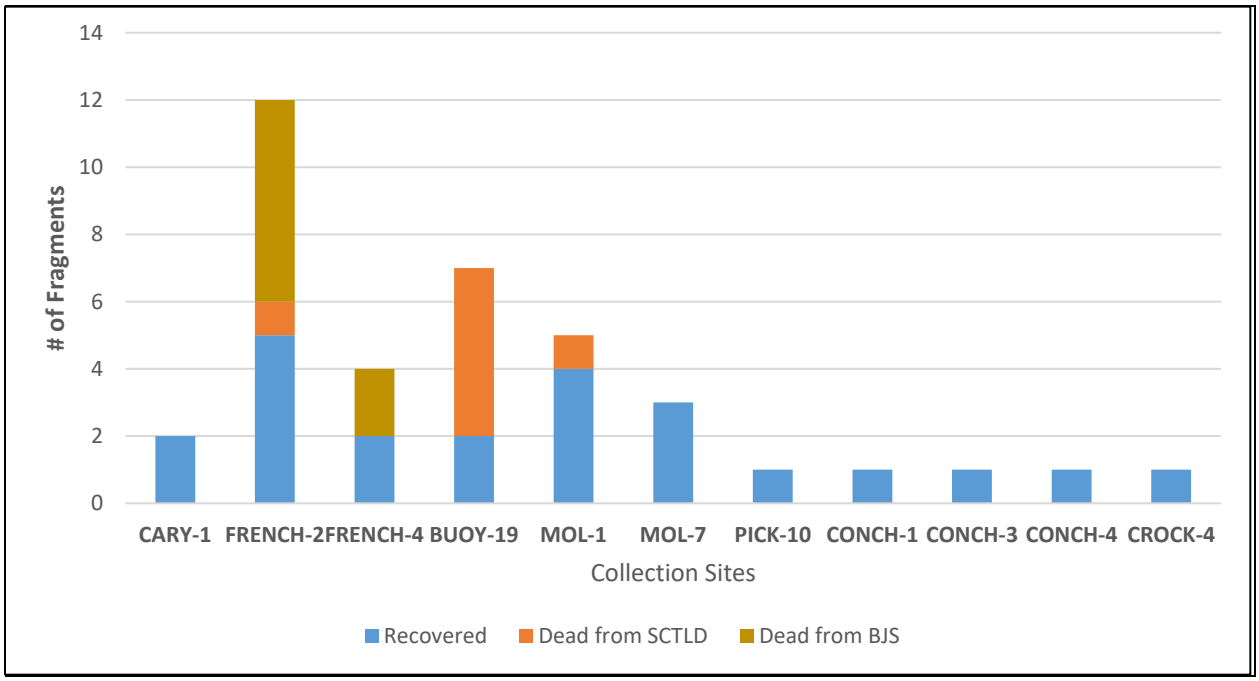


**Figure 11. Survivorship of diseased *Dendrogyra cylindrus* fragments collected in February 2017. Of 38 fragments, 7 (18 %) died from SCTLD, and 8 (21 %) were lost due to brown jelly syndrome.**

### *Bleaching event and initial mortality*

Treatments were confounded by a timer malfunction causing the lights over the coral treatment tanks to remain on continuously for 60+ h, which resulted in bleaching for some fragments (those most centered under the light fixtures). Once the problem was detected (the morning of 2/21/2017) lights were shut off for 24 h to allow corals to recover. Five fragments (DC-177, DC-178, DC-179, DC-183, DC-184) all showed signs of significant bleaching. The timer malfunction might have also contributed to mortality of several fragments within the first few days of treatment (except DC-185, which was not under these lights):

- DC-185 (treated in a beaker with 50 mg/mL amoxicillin in CDP) had almost no healthy tissue when fragmented on 2/17/2017, and died 2/20/2017
- DC-173A and DC-173B (25 mg/L ampicillin) were very small fragments that seemed to have little healthy tissue, and died 2/21/2017
- DC-175 and DC-176 (50 mg/mL amoxicillin in CDP) died 2/21/2017

In summary, five fragments succumbed to the SCTLD (four from site Buoy-19 and one from Molasses-1) during initial treatment regimens. After initial treatment regimens were completed, of the 33 remaining fragments, 25 appeared to be recovering and eight still exhibited signs of disease. By 2/28/2017 (10 days after initial treatment and one day prior to BJS detection), almost all remaining colonies appeared to have stopped actively sloughing tissue and many seemed to show signs of recovery (darkening tissue, a more defined tissue margin and better polyp extension). However, some had very little live tissue remaining. Fragment DC-164 was unable to recover having only a small amount of tissue (one polyp); that remaining tissue was lost on 3/27/2017. DC-177 had more tissue but continued to bleach and slowly lost all tissue by 4/3/2017. The reduced ampicillin treatments (25-50 mg/L in TMASW) were not effective in treating more severe cases of SCTLD, but subsequent applications of amoxicillin in CDP had a positive health effect for the *D. cylindrus* fragments. The four Category 1 and 2 fragments receiving ampicillin at 25 mg/L did not develop SCTLD signs and all survived.

### *Brown Jelly Syndrome*

The first signs of BJS were confirmed on 3/1/2017 (11 days after arrival) in one treatment tank containing fragments DC-161, DC-163, and DC-168. On 3/15/2017, a fragment (DC-170) in a second treatment tank also contracted BJS, and six days later BJS was detected on three fragments (DC-169, DC-171, DC-172) in a third treatment tank. On 3/27/2017, fragments DC-162 and DC-166 were very pale with little polyp extension, but no sign of BJS was observed. Therefore, these fragments were transferred from their treatment tank into a permanent aquaculture system with other fragments to provide a more stable environment. Unfortunately, on 3/31/2017, BJS was detected on fragment DC-166, and it was moved back into a treatment tank. However, it was later evident that all other fragments in the larger aquaculture system were infected with BJS (Table 10). Hence, all fragments were removed for BJS treatment. The larger aquaculture system was completely dismantled and sterilized. In total, 15 of the original

38 fragments were exposed to BJS. All 15 fragments of *D. cylindrus* that contracted BJS originated from two French Reef locations (French-2 and French-4). It is unclear whether French Reef is a reservoir for BJS, or if the fragments from these sites were infected at a later date. With two exceptions (DC-174-2b and DC-198b), all subfragments created during this rescue were a result of attempts to stop BJS advancement (Table 11).

Detection of BJS occurred at a much earlier stage because previous experience provided insights to initial disease signs which manifested in *D. cylindrus* as a slight 'webbing' around the tissue margin. Successful transmission of BJS was demonstrated in the laboratory by exposing several other healthy coral species (both Atlantic and Pacific species, e.g., *Acropora cervicornis, Pocillopora damicornis*, and *Fungia* sp.) to a diseased tissue slurry. Specimens that had been fragmented (i.e., open wounds) were consumed rapidly (1-2 days) whereas those with no obvious wounds were consumed at a much slower rate (~5-7 days). Observation under a dissecting scope revealed areas of infection that were dominated by ciliate-like organisms, which is consistent with other reports of BJS (Sweet 2013). These organisms were the likely cause of BJS.

Several treatments were used to combat BJS (Table 11). Before a treatment was administered, fragments were thoroughly rinsed under 36 ppt TMASW and in cases of a more severe infection, excess dead skeleton was trimmed away and the fragment was rinsed a second time. Due to the detection of a large population of ciliate-like organisms (Figure 16) around areas of infection, paromomycin was initially chosen as a treatment due to its antiprotozoal activity. A 200 mg/L dose for seven days was chosen, because the compound did not seem to have any ill effect on the coral fragments in previous tests. Subsequent treatments included immersing corals for 10-15 min in concentrations of Lugol's solution or various commercial coral dips formulated for the aquarium trade (ReVive® Coral Cleaner, Two Little Fishies, Miami Gardens, FL; Coral Rx® Coral Dip, Blue Ocean Corals, Deerfield Beach, FL; Reef Pure® Coral Dip & Conditioner, Warner Marine, Simi Valley, CA; Pro-Coral Cure®, Tropic Marin, Montague, MA).

Of the 26 fragments and subfragments affected by BJS, only nine survived after various treatments. Of the nine survivors, two were subfragments. This may be due to the smaller size of the subfragments and having unhealed tissue margins (i.e., disease entry points). Paromomycin (Treatment C), known for its antiprotozoal activity, was largely ineffective at eliminating BJS (1/8 surviving fragments). Six treatments had no effect against BJS: (1) TMASW rinse (Treatment B), (2) 15 min Lugol's immersion (1 mL/L; Treatments D), (3) 15 min Lugol's immersion (2 mL/L; Treatment E), (4) 5 consecutive daily 15 min immersion treatments of ReVive Coral Cleaner (10 mL/L; Treatment H), (5) 10 min immersion in Coral Rx Coral Dip (8 drops/L; Treatment I) and (6) 10 min immersion in Reef Pure Coral Dip and Conditioner (10 drops/L; Treatment J). One 15 min immersion in ReVive Coral Cleaner (10 mL/L; Treatment F) seemed to be effective against BJS; however, this was tried only on fragments with very mild infection (n=3). ReVive Coral Cleaner (10 mL/L) followed by Pro-Coral Cure (5 mL/L) (12 min immersion in each; Treatment G) was only partially effective (4 of 11 – 36 % recovered).

**Table 11. *Dendrogyra cylindrus* fragments treated for brown jelly syndrome.**

| Frag # | Date of First BJS Detection | Location of First Detection* | BJS Treatment(s) and Date(s)** | Status |
|---|---|---|---|---|
| DC-157 | 4/11/2017 | 3 | F. 4-11-2017 | **Fully recovered** |
| DC-158 | 4/3/2017 | 3 | D. 4-7-2017, G. 4-11-2017, 4-17-2017, 4-23-2017 | DEAD, 5-2-2017 |
| ^DC-158b | 4/3/2017 | 3 | B. 4-3-2017 | DEAD, 4-7-2017 |
| DC-159 | 4/1/2017 | 3 | A. | DEAD, 4-1-2017 |
| ^DC-159b | 4/3/2017 | 3 | D. 4-3-2017 | DEAD, 4-11-2017 |
| ^DC-159c | 4/11/2017 | 3 | G. 4-11-2017, D. 5-2-2017, E. 5-13-2017, H. 5-23-2017, I. 5-30-2017, J. 6-2-2017, 6-5-2017 | DEAD, 6-8-2017 |
| DC-160 | 4/11/2017 | 3 | G. 4-11-2017 | **Fully recovered** |
| ^DC-160b | 4/11/2017 | 3 | G. 4-11-2017 | DEAD, 5-13-2017 |
| ^DC-160c | 4/11/2017 | 3 | G. 4-11-2017 | **Fully recovered** |
| ^DC-160d | 4/11/2017 | 3 | G. 4-11-2017 | DEAD, 6-8-2017 |
| ^DC-160e | 4/11/2017 | 3 | G. 4-11-2017 | DEAD, 4-30-2017 |
| ^DC-160f | 4/11/2017 | 3 | G. 4-11-2017 | DEAD, 4-30-2017 |
| DC-161 | 3/1/2017 | 1 | B. 3-1-2017, 3-8-2017, C. 3-13-2017 | DEAD, 3-28-2017 |
| DC-162 | 3/31/2017 | 1 | A. | DEAD, 4-1-2017 |
| DC-163 | 3/1/2017 | 1 | B. 3-1-2017, 3-13-2017 | **Fully recovered** |
| DC-165 | 4/11/2017 | 3 | F. 4-11-2017 | **Fully recovered** |
| DC-166 | 3/31/2017 | 1 | B. 3-31-2017, C. 4-1-2017, D. 4-3-2017 | DEAD, 4-19-2017 |
| DC-167 | 4/11/2017 | 3 | F. 4-11-2017 | **Fully recovered** |
| DC-168 | 3/1/2017 | 1 | B. 3-1-2017, 3-13-2017 | DEAD, 3-21-2017 |
| DC-169 | 3/21/2017 | 1 | C. 3-21-2017, D. 3-23-2017 | DEAD, 3-30-2017 |
| DC-170 | 3/15/2017 | 2 | C. 3-15-2017, G. 4-17-2017, 4-24-2017 | **Fully recovered** |
| ^DC-170b | 3/15/2017 | 2 | C. 3-15-2017, G. 4-17-2017, 4-24-2017 | **Fully recovered** |
| ^DC-170c | 3/15/2017 | 2 | C. 3-15-2017, G. 4-17-2017 | DEAD, 4-24-2017 |
| ^DC-170d | 3/15/2017 | 2 | A. | DEAD, 3-15-2017 |
| DC-171 | 3/21/2017 | 1 | C. 3-21-2017 | DEAD, 3-30-2017 |
| DC-172 | 3/21/2017 | 1 | C. 3-21-2017 | **Fully recovered** |

^denotes a subfragment.

*Location Key:
1. BJS was detected when fragment was in the initial treatment tank grouped with other fragments
2. BJS was detected when fragment was in the initial treatment tank with only itself and its subfragments
3. BJS was detected only after fragment was placed in a permanent aquaculture system with other fragments

**Treatment Key:
A. Dead or almost dead when BJS was detected so no treatment initiated
B. Rinsed with TMASW and put in new sterile treatment tank
C. Treated with 200 mg/L paromomycin daily for seven days
D. Treated with 1 mL/L Lugol's solution in 36 ppt TMASW for 15 min
E. Treated with 2 mL/L Lugol's solution in 36 ppt TMASW for 15 min
F. Treated with ReVive Coral Cleaner at 10 mL/L TMASW for 15 min
G. Treated with ReVive Coral Cleaner at 10 mL/L TMASW for 12 min, then Pro-Coral Cure at 5 mL/L TMASW for 12 min
H. Treated with ReVive Coral Cleaner at 10mL/L TMASW for 15 min for 5 consecutive days
I. Treated with 8 drops/L (with supplied dropper) TMASW of Coral Rx Coral Dip for 10 min
J. Treated with 10 drops/L (with supplied dropper) of Reef Pure Coral Dip & Conditioner for 10 min

## Conclusions

- Antibiotic treatments effectively cured 23 of the 38 pillar coral fragments (61 % survivorship; Figure 11; Table 10). Four replicate subfragments also survived.

- Paromomycin was ineffective in treating the SCTLD.

- The treatment regimen with 25 mg/L of ampicillin was an effective preventive measure for Category 1 and Category 2 corals and may be considered as a prophylactic treatment.

- While some of the reduced ampicillin treatments (25 and 50 mg/L) seemed to be slowing SCTLD on Category 3 fragments, the ultimate goal was to save as much living tissue as possible. Thus, these fragments were switched to 50 mg/mL amoxicillin in CDP within 6 days of treatment initiation. Compared to other tested treatments, 50 mg/mL amoxicillin in CDP was most effective against SCTLD.

- Excising necrotic tissue, rinsing fragments in fresh TMASW, and pretreating with Lugol's solution increased the effectiveness of the amoxicillin/CDP treatment. Higher concentrations (>0.5 mL/L) of Lugol's solution may be more effective against infections, but can cause stress responses. For example, a concentration of 1 mL/L with a 15 min immersion resulted in tissue retracting from the apical septa coupled with polyp retraction. Thus, higher concentrations should be considered only as a last resort.

- The secondary infection with BJS hampered our ability to determine the overall success of SCTLD treatments. Additional treatments for BJS described here showed no efficacy. BJS continues to be a threat during *D. cylindrus* rescue and rehabilitation.

# Chapter 5. *Dendrogyra cylindrus*: Implementing Best Treatment Practices

From April 2018 – October 2019, four additional field collections were conducted by Dr. Karen Neely (NSU) and her team to retrieve specimens from genetically distinct *D. cylindrus* colonies before they succumbed to SCTLD. In all, 102 fragments representing a possible 45 genotypes were collected in various stages of disease. Two additional trips were made to obtain pillar coral fragments being held at Keys Marine Laboratory (9/6/2018 and 6/5/2019) and Mote Marine Laboratory (6/5/2019) due to declining health status. These two trips yielded an additional 43 fragments representing 27 genotypes. All 145 fragments received treatment with ampicillin and/or amoxicillin at the NOAA NCCOS Coral Health and Disease laboratory. Due to varying circumstances surrounding each trip, (e.g., disease category of fragments), treatment methods evolved from one rescue trip to the next. Therefore, the sections that follow have been sub-divided by rescue trip.

## Methods

### *Field Rescue Collections*

#### ***Rescue III:***

Eighteen fragments of *D. cylindrus* were collected from six sites between Key Largo and Big Pine Key, FL on April 17 and 18, 2018 (Figure 12; Table 12). All fragments had obvious signs of disease (Category 3). Coral fragments were transported to KML where treatment was initiated. Due to the effectiveness of the amoxicillin/CDP treatment for previous rescues, this antibiotic treatment was selected and initiated prior to transport to the NOAA NCCOS Charleston coral facility. Included in the collection was a single diseased fragment of maze coral (*Meandrina meandrites*) from Looe Key 17. The maze coral fragment was used to test the efficacy of the amoxicillin/CDP treatment on species other than *D*. *cylindrus*.

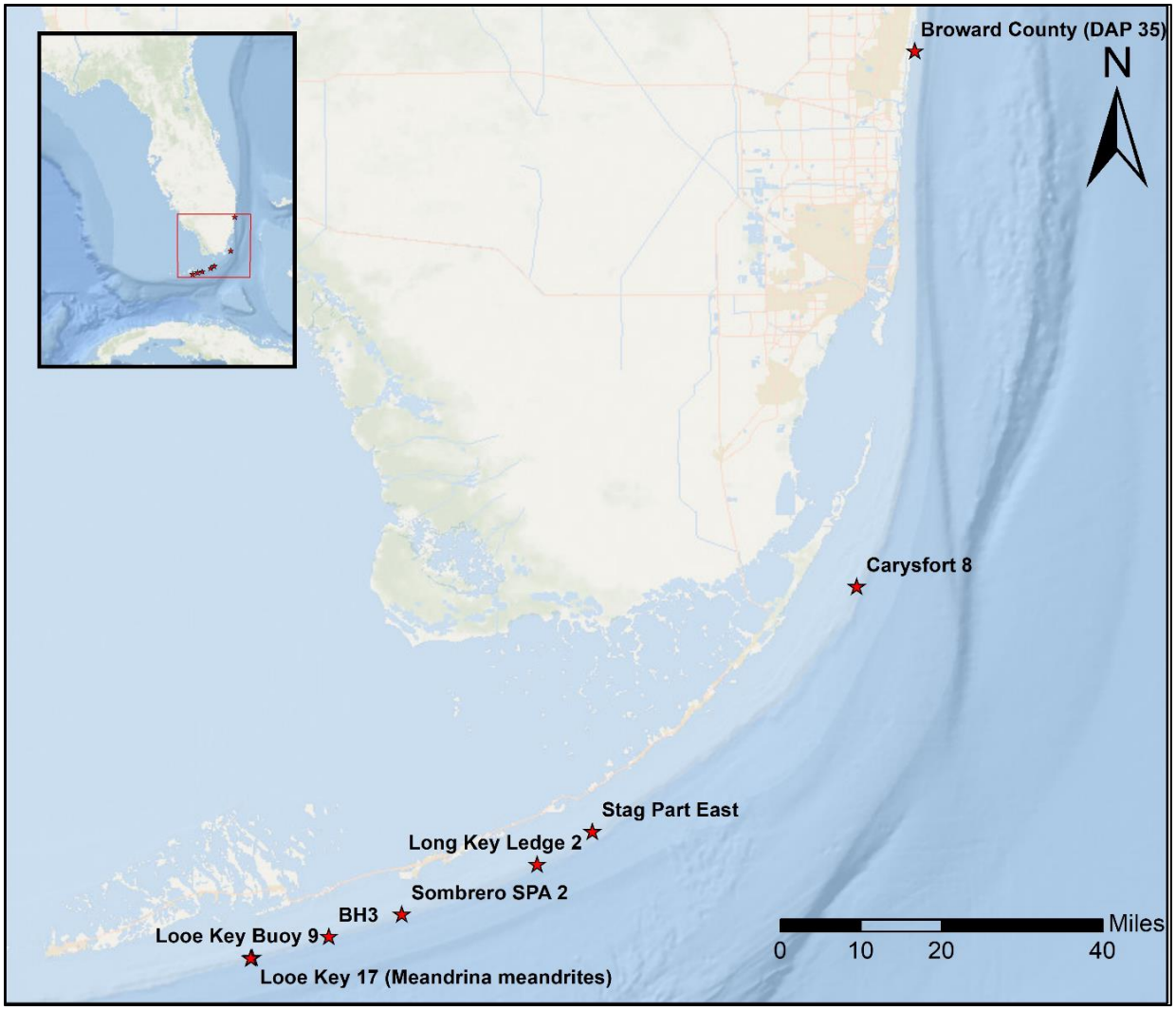


**Figure 12. Rescue III: Coral collection sites along the Florida Reef Tract.**

**Table 12. Rescue III: GPS coordinates of diseased coral collections.**

| Collection Site | Latitude | Longitude |
|---|---|---|
| Broward County (DAP 35) | 26.209 | -80.085 |
| Carysfort 8 | 25.226 | -80.208 |
| Stag Part East | 24.778 | -80.733 |
| Long Key Ledge 2 | 24.718 | -80.842 |
| Sombrero SPA 2 | 24.626 | -81.109 |
| BH3 | 24.585 | -81.253 |
| Looe Key Buoy 9 | 24.546 | -81.404 |
| Looe Key 17 (*M. meandrites*) | 24.545 | -81.407 |

At KML on 4/19/2018, visibly diseased tissue for each coral fragment was excised using a tile saw or Dremel tool and sub-samples were taken for future biological or histological analyses (Table A21). Mucus swabs from the diseased margin and apparently healthy tissue were collected, placed in a mixture of 50 % glycerol and 50 % glycerol artificial seawater media (GASW) (Smith and Hayasaka 1982), and then cryopreserved in a nitrogen vapor shipper. Diseased tissue samples were collected from the excised tissue margin. If sufficient diseased tissue was available on a given fragment, 1-3 subsamples were taken: one wrapped in foil and placed in a nitrogen vapor shipper, and the others were archived for histological analysis (zinc-formalin, Z-fix) for light microscopy; and modified Karnovsky's fixative (2.5 % glutaraldehyde, 2 % paraformaldehyde in 35 ppt TMASW) for transmission electron microscopy. Immediately following removal of the diseased margin, fragments were rinsed in TMASW and treated with a 15-min immersion in 0.5 mL/L Lugol's solution in TMASW (36 ppt). Fragments were again rinsed with fresh TMASW, and amoxicillin in CDP (50 mg/mL) was applied to the entire tissue margin. Corals were placed in 10-gallon coolers half-filled with 100 % TMASW for transport. Three test fragments (261b, 261c and 261d) received the Lugol's treatment and rinse only before being placed in a separate transport cooler as they were designated for experimental treatment at the NOAA NCCOS Charleston laboratory. The process yielded 18 individual *D. cylindrus* fragments and one *M. meandrites* fragment (Table 13).

**Table 13. Rescue III: Coral fragments with collection data and treatment.**

| Fragment ID | Parent Colony Tag # | Date Collected | Site Name | Genotype | Health Category* | Treatment** |
|---|---|---|---|---|---|---|
| **DC-257** | 1 | 4/19/2018 | LKLD-2 | D1076 | 3 | 1, 2 |
| **DC-258** | 1 | 4/19/2018 | LKLD-2 | D1076 | 3 | 1,2 |
| **DC-259** | 1 | 4/19/2018 | LKLD-2 | D1076 | 3 | 1,2 |
| **DC-260** | 1 | 4/19/2018 | LKLD-2 | D1076 | 3 | 1,2 |
| **DC-261a** | 3 | 4/19/2018 | SOMB-SPA2 | D1172 | 3 | 1,2 |
| **DC-261b** | 3 | 4/19/2018 | SOMB-SPA2 | D1172 | 3 | 3, 4, 1*** |
| **DC-261c** | 3 | 4/19/2018 | SOMB-SPA2 | D1172 | 3 | 3 |
| **DC-261d** | 3 | 4/19/2018 | SOMB-SPA2 | D1172 | 3 | 3, 4, 1*** |
| **DC-266** | 3 | 4/19/2018 | SOMB-SPA2 | D1172 | 3 | 1, 2 |
| **DC-267** | 2 | 4/19/2018 | CARY-8 | D1367 | 3 | 1 |

| Fragment ID | Parent Colony Tag # | Date Collected | Site Name | Genotype | Health Category* | Treatment** |
|---|---|---|---|---|---|---|
| **DC-268** | 2 | 4/19/2018 | CARY-8 | D1367 | 3 | 1 |
| **DC-269a** | 1 | 4/19/2018 | STAG-E | unknown | 3 | 1 |
| **DC-269b** | 1 | 4/19/2018 | STAG-E | unknown | 3 | 1 |
| **DC-270** | 1 | 4/19/2018 | BHP-3 | D1183 | 3 | 1, 2 |
| **DC-271** | 1 | 4/19/2018 | BHP-3 | D1183 | 3 | 1 |
| **DC-272** | 1 | 4/19/2018 | BHP-3 | D1183 | 3 | 1, 2 |
| **DC-273** | 1 | 4/19/2018 | LOOE B-9 | D1384 | 3 | 1, 2 |
| **DC-274** | 1 | 4/19/2018 | LOOE B-9 | D1384 | 3 | 1 |
| ***Meandrina*** | unknown | 4/19/2018 | LOOE KEY | unknown | 3 | 1 |
| **DC-290a** | 1 | 4/19/2018 | DAP35 | unknown | 3 | 1 |
| **DC-290b** | 1 | 4/19/2018 | DAP35 | unknown | 3 | 1, 2 |
| **DC-290c** | 1 | 4/19/2018 | DAP35 | unknown | 3 | 1, 2 |
| **DC-290d** | 1 | 4/19/2018 | DAP35 | unknown | 3 | 1, 2 |
| **DC-290e** | 1 | 4/19/2018 | DAP35 | unknown | 3 | 1, 2 |
| **DC-290f** | 1 | 4/19/2018 | DAP35 | unknown | 3 | 1, 2 |

*Health category 3: actively diseased fragments

**Treatment Key
Treatment 1: Excise diseased tissue, immerse in Lugol's solution, apply 50 mg/mL amoxicillin/CDP
Treatment 2: 25 mg/L ampicillin in TMASW with 100 % daily treatment changes
Treatment 3: Excise diseased tissue, immerse in Lugol's solution, apply Chelex 100 mg/mL in CDP to tissue margin
Treatment 4: 35 mg/mL 2, 2'-bipyridyl in CDP

***Fragments received Treatment 1 when tissue loss continued with Treatment 3 and 4; Fragment 261b survived

Coral fragments were transported by van in coolers with aeration driven by a Danner AP-20 air pump and air manifold. The van with coral was parked in an enclosed garage overnight (temperature was maintained at 23-26 °C) with the air pump running. On 4/20/2018, fragments were transported under temperature-controlled conditions (24 °C) with constant aeration to the NOAA NOS NCCOS Charleston coral facility. During transport, a stop was made in Broward County, FL, to acquire six additional small fragments of *D. cylindrus* from that area (Ken Banks, point of contact). The Broward County samples were maintained in a separate cooler during transport and were not treated until arrival in Charleston, SC (Table 13).

Upon arrival at the NOAA facility in Charleston, SC, larger fragments of *D. cylindrus* and the single *M. meandrites* were placed into individual 2.5-gallon tanks within the treatment system as described in Chapter 2. The small Broward County fragments were first trimmed of dead skeleton, underwent a 15-min immersion in 0.5 mL/L Lugol's solution in TMASW (35 ppt), and were rinsed with TMASW. Amoxicillin/CDP (50 mg/mL) then was applied around their entire tissue margin. After treatment application, they were placed into two treatment tanks, with the largest fragment (DC-290a) in a tank by itself and the five smallest fragments (DC-290b-f) in a separate tank. Some tissue loss was noted for four FL Keys fragments (DC-258, DC-259, DC-260, DC-266) on arrival, so in addition to the initial amoxicillin/CDP treatment, a supplementary low-dose antibiotic was added to the artificial seawater (25 mg/L ampicillin) and continued for 10 days with daily 100 % replenishment. All treatment tanks received daily 100 % TMASW changes (with antibiotic as designated). Spot treatments with amoxicillin/CDP occurred for fragments with areas of paling tissue or observed tissue loss on an 'as needed' basis. Between 4/23/2018 and 4/24/2018, 10 additional *D. cylindrus* fragments (DC-257, DC-261a, DC-270, DC-272, DC-273, DC-290b-f) and the *M. meandrites* fragment received a supplementary 25 mg/L ampicillin in seawater with daily 100 % replenishment for 10 days.

Alternatives to antibiotic therapy were investigated by using metal-chelating compounds that disrupt essential metal metabolism, targeting transition metal ions (e.g., iron copper, zinc, magnesium, cobalt and nickel). Three small test fragments (DC-261b, c, d) were used for experimental treatment with a metal-chelating agent, Chelex® 100 (BioRad, Hercules, CA). Chelex 100 has an exceptionally high preference (5000:1) for divalent cations (e.g., copper, iron and cadmium, zinc) over monovalent cations (e.g., sodium and potassium; BioRad Instruction Manual). This was mixed with CDP (100 mg/mL) and applied to the tissue margin. The test fragments were placed in two treatment tanks (DC-261b and DC-261c together).

A second chelating compound, 2,2'-bipyridyl (BIP) is highly membrane permeable and has been shown to interact with iron pools within eukaryotic cells that are used by regulatory factors (Romeo *et al.* 2001; Thompson & Carabeo 2011). Subsequently, the two surviving fragments of Chelex treatment, underwent an application of BIP (35 µg/mL) mixed in CDP.

### *Rescue IV:*

*Dendrogyra cylindrus* fragments (n=17) were collected from nine different reef sites in the lower Florida Keys (K. Neely) (Figure 13; Table 14) on 4/12/2019, and transported in 5-gallon buckets to rendezvous in Orlando, FL. Shortly after midnight, fragments were transferred to NOAA Coral Health and Disease staff and placed into 10-gallon coolers half-filled with 36 ppt TMASW. Water temperature was recorded at 26 °C and all coolers were vigorously aerated in the van for 30 min via airstones driven by a Danner AP-20 air pump, which was then shut off for 4 hours. Nighttime temperatures in Orlando were forecasted to be between 24 °C and 27 °C, so temperature regulation was not a concern. Transportation to the Charleston, SC, began before daylight on 4/13/2019 and treatments were initiated on arrival at the laboratory.

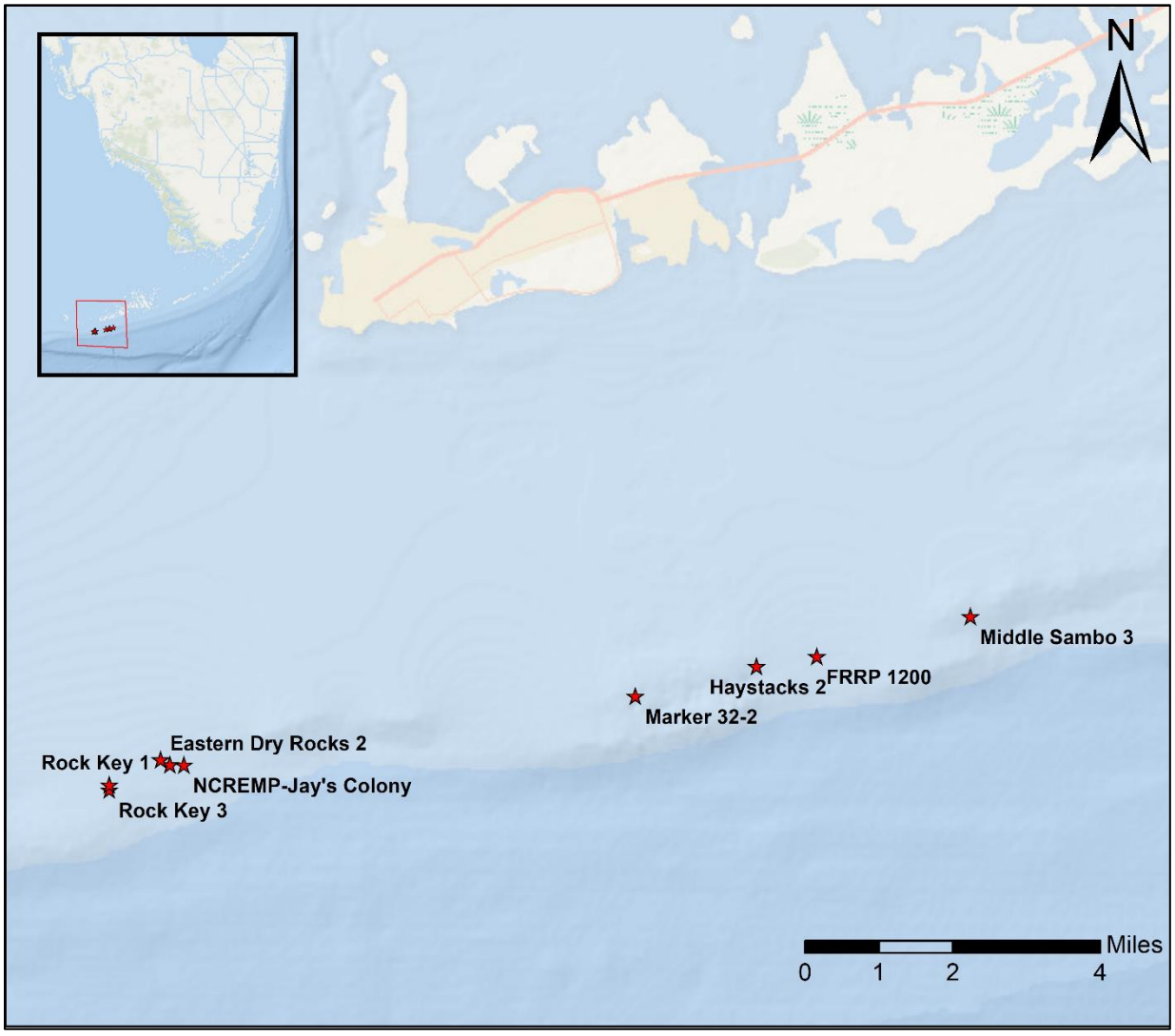


**Figure 13. Rescue IV: *Dendrogyra cylindrus* collection sites along the Florida Reef Tract.**

**Table 14. Rescue IV: GPS coordinates of diseased *Dendrogyra cylindrus* collections.**

| Collection Site | Latitude | Longitude |
|---|---|---|
| Middle Sambo 3 | 24.489 | -81.672 |
| FRRP 1200 | 24.481 | -81.705 |
| Haystacks 2 | 24.479 | -81.718 |
| Marker 32-2 | 24.473 | -81.744 |
| Eastern Dry Rocks 2 | 24.46 | -81.846 |
| NCREMP-Jay's Colony | 24.459 | -81.841 |
| Eastern Dry Rocks Ball 1-2 | 24.459 | -81.844 |
| Rock Key 1 | 24.455 | -81.857 |
| Rock Key 3 | 24.454 | -81.857 |

Fifteen fragments came from diseased reef sites but from colonies that exhibited no obvious signs of disease (Category 2). Two fragments (DC-362 and DC-363; NCREMP-Jay's Colony) were collected from a diseased colony but from an area distal to the active disease lesion. This was classified as a new disease category (Category 2.5) for *D. cylindrus* fragments. Initially, dead skeleton and associated live rock was trimmed from the Category 2 fragments. All 17 fragments were rinsed in TMASW and immersed in a 0.5 mL/L Lugol's solution in 36 ppt TMASW for 15 min, rinsed in TMASW again, and added to the treatment system detailed in Chapter 2. Because most fragments from this collection were Category 2, a less stringent antibiotic treatment was used. Thirteen Category 2 fragments were added to 2.5-gallon aquaria, in most cases grouped together by reef site. The two larger Category 2 fragments were placed individually into 5-gallon aquaria. All 15 of these fragments were given a daily 50 mg/L dose of ampicillin in TMASW for seven days (Table 15). Water changes (100 %) were performed with 36 ppt TMASW daily before each new dose was administered. The two fragments from NCREMP-Jay's Colony were given a treatment of amoxicillin in CDP (50 mg/mL) applied to the entire tissue margin (Table 15) due to having an increased disease risk (Category 2.5). Additional spot treatments of 50 mg/mL amoxicillin in CDP were administered to areas of concern (e.g., localized bleaching, tissue damage along margin possibly from transport) on seven fragments receiving the 50 mg/L ampicillin treatment (Table 15; DC-360, 361, 368, 369, 365, 355, 359).

**Table 15. Rescue IV: *Dendrogyra cylindrus* fragments with collection data and treatment.**

| Fragment ID | Parent Colony Tag # | Collection Date | Site Name | Genotype | Health Category* | Antibiotic Treatment(s)** |
|---|---|---|---|---|---|---|
| **DC-354** | 1 | 4/12/2019 | Middle Sambo 3 | unknown | 2 | 1 |
| **DC-355** | 1 | 4/12/2019 | Middle Sambo 3 | unknown | 2 | 1,3 |
| **DC-356** | 1 | 4/12/2019 | FRRP 1200 | unknown | 2 | 1 |
| **DC-357** | 1 | 4/12/2019 | FRRP 1200 | unknown | 2 | 1 |
| **DC-358** | 1 | 4/12/2019 | Haystacks 2 | unknown | 2 | 1 |
| **DC-359** | 1 | 4/12/2019 | Haystacks 2 | unknown | 2 | 1,3 |

| Fragment ID | Parent Colony Tag # | Collection Date | Site Name | Genotype | Health Category* | Antibiotic Treatment(s)** |
|---|---|---|---|---|---|---|
| **DC-360** | 1 | 4/12/2019 | MK32-2 | D1402 | 2 | 1,3 |
| **DC-361** | 1 | 4/12/2019 | MK32-2 | D1402 | 2 | 1,3 |
| **DC-362** | 1 | 4/12/2019 | NCREMP-Jay's | unknown | 2.5 | 2 |
| **DC-363** | 1 | 4/12/2019 | NCREMP-Jay's | unknown | 2.5 | 2 |
| **DC-364** | 1 | 4/12/2019 | EDR B1&2 | unknown | 2 | 1 |
| **DC-365** | 1 | 4/12/2019 | EDR2 | D1400 | 2 | 1,3 |
| **DC-366** | 1 | 4/12/2019 | EDR2 | D1400 | 2 | 1 |
| **DC-367** | 1 | 4/12/2019 | EDR2 | D1400 | 2 | 1 |
| **DC-368** | 1 | 4/12/2019 | Rock Key 1 | D1407 | 2 | 1,3 |
| **DC-369** | 1 | 4/12/2019 | Rock Key 1 | D1407 | 2 | 1,3 |
| **DC-370** | 1 | 4/12/2019 | Rock Key 3 | unknown | 2 | 1 |

*Health Category Key:
Category 2: Healthy colony in area of diseased colonies
Category 2.5: Unaffected fragment from a colony showing signs of disease

**Treatment Key:
1. 50 mg/L ampicillin for 7 days with daily 100% treatment change
2. 50 mg/mL amoxicillin in CDP applied to the entirety of the tissue margin
3. Spot treatment of 50 mg/mL amoxicillin in CDP applied to areas of concern

### *Rescue V and Rescue VI:*

*Dendrogyra cylindrus* Rescues V and VI collections commenced on 6/23/2019 and 10/22/2019, respectively. Disease treatments were initiated on 6/25/2019 and 10/23/2019 while still in the Florida Keys. Rescue V yielded 32 fragments from 16 different colonies from the lower Florida Keys (Figure 14; Table 16). Rescue VI yielded 29 fragments from 16 colonies also found at lower Florida Keys reef sites (Figure 14; Table 16). Since the two Lost Reef (colony 1) fragments from Rescue V died, additional fragments from colony 1 were collected during Rescue VI. In total, 61 fragments were collected from 31 different pillar coral colonies over both rescues (Table 17). These two collections have been grouped together because identical treatment methods were used. On both occasions, the *D. cylindrus* fragments collected represented all health categories. Rather than trying to treat each health category with a slightly different regimen of antibiotics, treatments were administered based primarily on the results of Rescue III.

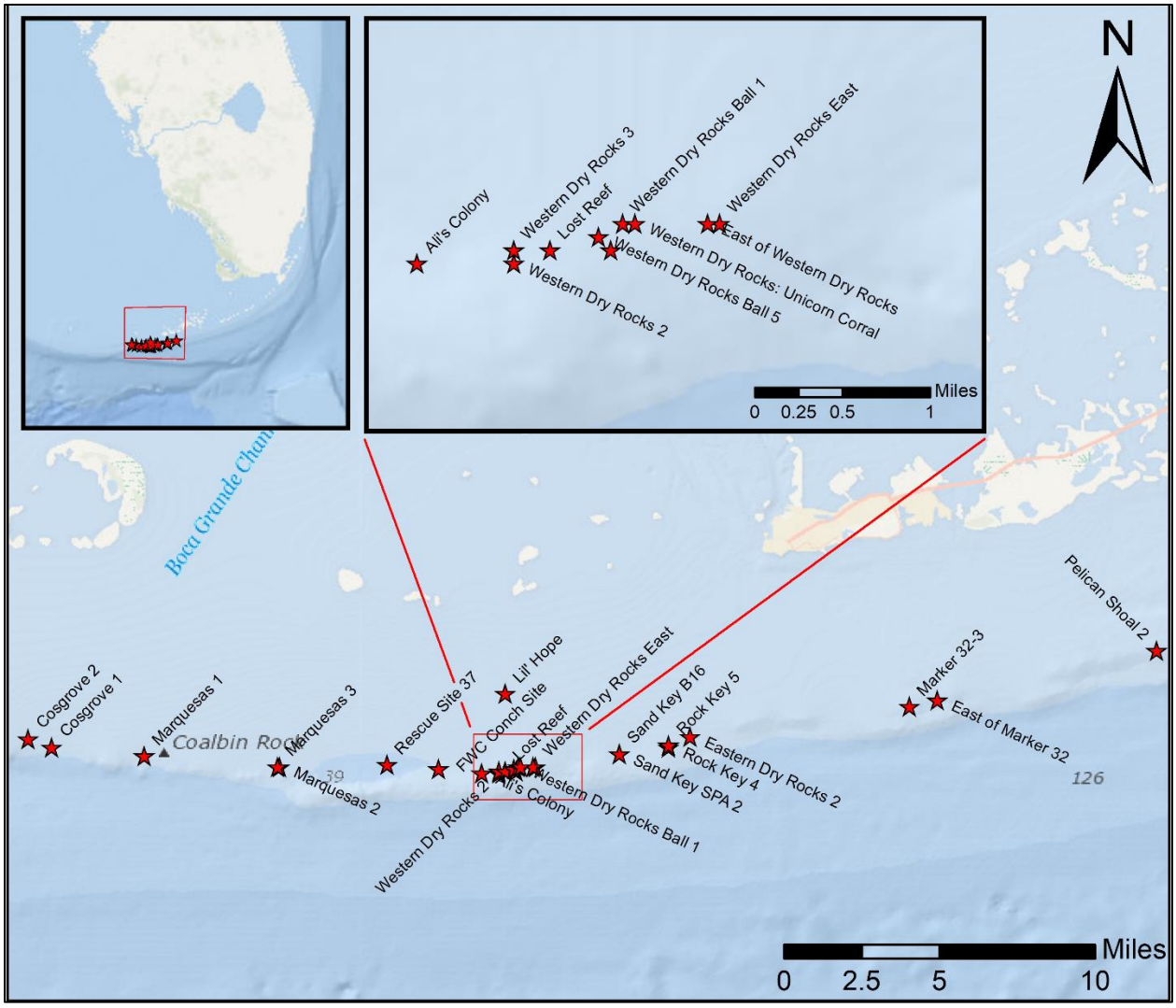


**Figure 14. Rescue V and VI: *Dendrogyra cylindrus* collection sites along the Florida Reef Tract.**

**Table 16. Rescue V and VI: GPS coordinates of diseased *Dendrogyra cylindrus* collections.**

| Collection Site | Colony # | Latitude | Longitude |
|---|---|---|---|
| Pelican Shoal 2 | 2 | 24.5 | -81.629 |
| East of Marker 32 | 1 | 24.477 | -81.731 |
| Marker 32-3 | 5 | 24.474 | -81.744 |
| Eastern Dry Rocks 2 | 2 | 24.46 | -81.846 |
| Rock Key 5 | 1 | 24.456 | -81.856 |
| Rock Key 4 | 1 | 24.455 | -81.856 |
| Sand Key SPA 2 | 1 | 24.452 | -81.879 |
| Sand Key B16 | 1 | 24.452 | -81.879 |
| Western Dry Rocks East | 1 | 24.446 | -81.918 |
| East of Western Dry Rocks | 2 | 24.446 | -81.919 |
| Western Dry Rocks: Unicorn Corral | 3,6 | 24.446 | -81.925 |

| Collection Site | Colony # | Latitude | Longitude |
|---|---|---|---|
| Western Dry Rocks Ball 1 | 1 | 24.446 | -81.926 |
| Western Dry Rocks Intermediate | 1 | 24.444 | -81.927 |
| Western Dry Rocks Ball 5 | 1, 2, 3 | 24.445 | -81.928 |
| Lil' Hope | 1 | 24.48 | -81.932 |
| Lost Reef | 1,2 | 24.444 | -81.932 |
| Western Dry Rocks 3 | 1 | 24.444 | -81.935 |
| Western Dry Rocks 2 | 1 | 24.443 | -81.935 |
| Ali's Colony | 1 | 24.443 | -81.943 |
| FWC Conch Site | 1 | 24.445 | -81.963 |
| Rescue Site 37 | 1 | 24.447 | -81.987 |
| Marquesas 2 | 1 | 24.446 | -82.037 |
| Marquesas 3 | 1 | 24.446 | -82.038 |
| Marquesas 1 | 1 | 24.451 | -82.1 |
| Cosgrove 1 | 1,2 | 24.455 | -82.143 |
| Cosgrove 2 | 1 | 24.459 | -82.154 |

Rescue V fragments were transferred to NOAA staff by the Neely team in Islamorada, FL. Rescue VI fragments were brought to Keys Marine Laboratory. Both sets of fragments were held in a NOAA van in 10-gallon coolers half-filled with 100 % TMASW and equipped with airstones driven by an air pump and manifold, which provided circulation and aeration during the treatment process.

For the initial treatment, diseased tissue and excess skeleton were resected from the base of fragments, followed by a TMASW rinse and immersion for 15 min in 0.5 mL/L Lugol's solution in TMASW (36 ppt). Each fragment was rinsed again with fresh TMASW, and amoxicillin in CDP (50 mg/mL) was applied to the tissue margin and other discrete areas of disease. Treated corals were grouped by health category in aerated 10-gallon coolers. The initial treatment process took up to 10 h for each rescue, during which time the temperature was maintained between 25-27.5 °C by idling the van engine to run the air conditioning. The engine was shut off overnight since local temperatures did not exceed 28 °C and aeration/circulation was maintained by using an extension cord for the air pump. For both rescues, transportation to Charleston, SC, started at 6:00 a.m. (before day-time temperatures elevated), using van air conditioning to control water temperatures.

In Charleston, all fragments were immediately added to the treatment system described in Chapter 2. Upper Keys *Dendrogyra cylindrus* Rescue I. Smaller fragments were added to 2.5-gallon treatment tanks, and larger fragments were added to 5-gallon treatment tanks. Due to space limitations, some duplicate fragments (those originating from the same colony) had to be placed together in a single treatment tank. As was noted in Rescue III, during transport, much of the amoxicillin/CDP sloughed off and created very cloudy water conditions. Despite many fragments in Rescue III only receiving this initial treatment and completely recovering, a secondary regimen of ampicillin (50 mg/L) in TMASW was administered to all 61 fragments for seven days

with 100 % daily renewal. Since many of these newly treated fragments ultimately would be added to aquaculture systems containing healthy pillar coral fragments from previous rescues, this additional treatment was an added precaution against potential disease introduction.

**Table 17. Rescue V and VI: *Dendrogyra cylindrus* fragments with collection data and treatment.**

| Fragment ID | Parent Colony Tag # | Collection Date | Site Name | Genotype | Health Category* | Antibiotic Treatment** |
|---|---|---|---|---|---|---|
| **DC-371a** | 1 | 6/23/19 | Sand Key SPA 2 | D1408 | 2 | 1, 2 |
| **DC-371b** | 1 | 6/23/19 | Sand Key SPA 2 | D1408 | 2 | 1, 2 |
| **DC-371c** | 1 | 6/23/19 | Sand Key SPA 2 | D1408 | 2 | 1, 2 |
| **DC-372** | 1 | 6/23/19 | Sand Key B16 | unknown | 3 | 1, 2 |
| **DC-373a** | 1 | 6/23/19 | Cosgrove 2 | unknown | 1 | 1, 2 |
| **DC-373b** | 1 | 6/23/19 | Cosgrove 2 | unknown | 1 | 1, 2 |
| **DC-374a** | 1 | 6/23/19 | Cosgrove 1 | unknown | 1 | 1, 2 |
| **DC-374b** | 1 | 6/23/19 | Cosgrove 1 | unknown | 1 | 1, 2 |
| **DC-375a** | 1 | 6/23/19 | Marquesas 1 | unknown | 1 | 1, 2 |
| **DC-375b** | 1 | 6/23/19 | Marquesas 1 | unknown | 1 | 1, 2 |
| **DC-375c** | 1 | 6/23/19 | Marquesas 1 | unknown | 1 | 1, 2 |
| **DC-375d** | 1 | 6/23/19 | Marquesas 1 | unknown | 1 | 1, 2 |
| **DC-376a** | 1 | 6/23/19 | Western Dry Rocks 3 | D1411 | 2 | 1, 2 |
| **DC-376b** | 1 | 6/23/19 | Western Dry Rocks 3 | D1411 | 2 | 1, 2 |
| **DC-377a** | 1 | 6/23/19 | Western Dry Rocks 2 | D1194 | 3 | 1, 2 |
| **DC-377b** | 1 | 6/23/19 | Western Dry Rocks 2 | D1194 | 3 | 1, 2 |
| **DC-378a** | 1 | 6/23/19 | Lost Reef | unknown | 2 | 1, 2 |
| **DC-378b** | 1 | 6/23/19 | Lost Reef | unknown | 2 | 1, 2 |
| **DC-379a** | 2 | 6/23/19 | Lost Reef | unknown | 2 | 1, 2 |
| **DC-379b** | 2 | 6/23/19 | Lost Reef | unknown | 2 | 1, 2 |
| **DC-380a** | 1 | 6/23/19 | Lil' Hope | unknown | 1 | 1, 2 |
| **DC-380b** | 1 | 6/23/19 | Lil' Hope | unknown | 1 | 1, 2 |
| **DC-380c** | 1 | 6/23/19 | Lil' Hope | unknown | 1 | 1, 2 |
| **DC-381a** | 2 | 6/24/19 | W. Dry Rocks B5 | unknown | 2 | 1, 2 |
| **DC-381b** | 2 | 6/24/19 | W. Dry Rocks B5 | unknown | 2 | 1, 2 |
| **DC-382** | 1 | 6/24/19 | W. Dry Rocks B5 | unknown | 2 | 1, 2 |
| **DC-383a** | 3 | 6/24/19 | W. Dry Rocks B5 | unknown | 3 | 1, 2 |
| **DC-383b** | 3 | 6/24/19 | W. Dry Rocks B5 | unknown | 3 | 1, 2 |
| **DC-384** | 1 | 6/24/19 | W. Dry Rocks B1 | unknown | 2 | 1, 2 |
| **DC-385** | 1 | 6/24/19 | W. Dry Rocks East | unknown | 2 | 1, 2 |
| **DC-386a** | 1 | 6/24/19 | Rock Key 5 | unknown | 3 | 1, 2 |
| **DC-386b** | 1 | 6/24/19 | Rock Key 5 | unknown | 3 | 1, 2 |
| **DC-387** | 2 | 10/22/19 | Cosgrove 1 | unknown | 2 | 1, 2 |

| Fragment ID | Parent Colony Tag # | Collection Date | Site Name | Genotype | Health Category* | Antibiotic Treatment** |
|---|---|---|---|---|---|---|
| **DC-388A** | 2 | 10/22/19 | Pelican 2 | D1396 | 2.5 | 1, 2 |
| **DC-388B** | 2 | 10/22/19 | Pelican 2 | D1396 | 2.5 | 1, 2 |
| **DC-389A** | 1 | 10/22/19 | East of MK32 | unknown | 2.5 | 1, 2 |
| **DC-389B** | 1 | 10/22/19 | East of MK32 | unknown | 2.5 | 1, 2 |
| **DC-390A** | 5 | 10/22/19 | Marker 32-3 | D1120 | 3 | 1, 2 |
| **DC-390B** | 5 | 10/22/19 | Marker 32-3 | D1120 | 3 | 1, 2 |
| **DC-391A** | 1 | 10/22/19 | Rock Key 4 | unknown | 2.5 | 1, 2 |
| **DC-391B** | 1 | 10/22/19 | Rock Key 4 | unknown | 2.5 | 1, 2 |
| **DC-392A** | 2 | 10/22/19 | E of WDR | unknown | 2 | 1, 2 |
| **DC-392B** | 2 | 10/22/19 | E of WDR | unknown | 2 | 1, 2 |
| **DC-393** | 6 | 10/22/19 | Unicorn Corral | unknown | 3 | 1, 2 |
| **DC-394** | 3 | 10/22/19 | Unicorn Corral | unknown | 3 | 1, 2 |
| **DC-395A** | 1 | 10/22/19 | WDR Intermediate | unknown | 3 | 1, 2 |
| **DC-395B** | 1 | 10/22/19 | WDR Intermediate | unknown | 3 | 1, 2 |
| **DC-396A** | 1 | 10/22/19 | Lost Reef | unknown | 3 | 1, 2 |
| **DC-396B** | 1 | 10/22/19 | Lost Reef | unknown | 3 | 1, 2 |
| **DC-397A** | 1 | 10/22/19 | FWC Conch | unknown | 3 | 1, 2 |
| **DC-397B** | 1 | 10/22/19 | FWC Conch | unknown | 3 | 1, 2 |
| **DC-398A** | 1 | 10/22/19 | Rescue 37 | unknown | 2.5 | 1, 2 |
| **DC-398B** | 1 | 10/22/19 | Rescue 37 | unknown | 2.5 | 1, 2 |
| **DC-399A** | 1 | 10/22/19 | Marquesas 2 | unknown | 2.5 | 1, 2 |
| **DC-399B** | 1 | 10/22/19 | Marquesas 2 | unknown | 2.5 | 1, 2 |
| **DC-400A** | 1 | 10/22/19 | Marquesas 3 | unknown | 2.5 | 1, 2 |
| **DC-400B** | 1 | 10/22/19 | Marquesas 3 | unknown | 2.5 | 1, 2 |
| **DC-401A** | 2 | 10/23/19 | EDR 2 | D1399 | 3 | 1, 2 |
| **DC-401B** | 2 | 10/23/19 | EDR 2 | D1399 | 3 | 1, 2 |
| **DC-402A** | 1 | 10/23/19 | Ali's Colony | unknown | 2 | 1, 2 |
| **DC-402B** | 1 | 10/23/19 | Ali's Colony | unknown | 2 | 1, 2 |

*Health Category Key:
Category 1: Healthy colony, no disease at site
Category 2: Healthy colony in area of diseased colonies
Category 2.5: Unaffected fragment from a colony showing signs of disease
Category 3: Visibly diseased fragment

**Treatment Key:
1. 50 mg/mL amoxicillin in CDP applied to the entirety of the tissue margin
2. 50 mg/L ampicillin for 7 days with daily 100% treatment change

### *Collections from Laboratories*

In addition to field rescue collections, two separate trips were made to acquire previously collected pillar coral fragments from laboratories that could no longer house them. On 9/6/2018, 11 pillar coral fragments were brought from KML, and on 6/5/2019 an additional 32 fragments were retrieved from KML (14 fragments) and Mote (18 fragments). Original sites and dates of collection were documented for each fragment (Figure 15; Table 18). All 43 fragments were transported by van in 10-gallon coolers half-filled with 36 ppt TMASW with aeration.

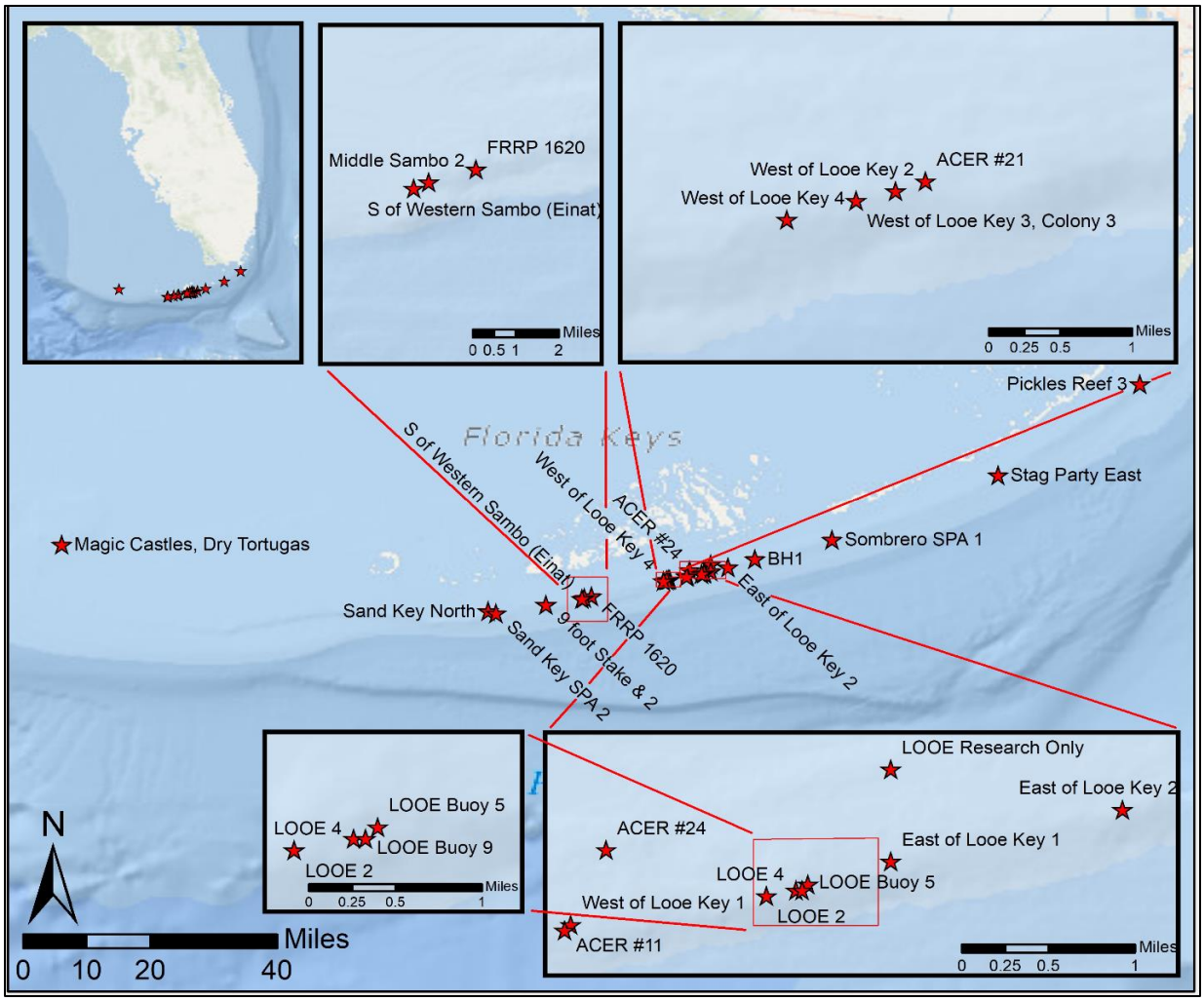


**Figure 15. *Dendrogyra cylindrus* collection sites for fragments originally housed at Keys Marine Laboratory and Mote Marine Laboratory.**

**Table 18. GPS coordinates and collection dates for fragments originally housed at KML and Mote.**

| Collection Site | Collection Date | Latitude | Longitude |
|---|---|---|---|
| Pickles Reef 3 | 5/15/2018 | 24.992 | -80.409 |
| Stag Party East | 4/18/2018 | 24.778 | -80.733 |

| Collection Site | Collection Date | Latitude | Longitude |
|---|---|---|---|
| Sombrero SPA 1 | 8/9/2018 | 24.625 | -81.112 |
| BH1 | 8/1/2018 | 24.58 | -81.288 |
| East of Looe Key 2 | 8/1/2018 | 24.56 | -81.35 |
| LOOE Research Only | 12/18/2018 | 24.567 | -81.389 |
| East of Looe Key 1 | 8/1/2018 | 24.551 | -81.389 |
| LOOE Buoy 5 | 12/18/2018 | 24.547 | -81.403 |
| LOOE Buoy 9 | 8/1/2018 | 24.546 | -81.404 |
| LOOE 1 | 8/1/2018 | 24.546 | -81.405 |
| LOOE 2 | 8/1/2018 | 24.545 | -81.41 |
| LOOE 4 | 8/1/2018 | 24.545 | -81.41 |
| ACER #24 | 11/2/2018 | 24.553 | -81.437 |
| West of Looe Key 1 | 11/2/2018 | 24.54 | -81.443 |
| ACER #11 | 11/2/2018 | 24.539 | -81.444 |
| ACER #21 | 11/29/2018 | 24.532 | -81.483 |
| West of Looe Key 2 | 6/22/2016 | 24.531 | -81.486 |
| West of Looe Key 3, Colony 2 | 12/18/2018 | 24.53 | -81.49 |
| West of Looe Key 3, Colony 3 | 6/22/2016 | 24.53 | -81.49 |
| West of Looe Key 4 | 11/7/2018 & 12/18/2018 | 24.528 | -81.497 |
| FRRP 1620 | 12/18/2018 | 24.492 | -81.662 |
| Middle Sambo 2 | 12/18/2018 | 24.488 | -81.678 |
| South of Western Sambo (Einat) | 9/13/2018 | 24.486 | -81.683 |
| 9 foot Stake & 2 | 9/13/2018 | 24.472 | -81.765 |
| Sand Key SPA 2 | 6/26/2016 | 24.452 | -81.879 |
| Sand Key North | 6/26/2016 | 24.457 | -81.897 |
| Magic Castles, Dry Tortugas | 7/13/2017 | 24.614 | -82.871 |

The disease status of the 11 pillar coral fragments received on 9/6/2018 was unclear. All fragments were initially labelled as either health Category 2 or 3 (Table 19) at the time of collection. Each fragment also received various disease intervention treatments while still being held at KML, some within two weeks of transport (8/22/2018). However, the success of these treatments was not determined before they were transported to the NOAA NOS NCCOS Charleston coral facility. Due to this uncertainty, all 11 fragments were removed from their clay tile bases and placed in separate treatment tanks within the treatment system described in Chapter 2. Upper Keys *Dendrogyra cylindrus* Rescue I. A low dose ampicillin treatment (25 mg/L in TMASW for 10 days with 100 % daily renewal) was initiated for all fragments on the day of arrival (9/6/2018).

Prior to transport, Dr. Cindy Lewis (Deputy Director, KML) communicated health concerns for fragment DC-331. This fragment was significantly lighter than the other

fragments, an indication of possible bleaching or a precursor to SCTLD onset. However, during the ampicillin treatment, no further signs of bleaching or tissue loss were observed, so DC-331 received no further treatment. By day six of the ampicillin treatment, two other fragments (DC-269d, DC-304a) were showing signs of stress and/or disease. Fragment DC-269d had clear tissue loss around its base and DC-304a had a few small spots that appeared to be slowly receding. Dead skeleton was resected from DC-269d and amoxicillin in CDP (50 mg/mL) was applied to the entire tissue margin. Fragment DC-304a received three small spot treatments of amoxicillin in CDP (50 mg/mL) around the areas of minor tissue recession. Both DC-269d and DC-304a continued their daily ampicillin regimen.

**Table 19. *Dendrogyra cylindrus* fragments from KML and Mote with collection data and treatment.**

| Fragment ID | Parent Colony Tag # | Date of Transport to NOAA | Site Name | Genotype | Health Category* | Antibiotic Treat-ment(s)** |
|---|---|---|---|---|---|---|
| **DC-269D** | 1 | 9/6/2018 | Stag Party East | unknown | 3 | 1, 2 |
| **DC-304A** | 22 | 9/6/2018 | Pickles Reef 3 | D1259 | 2 | 1, 3 |
| **DC-314** | 1 | 9/6/2018 | BH1 | D1028 | 2 | 1 |
| **DC-318C** | 1 | 9/6/2018 | East of Looe Key 2 | D1388 | 2 | 1 |
| **DC-319B** | 1 | 9/6/2018 | East of Looe Key 2 | D1388 | 2 | 1 |
| **DC-321B** | 1 | 9/6/2018 | East of Looe Key 1 | D1382 | 3 | 1 |
| **DC-323** | 1 | 9/6/2018 | LOOE Buoy 9 | D1384 | 2 | 1 |
| **DC-326** | 1 | 9/6/2018 | LOOE 1 | D1084 | 2 | 1 |
| **DC-328** | 1 | 9/6/2018 | LOOE 4 | unknown | 3 | 1 |
| **DC-329B** | 1 | 9/6/2018 | LOOE 2 | D1044 | 2 | 1 |
| **DC-331** | 1 | 9/6/2018 | Sombrero SPA | D1066 | 3 | 1 |
| **DC-333A** | 1 | 6/5/2019 | 9 foot Stake 2 | unknown | 1 | 1 |
| **DC-334A** | 1 | 6/5/2019 | S of W. Sambo (Einat) | unknown | 1 | 1 |
| **DC-336** | 1 | 6/5/2019 | West of Looe Key 1 | D1079 | 2 | 1 |
| **DC-339** | 1 | 6/5/2019 | ACER #24 | D1386 | 2 | 1 |
| **DC-341A** | 1 | 6/5/2019 | ACER #11 | D1387 | 3 | 1 |
| **DC-342C** | 1 | 6/5/2019 | ACER #21 | D1389 | 3 | 1 |
| **DC-346B** | 1 | 6/5/2019 | West of Looe Key 4 | D1394 | 2 | 1 |
| **DC-347A** | 1 | 6/5/2019 | LOOE Research Only | unknown | 3 | 1 |
| **DC-348C** | 1 | 6/5/2019 | LOOE Buoy 5 | unknown | 2 | 1 |
| **DC-348D** | 1 | 6/5/2019 | LOOE Buoy 5 | unknown | 2 | 1 |
| **DC-350A** | 2 | 6/5/2019 | West of Looe Key 3-1,2 | D1392 | 2 | 1 |
| **DC-351A** | 1 | 6/5/2019 | West of Looe Key 4 | D1394 | 2 | 1 |
| **DC-352A** | 1 | 6/5/2019 | FRRP 1620 | D1405 | 2 | 1 |
| **DC-353A** | 1 | 6/5/2019 | Middle Sambo 2 | unknown | 2 | 1 |
| **DRTO-12** | 3 | 6/5/2019 | Magic Castles, DRTO | D1153 | 1 | 1 |
| **DRTO-13** | 3 | 6/5/2019 | Magic Castles, DRTO | D1153 | 1 | 1 |

| Fragment ID | Parent Colony Tag # | Date of Transport to NOAA | Site Name | Genotype | Health Category* | Antibiotic Treat-ment(s)** |
|---|---|---|---|---|---|---|
| **DRTO-14** | 3 | 6/5/2019 | Magic Castles, DRTO | D1153 | 1 | 1 |
| **DRTO-15** | 3 | 6/5/2019 | Magic Castles, DRTO | D1153 | 1 | 1 |
| **DRTO-16** | 3 | 6/5/2019 | Magic Castles, DRTO | D1153 | 1 | 1 |
| **DRTO-17** | 3 | 6/5/2019 | Magic Castles, DRTO | D1153 | 1 | 1 |
| **DRTO-18** | 3 | 6/5/2019 | Magic Castles, DRTO | D1153 | 1 | 1 |
| **DRTO-19** | 3 | 6/5/2019 | Magic Castles, DRTO | D1153 | 1 | 1 |
| **DRTO-20** | 3 | 6/5/2019 | Magic Castles, DRTO | D1153 | 1 | 1 |
| **DRTO-21** | 3 | 6/5/2019 | Magic Castles, DRTO | D1153 | 1 | 1 |
| **DRTO-22** | 3 | 6/5/2019 | Magic Castles, DRTO | D1153 | 1 | 1 |
| **DRTO-23** | 3 | 6/5/2019 | Magic Castles, DRTO | D1153 | 1 | 1 |
| **SKN-16** | 1 | 6/5/2019 | Sand Key North | D1102 | 1 | 1 |
| **SSPA2-1** | 1 | 6/5/2019 | Sand Key SPA 2 | D1408 | 1 | 1 |
| **WL2-1** | 1 | 6/5/2019 | West of Looe Key 2 | D1390 | 1 | 1 |
| **WL3 -10** | 3 | 6/5/2019 | West of Looe Key 3-3,4 | D1391 | 1 | 1 |
| **WL3 -11** | 3 | 6/5/2019 | West of Looe Key 3-3,4 | D1391 | 1 | 1 |
| **WL3 -12** | 3 | 6/5/2019 | West of Looe Key 3-3,4 | D1391 | 1 | 1 |

*Health Category Key:
Category 1: Healthy colony, no disease at site
Category 2: Healthy colony in area of diseased colonies
Category 3: Visibly diseased fragment

**Treatment Key:
1. 25mg/L ampicillin for 10 days with daily 100% water change
2. 50mg/mL amoxicillin in CDP applied to the entirety of the tissue margin
3. Spot treatment of 50mg/mL amoxicillin in CDP applied to areas of concern

All fragments received on 6/5/2019 (Table 19) were placed immediately in the treatment system described in Chapter 2. Upper Keys *Dendrogyra cylindrus* Rescue I. Those from Mote were removed from their bases, due to infestation with large populations of a pest gastropod species (Vermetidae). Although none of these fragments showed signs of disease, a 10-day, 25 mg/L ampicillin dose with 100 % daily renewal was administered as part of the quarantine process.

## Results

### *Field Rescue Collections*

#### *Rescue III, Experimental Chelex resin treatment:*

Iron availability is well known to facilitate many pathogenic diseases and important in the production of virulence in most microbes (Artis *et al.* 1983; Santos *et al.* 2012; Falconer *et al.* 2014). Generally, infections by protozoa, fungi, and bacteria are

promoted through sequestering growth-essential iron from hosts (Santos *et al.* 2012). Thus, iron chelation therapy is potentially a broad spectrum chemotherapeutic; this is an alternative to antibiotic treatment and an alternative strategy to overcome antibiotic resistance (Santos *et al.* 2012). Limited testing was conducted with two chelating compounds, Chelex 100 and 2,2-bipyridyl (BIP).

Treatment with the metal-chelating Chelex resin did not slow disease progression within the first three days. Test fragment DC-261c was dead by 72 h and DC-261b had lost approximately 80 % tissue. There was no obvious change in DC-261d at 72 h post-treatment, however slow tissue loss continued for both remaining fragments. An increased dose of Chelex in CDP (500 mg/g CDP) was applied to the remaining tissue margin of each test fragment 10 days after the initial treatment. On May 4, 2018 (14 days after initial treatment), an iron chelator (2,2'-bipyridyl, Sigma) was obtained. It was mixed with CDP (35 µg/mL) and applied to the remaining tissue margin of DC-261d, as it appeared to be in worse condition. The consistency of the CDP changed with the addition of BIP, suggesting a chemical reaction with an ingredient in the paste. After 5 h of treatment, DC-261d appeared marginally improved, while DC-261b appeared worse. The BIP in CDP was added to DC-261b tissue margins. Three days after treating with BIP and with no additional treatment, there did not appear to be much change in either test fragment. On May 11, 2018 (one week after BIP treatment was started), test fragment DC-261d started to bleach and DC-261b was unchanged. Since there was no improvement in the health of either, both were treated with the amoxicillin/CDP in an attempt to save the fragments. DC-261d died shortly after treatment, while DC-261b eventually made a full recovery. Though this very limited test did not appear successful, there was not sufficient material (diseased test fragments) to test a dose range for efficacy. There is also a possibility that daily 100 % TMASW changes could have contributed to the ineffectiveness of the chelators or exhausting the binding capacity. Research with this alternative antimicrobial strategy warrants further investigation.

### *Rescue III, Amoxicillin in CDP treatment:*

A 100 % survival rate was achieved for 22 fragments (*D. cylindrus*, n=21 and *M. meandrites,* n=1) treated with amoxicillin/CDP. No instances of brown jelly syndrome (BJS) were observed. This is an improvement over the 55 % and 61% survival rates from the two previous genetic rescues. Early antibiotic treatment immediately following resection of diseased tissue and Lugol's treatment can improve survival rates. Fifteen fragments were dosed with a supplemental treatment of 25 mg/L ampicillin in TMASW, which may have provided additional protection from disease recurrence. No negative effects were observed with the supplemental ampicillin treatment. All 22 fragments and DC-261b (treated with chelating agent) were moved to permanent culture systems (see Chapter 2. Upper Keys *Dendrogyra cylindrus* Rescue I for details) and have shown no further signs of disease as of July 2020.

Amoxicillin in CDP also was an effective treatment for tissue loss disease in *M. meandrites*, indicating that it may be a successful laboratory treatment for the more than 20 coral species currently affected by the SCTLD disease outbreak.

### *Rescue IV:*

All 17 *D. cylindrus* fragments treated from this collection showed no signs of disease throughout a two-week monitoring period after all antibiotic treatments were completed. Disease free status of these fragments continued after they were transferred to small 40-gallon permanent aquaculture systems. Subsequently all have been added to larger aquaculture systems and none have exhibited any signs of SCTLD or any other disease as of July 2020.

The 15 fragments receiving a 50 mg/L ampicillin regimen exhibited no clinical signs of disease, although originating from a diseased site (Category 2). Despite full recovery of all Category 2 fragments, seven received spot treatments with amoxicillin/CDP. Thus, we cannot conclude that this ampicillin concentration alone is a sufficient treatment for SCTLD.

### *Rescue V and Rescue VI:*

Of the 61 *D. cylindrus* fragments treated during Rescues V and VI, 58 have shown no new signs of SCTLD (95 % cure rate) and have been added to custom 200 gallon permanent NCCOS coral aquaculture systems. Three fragments did not survive: two were from Rescue V (DC-378a and DC-378b) and one was from Rescue VI (DC-401a).

DC-378a and DC-378b were both larger fragments from Lost Reef Colony 1 that were paired together in a 5-gallon treatment tank and had significant amounts of amoxicillin/CDP on them after being transferred from the transport cooler to their treatment tank. This coupled with an additional 7-day 50 mg/L ampicillin treatment created a more concentrated antibiotic dose that likely confounded recovery.

Signs of BJS were discovered on DC-401a, DC-401b and DC-394 five days into their ampicillin treatment on 10/29/2019. A small sample biopsy of BJS-affected tissue was taken from each fragment and placed into a small crystallizing dish with a small volume of TMASW. Fragments were then rinsed thoroughly with TMASW and placed into clean treatment tanks. Tissue biopsies were viewed with a macroscope (Olympus® MVX10, research macro zoom microscope 0.63X objective equipped with a DP71 digital camera, Olympus, Melville, NY). All three biopsies were infested with large numbers of ciliate-like organisms (Figure 16). Biopsies were dosed with Two Little Fishies ReVive Coral Cleaner as per manufacturer's protocol (10 mL/L in TMASW) to test its efficacy on BJS after results mentioned in Chapter 4. Upper Keys *Dendrogyra cylindrus* Rescue II. The protocol indicates a 15-min exposure, however after 30-min, the ciliate-like organisms showed no signs of impairment. Hence, this treatment was not administered to the affected fragments.

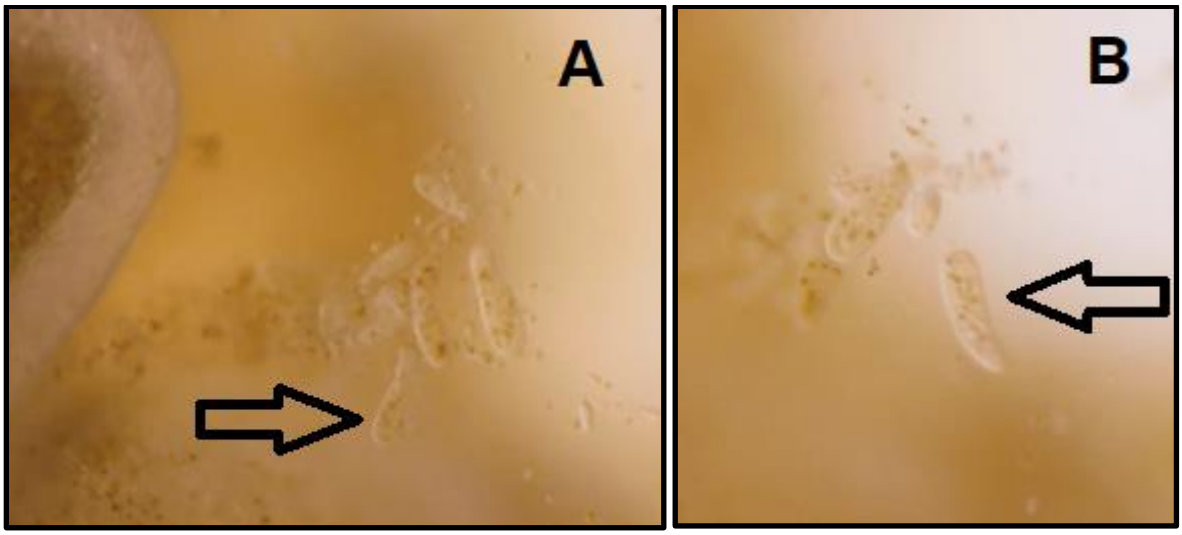


**Figure 16. Photomicrographs of ciliate-like organisms in tissue biopsies from fragments DC-394 (A) and DC-401a (B).**

Following the TMASW rinse and placement into new treatment tanks, no further signs of BJS were detected on either DC-401b or DC-394. DC-401a continued to exhibit signs of BJS, therefore on 11/19/2019, a new treatment was attempted: 1) the tissue margin was sprayed carefully with TMASW, 2) the bare skeleton and tissue margin were dried with paper towels, 3) 70% isopropanol was dripped along the entire tissue margin, 4) the fragments were left to air dry for 10 min, and 5) the tissue margin was rinsed again with TMASW to remove any traces of isopropanol. During both rinses, it was observed that tissue would detach from any point on the skeleton. The detached tissue was examined microscopically showing the same ciliate-like organisms. In addition, the detached tissue was fragile, lysing and releasing symbiotic zooxanthellae. DC-401a received no further treatment and by 11/27/2019 no tissue remained.

### *Collections from Laboratories*

All *D. cylindrus* fragments (n=43) transferred to NOS from KML and Mote underwent antibiotic treatment (Table 19) as part of the quarantine process, even though most were considered disease-free. Forty-two survived and are now in permanent culture systems. Minor tissue recession was halted on DC-304a after three consecutive spot treatments of amoxicillin in CDP (50 mg/mL) while continuing the ampicillin regimen. DC-269d continued to experience tissue loss during a 10-day regimen of ampicillin (25 mg/L). On day 6, dead skeleton was resected, amoxicillin in CDP (50 mg/mL) was applied to the entire tissue margin, and DC-269d was returned to the ampicillin regimen. After completing both treatments, DC-269d lost all tissue by 10/9/18.

## Conclusions

- Metal chelation therapy (particularly iron starvation) is becoming a viable alternative to antibiotics in combating a number of pathogenic diseases in human

medicine. There were slight indications that the Chelex and subsequent 2,2-bipyridyl treatments may have slowed disease progression, but significant tissue loss continued. It was later learned that our artificial seawater salts supplier had started incorporating an iron-containing anti-caking agent, which may have contributed to the ambiguous results obtained with daily water changes replenishing trace iron levels. Therefore, no firm conclusions could be made from this very limited trial (3 very diseased, small fragments).

    - Based on success in other studies of this strategy in arresting growth, replication and virulence of a number of bacteria, fungi, protists, and some DNA viruses, further research to determine dosage and efficacy for coral diseases is warranted.

- Immediate treatment with amoxicillin in CDP after tissue resection (versus waiting >24 h) improved survival of *D. cylindrus* fragments. Survival rates were 55-61 % without early intervention and improved to 97 % (100 % Rescue III, 100 % Rescue IV, 94 % Rescue V, 97 % Rescue VI).

    - When used in conjunction with amoxicillin/CDP treatment, additional spot treatments with amoxicillin in CDP and/or incorporating either 25 mg/L (Rescue III) or 50 mg/L (Rescue IV, V, and VI) ampicillin in ambient seawater for 7-10 days likely contributed to survival.

- While the 25 mg/L ampicillin regimen does not stress *D. cylindrus*, it was an insufficient standalone treatment for SCTLD.

- A 50 mg/L ampicillin regimen (7 days) may be a sufficient prophylactic for SCTLD exposure without causing additional stress to the fragment. Category 2 fragments, potentially exposed to SCTLD, never exhibited signs of disease after receiving this treatment.

- The 50 mg/L ampicillin regimen has been added to the quarantine process for all organisms added to NOAA NCCOS *D. cylindrus* aquaculture systems to guard against potential contamination from wild-collected organisms deemed beneficial to co-culture with *D. cylindrus* (snails, crabs, shrimp, and macroalgae).

- Amoxicillin/CDP was a successful treatment for SCTLD in *Meandrina meandrites*, indicating that other coral species with SCTLD also may benefit from this treatment regimen.

- There is a potential to over-treat *D. cylindrus* with antibiotics. Be cognizant that larger fragments with lengthy tissue margins treated with amoxicillin/CDP should be put into individual tanks with larger volumes of seawater. Some examples of potential over-treatment are:

- DC-261a was a large fragment (>70 cm in linear tissue margin) placed in a 5-gallon treatment tank and received 50 mg/mL amoxicillin/CDP treatment to the entire tissue margin, and three additional spot treatments to large areas of tissue recession and 25 mg/L ampicillin for 10 days. The health of the colony continued to decline with increased polyp retraction, minor tissue sloughing and paling. Antibiotic treatments were halted and aquarium water (50 %) was replaced with water from a permanent pillar coral aquaculture system, in an attempt to re-seed healthy bacteria. Ultimately, tissue recession stopped and the fragment slowly made a full recovery.

- DC-378a and DC-378b were large fragments (>100 cm in combined linear tissue margin) placed together in a 5-gallon treatment tank and received 50 mg/mL amoxicillin/CDP treatment to the whole tissue margin and 50 mg/L ampicillin renewed daily for 7 days. Fragments were transferred into a 40-gallon fully-cycled aquarium system post-treatment, to reintroduce beneficial bacterial communities. However, both fragments ultimately died.

- DC-269d may have succumbed to disease, but a potential antibiotic overdose is also possible. Pillar coral fragments that perished after undergoing multiple antibiotic treatment regimens in Chapter 1, Chapter 2 and Chapter 4 may also have been stressed by antibiotic overdoses rather than disease.

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**Appendix I: Record of *D. cylindrus* swab and tissue samples collected during rescues.**

**Table A20. Rescue II: List of *Dendrogyra cylindrus* swab and tissue samples.**

| Site Name | Parent Colony Tag # | Frag # | Date Collected | Health Category | Genotype ID | Histology Sample # | Diseased Swab # | Healthy Swab # | Cryo-preserved Tissue # |
|---|---|---|---|---|---|---|---|---|---|
| CARY-1 | 3 | DC-156a | 2/14/17 | 3 | D1368 | 38* | 38* | 39* | 156* |
| CARY-1 | 3 | DC-156b | 2/14/17 | 3 | D1368 | 38* | 38* | 39* | 156* |
| FRENCH-2 | 1 | DC-157 | 2/16/17 | 3 | D1371 | 18 | none | none | 157 |
| FRENCH-2 | 1 | DC-158 | 2/16/17 | 3 | D1371 | 1 | 1 | 2 | 158 |
| FRENCH-2 | 1 | DC-159 | 2/16/17 | 3 | D1371 | 3 | 3 | 4 | 159 |
| FRENCH-2 | 1 | DC-160 | 2/16/17 | 3 | D1371 | 5 | 5 | 6 | 160 |
| FRENCH-2 | 1 | DC-161 | 2/16/17 | 3 | D1371 | 19 | none | none | 161 |
| FRENCH-2 | 1 | DC-162 | 2/16/17 | 3 | D1371 | 11 | 11 | 12 | 162 |
| FRENCH-2 | 1 | DC-163 | 2/16/17 | 3 | D1371 | 7 | 7 | 8 | none |
| FRENCH-2 | 1 | DC-164 | 2/16/17 | 3 | D1371 | 13 | 13 | 14 | 164 |
| FRENCH-2 | 1 | DC-165 | 2/16/17 | 3 | D1371 | 15 | 15 | 16 | none |
| FRENCH-2 | 1 | DC-166 | 2/16/17 | 3 | D1371 | 9 | 9 | 10 | 166 |
| FRENCH-2 | 1 | DC-167 | 2/16/17 | 3 | D1371 | 17 | 17 | none | none |
| FRENCH-2 | 1 | DC-168 | 2/16/17 | 3 | D1371 | 20 | none | none | 168 |
| FRENCH-4 | 1 | DC-169 | 2/16/17 | 3 | D1370 | 24 | 24 | 25 | none |
| FRENCH-4 | 1 | DC-170 | 2/16/17 | 3 | D1370 | 21* | 21* | 22* | 170* |
| FRENCH-4 | 1 | DC-171 | 2/16/17 | 3 | D1370 | 21* | 21* | 22* | 170* |
| FRENCH-4 | 1 | DC-172 | 2/16/17 | 3 | D1370 | 21* | 21* | 22* | 170* |

| Site Name | Parent Colony Tag # | Frag # | Date Collected | Health Category | Genotype ID | Histology Sample # | Diseased Swab # | Healthy Swab # | Cryo-preserved Tissue # |
|---|---|---|---|---|---|---|---|---|---|
| BUOY-19 | 4 | DC-173a | 2/16/17 | 3 | D1373 | none | none | none | none |
| BUOY-19 | 4 | DC-173b | 2/16/17 | 3 | D1373 | none | none | none | none |
| BUOY-19 | 1 | DC-174b | 2/16/17 | 3 | D1373 | 28* | 28* | 29* | 174* |
| BUOY-19 | 1 | DC-174c | 2/16/17 | 3 | D1373 | 28* | 28* | 29* | 174* |
| BUOY-19 | 1 | DC-175 | 2/16/17 | 3 | D1373 | 26 | 26 | 27 | 175 |
| BUOY-19 | 1 | DC-176 | 2/16/17 | 3 | D1373 | 30 | none | none | 176 |
| BUOY-19 | 1 | DC-177 | 2/16/17 | 3 | D1373 | none | none | none | none |
| MOL-1 | 1 | DC-178 | 2/16/17 | 3 | D1374 | none | none | none | none |
| MOL-1 | 1 | DC-179 | 2/16/17 | 3 | D1374 | 36 | 36 | 37 | none |
| MOL-7 | 1 | DC-180 | 2/16/17 | 3 | D1372 | 33 | 33 | 34 | 180 |
| MOL-7 | 1 | DC-181a | 2/16/17 | 3 | D1372 | 31 | 31 | 32 | 181 |
| MOL-7 | 1 | DC-182 | 2/16/17 | 3 | D1372 | 35 | none | none | 182 |
| MOL-1 | 1 | DC-183 | 2/16/17 | 3 | D1372 | none | none | none | none |
| MOL-1 | 1 | DC-184 | 2/16/17 | 3 | D1372 | none | none | none | none |
| MOL-1 | 1 | DC-185 | 2/16/17 | 3 | D1372 | none | none | none | none |
| PICK-10 | 1 | DC-186 | 2/17/17 | 3 | D1286 | 40 | 40 | 41 | 186 |
| CONCH-1 | 2 | DC-188 | 2/17/17 | 1 | D1375 | none | none | none | none |
| CONCH-3 | 3 | DC-193 | 2/17/17 | 2 | D1042 | none | none | none | none |
| CONCH-4 | 1 | DC-198 | 2/17/17 | 1 | D1372 | none | none | none | none |
| CROCK-4 | 3 | DC-202 | 2/17/17 | 1 | D1376 | none | none | none | none |

**Table A21. Rescue III: List of *Dendrogyra cylindrus* swab and tissue samples.**

| Site Name | Parent Colony Tag # | Fragment ID | Date Collected | Health Category | Z-fix Histology Sample # | Karnovsky's TEM Sample # | Healthy Swab # | Diseased Swab # | Cryo-preserved Tissue |
|---|---|---|---|---|---|---|---|---|---|
| LKLD-2 | 1 | DC-257 | 4/19/18 | 3 | 1 | 1 | 1 | 2 | Y |
| LKLD-2 | 1 | DC-258 | 4/19/18 | 3 | none | none | 3 | 4 | N |
| LKLD-2 | 1 | DC-259 | 4/19/18 | 3 | none | none | 5 | 6 | N |
| LKLD-2 | 1 | DC-260 | 4/19/18 | 3 | 2 | 2 | 7 | 8 | Y |
| SOMB-SPA2 | 3 | DC-261a | 4/19/18 | 3 | 3 | 3 | 9 | 10 | Y |
| SOMB-SPA2 | 3 | DC-261b | 4/19/18 | 3 | none | none | none | none | N |
| SOMB-SPA2 | 3 | DC-261c | 4/19/18 | 3 | none | none | none | none | N |
| SOMB-SPA2 | 3 | DC-261d | 4/19/18 | 3 | none | none | none | none | N |
| SOMB-SPA2 | 3 | DC-266 | 4/19/18 | 3 | none | none | none | none | N |
| CARY-8 | 2 | DC-267 | 4/19/18 | 3 | 5 | 5 | 15 | 16 | Y |
| CARY-8 | 2 | DC-268 | 4/19/18 | 3 | 6 | 6 | 17 | 18 | Y |
| STAG-E | 1 | DC-269a | 4/19/18 | 3 | 7 | 7 | 19 | 20 | Y |

| Site Name | Parent Colony Tag # | Fragment ID | Date Collected | Health Category | Z-fix Histology Sample # | Karnovsky's TEM Sample # | Healthy Swab # | Diseased Swab # | Cryo-preserved Tissue |
|---|---|---|---|---|---|---|---|---|---|
| STAG-E | 1 | DC-269b | 4/19/18 | 3 | none | none | none | none | N |
| BHP-3 | 1 | DC-270 | 4/19/18 | 3 | none | none | 24 | 25 | N |
| BHP-3 | 1 | DC-271 | 4/19/18 | 3 | none | none | 26 | none | N |
| BHP-3 | 1 | DC-272 | 4/19/18 | 3 | 8 | 8 | 27 | 28 | N |
| LOOE B-9 | 1 | DC-273 | 4/19/18 | 3 | 9 | none | 21 | 22 | Y |
| LOOE B-9 | 1 | DC-274 | 4/19/18 | 3 | none | none | 23 | none | N |
| LOOE KEY | unk | *Meandrina* | 4/19/18 | 3 | 10 | 9 | 29 | 30 | N |
| DAP35 | 1 | DC-290a | 4/19/18 | 3 | none | none | none | none | N |
| DAP35 | 1 | DC-290b | 4/19/18 | 3 | none | none | none | none | N |
| DAP35 | 1 | DC-290c | 4/19/18 | 3 | none | none | none | none | N |
| DAP35 | 1 | DC-290d | 4/19/18 | 3 | none | none | none | none | N |
| DAP35 | 1 | DC-290e | 4/19/18 | 3 | none | none | none | none | N |
| DAP35 | 1 | DC-290f | 4/19/18 | 3 | none | none | none | none | N |

# Exploratory Treatments for Stony Coral Tissue Loss Disease: Pillar Coral (*Dendrogyra cylindrus*)

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