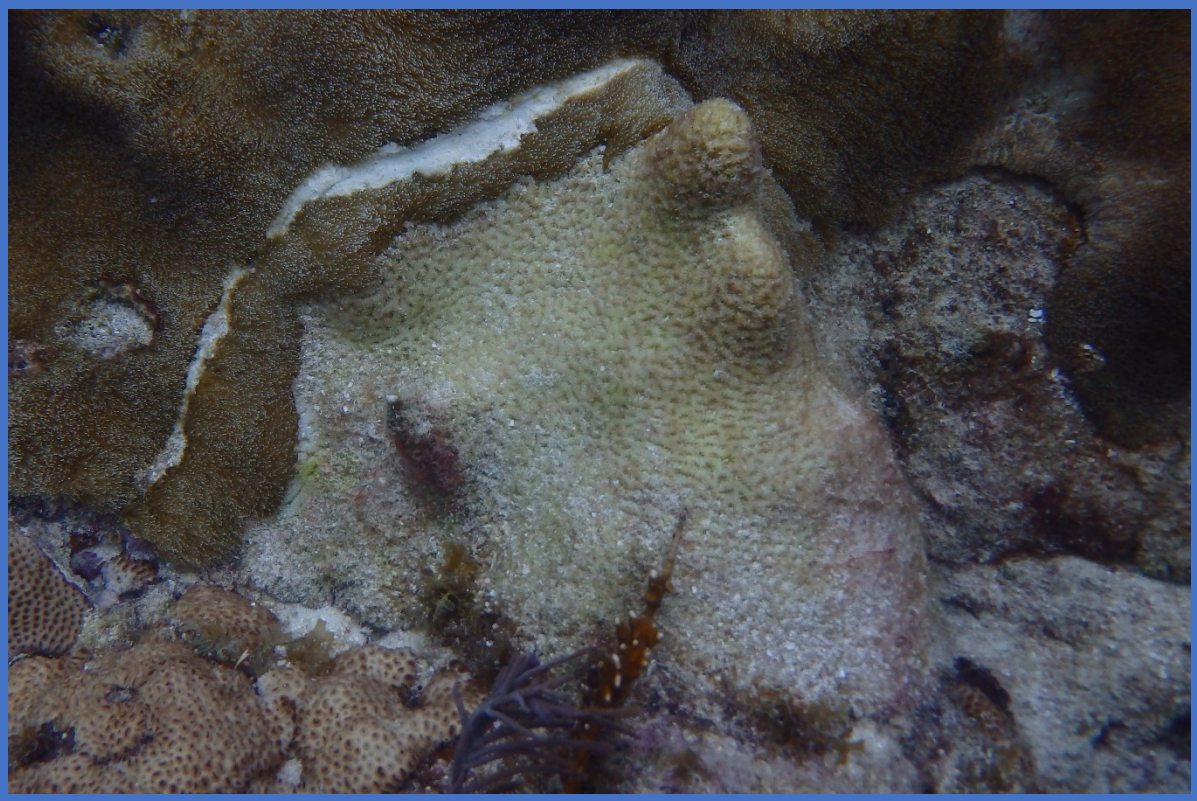
IN-SITU DISEASE INTERVENTION



Florida Department of Environmental Protection
Coral Reef Conservation Program

In-Situ Disease Intervention

Final Summary Report

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Background:

Since 2014, a multi-year, multi-species disease outbreak has progressed geographically along the Florida Reef Tract from an origin near Virginia Key. Over half of the hard coral species on the reef are susceptible, and the disease often results in 100% infection rates and 100% subsequent mortality. Susceptible species include five of the seven ESA-listed Caribbean coral species and most of the reef-building species. The already uncommon pillar coral (*Dendrogyra cylindrus*) is highly susceptible, with 99% of the population from Crocker Reef to the northern end of the reef tract extinct as a result of the disease.

Treatment options in laboratory settings have been tested for effectiveness in halting advancing disease margins. Treatments have included barriers such as trenching or application of clay or epoxy bands. They have also included the addition of high chlorine concentrations or antibiotics to those barriers. Most treatments have proven ineffective in the laboratory setting. The most promising laboratory results have come from the use of antibiotic applications combined with physical trenching to firebreak the healthy tissue from the diseased tissue. Trials in May and June 2018 showed some promise of this method when applied in permeable materials that allow for the metered dissolution of the drug.

Based on these early successes, an initial pilot project on a single *D. cylindrus* colony was conducted in late May 2018. Further testing at a multi-colony location is upcoming.

Project Design:

Single Colony

A lesion on a large colony of *D. cylindrus* at Sombrero Reef was first observed May 18, 2018 and treated on May 23, 2018. Based on relative successes of laboratory trials to date, the wild colony was treated by chiseling a 0.5-1.0 cm deep trench into healthy tissue approximately 10 cm from the disease margin. The trench was then packed with an amoxicillin/shea butter treatment. Shea butter was heated in a warm water bath, and the softened product was mixed with amoxicillin powder in a 1:16 weight ratio. The mixture was packed into syringes, transported on ice to the site, and applied at ambient seawater temperature (26°C-27°C). The mixture adhered moderately well within the trench, but because of inconsistent trench depth using hammer and chisels, high wave action, and the treatment's positive buoyancy, some treatment areas were covered with modeling clay to secure it. The progression of the disease, adherence of the treatment, and status of the colony continues to be monitored weekly to bi-weekly.

Multi-colony Site

The last remaining high-density stand of *D. cylindrus* in Florida is at Coffins Patch off of Marathon. Mapping of the site in 2014 identified 51 colonies; subsequent genetic testing showed them to be ramets of a single genet. The site has been heavily diseased and will be visited in late June/early July for treatment.

The most recent laboratory-based trials show the highest success rates when a trench is packed with an amoxicillin-containing silicone-based paste. The paste has been developed by Core Rx to release the antibiotic over a 36-hour period. Amoxicillin is mixed in by hand in a 1:16 by weight ratio. In the lab, this treatment stopped disease progression at the treatment margin on 3/3 *Dichocoenia stokesii*, 3/3 *Colpophyllia natans*, and 1/3 *Pseudodiploria strigosa*. As the current best practice, this procedure will be applied on affected *D. cylindrus* at the field site.

Treatment will be conducted using an underwater angle grinder to create a 1cm deep trench approximately 5-10 cm away from the disease margin, then packing the antibiotic mixture into the trench. Depending on adherence, modeling clay may be applied over the treatment to keep it in place. The number of colonies treated will depend on the number of colonies still alive and showing signs of disease.

In cooperation with USGS, samples will be taken to determine the impact of antibiotic application on the development of antibiotic-resistant genes. Samples from water, sediment, and coral mucus will be taken before and after treatment for analysis by USGS.

Treatment sites will be monitored weekly for one month, bi-weekly for the following two months, and monthly thereafter. Monitoring will include disease progression, overall colony status, and observations of surrounding corals and other benthos.

Results:

On the *D. cylindrus* colony at Sombrero Reef, the disease margin continued to progress towards the treatment line in the week following treatment to a point approximately 2-5 cm from the treatment. In the two subsequent weeks, disease progression appears to have halted. While we caution that this is a single sample with incomplete results, this may indicate a degree of disease resistance in the tissues near the antibiotic. Further monitoring may help determine whether this resistance is maintained over time.

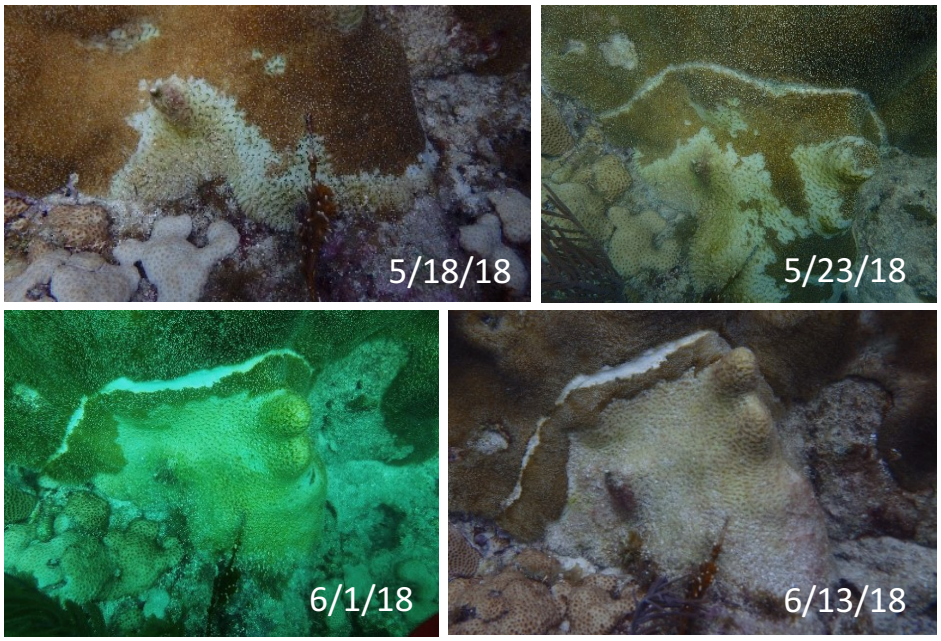

Fig 1: Progression of disease towards the treatment line on a *D. cylindrus* colony. Disease was first observed on 5/18/18 and treatment was conducted on 5/23/18. Disease appears to have progressed for a week following the treatment, and then halted.

Though this treatment may confer resistance near the treatment line and thus potentially prevent the progression of disease throughout the colony, this particular colony began exhibiting many more lesions

in other regions. We conclude that advantage conferred by the treatment is not transmitted widely throughout the colony and does not provide any colony-level resistance to infection.

Recommendations:

At this point, laboratory trials still show only moderate rates of success at halting disease progression. Continued laboratory trials are needed to look at improving delivery mechanisms, determining the most effective antibiotic, and examining dosage. As the trials lead to improved success rates, the best practices can be tested in the field to determine effectiveness in wild colonies. This may be effective in preserving individual colonies but is likely to require regular and repeated treatments over long periods of time.

Trials to date have only looked to arrest the advancing disease margin; field conditions suggest that infection and spread from multiple other areas of colonies is likely. Future methodologies may need to consider treatment across an entire colony, perhaps through antibiotic injections, medicated feed, or controlled water dosing.

At this point, few reef- or population-scale options have even been proposed. One potential option might be phage therapy, in which populations of naturally occurring viruses that target the pathogenic bacteria are elevated to consume the pathogens causing the disease. This work to date has been experimental but has been successful on coral diseases in laboratory conditions. However, identifying the pathogen is an important component of this process.

It is recommended that laboratory and field trials continue to identify and improve the success of the best practices available to date. It is recommended that additional trials consider other options for administering antibiotics at a colony scale. And it is recommended that the scientific, management, and regulatory communities consider the options for unprecedented initiatives to address disease at a reef-wide scale through experimental means like phage therapy.