FLORIDA KEYS CORAL DISEASE STRIKE TEAM: FY 2021/2022 FINAL REPORT

Florida Department of Environmental Protection
Office of Resilience and Coastal Protection

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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 (Precht et al. 2016). Termed Stony Coral Tissue Loss Disease (SCTLD) (Florida Coral Disease Response Research & Epidemiology Team 2018), it affects over half of the stony coral species on the reef and generally results in 60-100% infection rates and 100% subsequent mortality. At least 25 coral species are known to be susceptible, including five of the seven ESA-listed Caribbean coral species and most of the reef-building species.

A response priority has been active treatments of diseased corals to allow for the survival of priority sites and colonies. As such, a coral disease response strike team was established by Nova Southeastern University to treat and monitor diseased corals in the Florida Keys. The strike team was contracted for 75 field days (200 diver days) of in-water work, with an additional 10 field days to assist with Smithsonian-led probiotics work.

This report outlines the activities undertaken and results of work conducted July 1, 2021 to May 28, 2022.

Permitting

Permitting to conduct diseased coral treatments using antibiotic pastes and chlorinated epoxies was federally authorized on November 30, 2018 under permit FKNMS-2018-141. The permit approved activity within Upper Keys Sanctuary Preservation Areas (SPAs). Revisions to the permit on January 28, 2019 and March 26, 2019 incorporated additional sites by authorizing treatment at all locations northeast of and including Looe Key SPA. Experimental work at Sand Key was authorized on September 24, 2019 under FKNMS-2019-115. All work was reauthorized on July 16, 2020 under permit FKNMS-2020-077. Changes to the monitoring protocol were authorized on February 15, 2022 under FKNMS-2020-077-A1. Permission to apply antibiotics was separately authorized by the FDA's Office of Minor Use and Minor Species.

Protocols and Quality Assurance

Beginning in January 2019, large-scale field interventions were conducted using two methodologies: antibiotic paste and chlorinated epoxy. Subsequent monitoring events showed chlorinated epoxy to be ineffective, and conversations with the Disease Advisory Council, DEP staff, and ultimately the 2019 coral disease workshop resulted in agreement to proceed using only amoxicillin paste.

Powdered amoxicillin is mixed with a paste termed Base2b developed by Ocean Alchemists / Core Rx that delivers topical, targeted application to the coral tissue while minimizing transmission to the surrounding water. Amoxicillin powder is mixed into this paste in a 1:8 by weight ratio, then packed into syringes for direct application to disease lesions. Full protocol are available in Appendix I.

The Coral Disease Intervention Action Plan (Neely 2018) and the Quality Assurance (QA) plan for intervention teams (Neely 2019) were developed to lay out the site selection process, work plan, and monitoring guidelines. Site selection was guided by the Florida Keys National Marine Sanctuary staff, management team, and ECT team which initially prioritized Upper and Middle Keys Sanctuary Preservation Areas (SPAs). SPAs were selected because of their increased stakeholder use, high-profile status, and ease of access (all have mooring buoys), as well as potentially easier mapping and monitoring conditions. After the selected SPAs were treated or found to have no treatable corals, permitting and site prioritization shifted to Looe Key and Newfound Harbor SPAs at the eastern end of the Lower Keys. A small number of experimental treatments were later authorized at Sand Key SPA. When these experimental treatments were unsuccessful, full

authorization to treat Sand Key using best practices was authorized. Additional mid-channel patch reefs in the Middle and Upper Keys were added in spring 2020. With FKNMS approval, another Upper Keys inshore site was added in August 2021.

Within sites, priority corals were selected by divers utilizing the guiding principles (Appendix II) outlined in the Coral Disease Intervention Action Plan (Neely 2018). Standard operating procedures at each priority coral were as follows:

- Affix a numbered tag (which includes instructions for citizen scientists) to dead coral skeleton or adjacent substrate.
- Take photos of the coral and the lesions
- Take diameter and height measurements of the coral
- Apply the amoxicillin treatment to diseased lesions
- Take photos of treated lesions
- Get distance/bearings from other tagged corals or fixed points in order to build a map for subsequent monitoring

The Coral Disease Intervention Action Plan recommends monitoring at a level commensurate with the monitoring goals and logistical capacity. A minimum of 10 lesions or 10% of treated lesions is suggested. No recommended time periods are outlined. Strike team work to date has identified monitoring every 2-3 months as a recommended interval to treat newly infected corals or new lesions on previously treated corals; similar work in southeast Florida identifies similar intervals (Shilling et al. 2021). Revisitation prior to 1-month can result in incorrect assessments for two reasons: 1) on species or individuals with slower disease progression rates, disease may have not passed the treatment line, and 2) the area beneath the treatment can appear bright white, suggesting disease where there is none.

The strike team identified the time period for monitoring and retreatment as outlined above and, where practicable, revisited sites every two months for monitoring and touch-ups as required. The strike team has greatly exceeded the 10% minimum monitoring and has made efforts to revisit every treated colony during each monitoring period. During each visit, failed lesions or new lesions are treated with Base2b + amoxicillin and the number of such treatments is recorded.

All treatment sites established between January 2019 and September 2019 received lesion-level monitoring for one year. This included applying nails to treated lesions and using photographs to assess effectiveness through time. Results showed a greater than 90% effectiveness rate (Neely et al. 2020; Neely et al. 2021). Monitoring efforts subsequently switched to colony-level assessments.

Sites visited for longer than one year as well as sites established after September 2019 were assessed using an alternate monitoring protocol (outlined in Neely (2020) and permitted through FKNMS-2020-077). Lesions were no longer marked with nails, and lesion-level effectiveness was no longer tracked. Instead, each colony was assessed in the field with a code identifying its status during each monitoring event. Codes were: no active disease, treated, active lesions but not treated, and dead. The number of treated lesions continued to be recorded and colony-level and new lesion photographs continued to be taken.

Following two to three years of monitoring nearly every coral every two months at each site, the strike team worked in collaboration with FKNMS and DEP to develop new monitoring protocol that would allow for increased focus and effort on SCTLD-affected corals. Rather than visiting every previously tagged and treated

coral at each site, most sites would be surveyed holistically looking only for colonies with SCTLD lesions. These affected corals would be treated as before, with new colonies being tagged, measured, and mapped during treatment, and any previously tagged corals being recorded as requiring additional treatments. This new protocol was approved and implemented in February 2022.

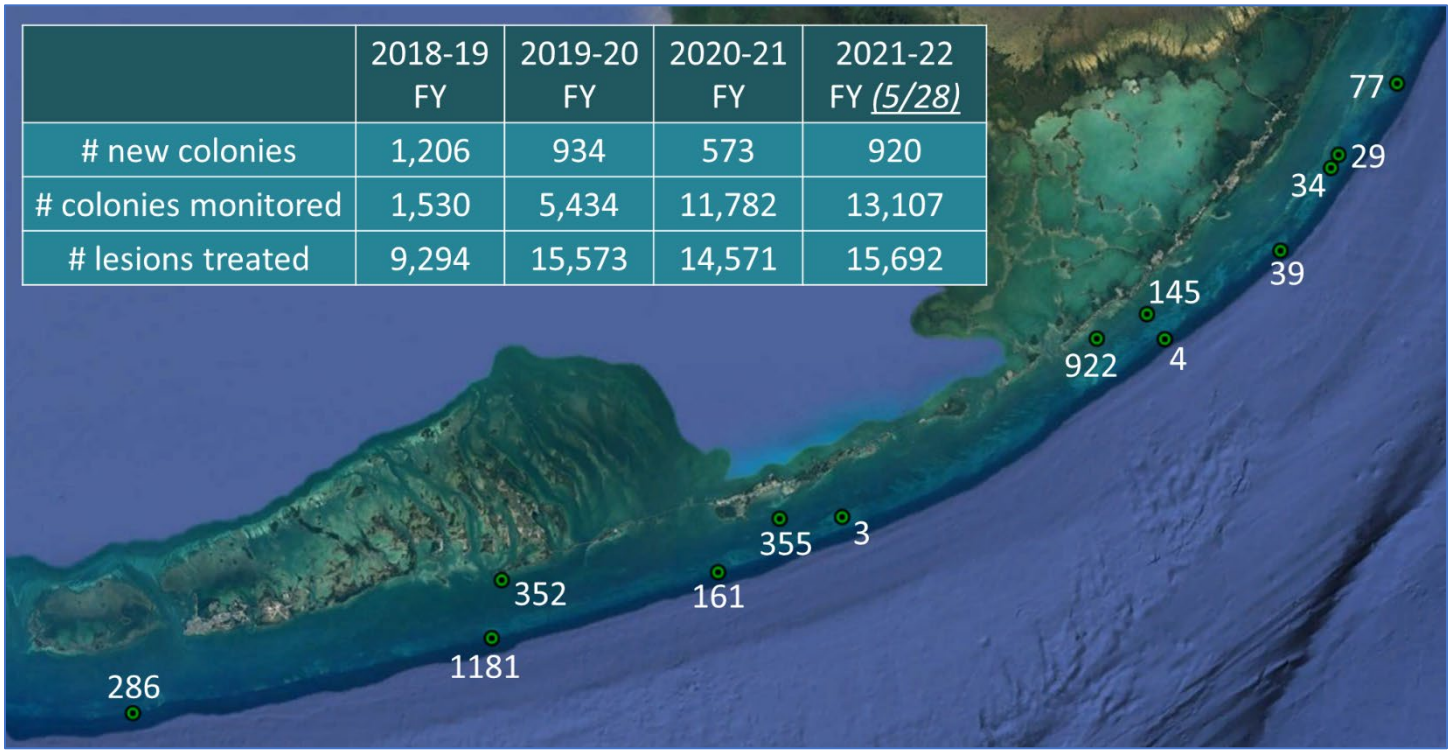
Work Accomplished

Strike team work conducted under this project from July 1, 2021 to May 28, 2022 included 96 in-water boat days, equating to 237 diver days. The strike team conducted 729 intervention/monitoring dives, totaling 980 in-water hours.

Probiotics work, led by the Smithsonian Institution, occurred over eight additional days. This work involved three days of probiotics treatments (September 2021 and December 2021), monitoring of those experimental colonies (October 2021 and January 2022), collections of colonies for laboratory probiotics experiments (May 2022), and scouting for sites for additional experiments (March 2022). Discussions for two new experimental designs were held along with DEP and FKNMS managers in February 2022. Proposed experiments were direct comparisons of antibiotic and probiotic applications and also the application of probiotics to colonies that frequently reinfect, applied within a few weeks after antibiotics were applied to halt active lesions. Sites were scouted for these experiments, but Smithsonian requested waiting until the new fiscal year for implementation.

Disease Treatment

Across the 237 strike team diver days, 920 new priority corals were treated (3851 lesions). Additionally, 13,107 individual coral monitoring events occurred (almost all corals were monitored approximately every two months), with touch-up treatments occurring on 2191 (16.7%) of these (11,841 lesions). In total since January



	2018-19 FY	2019-20 FY	2020-21 FY	2021-22 FY *(5/28)*
# new colonies	1,206	934	573	920
# colonies monitored	1,530	5,434	11,782	13,107
# lesions treated	9,294	15,573	14,571	15,692

Fig 1. Map identifying treatment sites and number of colonies treated at each site through May 28, 2022. The number of colonies monitored, new colonies treated, and lesions treated are shown for each fiscal year.

2019, 3670 unique corals and 55,350 SCTLD lesions have been treated within the FKNMS by NSU strike teams (Figure 1).

In July 2021, strike teams treating heavy disease at Cheeca Rocks (Upper Keys inshore reef) also looked at the nearby Hen & Chickens reef. Hen & Chickens is a highly diverse patch reef with high coral cover, and authorization to immediately treat corals at this reef was granted by FKNMS. Of the 920 new priority corals treated through May 28 in the 2021-22 fiscal year, 145 were at this newly authorized reef. Additional reefs of similar high quality are also known to be experiencing SCTLD outbreaks, but under the budget and monitoring constraints of the year, these were unable to be treated.

Across all treated sites, the average diameter of treated colonies was 114 cm. Over 450 treated corals were larger than 200 cm in diameter. A total of 17 coral species were treated. The most treated species were *Orbicella faveolata* (37%), *Montastraea cavernosa* (21%), and *Colpophyllia natans* (18%). However, composition of species treated varied by region. In the endemic region of the Upper Keys, the primary remaining susceptible corals were *Orbicella faveolata*. Sites in the Middle and Lower Keys, and also nearshore patch reefs, which were treated earlier in their infection phases, had more diverse species assemblages remaining for treatment. Newfound Harbor was particularly anomalous as a nearshore patch reef that was treated when disease first appeared; the species treated reflect these differences.

Since April 2020, the number and locations of treated corals have been provided monthly to FWC for inclusion in an open-access intervention dashboard (Florida Fish and Wildlife Research Institute 2019). The dashboard provides an interactive visualization of interventions conducted by all Florida practitioners and is available at http://arcg.is/84Cej (Figure 2).

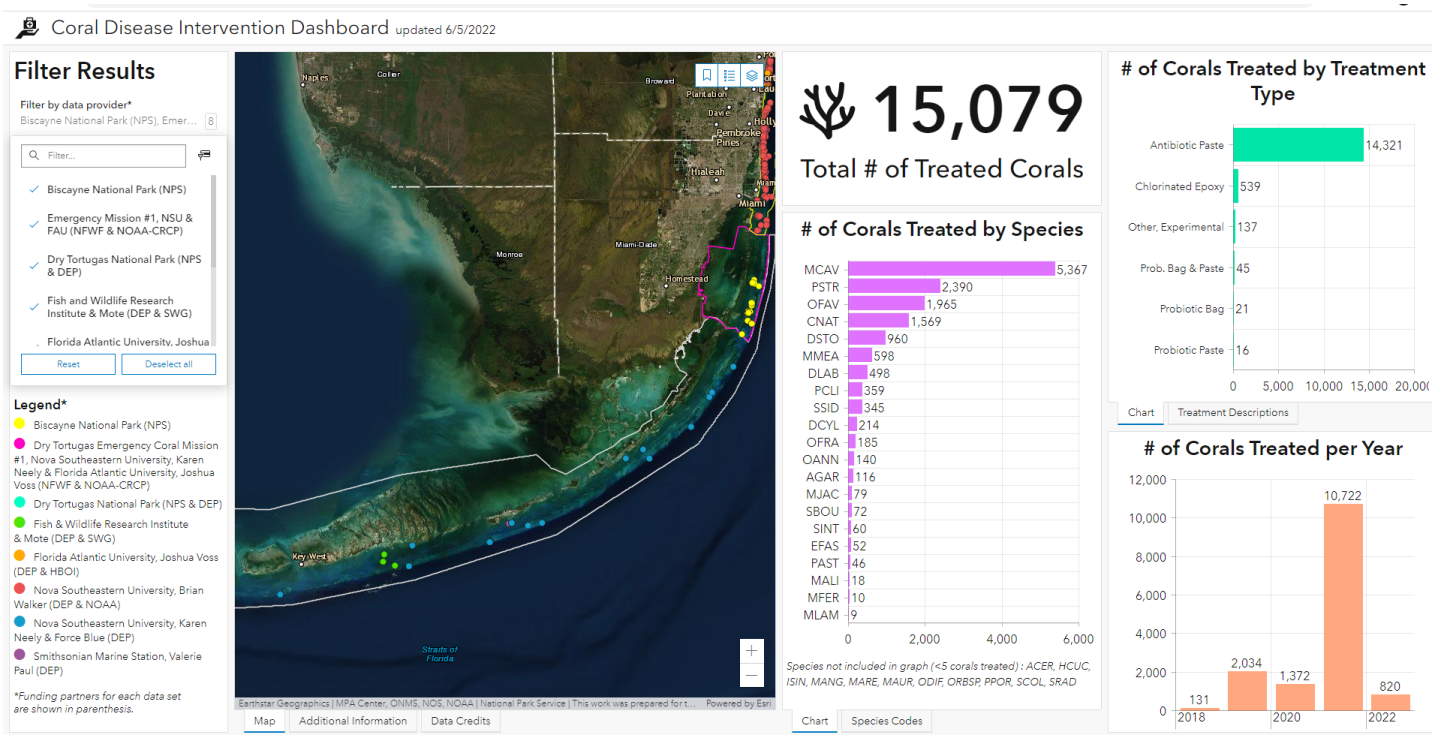


Fig 2. FWC Intervention dashboard showing locations, species, treatment types, treatment dates, and total number of corals treated by all practitioners. Accessed 6/6/2022.

Value Added Components

We were able to leverage DEP funding as match for National Fish and Wildlife Federation CRCP funding, which provides additional support for intervention at Mission: Iconic Reefs sites. We are grateful to both programs and the collaborative match that allows this work to continue.

The fate-tracking data from this ongoing project, along with the intervention efforts that have kept these corals alive when they would otherwise not have been, have allowed for experimentation. Two projects have utilized these corals and intervention efforts: RRC (Reef Resistance Consortium) and CISME (Coral In Situ Metabolism). Both projects have utilized the fate-tracked history of intervention corals to look at factors that may be impacting SCTLD resistance (RRC) and how disease and treatment may be impacting coral metabolic factors (CISME).

The experience and methodologies developed through this project have also been used in scaled-up intervention efforts in Dry Tortugas National Park. The Keys Strike Team has led two collaborative liveaboard cruises within the Park. These cruises resulted in nearly 9,500 SCTLD-afflicted corals being treated in order to preserve coral cover and species richness within the affected areas.

Results

Mortality rates

Of the 2955 colonies treated with amoxicillin through 5/28/2022, 2745 (93%) are still alive. The "intermediately susceptible" species which often have slower-moving lesions had notably lower mortality rates (OANN: 0%, OFAV: 2%, MCAV: 2%) than "highly susceptible" species (PSTR: 13%, CNAT: 16%, DLAB: 24%). Highly susceptible species generally have more rapidly progressing lesions and are often smaller corals; mortality could occur if a new lesion developed and consumed the colony between monitoring events.

To provide context to the amoxicillin-treated mortality rates, we offer the following comparisons:

- Thome et al. (2021) tagged 96 *P. strigosa* colonies on an SCTLD-affected reef in Mexico; within 306 days, survival was less than 16%, with nearly half of the surviving actively diseased at that point.
- Aeby et al. (2019) tagged 5 *P. strigosa* and 5 *D. labyrinthiformes* in the Middle Keys; within 7 months, 100% of these were dead.
- Though not tracking individual colonies, Walton et al. (2018) documented declines in density of 50% in *M. cavernosa* over two years through the SCTLD outbreak.
- Fixed survey sites in the upper Keys documented substantial losses in colonies between pre-SCTLD years (average of 2014 – 2016) and post-SCTLD (2018): 18% of *Orbicella* colonies, 52% of *M. cavernosa*, 78% of *P. strigosa*, 91% of *D. labyrinthiformes*, and 100% of *C. natans* (CREMP, unpublished data)

Colony Health Through Time

Because we fate-tracked each treated coral through time, we were able to observe the prevalence of SCTLD on previously-treated corals across multiple years. At four sites (Upper Keys, Sombrero Reef, Looe Key, and Newfound Harbor), this monitoring occurred every two months from early 2019-early 2022. Other sites were added from late 2019 to late 2021 and fate-tracked accordingly.

Fig 3. Metrics of SCTLD at different treatment sites. For each offshore (top row) and inshore (bottom row) treatment site, we show 1) the number of new coral colonies treated during each monitoring period (yellow bars), the proportion of previously treated corals still alive (red line), and the proportion of living previously-treated corals with no active disease (green line). Note that at all offshore sites, experimental treatments were used during the first few months which resulted in rapid initial mortality rates.

At all sites, the proportion of previously-treated colonies without any active lesions continues to increase with time, and the number of new colonies needing to be treated continues to decrease (Figure 3). Some notable patterns between offshore and inshore reefs are apparent in these metrics. Offshore reefs display a relatively slow but steady increase in the proportion of previously-treated colonies that do not have active lesions. After 2.5 – 3 years of treatments at a site, 84-90% of the live previously-treated corals appear healthy. At inshore reefs, the proportion of previously-treated colonies with no disease increases more rapidly and to higher levels. After 2-3 years, 87-100% of colonies had no active disease.

Spatial and Temporal Infection Patterns

A temporal trend in SCTLD prevalence is apparent at inshore reefs, but not offshore reefs. Inshore sites showed seasonal increases in SCTLD corresponding with early summer (March – August) (Figure 4). We hypothesize that summer bleaching events at these inshore sites assists in halting disease during the September – November timeframe, with disease reappearing in greater amounts with temporal distance from these events each year. The pattern is not seen at offshore reefs, which have not bleached during the time period of intervention at the sites. Further analyses of these patterns are recommended.

During monitoring events, we have also noted that at inshore reefs, SCTLD-affected colonies tend to be spatially clumped, while at offshore reefs this pattern is not apparent. This was reported in Neely (2021), appeared to remain true through this fiscal year, and is recommended for proper spatial analysis.

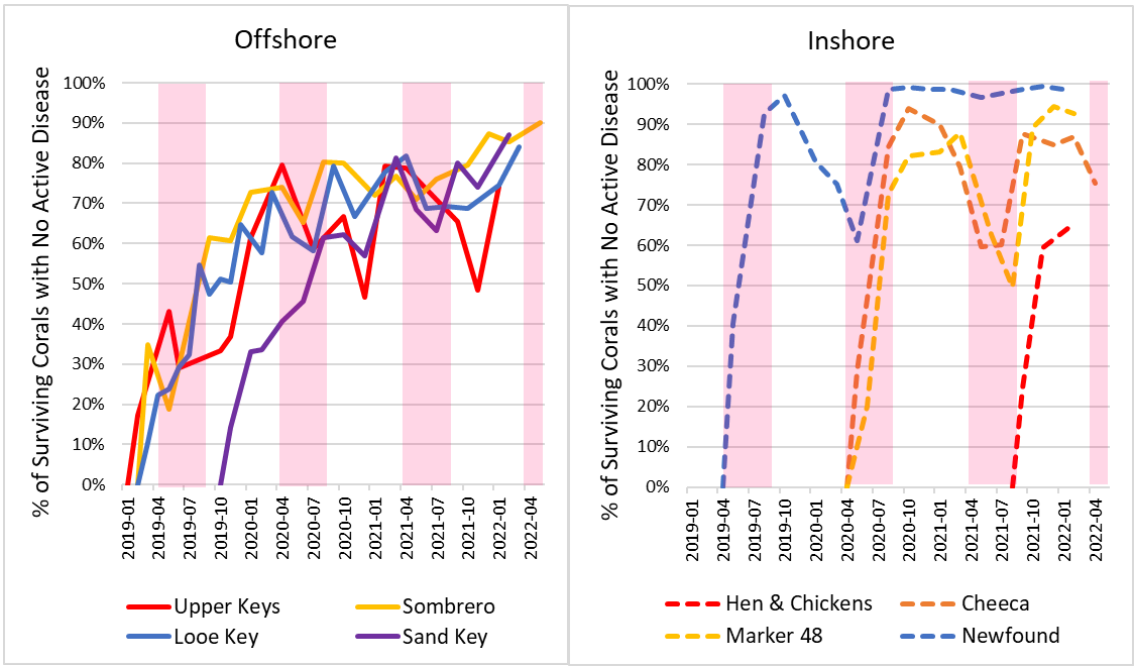


Fig 4. Percentage of live, previously-treated corals with no signs of SCTLD during each monitoring period. Periods of increased SCTLD (lower values on the line graph) correspond with the April – September timeframe (red bars) at inshore reefs (right) but not offshore reefs (left).

Comparing Two Revisitation Protocol

Initial permitting and monitoring protocol were to revisit every previously tagged coral every two months. As the number of treated colonies increased, and as sites moved progressively towards lower prevalence of SCTLD on previously treated colonies, the vast majority of underwater time was spent attending to non-SCTLD colonies (Figure 5), detracting from the goal of preventing coral mortality.

In February 2022, new monitoring protocol were implemented at all sites except Sombrero and Cheeca Rocks (where every colony continues to be monitored). These protocol were designed to focus efforts on SCTLD-affected corals, thus allowing more area to be covered and potentially more time to be available for additional sites.

The first monitoring event at each site involved expanding the area of visitation at the sites (Figure 6). Areas where no SCTLD-susceptible corals were found were henceforth shaded on the monitoring maps and will not be visited again. During the second monitoring event using the new protocol at each site (currently ongoing), we were able to assess the increase in efficiency. At sites with few corals, the decrease in effort is minimal (for example: sites in the Upper Keys generally averaged 1 diver day to

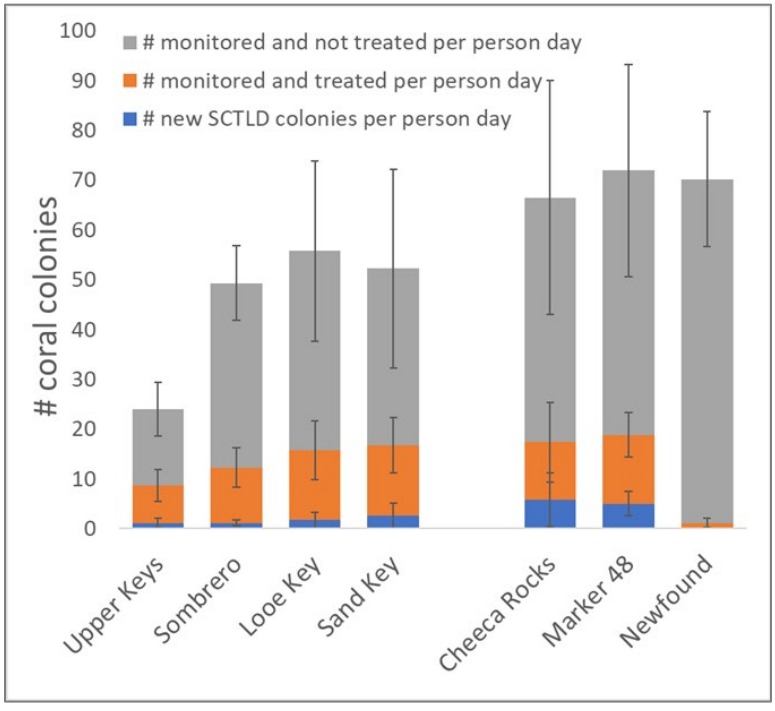


Fig 5. Distribution of coral colonies attended to during monitoring events at each site under the 2019-2022 protocol. The majority of visited colonies had no active disease.

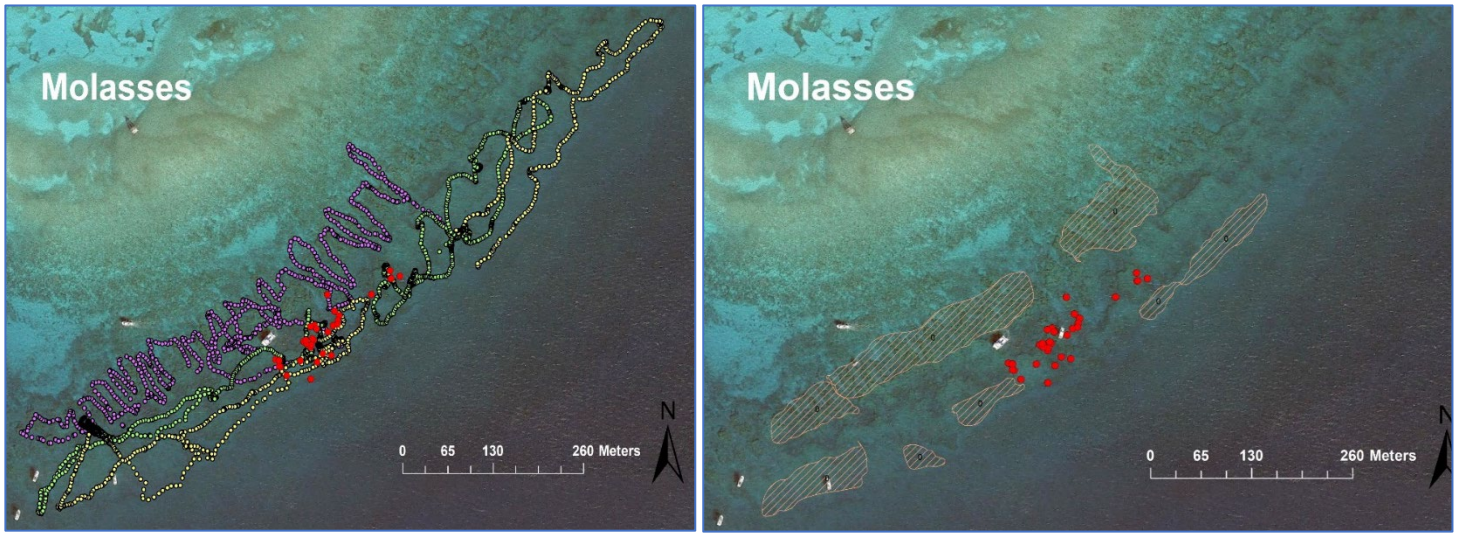


Fig 6. An example of the new monitoring protocol. During the first event with the new protocol, we expanded the survey area from the area of known SCTLD-impacted corals (red) to the entire reef. Lines on the lefthand figure represent GPS tracks of surveyors. Surveyors shaded areas (orange hatch on the righthand figure) where no SCTLD-susceptible species exist to focus future survey efforts.

complete under the old protocol, and now average 2/3 of a diver day under the new protocol. However, at sites with many corals, the increases in efficiency are notable. For example, Looe Key generally required 15-20 diver days under the former protocol, but required only 7 diver days under the new methodology. We remain hopeful that these changed protocols will free up diver days to incorporate new sites in the next fiscal year.

Recommendations

- Many Florida Keys reefs, particularly offshore sites, can be considered "endemic" in that most susceptible individuals have died and infection rates are low. However, coral cover and diversity remains high on many mid-channel patch reefs, and SCTLD prevalence on these can be exceedingly high, particularly on highly susceptible species. These reefs would benefit substantially from rapid and thorough intervention efforts, which should be prioritized over maintaining intervention at low-quality offshore sites.
- Treatments should be scaled up through additional funding or additional partners. Other groups throughout the Caribbean utilize volunteer strike teams or "Adopt a Reef" strategies by various dive shops or other stakeholders that allow for much greater impact from intervention.
- Colony-level monitoring has highlighted interesting temporal and spatial patterns that are yielding information on SCTLD. This dataset should be further explored.
- We recommend prioritizing and funding experiments that will determine the reef-scale impacts of intervention. For example, does intervention on diseased corals at a reef scale minimize infection on neighboring corals as would be suggested by the patchy distribution of infected corals at inshore sites? Even within Florida, these experiments could be conducted on affected patch reefs.
- We recommend identifying specific concerns about the use of antibiotics and prioritize hypothesis-driven experiments to determine the actual risks. In addition, determine acceptable risk thresholds so that intervention activities can work within them.

APPENDIX I: Protocol for Topical Antibiotic Treatment

1. Create a treatment paste using powdered amoxicillin (example: Phytotechnology amoxicillin trihydrate, purity >95%) and Ocean Alchemist / Core Rx Base 2b.
    a. Amoxicillin and Base2b should be refrigerated before use to increase shelf life.
    b. Once amoxicillin and Base2b are mixed, degradation of amoxicillin occurs at approximately 1-2% per day. Ingredients should not be mixed more than a few days in advance of field application.
    c. Take appropriate precautions for working with chemicals/pharmaceuticals.
2. Mix powdered amoxicillin into the Base in a 1:8 by weight ratio. Mixing can be done by hand using a metal spatula or butter knife in a large pot.
    a. For a single small coral (e.g. in a nursery or a single target), 2.5 g amoxi + 20 g of Base is appropriate. For these smaller amounts, a balance is advised to weigh out the correct ratio.
    b. For larger applications, jars of Base2b come in 400g amounts, so two jars can be mixed with one jar of 100g amoxicillin and stirred on the boat. For field treatments at a high-density site, a single experienced diver (~ 6 hours of bottom time) will average ~50 g amoxi + 400 g of Base2b.
3. Pack the mixture into the back of syringes for application using a small spatula or butter knife.
    a. 60cc syringes are recommended for ease of application over multiple corals. Syringes can be reused
    b. Catheter (tapered) syringes are recommended as they can be cut to increase tip diameter if application is difficult.
    c. Syringes are positively buoyant. Sticking a lump of modeling clay onto each syringe is recommended to provide weight and prevent syringe loss.
4. At the SCTLD lesion, use the syringe to apply the treatment mixture over the lesion margin. Use a finger to press the product into the margin area. The treatment will be ~1 cm wide, with approximately half of that anchoring onto recently dead skeleton and the other half overlaying the live tissue. It adheres better to the skeleton than to the tissue, and should be pressed with moderate force for adherence. Small pieces may detach during application, but can generally be caught and remolded into to the application.
    a. Compound adheres to nitrile gloves and neoprene gloves, which are not recommended. Other glove materials may be effective.
5. Alternative or additional intervention can be accomplished by creating and applying the compound to a firebreak about 5 cm away from the disease margin. An underwater angle grinder provides a rapid and clean trench, but this can also be accomplished with a hammer/chisel. Use the syringe to squeeze the amoxicillin mixture into the resulting trench. This provides a moderate increase in effectiveness but also substantially increases treatment time.
6. In rare instances, the treatment mixture will not adhere. In such cases, modeling clay can be used to strategically anchor the Base2b into place. Do not cover the entire treatment, but rather use small pieces of clay to weight or bridge the treatment into place.

Product	Weight	Price	Notes	Weblink for products that have been used in past efforts
Antibiotic (Amoxicillin)	25g	$55.95		https://phytotechlab.com/amoxicillin.html. Contact company directly for 100g jars and bulk discount. Veterinary/ranching alternatives may be cheaper, but effectiveness has not been tested.
Base 2b	400g	$50.00	This amount will fill ~7 60cc syringes	Contact Ocean Alchemists. oceanalchemists@gmail.com
Catheter Syringe	10 syringes	$10.99		https://www.amazon.com/Catheter-Syringe-Syringes-Care-Touch/dp/B01M1R392V/ref=sr_1_1_sspa?ie=UTF8&qid=1537552151&sr=8-1-spons&keywords=catheter+syringe&psc=1
Modeling clay	2 lb	$5.94		https://www.amazon.com/Sargent-Art-Plastilina-Modeling-2-Pound/dp/B00FR7TQOM/ref=sr_1_16?dchild=1&keywords=modeling+clay&qid=1591715628&sr=8-16

APPENDIX II: Guiding principles for determining priority coral colonies (section from Florida's Coral Intervention Action Plan)

Ecological:

- Structure builder: Some susceptible species contribute substantially to reef-building and the associated ecosystem services that provides (*Orbicella* spp., *Montastraea cavernosa*, *Colpophyllia natans*). These species may be prioritized over others that are not primary structure builders.

- Size: Larger colonies are likely to have greater reproductive capacity and provide more habitat. Corals larger than 2 meters may be prioritized for these features.

- Relative size: Colonies that are large for their species are likely to be older and thus more resilient to long-term environmental conditions. They also likely contribute more substantially to reproduction within their species. Corals in the top 5% of size for their species may be prioritized.

- Localized reproductive capacity: A coral surrounded (in the same general reef area) by other live colonies of the same species may have greater reproductive capacity because fertilization rates are likely to be greater.

Regulatory:

- Iconic coral: Corals identified by stakeholders as important for historical, educational, or economic reasons. This could include colonies popular at dive sites.

- Within an MPA: Corals within zones of extra protection may be living under better environmental conditions.

- Within a recreational area (within FKNMS – on a reef with mooring balls): Corals near mooring balls likely have more visitors who utilize the resource. This could provide additional awareness of treatment action and potentially greater involvement through citizen engagement.

- An ESA-listed species.

Treatability:

- Portion of colony unaffected: Treatment is likely to be more effective if the majority of the coral survives as a result. A recommended guideline is if greater than 75% of colony is still alive.

- Number of active SCTLD lesions: Each lesion requires initial treatment as well as follow-up. A greater number of lesions may also signify poorer overall health of a colony and thus a higher chance of new lesions developing. Colonies with fewer than 5 lesions are more treatable than those with more.

- Monitoring efficiency: Colonies in proximity to other treated corals, sites, or other ongoing projects will ease subsequent monitoring and re-treatment events.

- Suitability for treatment: Certain colonies may be disqualified for treatment for external reasons. For example, certain treatments (e.g. removal) may not be practicable if the coral is attached to a cultural resource. Individual sites and projects should consider these additional factors.

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