FLORIDA KEYS CORAL DISEASE STRIKE TEAM: FY 2019/2020 FINAL REPORT

Florida Department of Environmental Protection
Coral Reef Conservation Program

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FY 2019/2020 FINAL 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. Termed Stony Coral Tissue Loss Disease (SCTLD), it affects over half of the stony coral species on the reef and generally results in 60-100% 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.

A response priority has been active in-water intervention to treat diseased corals to allow for the survival of priority colonies. As such, a coral disease response strike team was established by Nova Southeastern University to treat and monitor diseased corals. Additional duties of this team included training other regional strike teams, collecting colonies for laboratory trials, conducting coral spawning activities, and providing additional field support. The strike team was contracted for 100 days of in-water work.

This report outlines the activities undertaken and results of work conducted July 1, 2019 to June 15, 2020.

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. Permission to apply antibiotics was separately authorized by the FDA's Office of Minor Use and Minor Species. Collections of corals for laboratory experiments were authorized under FKNMS-2018-007-A1 and FKNMS-2019-135.

Training

Disease intervention training was provided to Biscayne National Park staff on 10/1/2019 (land-based) and 10/15/2019 (in-water). Nine National Park Service staff were trained in identification of priority corals, disease identification, preparation and application of antibiotic treatments, and subsequent monitoring and touch-up protocols. During the in-water training, staff from the educational facility Marine Lab also participated in identification and monitoring. As a result of this training, Biscayne National Park has initiated disease treatment on priority corals within their jurisdiction. Additionally, students and staff of Marine Lab have undertaken regular monitoring of treated corals at some Upper Keys sites.

Fig 1. An NSU strike team member demonstrates a firebreaking technique to Biscayne National Park Service staff during training (left). A DiveN2Life student conducts a supervised in-water intervention on an active disease lesion.

In December 2019, students and staff with the youth STEM program DiveN2Life were also trained in the process and protocols of disease intervention. Though not permitted to undertake treatment independently, the training raised awareness and promoted citizen science monitoring.

An additional training was provided to Florida Keys National Marine Sanctuary personnel on 1/30/2020 (land-based) and 3/5/2020 (in-water). Four staff completed the land-based training on identification of priority corals, treatment protocols, and post-dive time-series and data management. Three staff completed the in-water training. The goal for these trainings was to turn over the required monitoring and touch-ups for approximately 30% of the corals at Looe Key. However, personnel and COVID-related dive shutdowns within FKNMS made this impracticable.

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 (Figure 2). Full protocol are available in Appendix I.

A Quality Assurance (QA) plan was developed to lay out the site selection process, work plan, and monitoring guidelines (Neely 2019a). 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.

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

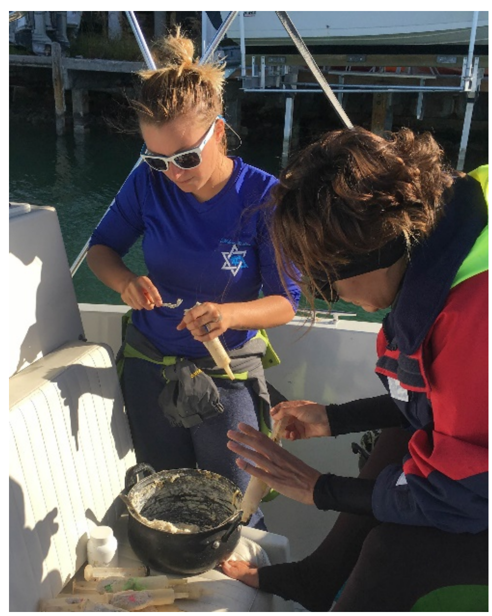


Fig 2. Strike team members mixing amoxicillin into the CoreRx/Ocean Alchemists base and packing the application into syringes.

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 1-month monitoring as a recommended interval. 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 (Figure 3). At one month, these problems have largely abated, and identification of treatment success and failure is more definitive. One-month visitation also provides an opportunity to identify failed lesions or new lesions and conduct touch-ups in a timely manner. After the initial one-month assessment, monitoring every two months is advised to treat new lesions and assess newly diseased priority corals at the site.

The strike team identified the time period for monitoring and retreatment as outlined above and where practicable has adopted a one-month revisit to newly established sites, followed by monitoring and touch-ups as required every two months. 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.

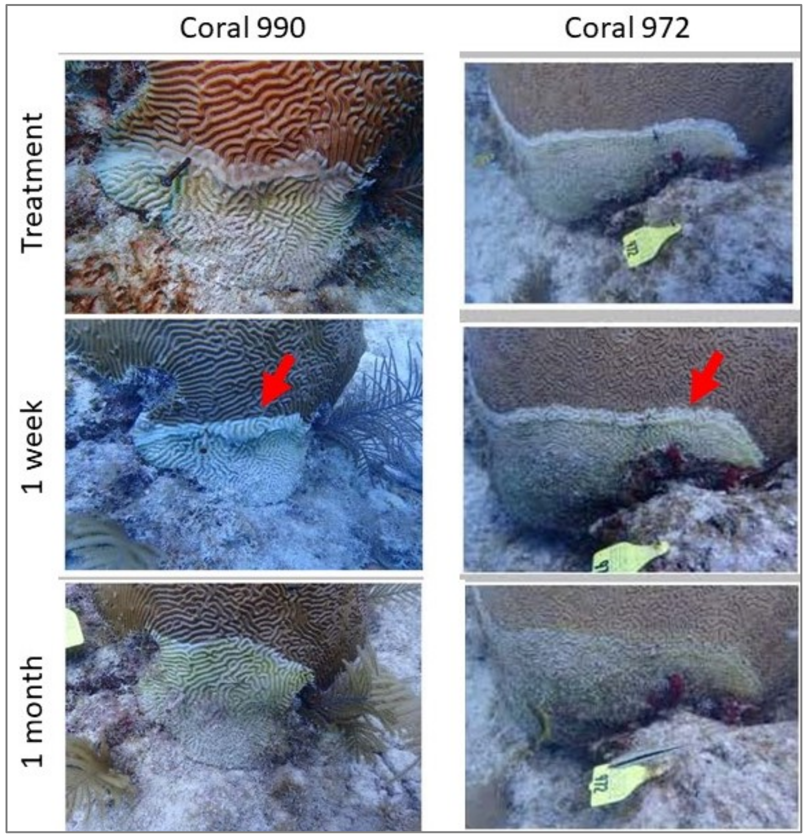


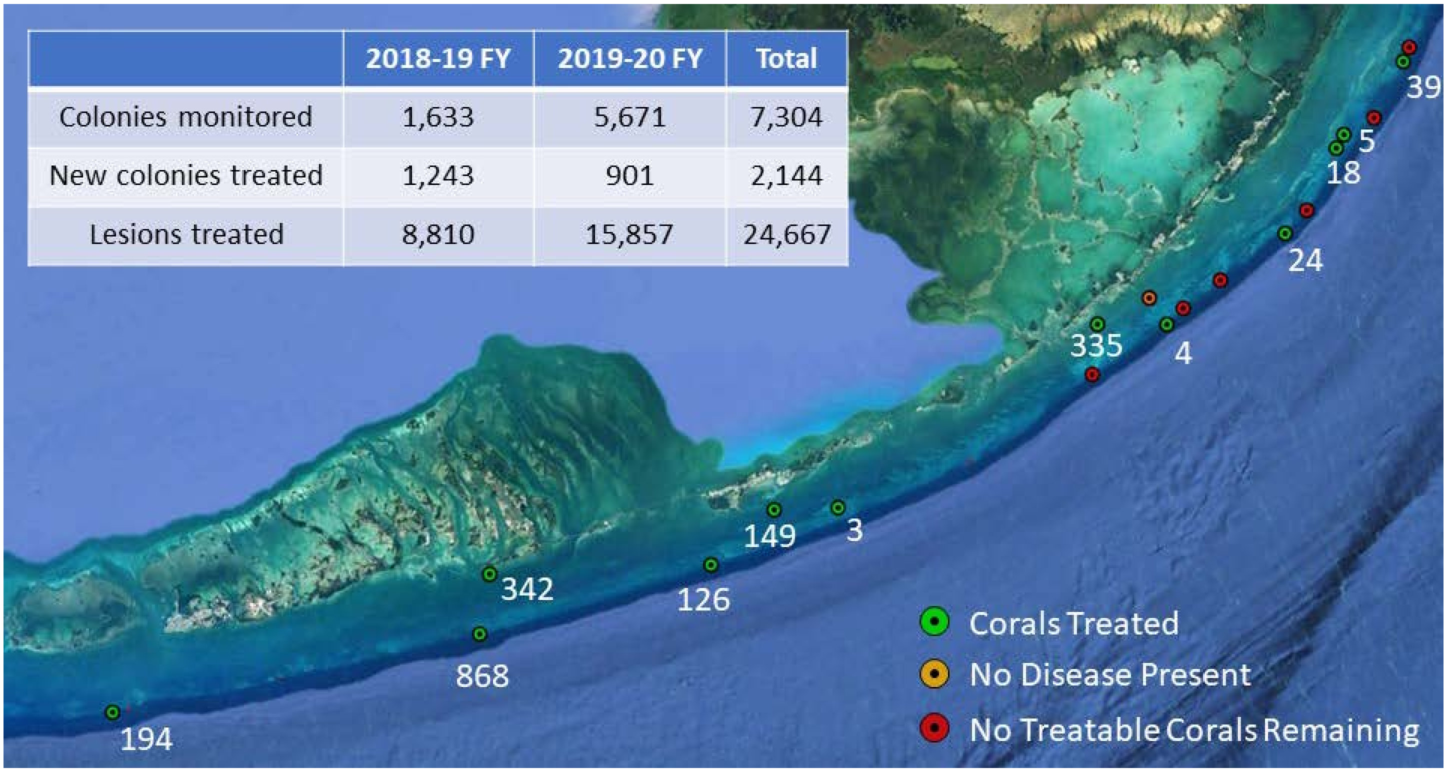
Fig 3. Monitoring photos of two corals at one week and one month after treatment. At one week, both lesions (red arrows) look similar. At one month, it is clear that the treatment was ineffective on Coral 990 as disease continued to progress, and that treatment was effective on Coral 972 as disease halted at the treatment line.

All treatment sites established between January 2019 and September 2019 received lesion-level monitoring for one year. At each monitored coral, photographs were taken of the whole colony as well as each previously treated lesion marked by a nail. The total number of effective and ineffective treatments were tallied in the field, and new lesions were marked with nails. Each lesion was tracked through time using photographic time-series, with each lesion analyzed for effectiveness through time (see example in Appendix III). These time-series photos were used to quantify failure rates by assessing the change in the disease margin between each treatment/monitoring period. Each lesion was classified as either "ineffective" (disease progressed past the treatment and proceeded unimpeded across the tissue) or "effective" (disease progression halted at the treatment line). To determine consistency in using photos for analysis, three NSU team members conducted full independent photo assessments of 433 amoxicillin lesions and 325 chlorine lesions and trenches. Interobserver differences were found to average less than 10%, and subsequent assessments have been conducted by a single team member for consistency. Over 10,000 lesions were tracked for up to one year after application to determine lesion-level effectiveness.

In order to improve underwater efficiency and focus data collection on questions regarding colony-level effectiveness, sites visited for longer than one year as well as sites established after September 2019 were assessed using an alternate monitoring protocol. Lesions were no longer marked with nails, and tallies of effectiveness were no longer determined in the field or through photographic time series. 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.

Work Accomplished

Work conducted from July 1, 2019 to June 15, 2020 included 100 in-water days of two to four divers each (896 dives totaling 1017 in-water hours). Of these 100 days, 3 were dedicated to coral spawning (Neely 2019b), 9 to disease scouting and coral collections, 3 to training of other partners, and 85 to disease treatment and monitoring. Across the treatment



	2018-19 FY	2019-20 FY	Total
Colonies monitored	1,633	5,671	7,304
New colonies treated	1,243	901	2,144
Lesions treated	8,810	15,857	24,667

Fig 4. Map showing all visited and treated sites. Sites where no corals were treated are indicated in red (no treatable colonies remaining) or orange (susceptible species present, but no active disease). Sites where colonies were treated are green, and numbers indicate the number of colonies treated at each site through June 15, 2020.

days, 901 new priority corals were treated (6296 lesions). Additionally, 5671 corals were monitored, and 1760 of them were re-treated on failed or new lesions (9550 lesions). In total since January 2019, over 2100 corals and 24000 lesions have been treated (Figure 4).

Three new sites were added between July 2019 and June 2020: Sand Key, Cheeca Rocks, and Marker 48. Sand Key was authorized by FKNMS as an “experimental site” for testing alternate products (discussed further below). When those products failed, retreatment with amoxicillin was authorized and conducted to save remaining tissue. Cheeca Rocks and Marker 48 were both regions containing multiple mid-channel patch reefs in the Middle Keys. When these were scouted in early 2019, they did not have active disease. At that time, patterns of old mortality suggested that some corals at those sites had most likely been SCTLD-affected, and it was assumed that active disease halted following the 2018 inshore bleaching event as was documented in other nearby inshore reefs. When Cheeca Rocks and Marker 48 were re-assessed in April 2020, they were found to again be in the early stages of active SCTLD infection. Seven patch reefs in the Marker 48 region were assessed using roving diver surveys (Figure 5). All were found to be in similar stages of infection and so the one with the highest diversity and coral cover was selected for intervention. At Cheeca Rocks, infections on each small patch reef displayed radiating patterns of infection, with small portions of most patches heavily infected and surrounded with a gradient to undiseased areas. Across all patches, the most heavily affected areas were on the northeast side, with more recently affected corals to the west of those areas (Figure 6). All of these patches were treated.

Across all sites, the average diameter of treated colonies was 110 cm. The most treated species were *Orbicella faveolata* (35%), *Montastraea cavernosa* (22%), and *Colpophyllia natans* (15%). 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, which were more recently infected, had more diverse species assemblages remaining for treatment (Figure 7). Newfound Harbor was particularly anomalous as a nearshore patch reef that was treated when disease first appeared; the species treated reflect these differences.

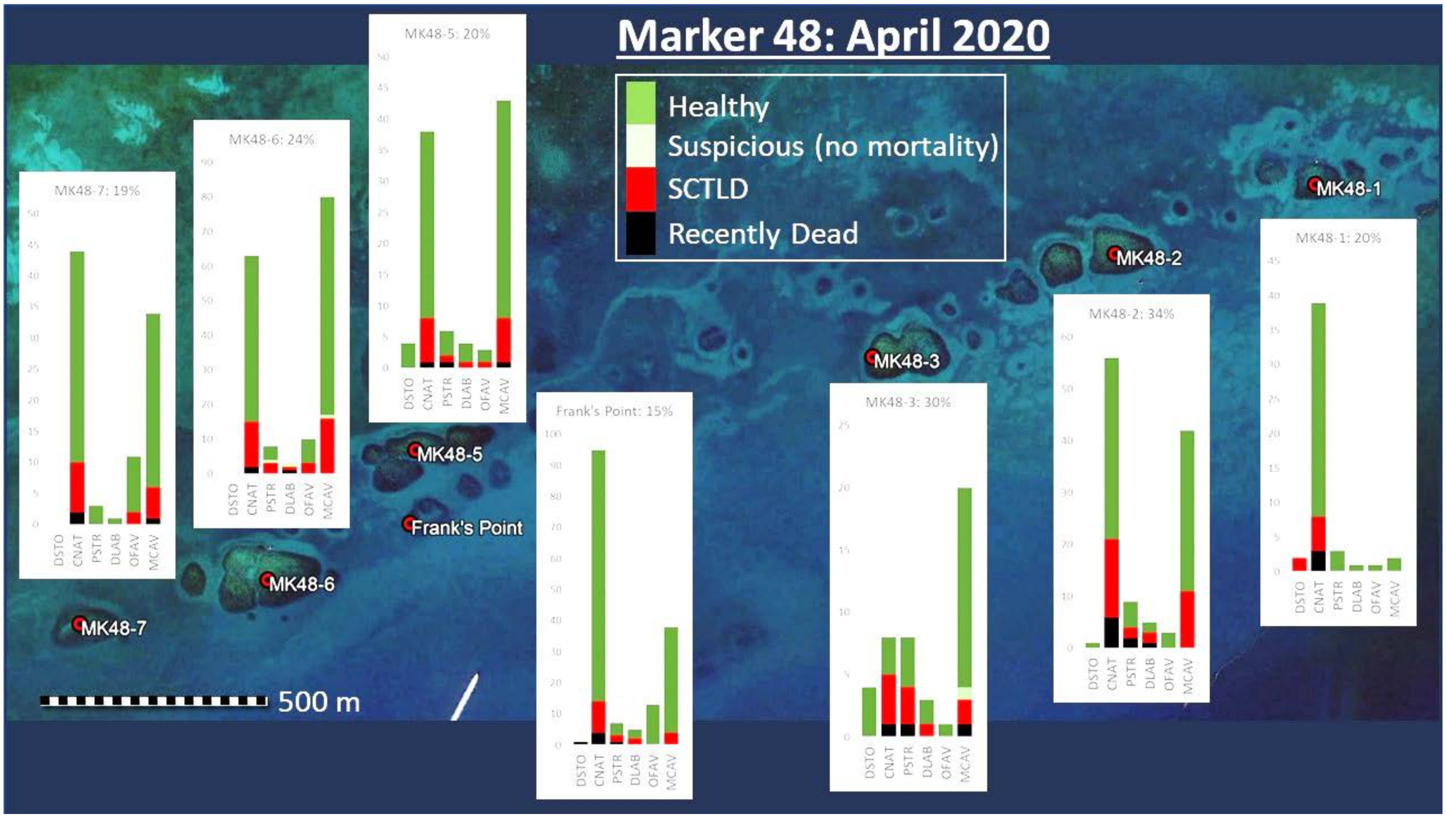


Fig 5. Results of roving diver surveys at patch reefs near Marker 48 (Middle Keys) in April 2020. Bars represent the number of colonies of each species falling into each disease category (recently dead, diseased, suspicious but no recent mortality, and healthy). All sites showed similar levels of disease prevalence across a variety of species.

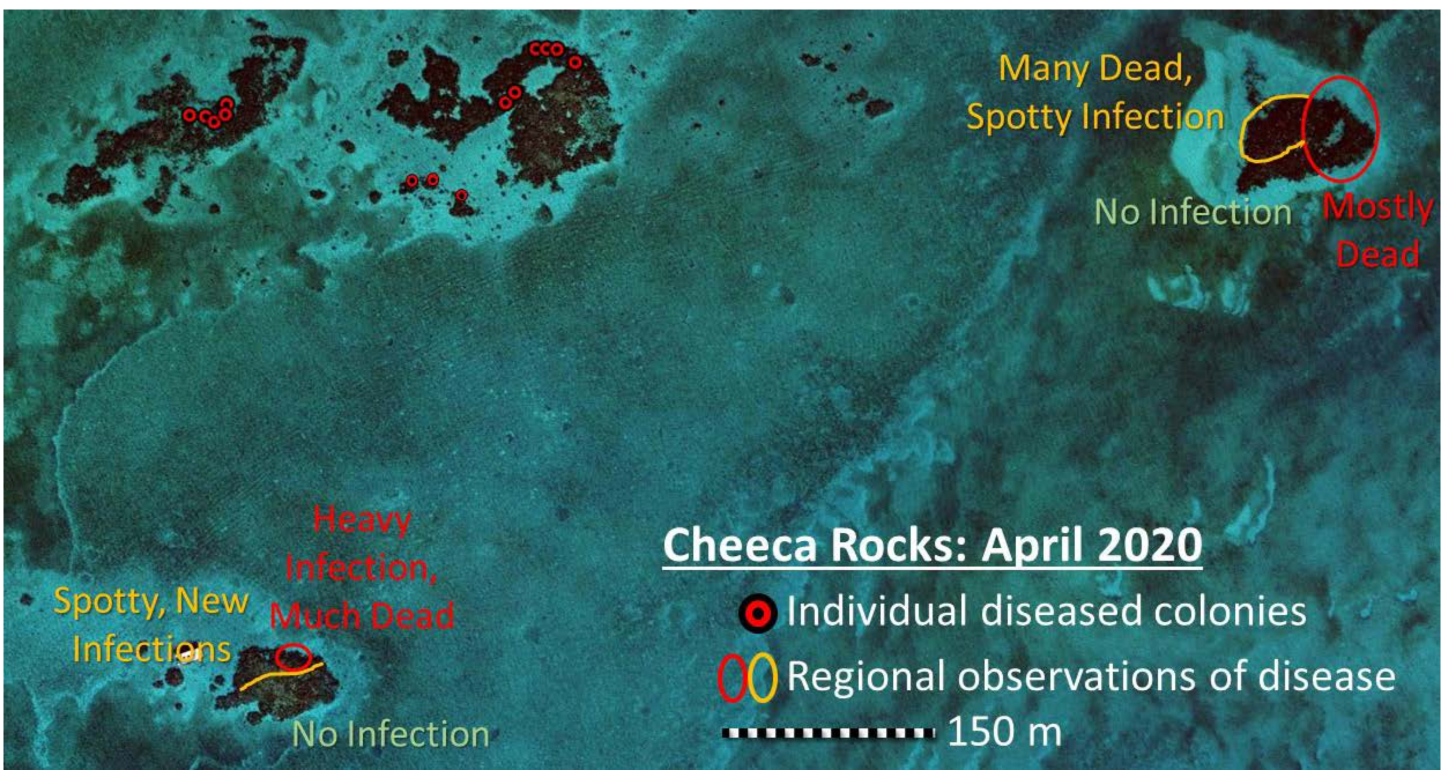


Fig 6. SCTLD presence on patch reefs at Cheeca Rocks. On the northwest patches, SCTLD was rare and each affected colony is shown. On the eastern and southern patches, SCTLD was more common, and heavily-, lightly-, and non-affected regions are delineated. On all reefs, infection was heaviest on the north or northeast side and appeared to have spread primarily to the west.

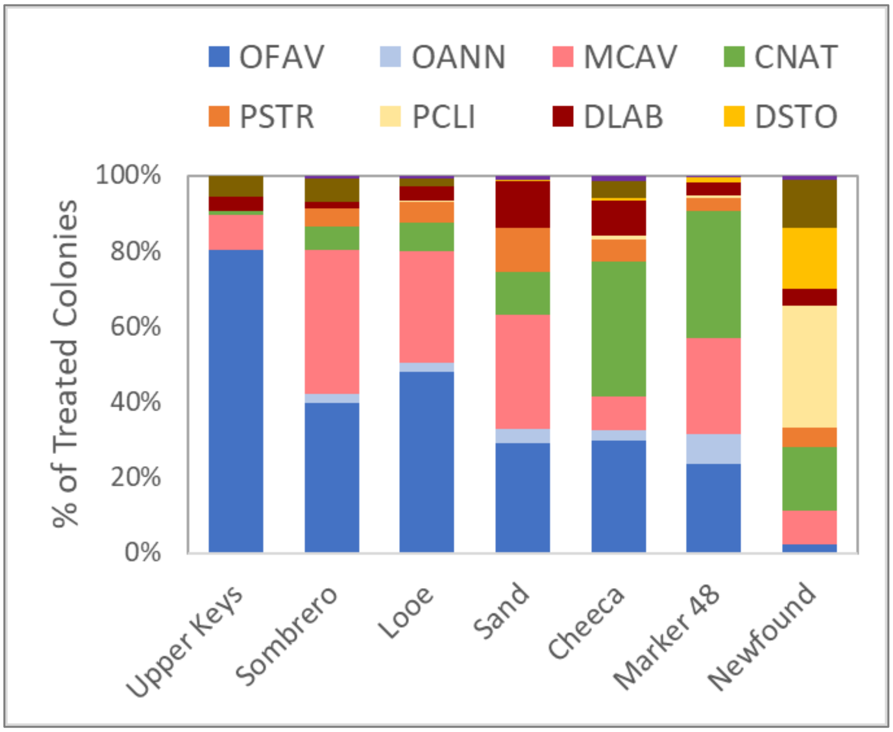


Fig 7. Percentage of treated colonies by species at treatment sites. Labels in the legend represent four-letter species codes. Upper Keys treatments were limited mostly to the remaining *Orbicella faveolata* colonies while other sites had more diversity. Cheeca and Marker 48 are mid-channel patch reefs. Newfound is a nearshore patch reef.

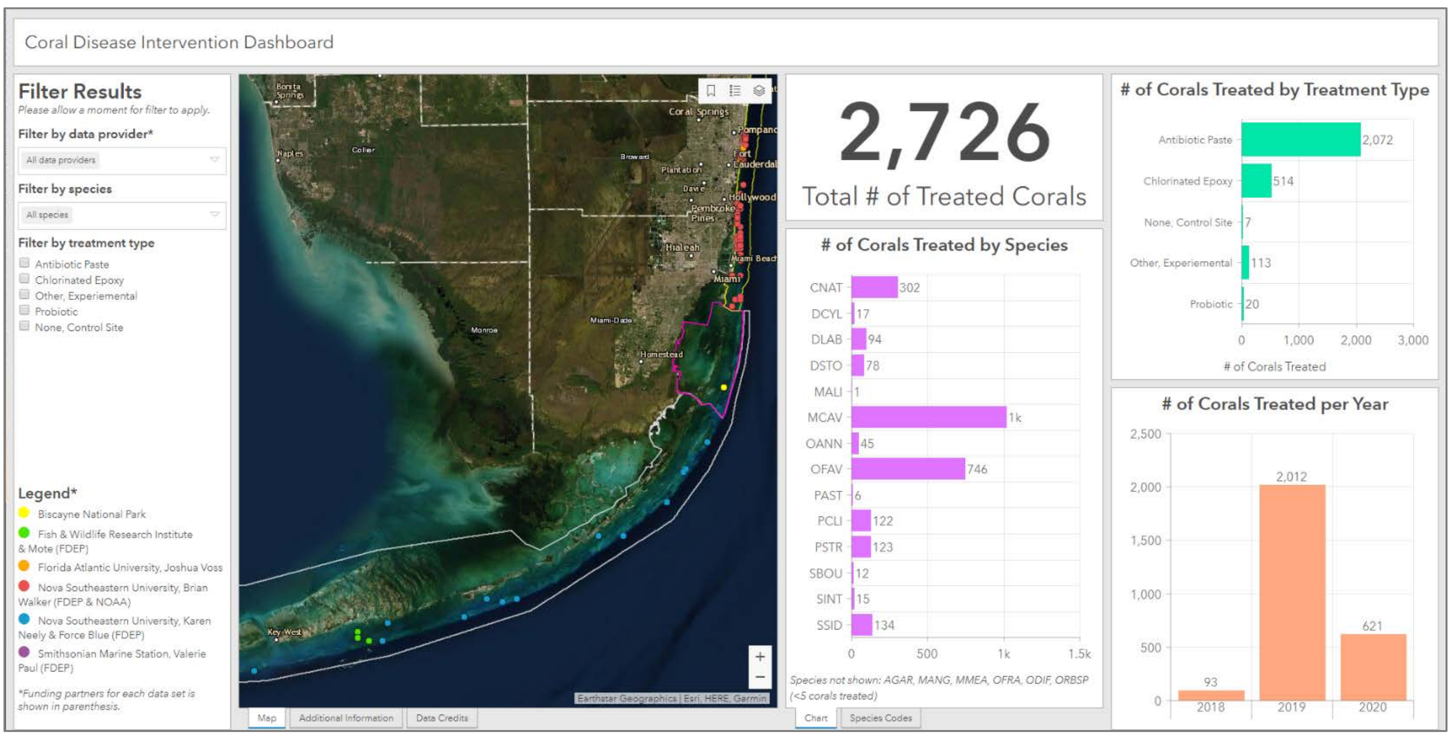


Fig 8. The intervention dashboard developed and maintained by FWC showing ongoing intervention activities from all Florida practitioners. The dashboard is updated monthly (this screenshot is from May 2020).

Since April 2020, the number and locations of treated corals have been provided monthly to FWC for inclusion in an open-access intervention dashboard. The dashboard provides an interactive visualization of interventions conducted by all Florida practitioners and is available at http://arcg.is/84Cej (Figure 8).

Citizen Science

Each treated coral was tagged with a unique identification number and instructions directing citizens to an FWC-developed database (www.seafan.net/tags) to upload photos. NSU staff used these photos alongside the formal monitoring data to build photo time-series that follow the fate of treated lesions over time. Approximately 200 flyers distributed in dive shops, marinas, and local businesses, as well as numerous social media posts, alerted citizens to this project. Since July 1, 2019, a total of 178 citizen science reports have been submitted. Of these, 90% were of usable quality. However, the majority of these were submitted directly after NSU monitoring had occurred (and tags had been cleaned), so were not useful in providing additional data. However, 32 of them (18%) were submitted during periods of non-NSU monitoring and were used in lesion effectiveness analyses. Comments associated with the submissions were overwhelmingly positive and confirm this as a tool for engaging the public. Though a valuable way of raising awareness, current citizen science efforts represented only 0.6% of monitoring efforts and thus cannot be relied upon to replace regularly scheduled monitoring trips.

Results

Chlorinated Epoxy:

Monitoring rapidly identified chlorinated epoxy treatments to be largely ineffective. Most disease lesions progressed past both the margin treatment and the trench treatment within three months. Progression was particularly rapid on brain corals (*C. natans*, *P. strigosa*, *D. labyrinthiformes*), with 60% failure after 1 month and 95% failure after 3 months. But complete failure was also high on *O. faveolata* (68%) and *M. cavernosa* (73%) after three months (Figure 9). As a result of these failures, conversations among partners including DEP staff, the Disease Advisory Council, and the 2019 coral disease workshop participants agreed that chlorinated epoxy treatments were ineffective and should be halted. Live but actively diseased chlorine-treated corals were retreated using amoxicillin starting between 2 – 5 months after initial treatment. In total, 10% of chlorine-treated corals experienced complete mortality.

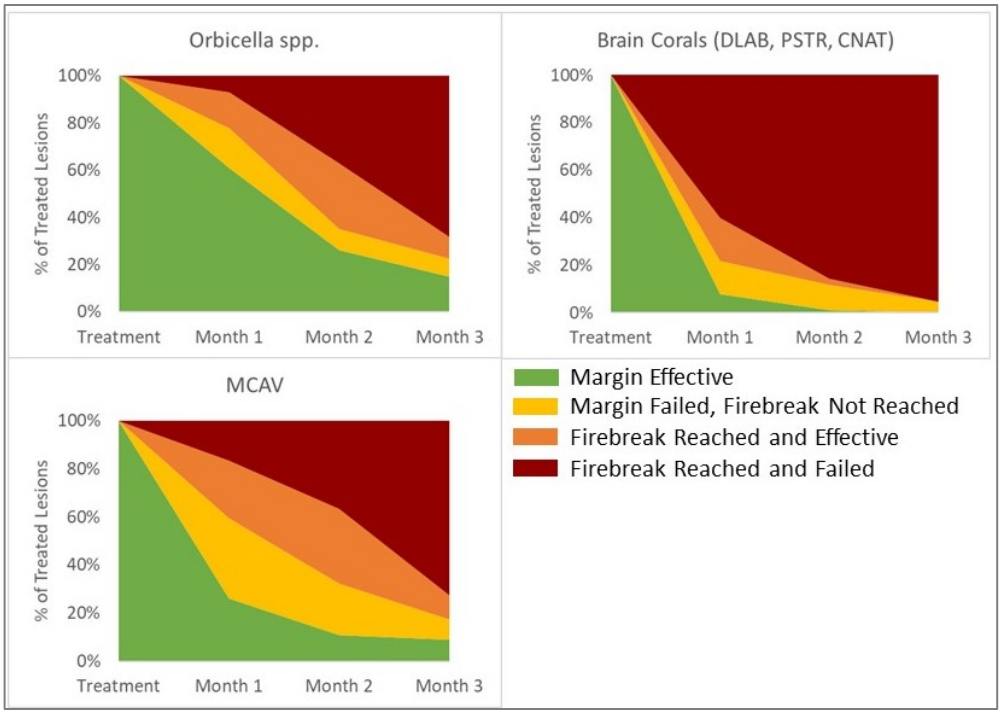


Fig 9. Status of chlorine-treated margins and trenches at 1, 2, and 3 months after treatment on *Orbicella* spp, *Montastraea cavernosa*, and brain corals (*Diploria labyrinthiformes, Pseudodiploria strigosa,* and *Colpophyllia natans*).

Non-antibiotic products:

Experimental products were trialed at Sand Key in October 2019. These included six different “natural products” whose ingredients are proprietary (CoreRx / Ocean Alchemists) and also included placebos of Base2b and an alternative base also developed by CoreRx / Ocean Alchemists (Figure 10). All experimental products were compared with the traditional Base2b + amoxicillin, the alternative base with amoxicillin, and untreated controls.

Full results are presented in the final novel trials report (Neely 2020), and also in PeerJ (Neely et al. 2020). Briefly, none of the placebos or non-antibiotic products halted disease. Following the one-month results, FKNMS staff authorized all surviving treated corals to be treated with Base2b + amoxicillin. In total, 21% of non-antibiotic treated corals died before such retreatments occurred.

Fig 10. Non-antibiotic products developed by CoreRx / Ocean Alchemists.

Amoxicillin:

In contrast to chlorinated epoxy and alternative product treatments, amoxicillin paste has proven effective at halting disease lesions. Over 8200 amoxicillin-treated lesions were analyzed from time-series photographs to show success rates across seven species. Only sample sizes larger than 15 lesions were assessed, and thus the maximum assessment period ranged from nine to twelve months depending on species (Figure 11). Average lesion treatment success across all species and time periods averaged 91%. The lowest recorded success rate was 71% for *Colpophyllia natans* seven months after treatment. Topical Base2b + amoxicillin treatments are thus considered effective at halting disease lesions across all tested species and for extended periods of time (at least nine months).

During monitoring events, amoxicillin touch-ups on failed treatments and new lesions helped to maintain live colonies; these touch-ups were tallied as either retreatments (on failed lesions) or new treatments. One month after the initial treatment, 69% of touch-ups were on newly developed lesions. This percentage increased through time to over 85% of touch-ups after eight months (Figure 12). We conclude that the majority of touch-ups are for new lesion development and that the majority of failed lesion treatments can be corrected with 1-month touchups.

Because new lesions develop, corals need to be revisited periodically for assessments and touch-ups. We conducted colony-level analyses to determine the long-term trajectory of treated corals. During each monitoring event, every coral was classified as one of the following: 1) no active disease (NAD), 2) treated, 3) active lesions (but not treated) or 4) dead. We assessed the percentage of NAD-coded corals through time across sites and species. Across all corals and sites, only 43% of colonies were not actively diseased one month after the initial treatment. This percentage varied across species and sites (Figure 13). However, the percentage of corals with no active disease continued to increase over subsequent months, with increases seen for all species and all sites. By month 14 after initial treatment, 60% of treated corals had no active disease. Among sites, Newfound Harbor showed the most rapid increase in NAD-coded corals. Among species, *O. faveolata* were the most likely to have new lesions through time. The overall decline in new infections through time may be the result of reduced susceptibility of treated corals to new infections and/or to decreased pathogen load and potential for infection at a site over time.

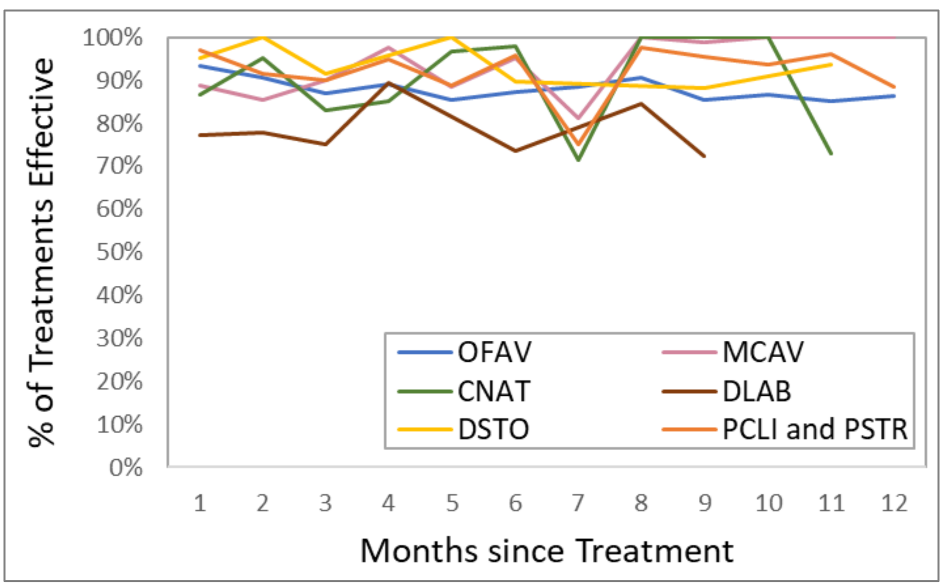


Fig 11. Effectiveness rates of Base2b + amoxicillin treatments on multiple species over time. For each species and time period, only results with more than 15 assessed lesions are presented (average sample size = 279 ± 193 SD).

We also assessed individual corals to determine whether the reinfection patterns by site and species are driven by specific individuals that are continually reinfected, or whether reinfection occurs across all colonies. By using color plots to group together colonies with similar monitoring histories, patterns of infection can be identified (Figure 14). Using these color plots, the percentage of treated corals experiencing no active disease for at least six months and the percentage experiencing no further disease after one initial treatment at the 1 – 2 month monitoring date were calculated across species and sites.

Overall, 34% of corals showed no sign of disease for at least six months after the initial treatment. An additional 12% required a touch-up at the first monitoring event, but subsequently did not show disease. These patterns varied by site and by species however (Figure 15). For example, at Looe Key, less than 30% of *O. faveolata* colonies remained undiseased after the initial treatment or one additional touchup. In contrast, over 70% of brain corals at Newfound Harbor remained without disease under the same scenarios. These metrics provide a measure of the hypothetical fate and mortality rates of colonies had they not received regular monitoring and touchups. Combined with ongoing monitoring and further analysis, these data could provide species- and site-specific information on the future maintenance requirements of treated corals.

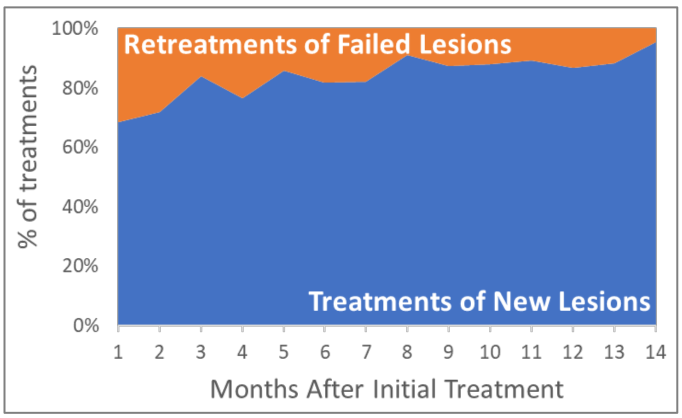


Fig 12. Proportion of touch-ups during each month after the initial colony treatment that are either retreatments of failed lesions (orange) or treatments on newly developed lesions (blue).

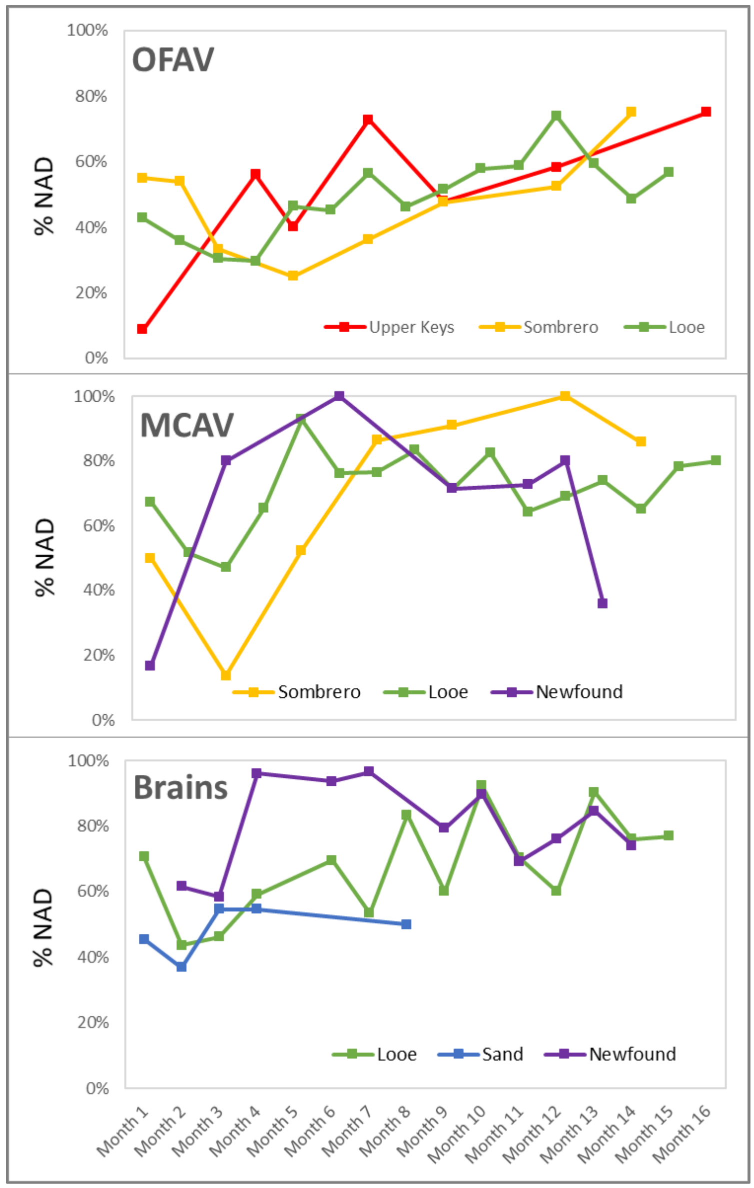


Fig 13. Percentage of treated colonies with no active disease (NAD) during the months following initial treatment. Months in which there were fewer than 10 observations per species:site are omitted. “Brains” includes DLAB, CNAT, and *Pseudodiploria* species.

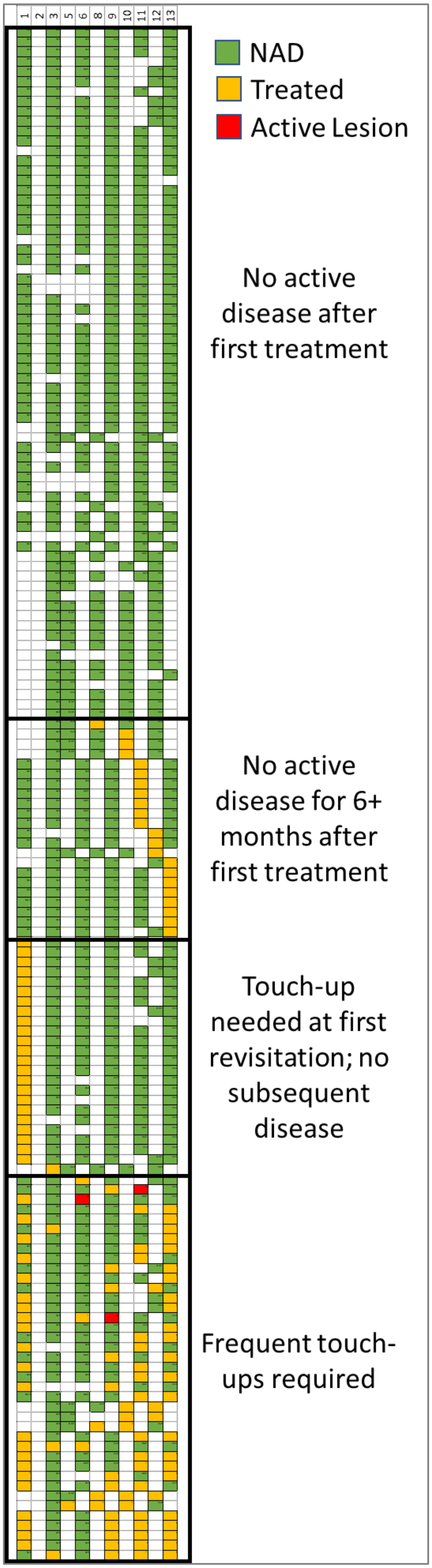
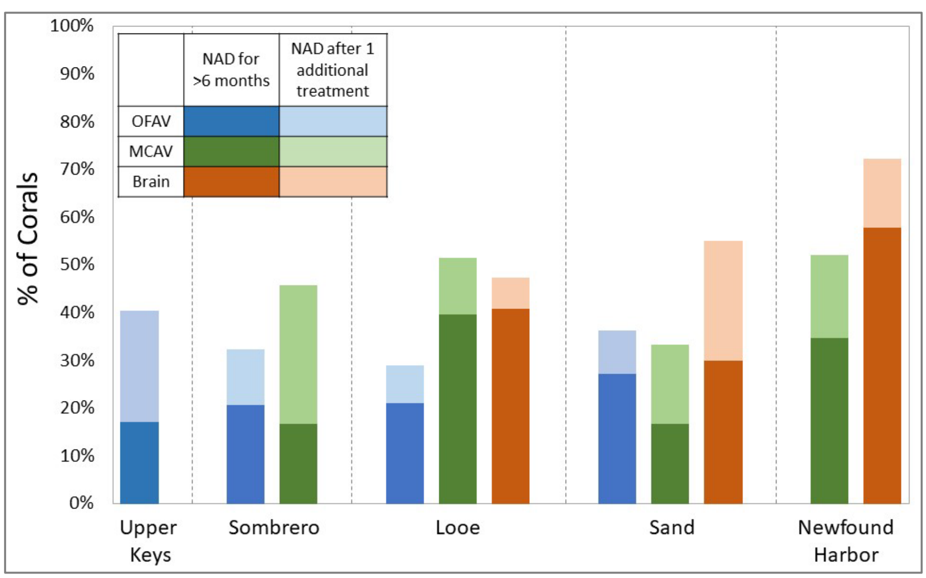


Fig 14. A representative color plot of colony status during monitoring events. This plot shows brain corals (DLAB, CNAT, and *Pseudodiploria* species) at Newfound Harbor. Each row represents a unique coral colony. Each column represents a subsequent month since the initial treatment. Colonies are sorted by similar patterns of treatment (Treated: yellow) or absence of disease (NAD: green). In some cases, active lesions were present but not treated (red) because they were on small or nearly dead isolates.

Fig 15. Percentage of treated corals categorized through color plots as having no active disease (NAD) for 6+ months after initial treatment (dark hues), and as having no additional disease after the initial treatment plus a touch-up during the first monitoring event (pastel hues). "Brain" category includes DLAB, CNAT, and *Pseudodiploria* species.



Calculation of Resources

In order to provide information for other practitioners, calculations of required resources and effort were done based on the Florida Keys treatments.

The amount of Base2b applied to colonies during initial treatments at Sand Key was measured for each coral. On average, 12 mL of base and 1.6 grams of amoxicillin were applied to corals. However, this varied greatly depending on the size and species of the coral as well as the size and number of disease lesions (Figure 16).

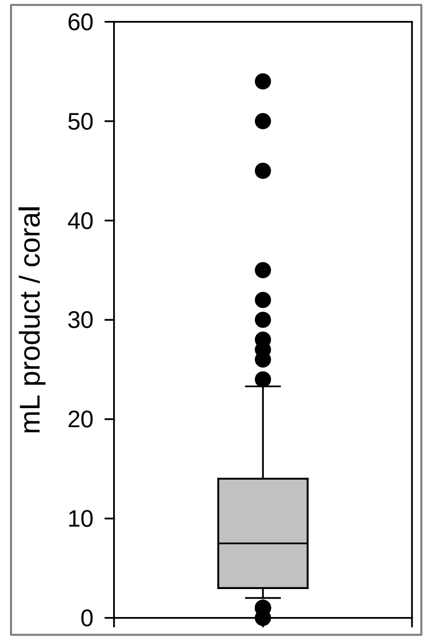


Fig 16. Amount of Base2b applied to each treated coral at Sand Key. The average application was 12 mL. Box shows the quartiles with the median. Whiskers encompass the 10th-90th percentile. Dots are outliers.

Additionally, the total amount of Base2b used per day during fifteen monitoring/touch-up days during May/June 2020 was tallied. The days included visits to four different sites: Looe Key, Newfound Harbor, Dustan Rocks, and Cheeca Rocks. The amount of product used was divided by the number of corals treated as well as the number of corals visited (which accounts for corals not requiring retreatment) to estimate per coral usage (Figure 17). On average, 17.8 mL of product were used per treated coral, and 7.9 mL were used per visited coral. The discrepancy between Sand Key coral treatments (12 mL/coral) and these retreatment averages is likely accounted for by: 1) the larger number of *Colpophyllia natans* at the retreatment sites, which require more product to fill the rugose skeletal grooves, and 2) the average larger size of corals at the monitored sites. In particular, some of the heavily diseased corals at Cheeca Rocks were in excess of 4 m diameter.

The time required to do initial treatments on corals is impacted by the coral size, species, site, and experience of the diver. In order to develop a standard metric for comparison, the total bottom time of NSU divers each day was divided by the number of corals treated during new site setup (no monitoring). These estimates include search time, tagging, measuring, photographing, treating, and mapping each coral. On average, the time spent on each coral was 16 minutes. Two factors that clearly influenced this were average colony size, which positively correlated with the the amount of time spent per coral ($R^2 = 0.30$, $p < 0.001$), and the percent coral cover of susceptible species, which negatively correlated with time spent per coral ($R^2 = 0.31$, $p < 0.001$) (Figure 18). Areas with low coral cover, such as sites in the endemic zone where most corals have already died, require much greater search time for treatable corals; in Upper Keys sites where corals were uncommon, average time expended per coral sometimes exceeded 40 minutes. Maximum efficiency is achieved where treatable corals are in close proximity to each other and lesions are small in number and size. These characteristics are most commonly found in the outbreak zone.

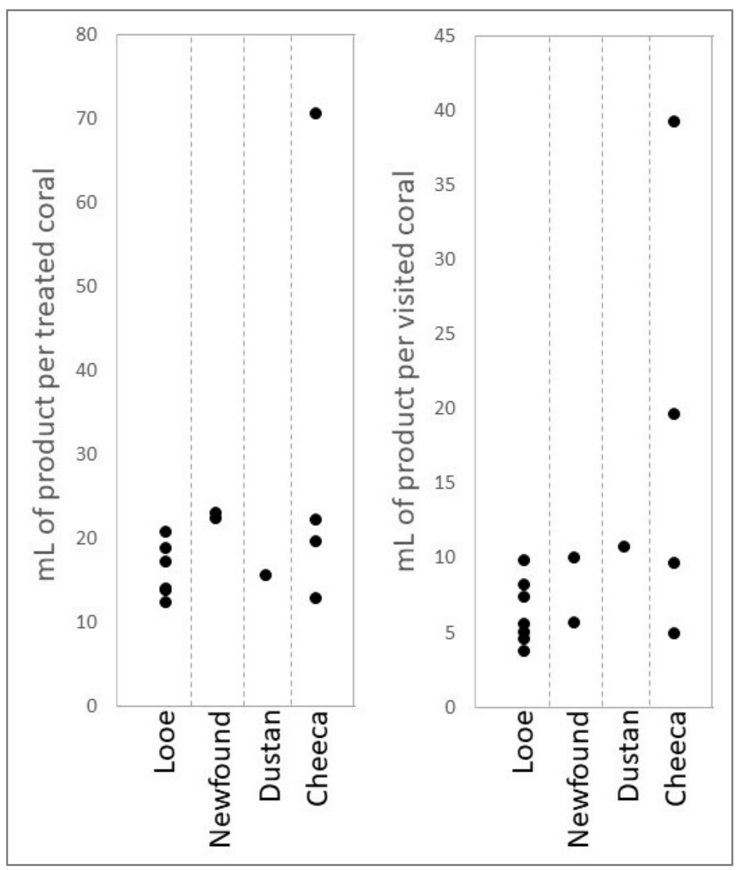


Fig 17. The average amount of Base2b used per treated coral (left) and per monitored coral (right) during monitoring/touch-up days. Values are calculated as the total amount of product used each day divided by the number of corals. The high value at Cheeca represents a day when several heavily diseased corals in excess of 4 meters in diameter were treated.

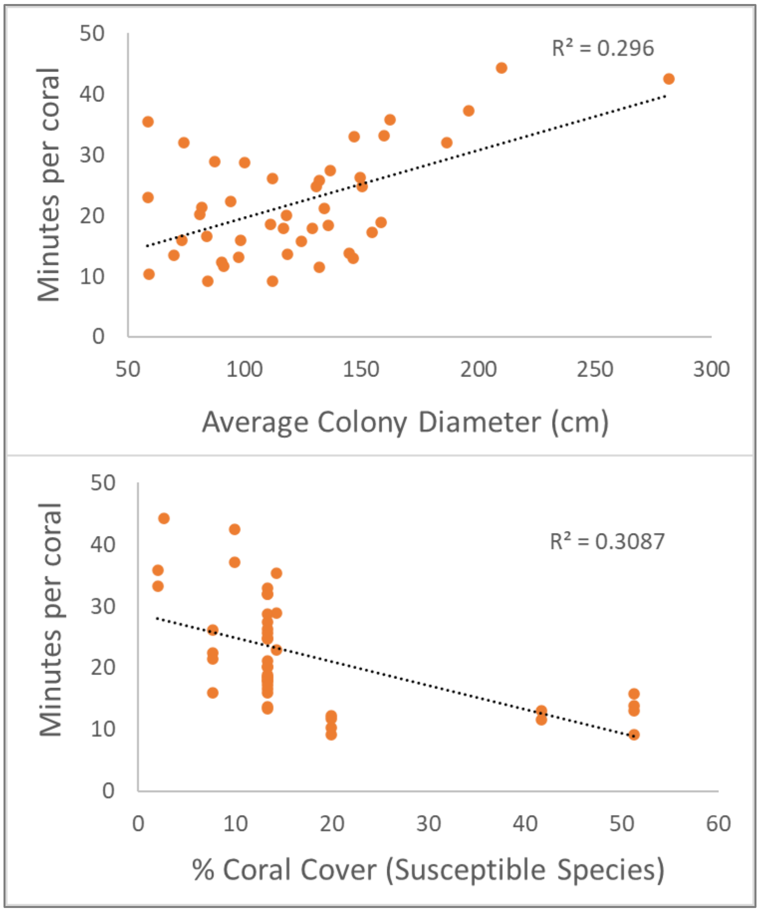


Fig 18. The average amount of time spent per coral on days when new sites were set up. The amount of time expended per coral increased with average colony diameter as large corals required more time and effort. The amount of time per coral also decreased with the coverage of susceptible species as search time increased as a result of rarity.

Recommendations

- Implement large-scale treatment of corals using amoxicillin paste in order to preserve priority corals and also specific sites with high diversity and intact ecosystem structure. Increase capacity by expanding existing strike teams or considering additional partners who could scale-up in-water intervention.
- Consider efficiency and effectiveness as part of priority site selection. Treatments in the endemic zone require extensive search time, respond less well as they are primarily conducted on *Orbicella faveolata*, and are unlikely to result in enhanced reproductive capacity. Treatments in more recently infected areas maximize in-water efficiency, preserve species diversity, and result in more intact ecosystems with greater potential for successful reproduction.
- As new Caribbean regions are first affected by SCTLD, they look to Florida for guidance. However, Florida practitioners have never been permitted to work in outbreak zones similar to what new regions are experiencing. Consider permitting work within the outbreak zone (particularly isolated patch reefs) to answer questions about treatment of highly susceptible species (particularly *Meandrina meandrites*) and effectiveness of very early intervention in halting reef-scale infection.
- Consider 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? Does intervention have any impacts on reproductive capacity? Does intervention affect other reef species?
- Identify 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.
- Continue searching for new disease treatments that may be effective at the colony level such as antibiotic feed, probiotics, etc. Ultimately, a vaccine should be developed to prevent re-infections and halt continued disease spread.

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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.

APPENDIX III: Example time series of treated lesions on a colony over time

Date Pre-treatment

2019_04_22

Post-treatment a b (bottom) c d

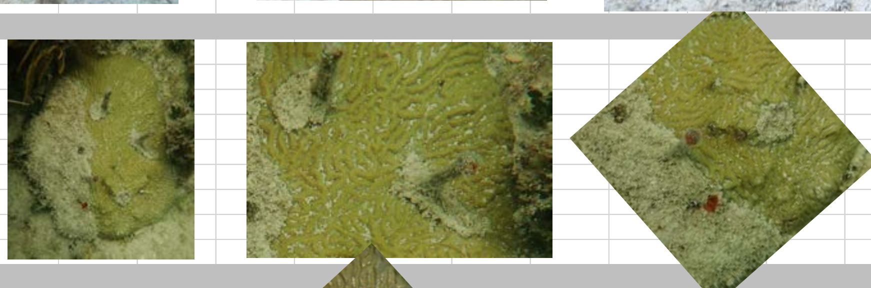
2019_04_22

1 Month

2019_05_21

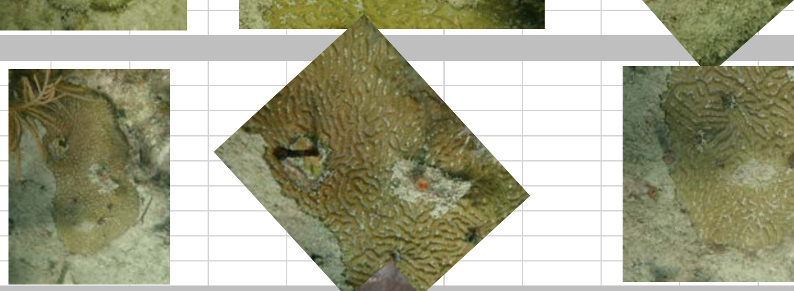
4 MONTH

2019_08_04



6 MONTH

2019_10_16



9 MONTH

2020_01_25

12 MONTH

2020_04_01