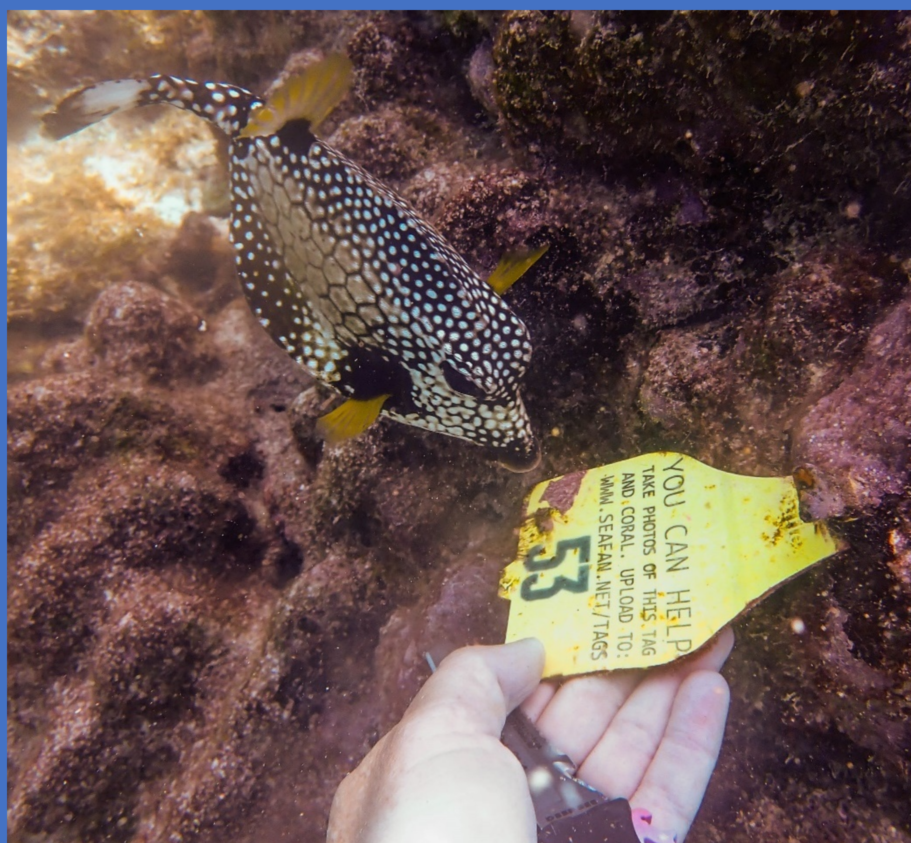
FLORIDA KEYS CORAL DISEASE STRIKE TEAM: FY 2020/2021 FINAL REPORT



Florida Department of Environmental Protection
Office of Resilience and Coastal Protection

FLORIDA KEYS CORAL DISEASE STRIKE TEAM:
FY 2020/2021 FINAL REPORT

Prepared By:

Karen Neely, Ph.D.

Nova Southeastern University
Halmos College of Natural Science and Oceanography
8000 N. Ocean Drive
Dania Beach, FL 33004-3078

June 15, 2021

Completed in Fulfillment of PO B77D91 for

Florida Department of Environmental Protection
Office of Resilience and Coastal Protection
1277 N.E. 79th Street Causeway
Miami, FL 33138

This report should be cited as follows:
Neely K. 2020. Florida Keys Coral Disease Strike Team: FY 2019/2020 Final Report. Florida DEP. Miami, FL. Pp. 1-19.

This report was prepared for the Florida Department of Environmental Protection, Office of Resilience and Coastal Protection by Nova Southeastern University. Funding was provided by the Florida Department of Environmental Protection. The views, statements, findings, conclusions and recommendations expressed herein are those of the author and do not necessarily reflect the views of the State of Florida or any of its sub-agencies.

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 treat 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. Additional duties of this team included training other regional strike teams, conducting coral spawning activities (with summary and results of work available in Neely (2020a)), and providing additional field support. The strike team was contracted for 100 days of in-water work, with an additional nine days added in May 2020 as work was completed early.

This report outlines the activities undertaken and results of work conducted July 1, 2020 to June 15, 2021.

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. Permission to apply antibiotics was separately authorized by the FDA's Office of Minor Use and Minor Species. Spawning work was conducted under permit FKNMS-2020-078, authorized on July 16, 2020.

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.

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. At each monitored coral, photographs were taken of the whole colony as well as each previously treated lesion as marked by a nail. The total number of effective and ineffective treatments were tallied in the field, and new lesions were marked with nails. Field tallies were found to be highly ineffective. One month after treatment, only 51% of known treated and nail-tagged lesions could be identified in the field. For only 39% of monitored corals did the tallied lesions match the number of treatments conducted the previous month. Additionally, determining effectiveness from nails is complicated by the appearance of new lesions. Examples are shown in Figure 1.

Instead, photographic time-series were used to analyze each lesion 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). Over 10,000 lesions were tracked with these photo timeseries for up to one year after application. A subset of these that had consistent time-series photos and clear SCTLD appearance

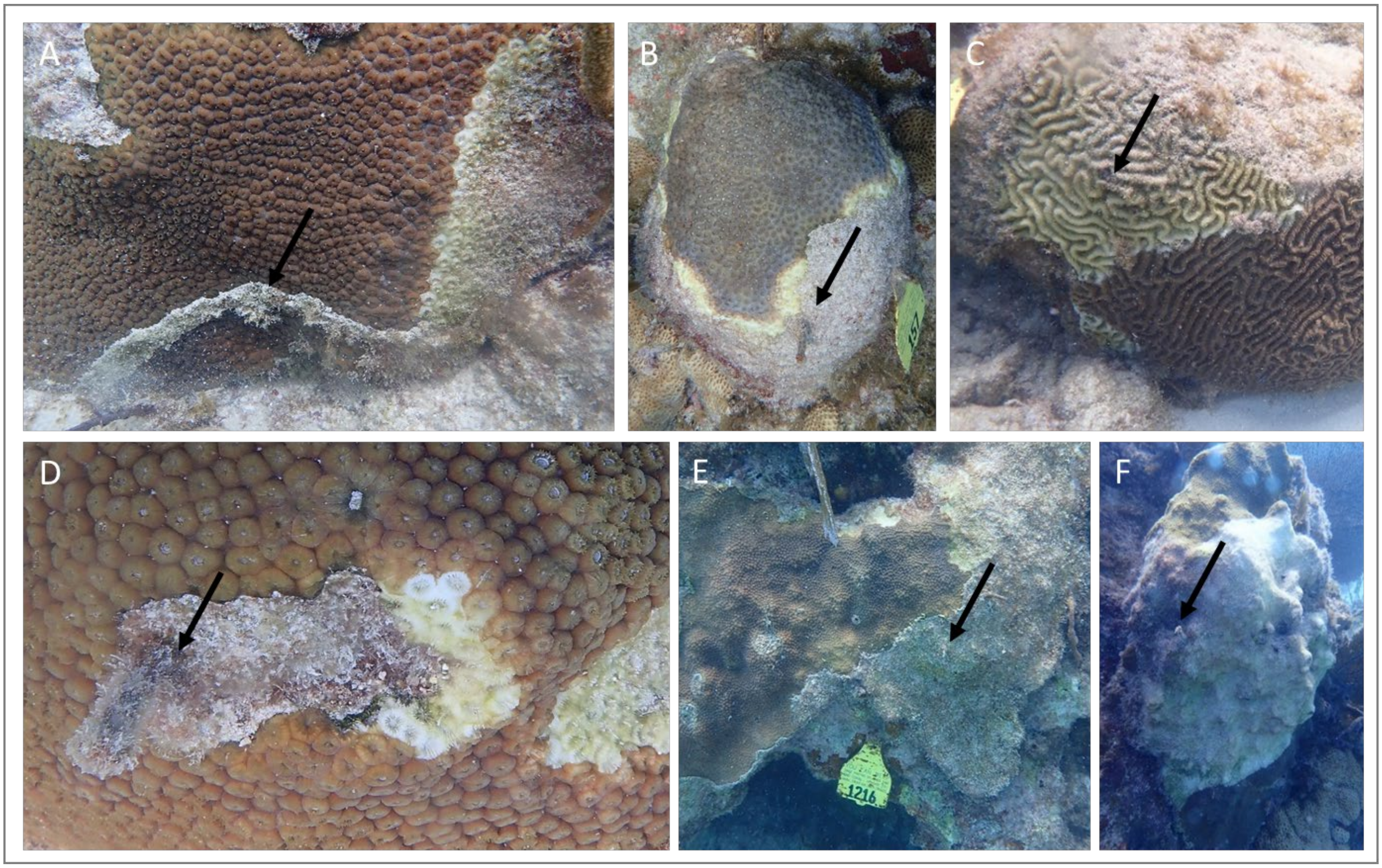


Fig 1. Examples of nails (marked by arrows) applied during previous disease treatments and revisited during subsequent monitoring events. These highlight the challenges that new lesions pose in evaluating previous treatment efficacy. A) Lesion is touching previous treatment line (bottom right), but is clearly a new lesion. B) Disease lesion is near the marker nail, but is widespread and includes areas far from the nail and separated by non-diseased tissue. C)Lesion appears to be stemming from the nail; however this photo shows the coral 3 months after monitoring, and at one month the lesion had clearly healed. D) Lesion appears to be stemming from a previous lesion, but only from the side opposite the nail; additionally, this coral was disease-free with a healed lesion at 1 month. E) The nail is some distance from the live tissue, but the adjacent margin is healed while another active lesion is visible above. F) Live tissue is quite far from the nail, but it is unclear where the rapidly-progressing lesion originated from.

were further analyzed. Further discussion is available in the “Results” section of this report and in Neely et al. (2021)

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 (outlined in Neely (2020b) and permitted through FKNMS-2020-077). 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 under this project from July 1, 2020 to June 14, 2021 included 110 in-water days of two to four divers each. The strike team conducted 876 dives, totaling 1073.5 in-water hours. Of the 110 days, 2 were dedicated to coral spawning (Neely 2020a), 4 to disease scouting, 2 to coral collections for Smithsonian experiments and coral collections, 2 to probiotics trials, and 100 to disease treatment and monitoring.

Training

Disease intervention training was provided to Dry Tortugas National Park staff on 2/1/2021 (land-based) and 2/9/2021 (in-water). Eight 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 (Figure 2). Time was also allocated for goal-setting and protocol development. As a result of this training, Dry Tortugas finalized an intervention plan, actively monitored for SCTLD within their jurisdiction, and when SCTLD was observed in June 2021 responded rapidly and aggressively with treatments.

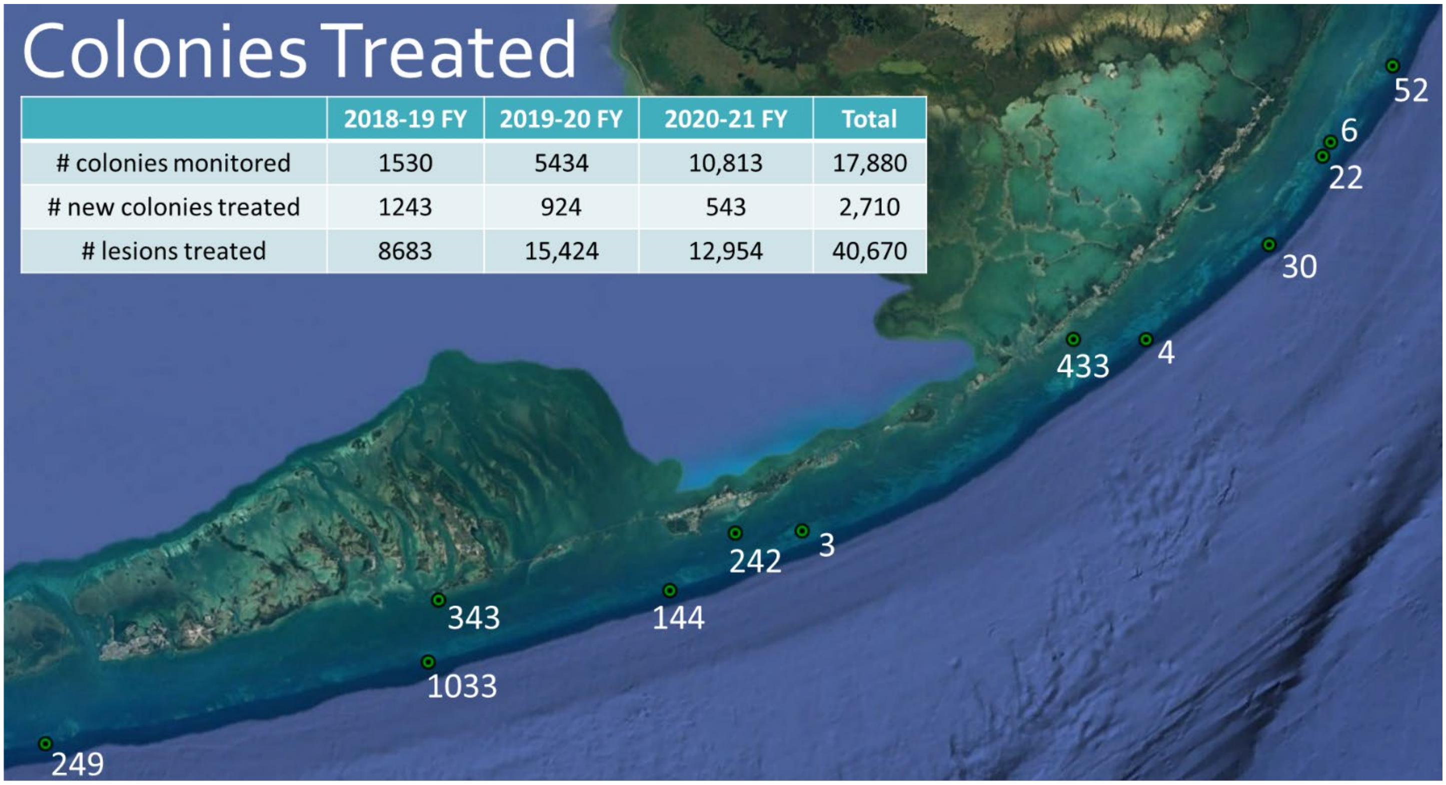
Fig 2. An NSU strike team member guides Dry Tortugas National Park staff in the sample application of treatment materials during land-based training.

During the course of regular treatment work, additional training opportunities included cross-training with Brian Walker (NSU), and hosting a scientist from the Bahamas on who used the information and experience gathered to develop and implement intervention actions within the Bahamas.

Disease Treatment

Across the 100 treatment days, 543 new priority corals were treated (2273 lesions). Additionally, 10,813 previously treated corals were monitored (all corals were monitored approximately every two months), and 1886 (17%) were retreated (10,681 lesions). In total since January 2019, 2710 unique corals and 40,670 lesions have been treated (Figure 3).

The monitoring burden at these sites has exceeded strike team capacity, and no new sites were added between July 2020 and June 2021. Scouting opportunities have identified that patch reefs throughout the Marquesas, Lower, Middle, and Upper Keys continue to be ravaged by SCTLD; many of these represent the last regions of species rich, high coral cover sites in Florida, and many have been found in "hotspot" conditions with extremely high SCTLD prevalence. These include: Marquesas and Lower Keys sites scouted in August 2020 (Figure 4), as well as Hens and Chickens in the Upper Keys and the patch reefs off of Marathon which were observed with high prevalence in May-June 2021. Under current funding and monitoring protocol (as well as the monitoring and funding landscapes for FY 2021-22), we do not have the ability to save these corals and reefs.



	2018-19 FY	2019-20 FY	2020-21 FY	Total
# colonies monitored	1530	5434	10,813	17,880
# new colonies treated	1243	924	543	2,710
# lesions treated	8683	15,424	12,954	40,670

Fig 3. Map identifying treatment sites and number of colonies treated at each site (as of June 14, 2021). The number of colonies monitored, new colonies treated, and lesions treated are shown for each fiscal year and as totals.

Fig 4. Map of Lower Keys sites surveyed in August, 2020 showing coral density, active SCTLD presence/absence, and evidence of previous SCTLD at 44 reefs. Note that 30 of these (68%), including very high density reefs, had active SCTLD.

Across all treated sites, the average diameter of treated colonies was 112 cm. Over 450 treated corals were larger than 200 cm in diameter. A total of 16 coral species were treated. The most treated species were *Orbicella faveolata* (37%), *Montastraea cavernosa* (20%), 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 more recently infected, 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 5).

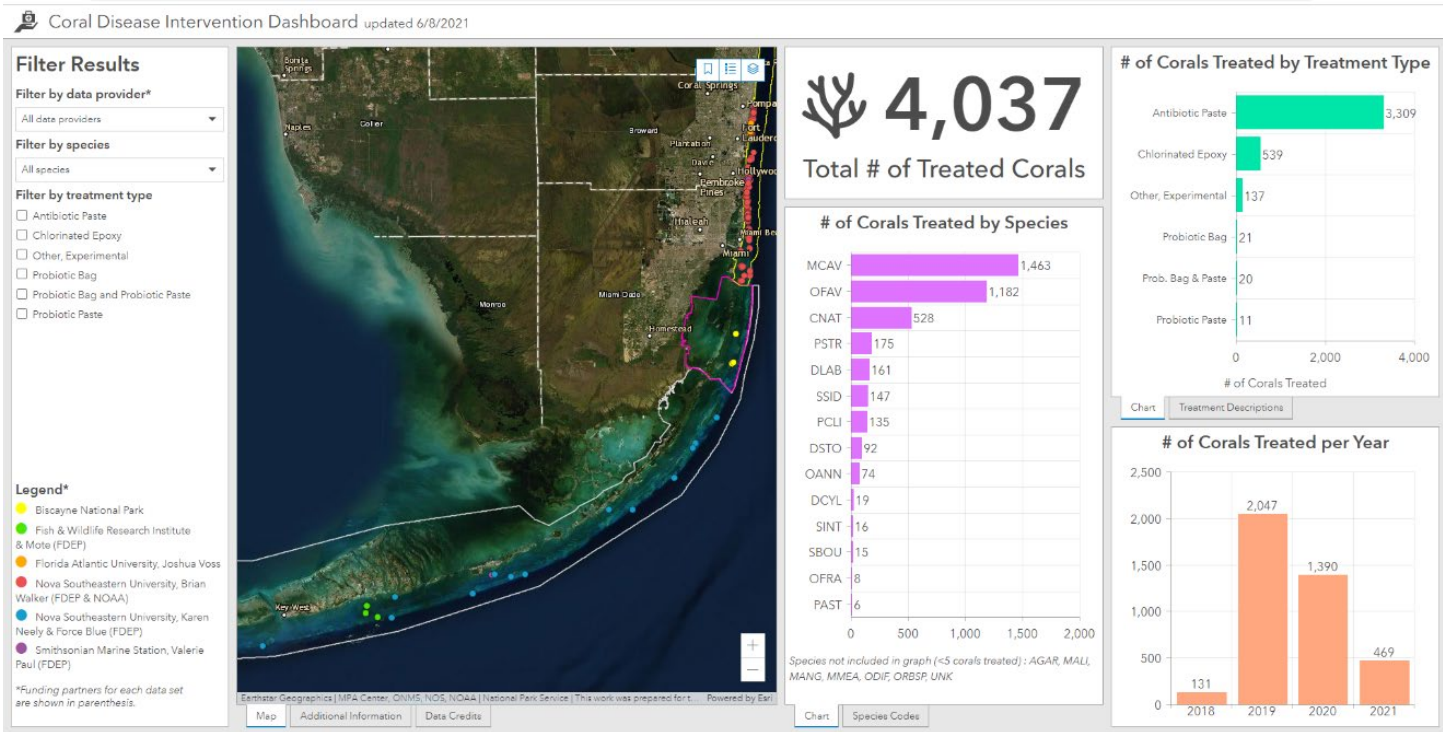


Fig 5. FWC Intervention dashboard showing locations, species, treatment types, treatment dates, and total number of corals treated by all practitioners. Accessed 6/14/2021.

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 followed 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. Between February 1, 2019 and May 17, 2021, a total of 574 citizen science reports were submitted for tagged corals in the Florida Keys. Of these, 81% 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. 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, citizen science efforts to date represented only 1% of monitoring efforts and thus cannot be relied upon to replace regularly scheduled monitoring trips.

Results

Mortality rates

Of the 1998 colonies treated with amoxicillin through 6/14/2021, 1882 (94%) are still alive. Mortality rates of chlorinated epoxy and experimental products approached 19% before subsequent treatments were switched to amoxicillin (Figure 6). 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).

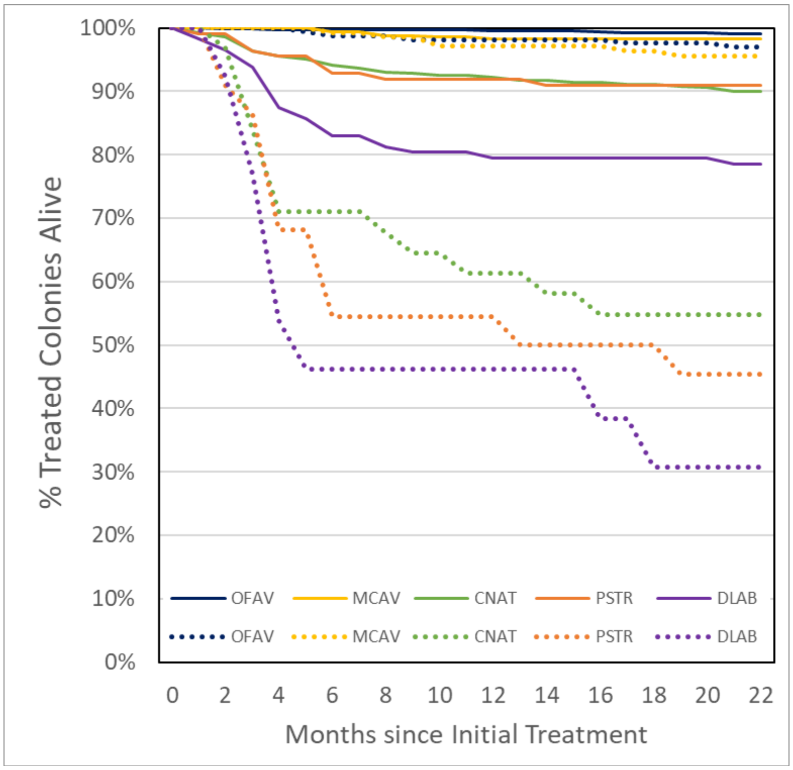


Fig 6. Percent of SCTLD colonies treated that remain alive up to 22 months after initial treatment. Solid lines represent colonies treated with amoxicillin + Base 2b. Dashed lines indicate colonies initially treated with chlorinated epoxy. All chlorinated epoxy treatments were switched to amoxicillin treatments within 4 months after initial treatment.

When these values are compared to amoxicillin-treated and initially chlorine-treated coral mortality rates by species, the ability of treatments to improve coral survival during SCTLD is clear (Figure 7).

Lesion level effectiveness

A total of 2,379 lesions from the initial chlorine and amoxicillin treatments (February – April 2019) were tracked using time series photographs taken during subsequent monitoring events. These lesions all had at least two monitoring events within the 140 days following treatments, and all were confirmed with photos to present as SCTLD. Success or failure of each lesion was assessed during each monitoring event during the subsequent 140 days, and the data were input into a mixed effects logistic regression analysis. Sample sizes were sufficient for analyses across five coral species: *Colpophyllia natans*, *Pseudodiploria strigosa*, *Diploria labyrinthiformis*, *Montastraea cavernosa*, and *Orbicella faveolata*. Full methods are available in Neely et al. (2021). At 109 days, modeled treatment effectiveness exceeded 95% among all tested species at Looe and the Upper Keys (Figure 8). Effectiveness was slightly lower for *D. labyrinthiformis* and *O. faveolata* at Sombrero, but still exceeded 75%. Across regions, amoxicillin treatments had similar effectiveness with the exception of treatments at Sombrero which were not as effective as those at Looe. However, the magnitude of differences was minor as effectiveness was high at both sites. As reported in the past, chlorinated epoxy treatments were ineffective; effectiveness was equally poor across all regions. Among species, chlorine-treated brain corals failed quickly, but boulder coral treatments continued to fail throughout the 109-day period. Full methods and analyses are available in Neely et al. (2021).

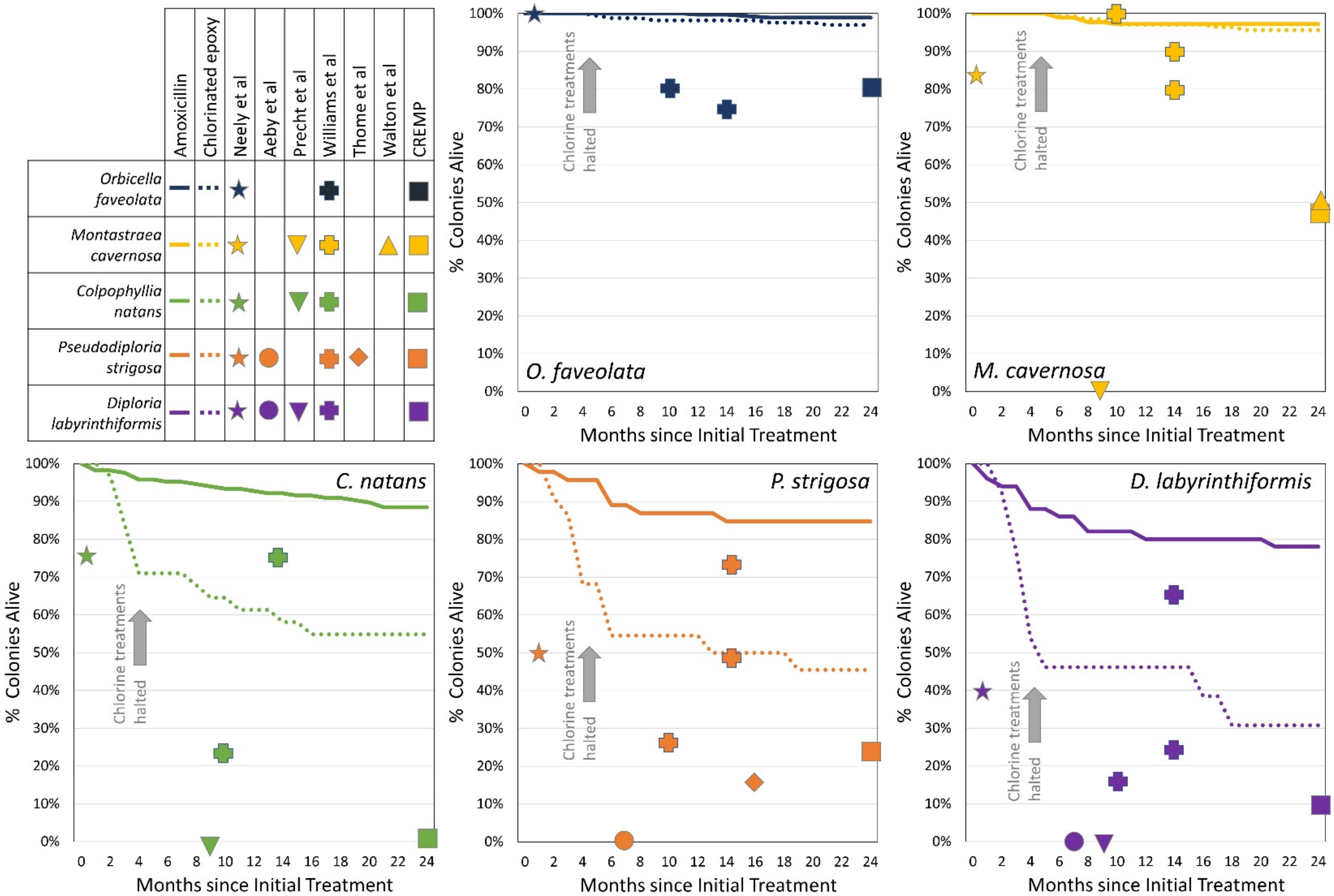


Fig 7. Survival curves for amoxicillin-treated colonies (solid line) and colonies initially treated by chlorinated epoxy (dashed line) by species. Shapes represent known mortality rates of species at different time points within published or publicly available studies.

Colony level effectiveness

Observations of reinfections following initial treatments identified the importance of colony-level monitoring to assess health over time. A total of 1664 amoxicillin-treated corals representing eight species (*C. natans*, *D. labyrinthiformis*, *D. stokesii*, *M. cavernosa*, *O. annularis*, *O. faveolata*, *P. strigosa*, and *S. siderea*) were visited a total of 9956 times ranging from 8 to 710 days after initial treatment and treated as needed during those monitoring events. We used a mixed effects logistic regression to calculate the probability of a colony showing no signs of disease at up to 24 months after treatment. For all regions, habitats, and species, the proportion of colonies with no disease signs increased with time (Figure 9). Two years after initial treatment, the modeled proportion of colonies with no disease exceeded 95% for most species at inshore sites. In contrast, modeled proportion of colonies with no disease was less than 80% for some species at offshore sites. Full methodologies and analyses are available in Neely et al. (2021).

Colony Resilience

Intervention teams throughout Florida have noted that some coral colonies respond better to treatment than others (Neely 2020b; Walker et al. 2020), while some corals remain unaffected by SCTLD even years after the arrival of disease. We used reinfection rates over time to categorize five species' responses to treatment and reinfection patterns. Colonies that had been monitored for a full year after initial treatment were identified as 1) never again exhibiting SCTLD, 2) having one subsequent SCTLD event but only within the first 5 months after the initial treatment, 3) having one subsequent SCTLD event, but at least 5 months after the initial treatment, 4) having two subsequent SCTLD events, but only within the first 5 months after the initial treatment, and 5) having two or more subsequent events including some at least five months after initial treatment. Corals that did not show reinfection or ones that only exhibited a single reinfection shortly after the initial treatment are

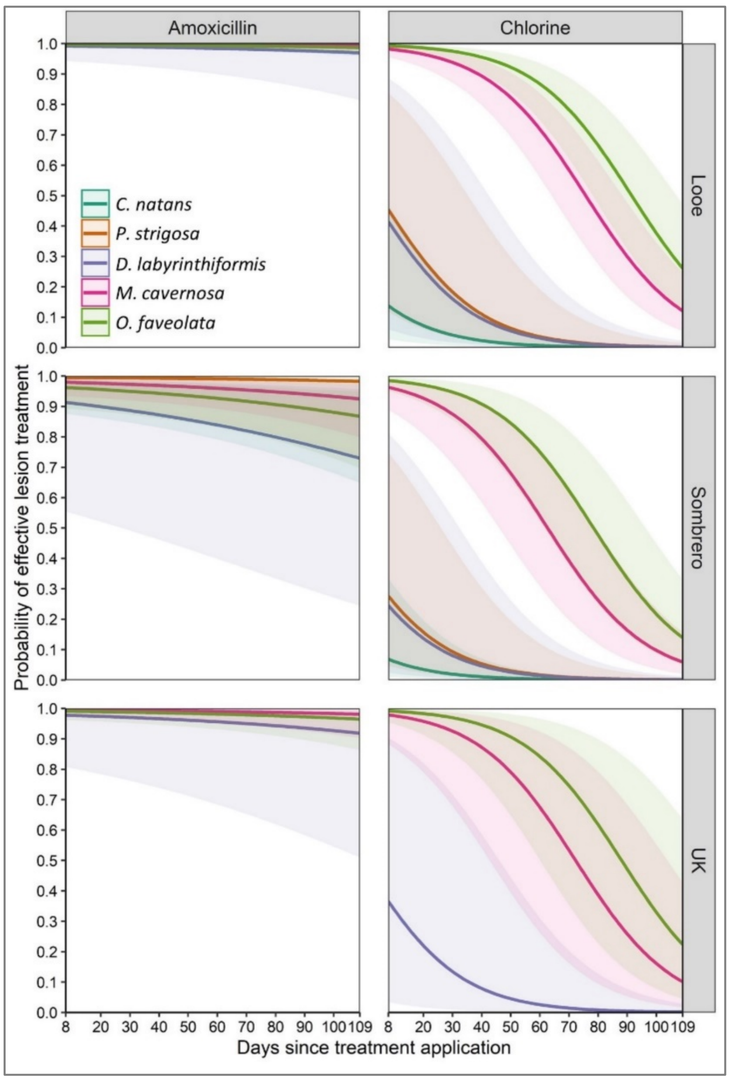


Fig 8. Mean predicted probability (+ 95% CI) of effective lesion treatment using amoxicillin and chlorinated epoxy, in relation to region, species, and the number of days since treatment.

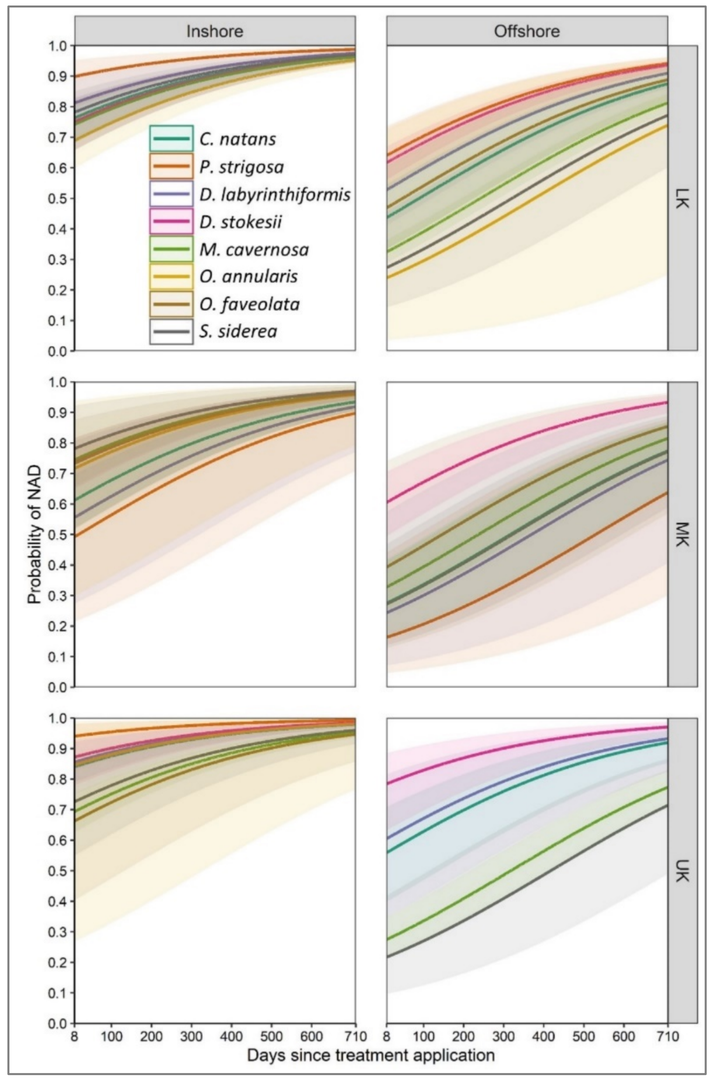


Fig 9. Mean predicted probability (+ 95% CI) of a coral exhibiting no active disease (NAD) over time since initial treatment. Corals represent only those initially treated with amoxicillin and retreated as necessary during monitoring events. Results are shown for eight species across two geographic variables: inshore/offshore and upper/middle/lower Keys (UK/MK/LK).

considered "highly treatable" as minimal effort is required to keep them disease free for at least the first year. The other categories become increasingly difficult to successfully save, as additional monitoring and treatment events are required.

The proportion of coral colonies within the different categories varied by species, but also by inshore/offshore habitat. Overall, less than 50% of corals of the five analyzed species fell within the "highly treatable" categories at offshore sites. In contrast, at inshore sites at least 70% of colonies across all five species were considered "highly treatable," either never reinfecting or requiring only one additional treatment within five months (Figure 10).

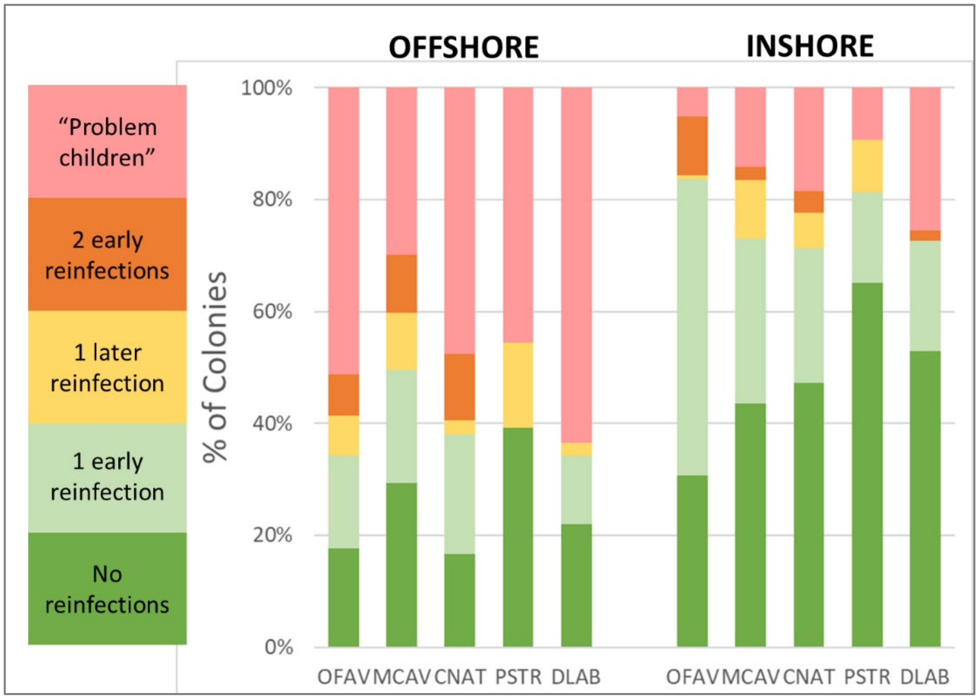


Fig 10. Resilience to reinfection of colonies at offshore and inshore treatment sites within 1 year after initial treatment. At both sites, fewer than 50% of colonies remained uninfected for a year after treatment. But at inshore sites, the proportion of colonies that either never reinfected or required only one subsequent treatment exceeded 75% for all five dominant treated species.

Temporal Infection Patterns

Infection rates, as evidenced by the proportion of new/reinfected colonies compared to all visited colonies, do not show temporal patterns at offshore sites (Figure 11). At all sites, the proportion of

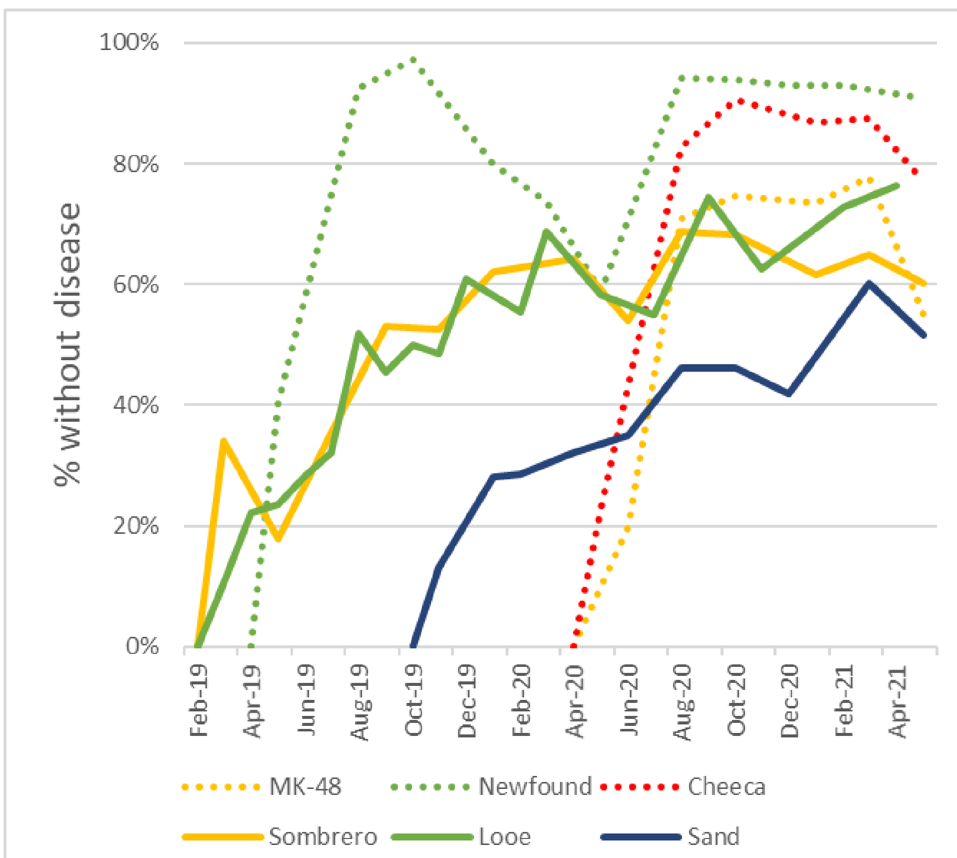


Fig 11. The percentage of previously-treated colonies showing no signs of disease from February 2019 – May 2021. At all sites, values start at 0 as all treated colonies exhibited disease. Over time, the proportion of colonies with no disease increases at all sites. Offshore sites (solid lines) have a lower proportion of non-diseased corals across time, while inshore sites respond more rapidly but suggest an increase in disease in spring 2020 and 2021.

previously-treated colonies without disease signs continues to increase with time, and without any seasonal fluctuations.

Inshore sites responded more quickly to disease treatment via decreased proportion of colonies with disease. These low prevalence values across previously-treated corals at inshore sites were maintained for many months. However, the inshore reef treated before spring 2020, and all three inshore reefs treated before spring 2021 exhibited increased diseased prevalence during those spring seasons (starting December 2020 and March 2021). We propose that the bleaching of these sites in summer 2019 and summer 2020, along with treatments leading into those seasons, curtailed or eliminated community spread. With little infection potential, the communities remained largely healthy for months after the bleaching event. Without the bleaching events at the offshore sites, infections remained low but consistent. We point out that the patterns of infections that correlate with the onset of the rainy season in southeast Florida (Walker et al. 2020) are not present at any of the Florida Keys sites.

Spatial Infection Patterns

As sites are monitored over time, observers have noticed that corals on each offshore reef appear to reinfect with no spatial pattern. Even at sites as large as Looe Key (0.8 km), infected corals are scattered throughout the reef with lots of uninfected corals interspersed (Figure 12a). In contrast, at inshore patch reefs, diseased corals appear to cluster (Figure 12b). We have not conducted analyses on these data, but they do suggest that reinfection or community spread may be acting differently between the habitats.

We here highlight some of the differences between inshore and offshore infections and examine some hypotheses to explain these differences.

Differences in disease patterns:

1. Inshore corals of all species are less likely to reinfect than their offshore counterparts.
2. More inshore corals are “highly treatable” in that they do not reinfect or only infect once after initial treatment during their first year.
3. Reinfections on inshore corals are clumped, while infected inshore corals are spread throughout the reef.

These differences all suggest that the disease/coral dynamic is different between inshore and offshore reefs. Differences 1 & 2, which speak to reinfection rates, could be interpreted as inshore corals/reefs being more resilient than their offshore counterparts. Certainly, inshore corals have a history of experiencing greater annual temperature fluctuations as well as higher turbidity and nutrient loads. An alternate hypothesis stems from inshore corals bleaching during the summers of 2019 and 2020 while offshore treatment sites did not. Bleaching is known to correlate with slowed/halted disease lesions (Meiling et al. 2020; Sharp et al. 2020). It may be that these bleaching events were the cause of the perceived resiliency of inshore corals by reducing their susceptibility to SCTLD during the warm-water months of each year.

However, the patchiness outlined in difference 3, which shows clumping of diseased corals at inshore sites but not at offshore sites, suggests the difference may also lie in reinfection patterns between habitat types. Using the Unified Reef Map (Florida Fish and Wildlife Research Institute 2014), we calculated the amount of reef habitat within a 4 kilometer radius of our inshore and offshore treatment sites (Figure 13). At offshore sites, between 4 – 9% of the nearby habitat was reef habitat. At inshore sites, less than 1% of the surrounding area was reef habitat. We suggest that the isolation of the inshore sites makes them less susceptible to infection from areas outside the treatment zone.

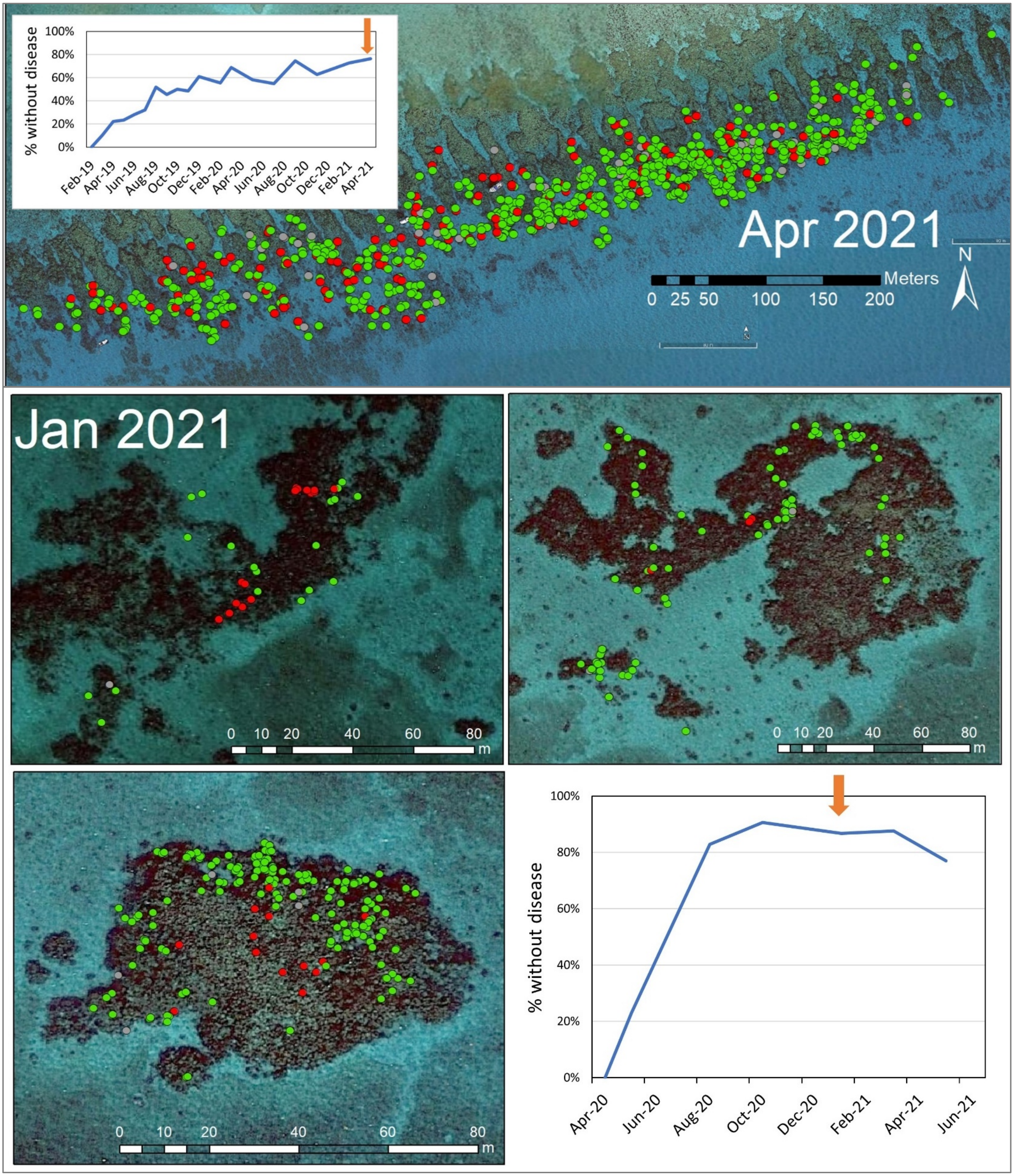


Fig 12. Distribution of previously-treated corals that are diseased (red) and non-diseased (green) in Spring 2021. At the offshore Looe Key (top), just over 20% of corals were diseased, and these were distributed throughout the reef. At the nearshore Cheeca Rocks (bottom), just over 10% of corals were diseased, but these were clumped at the reef sites such that diseased corals were generally near to other diseased corals.

It is probable that infections at any site are a combination of community spread (from nearby pathogen sources like other diseased corals) and from infections that have traveled from elsewhere. Based on the higher reinfection rates and the relatively continuous habitat of the forereef, we suggest that even if disease were eradicated within the treated zone, the surrounding habitat is continually providing new sources of infection. This would account for the constant and dispersed nature of reinfected corals. In contrast, we suggest that treatment of isolated patch reefs may curtail or eliminate community spread (likely assisted by summer bleaching events), thus creating a disease-free zone until pathogens breach the surrounding non-reef habitats and infect a colony(ies) at the inshore sites. This infection then triggers surrounding corals to become reinfected, resulting in the clumped distribution patterns.

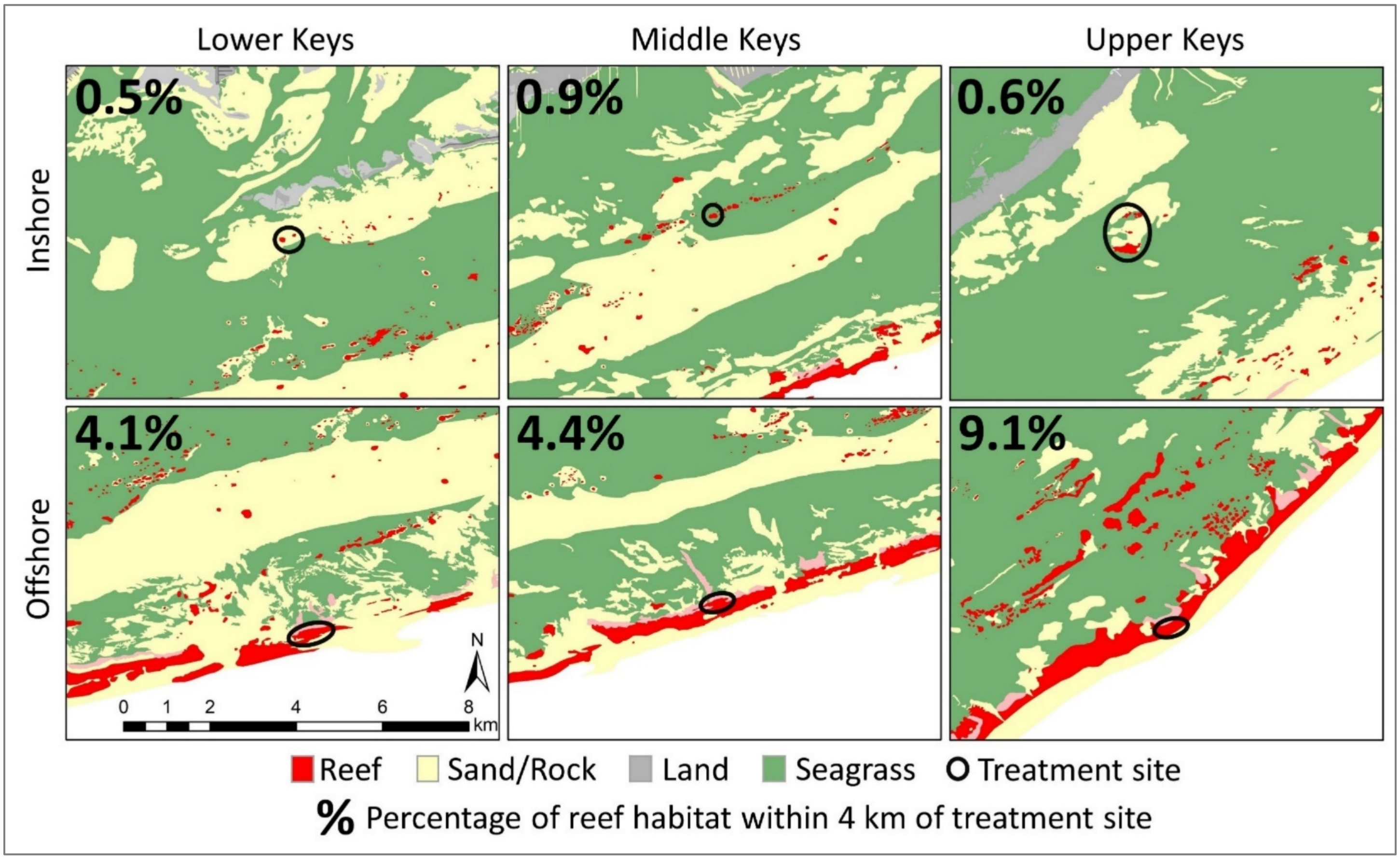


Fig 13. Habitat maps surrounding six treatment sites (inshore and offshore within each region) indicating the relative isolation of inshore sites from surrounding reef environments compared to offshore sites. Red indicates reef environment, and black circles indicate treatment sites. All maps are to the same scale. Percentage values in each map indicate the percentage of habitat within a 4 km radius of each site that is reef habitat. Habitat classifications are derived from the Florida Unified Reef Map

Recommendations

- There are many high-priority reefs with currently active SCTLD infection. It is possible to save these reefs, but only if immediate and directed action is taken to do so.
- Treatments could be scaled up through additional funding, additional partners, or through reduction of monitoring burden.
- Lesion-level treatment efficacy is well-documented throughout Florida and the Caribbean, within all disease zones, and over multiple years. Correctly assessing lesion-level efficacy is only possible through repeated photographic timeseries and is extremely time consuming. We recommend an immediate halt to any lesion-level monitoring, particularly field assessments due to inaccuracy as well as the time they take away from the primary goal of saving corals.
- Colony-level monitoring has highlighted interesting temporal and spatial patterns that are yielding information on SCTLD. This monitoring is time-consuming (although not as much as lesion-level monitoring), but does continue to provide data on long-term trends and health of treated corals. We recommend this as capacity allows.
- 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 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.
- 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.

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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

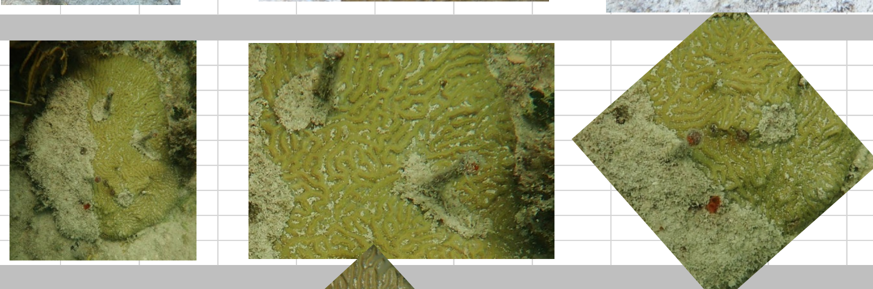
2019_04_22

1 Month

2019_05_21

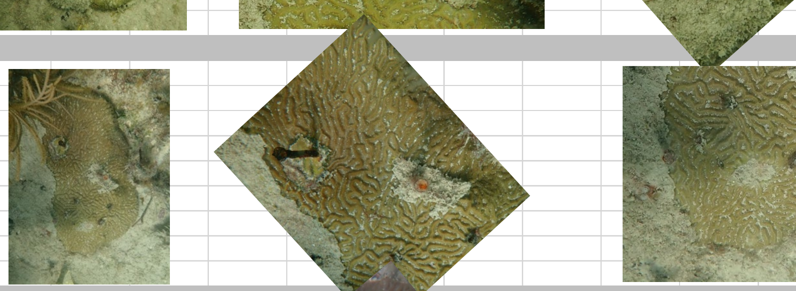
4 MONTH

2019_08_04



6 MONTH

2019_10_16



9 MONTH

2020_01_25

12 MONTH

2020_04_01