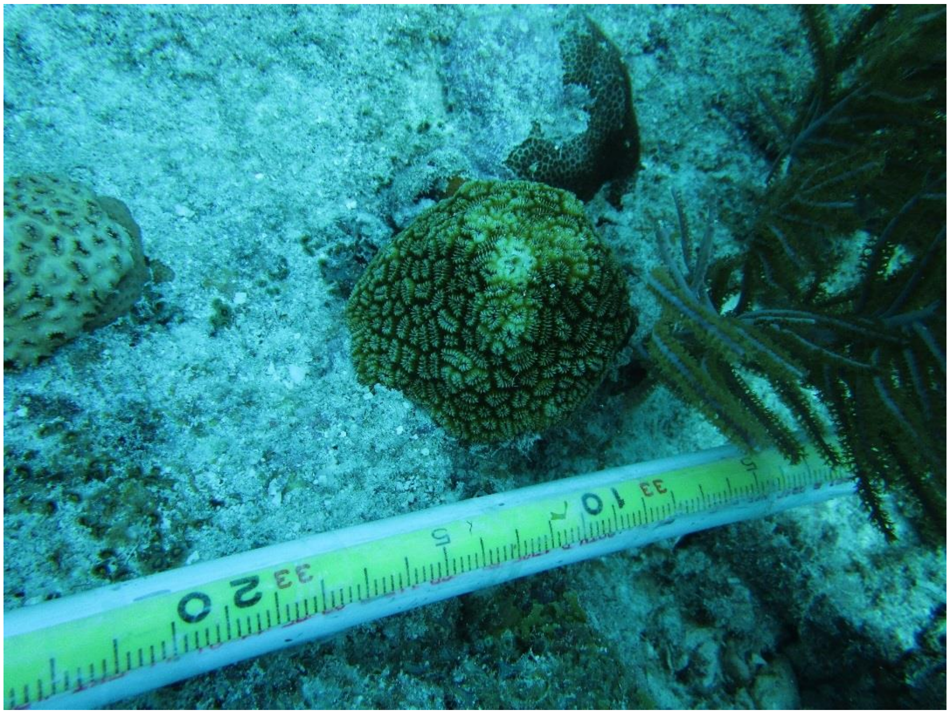

Investigating the ongoing coral disease outbreak in the Florida Keys: Evaluating its small-scale epidemiology and mitigation techniques

Final Report

Prepared for:

Florida Department of Environmental Protection,
Coral Reef Conservation Program

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Florida Fish & Wildlife Conservation Commission
Fish & Wildlife Research Institute

June 11, 2019

January 1, 2020
This is an update to the original report
Figure 13 has been corrected

Project Title:

Investigating the ongoing coral disease outbreak in the Florida Keys: Evaluating its small-scale epidemiology and mitigation techniques

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Project Period:

8 August 2018 – 30 June 2019

Report Period:

8 August 2018 – 9 June 2019

FWC Project #: 4406-18-F

This report should be cited as follows:

Sharp W.C. Maxwell, K.E. and Hunt JH. 2019. Investigating the ongoing coral disease outbreak in the Florida Keys: Evaluating its small-scale epidemiology and mitigation techniques. Final Report. Florida Department of Environmental Protection Award. Pp. 1-34.

This report was prepared for the Florida Department of Environmental Protection, Office of Resilience and Coastal Protection by The Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute. Funding was provided by the Florida Department of Environmental Protection PO B3AA73. The views, statements, findings, conclusions and recommendations expressed herein are those of the authors and do not necessarily reflect the views of the State of Florida or any of its sub-agencies.

Background:

Disease is recognized as a major cause of the progressive decline in reef-building corals that has contributed to the general decline in coral reef ecosystems worldwide. The first reports of coral disease in the Florida Keys emerged in the 1970's and have been documented with increasing frequency. Presently, the Florida Reef Tract is experiencing one of the most widespread and virulent disease outbreaks on record. This outbreak has resulted in the mortality of many thousands of colonies of at least 20 species of scleractinian coral, including primary reef builders and species listed as Threatened under the Endangered Species Act. First reported near Key Biscayne in 2014, this outbreak, recently described as "Stony Coral Tissue-Loss Disease" (SCTLD), has progressed southward along the Florida Reef Tract and by December 2017 had reached the vicinity of Coffins Patch Reef in the middle Florida Keys. The FWC began assessing its progression as it approached the reefs off Marathon in the middle Keys. In January 2018, the FWC initiated a DEP-funded project to collect tissue samples to identify the causative pathogens of this disease and establish sentinel monitoring sites to evaluate disease transmission rates and its small-scale epidemiology. We collected more than 700 tissue samples for histological evaluation and established seven sites (four sentinel sites and three sites that contained individually tagged corals) that tracked the fate of approximately 1,400 individual coral colonies at two-week intervals (Sharp and Maxwell, 2018).

By July 2018, the incidence of SCTLD at FWC's sentinel sites remained in the 'Epidemic Zone' (*sensu* Coral Disease Workshop, Key Largo, FL July 10-13, 2018), but we anticipated that its rate would slow to the point that these sites could be considered 'endemic' within the next year. Similar sentinel sites established by Mote Marine Laboratory (MML) in the lower Florida Keys remained disease-free in July 2018 (Erinn Muller, pers. comm.), though SCTLD had been reported in the region.

Given the location of FWC's and MML's sentinel sites, we were initially funded to continue monitoring at the FWC middle Keys sentinel sites, and once the prevalence of SCTLD had decreased to the point that they were within the endemic zone, conduct experimental coral outplanting. We would also test colony-specific intervention techniques at MML's lower Keys sentinel sites once SCTLD was detected. During the course of the study, the experimental outplanting deliverable was removed in order to extend the capacity to conduct intervention.

The original objectives of this project were:

i) Conduct active intervention trials to treat diseased coral colonies to reduce the rate of SCTLD progression at localized sites;

ii) Continue collecting small-scale epidemiology information from the existing FWC sentinel sites and develop a model that examines the small-scale spatio-temporal dynamics of SCTLD progression to identify when these sites have entered the 'endemic 'stage'. Once that is determined;

iii) Outplant coral colonies onto previously established FWC's sentinel sites.

Methods

Task 1: Conduct active coral intervention activities to reduce the rate of disease progression.

In May 2018, Mote Marine Laboratory (MML) established three sentinel site locations in the lower Keys, each composed of three 10 x 10m plots to assess disease spatial epidemiology to complement FWC's middle Keys sentinel sites described below (Figure 1). When the project was conceived, these sites were in the "Pre-Invasion Zone" (*i.e.*, no active SCTLD present). MML approached the FWC and offered the use of those sentinel site locations to conduct active disease intervention trials (Erinn Muller, MML). This intervention activity was conceived to determine the feasibility of altering location-specific disease prevalence through the repeated direct treatment of infected colonies at that location.

We selected one plot at each of the three sentinel site locations (near-shore, mid-channel, and off-shore) to field test intervention techniques discussed at the Coral Disease Workshop (Key Largo, FL July 10-13, 2018). The intent was that once SCTLD was observed on any of the selected plots (MML began monitoring them during July 2018), we would begin intervention trials. This would occur in a tiered fashion, beginning with the least invasive method. We decided that the initial treatment method would entail covering each disease lesion as they appeared with a topical barrier composed of amoxicillin impregnated within shea butter, then covered with modeling clay *(see* Appendix 1 for protocol). Prior to the treatment, the selected diseased colony was photographed, and the proportion of the colony affected by SCTLD (*i.e*., not apparently healthy tissue, older, non-tissue-loss disease related mortality) was recorded. If the disease at a colony was observed to have progressed after the topical barrier treatment, we would then affect the more invasive "trenching" technique. This involved cutting a groove through the coral tissue several centimeters into the skeleton in a perimeter around the active lesions, then filling it with a topical barrier of the amoxicillin/Shea butter. If a disease lesion was located along the edge of a colony and had breached the barriers, we would amputate the section of the colony and bring it back to the vessel for safe disposal. Once SCTLD was observed, we would revisit each plot as frequently as logistically feasible, with an initial goal to treat the sites twice weekly. As this work proceeded, it became evident that weekly visits were sufficient to treat affected coral colonies. However, after observing a rapid increase in disease incidence during April 2019, we once again began re-visiting sites twice weekly until intervention efforts ceased on May 13, 2019.

Throughout the course of this work, MML personnel conducted regular surveys at the treated and untreated sites. These surveys were intended to provide the site-level data for us to evaluate the effectiveness of treating individual coral colonies. MML graciously provided these monitoring data to the FWC to evaluate this intervention effort.

Task 2: Continue collecting small-scale epidemiology information at the existing sentinel sites and develop a model to examine the small-scale spatio-temporal dynamics of disease.

During January 2018, the FWC established four sentinel sites off Marathon (Figure 1) to evaluate the spatial epidemiology of SCTLD and assess species-specific disease progression rates. In brief, two locations were located on offshore bank reef habitat and two were located within near-shore patch reef habitat. At each of the four selected sites, we established two plots (either 5 x 5m, 7 x 7m, or 10 x 10m area, depending of the coral density at the location) and measured and mapped the location of each coral colony within the plots. Every two weeks, divers surveyed the site for the presence of SCTLD. If disease was observed on a colony, a photograph of the colony was taken, and the proportion of the colony affected by SCTLD was recorded. (Figure 2 and *see* Appendix 2 for detailed monitoring protocols). A summary of this monitoring effort through early June 2018 was provided to the DEP (Sharp and Maxwell 2018).

Beginning in August 2018, we reduced our sampling frequency to monthly, which we deemed sufficient to determine when the rate of disease progression had slowed to the point where SCTLD at these sites could be deemed 'endemic'. For the purposes of this study, we defined endemic as that point at which nearly all the coral colonies of the most highly susceptible SCTLD-susceptible species had succumbed and the disease progression in the remaining colonies slowed or stopped for several months. This report includes results of monthly monitoring through May 2019, although those efforts are ongoing.

Task 3: Evaluate the potential for coral community recovery at endemic sentinel sites by susceptibility and survival of experimental coral outplants.

At the outset of this project, the FWC sentinel sites were determined to be ideal sites to test the susceptibility of restoration coral outplants while in the endemic zone and evaluate additional parameters related to restoration while providing key information about the disease outbreak. Such information would include: whether the pathogen is still present, if nursery-reared colonies of the susceptible species are vulnerable to disease, whether the introduction of new colonies exacerbates disease, and when and where future restoration activities might have the most success. The intent was that once it was determined that the SCTLD incidence had sufficiently decreased at the four sentinel sites, we would initiate a coral restoration effort using micro-fragmented coral colonies obtained from MML and larger colonies from FWC's *in situ* nursery. We previously consulted with Erinn Muller (Program Manager and Science Director of the Elizabeth Moore International Center for Coral Reef Research and Restoration) regarding the use of MML corals for restoration and she agreed that MML would provide corals for this purpose. However, by agreement between the FWC and DEP, this task was removed as a deliverable for this project so that effort could be focused on the work associated with Task 1.

<u>Results</u>:

Task 1: Conduct active coral intervention activities to reduce the rate of disease progression.

All coral colonies at the three lower Keys intervention plots were mapped on August 7, 2018 as FWC staff had previously done when establishing the middle Keys sentinel sites earlier in 2018 (*see* Appendix 1). In all, 1,005 colonies encompassing 19 species were identified (Figure 3). Beginning on August 22, 2018 sites were visited at approximately one-week intervals until SCTLD was observed. We believed that SCTLD was first observed at "Porky's Reef", the most offshore of the intervention sites in late October 2018, and intervention efforts began (Figure 4). Accordingly, we began twice-weekly monitoring at all three sites. By mid-November 2018, we had begun treating coral colonies suspected of being infected with SCTLD at all three sites.

MML disease monitoring efforts across both our intervention and the control plots revealed a noticeable difference in the incidence of disease between the treatments. The incidence of disease at the control plots was much lower relative to the FWC intervention plots and their monitoring did not pick up SCTLD-affected corals until mid-November at the offshore sites, and late November at the mid-channel sites. As of January 18, 2019, no disease had been observed at the inshore control plots (Figure 5). We believe this discrepancy was due to our initial approach to intervention. Based on our experience with the middle Keys sentinel sites during 2018, we anticipated that once disease occurred on the intervention plots, the rate of progression would overwhelm our efforts. Consequently, our approach was to treat any colony we believed was exhibiting the early manifestations of SCTLD. However, the differences between the treatment and control sites suggested that we had almost certainly been treating colonies that were not actually affected by SCTLD. Indeed, examining the coral species that we had treated at the inshore sites revealed many were *Solenastrea bournoni*, *Stephanocoenia intersepta*, and *Siderastrea siderea* (Figure 6). Although these species are susceptible to SCTLD, they typically do not exhibit infection until after the maze, brain, and star corals species show signs of infection. *S. siderea* can be difficult to assess for SCTLD, particularly early in the infected stage.

Examining our time series of treated coral colonies focusing on a subset of the more SCTLD-susceptible species at the intervention sites (*Colphyllia natans, Diploria labyrinthiformis, Dichocoenia stokesii, Eusmillia fastigiata, Montrastraea cavernosa, Meandrina meandrites, Orbicella annularis, O. faveolata, Pseudodiploria clivosa* and *P. strigosa*) suggests, as MML observed, that SCTLD began to affect the offshore and mid-channel plots during November 2018, but had not yet affected the nearshore site. The number of colonies that were exhibiting signs of SCTLD and treated increased progressively at those two sites through January 2019. In contrast, the number of colonies treated for SCTLD at the inshore site did not show similar progression, and we believe those colonies that had been treated were likely not yet affected by SCTLD (Figure 7).

In late January 2019, we reduced our monitoring effort to weekly site visits. However, by April 2019 the number of coral colonies requiring treatment began to rapidly increase at the nearshore site, and it was clear that the disease progression was reaching the epidemic stage across the sampling area. Consequently, we mounted a concerted effort to treat all affected colonies and began visiting the plots twice a week. During the last month of the project the number of new coral colonies infected increased rapidly despite the increased effort. By May, more than half of the more susceptible coral species (listed above) across the three plots were infected (Figure 8). Moreover, many colonies, once treated with one or more topical "patches" during a site visit, required repeated patches of new lesions presenting on different areas of the colony (Figure 9 and 10). In the most extreme case, a large *O. faveolata* had 199 separate lesions that required treatments by the time the field trials ended (Figure 11). Also, by the cessation of field activities approximately 45% of those colonies that were treated with the topical patch had required further treatment *via* the more invasive trenching technique.

MML's monitoring of our intervention sites and associated control sites revealed differences in the proportion of coral colonies affected by SCTLD. Figures 12, 13, and 14 compare a time series of the mean (±1 SE) colony-specific proportion of SCTLD-related mortality of the more SCTLD-susceptible coral species (listed above) between the treatment and control plots. We note the three treatment plots exhibited a lower mean proportion of mortality.

This encouraging result must be viewed with the lens of the extensive manpower necessary to achieve this result on this limited spatial scale. Recognizing that such an effort is not sustainable, this intervention technique is not feasible to meaningfully affect the disease progression in areas of the reef that are experiencing epidemic rates of SCTLD infection.

Task 2: Continue collecting small-scale epidemiology information at the existing sentinel sites and develop a model to examine the small-scale spatio-temporal dynamics of disease.

As mentioned above, bi-monthly sampling was conducted at each of the sentinel sites from January to mid-August 2018. At that time, it became apparent that the progression of the disease had slowed (Sharp *et al*. 2018). Therefore, after completing the mid-August monitoring, monitoring frequency was reduced to once monthly.

The progression of disease and new infections on previously uninfected colonies slowed or stopped beginning in mid-July and remained similar through November (Sharp *et al* 2018). However, by the December 2018 monitoring period, it was evident that the percentage of new colonies infected began to increase at the two offshore sites, Grouper and Sombrero Reef (Figure 15). These patterns in the progression of the disease at all four sites is evident when the data are viewed as the mean percentage of live tissue per colony over the time series (Figure 16). Despite the ongoing disease activity, it remained low enough that monthly monitoring remained sufficient to track and evaluate its progress. The FWC remains committed to monitoring these four sites to track the persistence of SCTLD in the area.

Figure 17 summarizes the status of coral colonies by species across the four sentinel sites (*i.e.*, colonies unaffected and affected by SCTLD and dead colonies). Most species were affected to varying extents, and the relative species-specific susceptibility was consistent with previous observations of SCTLD-affected coral communities, with maze, brain, and star coral species being particularly impacted.

Progress has been made toward refining a model to evaluate the small-scale spatial epidemiology of SCTLD using information collected from the middle Keys sentinel site monitoring. To build this model, we first calculated the Euclidian distance of each pair of coral colonies such that the distance of a colony from every other colony within each of the eight experimental plots was identified (Sharp *et al*. 2018a). Using these data, we then developed a Bayesian dynamic multi-state model. This model estimated the probability of each coral colony changing conditions (*i.e.*, uninfected, infected, dead) between monitoring periods based on the condition and distance to its nearest neighbor. The model makes a simplifying assumption that once infected, a coral colony did not become uninfected.

The results of the model are summarized in Sharp *et al*. 2018b. That model incorporated sentinel site monitoring data through mid-July 2018, just prior to the decrease in disease prevalence detailed above. At that point, the model detected little evidence that there was a positive relationship between probability of infection of one colony based on the condition of its neighboring colony and there was less evidence of a relationship between the condition of a colony and the distance to its nearest neighbor.

The model was updated to include data through May 2019. A logistic regression with two predictor variables was used to evaluate the association of the status of a coral colony at a given sampling period to its nearest neighbor (i.e., uninfected, infected, dead) and the distance to that nearest neighbor. The results of this most recent model remain almost identical to its previous iteration. Results are summarized as the odds ratios (± 95% C.I.) calculated from the model's parameter estimates (Figure 18). In simple terms, the Odds Ratio can be thought of as is "a measure of association that compares the odds of an individual becoming diseased after being exposed to the disease vs. the odds of an unexposed individual becoming diseased." Again, the model detected little evidence that there was a positive relationship between the condition of a colony (uninfected, infected, dead) based on the condition or distance of its neighboring colony. These results suggest that as SCTLD moved into the middle Florida Keys in 2018, it was affecting the reef tract at a larger spatial scale than we could detect through the within-site monitoring. The absence of a nearest neighbor effect was further supported by our observations of SCTLD first occurring across our sentinel sites simultaneously in late February 2018.

Task 3: Evaluate the potential for coral community recovery at endemic sentinel sites by susceptibility and survival of experimental coral outplants.

Because the information that was collected through the intervention efforts associated with Task 1 was the most urgent priority, it necessitated a more protracted effort than originally estimated. Consequently, it was decided by agreement that the FWC and DEP funding for Task 3 would be shifted to Task 1. It is also worth noting that we had anticipated that the disease would have moved through the intervention plots such that they would be considered within the endemic zone, but by the end of this project the numbers of colonies becoming infected with SCTLD was continuing to increase at those plots.

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Figure 1. Map showing locations of the Florida Fish and Wildlife Conservation Commission's (middle Keys) and Mote Marine Laboratory's (lower Keys) sentinel sites.

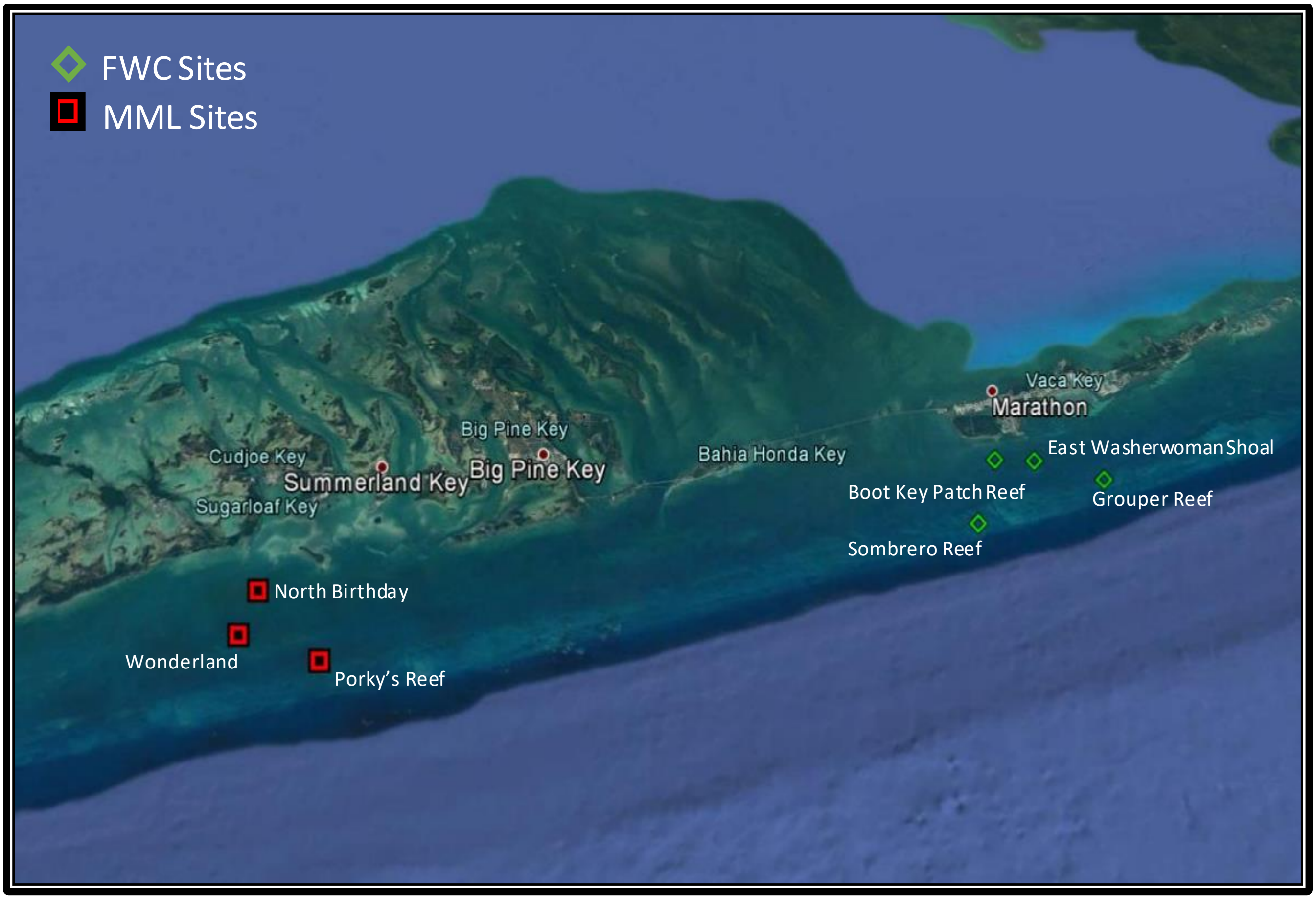


Figure 2. FWC researcher recording the proportion of a coral colony infected by SCTLD on one of FWC's sentinel monitoring sites.

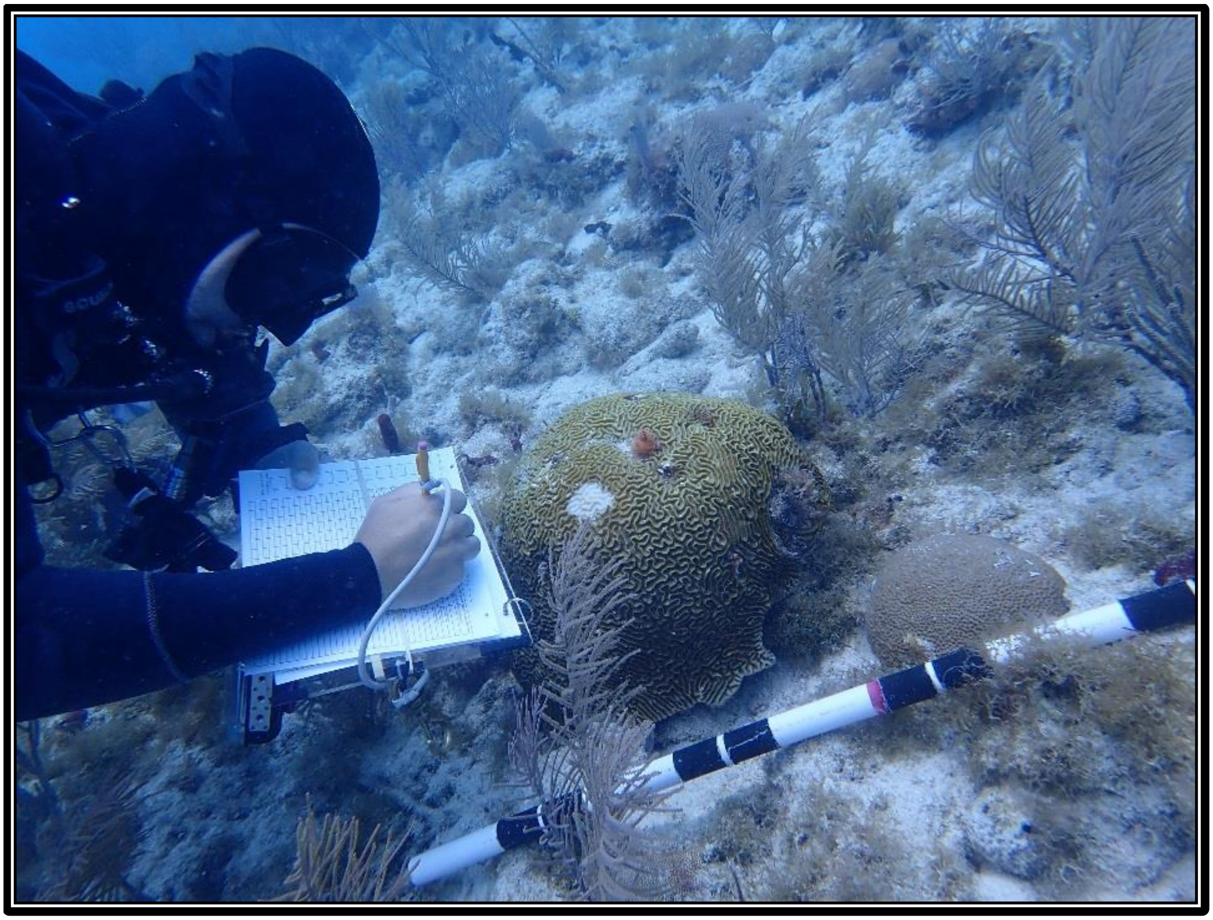


Figure 3. Number of colonies identified and mapped at each of Mote Marine Laboratory's lower Keys sentinel plots selected by the FWC for SCTLD intervention trials.

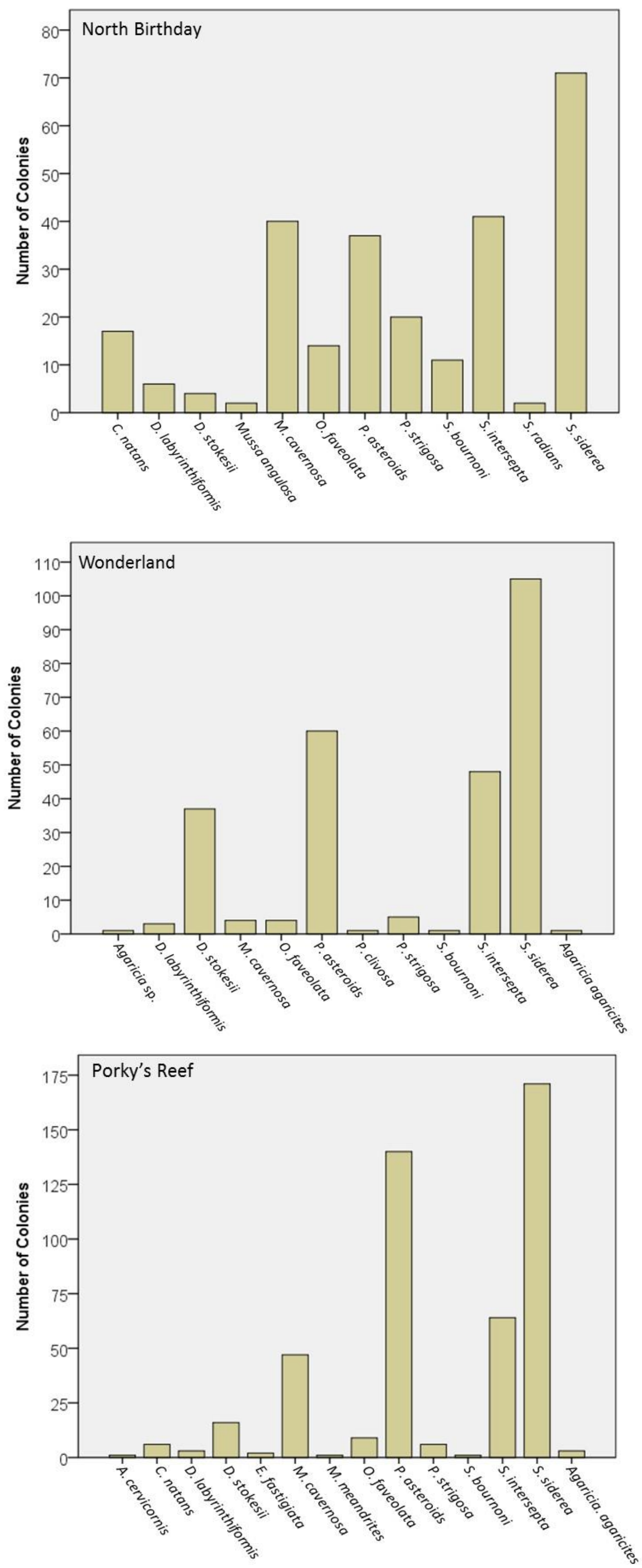


Figure 4. (A) FWC researcher inspecting a coral colony for SCTLD and (B) a coral colony that was initially treated with a topical barrier of Shea butter and amoxicillin and subsequently by trenching. Trenching entails cutting a drove into the colony's skeleton and inserting the Shea butter/amoxicillin mixture.

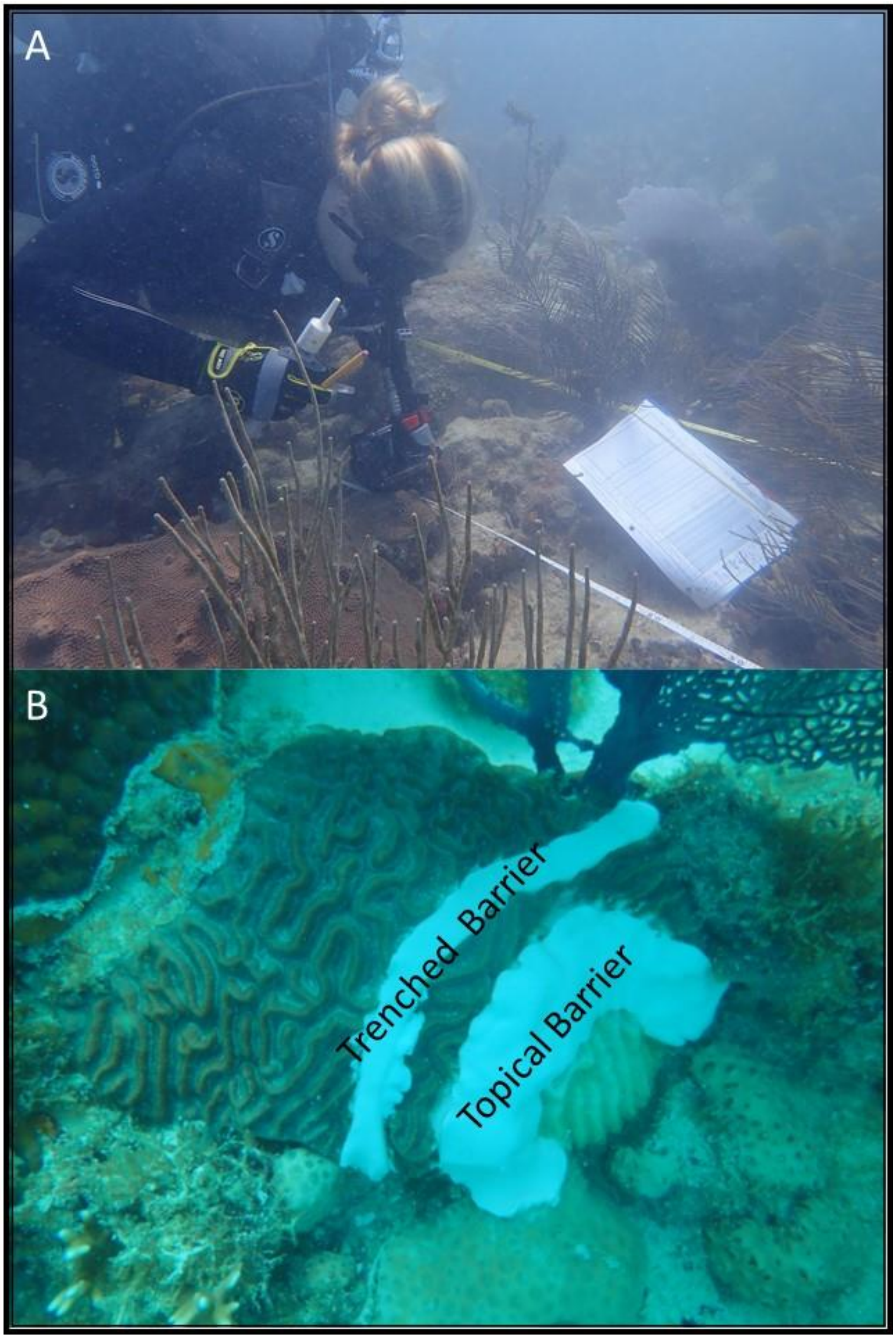
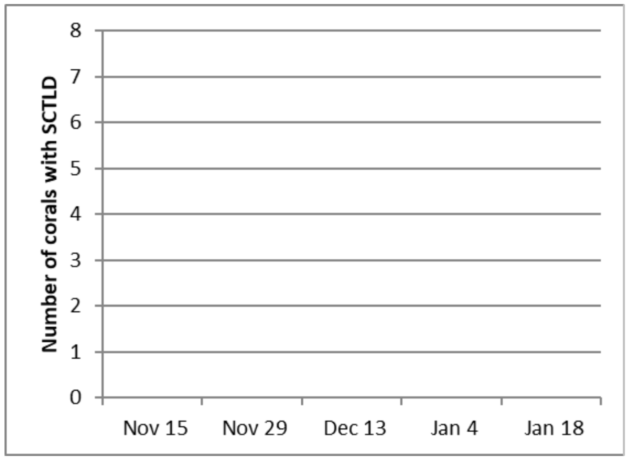
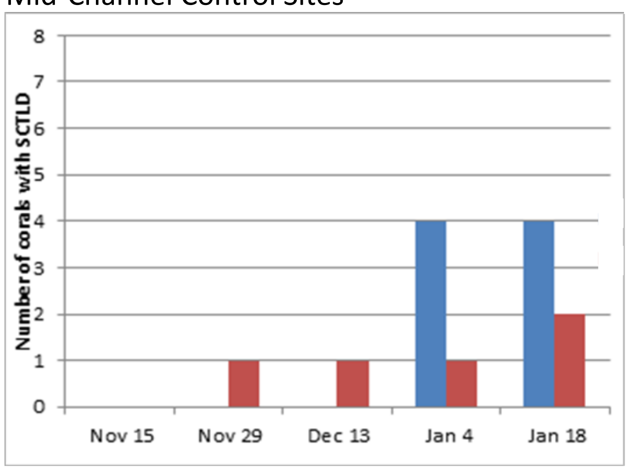


Figure 5. Number of coral colonies exhibiting SCTLD on the two control plots across the three lower Keys sites monitored by MML from mid-November 2018 through mid-January 2019. The red and blue bars differentiate the two plots.

Inshore Control Sites



Mid-Channel Control Sites



Offshore Control Sites

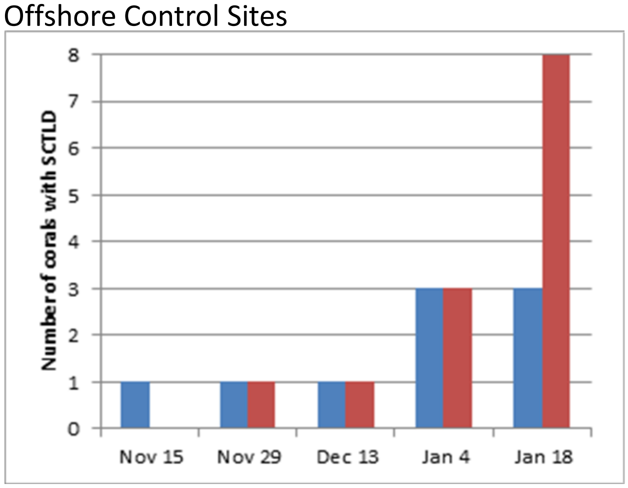


Figure 6. Number of treated and untreated colonies at the North Birthday (nearshore) intervention plot by mid-January 2019.

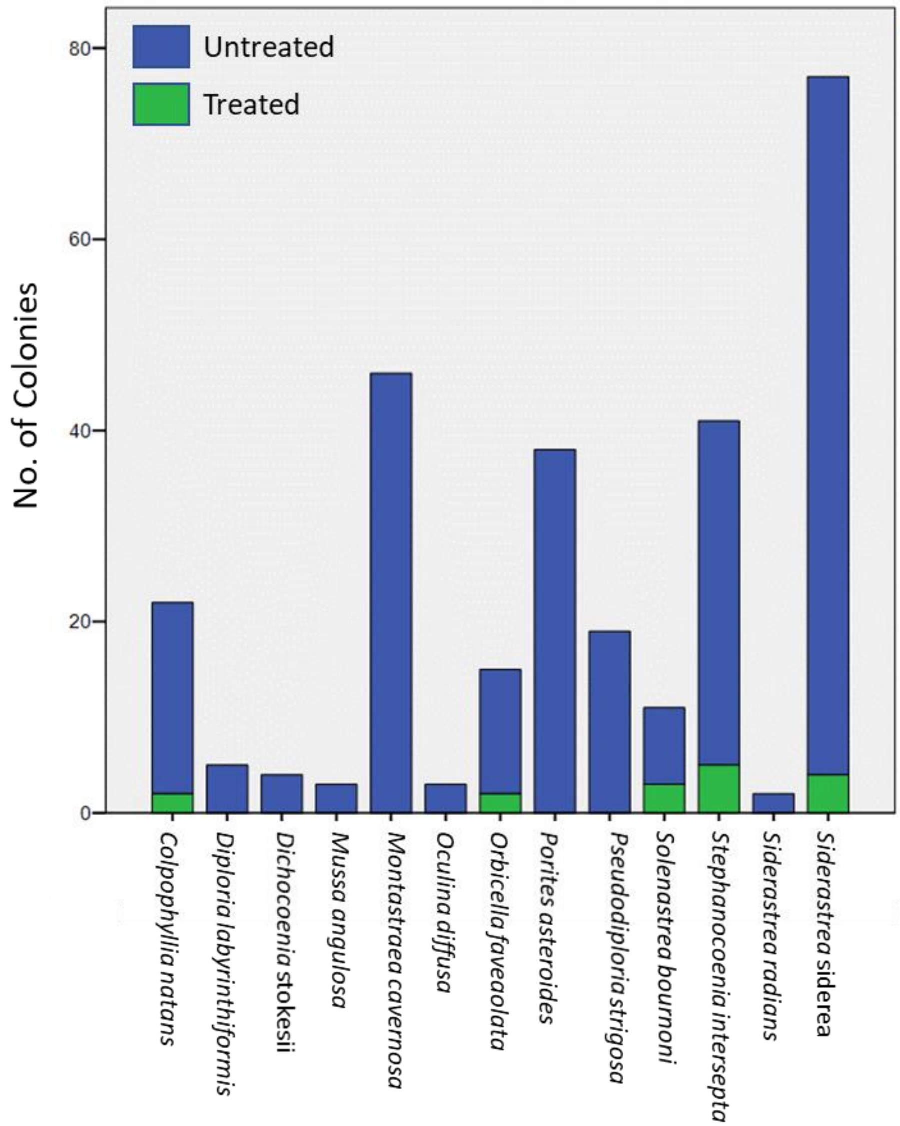


Figure 7. Time series summarizing the percentage of coral colonies that have exhibited SCTLD and required intervention from late August through late January. A= the North Birthday (nearshore) site; B= the Wonderland (mid-channel) site; C = the Porky's Reef (offshore) site.

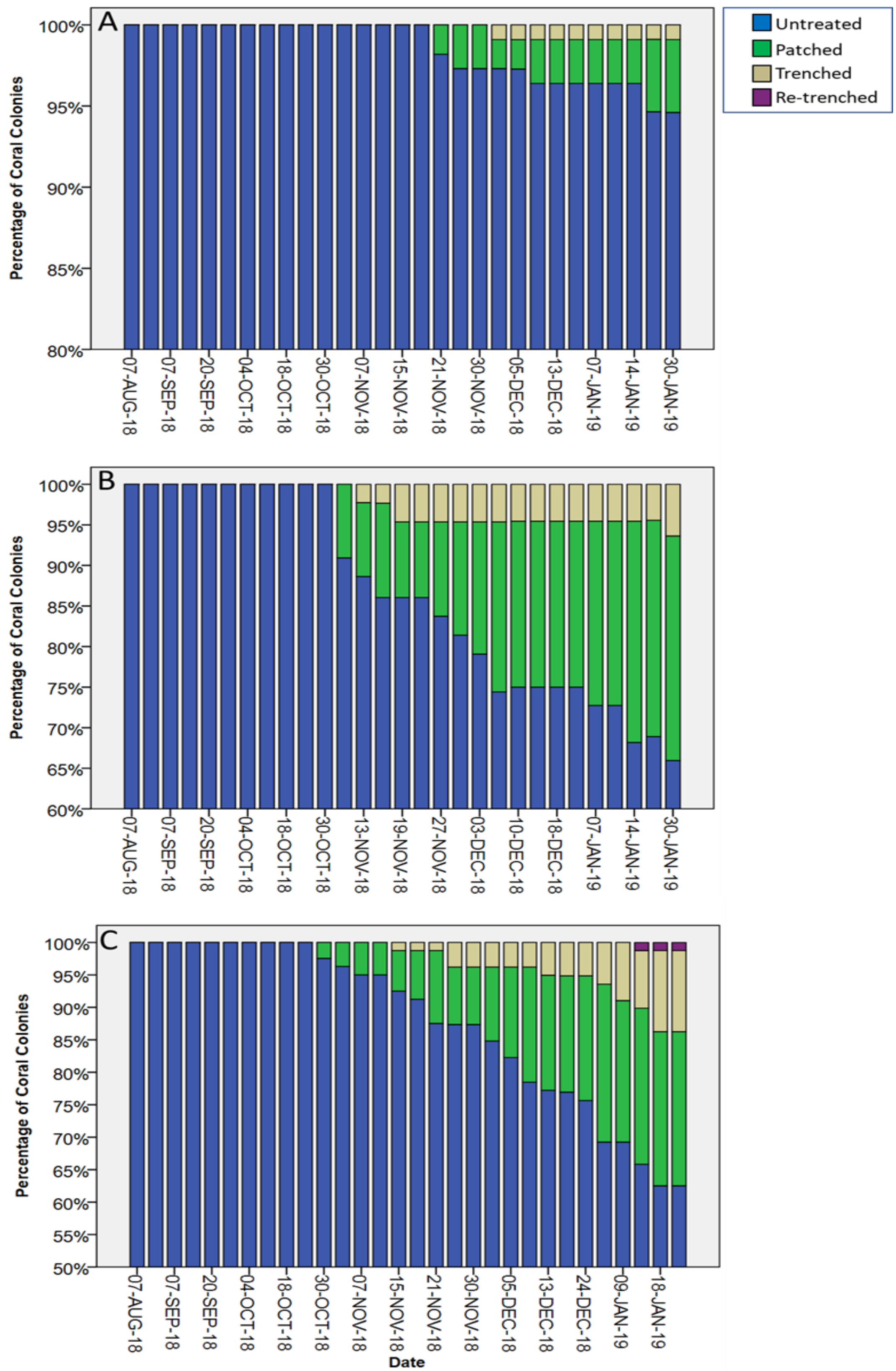


Figure 8. Time series summarizing the percentage of coral colonies that have exhibited SCTLD and required intervention from late August 2018 through May 2019. A= the North Birthday (nearshore) site; B= the Wonderland (mid-channel) site; C = the Porky's Reef (offshore) site.

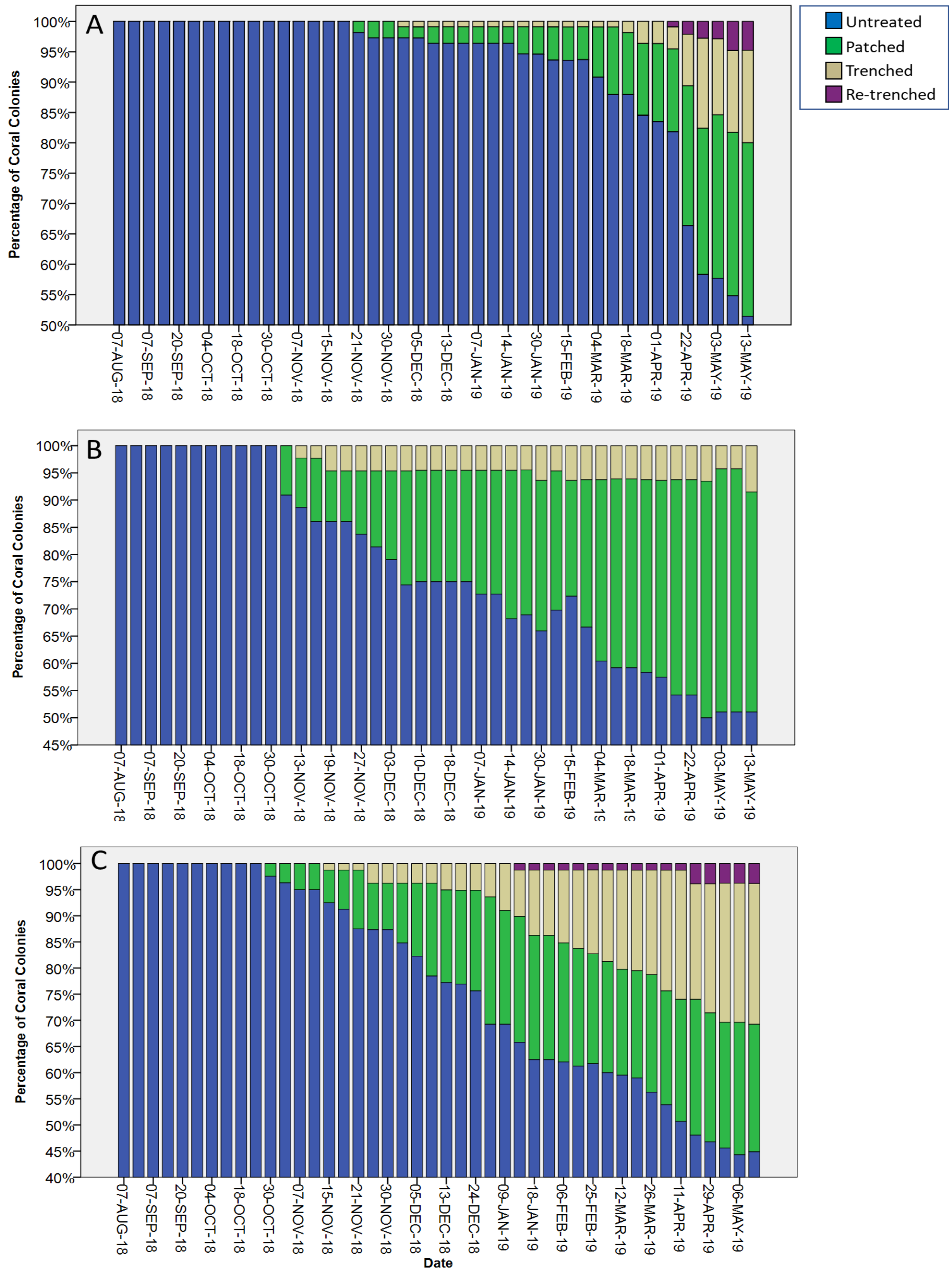


Figure 9. Summary of the number of coral colonies that have required additional patches of amoxicillin on new SCTLD lesions. These data only include colonies where the patches have not failed, necessitating trenching.

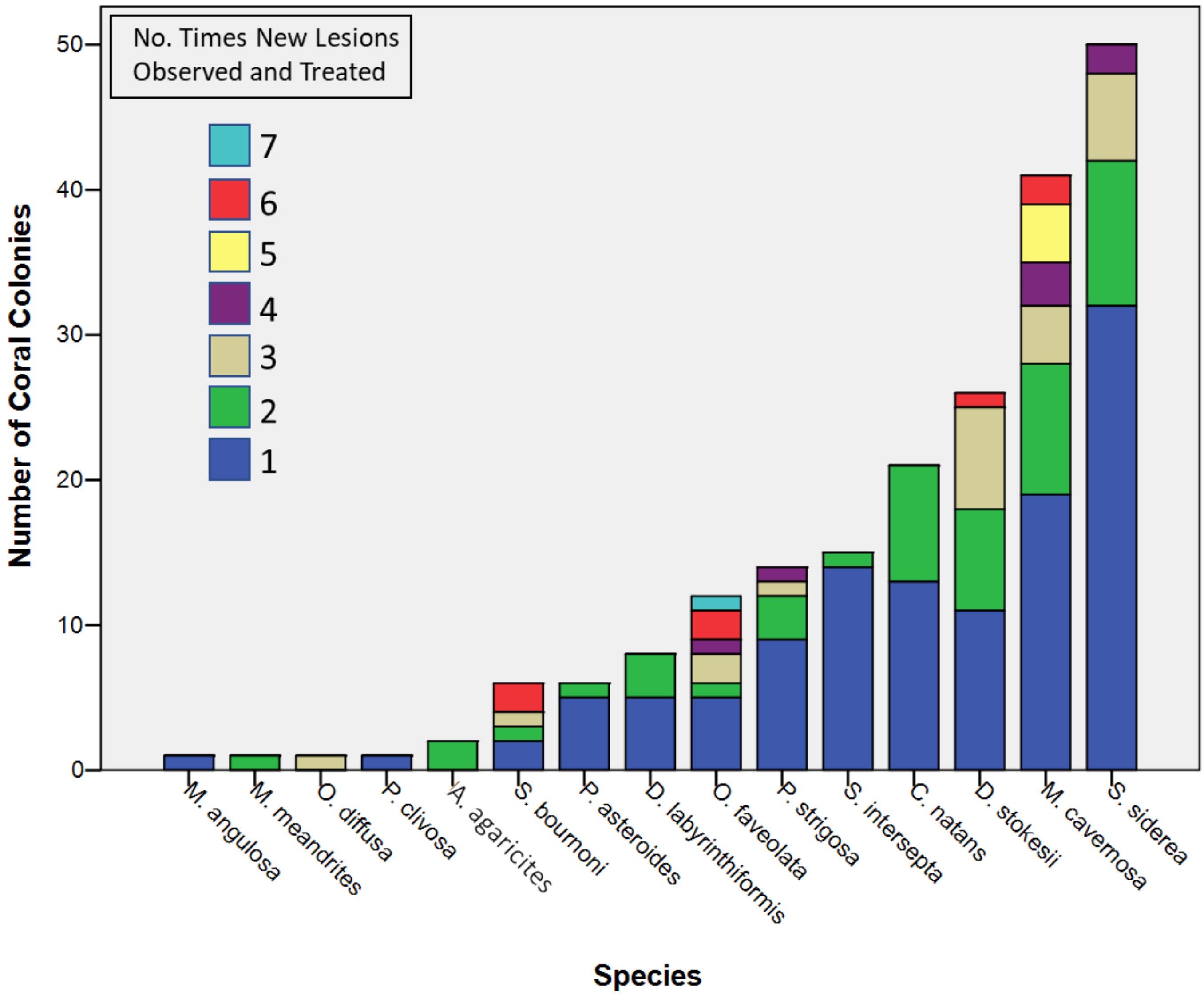


Figure 10. Photos of coral colonies at MML/FWC lower Keys intervention plots showing multiple topical patches that have been applied to SCTLD lesions.

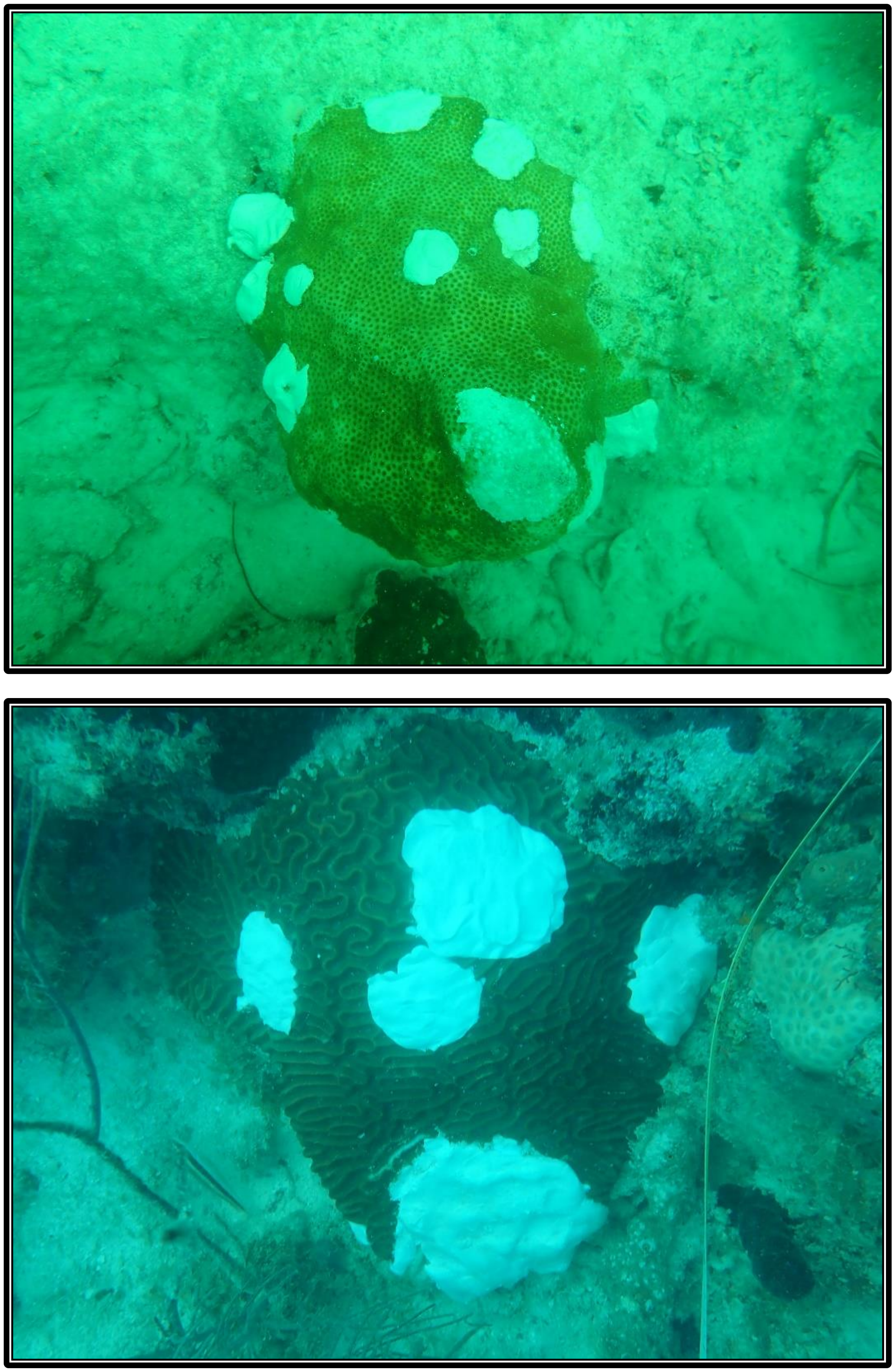


Figure 11. *Orbicella faveolata* colony at one of the FWC/MML lower Keys intervention sites showing several topical patches covering SCTLD lesions. By the end of the project, 199 separate patches had been applied to this colony. Several yet-to-be treated lesions can be seen in this photograph.

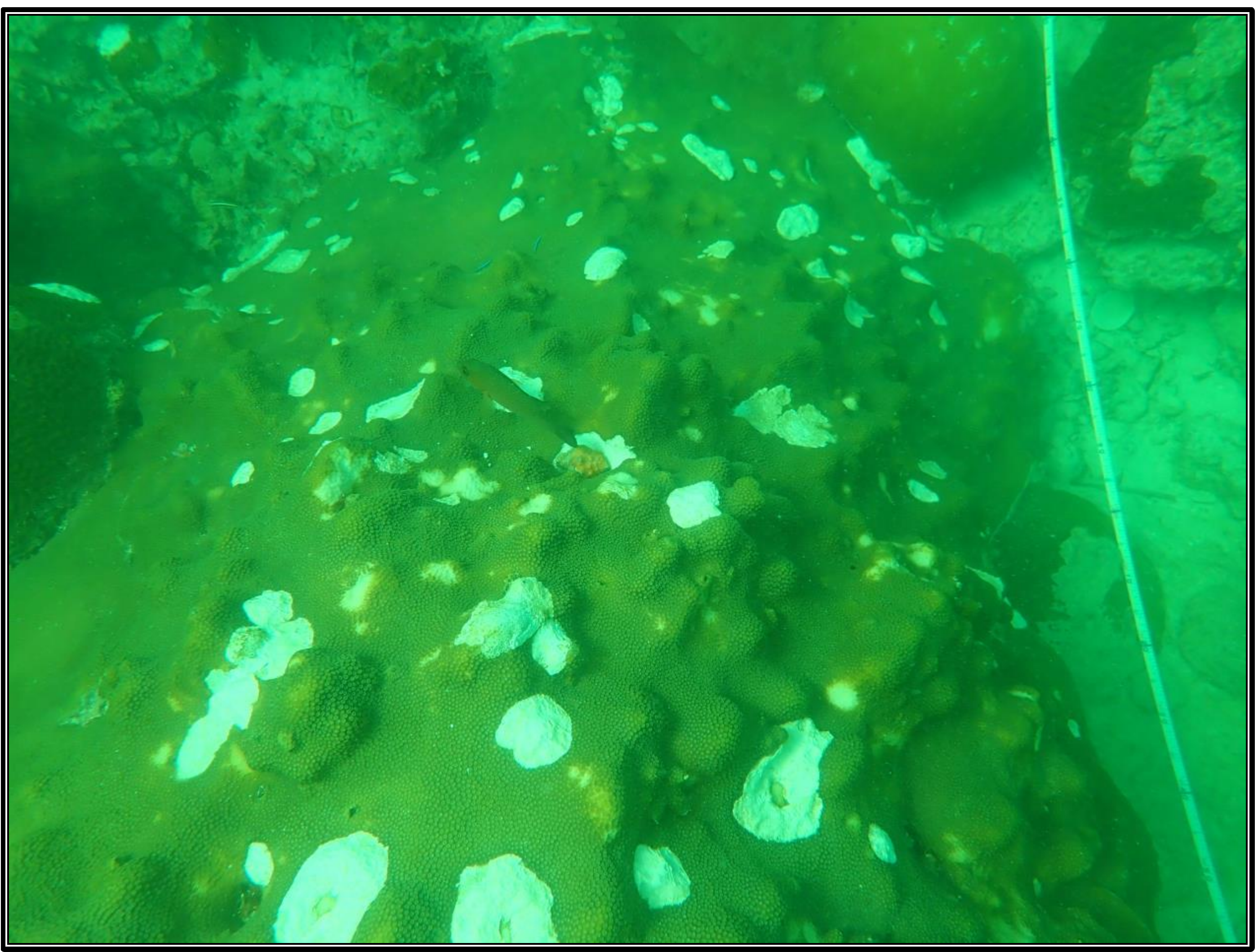


Figure 12. Time series comparing the mean (±1 SE) percentage of diseased tissue per colony at the three offshore MML plots (Porky's Reef). Surveys conducted by MML staff.

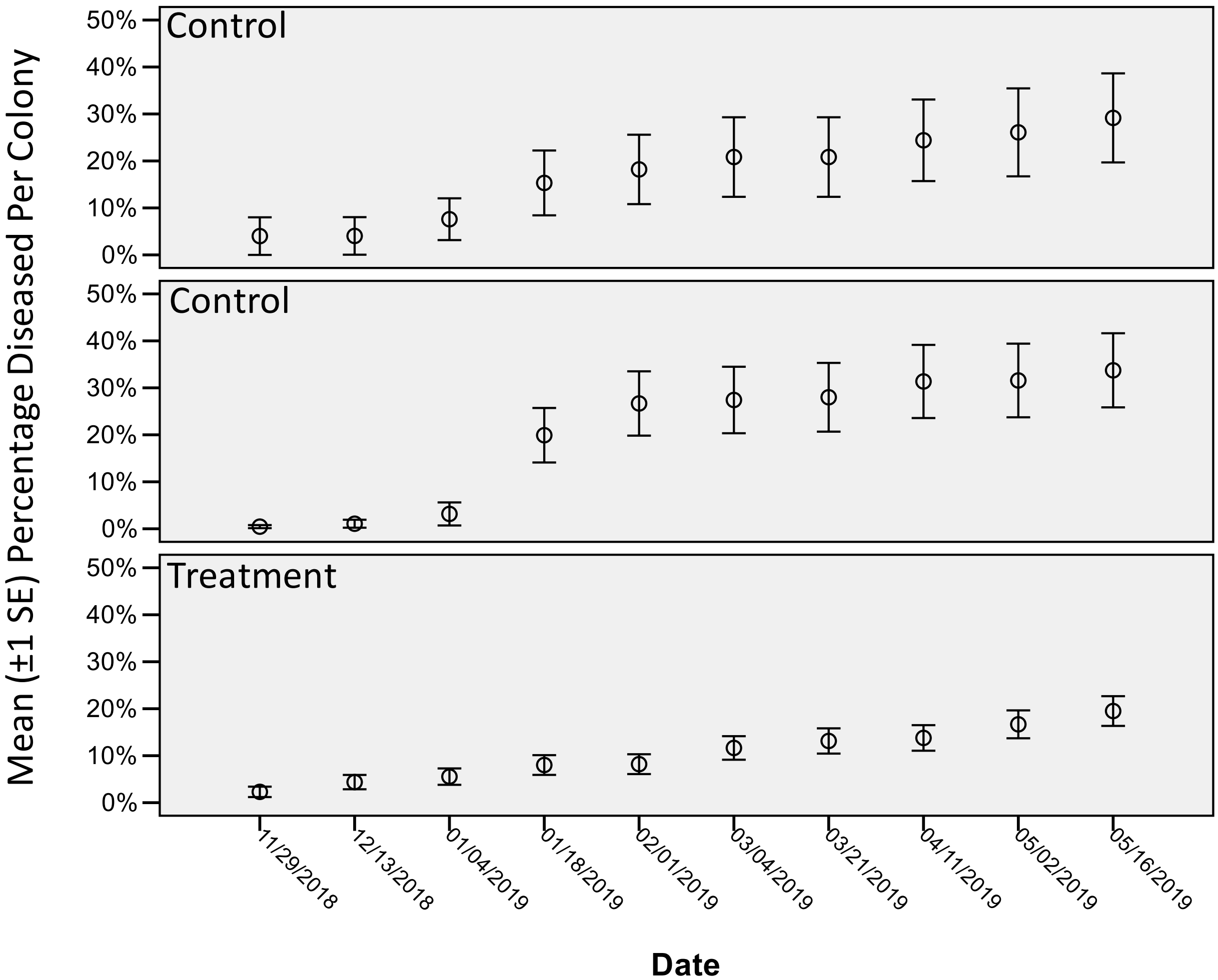


Figure 13. Time series comparing the mean (±1 SE) percentage of diseased tissue per colony at the three mid-channel MML plots (Wonderland). Surveys conducted by MML staff.

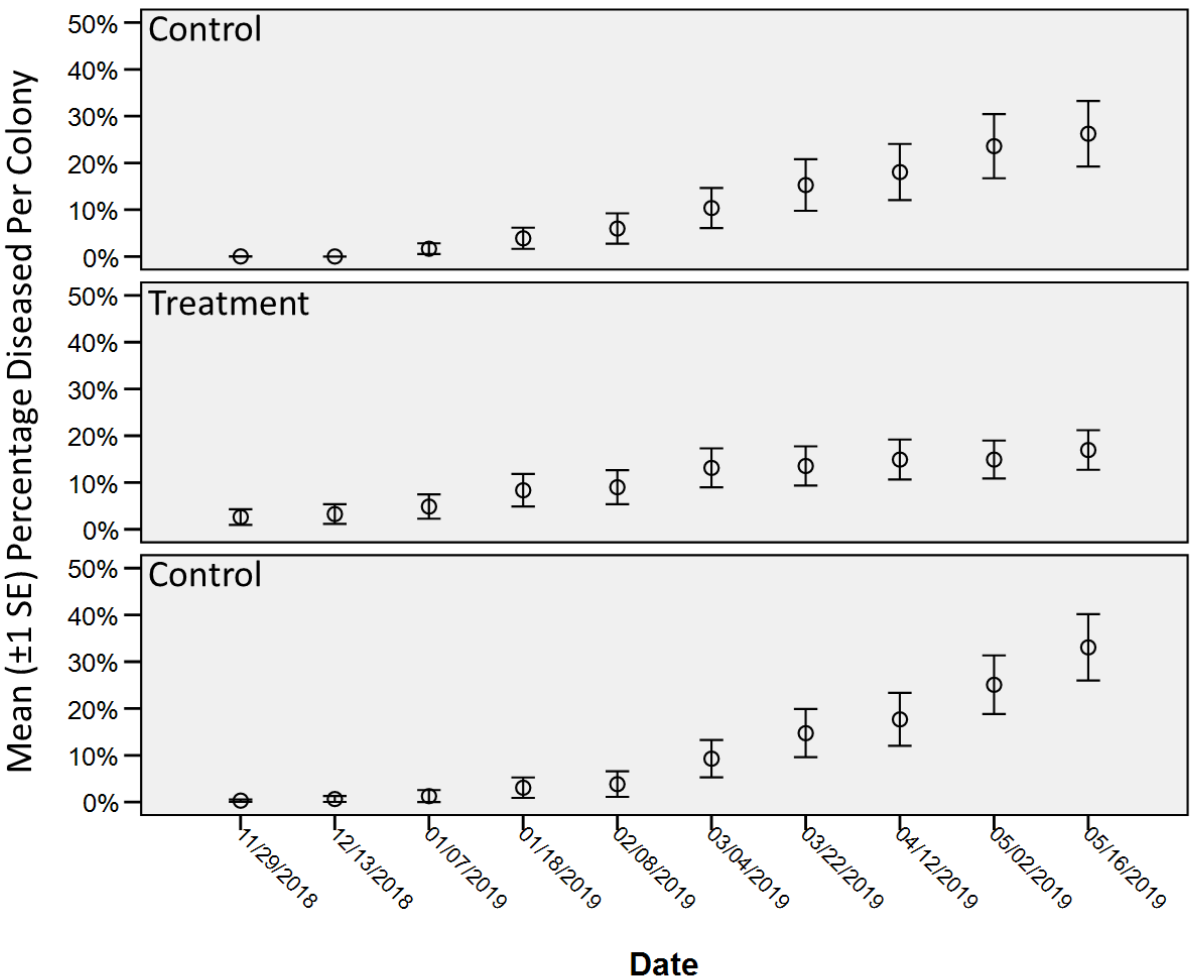


Figure 14. Time series comparing the mean (±1 SE) percentage of diseased tissue per colony at the three inshore MML plots (North Birthday). Surveys conducted by MML staff.

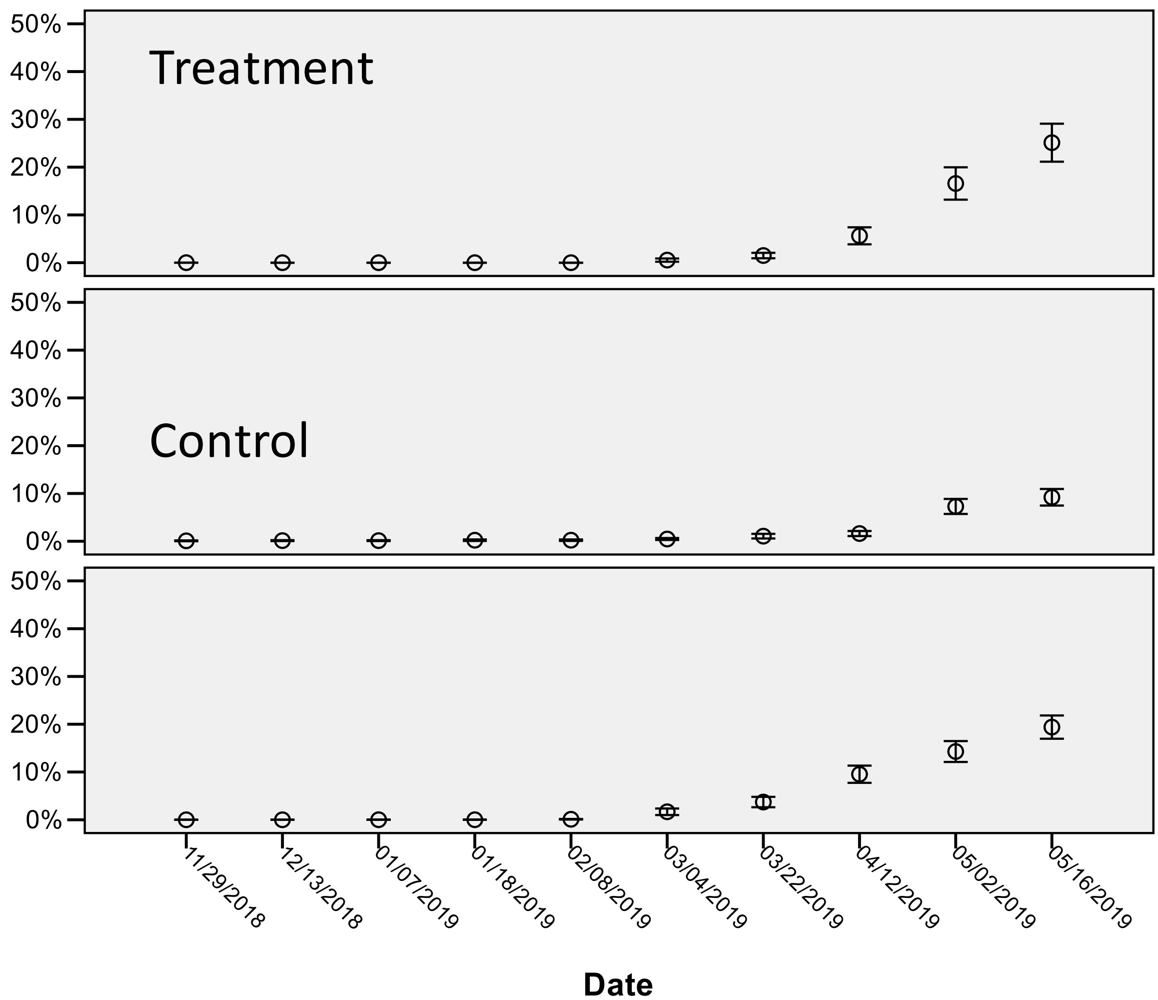


Figure 15. Time series of the mean (± 1 SE) percentage of living tissue per coral colony at the four middle Keys sentinel sites from late January 2018 through mid-May 2019.

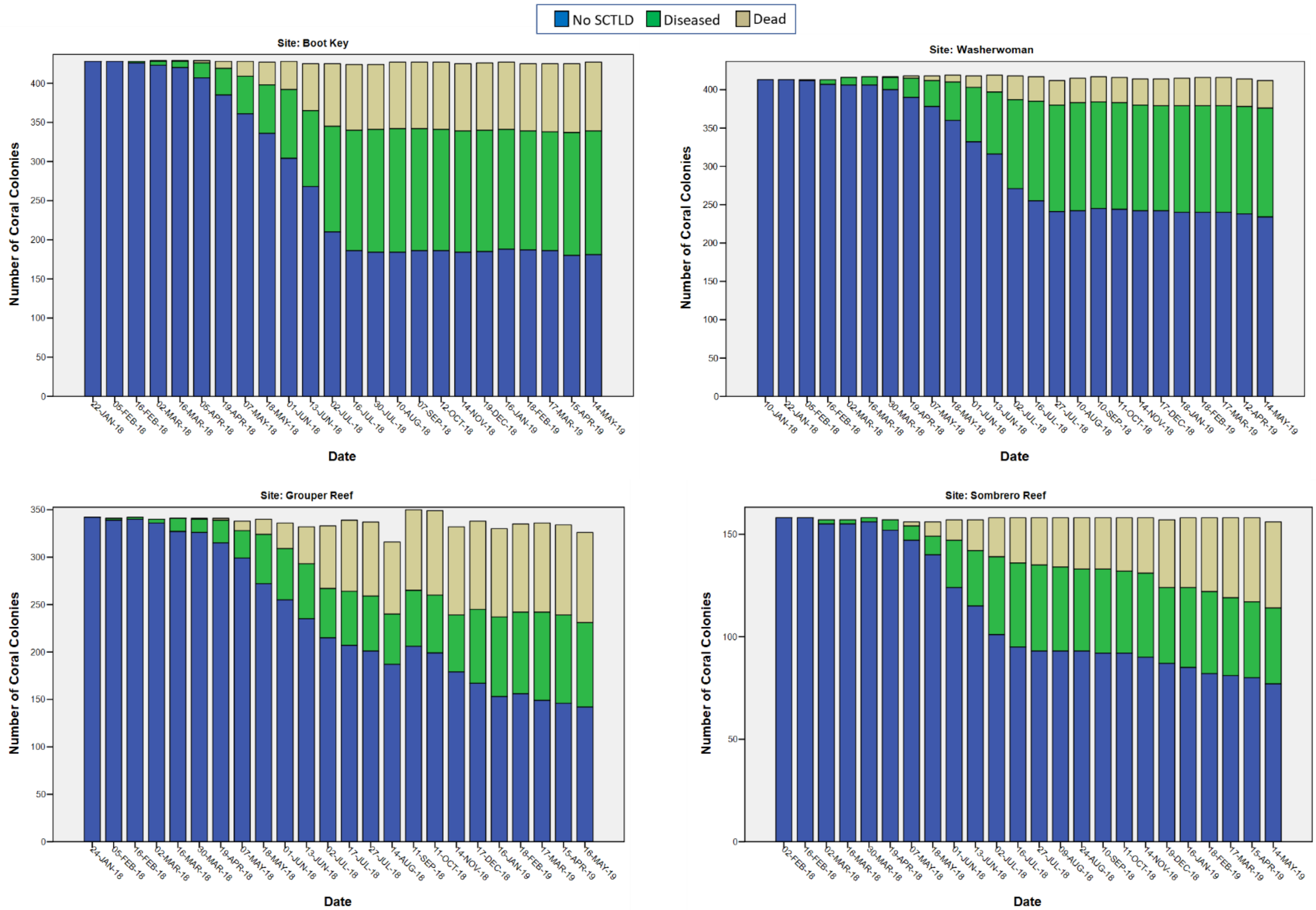


Figure 16. Time series of the mean (± 1 SE) percentage of living tissue per coral colony at the four middle Keys sentinel sites from late January 2018 through mid-May 2019.

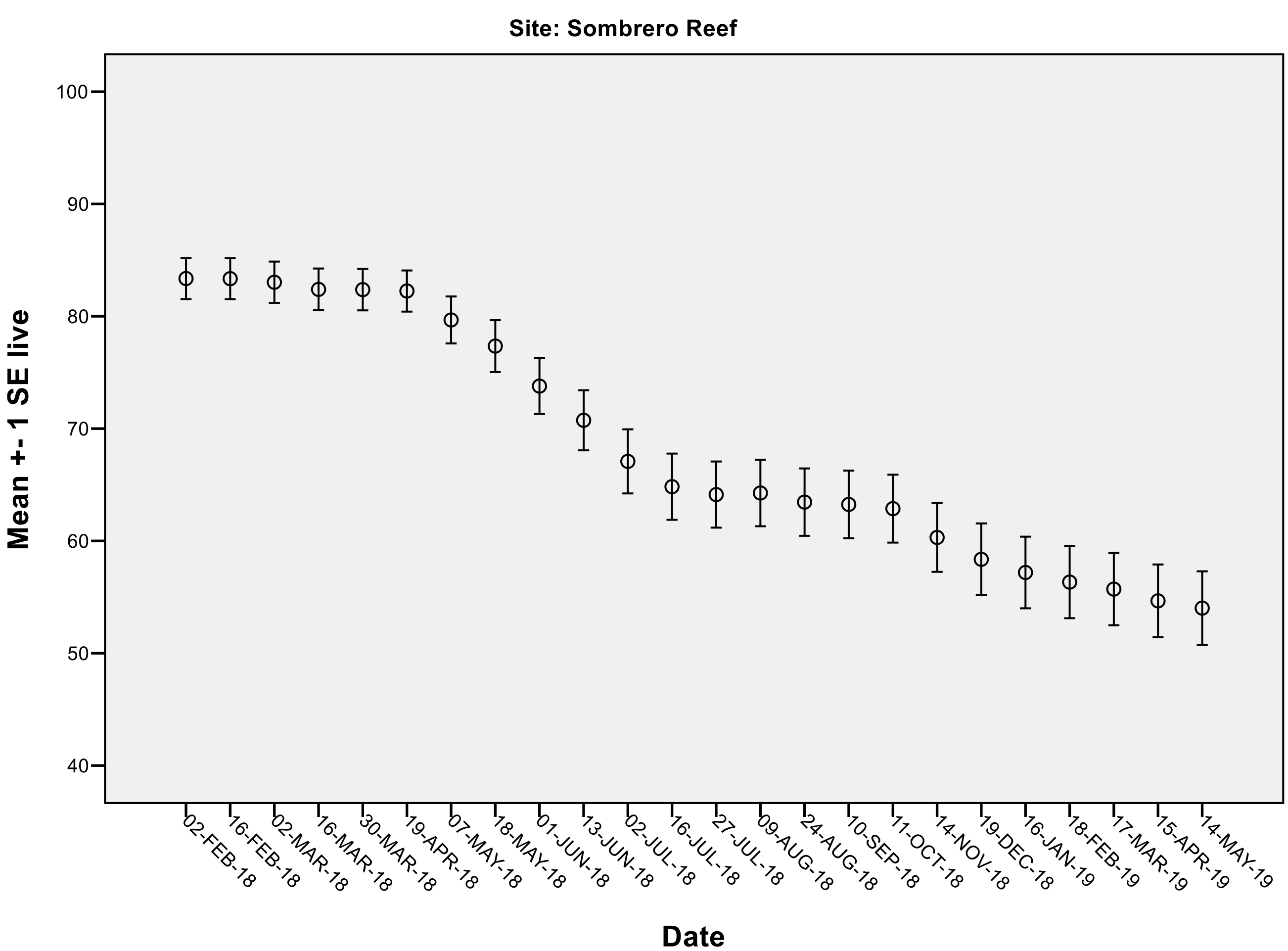


Figure 17. Status of coral colonies by species across the four middle Keys sentinel sites, May 2019.

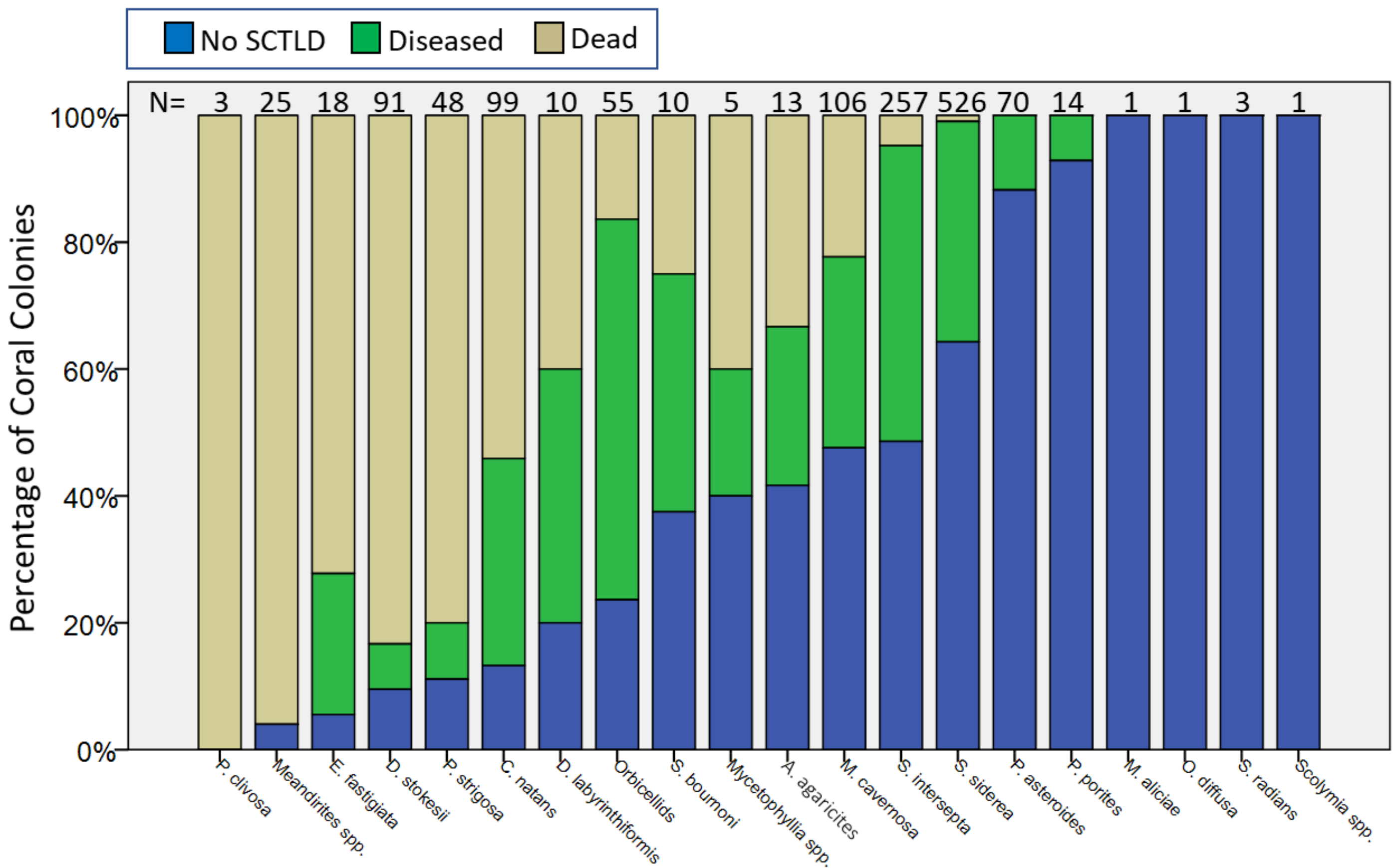
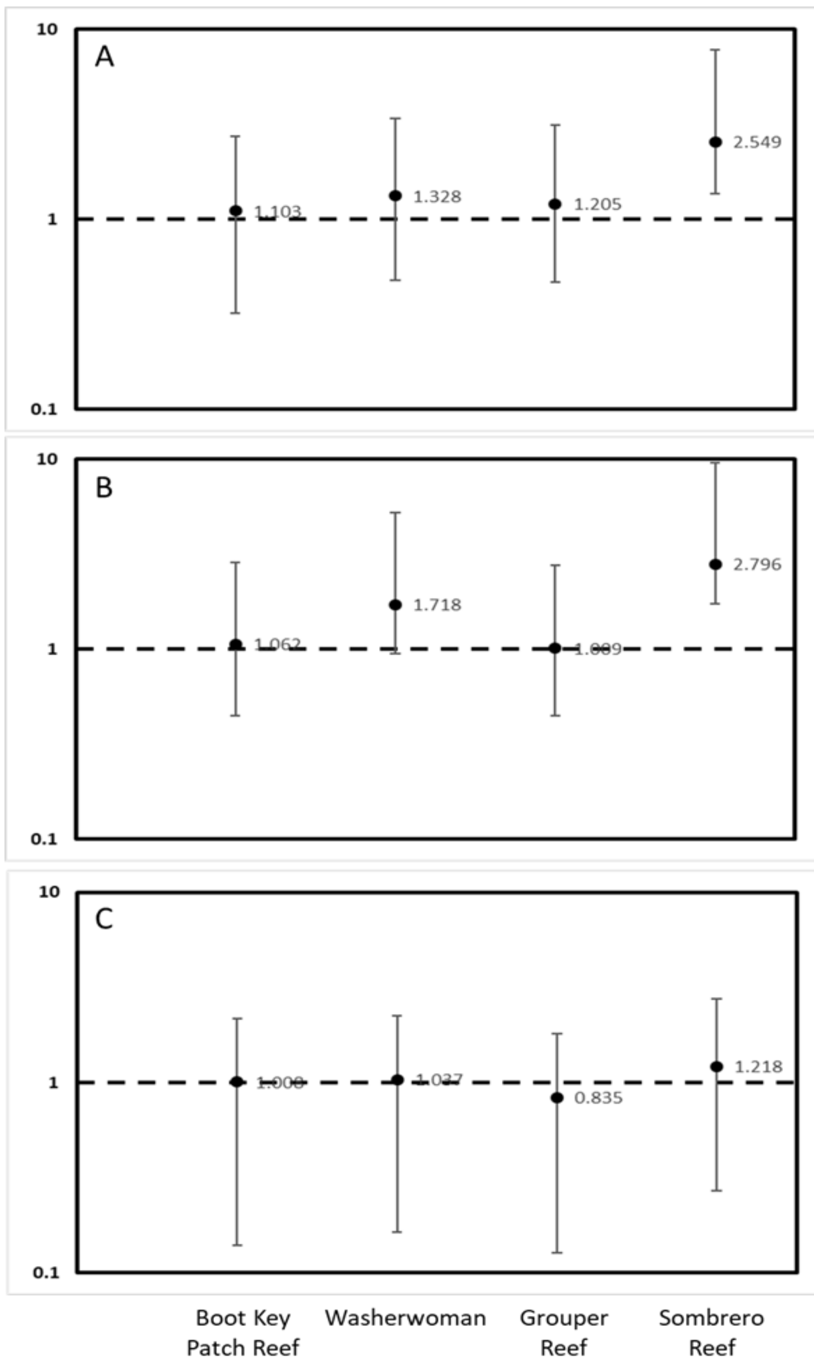


Figure 18. Odds ratios and 95% confidence intervals calculated from the logistic regression applied to the dynamic multi-state model that describe the probability of a coral colony becoming infected based on its nearest-neighbor's status (*i.e.* infected, or dead) and the distance to that neighboring colony. Odds ratios are shown for each of the four FWC middle Keys sentinel sites and included bi-monthly to monthly monitoring data collected from January 2018 through May 2019. (A) Nearest-neighbor's status =infected. (B) Nearest-neighbor's status = dead. (C) Distance to nearest-neighbor.



<u>Appendix 1</u>: Monitoring protocols associated with disease intervention activities

Disease intervention protocol

Project back ground:

Mote Marine Laboratory (MML) divers established three sentinel site locations in the lower Keys in May 2018, each composed of three 10 x 10m plots to assess disease spatial epidemiology to complement the efforts conducted by the FWC in the middle Keys. FWC selected one plot at each of the three sentinel site locations (nearshore, mid-channel, and offshore) to field test intervention techniques discussed at the Coral Disease Workshop (Key Largo, FL July 10-13, 2018). The nearshore site is named "North Birthday" (24.584930° 81.496830°), the mid-channel site "Wonderland" (24.560790° 81.501200°), and the offshore site "Porky's Reef" (24.547630° 81.457490°). Once SCTLD is observed on any of the MML plots (FWC is monitoring them weekly) FWC will begin intervention treatments at the selected plots in a tiered fashion, beginning with the least invasive treatments and progressing to more invasive treatments if the SCTLD has not been arrested. As FWC treats colonies, MML will be utilizing a methodology like that which FWC employed at the middle Keys sentinel sites to track the survival of colonies within treated and untreated plots. These intervention activities will be a first attempt to determine the likelihood of altering the trajectory of the disease at a location.

Methodology for monitoring plots for disease:

FWC divers will visit MML sentinel sites weekly to monitor for the presence of SCTLD. Once on site, divers will mark the plot to be surveyed with a dive flag and will outline the boundary with surveyor's tapes (See Appendix II: Sentinel Site Monitoring Protocols; Monitoring Plot Set-Up Methodology). Divers will then evaluate each coral colony within the plot for the presence of SCTLD. If SCTLD is observed, intervention efforts will begin and MML will be notified so that they can begin tracking survival of colonies.

Methodology for disease treatments:

When disease is observed, active treatment will begin in a tiered fashion. The initial response will be a patch (regardless of lesion size) consisting of a chlorine or antibiotic-impregnated medium covering the entire disease lesion and extending 1 cm into healthy tissue. Our original protocol involved applying a second topical barrier if the disease had expanded beyond the boundaries of the initial topical patch. ***NOTE: After discussion with Andrew Bruckner, Science Coordinator for the FKNMS, it was decided to omit the second application of the topical barrier and move directly to the more invasive technique described in the next paragraph.*** If a small lesion is observed on the edge of a colony and is easily removed it may be amputated. The amputated portions of the colony will include some live tissue ahead of the lesion and will be bagged and removed from the water.

If the disease moves past the topical barrier treatment, we will affect the more invasive trenching technique. This involves cutting a deep groove through the coral tissue several centimeters into the skeleton in a perimeter around the active lesions, then filling the groove with the treatment medium. If applied intervention techniques are not effective, more invasive interventions may be implemented after discussions with the disease intervention steering group.

Our protocol stated that we would use the best technique (i.e., the chlorine or the antibiotic treatment), and treat disease lesions at one of the two sites farthest offshore with Aves Apoxie®

impregnated with chlorine and the other with Shea butter impregnated with antibiotics and covered with modelling clay. The site nearest shore will be treated with Aves Apoxie® impregnated with chlorine. ***NOTE: We simplified our protocol to employ an antibiotoic within a Shea butter matrix to treat lesions at all three of the plots.***

MML personnel will be conducting their bi-monthly surveys at the treated and untreated sites. These surveys will provide the site-level data for us to evaluate the effectiveness of treating corals. We have developed a conceptual model that depicts a hypothetical experimental plot-level difference in the percentage of dead corals through time at untreated and treated sites. If treatment is successful at the site level, then these lines when calculated should diverge through time. We will continue treatment activity at each site based upon this model and our capacity to continue treatments if the actual number of corals requiring treatment becomes too large.

<u>Appendix 2</u>: Monitoring protocols associated with sentinel site monitoring.

Sentinel site monitoring protocols

Monitoring Plot Set-Up: Methodology

One diver will survey the site to identify two locations to place plots. If there is any disease present at the site, choose a new site further west. The locations should be 10-30m apart- far enough to be distinct, but close enough to easily swim between the plots. The locations should have a high density of the coral species that are most impacted by the disease. Avoid areas directly over CREMP sites, outplant sites, or other research sites. Install a large cow-ear tag in the location that will serve as the south-west corner of the plot (Figure 1). From this starting point, two divers will swim tapes due east and due north 5 m (or up to 10 m at low coral density sites). Lay out a diagonal transect (7.07 m for a 5m^2 plot and 14.14 for 10 m^2 plot) to ensure the first two sides are at a 90-degree angle and install nails at the plot corners. Then the divers will turn 90 degrees (north diver turns east, and east diver turns north) so that they meet at the northwest corner of the plot and install a nail. Then, divers will install nails and tags every meter on the north and south sides of the plots. Tags will be labelled 0-5 at the high-density plots and 0-10 at the low density plots, so that divers can easily extend a tape from the “1” tag to the “1” tag at 1 m for transect 1, and so forth. All nails should extend several inches out of the reef so that a clip can attach to it. Divers will extend tapes (clipped to the nails on the southern side) at one-meter intervals from the south side of the plot to the north side of the plot. Wrap the tape gently around the nails on the northern side. The area between the tapes will be referred to as transects.

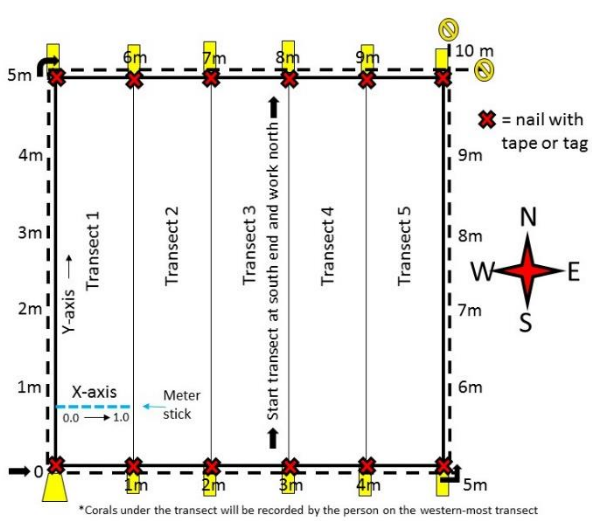
Divers will record data on every coral ≥ 10 cm within the transect, but because DSTO and EFAS can be small and MMEA are uncommon, we will include corals ≥ 4 cm for DSTO, EFAS, and MMEA. If a coral lies under a tape along the perimeter of the plot, it should be recorded if ≥ 50% of the coral is within the plot. If a coral lies under a tape that separates two transects, that coral will be recorded by the diver that has ≥ 50% of the coral. If it’s unclear which transect the coral lies in, the diver in the western-most transect will record it. Quickly sketch a shape representing the coral within the appropriate mapping square and assign the coral a sequential number. Record the number within the mapping square and in the Coral # column. For each coral, identify the center of the colony. For the distance on the Y-axis, record the distance along the meter tape from 0 to the center of the colony (0.0-5.0 or 0.0-10.0 m). For the distance on the X-axis, use the meter stick to record the distance across the transect (0.0-1.0 m) from the west side of the transect to the center of the colony. If the center of the colony is outside the transect, the distance may be negative or greater than 5.0 m for the Y-axis distance or greater than 1.0 m for the X-axis distance (unlikely if ≥ 50% of the coral is within the plot). The X/Y distances should be recorded to the nearest 0.1 m.

Follow the general FRRP protocol for identifying and measuring corals, identifying tissue isolates, and assessing bleaching. Record the four letter FRRP code for the species. Measure the coral using the meter stick in three dimensions- maximum width, perpendicular width, and maximum height. Coral measurements should be to the nearest 1.0 cm. Record the number of tissue isolates (the number of areas of live tissue separated by dead tissue on the same colony). If the number of tissue isolates is greater than 5, estimate the number. If it is unclear whether multiple isolates were originally the same colony or separate individuals, err towards recording them as separate individuals. Record the percentage of the entire colony that comprises old dead tissue and new dead tissue. Old dead tissue is defined as any non-living parts of the coral in which the corallite structures are either gone or covered over by organisms that are not easily removed (certain algae and invertebrates). New dead tissue is defined as any non-living parts of the coral in which the corallite structures are either white (and no clear polyps are present) and still intact or slightly eroded but easily identifiable to species. Recently dead skeletons may be covered by sediment or a thin layer of algae. The percentage of new dead tissue (there should be very little new dead tissue during site set up) is divided into mortality attributed to disease and mortality attributed to other sources. We expect most of the new dead tissue to be caused by disease and should be recorded in that column, but if new dead tissue can be attributed to other sources (predation), record that percentage in the "other" column and specify the source of mortality in the notes section. Record P for pale, PB for partial bleach, or B for bleached under the Bleach or Pale column. Visually inspect corals for snails and record observed snails in the notes section. Avoid feeling the edges of corals for snails to reduce the likelihood of enabling disease transmission. Add other notable details on disease observations to the notes section as well.

When you complete the data collection, choose up to 10 corals in the transect to photograph. Select species that are likely to be impacted by disease. Take the photos starting at the 0 m and end at the north side of the transect. Ensure that a marked meter stick is present on the southern side of the corals as a reference object. Put a star next to the shape representing the photographed coral and in the notes section of the data. Photos should be labelled as follows; site name-plot number-transect number-coral number (Sombrero-1-4-3)

Leave a floating dive buoy at the start tag and collect GPS coordinates.

Figure 1. Schematic of plot set-up at high density coral density sites. Cow ear tag installed at the arrow at the southwest corner of the plot.



Monitoring Plots: Monitoring Methodology

Every two weeks monitor plots for disease. Throw a floating dive flag to mark each plot. Lay out tapes out exactly as described in the site set-up. The map drawing, and data for coral #, distance on X-axis, and distance on Y-axis, species, maximum width, perpendicular width, height, # of tissue isolates, and % old dead will be filled in on the datasheets and unless the data have changed, they do not need to be rerecorded. These data will also assist in identifying individuals for fate tracking. Slowly swim over the transects to determine if disease is present at the plot.

If there is no disease:

If no disease or recent mortality is present on the transect, write None in the New Dead disease column and an arrow down the column to indicate the absence of disease for the entire transect. No other data collection or photos are required. Even though no new data is recorded, it is important to have a datasheet with the site, date, and confirmation that there is no disease.

If there is disease:

If there is disease or recent mortality present on the transect, complete a full assessment of the corals in each transect. Use the sketched map with numbered corals to ID the corals. Coral #, distance on x-axis, dist. On Y-axis, species, max width, perp width, height, and number of isolates will already be filled out. Old mortality will be filled out but write a new number if necessary. For corals with no disease- write a dash in the new dead columns; there is no need to record further data unless it is noteworthy (snail predation or bleaching/paling). If there is mortality that is attributed to disease, record the percentage of the entire colony with new dead tissue in the disease column. If there is mortality that can be attributed to other sources, such as predation, record that percentage under the "other" column and specify the source of mortality in the notes section. Write any other notable observations related to the disease in the notes section. Take photos of any "starred" diseased corals.

Project Sampling Gear:

- 2 Floating dive flags
- Cleaning tools (to clean tags)
- 4 Clip boards
- Data sheets
- Site maps
- Coral cheat sheets - laminated
- Six 30 m tapes
- 5 PVC 1m meter sticks
- Cameras
- Working pencils/rubber bands and extras
- Field notebooks
- Dive Gear

Sentinel Site Locations

Site	Date Established	Lat	Long	Plot	Name in GPS
Washerwoman	1/15/2018	24.66426	-81.07385	1	CD17-WASH1
Washerwoman	1/15/2018	24.66417	-81.07378	2	CD17-WASH2
Boot Key Patch	1/22/2018	24.66489	-81.09616	1	CD17-BOOT1
Boot Key Patch	1/22/2018	24.6649	-81.09633	2	CD17-BOOT2
Grouper Reef	1/24/2018	24.65257	-81.03652	1	CD17-GROU1
Grouper Reef	1/24/2018	24.65259	-81.03615	2	CD17-GROU2
Sombrero	2/2/2018	24.62536	-81.1114	1	CD17SOMBD1
Sombrero	2/2/2018	24.62539	-81.11155	2	CD17SOMBD2