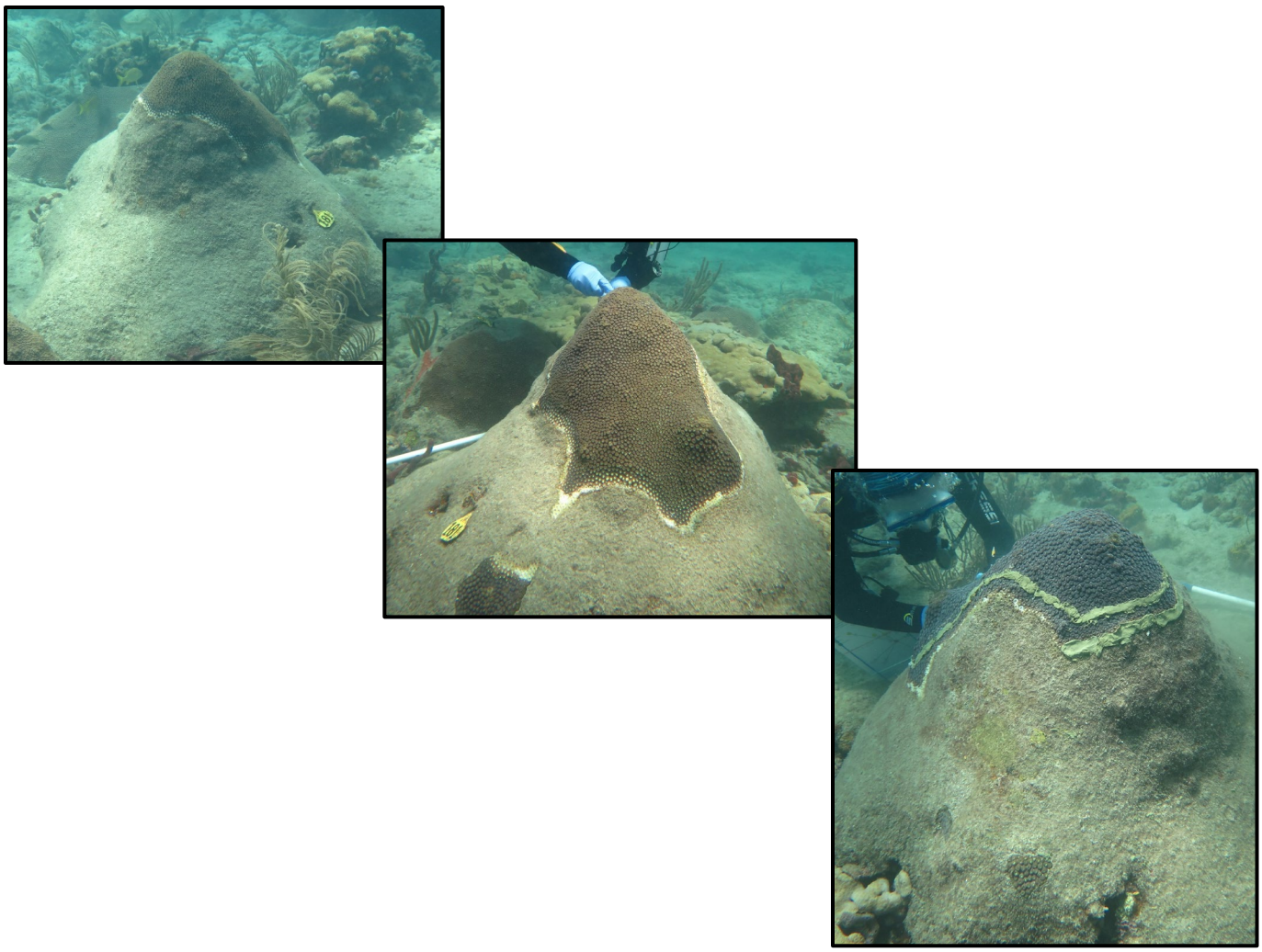
# Intervention and fate tracking for corals affected by stony coral tissue loss disease in the northern Florida Reef Tract



Final Summary Report

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## 1. BACKGROUND

Florida's coral reefs are currently experiencing a multi-year outbreak of a coral disease described as "stony coral tissue loss disease (SCTLD)." While disease outbreaks are not unprecedented, this event is unique due to the presence of multiple symptoms and etiologies that have affected at least 21 species of coral across the Florida Reef Tract (FRT). The disease(s) are highly prevalent and are estimated to have resulted in the mortality of millions of corals across the newly designated Southeast Florida Coral Reef Ecosystem Conservation Area (Coral ECA), Biscayne National Park (BNP), and the Florida Keys. Hurricane Irma also recently impacted the entire FRT in September 2017, with subsequent freshwater discharge impacts particularly acute on coral reefs in Martin County. The efforts reported here focus within the Coral ECA as part of a larger effort to understand the impacts of disease on coral health and to determine mitigation efforts that may prevent losses of coral reef resources. This study leveraged funding from the Environmental Protection Agency (EPA) and NOAA Coral Reef Conservation Program to FAU Harbor Branch, as well as graduate student support from the Harbor Branch Oceanographic Institute Foundation.

### 1.1. Project Goals & Objectives

The purpose of this project was to continue monitoring coral disease incidence and prevalence in the northern portion of the Coral ECA and to experimentally test intervention strategies designed to 1) reduce coral tissue loss, 2) reduce the likelihood of total colony mortality, 3) reduce the probability of transmission to nearby colonies, and 4) reduce population declines in known areas of infection. This project was designed to improve understanding of the current spatial extent of the disease outbreak, prevalence, species affected, timeline of disease progression within colonies, likelihood of mortality due to disease among species, and the physiological responses of corals to disease. The outcomes of this project will contribute to on-going and future coral disease response efforts which seek to improve understanding about the severity and impacts of the Florida Reef Tract coral disease outbreak, identify management actions to remediate disease impacts, and, ultimately, prevent or mitigate the effects of future outbreaks. The project was designed with input from agency representatives and Martin County stakeholders to improve adaptive management regarding coral susceptibility to disease and impacts from infection. Finally, this project will improve the predictive capacity regarding coral susceptibility to disease and impacts from infection.

## 2. METHODOLOGY

This project combined repeated surveys, 3d imaging, experimental disease intervention, and coral sampling to address the objectives listed above. Table 1 below summarizes the operational activities at each of the project sites. Project sites on St. Lucie Reef were chosen from long-term monitoring sites in our lab with over 10 years of survey data, "SEFL" sites in Palm Beach County with the highest stony coral cover were selected from a larger number of Hurricane Irma impact survey sites used in 2017 to allow for continued monitoring, and the Broward County sites were chosen due to their relatively high stony coral and SCTLD abundance.

Table 1. List of operational activities at each project site.

| Operational Activities at Each Project Site | | | | | | |
|---|---|---|---|---|---|---|
| Site Name | Average Depth (m) | Lat | Long | County | Activity | Dates |
| SLR North | 3 | 27° 08.777' | -80° 08.350' | Martin | Surveys | 4/25/2019 |
| SLR Central (SEFL01) | 3 | 27° 07.900' | -80° 08.042' | Martin | Surveys | 3/1/2019 |
| SLR South (SEFL02) | 4 | 27 ° 07.286' | -80° 07.650' | Martin | Surveys | 3/1/2019, 4/25/19 |
| SLR Ledge | 5 | 27° 06.712' | -80° 07.531' | Martin | Surveys | 3/1/2019, 4/25/19 |
| SEFL04 | 18 | 26° 56.6225' | -80° 1.3183' | Palm Beach | Surveys | 12/18/2018, 3/11/19, 5/15/19 |
| SEFL05 | 17 | 26° 55.6467' | -80° 1.8060' | Palm Beach | Surveys | 12/18/2018, 3/11/19, 5/15/19 |
| SEFL06 | 16 | 26° 53.8641' | -80° 0.9830' | Palm Beach | Surveys | 12/18/2018, 3/11/19, 5/15/19 |
| SEFL08 | 12 | 26° 42.6260' | -80° 0.9490' | Palm Beach | Surveys | 2/5/2019, 3/12/19, 5/14/19 |
| SEFL11 | 11 | 26° 40.7100' | -80° 1.0950' | Palm Beach | Surveys | 2/5/2019, 3/12/19, 5/14/19 |
| SEFL12 | 13 | 26° 39.1432' | -80° 1.2409' | Palm Beach | Surveys | 2/5/2019, 3/12/19, 5/14/19 |
| T328 | 5 | 26° 10.567' | -80° 05.633' | Broward | Surveys[1], Intervention[2], & Samples[3] | 11/8/18[1], 12/17/18[1], 3/4/19[1], 3/26/19[2], 4/17/19[23], 5/7/19[123] |
| BC1 | 8 | 26° 08.855' | -80° 05.766' | Broward | Surveys[1], Intervention[2], & Samples[3] | 11/8/18[1], 12/17/18[1], 3/4/19[1], 3/26/19[2], 4/15/19[23], 5/6/19[123] |
| FTL4 | 5 | 26° 08.197' | -80° 05.843' | Broward | Surveys[1], Intervention[2], & Samples[3] | 11/8/18[1], 12/17/18[1], 3/4/19[1], 3/26/19[2], 4/16/19[23], 5/7/19[123] |

### 2.1. Roving Diver Surveys

Roving diver surveys were conducted in Martin, Palm Beach, and north Broward counties to record disease prevalence, species impacted, and disease incidence across sites. For 20 minutes, investigators swam across each site collecting data. For coral disease, the diver counted every coral species greater than 10 centimeters in diameter. These corals were tallied as either 'diseased' or 'not diseased'. Any coral disease was noted by general descriptors (e.g. SCTLD, Dark spot, White plague). Paling, partial bleaching and bleaching were also noted utilizing the following codes to indicate the severity of discoloration. Bleaching or paling directly associated with a disease (next to a margin of recent mortality) was not recorded as paling/bleaching, but this was difficult to distinguish in many cases of diffuse bleaching without decaying tissue. Any discoloration of coral tissue was considered pale (P). Patches of fully bleached or white tissue were considered partially bleached (PB), and totally white tissue with no visible zooxanthellae was considered bleached (BL). Diver propulsion vehicles were particularly useful for maintaining position and effectively conducting surveys (and later sampling) at sites such as Jupiter and Palm Beach Breakers where currents up to two knots were observed.

Roving Diver Code Legend:
UK = Unknown
DS = Dark Spot
BB = Black Band
RB = Red Band
YB = Yellow Band
WB = White Blotch
WP = White Plague
WS = White Syndrome
P = Paling
PB = Partially Bleached

BL = Bleached
SCTLD = Stony Coral Tissue Loss Disease**
**Not noted in the original 2017 Roving Diver Survey Data Sheets since this convention was not yet adopted. Early notes on the data sheets list these observations as "White Blotch"

### 2.2. QA/QC

All site data were entered into Excel where QA/QC and data summaries were performed. Once entered, data were reviewed to ensure consistency with data sheets. During the summary table creation, the data were once again reviewed for consistency between teams especially for coral species and disease identifications. In some cases, site pictures were reviewed to help this QA/QC process.

### 2.3. Coral Fate Tracking and Imaging

Corals at St. Lucie Reef (Martin County), including *Montastraea cavernosa* and *Pseudodiploria clivosa,* have been tracked since 2010 and were monitored throughout this project (until complete colony mortality occurred). Each coral was photographed and videoed using a Canon G16 camera in a Fantasea housing using underwater green laser arrays scaled at 15-centimeter spacing. The camera was oriented perpendicular to the colony at a linear distance suitable for capturing the entire colony with no zoom. If abnormalities were observed, more detailed close-up images of disease lines or bleached tissue were also collected. The objectives of this project also included tracking infected coral colonies in Palm Beach and later Broward counties based on the observed disease prevalence in 2017. However, no active diseases were observed in Palm Beach County during the course of the study. When active diseases were observed, we revisited the colonies for repeated imaging approximately every two months as weather and current conditions allowed. In addition, qualitative notes on the appearance of the lesions as well as the number of active lesions were recorded. If the colony had experienced complete mortality or if the colony had been removed from the reef, presumably due to strong wave events including those during Hurricane Irma, these were noted in our observations.

*3D Model Videography*
Fate-tracked colonies in Pompano (Broward County) were recorded using methods outlined in Young et al. (2018). Canon G16 Cameras in Fantasea Underwater housings shooting at 1080p and 60fps were used in filming. PVC scale bars scaled at 10cm increments were placed at opposing right angles to frame the designated colony. A diver on SCUBA swam approximately 1m above the highest point of each colony and recorded swimming a lawnmower pattern keeping the camera pointed in the same orientation throughout. The number of passes will vary depending on colony size, however, 60-70% overlap between passes is integral for downstream model generation. At the end of the first set of passes, the camera is rotated 90° and another set of passes is completed. A complete set of passes for one colony takes between 1-3 minutes depending on colony size.

A free software, FFmpeg (www.ffmpeg.org) was used to pull still frames from the *in situ* video using a simple command line prompt:
ffmpeg -i movie_filename.MP4 -vf fps=3 output_name_%d.png,
where -i denotes the input video filename, -vf deinterlaces the video to produce crisp still images, and fps=3 specifies that 3 frames should be taken per second of video.

Once the stills were generated, they were loaded into AgiSoft Metashape (AgiSoft LLC) which uses a proprietary algorithm, incorporating Structure from Motion and Brown's lens distortion model to generate 3D models from 2D images (C. Brown 1971; Westoby *et al*. 2012). Model generation in Metashape occurs in four steps: camera alignment, dense point cloud generation, mesh generation, and texture overlay. During camera alignment Metashape identifies common points within photographs and matches them. Camera alignment was repeated until the majority of the photographs were aligned. The second step is dense point cloud generation, built based on the estimated camera positions and pictures, and further identifies common points within each picture as well as calculating depth information to generate a three-dimensional dense point cloud of unique points and features. Following the point cloud, a three-dimensional polygonal mesh was constructed which represents the object surface based on the dense point cloud. Finally, photorealistic overlay was applied to the triangulated mesh. 3D models were exported as a .obj and loaded into Rhinoceros 3D for analysis.

Models were loaded into Rhinoceros 3D (Robert McNeel & Associates), scaled, and then measured for total colony surface area as well as disease lesion surface area. The models were scaled via the PVC scale bars that were placed during filming. Once scaled, the outline of the coral colony was traced using a polyline which adheres to the mesh, ensuring all of the tissue, both diseased tissue and apparently healthy tissue were captured within the polyline. A secondary mesh was overlaid onto the existing mesh and the parts outside of the polyline were trimmed away leaving a mesh that captures the tissue of the fate-tracked coral colony. From this, an area measurement ($m^2$) was generated. The same process was repeated for each individual disease lesion, laying a mesh, trimming the excess away and producing area measurements. The disease area measurements were tabulated and compiled for total area of disease tissue, total areas of healthy tissue, and total colony surface area.

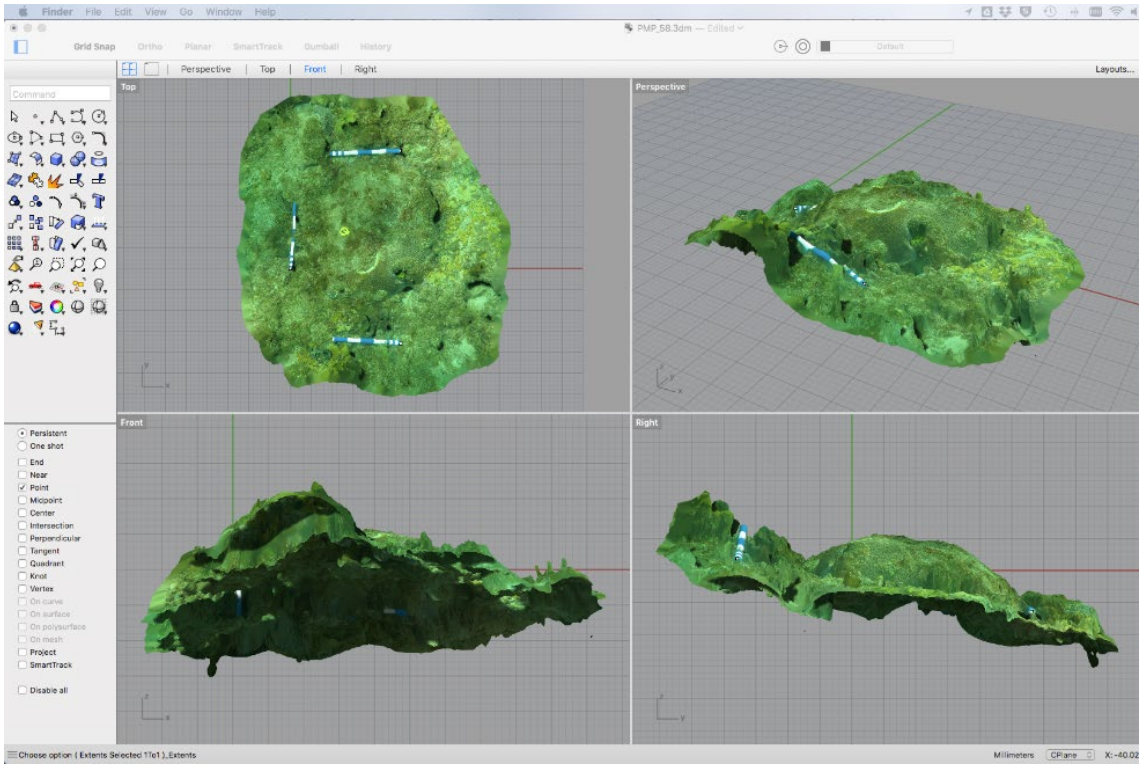



Figure 1. 3D models for each coral colony were constructed and surface areas for healthy tissue, lesions, and bare skeleton were calculated using Rhino 3D. An example model is shown above in the software module.

### 2.4. Coral and Disease Sample Collection

*Coral Gene Expression Samples*

To quantify the impacts of tissue loss disease on coral physiology, naturally infected *Montastraea cavernosa* and *Pseudodiploria clivosa* were sampled. For infected individuals of each species, 2.5-centimeter diameter core samples were collected at the disease margin and on the most distal unaffected area of the colony. Baseline non-infected transcriptomes have already been generated and analyzed on *M. cavernosa* at St. Lucie and Palm Beach. The samples were collected and preserved for future research. The optimized pipeline developed with recent FL Sea Grant funding will be used, including Tag-Seq transcriptomic analyses with Illumina HiSeq and DESeq2 to quantify differential gene expression.

*Contamination control*

Operational considerations for minimizing cross contamination or pathogen spread included sterilizing all sampling equipment before use and using separate numbered collection bags for each sample target and site. Each numbered collection bag, one for each colony to be sampled, contained sterile corer, a pair of nitrile gloves, and pre-labeled Whirl Packs. To minimize cross contamination between colonies, each pair of nitrile gloves was discarded in a separate designated sealable bag after each colony was sampled. To minimize cross contamination between sites, separate sets of sampling equipment were

used for each site. Dive gear and wetsuits were sterilized using a 10% Odoban solution between operations.

*Photogrammetry of Affected Corals*

In addition to videos for 3D modeling, before and after coral sampling laser-scaled digital still images were collected using custom cameras systems with Canon G16 cameras and Fanatasea underwater housings. For each sampled colony 90° overhead planer photographs, 45° colony profiles, and close ups of disease margins were included.

### 2.5. Intervention Strategies

Experimental interventions were focused across three sites (T328, BC1, and FTL4 Figure 2) located <1 mile offshore of Lauderdale-by-the-Sea and Pompano Beach in Broward County. The coral reefs at these sites are all <10 m in depth. SCTLD has not been observed at our other deeper sites (Table 1). The sites were originally chosen for a previous fate tracking experiment due to their relatively high disease abundance and coral cover, which allowed for the selection of enough diseased *M. cavernosa* colonies while maintaining a low likelihood of sampling clones. The fate-tracking experiment followed 32 SCTLD-affected *M. cavernosa* colonies from 24 August 2018 through 12 December 2018. To quantify lesion progression across individual colonies, the 3D modelling technique was adapted from Young et al. (2017) and optimized for this specific application (see above).

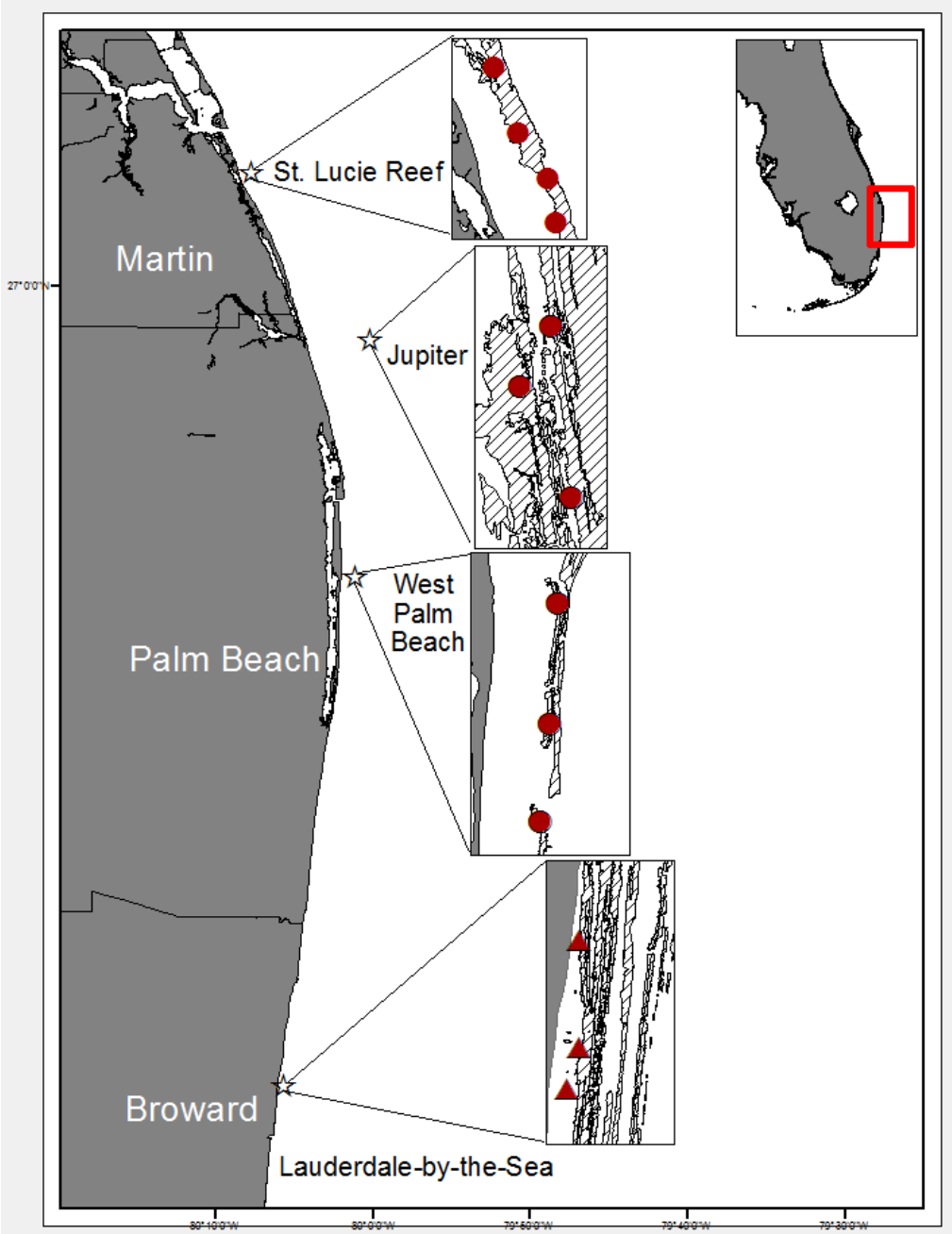



Figure 2. Research sites included in this project. Sites indicated with a circle included SCTLD reconnaissance and monitoring. Sites indicated with triangles included monitoring and experimental intervention with follow up fate tracking.

Of the original 32 tagged diseased *M. cavernosa* colonies included in the previous fate-tracking study initiated in July of 2018, by 26 March 2019 only 11 were still alive, diseased, and had sufficient tissue for experimental disease intervention (~30 $cm^2$). The remaining 22 had either suffered complete mortality, had insufficient tissue remaining for intervention, or the SCTLD previously affecting them originally had apparently quiesced. To supplement this sample group for this experiment, an additional 22 SCTLD affected *M. cavernosa* colonies were located and tagged (numbered cattle tags with the label "FAU

HBOI") across the three sites, as well as eight apparently healthy *M. cavernosa* colonies. Tagged diseased colonies were divided into three groups: 1) amoxicillin treatment (*n*=11); 2) chlorine treatment (*n*=12); 3) untreated control group (*n*=10). The remaining tagged apparently healthy colonies (*n*=8) were also left untreated as controls for use in the microbial portion of this study. A Kruskal-Wallis test indicated no significant difference in average colony size in each treatment group (p=0.85). Average colony size (as measured by total tissue surface area) at the initial intervention (mid-April dates) among amoxicillin treated colonies was 2,574 $cm^2$, chlorine colonies avg. was 3,277 $cm^2$, and control colony avg. was 2,598 $cm^2$. Size range of chlorine and amoxicillin treated colonies was 350 $cm^2$ to 9,422 $cm^2$. Additionally, starting in March 2019, duplicate HOBO Pendant® data loggers were also deployed at each of the three sites and will record temperature on a fifteen-minute interval throughout the course of the intervention experiment. Data loggers will be swapped and downloaded as necessary, with any data gaps from instrument swaps to be made in between sampling time points.

*Trenching*

Both direct intervention treatments began with trenches being created around all disease lesions present on the diseased colony. Approximately 1 cm deep by 1 cm wide trenches were made ~5 cm away from the disease margin using a Nemo™ Underwater Angle Grinder. If the polyps were deeper than one centimeter in the skeleton, the trench was made to a depth extending beyond the polyps.

*Treatment 1: Amoxicillin and Base 2b*

The 11 diseased colonies that received the amoxicillin treatment had an amoxicillin/Base 2b mixture packed into the trenches and applied to the lesion (see Figure 3). Base 2b (CoreRx Pharma; not currently market distributed) is a silicone-based product specifically formulated to be used in slow release (36 hr period post-application) antibiotic treatments. Base 2b was combined with powdered amoxicillin trihydrate (PhytoTechnology Laboratories; labelled "CB122" for this use). They were combined in an approximate weight ratio of 10:1 g Base 2b: amoxicillin and applied to fill the trench created by the angle grinder, as well as a patch over the disease lesion.

Amoxicillin Treated Colony #307

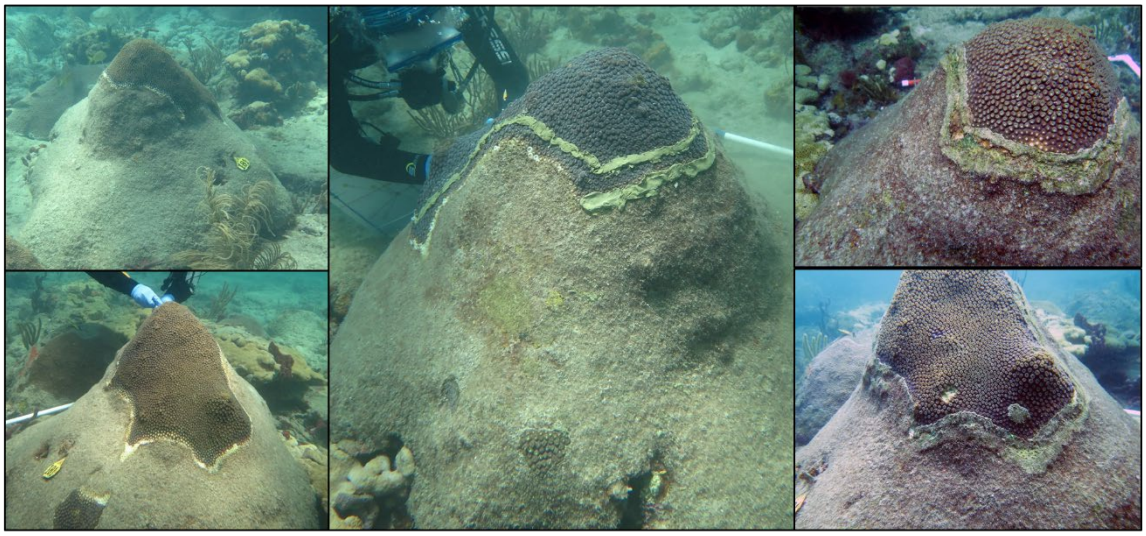

4/15/19 Pre-treatment | 4/15/19 Immediate Post-treatment | 6/21/19 Nine Weeks Post-treatment

Figure 3. Amoxicillin/ Base 2B treated colony example.

*Treatment 2: Chlorine*

The 12 diseased colonies that received the chlorine treatment in the trenches and along the lesion (see Figure 4). A combination of Poolife™ Turboshock© chlorine powder and ZSPAR A-788 Splash Zone™ two-part epoxy was mixed in an approximate volumetric ratio of 15:50 mL chlorine powder:part A epoxy. This compound was subsequently combined with the part B epoxy in equal proportions underwater and thoroughly mixed, immediately before application to the colony. This chlorine-epoxy mixture was applied in the same method as the amoxicillin-Base 2b mixture above.

Chlorine Treated Colony #181



4/15/19 Pre-treatment | 4/15/19 Treatment application | 6/21/19 Nine Weeks Post-treatment

Figure 4. Chlorine/ Z-spar epoxy treatment example.

Surface layer mucus was collected from all tagged treated and control *M. cavernosa* colonies immediately before initial intervention treatments. Approximately five cm from the disease lesion, a SCUBA diver wearing nitrile gloves aspirated a ~5 $cm^2$ area of the coral tissue using a sterile 10 mL syringe to encourage mucus production and then collected mucus with the syringe. Healthy colonies were sampled from the colony's center. Upon returning to the boat, mucus was filtered from the syringes through sterile 25 mm diameter, 0.2 µm pore size filters in Swinnex filter holders. Several samples were extremely viscous, and the pressure of filtering caused a break in the seal of the filter holder, allowing some mucus to escape rather than go through the filter. After this issue was realized, the technique was altered so that enough mucus was pushed through to saturate the filter, but not break the seal. Following filtration, the filter was placed into a sterile 2 mL cryovial, placed in CryoFlex wrap, immediately placed on ice, and flash frozen in liquid nitrogen once back on shore within 6 hours of collection. After flash freezing, filters were temporarily stored at -20° C, and transferred to long term -80° C storage upon arrival at Harbor Branch (within 72 hours of collection). All future mucus samples will be collected using the same methods, the exception being when the 5 cm away from disease margin area coincides with the previously created trenches, in which case the mucus will be sampled 1 cm away from the trench on the apparently healthy tissue side.

While they will not be utilized in this study, small (~5 $cm^2$) tissue samples were also collected from each tagged coral colony, directly after mucus sampling. Tissue samples were collected from the same area as the mucus to reduce additional wounding to the colony, and skeletal scars created from sampling tissue were subsequently filled with the respective treatment compound during intervention to prevent the site becoming another opportunity for infection.

Water samples were also collected in a 1 L wide-mouth Nalgene bottle at the bottom of the center of each sampling site ($n$=3). Upon returning to the boat, 100 mL of this water sample was filtered through a sterile 25 mm diameter, 0.2 µm pore size filter in a Swinnex filter holder. The filter was then preserved and stored in the same manner as filtered mucus samples. The remaining water sample was put on ice until storage in -20° C within six hours. All future sampling time points will include water samples collected in this manner at each site. The previously frozen water samples will be used to measure pH, salinity, nitrate, nitrite, ammonium, and phosphate levels. Salinity and pH will be measured in triplicate from each sample using a YSI™ Pro 2030 data sonde, while nitrate, nitrite, ammonium, and phosphate levels will also be measured in triplicate using a spectrophotometer.

## 3. RESULTS

### 3.1. Disease Observations and Sampling

The following quick look reports summarize our disease observations during the course of this project and provide preliminary statistical analyses regarding the relative abundance of coral species at each site where roving diver surveys were conducted.

On November 8, 2018, members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver surveys at each of the three sites (T328, BC1, & FTL4) within Broward

County, recording colonies observed and presence/absence of disease. A total of 325 colonies were observed, 52 (16%) of those were observed as diseased. Of the total colonies observed, 85% were *Montastraea cavernosa*, with the remaining 15% consisting of *Acropora cervicornis, Agaricia agaricites, Colpophyllia natans, Madracis auretenra, Orbicella faveolata, Porites astreoides, Pseudodiploria strigosa, Siderastrea siderea, Solenastrea bournoni,* and *Stephanocoenia intersepta*. Of the 52 diseased colonies observed, 48 were *Montastraea cavernosa*, with one diseased colony observed each of *Agaricia agaricites, Colpophyllia natans, Orbicella faveolata,* and *Porites astreoide*s. The 33 fate-tracked *Montastraea cavernosa* colonies at these sites were revisited and video was captured for 3D model generation and surface area analysis back at the lab.

On December 17, 2018, members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver surveys at each of the three sites (T328, BC1, & FTL4) within Broward County, recording colonies observed and the presence/absence of disease. A total of 484 colonies were observed, 43 (8.9%) of those were diseased. Of the total colonies observed, 75% were *Montastraea cavernosa*, with the second most abundant species being *Siderastrea siderea* at 10% of the total colonies observed. The remaining 15% consisted of *Acropora cervicornis, Agaricia agaricites, Colpophyllia natans, Madracis auretenra, Madracis formosa, Orbicella faveolata, Porites astreoides, Porites porites, Pseudodiploria clivosa, Pseudodiploria strigosa, Siderastrea siderea, Solenastrea bournoni,* and *Stephanocoenia intersepta*. Of the 43 diseased corals, 42 were *Montastraea cavernos*a and one was *Orbicella faveolata*. The 33 fate-tracked colonies at these sites were revisited and video was captured for 3D model generation and surface area analysis back at the lab.

On December 18, 2018, members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver survey at each of the three sites (SEFL04, SEFL05, & SEFL06) within Palm Beach County near Jupiter, FL. The divers recorded colonies observed >10cm in diameter, and the presence/absence of disease. A total of 456 colonies were observed, 7 (1.5%) of which were diseased. Of the total number observed, 77% were *Montastraea cavernosa*, 12% were *Porites astreoides*, and the remaining 11% consisted of *Agaricia agaricites, Colpophyllia natans, Dichocoenia stokesii, Madracis decactis, Siderastrea siderea, Siderastrea radians, Solenastrea bournoni,* and *Stephanocoenia intersepta.* All 7 of the diseased colonies were *Montastraea cavernosa*.

On February 5, 2019, members of the Voss Lab (FAU Harbor Branch) conducted two 20-minute roving diver surveys at each of the three sites (SEFL08, SEFL11, & SEFL12) within Palm Beach County near West Palm Beach, FL. The divers recorded colonies observed >10cm in diameter, and the presence/absence of disease. A total of 471 colonies were observed, none of which were diseased. Of the total colonies observed, 54% were *Montastraea cavernosa*, 15% were *Porites astreoides*, and the remaining 31% consisted of *Agaricia agaricites, Colpophyllia natans, Dichocoenia stokesii, Diploria labyrinthiformis, Eusmilia fastigiata, Favia fragum, Madracis auretenra, Madracis decactis, Meandrina meandrites, Millepora alcicornis, Mycetophyllia ferox, Orbicella annularis, Porites astreoides, Pseudodiploria clivosa, Pseudodiploria strigosa, Siderastrea siderea, Solenastrea bournoni,* and *Stephanocoenia intersepta*.

On March 1, 2019, members of the Voss Lab (FAU Harbor Branch) and Jeff Beal (FWC) conducted one 20-minute roving diver survey at each of the three (of four total) sites (SLR Central, SLR South, & SLR Ledge) within the St. Lucie Inlet State Park in Martin County (SLR North was skipped due to poor conditions). The divers recorded colonies observed >10cm in diameter and the presence/absence of disease. A total of 312 colonies were observed, none of which were diseased. Of the 312 colonies, 70% were *Porites astreoides*, 18% were *Siderastrea radians*, and the remaining 12 percent consisted of *Madracis decactis, Montastraea cavernosa, Oculina varicosa, Pseudodiploria clivosa, Siderastrea siderea,* and *Solenastrea bournoni.*

On March 4, 2019, members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver survey at each of the three sites (T328, BC1, & FTL4) within Broward County near Lauderdale-by-the-Sea, FL. The divers recorded colonies observed >10cm in diameter and the presence/absence of disease. A total of 300 colonies were observed, 23 (7.7%) of which were diseased and 1 was paling. Of the total colonies observed, 68% were *Montastraea cavernosa*. The remaining 32% consisted of *Acropora cervicornis, Agaricia agaricites, Colpophyllia natans, Dichocoenia stokesii, Diploria labyrinthiformis, Madracis auretenra, Orbicella annularis, Orbicella faveolata, Porites astreoides, Pseudodiploria strigosa, Siderastrea radians, Siderastrea siderea, Solenastrea bournoni,* and *Stephanocoenia intersepta*. Of the 23 corals afflicted with SCTLD, 48% were *Montastraea cavernosa*, 22% were *Porites astreoides*, and the remaining 30% consisted of *Agaricia agaricites, Dichocoenia stokesii,* and *Orbicella annularis*. The colony observed as paling was *Psuedodiploria strigosa*.

On March 11, 2019, members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver survey at each of the three sites (SEFL04, SEFL05, & SEFL06) within Palm Beach County near Jupiter, FL. The divers recorded colonies observed >10cm in diameter and the presence/absence of disease. A total of 163 colonies were observed, 14 (8.6%) of which were diseased. Of the total colonies observed, 64% were *Montastraea cavernosa*. The remaining 36% consisted of *Agaricia agaricites, Agaricia fragilis, Colpophyllia natans, Dichocoenia stokesii, Madracis decactis, Meandrina meandrites, Mycetophyllia aliciae, Orbicella faveolata, Porites astreoides, Pseudodiploria strigosa, Siderastrea siderea, Solenastrea bournoni,* and *Stephanocoenia intersepta.* Of the 14 diseased colonies, 8 were *Agaricia agaricites*, 5 were *Montastraea cavernosa,* and 1 was *Mycetophyllia aliciae*.

On March 12, 2019, members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver survey at each of the two (of three total) sites (SEFL11 & SEFL12) within Palm Beach County near West Palm Beach, FL (SEFL08 was skipped due to poor weather). The divers recorded colonies observed >10cm in diameter and the presence/absence of disease. A total of 91 colonies were observed, 4 (4.4%) of which were diseased. Of the total colonies observed, 55% were *Montastraea cavernosa*, 15% were *Porites astreoides*, and the remaining 30% consisted of *Dichocoenia stokesii, Madracis auretenra, Madracis decactis, Meandrina meandrites, Pseudodiploria strigosa, Siderastrea radians, Solenastrea bournoni,* and *Stephanocoenia intersepta*. The 4 diseased

colonies consisted of two colonies of *Montastraea cavernosa*, one colony of *Dichocoenia stokesii*, and one colony of *Porites astreoides*.

On March 26, 2019, two members of the Voss Lab and one volunteer (FAU Harbor Branch) dove at three sites (T328, BC1, & FTL4) within Broward County near Lauderdale-by-the-Sea, FL to monitor the 32 fate-tracked diseased *Montastraea cavernosa* colonies to assess how many would be suitable for the disease intervention experiment. An additional five diseased *Montastraea cavernosa* colonies were tagged, as well as three healthy *Montastraea cavernosa* colonies to supplement the intervention experiment. Ten of the original fate-tracked colonies were found to have quiesced and no longer had any apparent signs of disease, and five colonies had succumbed to the disease. HOBO data loggers were also deployed at each site to record water temperature over the course of the intervention experiment. They remain on site and will be recovered and temperature data downloaded in November 2019.

On April 15, 2019, members of the Voss Lab (FAU Harbor Branch) and Jeff Beal (FWC) conducted three dives at one site (BC1) within Broward County near Lauderdale-by-the-Sea, FL to begin the disease intervention experiment. Fifteen additional diseased *Montastraea cavernosa* colonies were tagged and three healthy *Montastraea cavernosa* were tagged. Nine diseased colonies were treated, four with chlorine epoxy and five with amoxicillin/Base 2b. Surface mucus and small tissue samples were collected from the nine treated colonies, four diseased untreated controls, and four healthy untreated controls, for a total of seventeen colony samples. An ambient water sample was collected at the time of coral sample collection. Videos for 3D models were taken for all diseased colonies which will be fate-tracked in the experiment.

On April 16, 2019, members of the Voss Lab (FAU Harbor Branch) conducted four dives at two sites (BC1 & FTL4) within Broward County near Lauderdale-by-the-Sea, FL to continue the initial intervention experiment. Four additional diseased *Montastraea cavernosa* colonies were tagged at one site. Eight diseased *Montastraea cavernosa* were treated, five with chlorine, three with amoxicillin/Base 2b. Mucus and small tissue samples were also collected from each of these colonies, for a total of eight tissue samples and eight mucus samples. An ambient water sample was collected at the time of coral sample collection. Videos for 3D models were taken for all diseased colonies which will be fate-tracked in the experiment.

On April 17, 2019, members of the Voss Lab and one volunteer (FAU Harbor Branch) conducted three dives at one site (T328) within Broward County near Lauderdale-by-the-Sea, FL to finish the initial interventions. Three additional diseased and three healthy *Montastraea cavernosa* colonies were tagged. Six diseased *Montastraea cavernosa* were treated, three with chlorine epoxy and three with amoxicillin/Base 2b. Small tissue samples and mucus samples were collected from the six treated colonies, six diseased and untreated controls, and four healthy controls, for a total of 16 tissue and 16 mucus samples. An ambient water sample was collected at the time of coral sample collection. Videos for 3D models were taken for all diseased colonies which will be fate-tracked in the experiment.

On April 25, 2019, members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver survey at each of the three (of four total) sites (SLR North, SLR Ledge, & SLR South) within St. Lucie Inlet State Park in Martin County. Divers recorded colonies observed >10cm in diameter and the presence/absence of disease. A total of 21 corals were observed, 4 of which were diseased and 1 was partially bleached. At the three sites the divers observed 1 *Montastraea cavernosa*, 7 *Pseudodiploria clivosa*, 12 *Siderastrea radians*, and 2 *Solenastrea bournoni*. Of the 4 diseased colonies, 3 were *Pseudodiploria clivosa* and 1 was *Solenastrea bournoni*. 1 *Siderastrea radians* was partially bleached.

On May 6, 2019, members of the Voss Lab (FAU Harbor Branch) and Jeff Beal (FWC) conducted two dives at one site (BC1) within Broward County near Lauderdale-by-the-Sea, FL to conduct the first follow up monitoring from disease intervention. Eighteen tissue and mucus samples were collected from the diseased treated, diseased untreated, and healthy *Montastraea cavernosa* colonies in the experiment. An ambient water sample was collected at the time of coral sample collection. Videos for 3D models were collected for all diseased colonies in the experiment.

On May 7, 2019, members of the Voss Lab (FAU Harbor Branch) conducted one dive at each of the other two sites, T328 & FTL4, involved in the intervention experiment in Broward County near Lauderdale-by-the-Sea, FL to continue the first round of monitoring/follow up from the initial interventions. Twenty-one tissue and mucus samples were collected from the diseased treated, diseased untreated, and healthy *Montastraea cavernosa* colonies in the experiment. An ambient water sample was collected at the time of coral sample collection. Videos for 3D models were collected for all diseased colonies in the experiment. At each of these sites as well, divers conducted one 20-minute roving diver survey. Divers recorded colonies observed >10cm in diameter and the presence/absence of disease. A total of 224 colonies were observed, 18 (8.0%) of which were diseased. Of the colonies observed, 63% were *Montastraea cavernosa*. The remaining 37% consisted of *Acropora cervicornis, Agaricia agaricites, Dichocoenia stokesii, Orbicella faveolata, Porites astreoides, Porites sp., Pseudodiploria clivosa, Pseudodiploria strigosa, Siderastrea siderea, Solenastrea bournoni,* and *Stephanocoenia intersepta*. Of the 18 diseased corals, 17 were *Montastraea cavernosa* and 1 was *Siderastrea siderea*.

On May 14, 2019, members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver survey at each of the three sites (SEFL08, SEFL11, & SEFL12) within Palm Beach County near West Palm Beach, FL. Divers recorded colonies observed >10cm in diameter and the presence/absence of disease. A total of 155 colonies were observed, none of which were diseased. Of the 155 colonies observed, 63% were *Montastraea cavernosa*, 25% were *Porites astreoides*, and the remaining 12% consisted of *Dichocoenia stokesii*, *Madracis auretenra, Madracis decactis, Meandrina meandrites, Mycetophyllia aliciae, Orbicella faveolata, Pseudodiploria strigosa, Siderastrea siderea,* and *Stephanocoenia intersepta*.

On May 15, 2019, members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver survey at each of the three sites (SEFL04, SEFL05, SEFL06) within

Palm Beach County near Jupiter, FL. Divers recorded colonies observed >10cm in diameter and the presence/absence of disease. A total of 342 colonies were observed, 1 (0.3%) of which was diseased. Of the 342 colonies observed, 74% were *Montastraea cavernosa*, the remaining 26% consisted of *Agaricia agaricites, Agaricia lamarcki, Dichocoenia stokesii, Helioseris cucullata, Madracis decactis, Oculina diffusa, Orbicella faveolata, Porites astreoides, Pseudodiploria strigosa, Siderastrea siderea,* and *Stephanocoenia intersepta*. The 1 diseased colony was *Siderastrea siderea*.

### 3.2. Fate Tracking Prior to Intervention

To understand how disease progression differed on each fate-tracked colony, we focused on *M. cavernosa* colonies in the Lauderdale-by-the-Sea/ Pompano sites. Proportions were created with disease area and heathy tissue divided by total colony area at $T_1$. Tissue loss was calculated by subtracting the sum of the proportion of diseased and healthy tissue at time n from the sum of the proportion of diseased and healthy tissue at $T_1$. Univariate repeated measures permutational multivariate analysis of variance showed significant differentiation across date of the proportion of healthy tissue (PERMANOVA, $df_{1.655}$, $p < 0.001$) and with tissue loss (PERMANOVA, $df_{1.653}$, $p < 0.001$). A permutational multivariate analysis of variance showed that the proportion of tissue area was differentiated through time (PERMANOVA, $R^2 = 0.15358$, $p = 0.001$). Further pairwise comparisons using Bonferroni corrections with 999 permutations showed that $T_1$ was significantly different from $T_3$ ($R^2 = 0.21057750$, $p = 0.003$, Fig. 3).

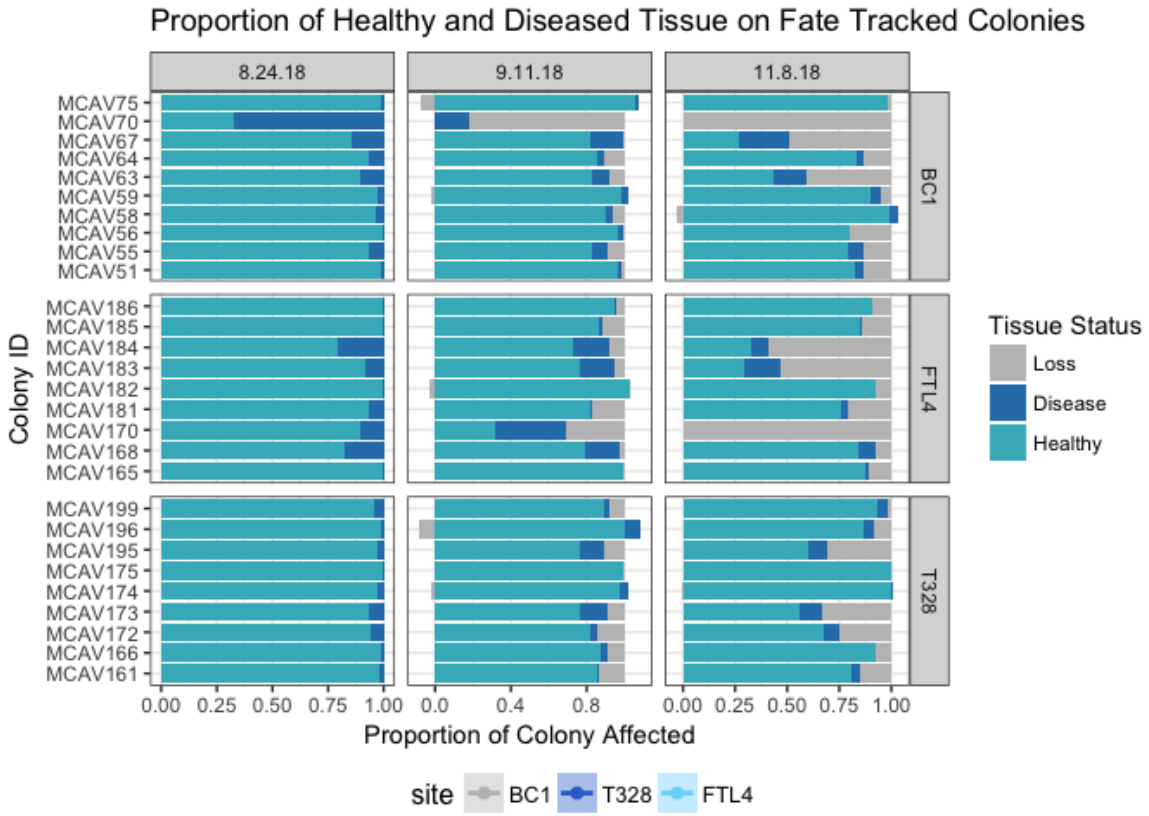



Figure 3. Proportion of healthy and diseased tissue on fate tracked colonies. Bar graphs indicating the relative proportions of disease tissue, healthy tissue, and tissue loss over time

for each colony among the three sites. Colonies with completed and verified 3D models are shown.

### 3.3. Intervention Trials

Due to challenges with sea state conditions and weather, follow up monitoring of the intervention experiment at the Lauderdale-by-the-Sea sites was completed on May 6-7, May 20-21, and June 21, three, five, and nine weeks after interventions, respectively.

At the 3-week time interval three of the eleven amoxicillin treated corals still had active lesions, and eight of eleven chlorine treated still had active lesions. All the lesions present on these corals were still contained within the trench barrier. One chlorine treated colony was unable to be relocated on this date.

At 5 weeks, again the same three out of the eleven amoxicillin treated corals still had active lesions, and all the lesions present on these corals were still contained within the trench barrier. One of those three amoxicillin treated corals had a new lesion outside from the treatment area. Nine of eleven chlorine treated still appeared to have active lesions. Three of those nine chlorine treated colonies had new lesions outside of the treatment areas. One chlorine treated colony was unable to be relocated on this date.

At 9 weeks, three of the eleven amoxicillin treated colonies appeared to have new active lesions outside of the treatment areas, however all treated lesions which had been trenched were all fully healed. Four of the twelve chlorine treated colonies appeared to have new active lesions outside of the treatment areas, and ten of the twelve's trenches had failed, with the lesions surpassing them in at least one area by at least 1 cm.

A chi-square analysis testing the assumption that each treatment would have equal rates of success/failure indicated that the probability of success or failure (i.e. the lesion progression beyond the trench) is not similar between treatments ($\S_2^{(1)} = 8.17$, $p < 0.01$), with amoxicillin treated coral colonies having a greater likelihood. Similarly, amoxicillin treated colonies were less likely to demonstrated active lesions ($\S_2^{(1)} = 6.39$, $p < 0.05$).

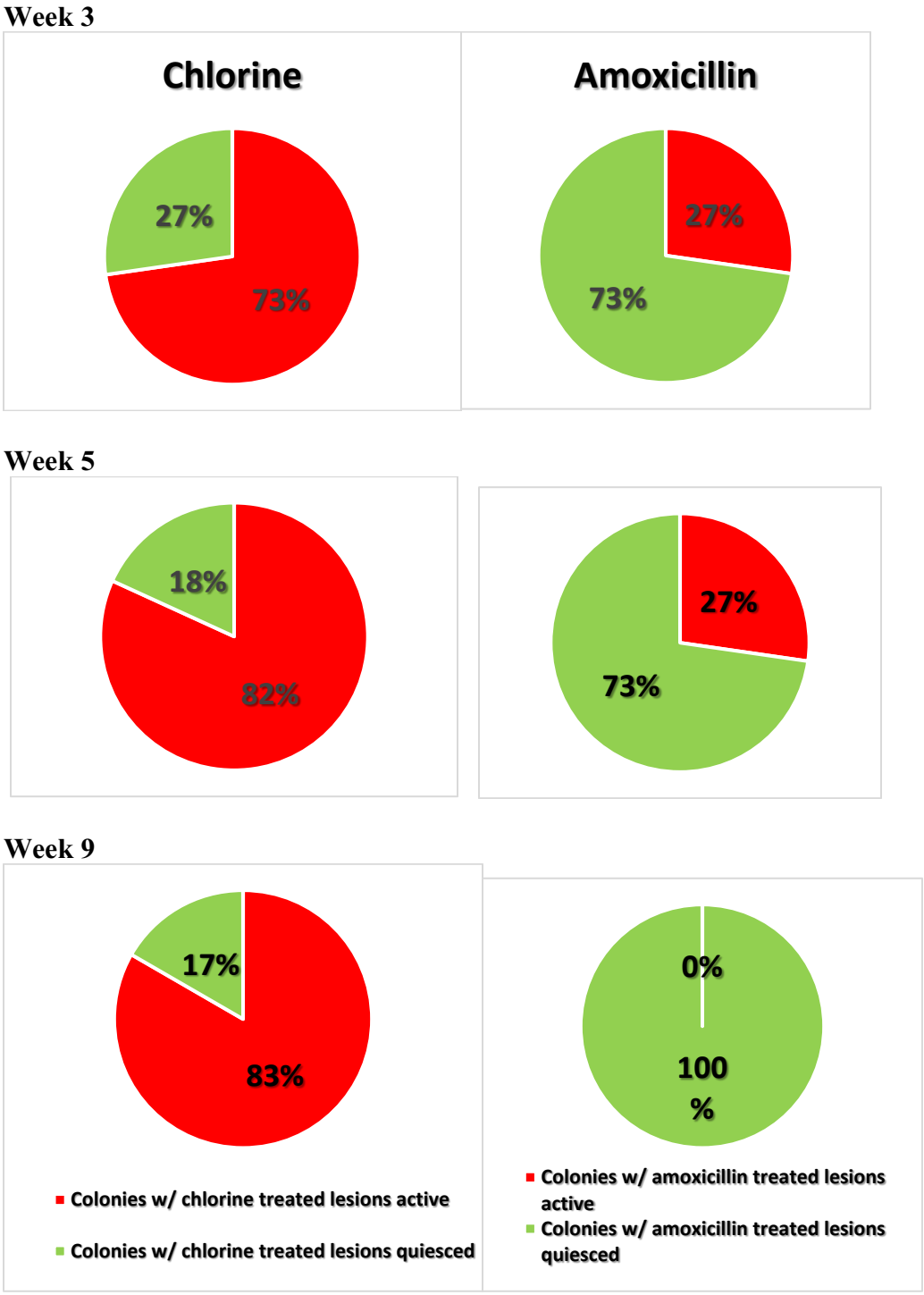



Figure 4 Proportion of chlorine- (left hand charts) and amoxicillin-treated (right hand charts) coral colonies at each monitoring time point, three weeks after intervention, five weeks after intervention, and nine weeks after intervention.

### 3.4. Coral and Disease Sample Summary

Coral tissue and mucus samples were collected at the initial and 3-week time points for each of the experimental and control colonies in the intervention experiment. As a result 80 tissue samples and 82 coral mucus samples have been preserved. Diseased samples will be analyzed using a Tag-Seq transcriptomic analyses with Illumina HiSeq and DESeq2 to quantify differential gene expression. Mucus samples will be analyzed to determine potential effects of the intervention treatments on coral microbial communities. These analyses, funded through support from a Harbor Branch Foundation Fellowship to Erin Shilling, are ongoing and complementary to the scope of work for this FDEP project.

## 4. PRELIMINARY CONCLUSIONS

This study demonstrated that tissue loss disease incidence and prevalence may be highly variable over space and time on coral reefs in SE FL. For example, while stony coral tissue loss disease and bleaching were observed continually throughout the project period among corals at St. Lucie Reef and Lauderdale-by-the-Sea, for most of 2019 disease prevalence was relatively low in the Palm Beach County sites. Perhaps these observations are indicative of the timing and progression of this disease. While coral populations in Palm Beach County sites appear to maintain some diversity in the coral communities after disease, the relative loss of corals and coral cover at St. Lucie Reefs is far greater based on analyses of the roving diver surveys.

Previously, we hypothesized that St. Lucie Reef may have been buffered from tissue loss impacts by 1) relative distance from other infected coral communities, and/or 2) stress hardened coral colonies resistant to disease. However, the observation of high disease prevalence and up to 83% losses of coral colonies counter these hopeful hypotheses. The losses at St. Lucie Reef cannot be attributed to disease impacts alone. During the time of these losses impacts from Hurricane Irma and subsequent discharges from the St. Lucie Estuary were also critical drivers that contributed to a severe multiple stressors scenario. The temporal confounding of these events makes interpretation of the proximal causes of coral loss difficult.

With respect to the goals of 1) reducing coral tissue loss, 2) reducing the likelihood of total colony mortality, the intervention trials have demonstrated that the amoxicillin/ Base 2b treatments were far more effective than the chlorine/ epoxy treatment against SCTLD lesions on *M. cavernosa*, and more effective than untreated controls (analyses ongoing). While there are challenges in applying this treatment, including a 15-20 minute time investment per coral and the neutral buoyancy of the base, the treatment appears effective. However, the physiological mechanism making this treatment effective is unknown at this time. The Base 2b by itself was not tested in this design and may be successful if applied alone with antibiotics. Though the amoxicillin treatment was effective, broad scale application of antibiotics on coral reefs may prove challenging given the scope and scale required. At this time it is unclear if the intervention treatments assessed in this study achieve the additional goal of 3) reducing the probability of transmission to nearby colonies. We have observed a drop in disease incidence over time at our study sites, but this may be attributed to loss of potential hosts rather than intervention directly reducing the likelihood of disease transmission. Additional studies to assess modes of transmission coupled with intervention tests may address goal 3. At least in the short term, the Base 2B

plus amoxicillin treatments appear promising with respect to goal 4) reducing population declines in known areas of infection.

## 5. RECOMMENDATIONS

***Recommendation 1****: Ongoing efforts to identify tissue loss disease agent(s) should be coupled with efforts to identify the etiological mechanisms driving pathogenicity.* Coordinated efforts to share both environmental and experimental samples among multiple researchers aid these complementary goals and can be facilitated by the DAC email communications and calls. We will be using EPA and NOAA OAR support to investigate the transcriptomes of affected corals and potentially identify signatures of physiological responses of the corals when affected by disease. Ideally the same samples will also be assessed using histological and bacteriological methods.

***Recommendation 2****: Because the prevalence of many coral diseases is known to correlate positively with temperature, high frequency monitoring at key sites during periods of thermal stress should be a priority.* Given the speed and severity of tissue loss disease, more frequent monitoring is needed to understand the impacts on Florida's coral communities and to direct any potential mitigation efforts (see below).

***Recommendation 3****: Continue investment in disease mitigation strategies and testing to reduce losses of key ecosystem components.* Base 2b plus amoxicillin demonstrated success against SCTLD lesions on *M. cavernosa* with a 100% success rate after 9 weeks. However, new lesions can arise, and broad scale application of antibiotics may not be advisable or scalable. We recommend testing Base 2b alone in *ex situ* and *in situ* settings over a minimum eight-week period (based on preliminary observations from the current intervention experiment), and testing euthanizing infected colonies in areas where SCTLD has just begun to infect coral communities.

***Recommendation 4****: Advance coral conservation initiatives with support from Magnuson-Stevens Act and implement actions/regulations for the Southeast Florida Coral Reef Ecosystem Conservation Area.* The threat posed to Florida's coral reefs by the tissue loss disease are severe. Any additional efforts to reduced stressors or known impacts to coral reef communities should be implemented to enhance the likelihood of coral resilience and recovery, particularly with respect to water quality. Furthermore, efforts to develop more robust coral restoration programs should include research toward sexual propagation, *ex situ* and *in situ* nurseries, and subsequent outplanting. However, until methods to mitigate the effects of SCTLD are determine, we recommend against broad scale outplanting efforts. It would not be worthwhile to outplant numerous susceptible fragments onto any of Florida's coral reefs.

***Recommendation 5:*** *To support effective management for coral reef populations and communities in Florida, additional information on population connectivity and source-sink dynamics is needed.* After severe disturbance events like the tissue loss disease outbreak, allocated effort/ resources to particular regions should be based on predicted coral recruitment and recovery. Information about natural recruitment rates and recruit

survivorship are needed to assess population dynamics and inform recovery strategies. Likewise, effective coral restoration strategies will require knowledge of genetic stocks among various coral populations.

## 6. LITERATURE CITED

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