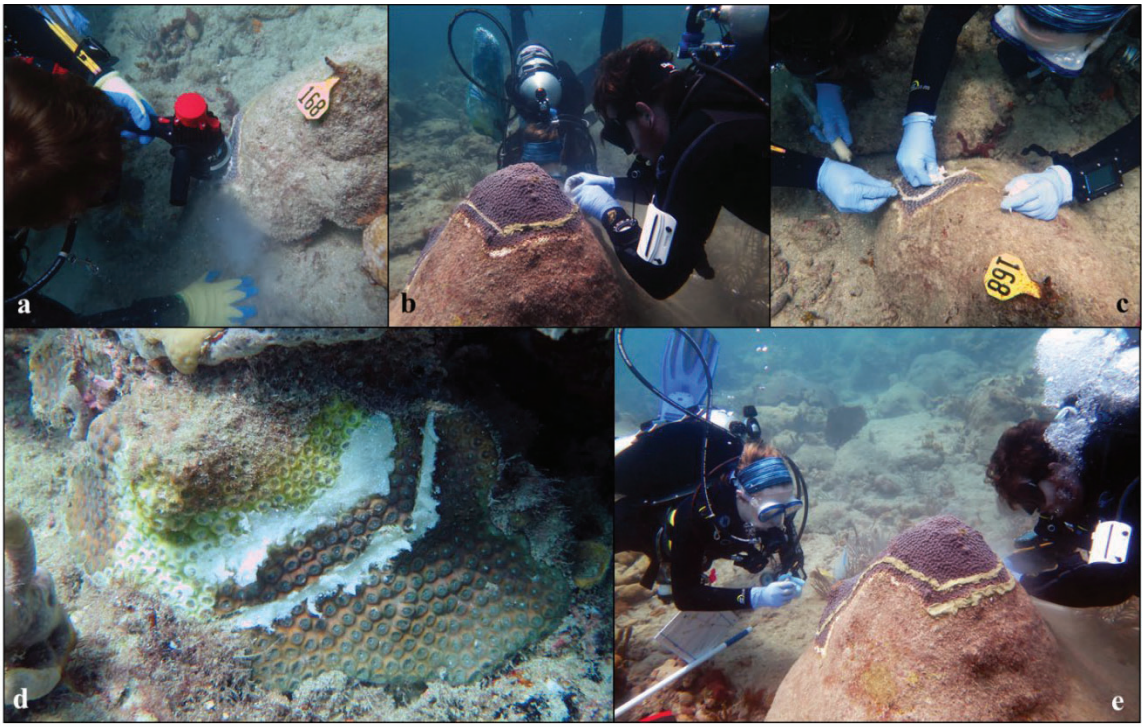
2020
Intervention and fate tracking for corals affected by stony coral tissue loss disease in the northern section of Florida's Coral Reef



Florida Department of Environmental Protection
Office of Resilience and Coastal Protection

Intervention and fate tracking for corals affected by stony coral tissue loss disease in the northern section of Florida's Coral Reef

Final Report

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1. EXECUTIVE SUMMARY

Florida's Coral Reef is currently experiencing a multi-year outbreak of a coral disease described as stony coral tissue loss disease (SCTLD). In support of the local, state, and federal responses to SCTLD, since 2015 Florida Atlantic University's Harbor Branch Oceanographic Institute researchers have investigated the etiology and impacts of SCTLD, developed new tools to track and characterize SCTLD infections, and experimentally tested potential methods for treating SCTLD. The annual activities presented in this report include monitoring coral disease prevalence and impacts in the northern portion of Florida's Coral Reef and experimental tests of 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. Furthermore, through advanced molecular analyses of gene expression, this project was designed to determine the effects of SCTLD and antibiotic intervention treatments on both corals and their algal symbionts.

This study demonstrated that SCTLD incidence and prevalence may be highly variable over space and time on coral reefs in Southeast Florida. For example, while SCTLD and bleaching were observed continually throughout the project period among corals at our Lauderdale-by-the-Sea sites, for most of 2019 and early 2020 disease prevalence was relatively low in the Palm Beach County sites. Previously, we hypothesized that St. Lucie Reef may have been buffered from SCTLD 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 at St. Lucie Reef have countered these hopeful hypotheses.

Our SCTLD intervention experiment demonstrated that Base 2B plus amoxicillin treatment was significantly more effective at treating individual SCTLD lesions on *Montastraea cavernosa* coral colonies than chlorinated epoxy treatment or leaving the SCTLD-affected colonies untreated. In instances where time and effort underwater are constrained, application of Base 2B plus amoxicillin to more SCTLD affected colonies should be prioritized over supplementing the antibiotic treatments with trenching. However, the antibiotic treatment does not prevent the colony from developing new SCTLD lesions in other locations over time, reinforcing the need for repeated antibiotic treatments to effectively halt SCTLD impacts on a colony. Additional empirical research and controlled studies are needed to determine if there are regional, temporal, and/or species-specific influences on intervention treatment success.

The success of Base 2B plus amoxicillin treatments is encouraging in the face of a disease outbreak that is continuing to devastate Caribbean coral reefs. However, potential secondary impacts of amoxicillin treatments on SCTLD-affected corals remain uncharacterized. We recommend that future research efforts focus on assessing the potential unintended consequences of antibiotic treatments on corals, their microbial communities (including Symbiodinaceae), and neighboring organisms. Additionally, further efforts are needed to optimize dosing and delivery methods for antibiotic treatments on SCTLD-affected corals and scale up intervention treatments effectively.

2. 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's Coral Reef. 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 northern section of Florida's Coral Reef 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 NOAA OAR Omics program to FAU Harbor Branch, as well as graduate student support from the Harbor Branch Oceanographic Institute Foundation.

2.1. 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, and the physiological responses of corals to disease.

Four main objectives were established for FY20:

1. Continued monitoring of SCTLD dynamics in the northern section of Florida's Coral Reef.
2. Fate tracking of affected corals in areas with relatively high SCTLD prevalence. (Note that objectives 1 and 2 are combined under "Field Logistics" in project scope of work Task 2)
3. Continued experimental intervention efforts to determine the efficacy and impact of both chlorinated epoxy and Base 2b plus amoxicillin as compared to SCTLD controls and apparently healthy corals.
4. Investigation of gene expression profiles through holobiont transcriptome analyses to determine the effects of SCTLD and antibiotic intervention treatments on coral and *Symbiodinaceae* physiology.

The outcomes of this project contribute to on-going and future coral disease response efforts which seek to improve understanding about the severity and impacts of the Florida's Coral Reef 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 state and federal 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.

3. METHODOLOGY

This project combined repeated surveys, 3D imaging, experimental disease intervention, and coral sampling to provide data on SCTLD dynamics, intervention success, and corals' physiological responses. Table 1 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 continuous time-series monitoring in these locations. Broward County sites were chosen due to their relatively high stony coral and SCTLD abundance.

This project was significantly affected by the COVID-19 pandemic. Operational diving activities and laboratory access were limited by various state, local, and university procedures. Only university designated essential personnel and essential projects were permitted from March 2020-August 2020. As a result, we focused available staff and field capability on fate tracking, experimental intervention, and gene expression efforts during this time. During COVID restrictions, SCTLD roving diver surveys were deprioritized at Martin and Palm Beach county sites where SCTLD prevalence was low or absent as of Feb 2020.

Table 1.

Operational Activities at Each Project Site					
Site Name	Lat	Long	County	Activity	Dates
SLR North	27° 08.777'	-80° 08.350'	Martin	Surveys	7/19/19, 11/25/19
SLR Central (SEFL01)	27° 07.900'	-80° 08.042'	Martin	Surveys	7/19/19, 11/25/19
SLR South (SEFL02)	27° 07.286'	-80° 07.650'	Martin	Surveys	7/19/19, 11/25/19
SLR Ledge	27° 06.712'	-80° 07.531'	Martin	Surveys	7/19/19, 11/25/19
SEFL04	26° 56.6225'	-80° 1.3183'	Palm Beach	Surveys	7/3/19, 8/5/19, 10/22/19, 2/20/20
SEFL05	26° 55.6467'	-80° 1.8060'	Palm Beach	Surveys	7/3/19, 8/5/19, 10/22/19, 2/20/20
SEFL06	26° 53.8641'	-80° 0.9830'	Palm Beach	Surveys	7/3/19, 8/5/19, 10/22/19, 2/20/20
SEFL08	26° 42.6260'	-80° 0.9490'	Palm Beach	Surveys	10/22/19, 1/30/20
SEFL11	26° 40.7100'	-80° 1.0950'	Palm Beach	Surveys	10/22/19, 1/30/20
SEFL12	26° 39.1432'	-80° 1.2409'	Palm Beach	Surveys	10/22/19, 1/30/20
T328	26° 10.567'	-80° 05.633'	Broward	Surveys[1], Intervention Treatments[2], [4] Intervention Follow Up[3], & Samples	[4] [4] 7/26/19[13], 9/26/19[8], 10/10/19[1], 1/16/20[1], 3/4/20[3], 3/12/20[1], 4/21/20[2] , 5/4/20[3]
BC1	26° 08.855'	-80° 05.766'	Broward	Surveys[1], Intervention Treatments[2], [4] Intervention Follow Up[3], & Samples	[4] [4] 7/26/2019[13], 9/26/19[8] , 10/10/19[1], 1/16/20[1], 3/4/20[3], 3/12/20[1], 4/21/20[2] , 5/4/20[3]
FTL4	26° 08.197'	-80° 05.843'	Broward	Surveys[1], Intervention Treatments[2], [4] Intervention Follow Up[3], & Samples	[4] [4] [4] 7/26/2019[13], 9/26/19[8] , 10/10/19[1], 1/16/20[1], 3/4/20[3], 3/12/20[1], 4/21/20[2] , 5/4/20[3]

3.1. SCTLD Dynamics on the Northern Section of Florida's Coral Reef

Four locations across the northern section of Florida's Coral Reef were selected for coral health and disease surveys: St. Lucie Reef, Jupiter, Palm Beach, and Lauderdale-by-the-Sea (Fig 1) St. Lucie Reef is located in Martin County, Jupiter and Palm Beach are both located in Palm Beach County, and Lauderdale-by-the-Sea is located in Broward County. Following Hurricane Irma in September 2017, a rapid-response damage and disease survey effort was completed throughout Southeast Florida (Walker 2018). The resulting data from these initial surveys were used to inform decisions of which sites to target for continued monitoring and fate-tracking.

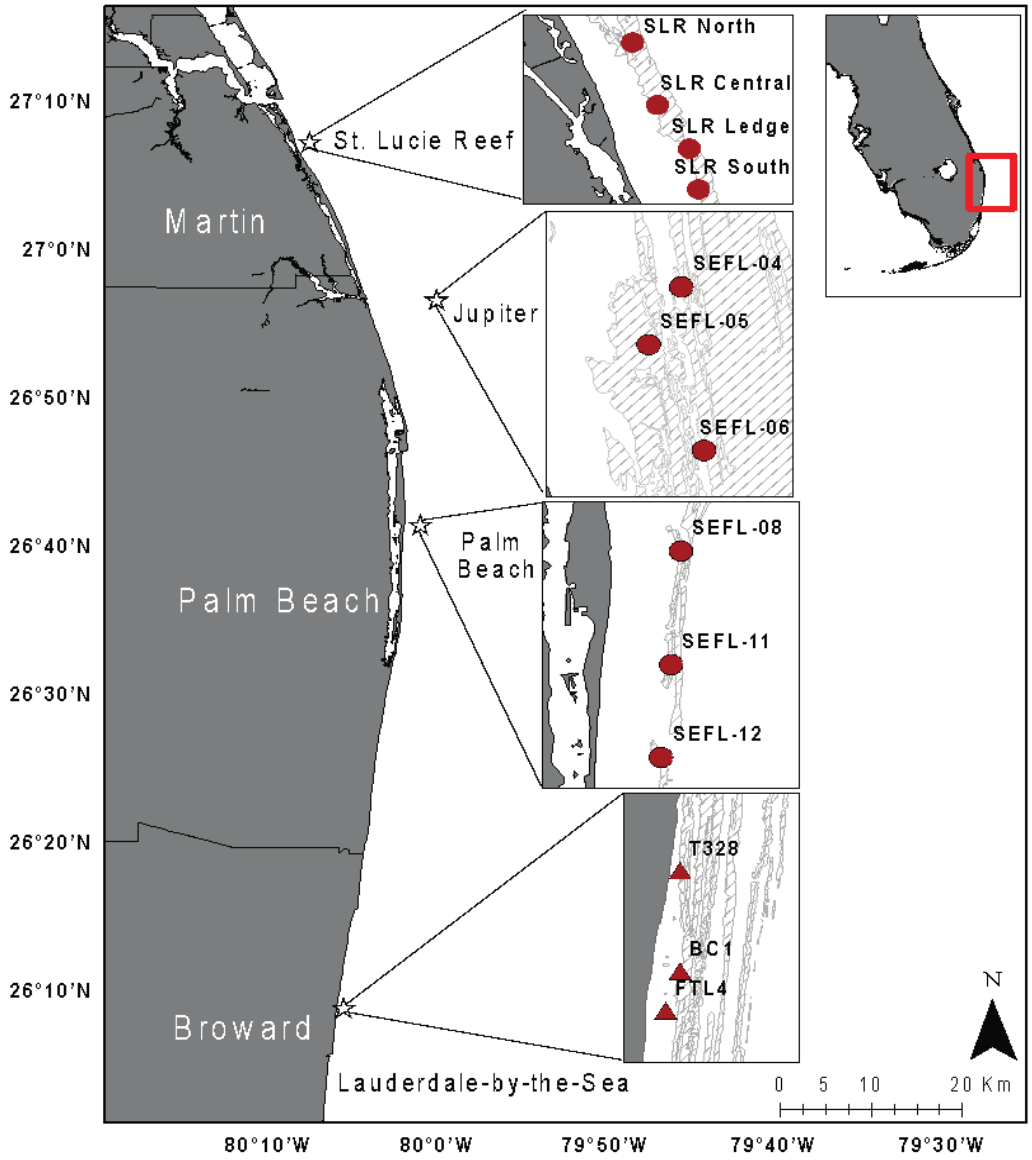


Fig 1. Map of study locations throughout the northern section of Florida's Coral Reef. Red circles indicate roving diver survey sites and red triangles indicate sites where both roving diver surveys and coral fate-tracking occurred.

Roving diver disease surveys were conducted to assess the greatest reef area possible, quantifying disease prevalence over an estimated range of 100–2000 m2 per survey based on conditions, principally underwater visibility. SCUBA divers swam for 20 min and recorded the species and disease status of every living coral colony ≥ 10 cm in diameter. Paling, partial bleaching, and bleaching were also noted within surveys. From those data, SCTLD abundance and prevalence, species diversity, and species richness were calculated. Statistical tests were run in the R statistical environment. Datasets were non-normal and normal distributions could not be achieved through transformation; therefore, non-parametric tests were implemented for all analyses unless otherwise noted. Permutational analysis of variance (PERMANOVA; 9999 permutations) was used in the package *vegan* to assess variation in disease prevalence among sites and survey times, with Bonferroni corrected pairwise comparisons using the package *pairwiseAdonis*.

3.2. Coral Fate Tracking and Imaging

Benthic survey data indicated that disease incidence was too low at Jupiter and Palm Beach sites, and that coral abundance was too low at sites in St. Lucie Reef, for a statistically robust fate-tracking study in these locations. Three fate-tracking sites (T328, BC1, and FTL4) were established in Lauderdale-by-the-Sea where sufficient disease incidence and coral abundance were present. These three sites are ~12 km from the nearby Hillsboro Inlet, less than 500 m from shore, and have been previously used for benthic and coral monitoring and surveys (Fig 1). *Montastraea cavernosa* was selected for targeted colony fate-tracking due to the abundance of infected colonies within the study sites. This coral species is considered intermediately susceptible to SCTLD, with onset of tissue loss occurring weeks to months later than highly susceptible species (e.g. *Dendrogyra cylindrus, Meandrina meandrites, Colpophyllia natans*). Lesions on infected *M. cavernosa* generally progress slower than highly susceptible species, with mortality occurring within months to years.

Colonies of *M. cavernosa* affected with SCTLD were tagged with individually numbered cattle tags across the three sites (T328, BC1, FTL4; Fig 1). *In situ* observations made for each colony included the presence/absence of disease, the number of disease lesions, and diver-based in situ estimates of percent mortality. Photographs were taken of each colony along with continuous video for 3D model generation to quantitatively measure total colony surface area and disease lesion area for each time point.

Fate-tracked colonies were filmed using methods outlined in Young et al. (2017), with the following modifications: Canon G16 cameras in Fantasea underwater housings were set on "Underwater mode," 1080p and 60 frames per second (fps), and exposure was adjusted as needed based on ambient light conditions. One-meter, L-shaped PVC scale bars marked at 10 cm increments were placed at opposing right angles to frame the designated colony. A SCUBA diver maintained approximately 1 m altitude above the highest point of each coral colony and recorded continuous video while swimming repeated linear, parallel, adjacent passes in a lawnmower pattern with the camera pointed directly downward. The number of adjacent passes varied depending on colony size, with 60–70% overlap between passes to aid in downstream model generation. The camera was rotated 90° at the end of the first set

of adjacent passes, then another set of passes was completed perpendicular to the first set. The two complete sets of passes for a single colony required between 1–3 min depending on colony size. Each sampling event produced an average of 14 GB of .mp4 video files, or approximately 425 MB of video files per coral colony.

Video processing and 3D model generation protocols are described in full in our GitHub repository (https://github.com/icombs2017/analysisOf3dModels). In summary, the free software package, FFmpeg (www.ffmpeg.org),was used to extract still frame images from videos of the fate-tracked colonies at a rate of 3 fps. Still images were then imported into AgiSoft Metashape (AgiSoft LLC) software, which uses a proprietary algorithm that incorporates SfM and Brown's lens distortion model to generate 3D models from 2D images. Model generation in Metashape was conducted according to the manufacturer's protocol in four general steps: 1) camera alignment, 2) dense point cloud generation, 3) mesh generation, and 4) texture overlay. Models were rendered on a 2018 Apple MacBook Pro with a 2.9 GHz processor, 16GB of RAM and a Radeon Pro Vega 16 4GB graphics card. A single model took approximately 40 min to render depending on the number of still images generated. Generated models were then exported as an .obj file and imported into the software Rhinoceros 3D (Robert McNeel & Associates) for analysis; the mean model file size was 64 MB.

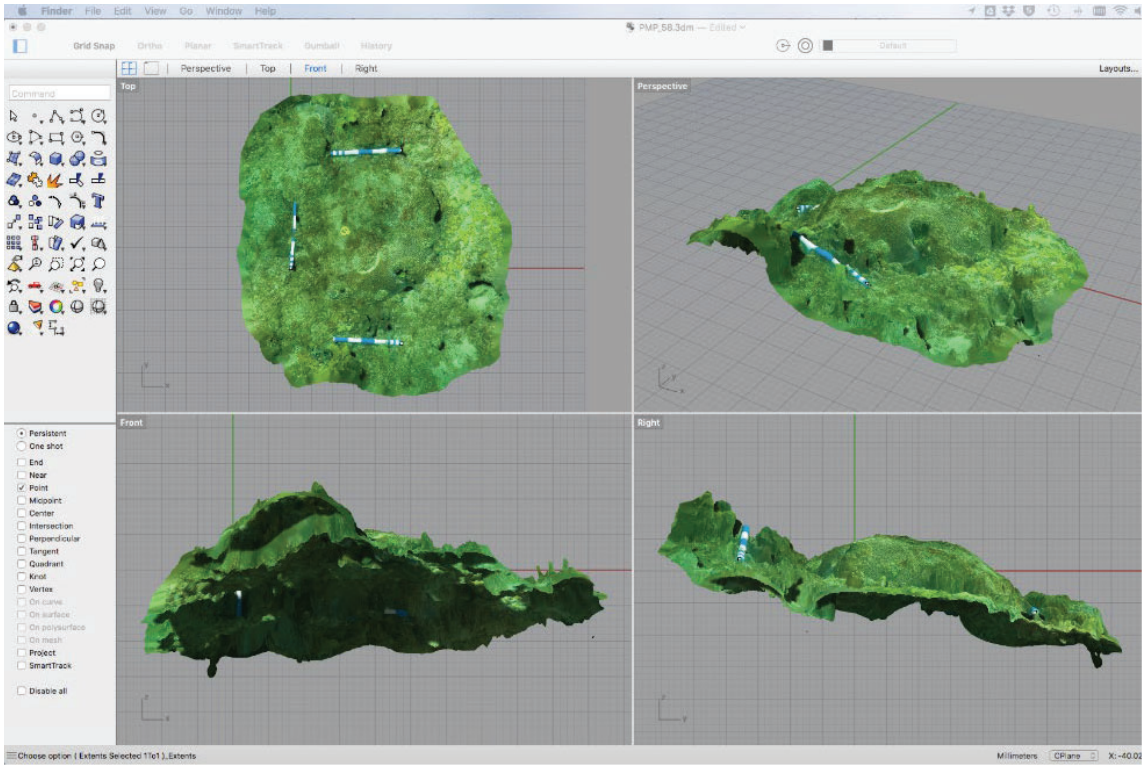


Figure 2. 3D models for each coral colony were constructed and surface areas for healthy tissue, lesions, and bare skeleton were calculated using Rhino 3D. An example model is shown above in the software module.

Models were scaled using the PVC scale bars, then total colony surface area and disease lesion surface area were measured with Rhinoceros 3D, where total colony area included both healthy and diseased coral tissue. SCTLD disease lesion area was considered as the stark white coral tissue, and both total colony area and disease lesion area were generated directly within Rhinoceros 3D, while healthy tissue area was calculated by subtracting disease lesion area from total colony area. Rate of tissue loss per week and change in disease tissue area per week were calculated for each pair of time points. Additionally, lesion count was derived from the number of polygons created when tracing the disease lesion area.

3.3. Experimental Interventions and Monitoring

Experimental interventions were focused across three sites (T328, BC1, and FTL4 Figure 2) located <500m offshore of Lauderdale-by-the-Sea and Pompano Beach in Broward County. In total, 41 *M. cavernosa* colonies were tagged for this experiment: 32 SCTLD-affected colonies and 9 apparently healthy colonies. Due to the varying abundances of SCTLD-affected and healthy colonies at each of the three sites, an attempt was made to balance the intervention treatment groups across sites. SCTLD-affected colonies were divided into three groups: 1) amoxicillin treatment (n = 11); 2) chlorine treatment (n = 11); 3) untreated control group (n = 10). The apparently healthy colonies (n = 9) were used as controls to assess incidence of SCTLD in the natural population and potential non-SCTLD-associated mortality in the region. Only visibly healthy corals that also showed no signs of previous tissue loss (characterized by bare or recently algae-colonized skeleton) were selected as healthy controls.

All intervention treatments were initiated with the creation of trenches around all SCTLD lesions on the colony. Trenches were cut approximately 1 cm deep x 1 cm wide and ~5 cm away from the disease margin using a Nemo™ underwater angle grinder (Figure 3a), creating a buffer of apparently healthy tissue to prevent potential SCTLD progression through sub-surface tissues (NOAA 2018).

The CoreRx/Ocean Alchemists Base 2B plus amoxicillin treatment was created by combining the Base 2B with the powdered amoxicillin trihydrate (Phytotech Labs) in a 10:1 mass ratio mixture. The amoxicillin was mixed into the Base 2B on the research vessels immediately before dives, as recommended by CoreRx. Underwater, the mixture was packed into the trenches and spread over the entirety of each SCTLD lesion (Figure 3c & d).

The chlorinated epoxy treatment included Poolife™ Turboshock© chlorine powder and ZSPAR A-788 Splash Zone™ two-part epoxy combined in an approximate volumetric ratio of 3:10 mL chlorine powder:part A epoxy (Figure 3e). This mixture was subsequently combined and thoroughly mixed with the part B epoxy in equal proportions underwater immediately before application to the colony's trenches and lesions as described above. Z-spar is designed to set within two hours after application and be completely hardened within 6–8 hours. Therefore, it is estimated that releases of chlorine from the epoxy occurred for a maximum 8-hour period following application.

Scaled photographs were taken of each experimental *M. cavernosa* colony immediately before and after the intervention treatments were applied. Additionally, videos were recorded for 3D model generation to measure initial colony size of all experimental colonies.

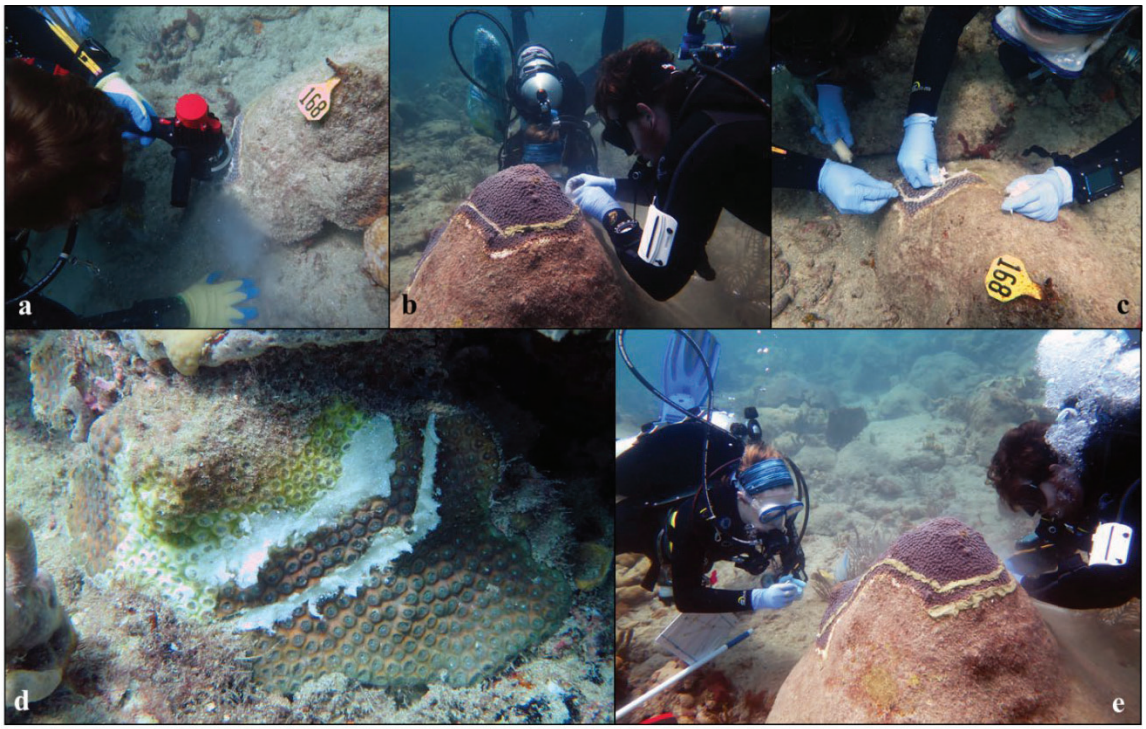


Figure 3. Process of treating SCTLD-affected coral colonies in situ. (a) Diver creating a trench around the SCTLD lesion using an angle grinder. (b) Filling a trench with chlorinated epoxy treatment. (c) Filling a trench with a Base 2B plus amoxicillin mixture. (d) A SCTLD-affected coral colony that has been treated with Base 2B plus amoxicillin mixture. (e) A SCTLD-affected coral colony partially treated with the chlorinated epoxy.

Experimental coral colonies were revisited six times over an 11-month period following the application of intervention treatments at 3, 5, 9, 14, 23, and 46 weeks after treatment. At each monitoring time point, SCTLD activity, number of lesions, and tissue presence or absence beyond the initially trenched area were recorded and scaled photographs were taken for each experimental colony. Colony SCTLD status was categorized as one of the following: a) “diseased”: visible lesions and active tissue loss occurring on the colony, b) “quiesced”: the colony was previously diseased but at the time of inspection had no visible lesions or active tissue loss, c) “dead”: no live tissue, healthy or diseased, was remaining from the original colony, or d) “healthy”: this only pertained to the healthy control colonies, as they were not currently diseased and never had been observed with disease signs. Lesions exhibiting signs consistent with SCTLD, including active tissue loss and/or paling or bleaching of coral tissue at the lesion margin (NOAA 2018), were classified as an active “diseased” lesion.

Videos of the colonies were recorded immediately before and after intervention and at all subsequent monitoring time points. 3D models were later generated using methods described in Young et al. (2017) and adapted by Combs et al. (in review), described above in section 2.2. Models and tissue areas were only generated for initially SCTLD-affected coral colonies in this experiment; healthy controls were not included in this analysis.

Details of sampling for transcriptomic analyses of gene expression associated with this experiment can be found in section 2.4. Though not a part of this original scope of work, samples were also collected/shared for several associated analyses. *M. cavernosa* tissue biopsies from each experimental sample were collected at 0, 3, 5, 23 weeks, preserved in molecular grade ethanol, and then stored at -80°C. Ethanol extract subsamples of these have been shared with Dr. Valeria Paul (Smithsonian Institution) and Dr. Neha Garg (Georgia Tech) for metabolomics analyses. Similarly, tissue biopsy subsamples from this set were shared with Dr. Andrew Baker (University of Miami) for algal symbiont analyses to assess both type and concentration of *Symbiodinaceae*. In addition to the coral tissue biopsies, surface layer mucus was collected from all tagged treated and control *M. cavernosa* colonies. Approximately 5 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 ~5 cm^2 from the colony's edge. 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). DNA from these samples has been extracted, prepared for sequencing analyses, and were submitted to Argonne National Laboratory in December 2019. Argonne has been working to optimize methods to eliminate mitochondrial DNA, but have experienced considerable delays as a result of COVID-19. They now estimate sequence data for the microbiome samples will be available in late September or early October 2020.

3.4. Gene Expression Response to SCTLD and Base 2B+Amoxicillin Treatment

During the initial *in situ* intervention experiment near Lauderdale-by-the-Sea, *M. cavernosa* tissue fragment samples for transcriptomic analyses were collected from treated (n=12 chlorine; n=11 amoxicillin), diseased but untreated (n=10), and healthy control (n=9) colonies at four time points: time zero, three weeks, five weeks, and five months. Samples for transcriptomics were preserved in-water in 100% ethanol, which was then replaced with fresh 100% ethanol at the surface. Preserved samples were stored at -80 ºC until RNA extractions.

An initial *ex situ* SCTLD transmission experiment was conducted in October 2019 at the University of Miami's Cooperative Institute for Marine and Atmospheric Studies (UM-CIMAS) Experimental Reef Laboratory (ERL). Coral fragments of *M. cavernosa* (n=15) and *Orbicella faveolata* (n=16) were obtained from lab-reared collections at Florida Atlantic University's Harbor Branch Oceanographic Institute (FAU-HBOI) and UM-CIMAS. Corals were cut in half for genotypic replication across healthy and diseased treatments. Half-corals were randomly-distributed in individual 0.5 L vessels for the duration of the experiment, with separate flow-through water sources and two common water baths for temperature regulation. One diseased *M. cavernosa* colony with apparent SCTLD lesions was collected from Lauderdale-by-the-Sea, fragmented into 31 1x2 cm fragments each containing infected tissue, and placed into half of the vessels to obtain direct-contact transmission to experimental fragments. Experimental corals were monitored for 11 days with twice-daily photographs and qualitative observations including the time to initial lesion. Diseased coral fragments and the corresponding genotypic replicate fragment in the healthy treatment were preserved in 100% ethanol once the disease lesion reached the approximate halfway point across the coral fragment. Any visibly uninfected fragments were preserved at the end of the 11-day experiment. Preserved samples were stored at -80 ºC until RNA extractions. Transmission rates were calculated from lesion observations to be 5.33±0.17 days for *M. cavernosa*, and 2.66±0.24 days for *O. faveolata*.

RNA extractions of samples from both the initial *in situ* and *ex situ* experiments were attempted in December 2019–February 2020. Unfortunately, these efforts were largely unsuccessful due to RNA degradation in all samples. Two extraction kits (Ambion RNAqueous Micro and Zymo Direct-zol/RNA Clean & Concentrator) with multiple optimization steps were attempted to recover any remaining RNA, but extracts were deemed too degraded and/or dirty for subsequent Tag-Seq library preparation and sequencing. We concluded that preservation in ethanol and lack of immediate freezing following preservation (e.g. freezing with liquid nitrogen) resulted in rapid RNA degradation that increased in severity with each freeze/thaw cycle needed for the extraction process. The decision was therefore made to repeat *in situ* and *ex situ* experiments for additional transcriptomics samples.

A second transmission experiment was conducted at the UM-CIMAS ERL in March 2020 using the same apparatus as previous, with the exception that coral fragments (*M. cavernosa*: n=20; *O. faveolata*: n=20) were sourced from Mote Marine Laboratory's land-based nursery. The experiment was also conducted over 14 days. Two small tissue subsamples were independently preserved in 1 ml of TRIzol and frozen using liquid nitrogen, while the remaining tissue was frozen 'dry' in liquid nitrogen, with all samples being stored at -80 ºC until future RNA extractions. Transmission rates were calculated from lesion observations to be 5.42±0.96 days for *M. cavernosa*, and 3.09±0.50 days for *O. faveolata*.

A second intervention experiment was conducted from April-May 2020 in Lauderdale-by-the-Sea, Florida to collect 'omics samples following amoxicillin intervention on SCTLD-infected *M. cavernosa* colonies. Tissue fragment samples for transcriptomic analyses were

collected from treated (n=15) and healthy control (n=19) colonies at two timepoints: time zero and two weeks. Two small tissue subsamples were independently preserved in 1 ml of TRIzol and frozen using liquid nitrogen, while the remaining tissue was frozen 'dry' in liquid nitrogen, with all samples being stored at -80 ºC until future RNA extractions. See more details on each portion of the methods for this experiment below.

Sample collection: All samples for the second intervention transcriptomic experiment were collected from a single reef site (BC1) offshore from Lauderdale-by-the-Sea, Florida (Fig. 1), where there was sufficient abundance of both diseased and apparently healthy (hereafter referred to as "healthy") *M. cavernosa* colonies. Pre-treatment sampling was conducted on 21 April 2020. Small tissue and skeletal fragments were taken from 14 diseased colonies and 20 healthy control colonies. Scaled photographs were taken pre- and post-tissue sampling, and colony locations were recorded with a surface towed GPS. Sampling depths ranged from 6.1–8.5 m, with an average depth of 6.8 m ± 0.1 (SEM). Following sampling, all diseased colonies were treated with a mixture of amoxicillin and Base2B (1:10 w/w), covering all infected tissue. Tissue samples were preserved in TRIzol and flash frozen in liquid nitrogen within 25 min of sampling and stored in a liquid nitrogen dry shipper for transport before long-term storage at -80 ºC. Post-treatment sampling was conducted on 4 May 2020, following the same procedures as pre-treatment sampling, with the exception of no further application of amoxicillin to diseased colonies.

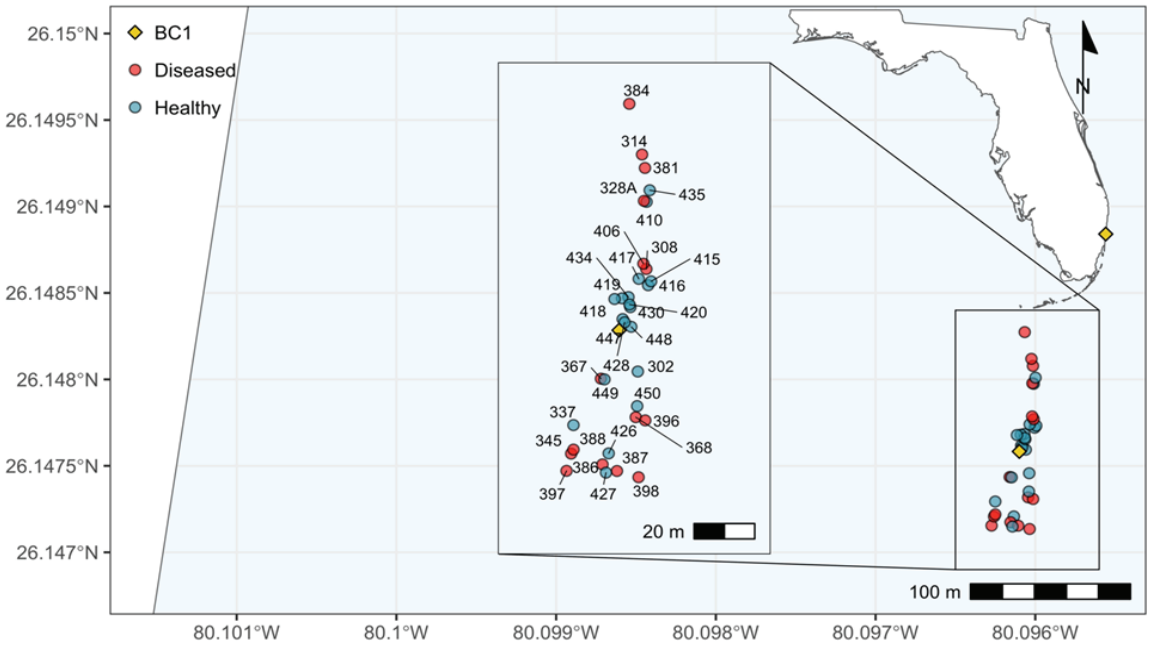


Figure 4. Map of sampled *Montastraea cavernosa* colonies. Red circles represent diseased colonies and blue circles represent apparently healthy colonies.

RNA library prep: To extract total RNA, each sample was homogenized in a 2.0 mL tube with 1 mL of TRIzol and ~0.075 g of 0.5 mm glass beads using a FastPrep-24 homogenizer at 6.0 m s-1 for two 60 s intervals with 5 min cool down between. Samples are then incubated for 5 min at room temperature and centrifuged at 16,000 x g for 2 min at room temperature. 800 µL of supernatant was then processed with the Zymo DirectZol Kit using

on-column DNase treatment, following the manufacturer's protocol. RNA was then eluted from the spin column with 50 µL of heated (60 ºC) nuclease free water (NFW). RNA extracts were then further purified using the Zymo RCC-5 kit following the manufacturer's protocol. Final, cleaned RNA was eluted with 20 µL of heated (60 ºC) NFW. RNA quality was checked using a NanoDrop-2000 (Thermo-Fisher), and the quantity and integrity was checked using Bioanalyzer 6000 RNA assay (Agilent Technologies, INC).

Sequencing: Complementary DNA (cDNA) libraries were prepared for sequencing and have been submitted for sequencing on the Illumina NovaSeq platform at the University of Texas Genome Sequencing and Analysis facility.

Sequence analysis: Returned sequences will be analyzed following the Tag-Seq analysis pipeline (https://github.com/z0on/tag-based_RNAseq) with modifications (Studivan and Voss 2020). Briefly, raw reads will be combined across sequenced duplicates and filtered using a custom perl script to remove PCR duplicates and reads with missing base calls in the degenerate header. Following quality filtering using fastx_toolkit (http://hannonlab.cshl.edu/fastx_toolkit), cleaned reads will be mapped using bowtie2 (Langmead & Salzberg, 2012) to a concatenated transcriptome including the *Cladocopium spp.* algal transcriptome using unique host/symbiont isogroup identifiers. The creation of a combined transcriptome for the two dominant eukaryotes in the holobiont has been used in previous studies to identify potential interactions between host and symbiont transcriptomic trends. Count data will be separated into host and symbiont datasets for subsequent analyses.

3.5. QA/QC

All roving diver, fate tracking, and intervention experiment data were entered into Access or 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.

4. RESULTS

4.1. Field Activities

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

<u>July 3rd Roving Diver Surveys – Palm Beach</u>

On July 3, 2019, members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver survey at each of the three sites (SEFL-04, SEFL-05, SEFL-06) in Jupiter, within Palm Beach County, recording colonies observed and presence/absence of disease or bleaching. A total of 184 colonies were observed, 1 (<1%) of those had some level of

bleaching. Of the total colonies observed, 70% were *Montastraea cavernosa*, with the remaining 30% (in order of prevalence) consisting of *Porites astreoides, Agaricia agaricites, Stephanocoenia intersepta, Siderastrea siderea, Orbicella annularis, Solenastrea bournoni, Eusmilia fastigiata, Mussa angulosa, Madracis decactis, Meandrina meandrites, and Siderastrea radians.* The one colony observed to have some level of bleaching was *Agaricia agaricites.*

July 19th Roving Diver Surveys – Martin

On July 19, 2019, members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver survey at each of the four sites (SLR North, SLR Central, SLR Ledge, SLR South) within Martin County, recording colonies observed and presence/absence of disease or bleaching. A total of 429 colonies were observed, 4 (<1%) of those were observed as diseased with Stony Coral Tissue Loss Disease (SCTLD), 1 (<1%) of those had some level of bleaching, and 1 had some other unidentifiable ailment. Of the total colonies observed 95% were *Porites astreoides*, with the remaining 5% (in order of prevalence) consisting of *Siderastrea siderea, Pseudodiploria clivosa, Montastraea cavernosa, Isophyllia sinuosa, and Solenastrea bournoni.* Of the 4 colonies observed to have SCTLD, 2 were *Pseudodiploria clivosa* with one colony observed each of *Montastraea cavernosa* and *Porites astreoides.* The one colony observed to have some level of bleaching was *Siderastrea siderea* and the unidentifiable observed ailment was observed on a *Porites astreoides* colony.

July 26th Roving Diver Surveys & First Intervention Experiment 14 Weeks Follow-up – Broward

On July 26, 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, recording colonies observed and presence/absence of disease or bleaching. A total of 486 colonies were observed, 17 (3%) of those were observed as diseased with SCTLD, and 1 (<1%) of those had some level of bleaching. Of the total colonies observed, 62% were *Montastraea cavernosa*, 12% were *Acropora cervicornis,* with the remaining 26% (in order of prevalence) consisting of *Porites astreoides, Siderastrea siderea, Orbicella faveolata, Madracis auretenra, Solenastrea bournoni, Stephanocoenia intersepta, Agaricia agaricites, Pseudodiploria strigosa, Dichocoenia stokesi, Colpophllia natans, Madracis decactis, Orbicella annularis, Porites porites,* and *Siderastrea radians.* Of the 17 colonies observed to have SCTLD, 14 were *Montastraea cavernosa,* with one SCTLD colony observed each of *Dichocoenia stokesii, Orbicella faveolata, and Solenastrea bournoni.* The one colony observed to have some level of bleaching was *Pseudodiploria strigosa.*

Also on July 26, 2019, four members of the Voss Lab (FAU Harbor Branch) conducted one dive at each of the three experimental intervention sites within Broward County to conduct the fourth monitoring follow up from the initial interventions. No mucus or tissue samples were collected at this time from any of the experimental colonies. Video for 3D models was collected for all diseased colonies in the experiment. At this 14-week post-intervention monitoring event, three of the eleven amoxicillin treated colonies appeared to have new active lesions outside of the treatment areas, however all but one of the treated

lesions which had been trenched were fully healed. Nine 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.

August 5th Roving Diver Surveys – Palm Beach

On August 5, 2019, members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver survey at each of the three sites (SEFL-04, SEFL-05, SEFL-06) in Jupiter, within Palm Beach County, recording colonies observed and presence/absence of disease or bleaching. A total of 202 colonies were observed, 1 (<1%) of those were observed to have an unidentifiable ailment. Of the total colonies observed, 79% were *Montastraea cavernosa*, with the remaining 22% (in order of prevalence) consisting of *Agaricia agaricites, Porites astreoides, Stephanocoenia intersepta, Siderastrea siderea, Solenastrea bournoni, Colphophyllia natans, Oculina diffusa, Orbicella faveolata, and Pseudodiploria strigosa.* The one colony observed to have an unidentifiable ailment was *Montastraea cavernosa.*

September 25th First Intervention Experiment 23 Weeks Follow-up – Broward

On September 25, 2019 four members of the Voss Lab (FAU Harbor Branch) conducted one dive at each of the three experimental intervention sites within Broward County to conduct the fifth monitoring follow up from the initial interventions. In total, 42 mucus samples and 40 tissue samples were collected from the diseased treated, diseased untreated, and healthy *Montastraea cavernosa* colonies in the experiment. Video for 3D models was collected for all diseased colonies in the experiment. A water sample from each site was collected as well. At this 23-weeks post-intervention monitoring event, five of the eleven amoxicillin treated colonies appeared to have new active lesions outside of the treatment areas, however all but one of the treated lesions which had been trenched were fully healed. Two other amoxicillin treated colonies had very small (2-3 polyp) white spots, and the remaining four amoxicillin treated colonies are apparently healthy. Ten of the twelve chlorine treated colonies appeared to have new active lesions outside of the treatment areas, and eleven of the twelve's trenches had failed, with the lesions surpassing them in at least one area by at least 1 cm.

October 10th Roving Diver Surveys – Broward

On October 10, 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, recording colonies observed and presence/absence of disease or bleaching. A total of 343 colonies were observed, 19 (6%) of those were observed as diseased with SCTLD, 6 (2%) of those were observed to have dark spot syndrome, 6 (2%) were observed to have some level of bleaching and 4 (1%) were observed to have some other unidentifiable ailment. Of the total colonies observed, 66% were *Montastraea cavernosa*, with the remaining 34% (in order of prevalence) consisting of *Acropora cervicornis, Porites astreoides, Agaricia agaricites, Stephanocoenia intersepta, Siderastrea radians, Siderastrea siderea, Orbicella faveolata, Pseudodiploria clivosa, Solenastrea bournoni, Solenastrea strigosa.* Of the 19 colonies observed to have SCTLD 17 were *Montastraea cavernosa,* with one SCTLD colony observed each of *Orbicella annularis* and *Orbicella*

franksi. Of the 6 colonies observed to have dark spot syndrome 5 were *Siderastrea siderea,* and 1 was *Siderastrea radians.* Of the 6 colonies observed to have some level of bleaching 4 were *Agaricia agaricites,* with one observed each of *Dichocoenia stokesi* and *Siderastrea siderea.* Of the 4 colonies observed to have some other unidentifiable ailment 3 were *Acropora cervicornis* and 1 was *Solenastrea bournoni.*

October 22nd Roving Diver Surveys – Palm Beach

On October 22, 2019, members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver survey at each of the three sites (SEFL-08, SEFL-11, SEFL-12) in West Palm Beach, within Palm Beach County, recording colonies observed and presence/absence of disease or bleaching. A total of 243 colonies were observed, 1 (<1%) of those were observed to be diseased with SCTLD and 1 (<1%) had some other unknown ailment. Of the total colonies observed, 53% were *Montastraea cavernosa*, 30% were *Porites astreoides,* with the remaining 17% (in order of prevalence) consisting of *Madracis decactis, Siderastrea siderea, Stephanocoenia intersepta, Agaricia agaricites, Pseudodiploria strigosa, Mycetophyllia aliciae, Meandrina meandrites, Mycetophyllia lamarckiana, Orbicella faveolata, and Pseudodiploria clivosa.* The colony observed to have SCTLD was *Montastraea cavernosa,* and the colony observed to have some other unidentifiable ailment was *Stephanocoenia intersepta.*

On October 22, 2019, members of the Voss Lab (FAU Harbor Branch) also conducted one 20-minute roving diver survey at each of the three sites (SEFL-04, SEFL-05, SEFL-o6) in Jupiter, within Palm Beach County, recording colonies observed and presence/absence of disease or bleaching. A total of 377 colonies were observed, 13 (3%) of those were observed as having some level of bleaching and 3 (<1%) were observed to be diseased with SCTLD. Of the total colonies observed, 59% were *Montastraea cavernosa*, 17% were *Porites astreoides,* 16% were *Agaricia agaricites,* with the remaining 8% (in order of prevalence) consisting of *Stephanocoenia intersepta, Madracis decactis, Meandrina meandrites, Orbicella faveolata, Colpophyllia natans, Mycetophyllia aliciae, Oculina diffusa,* and *Solenastrea bournoni.* Of the 13 colonies observed as having some level of bleaching 4 were *Montastraea cavernosa,* 4 were *Porites astreoides,* 4 were *Stephanocoenia intersepta,* and 1 was *Agaricia agaricites.* The 3 colonies observed to have SCTLD were *Montastraea cavernosa.*

November 25th Roving Diver Surveys – Martin

On November 25, 2019, members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver survey at each of the four sites (SLR North, SLR Central, SLR Ledge, SLR South) within Martin County, recording colonies observed and presence/absence of disease or bleaching. A total of 116 colonies were observed, 4 (3%) of those were observed to have some level of bleaching. Of the total colonies observed, 84% were *Porites astreoides*, with the remaining 16% (in order of prevalence) consisting of *Siderastrea siderea, Pseudodiploria clivosa, Montastraea cavernosa, Madracis decactis and Solenastrea bournoni.* Of the 4 colonies observed to have some level of bleaching 3 were *Porites astreoides* and 1 was *Siderastrea siderea.*

January 16th Roving Diver Surveys – Broward

On January 16, 2020, 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, recording colonies observed and presence/absence of disease or bleaching. A total of 351 colonies were observed, 12 (3%) of those were observed as being diseased with SCTLD. Of the total colonies observed, 60% were *Montastraea cavernosa*, 20% were *Acropora cervicornis* with the remaining 20% (in order of prevalence) consisting of *Porites astreoides, Siderastrea radians, Siderastrea siderea, Stephanocoenia intersepta, Orbicella faveolata, Solenastrea bournoni, Agaricia agaricites, Pseudodiploria strigosa,* and *Porites furcata.* Of the 12 colonies observed to have SCTLD 10 were *Montastraea cavernosa* and 1 was *Siderastrea siderea.*

January 30th Roving Diver Surveys – Palm Beach

On January 30, 2020, members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver survey at each of the three sites (SEFL-08, SEFL-11, SEFL-12) in West Palm Beach, within Palm Beach County, recording colonies observed and presence/absence of disease or bleaching. A total of 217 colonies were observed, 1 (<1%) of those were observed as being diseased with SCTLD. Of the total colonies observed, 51% were *Montastraea cavernosa*, 28% were *Porites astreoides,* with the remaining 21% (in order of prevalence) consisting of *Stephanocoenia intersepta, Siderastrea radians, Meandrina meandrites, Dichocoenia stokesi, Porites porites, Madracis decactis, Siderastrea siderea, Mycetophyllia lamarckiania, Oculina diffusa,* and *Pseudodiploria strigosa.* The one colony observed to have SCTLD was *Montastraea cavernosa.*

February 20th Roving Diver Surveys – Palm Beach

On February 20th, 2020 two members of the Voss Lab (FAU Harbor Branch) conducted one 20-minute roving diver survey at each of the three sites (SEFL-04, SEFL-05, SEFL-06) in Jupiter, within Palm Beach County, recording colonies observed and presence/absence of disease or bleaching. A total of 258 colonies were observed, 1 (<1%) of those were observed as being potentially diseased with SCTLD, and 1 (<1%) was observed as paling. Of the total colonies observed, 65% were *Montastraea cavernosa*, 12% were *Porites astreoides,* 11% were *Agaricia agaricites,* and the remaining 12% (in order of prevalence) consisted of *Stephanocoenia intersepta, Dichocoenia stokesii, Pseudodiploria strigosa, Oculina diffusa, Solenastrea bournoni, Orbicella faveolata, Siderastrea radians, Meandrina meandrites, Madracis decactis, Siderastrea siderea, Eusmilia fastigiata,* and *Helioseris cucullata.* The one colony observed to have SCTLD was *Montastraea cavernosa,* the one colony observed to be paling was *Dichocoenia stokesii*.

March 4th First Intervention Experiment 45 Weeks Follow-up - Broward

On March 4th, 2020 three members of the Voss Lab (FAU Harbor Branch) and one former lab member, who currently works at UMiami RSMAS, conducted 4 dives total across the three experimental intervention sites within Broward County to conduct the sixth and final follow up from the initial interventions. No mucus or tissue samples were collected at this time from any of the experimental colonies. Video for 3D models was collected for all diseased colonies in the experiment. Two SCTLD active *M. cavernosa* colonies were

collected for SCTLD transmission experiment. At this 45-weeks post-intervention monitoring event, three of the eleven amoxicillin treated colonies appeared to have active lesions outside of the treatment areas, however all but one of the treated lesions which had been trenched were fully healed. The remaining seven amoxicillin treated colonies are apparently healthy. Four of the twelve chlorine treated colonies appeared to have new active lesions outside of the treatment areas, and eleven of the twelve's trenches had failed, with the lesions surpassing them in at least one area by at least 1 cm. The remaining three chlorine treated colonies are apparently healthy.

March 12th Roving Diver Surveys - Broward

On March 12, 2020, 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, recording colonies observed and presence/absence of disease or bleaching. A total of 416 colonies were observed, 57 (14%) of those were observed as being diseased with SCTLD. Of the total colonies observed, 73% were *Montastraea cavernosa*, with the remaining 27% (in order of prevalence) consisting of *Siderastrea siderea, Stephanocoenia intersepta, Porites astreoides, Solenastrea bournoni, Orbicella faveolata, Agaricia agaricites, Orbicella annularis, Dichocoenia stokesi, Pseudodiploria clivosa,* and *Pseudodiploria strigosa.* Of the 57 colonies observed to have SCTLD, 52 were *Montastraea cavernosa,* with 2 colonies each of *Siderastrea siderea* and *Agaricia agaricites,* and 1 colony of *Solenastrea bournoni.*

April 21st Initiation of Second RNA/Omics Intervention Experiment - Broward

On April 21, 2020 four members of the Voss Lab (FAU Harbor Branch) conducted multiple dives at our BC1 monitoring site within Broward County to begin the RNA expression/response experiment on SCTLD affected colonies treated with Base2B/amoxicillin and healthy controls. All colonies were *Montastraea cavernosa*. Three diseased and 2 healthy from the previous disease intervention experiment at this site were used, 11 diseased and 18 healthy new colonies were tagged and mapped in addition. This resulted in a total of 20 apparently healthy *M. cavernosa* colonies and 14 SCTLD-affected *M. cavernosa* colonies for this experiment. Pre-treatment tissue samples were collected from the diseased tagged colonies at this time, and then all of the lesions on those diseased colonies were immediately treated with the Base2B/amoxicillin mixture. Tissue samples were also collected from all of the healthy controls. This resulted in a total of 14 SCTLD-affected and 20 apparently healthy tissue samples for later RNA extraction and gene expression analysis.

May 4th Second RNA/Omics Intervention Experiment Follow-up - Broward

On May 4, 2020 four members of the Voss Lab (FAU Harbor Branch) conducted multiple dives at our BC1 monitoring site within Broward County to collect a second, follow-up set of tissue samples from the tagged colonies from the RNA expression/response intervention experiment. All colonies from the April 21, 2020 setup and sampling date were sampled again, for a total of 14 tissue samples from Base2B/amoxicillin treated SCTLD colonies and 20 from apparently healthy untreated control colonies.

4.2. SCTLD Dynamics on the Northern Section of Florida's Coral Reef

SCTLD prevalence was significantly different between study locations during FY20 (PERMANOVA; Pseudo-$F = 4.5_{3,24}$, $p = 0.0139$) and over time in FY20 (PERMANOVA; Psuedo-$F = 3.65_{5,24}$, $p = 0.0123$, Fig. 5). Pairwise comparisons indicated that SCTLD prevalence in Jupiter was significantly lower than in Pompano (pairwise PERMANOVA; Pseudo-$F = 12.91$, $p = 0.0012$). The five most abundant species from our surveys were *Montastraea cavernosa* (0.53, n = 1798), *Porites astreoides* (0.24, n = 814) , *Acropora cervicornis* (0.045, n = 151), *Siderastrea siderea* (0.043, n = 145), *Agaricia agaricites* (0.039, n = 130) . The five species with the highest SCTLD prevalence were *Orbicella franksii* (1, n = 1), *Orbicella annularis* (0.143, n = 7), *Dichocoenia stokesi* (0.111, n = 9), *Pseudodiploria clivosa* (0.100, n = 20), and *Solenastrea bournoni* (0.067, n = 45). The SCTLD prevalence for *Montastraea cavernosa*, the most abundant species seen, was 0.05.

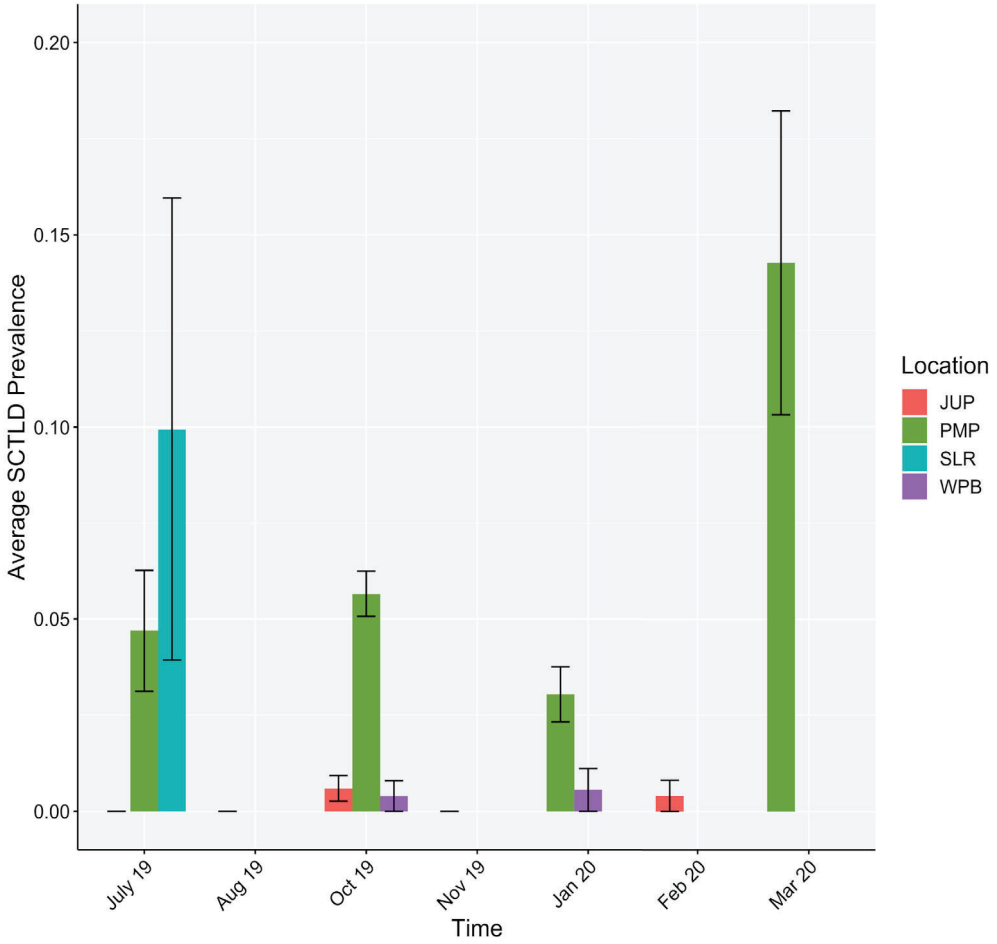


Figure 5. SCTLD mean prevalence ± SD from roving diver surveys between July 2019 and March 2020 at Jupiter (JUP), Lauderdale-by-the-Sea/Pompano (PMP), St. Lucie Reef (SLR), and West Palm Beach Breakers (WPB).

4.3. Coral Fate Tracking and Imaging

To understand how disease progression differed on each fate-tracked colony, we focused on 3D modeling of individual *M. cavernosa* colonies in the Lauderdale-by-the-Sea/ Pompano sites (Figure 6). We observed a significant decrease in total colony area (m^2) and healthy tissue area over time as a result of SCTLD (Figure 7; total Friedman $\chi^2 = 48.56$, $p < 0.001$; healthy Friedman $\chi^2 = 41.29$, $p < 0.001$). The number of disease lesions per colony differed by site (Kruskal-Wallis, H = 17.08 p < 0.001,), with T328 exhibiting more disease lesions per colony (9.57 ± 1.07) than both BC1 (4.31 ± 0.51) and FTL4 (4.65 ± 0.87; Dunn's test, both $p < 0.05$).

Correlation analyses between surface area and lesion metrics identified no significant correlation between colony size and disease lesion area (Spearman's rank correlation $r_s = 0.15$, $p = 0.154$). There was, however, a positive correlation between colony lesion number and disease lesion area ($r_s = 0.67$, $p < 0.001$), but no correlation between total colony size and the number of disease lesions ($r_s = 0.18$, $p = 0.076$).

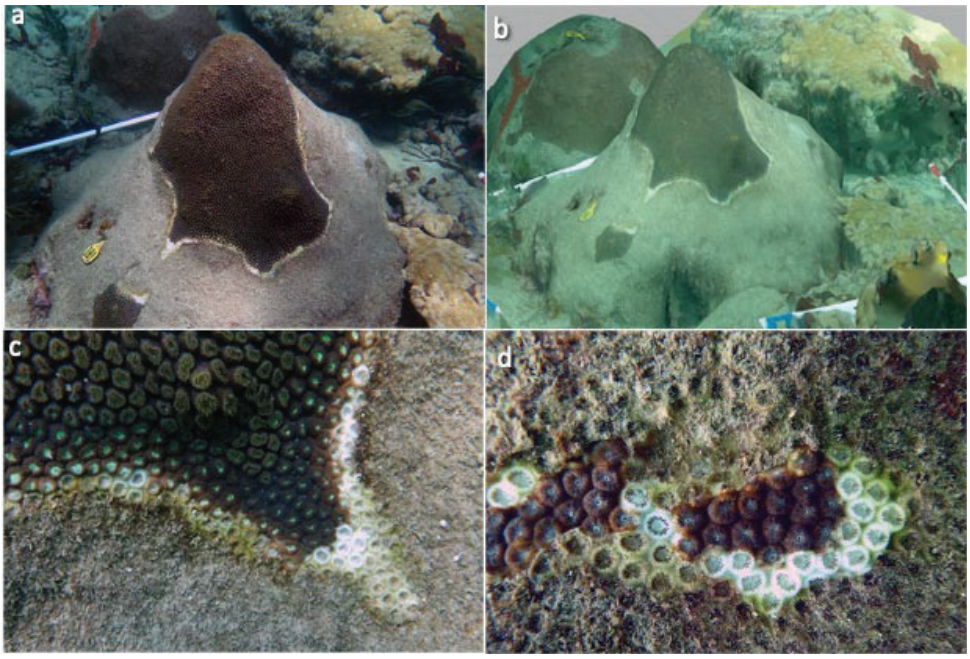


Figure 6. Representative fate-tracked, SCTLD-infected *Montastraea cavernosa* colony that was (a) photographed in situ (b) rendered as a 3D model, (c) examined for characteristic SCTLD lesions, and (d) verified with necrotic tissue.

The rate of change in disease area did not significantly differ over time (Friedman's rank sum test, $df_{2, 71}$, Friedman $\chi^2 = 2.58$, $p = 0.275$). There were significant differences between rates of tissue loss through time ($df_{2,71}$, Friedman $\chi^2 = 6.25$, $p = 0.044$). Rate of tissue loss was not significantly different among sites between any time point, and the rate of tissue loss was never correlated with colony size between any time point in the study.

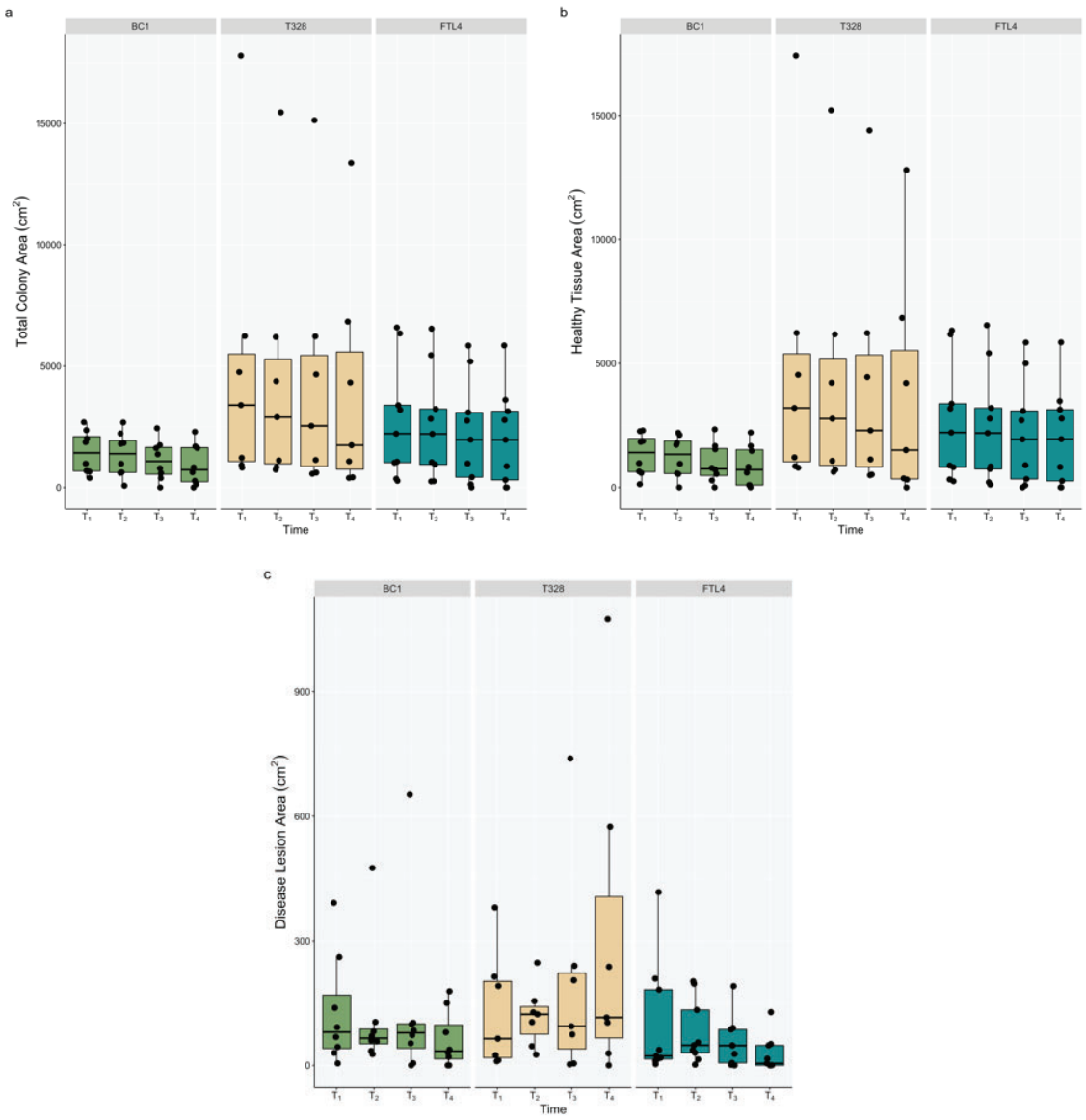


Figure 7. Box and whisker plots for fate-tracked *M. cavernosa* colonies at each site and through time including (a) mean total colony area (cm^2) (b) mean healthy tissue area (cm^2), and (c) mean disease lesion area at each site and through time for fate-tracked *M. cavernosa* colonies.

4.4. Experimental Interventions and Monitoring

All healthy control *M. cavernosa* colonies remained healthy throughout the course of the experiment, and therefore were not included in the following analyses. There was no significant difference among research sites in the development of new lesions on a colony over time, cumulative new lesion development over 46 weeks (11 months), the total lesions present on a colony at any time point, or SCTLD status of a colony at any time point (Kruskal-Wallis tests, all $p > 0.05$). There was no significant difference between the initial

numbers of lesions on experimental colonies between sites or treatment groups (Kruskal-Wallis tests, all $p > 0.05$). Three experimental colonies (one from each SCTLD-affected treatment group) were excluded from the initial colony size analyses because the generated models were of poor quality and did not accurately reflect the surface area of the colonies. Mean initial colony size of all SCTLD-affected experimental corals was 2,743.01 ± 377.24 cm^2 (with a range of 91.17–10,174.48 cm^2. Mean initial colony size did not differ between sites or treatment groups (Kruskal-Wallis tests, χ^2 0.322 and 1.90, $p = 0.851$ and 0.387, respectively). Initial colony size had no effect on the disease status of an initially SCTLD-affected coral at 46 weeks, even when blocked by treatment group (Kruskal-Wallis tests, all $p > 0.05$).

After 46 weeks, the amoxicillin treated lesions had the highest quiescence rate at 95%. The success of amoxicillin treated lesions was significantly higher than the quiescence rates for untreated lesions and chlorine treated lesions at this time point (Fisher's exact test, $p < 0.001$; pairwise Fisher's test, all $p < 0.001$; Figure 8). There was no significant difference detected between those quiescence rates of chlorine and untreated lesions at 46 weeks. Treatment significantly influenced the SCTLD status of a colony until the 46-week time point (Fisher's tests). From the first monitoring at three weeks to the third monitoring at 9 weeks, the amoxicillin treated colonies were more likely to be completely quiesced than the chlorine treated or untreated colonies.

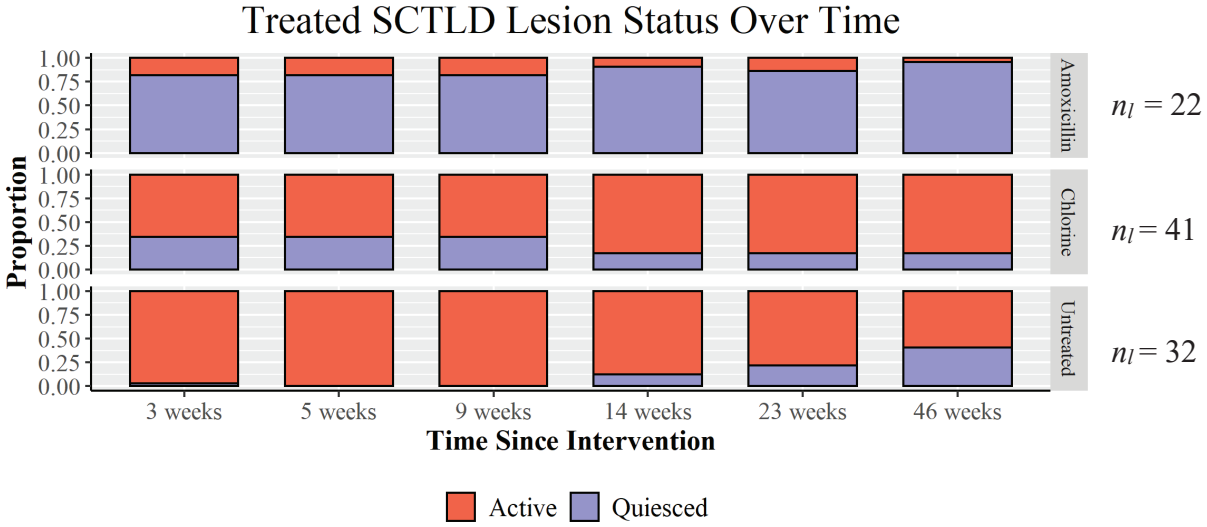


Figure 8. Status of initially treated disease lesions on colonies by treatment group at each monitoring event, shown in proportions of total, with n_l indicating total number of lesions present across all colonies in the treatment group. Untreated refers to untreated SCTLD-affected control colonies.

The initial number of SCTLD lesions present on a colony at the beginning of the experiment had no influence on its SCTLD status at any follow-up monitoring time point, even when blocked by treatment group (Kruskal-Wallis tests, all $p > 0.05$). Coral colonies that were in the Base 2B plus amoxicillin treatment group were more likely to have tissue

remaining behind the trenched barriers (71%) when compared to the chlorine treated coral colonies (6%, Fisher's exact test, $p < 0.001$) 46 weeks after treatment.

There was weak or no correlation detected between either the number of new lesions developed on colonies and time (Figure 9), or total lesions present on colonies and time, even when blocked by treatment group. There was a significant positive correlation between initial colony size and the initial number of lesions present (Spearman's rank correlation, $\rho = 0.465$, $p < 0.01$). However, the results from the correlation analyses are skewed by one outlier, specifically the largest colony which had almost four times as many lesions as any of the other colonies. With this individual excluded, the correlation remained significant but showed a weaker relationship (Spearman's rank correlation, $\rho = 0.405$, $p = 0.029$). There was also a significant positive relationship between initial colony size and number of new lesions accumulated during the course of the experiment (Spearman's rank correlation, $\rho = 0.40$, $p = 0.029$).

There was no significant effect of treatment on the number of new lesions developed on a colony over time, this was confirmed by conducting Kruskal-Wallis tests on new lesions developed between each time point by treatment group (all $p > 0.05$).

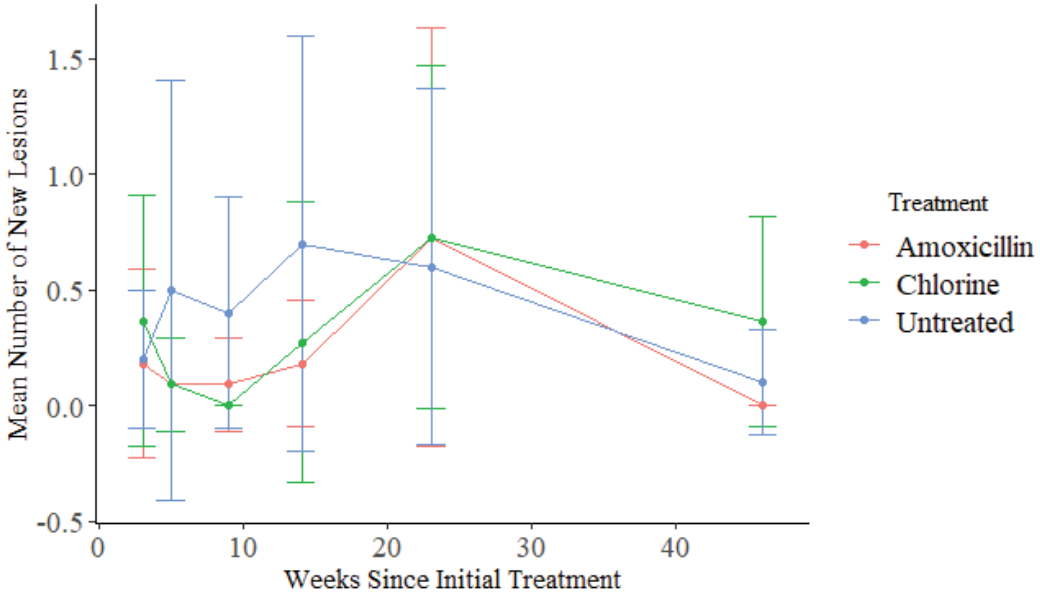
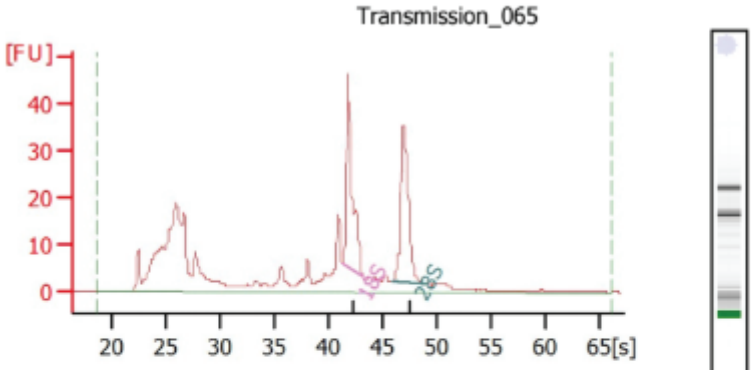


Figure 9. Mean number of new SCTLD lesions present on experimental colonies at each monitoring event, beginning from 3 weeks and ending at 46 weeks, separated by treatment group. Amoxicillin refers to the Base 2B plus amoxicillin, chlorine refers to the chlorinated epoxy, and untreated refers to untreated SCTLD-affected controls. Bars represent 95% confidence interval.

4.5. Gene Expression Response to SCTLD and Base 2B+Amoxicillin Treatment

RNA extraction from both the 2019 *in situ* intervention experiment and 2019 *ex situ* transmission experiment was compromised by RNA degradation from using ethanol as a preservative with 2+ freeze thaw cycles. Therefore, we repeated both an *in situ*

intervention experiment and an *ex situ* transmission experiment in 2020, this time storing tissue samples in TriZOL to preserved RNA based on successful extractions in previous experiments (e.g. Studivan and Voss 2020). The 2020 sample RNA extracts were sufficient for sequence analyses on the NovaSeq platform (see Figure 10). RNA integrity scores averaged 4.98 for the 2020 *in situ* transmission and 7.1 for the 2020 *ex situ* transmission experiment. RNA extracts averaged 425 ng/uL for the intervention experiment and 170 ng/uL for the transmission experiment. Details of the RNA extract metadata and quality can be found in deliverable T4b submitted to DEP. The prepared samples from gene expression transcriptomic analyses were submitted in Aug 2020 for sequencing on the Illumina NovaSeq platform at the University of Texas Genome Sequencing and Analysis facility. We anticipate sequence data will be available by Oct 2020. After quality filtering and cleaning the dataset, we will proceed with analyses, interpretation, and posting of the data in a publically available database.



Overall Results for sample 1 : Transmission_065

RNA Area:	474.9
RNA Concentration:	203 ng/µl
rRNA Ratio [28s / 18s]:	1.0
RNA Integrity Number (RIN):	7.6 (B.02.08, Anomaly Threshold(s) manually adapted)
Result Flagging Color:	
Result Flagging Label:	RIN: 7.60

Fragment table for sample 1 : Transmission_065

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.31	43.22	60.7	12.8
28S	45.86	49.17	59.8	12.6

Figure 10. Example Bioanalyzer output of an RNA extract processed from a *Montastraea cavernosa* sample in the *ex situ* transmission experiment indicating high RNA quality and quantity.

5. PRELIMINARY CONCLUSIONS

This study demonstrated that tissue loss disease incidence and prevalence may be highly variable over space and time on coral reefs in SEFL. For example, while stony coral tissue loss disease and bleaching were observed continually throughout the project period among corals at our Lauderdale-by-the-Sea sites, for most of FY20 disease prevalence was relatively low in the Palm Beach County sites.

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. 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. SCTLD prevalence at St. Lucie Reef dropped to an average of 10% in July 2019. Subsequent monitoring through Nov 2019 revealed that SCTLD was reduced to near zero at St. Lucie Reef. However, this appears to be a function of lack of susceptible hosts rather than any improvement in STLD status overall.

In our *in situ* SCTLD intervention experiment, the Base 2B plus amoxicillin treatment was significantly more effective at treating individual SCTLD lesions on *M. cavernosa* colonies than the chlorinated epoxy or leaving the colonies untreated. This study supports and reinforces previous reports of successful antibiotic application for the treatment of coral disease. This study and Neely et al. 2020 demonstrate that the Base 2B plus amoxicillin treatment is not only effective at treating SCTLD lesions, but that it can be used relatively easily by SCUBA divers. The chlorinated epoxy in this experiment was ineffective as a treatment for SCTLD lesions on *M. cavernosa.* SCTLD lesions treated with chlorinated epoxy were no more likely to quiesce than SCTLD lesions left untreated. In this experiment the effectiveness of the Base 2B plus amoxicillin resulted in living coral tissue being preserved behind trenches on most Base 2B plus amoxicillin treated colonies. When the chlorinated epoxy applied directly over the SCTLD lesion did not prevent progression, the trenches also did not prevent SCTLD from continuing to progress across the coral colonies. In instances where time and effort underwater are constrained, application of Base 2B plus amoxicillin to more SCTLD affected colonies should be prioritized over supplementing the antibiotic treatments with trenching. However, a controlled experiment comparing trenching and Base 2B plus amoxicillin treatments versus Base 2B plus amoxicillin alone is recommended to further support these observations and assess the relative risks and trade-offs associated with mechanical trenching.

Though Base 2B plus amoxicillin demonstrated high efficacy against SCTLD lesions in this experiment, the antibiotic treatment does not prevent the colony from developing new SCTLD lesions in other locations over time. This is consistent with other *in situ* and *ex situ* trials previously conducted on this disease, and reinforces the need for repeated antibiotic treatments to effectively halt SCTLD impacts on a colony (Aeby et al. 2019; Neely et al. 2020). The appearance of new lesions after all Base 2B plus amoxicillin treated lesions are healed suggest three potential scenarios: 1) the causative agent of SCTLD is still present in the environment and is re-infecting quiesced colonies, 2) the duration and dose of Base 2B plus amoxicillin is sufficient to arrest SCTLD at treated lesions, but insufficient for eliminating SCTLD pathogens from other areas of the coral colony, or 3) the coral immune system is compromised from this original SCTLD affliction and opportunistic bacteria are able to cause secondary infections observed as lesions.

Additional empirical research and controlled studies are needed to determine if there are regional, temporal, and/or species-specific influences on intervention treatment success. Nonetheless, the results of this study demonstrate that Base 2B plus amoxicillin treatments can be effective against SCTLD when combined with relatively frequent monitoring events. The observations of new lesions in this study suggest that follow-up evaluation should occur approximately two months after initial antibiotic application on SCTLD affected *M. cavernosa* colonies. Subsequent monitoring should be conducted every two months onward to treat new lesions as they develop. These timelines may apply to other "moderately susceptible" coral species as well, given that they are in part classified by disease progression rate, but further species-specific trials should be conducted to confirm this hypothesis. "Highly susceptible" species may need even more frequent follow-ups. This timeline also means we can tentatively determine if a treatment has offered colony-level benefits after nine weeks for these intermediately susceptible species. Lesion-level success should be determined by the halting of the treated lesion after one treatment, but colony-level success, which is more important in the long term, should be determined by the prevention of new lesion development on the treated colony.

The success of Base 2B plus amoxicillin treatments is encouraging in the face of a disease outbreak that is continuing to devastate Caribbean coral reefs. However, potential secondary impacts of amoxicillin treatments on SCTLD-affected corals remain uncharacterized. We recommend that future research efforts should focus on assessing the potential unintended consequences of antibiotic treatments on corals, their microbial communities (including Symbiodinaceae), and neighboring organisms. Additionally, further efforts are needed to optimize dosing and delivery methods for antibiotic treatments on SCTLD-affected corals and scale up intervention treatments effectively.

Finally, while *M. cavernosa* is a dominant species in the region and ideal for studies regarding connectivity and adaptation to various depth ranges, transcriptomic analyses for this species and other coral health studies in general may require individualized sample preservation and extraction protocols. For example, methods previously used successfully in *Orbicella faveolata* were less effective for *M. cavernosa.* Through several iterative efforts, we've identified a robust and effective transcriptomic methodology that will be used going forward and shared with colleagues as well.

6. 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. Ideally the same samples should be assessed using transcriptomic, genomic, microbial, histological, and metabolomics methods.

Recommendation 2*: Because the prevalence of many coral diseases are 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 or automated 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 95% success rate after 46 weeks. However, new lesions can arise, and broad scale application of antibiotics may not be advisable or scalable. We recommend testing Base 2B with various concentrations of amoxicillin and more stringent measurements of amoxicillin concentrations in the surrounding water column.

Recommendation 4*: Determine impacts of Base 2B plus amoxicillin treatments.* Since Base 2B plus amoxicillin appears to be the only treatment currently identified as effective in the field through controlled, year-long experiments (see above), we recommend continued use of this approach. However, both ethical and regulatory issues require that we systematically characterize the potential impacts of antibiotic treatments on the host coral, its algal symbionts, its microbiome, the microbiomes and macro organisms in the surrounding area, and the relative abundance and expression of antibiotic resistance genes.

Recommendation 5: *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 this tissue loss disease is severe. Any additional efforts to reduce 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, subsequent outplanting, and testing of outplant resilience to SCTLD.

Recommendation 6: *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 SCTLD 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.

7. RECENT PUBLICATIONS RESULTING FROM DEP FUNDED RESEARCH

[U] Indicates undergraduate student supervised by Voss
[G] Indicates graduate student supervised by Voss
[P] Indicates post-doctoral researcher supervised by Voss
Indicates senior authorship status

Dodge DL[G], Studivan MS[P], Eckert RJ[G], Chei E[U], Beal J, Voss JD . 2020. Population structure of the scleractinian coral, *Montastraea cavernosa*, in the northern Florida Reef Tract. *Bulletin of Marine Science* doi: 10.5343/bms.2019.0074.

Combs IR[G], Studivan MS[P], Voss JD . In review. Quantifying impacts of stony coral tissue loss disease on corals in Southeast Florida through surveys and 3D photogrammetry. *PLOS ONE.*

Shilling EN[G], Combs IR[G], Voss JD . In prep (submission planned for Sept 2020). Assessing the effectiveness of two intervention methods for stony coral tissue loss disease on *Montastraea cavernosa. Scientific Reports*.

Studivan MS[P], Shatters A[G], Dodge DL[G], Beal J, Voss JD . In prep (submission planned for Sept 2020). Synergistic effects of thermal stress and estuarine discharge on transcriptomic variation of *Montastraea cavernosa* corals in southeast Florida. *Molecular Ecology.*

8. ADDITIONAL PROJECT DELIVERABLES

A shared Google Drive folder includes the following in fulfillment of these deliverables for Tasks 2-5. Deliverables for Task 1 were submitted on January 10, 2020.

Folders:

1. T2c: Dive Logs

   All dive logs chronicling all fieldwork that was completed in fulfillment of the scope of work by members of the Voss Lab, collaborators, and volunteers.

2. T3b: Scaled Photos from Intervention Experiment

   All photos that were taken for the first fate-tracking 2019 intervention experiment on SCTLD-affected colonies.

3. T3d: 3D Model Stats

   All documents & figures pertaining to the analysis of the data from the 3D models generated as a part of the initial 2019 intervention experiment on SCTLD-affected corals.

Documents:

1. T2a: Operational Activities at Each Project Site

   Spreadsheet detailing the GPS location of each study site, activities completed, and the date on which those activities took place.

2. T2b: Sample Inventory

Spreadsheet detailing samples taken in FY20, including date collected, sample type, location, and which colony was sampled.

3. T2c: Collated SLR Dive Reports

Word document containing all pre- and post- dive quick look reports to St. Lucie Inlet Preserve State Park.

4. T2d: Roving Diver Survey Data Sheets Compiled

Scanned roving diver data sheets compiled as a PDF document.

5. T2e: Summaries of each field op

Summaries from each dive day conducted for various sampling or survey objectives.

6. T3a Demographic Info for Intervened Corals

Excel file with two worksheets, the first detailing the demographic information of the intervention experiment initiated in 2019 on STLD-affected coral colonies including date, site, diameter, status, and treatment application. The second worksheet includes demographic information for the second, 2020 transcriptomic-focused intervention experiment on SCTLD-affected coral colonies including date, site, status, and treatment application.

7. T3c: Intervention Response Variable Data

Excel file with eight worksheets, the first seven detailing each experimental coral colony's response to intervention treatments for the first fate-tracking 2019 intervention on SCTLD-affected colonies at each of the monitoring time points, and the eighth sheet detailing each experimental coral colony's response to intervention treatments for the second RNA/Omics 2020 intervention on SCTLD-affected colonies at the single follow-up monitoring event.

8. T3d: Time Series for Intervention Experiment Colonies PowerPoint

Compiled and organized time series photos of treated colonies at monitoring time points pre and post intervention.

9. T4a: SCTLD Transmission and Intervention 'Omics Methods

Word document containing the detailed protocols & methodologies for completed experiments and RNA extractions, as well as future analyses to be completed to assess gene expression from SCTLD transmission and intervention with Base 2B+amoxicillin.

10. T4b: SCTLD Omics Sample Metadata

    Excel file with details of treatments, location, sampling times for the 2019 and 2020 in situ intervention experiments and ex situ SCTLD transmission experiments as well as the quantity and quality of extracted RNA from the 2020 samples from each experiment.

11. T4b: Bioanalyzer Results

    This PDF file inclues all of the bioanalyzer electropherograms for RNA extracted from samples in both the 2020 ex situ transmission experiment and the 2020 in situ intervention experiment.

12. T5a: FAU SCTLD FY20 Summary Powerpoint

    This presentation has been scheduled by the DAC for Sept 16, 2020. The file will be uploaded in this Google Drive folder as well following the presentation.

13. T5b: FAU Draft FY20 Final Report to FDEP

    A copy of this report is provided in the Google Drive as well.