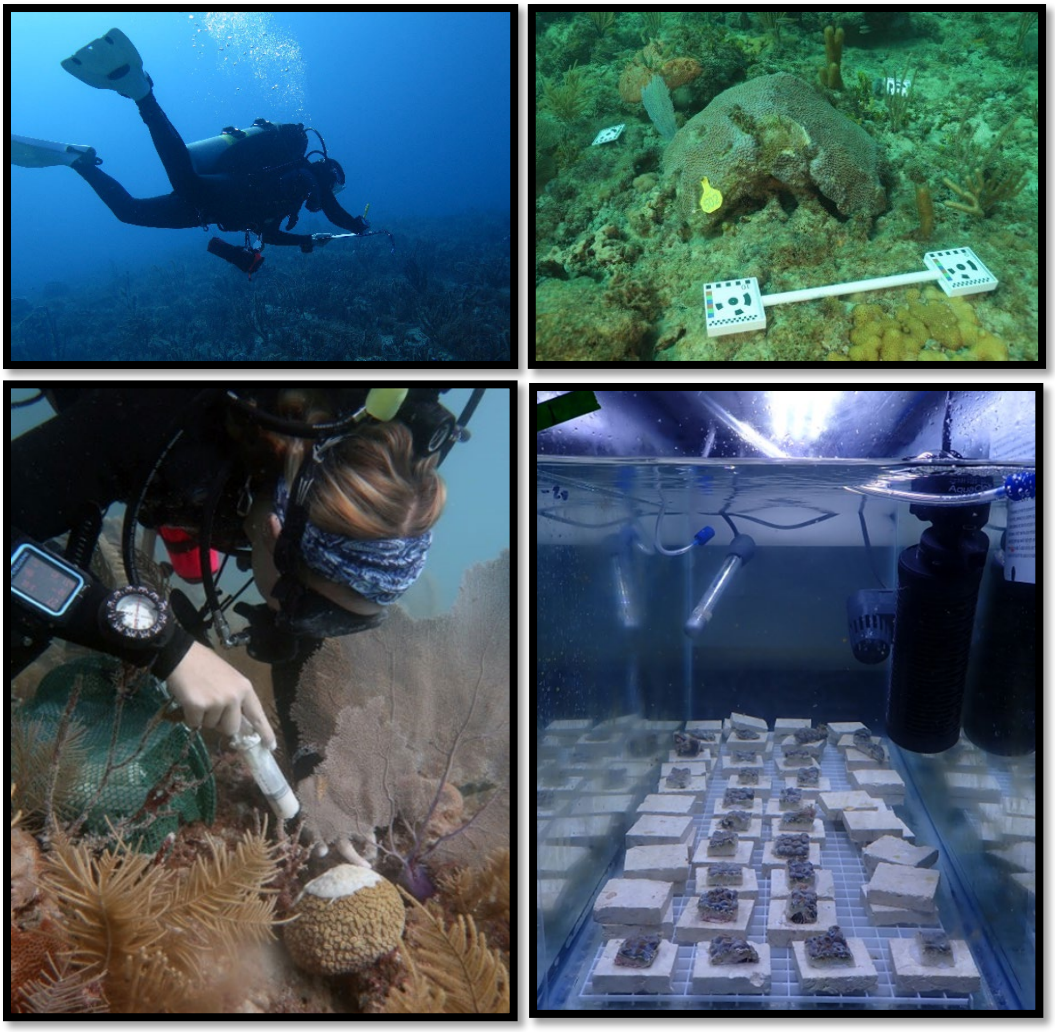
2022

Advancing Coral Reef Research and Resilience in Southeast Florida

Final Report



Advancing coral reef research and resilience in Southeast Florida

Final Report

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Executive Summary for Managers

Coral reefs in South Florida face severe challenges from coral disease, water quality, climate change, and other local anthropogenic impacts. This project applied multiple complementary approaches to help understand, reduce, and mitigate coral reef declines in southeast Florida. Continued monitoring of coral disease incidence and prevalence in the northern portion of the Coral ECA was coupled with ongoing disease intervention experiments, coral population genetics to inform population management and coral restoration, and experimental coral salinity thresholds tests. Collectively this project was designed to improve our overall understanding of the spatial extent and dynamics of this disease outbreak, prevalence, species affected, factors contributing to disease, and methods to reduce coral losses.

We found that reefs in Martin, Palm Beach, and Broward counties are in the endemic phase of stony coral tissue loss disease, with little disease observed at St. Lucie Reef or Palm Beach sites, but continued low levels of disease at Jupiter and Lauderdale-by-the-Sea sites. Long-term tracking demonstrated that diseased corals experimentally treated with antibiotics were approximately five times more likely to survive than corals not treated. In terms of coral genetics, we identified a high abundance of *Porites astroides* clones in southeast Florida, suggesting that asexual reproduction is important for this species. Boynton Beach is a key source population of *P. astreoides* in the region. Our results for *Stephanocoenia intersepta*, coupled with our previous studies on *Montastraea cavernosa*, indicate that mesophotic coral reefs may provide critical refuges for impacted shallow populations, and that including mesophotic populations in coral genetic assessments is important for understanding coral biodiversity. Finally, this project provides critical data on the effects of low salinity stress on corals in southeast Florida. Freshwater releases that expose corals to salinities lower than 20 PSU for even just 1 or 2 days risk severe impacts and coral mortality.

Acknowledgements

We appreciate the collaboration with Florida Department of Environmental Protection's Coral Protection and Restoration Program (DEP CPR) who supported this research. We thank the DEP CPR staff, particularly Kristi Kerrigan, Jennifer Coley, and Joanna Walczak for coordinating this award and providing critical suggestions to improve the quality and impact of the project. Kathy Fitzpatrick from Martin County has served as a key advisor on this project, particularly with regard to the coral salinity threshold experiments. Matt Roy and Jimmy Nelson at FAU Harbor Branch provided marina and logistic support. Karen Neely also at NSU provided collaborative support and advice on intervention approaches. Michael Studivan at CIMAS/AOML was our key collaborator on gene expression impacts related to SCTLD and intervention. Collaborator Ian Combs at Mote Marine Lab provided 3D modeling expertise and model generation. Stephanie Schopmeyer and Lisa Gregg at FWC, as well as Andrew Flanner and Scott Tedford at St. Lucie Inlet Preserve State Park helped to coordinate permitting for this study.

This project leveraged support from the NOAA OAR Omics program to JDV through CIMAS, a PADI fellowship award to Ashley Carreiro, and a Vero Beach Sunrise Rotary Club Dritenbas Memorial Fellowship to Haley Davis, and support from NOAA award NA14OAR4320260 to JDV through the Cooperative Institute for Ocean Exploration, Research, and Technology.

Samples within St. Lucie Inlet Preserve State Park were collected under permits 01221915 and 012102115 to Joshua Voss. Coral samples collected outside of the park in the Kristin Jacobs Coral Ecosystem Conservation Area were collected under Special Activity Licenses SAL-21-2332(A)-SRP and SAL-21-1702-SRP.

Contents

1. BACKGROUND

Florida's Coral Reef is 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 30 species of coral across Florida and the wider Caribbean. The disease is highly prevalent and estimated to have resulted in the mortality of millions of corals across the Kristin Jacobs Coral Reef Ecosystem Conservation Area (Coral ECA), Biscayne National Park (BNP), the Florida Keys, and Dry Tortugas National Park. 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.

2. PROJECT DESCRIPTION

This project included multiple complementary approaches to understand, reduce, and mitigate coral reef ecosystem declines in Southeast Florida (SE FL). Continued monitoring of coral disease incidence and prevalence in the northern portion of the Coral ECA was coupled with continued tracking of intervention experiments, coral population genetics to

inform restoration, and experimental coral salinity thresholds tests. Collectively this approach was designed to improve our overall understanding of the spatial extent and dynamics of this disease outbreak, prevalence, species affected, factors that contribute to disease, and methods to reduce coral losses. The research report here is part of a larger effort to understand the impacts of disease on coral health in the South Florida region and to determine optimized mitigation efforts that may prevent further losses of coral reef resources.

Five primary tasks were established for this period of performance:

Task 1: Project coordination and permitting
Task 2: SCTLD surveys and reconnaissance
Task 3: Disease intervention strategies
Task 4: Coral population genetics to inform restoration activities and management strategies
Task 5: Coral salinity threshold experiments
Task 6: Reporting

The outcomes of this project contribute to ongoing and future coral disease response efforts which seek to improve understanding of the severity of the coral disease outbreak and additional impacts to Florida's Coral Reef, 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 was designed to improve the predictive capacity regarding coral susceptibility to disease and impacts from infection.

Florida DEP funds awarded through PO#B9657D were used primarily to support coral disease surveys and reconnaissance, experimental disease interventions, field collections of corals and genomic sequencing for population genetics to inform management and restoration, and coral salinity threshold testing to inform freshwater release management criteria. This project leveraged support from several sources to provide additional analyses that complemented and extend our DEP project goals, including the NOAA OAR Omics program (coral gene expression associated with disease and intervention), Rotary Club (salinity thresholds and SCTLD), and NOAA OER through the Cooperative Institute for Ocean Exploration, Research, and Technology (additional sequencing for population genetics).

3. METHODOLOGY

Tables 1 and 2 below summarizes the operational activities at each of the project sites in this period of performance. Project sites, as shown in Figure 1, were chosen from long-

term monitoring sites in our lab with over 10 years of survey data at St. Lucie Reef. 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 a continuous monitoring time series in these locations. Broward County sites were chosen due to their relatively high stony coral and SCTLD abundance.

Operational activities in FY22 were severely affected by sea conditions and Covid. We were challenged by an abnormal number of windy days that restricted our field work to less field days than anticipated. In addition, over the course of the year several positive Covid cases within our team resulted in quarantine and restricted field activities.

In order to effectively overcome the limitations of weather windows and Covid, we started to send larger teams in the field and also extended the duration of our field days to accomplish all planned monitoring and research tasks. As a result, though we had fewer total days in the field than planned, with a larger team and marathon days we were able to accomplish the vast majority of our original goals.

In addition to our planned tasks and objectives, were we able to add additional sequencing analyses of *Porites astreoides*, including adding a site and additional samples from the expanding population at St. Lucie Reef.

Table 1. FAU Harbor Branch Project Sites

Site Name	Lat	Long	Region	County
SLR Central	27° 07.900'	-80° 08.042'	St. Lucie	Martin
SLR Ledge	27° 07.286'	-80° 07.650'	St. Lucie	Martin
SLR South	27° 06.712'	-80° 07.531'	St. Lucie	Martin
SEFL04	26° 56.6225'	-80° 1.3183'	Jupiter	Palm Beach
SEFL05	26° 55.6467'	-80° 1.8060'	Jupiter	Palm Beach
SEFL06	26° 53.8641'	-80° 0.9830'	Jupiter	Palm Beach
SEFL08	26° 42.6260'	-80° 0.9490'	West Palm Beach	Palm Beach
SEFL11	26° 40.7100'	-80° 1.0950'	West Palm Beach	Palm Beach
SEFL12	26° 39.1432'	-80° 1.2409'	West Palm Beach	Palm Beach
T328	26° 10.567'	-80° 05.633'	Pompano/ Lauderdale-by-the-Sea	Broward
BC1	26° 08.855'	-80° 05.766'	Pompano/ Lauderdale-by-the-Sea	Broward
FTL4	26° 08.197'	-80° 05.843'	Pompano/ Lauderdale-by-the-Sea	Broward

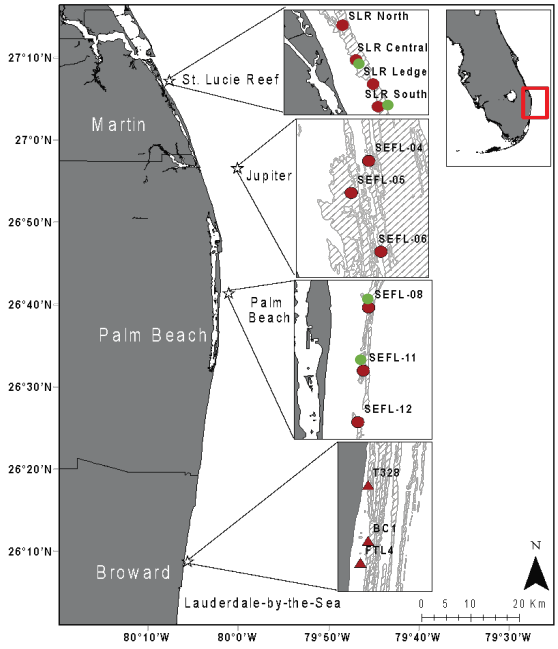

Figure 1. Map of study locations throughout Florida's Northern Coral Reef. Red circles indicate roving diver survey sites and red triangles indicate sites where both roving diver surveys and the SCTLD intervention treatment experiment occurred. SLR North surveys have been discontinued due to lack of live corals in the area. Sites selected as restoration candidate locations are indicated by green circles.

3.1. SCTLD Surveys and Reconnaissance

Roving diver disease surveys (see Table 2) 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 minutes and recorded all coral colonies to 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 incidence and prevalence, species diversity, and species richness were calculated. Statistical tests were run in the R statistical environment.

To inform restoration activities we also conducted reconnaissance dives in both our St. Lucie Reef and Palm Beach sites to identify potential coral restoration locations with suitable substrate, reef composition, and sufficient distance from known coral reef monitoring survey areas.

To understand the current condition of St. Lucie Reef, we supplemented the roving diver surveys with 3D mosaic imaging over 10x10 m plots in several locations across SLR. Though our initial plan in FY21 was to conduct reef wide 3D mosaics via diver propulsion vehicles, consistent visibility limitations required a change in our survey design.

Table 2. Operational Activities by Site

St. Lucie Reef			
	Central	Ledge	South
12/2/21	RD Survey	RD Survey	RD Survey
3/17/22	RD Survey	RD Survey	RD Survey
6/2/22	RD Survey	RD Survey	RD Survey
		Jupiter	
	SEFL-04	SEFL-05	SEFL-06
6/10/21	RD Survey	RD Survey	RD Survey
7/23/21	-	-	RD Survey
12/7/21	RD Survey	RD Survey	RD Survey
5/5/22	RD Survey	RD Survey	RD Survey
Palm Beach			
	SEFL-08	SEFL-11	SEFL-12
12/3/21	RD Survey	RD Survey	RD Survey
1/21/22	RD Survey	RD Survey	-
2/11/22	-	-	RD Survey
3/18/22	Salinity exp. coral collections	RD Survey	RD Survey
4/18/22	Salinity exp. coral collections	-	-
		Pompano/Lauderdale by the Sea	
	FTL4	BC1	T328
7/22/21	RD Survey	RD Survey	RD Survey
10/22/21	RD Survey	RD Survey	RD Survey
1/25/22	RD Survey & Intervention Follow-Ups	RD Survey & Intervention Follow-Ups	RD Survey & Intervention Follow-Ups
4/27/22	RD Survey & Intervention Follow-Ups	RD Survey & Intervention Follow-Ups	RD Survey & Intervention Follow-Ups

3.2. Experimental Disease Intervention Strategies

We continued our efforts to experimentally assess the effectiveness of intervention treatments on SCTLD-affected corals in southeast Florida. *Montastraea cavernosa* colonies treated with either chlorinated epoxy or amoxicillin combined with CoreRx/Ocean Alchemists Base 2B were compared against both disease and healthy controls. The experimental and control colonies were assessed in both January and April 2022 during this period of performance. Videos were recorded for 3D model generation to measure colony sizes and tissue loss over time. Briefly, stills were extracted from the videos using the software FFmpeg, and models were generated through a four-step process using the software Agisoft Metashape. All tracing and quantifying of tissue areas from coral colony models was conducted in the application software Rhinoceros 3D.

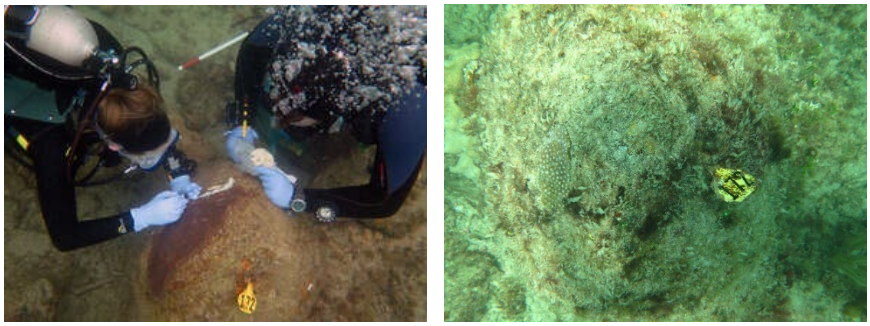

Figure 2. A SCTLD-affected *Montastraea cavernosa* coral colony is treated with Base 2B plus amoxicillin mixture in 2019 (left). In April 2022 this colony had lost additional tissue but was still surviving (right).

To further validate and improve our 3D photogrammetry methods, we created a 3D printed coral with a known surface area using engineering software and a 3D printer available at FAU Harbor Branch. With this and several other standard shapes of known areas, we collected replicated sets of videos for 3D model generation to assess error in the model generation methods across different image capture strategies. The standard "lawnmower" method recordings which have been used by our team were compared to other side angles of video recordings to determined which methods improved the overall accuracy and visual quality of the 3D models. Four imaging methods were assessed: Lawnmower, Lawnmower plus 45-degree, Lawnmower plus 90-degree, and Lawnmower with both 45- and 90-degrees. In total, 80 models were generated to test modeling accuracy and precision. These analyses were completed in May 2022 and will be used to improve 3D modeling methods for tracking tissue loss in intervention and other SCTLD experiments.

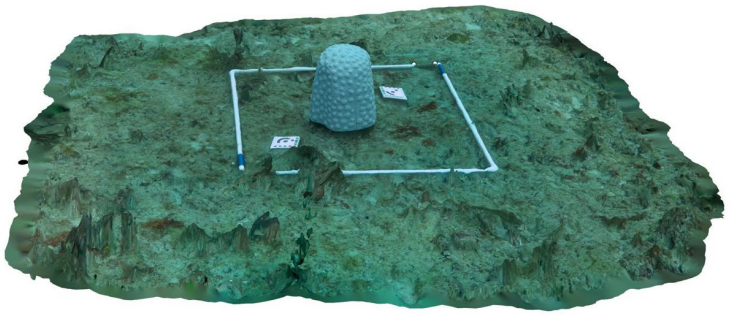

Figure 3. 3D model of 3D printed coral colony used to determine model precision in the field photogrammetry methods.

3.3. Coral Population Genetics to Inform Management and Restoration

During this period of performance, we focused on collection/analyses of additional samples of *Porites astreoides* (Figure 4) and *Stephanocoenia intersepta* (Figure 5). For all these samples, ~5 cm^2 tissue fragments were collected and preserved in either Trizol or Zymo DNA/RNA Shield. The samples were extracted using a modified dispersion buffer/phenol–chloroform-isoamyl alcohol extraction and cleaned using the Zymo DNA Clean and Concentrator Kit. DNA extracts were digested with BcgI enzyme and 2bRAD

libraries were prepared following Wang et al. (2012) including some modifications to optimize the libraries. Notably, 12 uniquely indexed 3' adaptors were incorporated, allowing 12 sample ligations to be pooled prior to amplification. Fully degenerate 5' adapters were also included, allowing PCR duplicate removal from downstream analyses. Additionally, triplicate libraries were prepared for three samples and used as a sequencing quality check and to identify natural clones. Sequencing was conducted on an Illumina NovaSeqS1 flow cell at the University of Texas at Austin's Genome Sequencing and Analysis Facility.

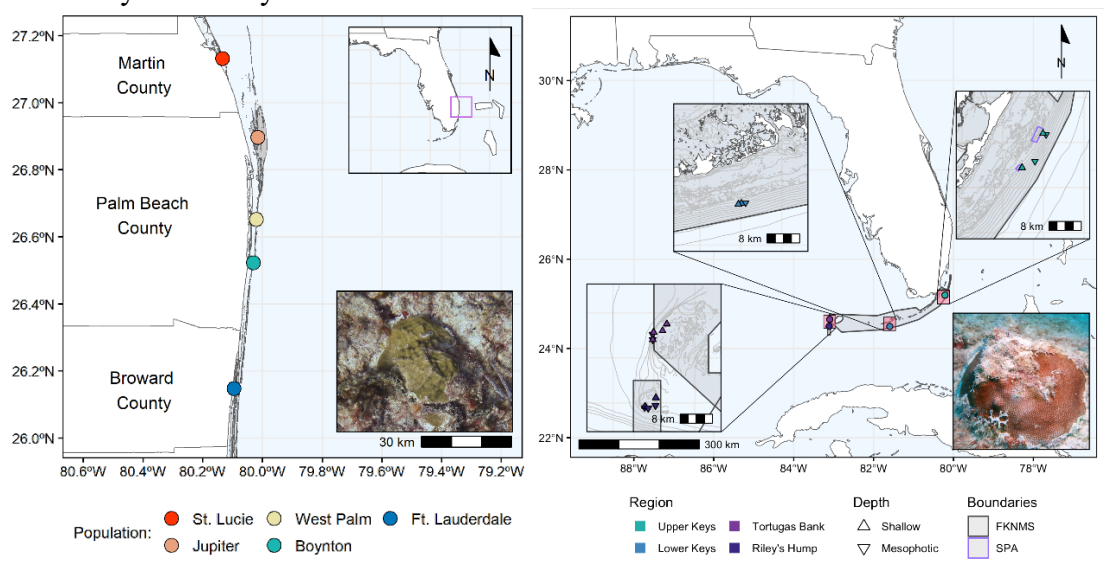

Figure 4. Collection locations for *Porites astreoides* (left) and *Stephanocoenia intersepta* (right) population genetics and connectivity assessment in the Coral ECA and FKNMS, respectively.

3.4. Coral Salinity Threshold Experiments

Both acute and chronic salinity threshold experiments were conducted in this period of performance. Coral collections for these experiments took place over a series of three dives in West Palm Beach where 10 colonies of *Montastraea cavernosa* and 10 colonies of *Porites astreoides* were collected. Due to the low abundance of suitably sized colonies in the region, *Siderastrea siderea* was ruled out as a study species. Sub-samples were collected and preserved in DNA shield and Trizol for potential future molecular analyses. Colonies were transported in coolers to Harbor Branch where they recovered for 48 hours prior to fragmentation. Corals cut into 3x3 cm fragments using a diamond blade tile saw and glued to labeled limestone tiles.

After coral collection and fragmentation, one fragment of each of 20 colonies were haphazardly placed into each of six ~100-liter aquaria. The acute salinity experiment began with a seven-day acclimation period followed by daily ~ 2 PSU /day reductions in each of the 3 randomly assigned experimental treatment tanks. Salinity was held at 36 PSU in the 3 control aquaria. Fragment condition was monitored by direct observation and scale, color corrected imaging. Fragment mortality was recorded to determine the LC50 or lethal concentration that cause mortality in 50% of the fragments for each species.

Based on the results of acute salinity experiment, 25 PSU was identified as an intermediate salinity stressor to test chronic salinity effects on coral mortality. Twenty fragments of naïve *M. cavernosa* (10) and *P. astreoides* (10) from the same coral collections were placed into each of 6 aquaria. For the chronic salinity stress experiment, following acclimation, salinity was reduced to 25 PSU in the 3 treatment aquaria and held at 25 PSU. Salinity in the control aquaria remained at 36 PSU. Time to mortality for 50% of the fragments was recorded for each species in each of the treatment aquaria. And again, scale imaging was used to track partial mortality or other changes in coral tissue.

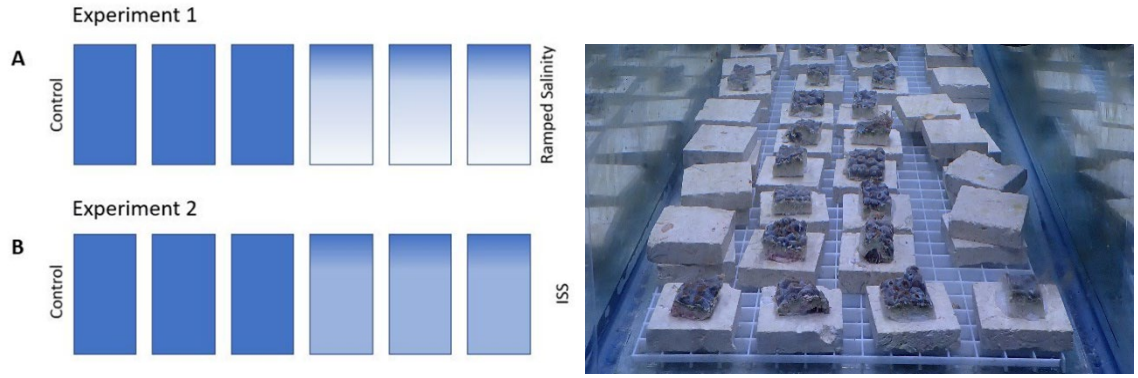

Figure 5. Experimental design (left) for acute salinity experiment (Experiment 1, A) and chronic intermediate salinity experiment (Experiment 2, B). Coral fragments mounted on limestone tiles and acclimating ahead of experiments (right).

3.5. QA/QC

All roving diver, fate tracking, intervention experiment, and salinity 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. Precision and accuracy in 3D modeling was assessed using 3D structures of known areas.

4. RESULTS

4.1. SCTLD Surveys and Reconnaissance

SCTLD prevalence from June 2021 to May 2022 was relatively low or absent at our St. Lucie Reef and Palm Beach sites. However, consistent levels of SCTLD were observed at our Lauderdale-by-the-Sea and intermittent SCTLD was observed in our Jupiter sites, and these were statistically greater than at St. Lucie or Palm Beach sites. Still, mean SCTLD prevalence levels observed in June 2021 to May 2022 ranging from 0-5% were significantly lower than levels observed in previous years. SCTLD prevalence was significantly different between locations (PERMANOVA; Pseudo-$F = 4.61$, $p < 0.01$) and over time (PERMANOVA; Psuedo-$F = 2.89$ $p < 0.03$).

Reconnaissance surveys to identify suitable coral restoration sites in the St Lucie Reef and Palm Beach areas identified two candidate sites in each location. All these sites had surrounding coral cover, evidence recent coral mortality, suitable substrate for adherence, and roughly level locations. We also selected sites based on suitable depth ranges for target restoration species, restricting our searches to areas between ~12ft (3.5 m) and 55 ft (17 m). Finally, we restricted restoration candidates sites so as to not overlap with existing monitoring program sites in both our monitoring and SECREMP. The candidate locations selected and recommended for restoration are below.

St. Lucie Reef: 27.1317 N, 80.1340 W
St. Lucie Reef: 27.1118 N, 80.1255 W
Palm Beach Breakers: 26.7104 N, 80.0158 W
Palm Beach Breakers: 26.6785 N, 80.0183 W

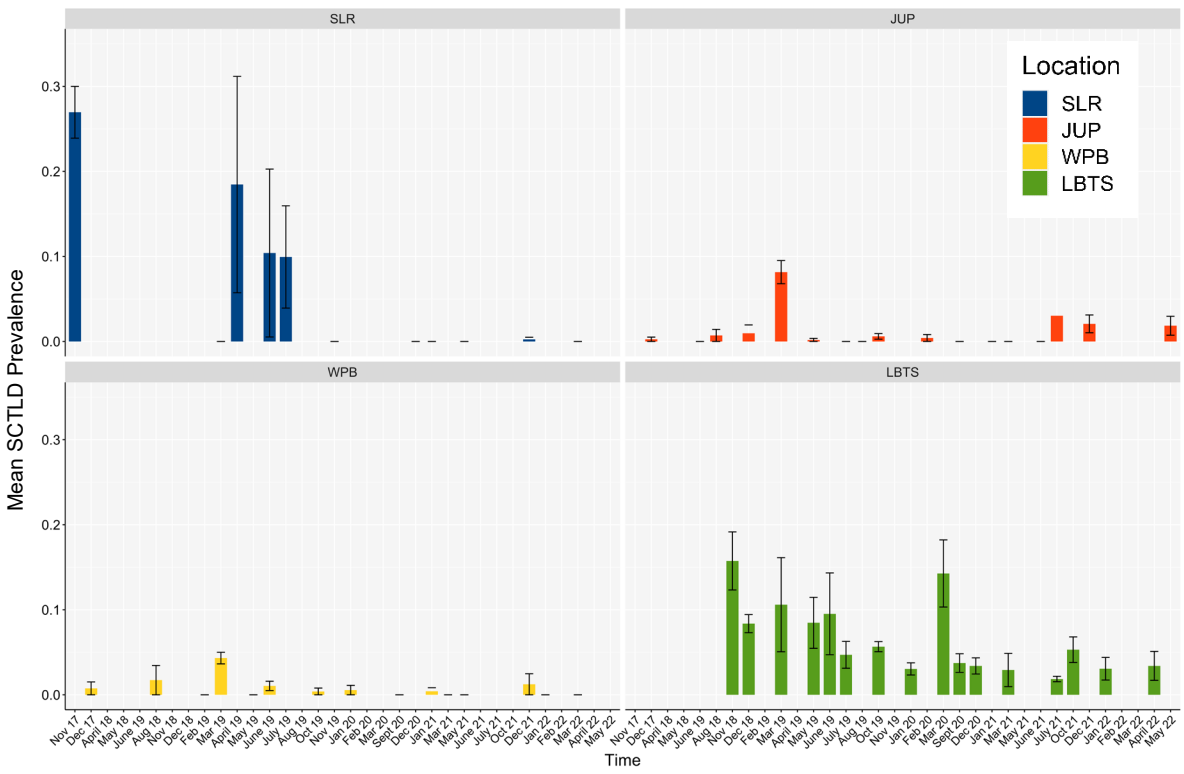

Figure 6. Mean SCTLD prevalence ± SD from roving diver surveys at St. Lucie Reef (SLR), Jupiter (JUP), West Palm Beach Breakers (WPB), and Lauderdale-by-the-Sea/Pompano (LBTS).

4.2. Experimental Disease Intervention Strategies

In this period of performance we tracked 45 colonies from previous experimental intervention efforts. The most recent comprehensive follow-up assessments on these colonies occurred on April 27, 2022 (Figure 7). Of the originally SCTLD affected but never treated colonies, five have quiesced, five had died, and one had active disease. Of the 24 fate-tracked *M. cavernosa* colonies that were treated with the amoxicillin/Base 2B treatment in either 2019 or 2020, in January and April 2022, 16 had quiesced, two were

dead, five had active lesions, and one could not be located. Of the ten fate-tracked colonies that were treated with the chlorinated epoxy treatment in the 2019 experiment, eight have quiesced, one is dead, and one had active lesions. During the April 2022 monitoring, all diseased corals were retreated with amoxicillin/base 2B.

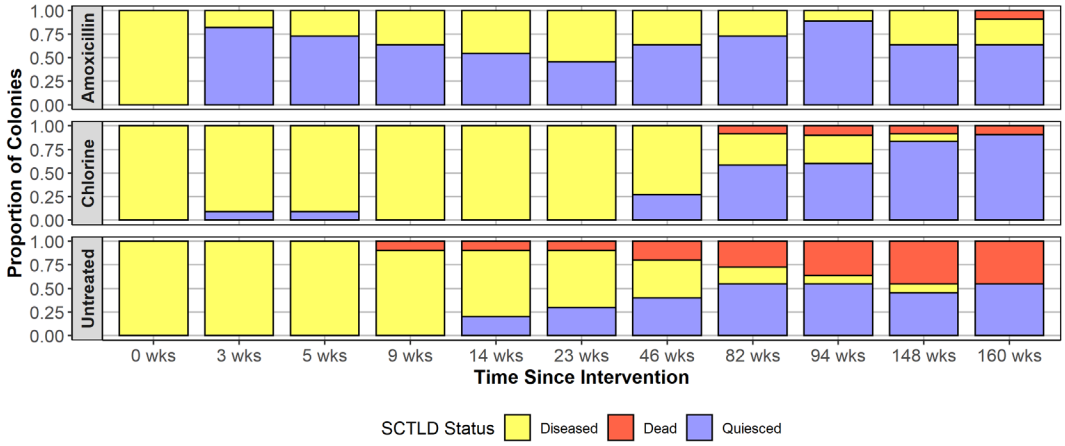

Figure 7. Disease status of colonies by treatment group at each time point shown in proportions of total. Amoxicillin refers to the Base 2B plus amoxicillin treatment, chlorine refers to the chlorinated epoxy treatment, and untreated refers to the SCTLD-affected controls.

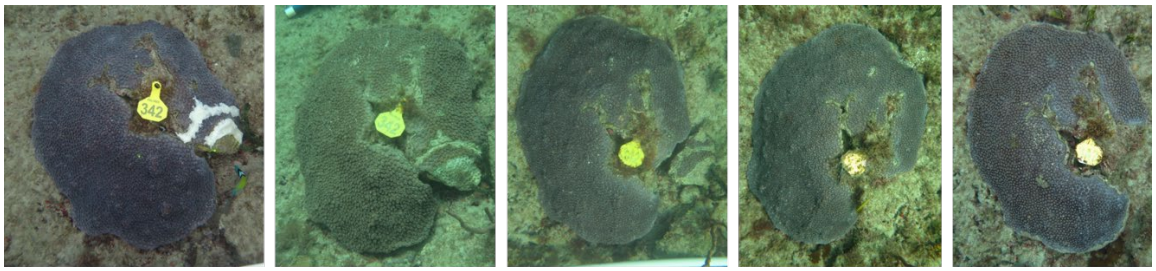

Figure 8. Selected time series photos of a *M. cavernosa* colony treated with amoxicillin and Base 2B.

Our efforts to determine error in 3D model generation indicated that for the 3D printed coral replica using the five separately filmed videos in the same lawnmower methods we have used in past modelling efforts, error was only 2%. Error was reduced to 0.9% when either 45-degree or 90-degree side angle recordings were added. Kruskal-Wallis tests also demonstrated that there was no significant differences among model replicates within each shape (Kruskal-Wallis tests, all $p > 0.05$, Table 8) nor were there significant differences among replica coral 3D models generated across several independent in silico model runs (Kruskal-Wallis tests, all $p > 0.05$).

4.3. Coral Population Genetic

4.3.1 *Porites astreoides*

To identify potential larval sources and *P. astreoides* population dynamics that may be contributing to observed coral community shifts in the Kristin Jacobs Coral ECA, we sampled 90 *P. astreoides* colonies across five locations in southeast Florida from St. Lucie Reef to Fort Lauderdale. Sequencing produced a total of 231 million raw reads before filtering, with an average of 2.4 million reads for each sample. After removal of PCR duplicates, trimming, and quality filtering, 148 million reads were left with an average of 1.5 million reads per sample. After re-running the clones and technical replicates-removed dataset in ANGSD, a total of 21,458 SNPs were identified. From the cluster dendrogram, nine clonal groups were identified for a total of 51 naturally occurring clones, demonstrating a high rate of clonality within and among across the sampling populations (Figure 9).

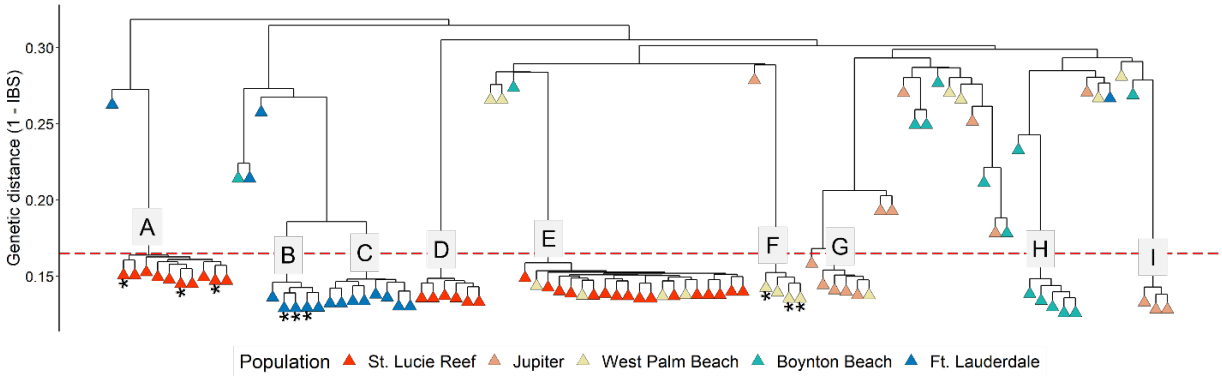

Figure 9. Dendrogram of *Porites astreoides* samples based on Identity-by-State matrix. Sample population denoted by color, asterisks denote technical replicates. The dashed red line indicates the minimum genetic distance threshold for calling clonal individuals or groups and was determined by the lowest level at which the technical replicate groups were present. Asterisks indicate technical replicates, and the nine clonal groups are labeled A–I directly above the corresponding cluster.

Despite the brooding reproductive strategy of *P. astreoides* coral species, there were relatively high levels of connectivity among populations, and varying levels of genetic structure correlated with geographic gradients across southeastern Florida (Figure 10).

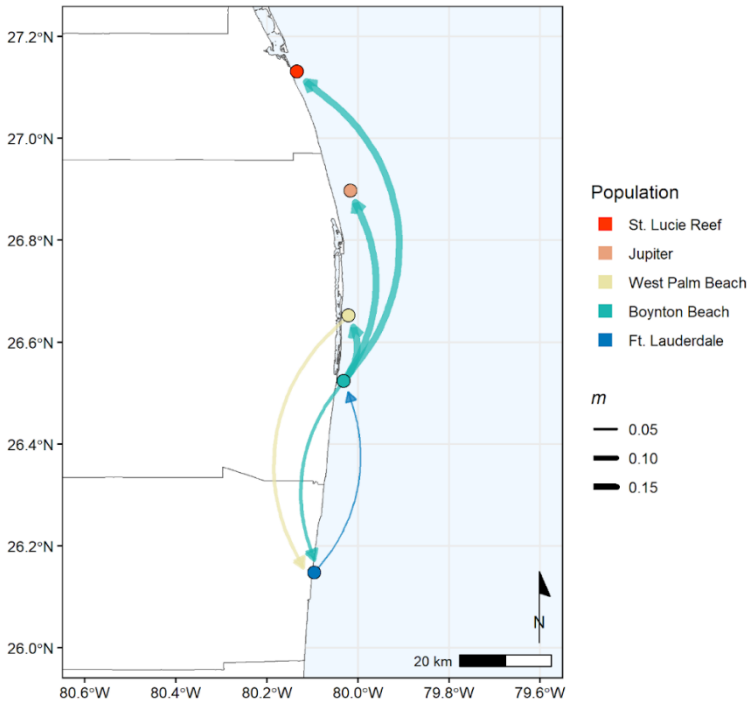

Figure 10. Map of *Porites astreoides* sample sites with arrows representing the levels of gene flow between populations as estimated by BayesAss analyses. Circles are colored by population, as are arrows, indicating which population they originate from. Direction of arrows indicates the direction of gene flow, and arrow width corresponds to the relative amount of gene flow. Only *m* values that had a >0 lower 95% confidence interval are displayed.

4.3.2 *Stephanocoenia intersepta*

To quantify the genetic connectivity and diversity of *Stephanocoenia intersepta* from shallow (<30 m) to mesophotic (30–45 m) depths across Florida Keys National Marine Sanctuary, we sampled 220 colonies using SCUBA and technical diving. These samples were sequenced on an Illumina NextSeq using a 2bRAD approach to generate a suite of 24,670 single nucleotide polymorphism (SNP) loci. AMOVA identified significant differentiation among sample populations (1.2%, $p = 0.01$), with both shallow and mesophotic populations demonstrating clustering (Figure 11). Throughout the region, shallow populations exhibited lower heterozygosity, higher minor allele frequencies, and significantly higher levels of intrapopulation relatedness and inbreeding relative to mesophotic *S. intersepta* populations.

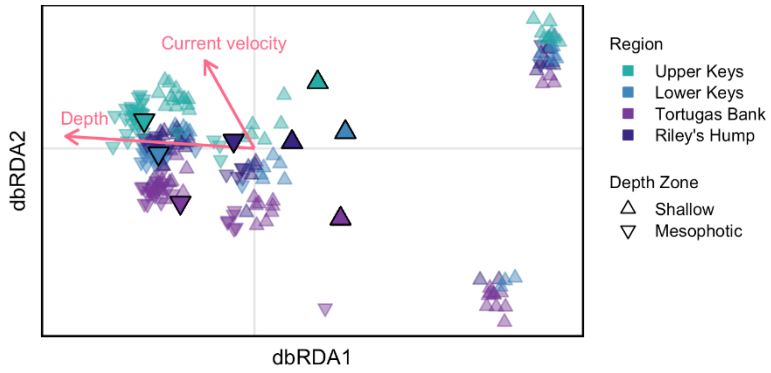

Figure 11. Distance-based redundancy analysis results for *S. intersepta* from the Identity-by-State matrix demonstrating clustering of samples by depth. Sites are coded by color and depths by shape. Individual samples are represented by transparent icons while larger, solid icons represent the population centroids. Vectors represent their corresponding environmental variables' relative contribution to the variation displayed on the axes.

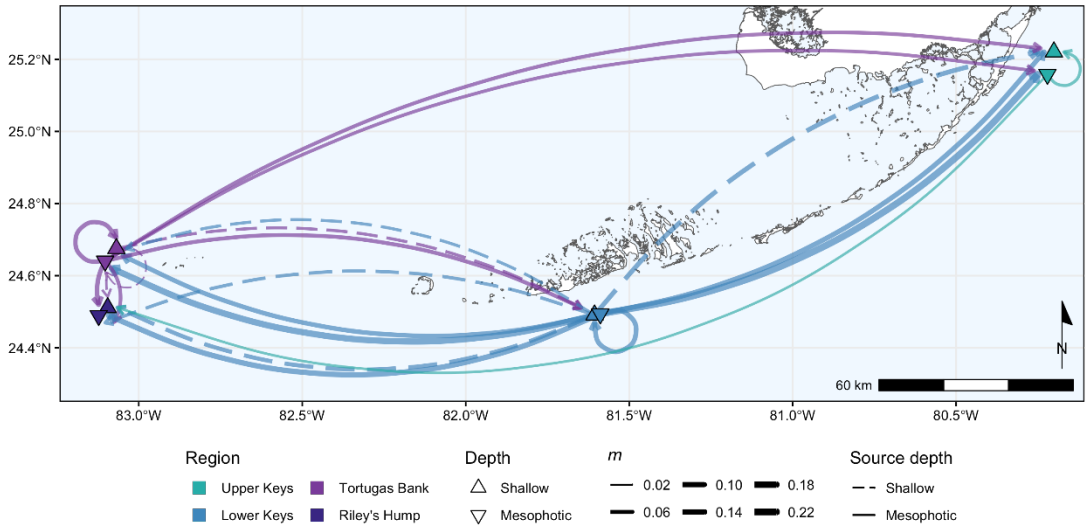

Figure 12. Map of *Stephanocoenia intersepta* sample sites with arrows representing the levels of gene flow between populations in the Florida Keys National Marine Sanctuary as estimated by BayesAss analyses. Sample icons and arrows indicating genetic sources are colored by location, icon shape indicates depth. Direction of arrows indicates the direction of gene flow, and arrow width corresponds to the relative amount of gene flow. Only *m* values that had a >0 lower 95% confidence interval are displayed.

4.4. Coral Salinity Threshold Experiments

Salinity was maintained with very little variation across individual tanks within an experimental condition (control or treatment). A ~2PSU reduction was successfully achieved across all treatment aquaria (Figure 13).

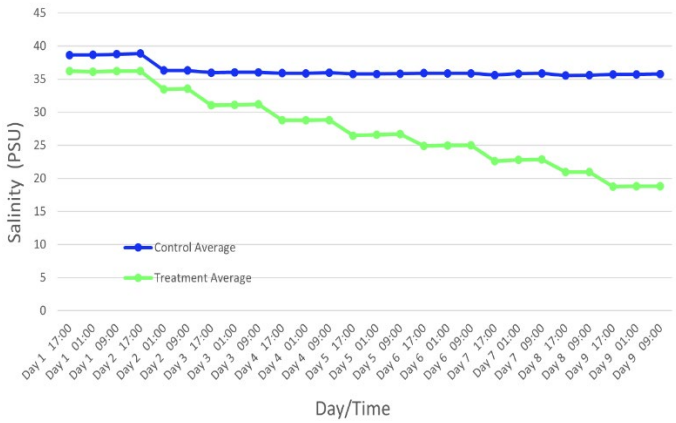

Figure 13. Salinities over time throughout the duration of the acute salinity experiment, beginning on the last day of acclimation. Blue represents the average of control tank salinities (PSU) at each timepoint, whereas green represents the same for the experimental treatment tanks with targeted 2 PSU per day reductions.

As salinities fell, *Porites astreoides* demonstrated initial mortality of a single fragment on day 4, with additional fragment mortality beginning on day 8 at 21 PSU. LC50 for both coral species was achieved at 19 PSU on day 9when ≥50% of fragments within each aquarium exhibited mortality.

ean percent survival for each species, *Porites astreoides* (solid lines) and *Montastraea cavernosa* (dotted lines). Controls are indicated by blue points and lines while acute salinity treatments are indicated by green points and lines.

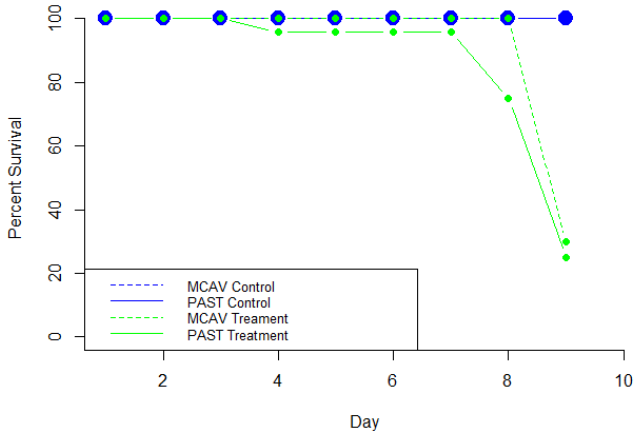

Figure 14. Mean percent survival for each species, *Porites astreoides* (solid lines) and *Montastraea cavernosa* (dotted lines). Controls are indicated by blue points and lines while acute salinity treatments are indicated by green points and lines.

In the chronic intermediate stress hyposalinity experiment, we found that the lethal duration 50 (LD 50) or time to result in mortality for 50% of the samples was 17 days for *P. astreoides* (Figure 15). For *M. cavernosa* fragments exposed to 25 PSU survived a full 26 days, at which time the experiment was terminated due to a scheduled expedition. No mortality was observed in 35 PSU controls for either species.

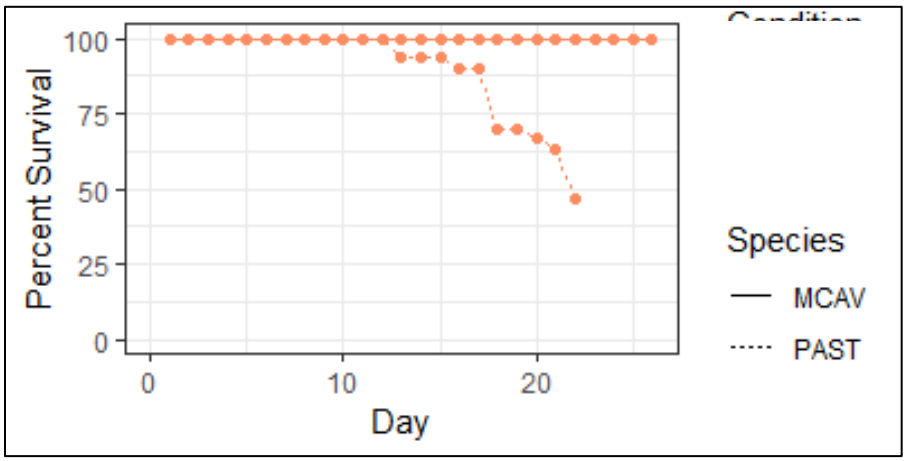

Figure 15. Mean percent survival for each species, *Porites astreoides* (dotted lines) and *Montastraea cavernosa* (solid lines). Controls are omitted for clarity; controls of both species had 100% survival through the trial.

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 SE FL. For example, stony coral tissue loss disease was observed continually throughout the project period among corals at our Lauderdale-by-the-Sea sites, while for June 2021–May 2022 SCTLD was essentially absent in the Palm Beach and Martin County sites. Overall, our regional surveys demonstrate that the Kristin Jacobs Coral ECA is well into the endemic phase of SCTLD, with few spikes or variations observed in SCTLD incidence and prevalence during the past year. Indeed, the low levels of SCTLD at St. Lucie Reef are almost entirely attributable to the loss of susceptible species in this area.

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. Even 3 years after a single treatment, *M. cavernosa* colonies treated with amoxicillin plus Base 2b were roughly 5 times less likely to die than corals with SCTLD lesions that were untreated. However, our results and those of others demonstrate that follow-up treatments are necessary for new lesions that may appear on previously treated corals. Furthermore, the potential secondary impacts of amoxicillin treatments on SCTLD-affected corals remain uncharacterized. We again recommend that targeted research efforts should focus on assessing the potential unintended consequences of antibiotic treatments on corals, their microbial communities (including Symbiodiniaceae), and neighboring organisms. Additionally, further efforts are needed to optimize dosing and delivery methods for antibiotic treatments on SCTLD-affected corals.

Our population genetics results indicated significant historical structuring of *P. astreoides* populations in the Coral ECA. However, there are also indications of relatively recent gene flow (over the past two-three generations) especially from Boynton to populations

northward. High rates of clonality were discovered across all sites but were highest at St. Lucie Reef which has several implications. First, it partially explains the rapid proliferation of *P. astreoides* colonies on St. Lucie Reef. We might assume based on relative geographic isolation and the dispersal distances of brooding *P. astreoides,* that St. Lucie Reef is likely not receiving a high influx of larvae from other populations recruiting to the reef. However, there does appear to be some level of multi-generational connectivity with the Boynton population. A significant amount of asexual reproduction via fragmentation or parthenogenesis or high rates of self-fertilization by a few successful individuals may have occurred over recent years. To further characterize the reproductive patterns driving the population dynamics and high rates of clonality at St. Lucie Reef we could employ histology to quantify sex ratios and fecundity of colonies and collect larvae *in situ* in combination with parentage analyses.

The level of clonality within *P. astreoides* resulted in a smaller usable sample set for this study due to many of the analyses requiring the exclusion of clones to meet the necessary assumptions. The presence of clones across adjacent sites (clonal groups were present across St. Lucie and West Palm as well as Jupiter and West Palm) are in themselves potential evidence that *P. astreoides* can successfully disperse distances of thousands of meters in this region. Whether this is occurring over a single generation through long-distance dispersal of parthenogenetically produced larvae or over many generations through a stepping-stone asexual methods, remains to be understood.

For *Stephanocoenia intersepta* in FKNMS, our results demonstrate that despite population genetic structuring across depth, mesophotic coral populations may provide refuge potential for shallow populations in the future. Mesophotic coral populations remain important contributors to the overall genetic diversity of *S. intersepta* throughout the region. This study highlights the importance of including mesophotic as well as shallow coral populations in population genetic analyses and provides information and data useful for future management and restoration efforts in Southeast Florida.

Few found that acute salinity exposures below 19 PSU risk severe mortality for both *M. cavernosa* and *P. astreoides* in south Florida. Further spatial/temporal salinity modeling is needed to determine the risks of this salinity level occurring during freshwater releases or severe precipitation events. In terms of chronic intermediate stress, it appears that *P. astreoides* is more susceptible to intermediate hyposalinity stress then *M. cavernosa.* If conditions near 25 PSU persist for more than two weeks, *P. astreoides* colonies will be at an elevated risk of mortality.

6. RECOMMENDATIONS

Recommendation 1: Prioritize disease mitigation/intervention efforts to reduce losses of key coral reef ecosystem components. Base 2B plus amoxicillin demonstrated success against SCTLD lesions on *M. cavernosa* with a 95% success rate. However, new lesions can arise, and broad scale application of antibiotics may not be advisable or scalable. We recommend continuing amoxicillin treatment activities to protect existing coral tissue/cover.

Recommendation 2*: 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 3: *Advance coral conservation initiatives with support from Magnuson-Stevens Act and implement actions/regulations for Kristin Jacobs Coral Reef Ecosystem Conservation Area.* 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 continued testing of outplant resilience to SCTLD.

Recommendation 4: *Advance coral population genetics to support effective management and restoration for coral populations and communities in Florida.* Additional effort and resources are needed to understand Florida's intraspecies genetic diversity. These assessments need sampling from the greatest number of locations and depths feasible. Likewise, successful coral restoration strategies will require knowledge of genetic stocks among various coral populations to design and implement effective restoration plans.

Recommendation 5: *Consider updating management criteria to keep salinities on coral reefs above 20 PSU for short term exposures (1-2 days), and above 25 PSU if freshwater releases will last longer than one week.* Currently corals' responses to freshwater releases and subsequent impacts on coastal nearshore salinities are currently not considered in management criteria. Our results suggest corals are more likely to perish when exposed to reduced salinity levels.