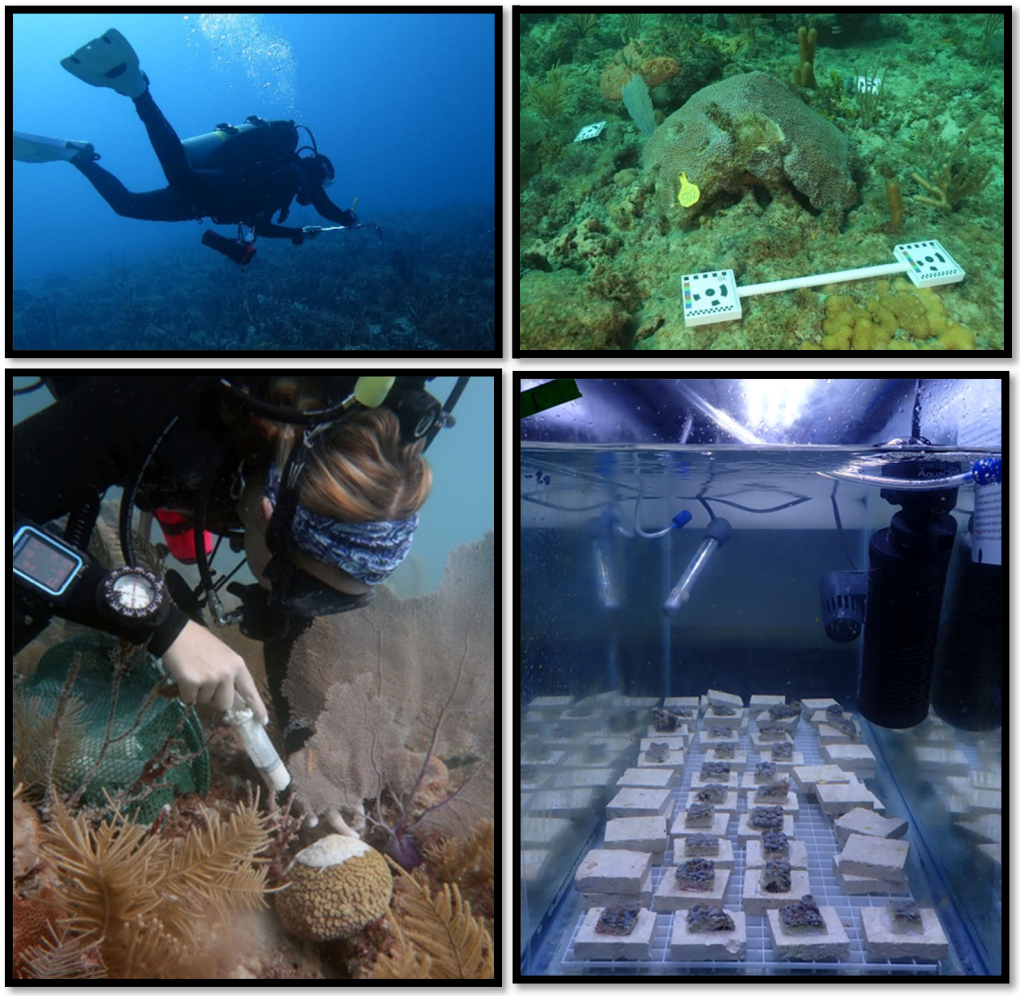
# 2023

# Advancing Coral Reef Research and Resilience in Southeast Florida

## Final Report



# Phase 3: Advancing coral reef research and resilience in Southeast Florida

## Final Report

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**Executive Summary for Managers**

Coral reefs in southeast Florida continue to face severe challenges from coral diseases, 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 (SE FL). Continued monitoring of coral disease incidence and prevalence in the northern portion of the Kristin Jacobs Coral Reef Ecosystem Conservation Area was coupled with ongoing disease intervention experiments, coral population genetics to inform population management and coral restoration, and experimental coral salinity thresholds tests. This integrative 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.

Reefs in Martin, Palm Beach, and Broward counties remain in the endemic phase of stony coral tissue loss disease (SCTLD), with low disease prevalence observed at our St. Lucie Reef, Palm Beach, and Jupiter sites. However, our Lauderdale-by-the-Sea sites continued to display SCTLD prevalence in the range of 2-4%. Long-term tracking demonstrated that diseased corals experimentally treated with antibiotics were approximately 4.5 times more likely to survive than corals not treated. After 4 years of monitoring, only 1 colony remains SCTLD active. In terms of coral genetics, 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. We added additional samples from SE FL for *S. intersepta* in FY23 and plan to integrate these into state-wide and region connectivity analyses. Finally, we've completed 3 experiments to examine the potential impacts of low salinity stress on corals in southeast Florida. Freshwater releases that expose corals to salinities lower than 20 PSU for even just 1 day risk severe impacts and coral mortality with >50% mortality observed in *Montastraea cavernosa* and *Porites astreoides*. Lower-volume, longer-duration releases of freshwater may help to preserve coral health in SE FL.

**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. 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, Chris Camargo, and Salena Alberti 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.

Samples within St. Lucie Inlet Preserve State Park were collected under permits 01221915, 012102115, 03152215 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, SAL-21-1702-SRP, and SAL-23-1702-SRP.

# Contents



## 1. BACKGROUND

Florida's coral reefs are currently experiencing a multi-year outbreak of a coral disease described as stony coral tissue loss disease (SCTLD). While coral disease outbreaks are not unprecedented, this event is unique due to 1) the presence of multiple symptoms and etiologies that have affected at least 21 species of coral across Florida's Coral Reef (FCR), 2) the persistence of the disease over multiple years, and 3) the high levels of mortality associated with infections. SCTLD is highly prevalent and is estimated to have resulted in the mortality of millions of corals across southeast Florida, Biscayne National Park, and the Florida Keys. Compounding SCTLD effects, Hurricane Irma impacted the entire FCR in early September 2017, with subsequent freshwater discharge impacts particularly acute on coral reefs in Martin County. The work herein focuses on southeast Florida (SE FL) within the Kristin Jacobs Coral Reef Ecosystem Conservation Area (Coral ECA) and is part of a larger effort to understand the impacts of disease on coral health in this region and to determine optimized mitigation efforts that may prevent further 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). A major ongoing effort included monitoring for potential new incidents of disease in corals in the northern part of Florida's Coral Reef and plans for intervention with amoxicillin plus Base 2B if needed. In addition, this project was designed to improve understanding of the current spatial extent of the disease outbreak, prevalence, and species affected. Our objectives for FY23 included:

1. Project coordination and permitting.
2. SCTLD monitoring, 3D imaging, and intervention activities in Martin, Palm Beach, and northern Broward counties, and supplemental reconnaissance surveys

for coral disease survivors in Palm Beach and Martin counties to help inform future endemic collections, propagation, and restoration.

3. Continued collaborative testing of intervention strategies including antibiotics, antiseptics, probiotics, etc.
4. Building critical data on population genetics and connectivity in Palm Beach, Martin, and northern Broward counties to support restoration planning for *Montastraea cavernosa, Porites astreoides, Stephanocoenia intersepta,* and *Xestospongia muta*.
5. Experimental assessment of coral salinity thresholds to inform freshwater management in Florida.

The outcomes of this project contribute to ongoing and future coral disease response efforts that seek to improve understanding about the severity and impacts of the SCTLD outbreak, identify management actions to remediate disease impacts, and, ultimately, prevent or mitigate the effects of future outbreaks. Finally, this project provides key information for coral species stock enhancement efforts to ensure that restoration efforts maintain community and population-level biodiversity in South Florida.

Florida DEP funds awarded through PO C01954 were used primarily to support coral disease surveys and reconnaissance, field collections of coral samples, 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 NCCOS (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. Monitoring 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. SE FL 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.

**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 |
| SEFL16 | 26°31.4131' | -80°1.9015' | Boynton Beach | Palm Beach |
| SEFL18 | 26°29.6155' | -80°2.2509' | Boynton Beach | Palm Beach |
| SEFL20 | 26°27.2298' | -80°2.7642' | Boynton 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 |

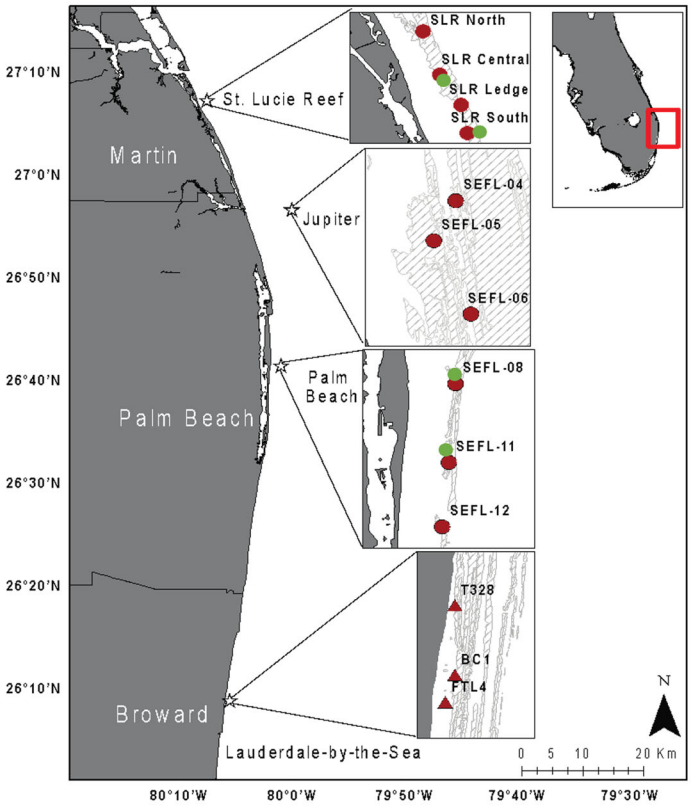


**Figure 1.** Map of monitoring locations throughout Florida's Northern Coral Reef. Red circles indicate roving diver survey sites and red triangles indicate sites where both roving diver surveys and the SCTLD intervention treatment experiment occurred. Sites selected as restoration locations are indicated by green circles.

### 3.1. Coral Health Surveys and Reconnaissance

Quarterly roving diver disease surveys (see Table 2) similar to Disease Response Monitoring surveys were conducted to assess the greatest reef area possible, quantifying disease prevalence over an estimated range of 900–2000 $m^2$ per survey based on conditions, principally limited by underwater visibility. SCUBA divers swam for 20 minutes and recorded all coral colonies to species level 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.

**Table 2.** Operational Activities by Site. RD = Roving Diver Survey.

| St. Lucie Reef | | | |
|---|---|---|---|
| | Central | Ledge | South |
| 09/06/22 | RD Survey | RD Survey | RD Survey |
| 01/05/23 | RD Survey | RD Survey | RD Survey |
| 03/07/23 | RD Survey | RD Survey | RD Survey |
| 06/09/23 | RD Survey | RD Survey | RD Survey |
| **Jupiter** | | | |
| | SEFL-04 | SEFL-05 | SEFL-06 |
| 08/11/22 | RD Survey | RD Survey | RD Survey |
| 12/09/22 | RD Survey | RD Survey | RD Survey |
| 02/03/23 | RD Survey | RD Survey | RD Survey |
| 06/14/23 | RD Survey | RD Survey | RD Survey |
| **Palm Beach** | | | |
| | SEFL-08 | SEFL-11 | SEFL-12 |
| 09/07/22 | - | RD Survey | RD Survey |
| 09/16/22 | RD Survey | - | - |
| 12/26/22 | RD Survey | RD Survey | RD Survey |
| 03/07/23 | RD Survey | RD Survey | RD Survey |
| 06/13/23 | RD Survey | RD Survey | RD Survey |
| **Pompano/Lauderdale by the Sea** | | | |
| | FTL4 | BC1 | T328 |
| 08/03/23 | RD Survey/Intervention Follow-Ups | RD Survey/Intervention Follow-Ups | RD Survey/Intervention Follow-Ups |
| 10/26/22 | RD Survey/Intervention Follow-Ups | RD Survey/Intervention Follow-Ups | RD Survey/Intervention Follow-Ups |
| 01/18/23 | RD Survey/Intervention Follow-Ups | RD Survey/Intervention Follow-Ups | RD Survey/Intervention Follow-Ups |
| 05/03/23 | RD Survey/Intervention Follow-Ups | RD Survey/Intervention Follow-Ups | RD Survey/Intervention Follow-Ups |

To understand the current condition of St. Lucie Reef (SLR), we supplemented the roving diver surveys with 3D mosaic imaging over 10x10 m plots in several locations across SLR. Photomosaics were generated for the St. Lucie reef sites Central and South. Briefly, a Canon 6D mkII DSLR camera with 24 mm lens in an Ikelite underwater housing was used to capture still images every second. Scale bars were placed on the sides of the 10 x 10 m reef area and the diver swam approximately 1 m above the highest point of the colony following a lawnmower pattern, following similar protocol to Young et al. (2018). Overlapping runs were oriented to achieve 60–80% overlap in the images. Images were imported into Agisoft Metashape Pro (Version 1.7.2, Agisoft LLC) for generation of 3D models through a four-step process: 1. Camera alignment, 2. Dense point cloud generation, 3. Mesh generation, 4. Texture overlay. The resulting 3D models were then imported into Rhinoceros 3D (Robert McNeel & Associates) for analysis

(Young et al. 2018; Combs et al. 2021). The models were scaled using the scale bars and verified using the two additional scale markers with fixed distances.

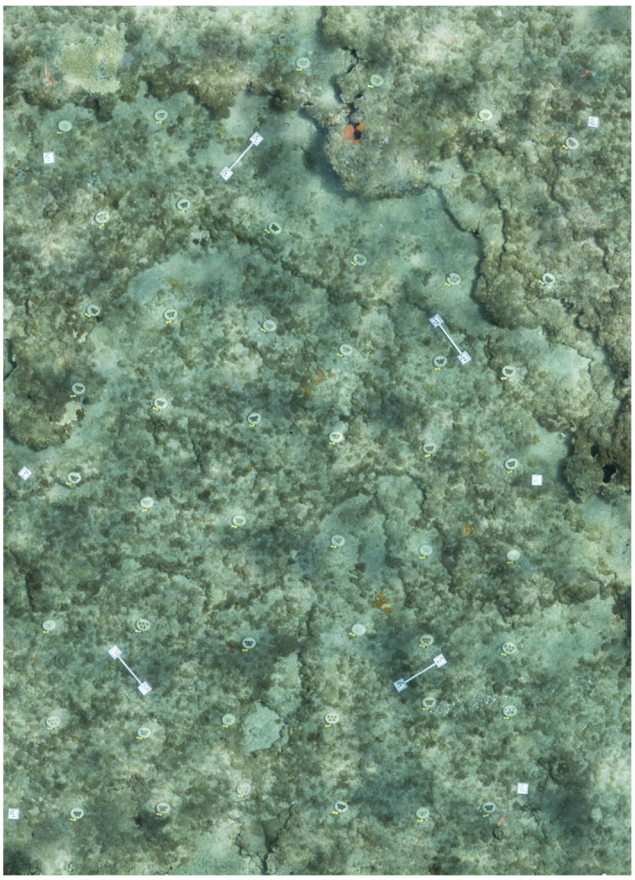

**Figure 2.** 3D model of St. Lucie South reef site.

### 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 over 4 years. *Montastraea cavernosa* colonies at our Lauderdale-by-the-Sea sites treated with either chlorinated epoxy or amoxicillin combined with CoreRx/Ocean Alchemists Base 2B were compared against both disease and healthy controls. Details of this experiment and analyses have been reported in previous annual reports and can be found in Shilling et al. 2021.

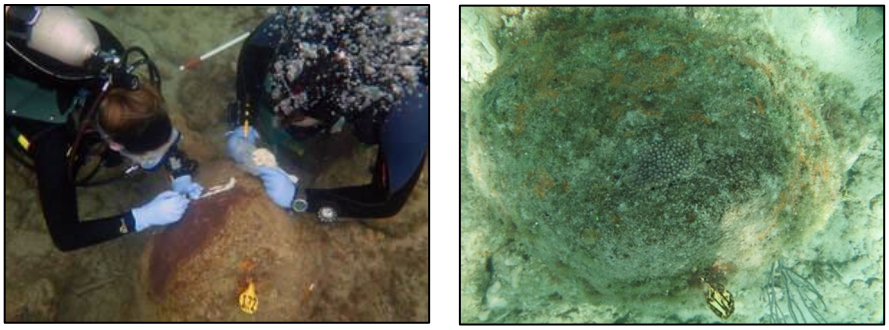

**Figure 3.** A SCTLD-affected *Montastraea cavernosa* coral colony is treated with Base 2B plus amoxicillin mixture in 2019 (left). As of May 2023 this colony had lost no additional tissue since 2022 and is still surviving (right).

### 3.3. Coral Population Genetics to Inform Management and Restoration

During this period of performance, we focused on analyses of samples fro the Florida keys and collection of additional samples of *Stephanocoenia intersepta* from Jupiter, Palm Beach, Boynton, and Lauderdale-by-the-Sea; there was insufficient abundance of this species for sampling from our St. Lucie Reef sites. These new SE FL samples are in our processing pipeline for sequencing but we do not yet have reads from these new samples.

For all coral population genetic samples, ~5 $cm^2$ tissue fragments were collected and preserved in 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 prepared for three samples provide sequencing quality check and to identify natural clones.

In FY23 we also employed new analytical methods for population connectivity analyses with previously sequenced *Porites astreoides* from our 5 Coral ECA sites and *S. intersepta* samples from the Florida Keys National Marine Sanctuary to build on results from FY22.

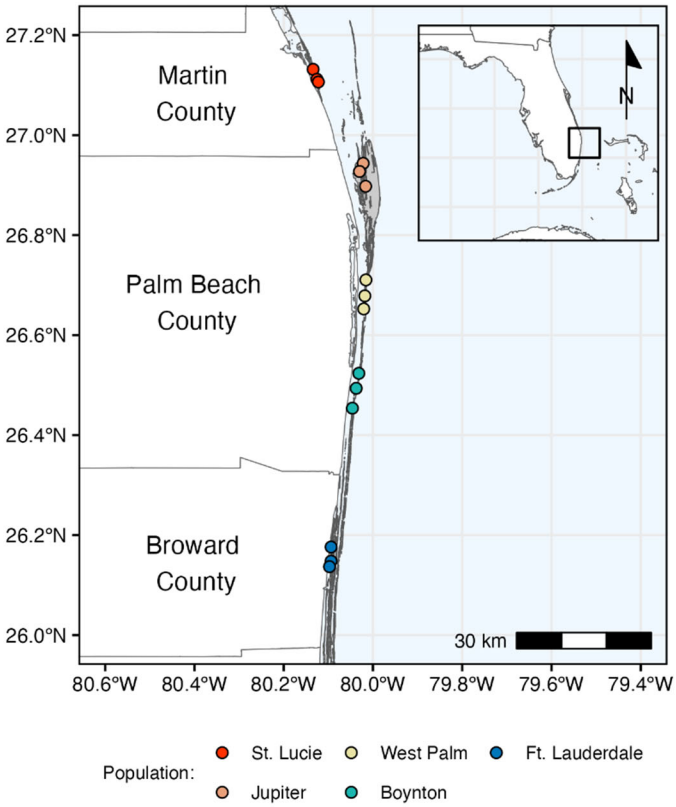


**Figure 4.** Collection locations for *Porites astreoides*, *Stephanocoenia intersepta*, *Montastraea cavernosa*, and *Xestospongia muta* population genetics and connectivity assessment in the Coral ECA.

### 3.4. Coral Salinity Threshold Experiments

Following experiments to evaluate acute and chronic salinity thresholds in FY22, in FY23 we completed final statistical analyses of these first two experiments and ran a third experiment combining chronic hyposalinity with SCTLD exposure. Coral collections for all 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 ~90-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.

Finally, combined stress tests were conducted using the same experimental design as the chronic hyposalinity test, but with the addition of a disease exposure as the treatment tanks reached 25 PSU. This involved the collection and fragmentation of a single SCTLD infected colony from Voss lab sites at Lauderdale-by-the-sea. On day 5 of the experiment, when treatment tanks reached 25 PSU, all aquaria were exposed to a single SCTLD-infected *M. cavernosa* fragment.

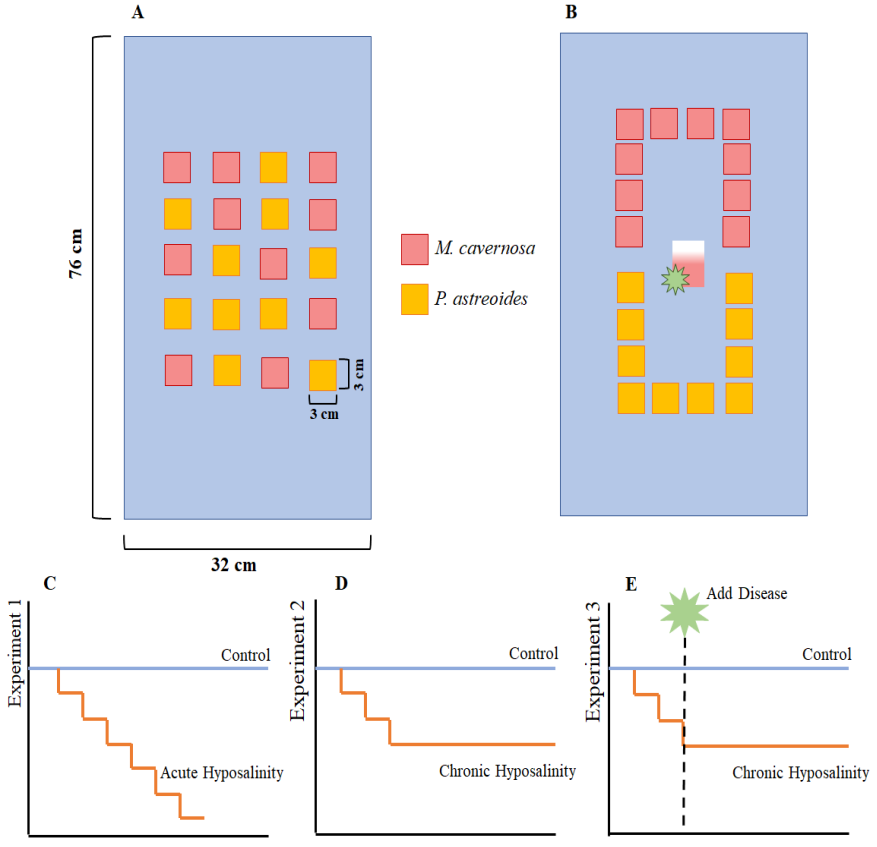


**Figure 5.** Representation of aquarium setups (A,B) and treatments (C,D,E) for a set of three experiments. A) Aquarium setup used for experiments 1 and 2 with 20 haphazardly placed coral fragments of roughly 3x3 $cm^2$ and color representing coral species. B) Aquarium setup used in experiment 3. C) Experiment 1, 35 PSU control and acute

hyposalinity stress, -2 PSU/day. D) Experiment 2, 35 PSU control and chronic hyposalinity stress at 25 PSU. E) Chronic hyposalinity plus SCTLD exposure.

### 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 2022 to June 2023 was relatively low or absent at our St. Lucie Reef, Palm Beach, and Jupiter sites (Figure 6). However, consistent levels of SCTLD were observed at our Lauderdale-by-the-Sea, and these were statistically greater than at St. Lucie, but not Palm Beach, and Jupiter sites. Still, mean SCTLD prevalence levels observed in June 2022 to June 2023 ranging from 0-3% were significantly lower than levels observed in previous years. Within the past 12 months, SCTLD prevalence significantly differed between locations (PERMANOVA; Pseudo-$F = 4.845$, $p = 0.003$) but not over time (PERMANOVA; Psuedo-$F = 0.663$, $p = 0.774$). Pairwise tests found significant differences in SCTLD prevalence among the Pompano and all other sites: Jupiter ($p = 0.03$), West Palm ($p = 0.023$), and St. Lucie ($p = 0.002$) but no significant differences among the other sites ($p > 0.05$).

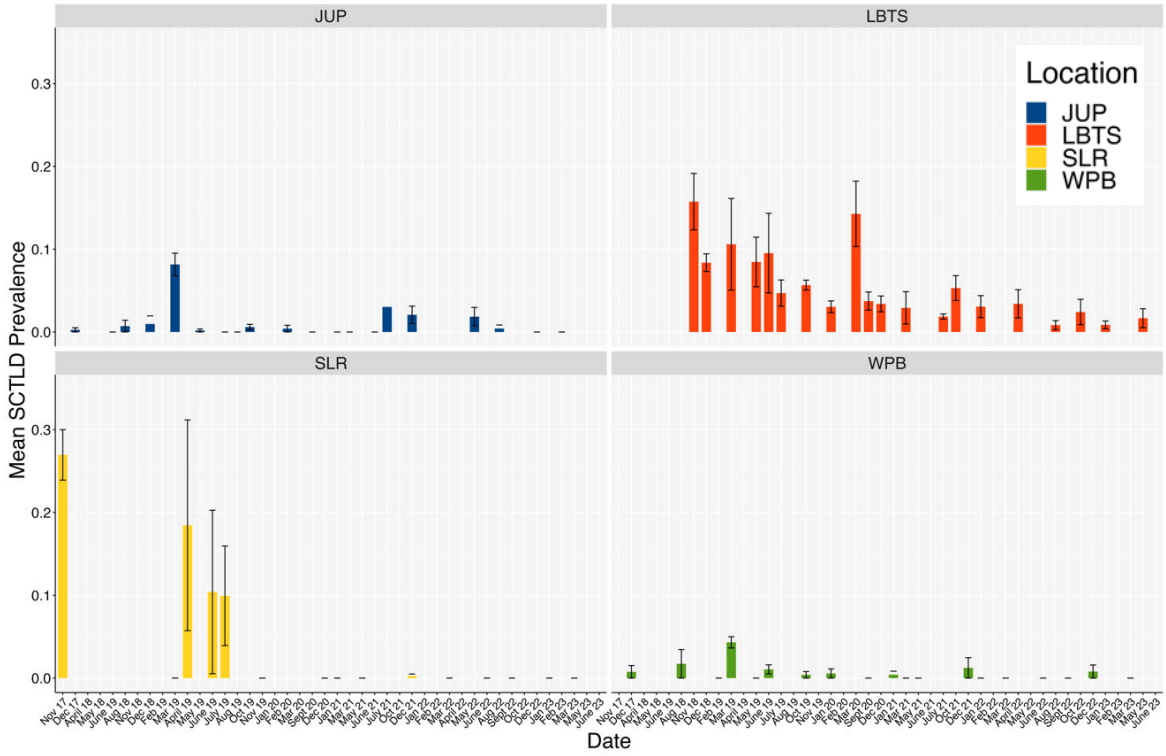


**Figure 6.** Mean SCTLD prevalence ± SD from roving diver surveys at St. Lucie Reef (SLR), Jupiter (JUP), West Palm Beach Breakers (WPB), and Lauderdale-by-the-Sea/Pompano (LBTS).

### 4.2. Experimental Disease Intervention Strategies

Due to very low/no SCTLD prevalence in our St. Lucie, Jupiter, and Palm Beach sites, no additional colonies were treated with amoxicillin/Base 2B in FY23.

In FY23 we tracked colonies from previous experimental intervention efforts at our Lauderdale-by-the-Sea sites. The most recent comprehensive follow-up assessments on these colonies occurred on May 03, 2023 (Figure 7). Of the originally SCTLD affected but never treated colonies one had active disease in the past year, but by May 2023 fourteen colonies)had quiescence (64%) or and eight colonies died (36%). Of the 24 fate-tracked *M. cavernosa* colonies that were treated with the amoxicillin/Base 2B treatment in either 2019 or 2020, throughout the past year, twenty one had quiesced (88%), two were dead (8%) , and one had active lesions (4%). Of the ten fate-tracked colonies that were treated with the chlorinated epoxy treatment in the 2019 experiment, throughout the year, eight were still healthy/quiesced (80%) and two died (20%).

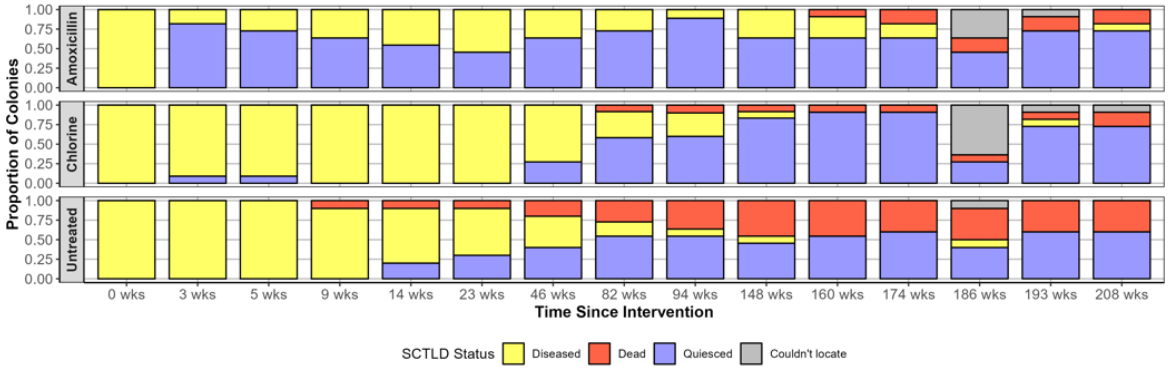


**Figure 7.** Disease status of colonies by treatment group at each time point shown in proportions of total. Amoxicillin refers to the BaseB plus amoxicillin treatment, chlorine refers to the chlorinated epoxy treatment, and untreated refers to the SCTLD-affected controls. Poor visibility limited data collection at 186 weeks.

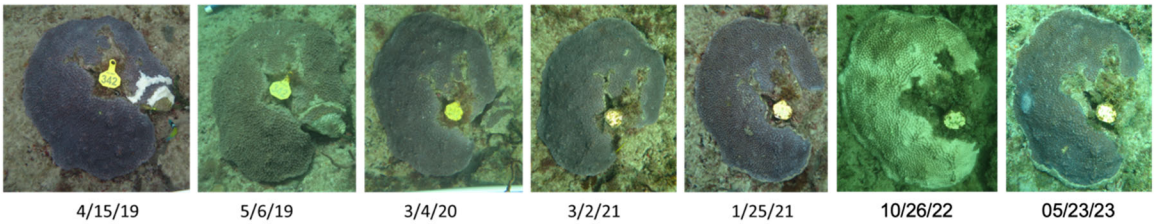


**Figure 8.** Selected time series photos of a *M. cavernosa* colony treated with amoxicillin and Base 2B.

### 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 remained 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. The overwhelming majority of the samples in each clonal group were from the same sampling site, however two of the groups did contain clones identified across different sites.

AMOVA indicated significant differences between populations, which explained 2.75% of the variation across samples (SS = 10,419.15, p = 0.001). The dbRDA showed some overlap but with distinct centroids for Jupiter, West Palm, and Boynton clustering more

and St. Lucie and Ft. Lauderdale being more distinct (Figure 9). Two statistically significant predictor variables were retained in the model: depth and latitude.

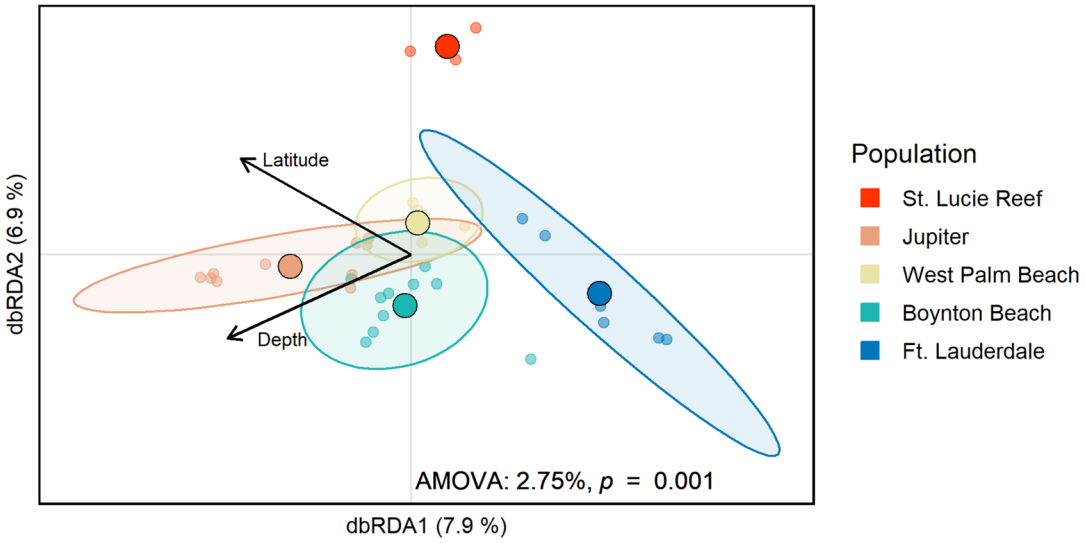


**Figure 9.** Distance-based redundancy analysis results from Identity-by-State matrix showing clustering of samples by population, coded by color. Individual samples represented by transparent circles while larger, solid circles represent the population centroids. Ellipses for each population (with the exception of the St. Lucie population due to low sample size) were calculated using multivariate t-distribution. The percent variation explained by population level differences calculated by the Analysis of Molecular Variance is listed in the bottom right. Total variation explained by each axis is indicated. Vectors represent their corresponding predictor variables relative contribution to the variation displayed on the axes: latitude of sample collection and depth.

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).

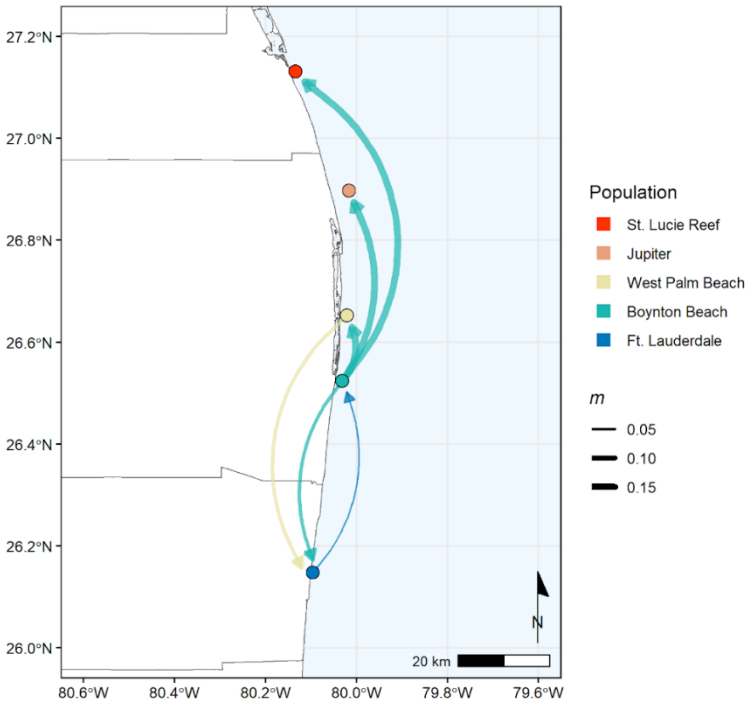


**Figure 10.** Map of *Porites astreoides* sample sites with arrows representing the levels of gene flow between populations as estimated by BayesAss analyses. Circles are colored by population, as are arrows, indicating which population they originate from. Direction of arrows indicates the direction 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.

CLUMPAK analyses identified three as the optimal number of genetic clusters, while two of StructureSelector's reported K estimators selected K = 2, and the other two selected K = 3 (Figure 11). After review of both NGSadmix plots, K = 3 was selected for subsequent interpretations and analyses given that it was the most frequently selected model. The first cluster (green) is relatively well distributed while the third (purple) is almost completely exclusive to Ft. Lauderdale. The northernmost site, St. Lucie, is dominated primarily by one genetic cluster (green), and this same cluster was also predominant in the West Palm and Boynton populations. Jupiter is almost equally split in dominance by the first (green) and second genetic cluster (pink), and the southernmost site in Ft. Lauderdale is primarily composed of the third (purple) genetic cluster.

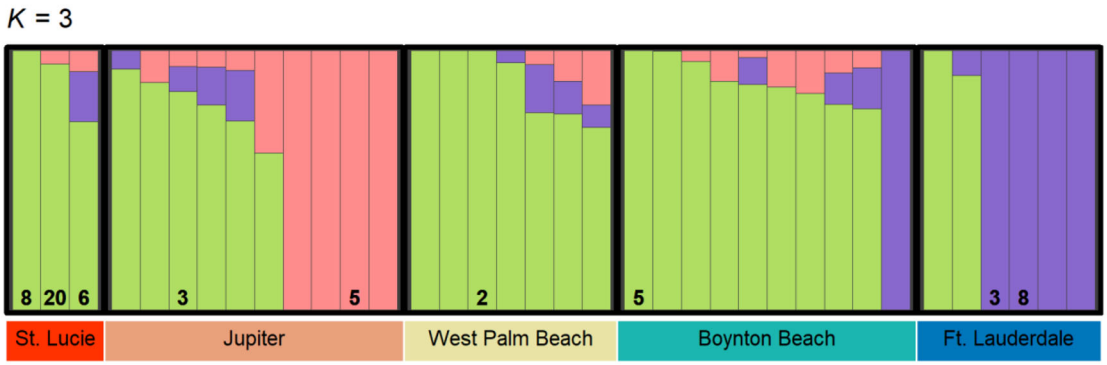


**Figure 11.** StructureSelector's and CLUMPAK's calculated K showing three genetic clusters (represented by green, pink, and purple) of *Porites astreoides* present among the populations sampled. Each column represents one sample, and the proportion of each column filled with the color representing each genetic cluster indicates the probability of membership to that cluster. Some individuals were from a clonal group, and the number of total individuals from that clonal group are represented by the numbers present at the base of the column; in some cases, this collapsed individuals from different sites into a column.

### 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 FKNMS, 220 samples were sequenced on an Illumina NextSeq using a 2bRAD approach to generate a suite of 24,670 single nucleotide polymorphism (SNP) loci. In FY23, we reanalyzed these data to assess difference among *S. intersepta* lineages and locations. *S. intersepta* populations in FKNMS exhibit strong population structuring across depth (Fig. 12 B, E, F). Our analyses uncovered four distinct genetic lineages throughout the Florida Keys (Fig. 12 C, D, E). Of these putative lineages, one is predominantly found in mesophotic sites (Blue lineage), and two lineages (Green, Yellow) are almost exclusively found in shallow populations (Fig. 13A).

Shallow lineages exhibited lower heterozygosities, higher mean nucleotide diversity, and were more inbred than lineages found to dominate mesophotic sites (Fig. 13B, C, D). Shallow lineages were also more distinct from one another than deeper lineages (Blue, Teal) were from one another ($F_{ST}$ = 0.211, 0.048, respectively; Fig. 13F). Reconstruction of effective population size ($N_e$) illustrate a similar pattern, with mesophotic lineages showing greater population sizes throughout time (Fig. 13E). In addition to greater effective population sizes, the dominant lineage in our samples experienced a large expansion 100 KYA, likely contributing to its continued success in present day even with all lineages experiencing relatively recent contractions (Fig. 13E).

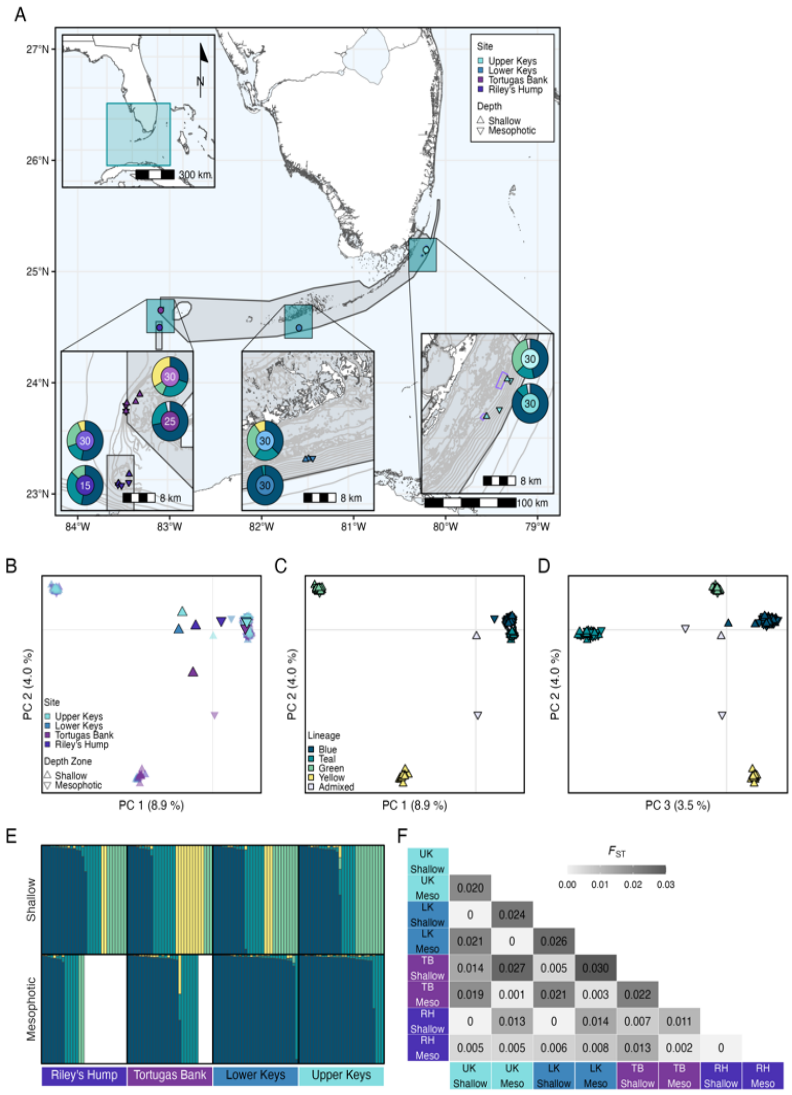


**Figure 12.** *Stephanocoenia intersepta* population structure across Florida Keys National Marine Sanctuary. **A)** Map of sampling sites. Color denotes sampling site and shape denotes depth zone (shallow, mesophotic). Donut plots in insets show population structure on the outer ring and number of samples inside ring, with the bottom plot of each pair representing the mesophotic site. FKNMS and SPA boundaries are shown as black and purple polygons, respectively. **B)** Principle component analysis based on IBS matrix. Color denotes sampling site and shape denotes depth zone. Larger, opaque triangles represent population centroids and smaller, transparent triangles are individual samples. **C)** Same plot as **(B)** but with samples coded by lineage assignment. Samples with < 75% assignment to a single lineage were considered admixed. **D)** Same plot as **(C)** rotated to show axis 2 and 3. **E)** Structure plot from NGSadmix analysisys. Each bar represents an individual sample and the proportion of each color is the probability of membership to that genetic lineage. **F)** Pairwise fixation index (FST) heatmap among sample populations. Increasing gray coloration indicates higher FST(i.e. greater differentiation).

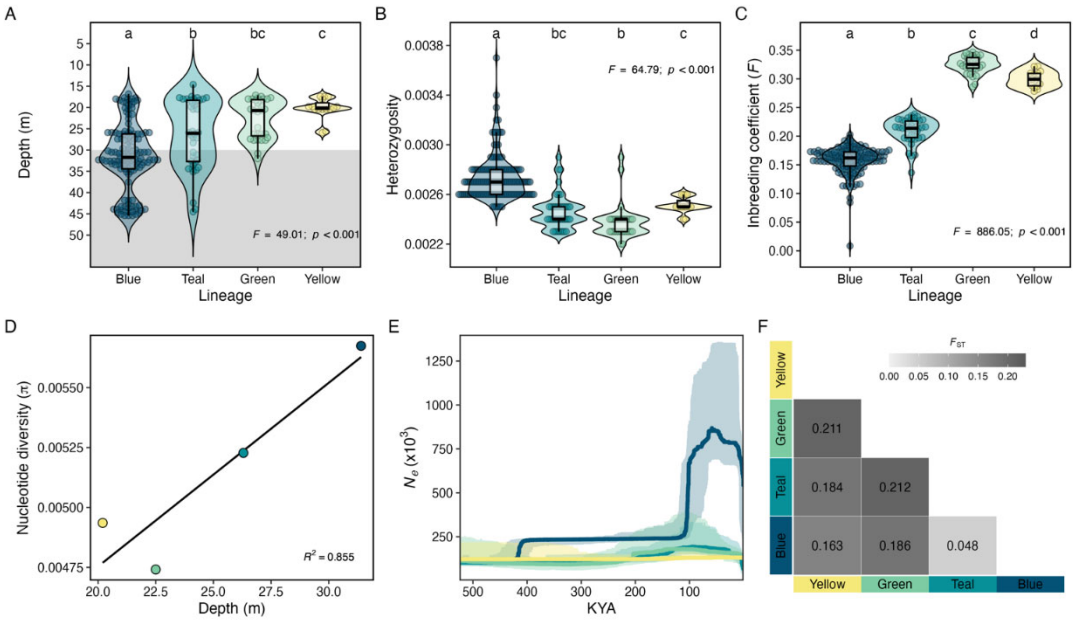


**Figure 13.** *Stephanocoenia intersepta* lineage demographics. A) Distribution of lineages across depth. B) Lineage heterozygosity across all RAD loci (variant and invariant sites). C) Inbreeding estimates from SNPs by genetic lineage. Violin plots represent the distribution and kernel probability density of the data points. Significant Welch's ANOVA results between population depth are listed and letters denote significant differences among lineages on (A,B C). D) Mean nucleotide diversity across all common SNPs plotted by mean sampling depth of each lineage $R^2$ value of linear regression shown. E) Historical changes in effective population sizes for each lineage. Bounding ribbons are 75% confidence intervals. F) Pairwise fixation index ($F_{ST}$) heatmap among lineages. Increasing gray coloration indicates higher $F_{ST}$ (i.e. greater differentiation). Colors depict lineage assignments in (A–F).

### 4.4. Coral Salinity Threshold Experiments

In response to acute hyposaline conditions, when salinity reached 19 PSU on the 9th day of experimentation, both *M. cavernosa* and *P. astreoides* fragments exhibited rapid mortality exceeding 50% of the fragments overall in each species (Figure 14). While both species encountered rapid mortality at 19 PSU, they began to show reduced health much earlier (Figure 15). Across both species, increases in health parameter scores were observed through the acclimation period. In *P. astreoides* the most prominent sublethal responses were polyp activity and color, in that order, which acted as indicators of stress beginning at 33 and 27 PSU, respectively. Similarly, sublethal stress responses were observed in *M. cavernosa*; most prominently, decreased coloration (increased bleaching) was an active indicator of hyposaline stress beginning around day two or 33 PSU, followed by decreased polyp activity beginning at day five or 27 PSU. Tissue integrity began to decrease in both species when compared to controls at 21 PSU, suggesting

mortality was imminent. Health score parameters varied significantly between control in stress conditions for both species.

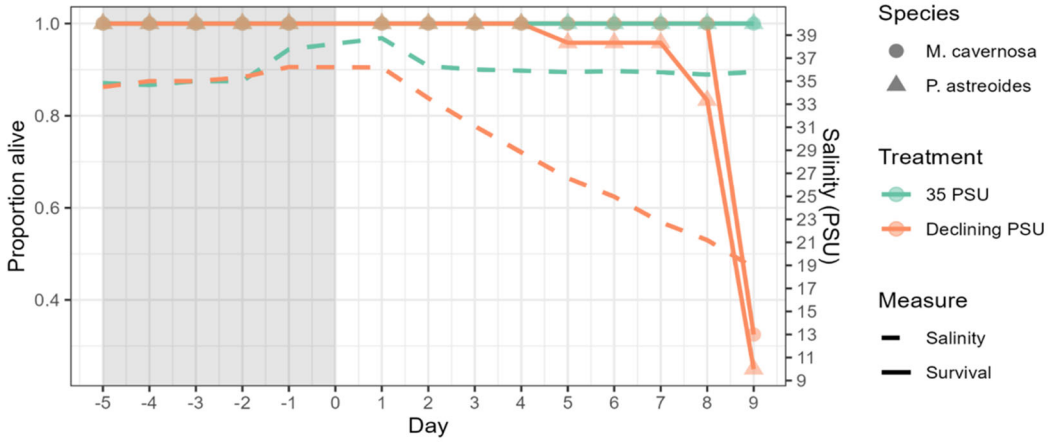


**Figure 14.** Experiment 1 measured the impacts of continuously declining salinity (dashed lines) on coral survival (solid lines).

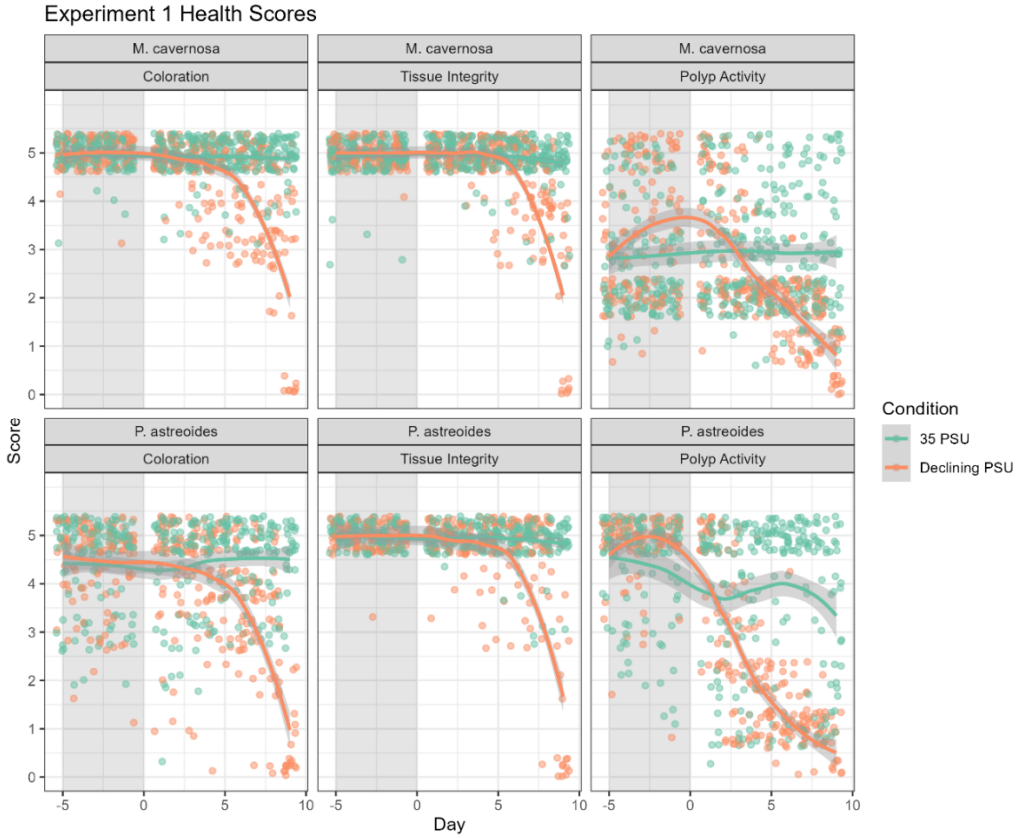


**Figure 15.** Coral health scores for fragments in 35 PSU "ambient" conditions (teal) and declining salinity conditions (orange). The gray box represents the acclimation phase prior to initiation of experimentation. Points represent individual data points jittered across their y-axis integer, where lines represent shifting averages calculated by the

predictdf function, and confidence intervals are calculated by the loess method based on T-distributions (Wickham, 2016).

Individual colonies varied in response to acute hyposalinity stress. *Montastraea cavernosa* fragments had variable polyp activity, coloration, and tissue integrity responses to acute hyposalinity based on colony ID. Polyp activity for *M. cavernosa* appears to have converged as stress became more intense.

In response to chronic hyposalinity stress, *Porites astreoides* fragments experienced mortality before *Montastraea cavernosa* fragments, beginning on the day 7 of chronic hyposalinity stress at 25 PSU (day 12 of the experiment; Figure 16). Some mortality occurred in *M. cavernosa* starting at 15 days at 25 PSU (day 20). The $LD_{50}$ (lethal duration for 50% of population) for *P. astreoides* was at 18 days of continuous exposure to 25 PSU.

Across species, stable physioscore values were observed through the acclimation period, except for *M. cavernosa* polyp activity which was highly variable and showed slight decline. *Montastraea cavernosa* colonies under chronic hyposaline conditions (25 PSU) had strongly reduced polyp activity and exhibited reduced coloration when compared to controls, with slight tissue loss occurring towards the end of the experiment. Health score parameters varied significantly between control in stress conditions for both species. Responses of each species to chronic hyposalinity stress varied across individuals in all three of the health score categories. Individual colonies had differing responses from one another where some had higher scores in coloration than others, while others had better polyp activity. This held true for both species.

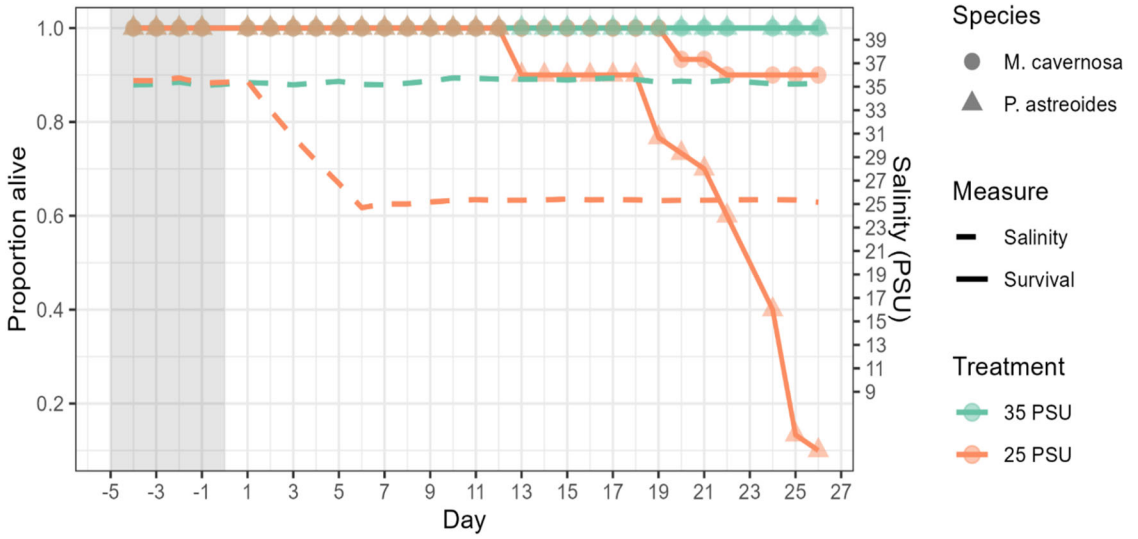


**Figure 16.** Experiment 2 measured the impacts of chronic hyposalinity salinity (dashed lines) on coral survival (solid lines).

Mortality in the combined chronic hyposalinty and SCTLD experiment followed similar trends to experiment 2, although there was higher variability in the mortality observed in

each tank, and slightly earlier mortality of *P. astreoides* fragments. The LD50 for *P. astreoides* was found to occur between day 16 and 17 at 25 PSU (day 21 and 22 of the experiment, Figure 15). No observable evidence of disease was noted on any fragment in any tank at any point during this experiment. Therefore, interactions between chronic hyposalinity stress and disease susceptibility could not be determined in this study. SCTLD introduction did not have any impact on mortality rate. Due to the complete lack of disease presentation on the experimental fragments, it was impossible to determine whether hyposalinity stress has an interaction with SCTLD susceptibility in corals. The lack of active SCTLD infection may be due to a high disease-tolerance of the collected colonies from Breakers Reef where SCTLD had been present for 3 years.

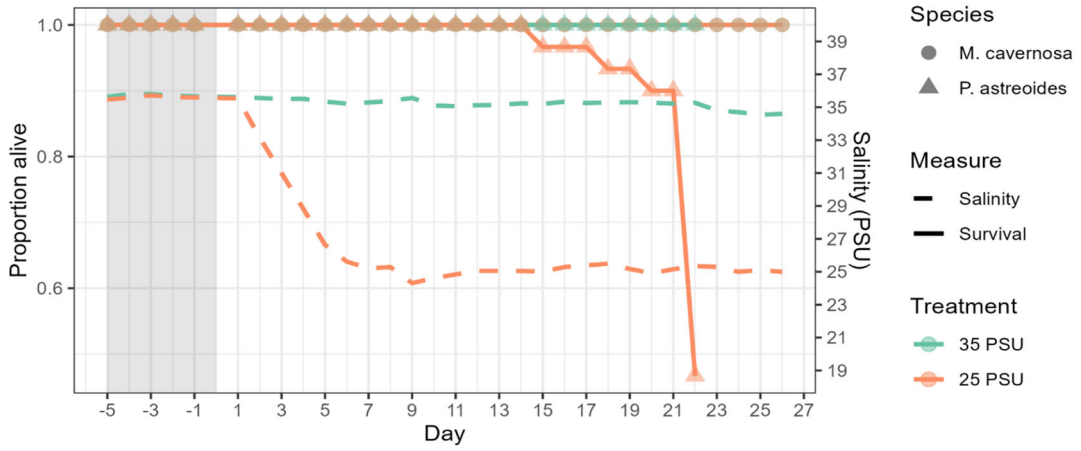


**Figure 7**. Experiment 3 measured the impacts of chronic hyposalinity salinity (dashed lines) on coral survival (solid lines).

## 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, and that SCTLD prevalence is declining in St. Lucie Reef, Jupiter, and Palm Beach sites. 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 2022–May 2023 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. The long-term successes of antibiotic treated corals continue to demonstrate the efficacy of this method for saving coral colonies.

Our population genetics results indicated significant historical structuring of *P. astreoides* populations in the Coral ECA. We found high rates of clonality of *P. astreoides* across all sites, with the highest proportional abundance of clones at St. Lucie. Significant historical structuring of populations from St. Lucie to Ft. Lauderdale was observed as well, and may be partially explained by associated variation in depth, latitude, and other spatial and environmental variables. However, there are also indications of relatively recent gene

flow (over the past two-three generations), especially from Boynton to northern populations. The average clonality rate found across sites in this study was 60%, which is high when compared to some other studies. High rates of self-fertilization by a few successful individuals, or increased asexual reproduction via fragmentation or parthenogenesis, may have occurred over recent years at St. Lucie. To further characterize the reproductive patterns driving the population dynamics and high rates of clonality at St. Lucie, histology could be utilized to quantify sex ratios and fecundity of colonies, as well as in situ larvae collection in combination with parentage analyses. These results indicate that 1) increasing *P. astreoides* abundance at a local site may be from asexual processes and may not necessarily result in increased biodiversity, and 2) that Boynton Beach represents an important source population for *P. astreoides* in the Coral ECA.

Both shallow and mesophotic populations of *S. intersepta,* an important reef-building coral species, exhibit variable population structure and are widely differentiated across depth throughout FKNMS. The lower diversity and higher levels of inbred individuals across shallow reefs may portend potential consequences moving forward, especially for conservation and restoration efforts. Though mesophotic reefs have been relatively understudied to date, they are critical to assessments of ecosystem biodiversity and genetic diversity. Mesophotic reefs harbor greater genetic diversity of a co-occurring depth-generalist species and mesophotic genets may offer an opportunity to increase lost shallow population diversity through restoration-based fragmentation and/or reproductive propagation, thereby increasing the likelihood of long-term population persistence throughout Southeast Florida. In FY24 we will complete the combined analyses to compare *S. intersepta* in the Coral ECA to those sampled in the FKNMS.

We found that acute salinity exposures below 19 PSU risk severe mortality for both *M. cavernosa* and *P. astreoides* in south Florida. Further, sustained salinity levels below 25 PSU for greater than 17 days can cause mortality above 50% for *P. asteroides.* Where mortality was observed, it seemed to decline beyond 50% within one day. Preceding mortality, fragments of both species showed reduced polyp activity, coloration, and tissue integrity, suggestion a state of increased stress and potential vulnerability. Additional spatial/temporal salinity modeling is needed to determine the risks of this salinity level occurring during freshwater releases or sever precipitation events.

## 6. RECOMMENDATIONS

**Recommendation 1**: Continue disease mitigation/intervention efforts to reduce losses of key coral reef ecosystem components, particularly in epidemic zones like the Dry Tortugas.

**Recommendation 2:** 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 continued testing of coral restoration activities in margin (St. Lucie) and deeper (Jupiter, Palm Beach) coral reef habitats.

**Recommendation 3:** 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 corals' intraspecies genetic diversity. Our ability to effectively sample, process, analyze, and interpret these result is advancing rapidly. Successful coral restoration strategies will require genetic data and genetic management plan to design and implement effective conservation and 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, and that even prior mortality, increased coral stress can result from prolonged freshwater release events. The rapid mortality observed after stress thresholds were reached, suggest that an abundance of caution should be used in these guidelines, and that more conservative thresholds should be used where feasible