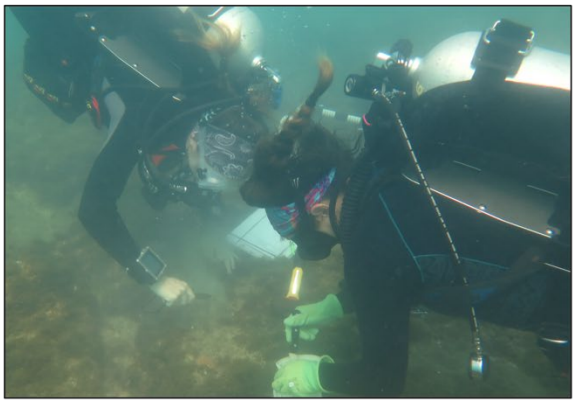
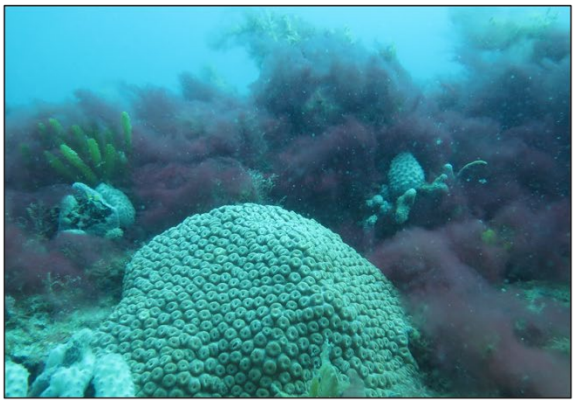
2025

Advancing Coral Reef Research and Resilience in Southeast Florida: Phase 5

Final Report



Advancing Coral Reef Research and Resilience in Southeast Florida: Phase 5

Final Report

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Executive Summary for Managers

This project applied multiple approaches to help understand, reduce, and mitigate coral reef declines in Florida. Continued monitoring of coral disease incidence and prevalence in the northern portion of Kristin Jacobs Coral Aquatic Preserve (KJCAP) was coupled with population genetics to inform population management and coral restoration, coral salinity stress experiments, and experimental restoration efforts.

For June 2024 to May 2025 stony coral tissue loss disease (SCTLD) prevalence remained low in Martin, Palm Beach, and Broward counties. We hypothesize fewer susceptible host corals rather than changing environmental conditions is driving this trend.

We discovered four cryptic lineages of *Stephanocoenia intersepta* throughout Florida, suggesting that restoration could be improved by identifying and planning for cryptic lineage diversity. *Xestospongia muta* exhibited less distinct lineage zonation across Florida. For both *S. intersepta* and *X.muta,* populations in Florida are distinct and well-differentiated from those in Flower Garden Banks National Marine Sanctuary.

In addition to direct impacts of hyposalinity on coral mortality, intermediate salinity stress (25 Practical Salinity Unit) significantly reduced corals' ability to heal from tissue damage, suggesting that recovery of damaged corals following storms/injuries can be improved by reducing freshwater discharges, and that coral restoration outplanting should avoid periods of <25 PSU.

Expanded coral restoration at St. Lucie Reef with transplants from Osborne tire reef resulted in 89% survival at 1 year. For two species, larger transplant colonies had a significantly greater survival probability than smaller colonies.

We resample Restoration Team Trials corals after ~ 2 years and again following the 2023 bleaching event to assess potential changes in their associated algal endosymbiont types.

Through DEP support, we have produced 22 peer-reviewed publications to date with 4 more in review. DEP support has also led to 3 PhDs and 9 master's degrees, several of whom now serve state and federal agencies.

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, Joanna Walczak, Kiley Morgan, and Kathleen Czaia for coordinating this award and providing critical suggestions to improve the quality and impact of the project. Jimmy Nelson and David Heuberger at FAU Harbor Branch provided marina and logistic support. Stephanie Schopmeyer and Lisa Gregg at FWC, as well as Chris Camargo at St. Lucie Inlet Preserve State Park helped to coordinate permitting for this study.

This project leveraged support from NOAA award NA14OAR4320260 to JDV through the Cooperative Institute for Ocean Exploration, Research, and Technology, a Vero Beach Sunrise Rotary Club Dritenbas Memorial Fellowship to Haley Davis, and an Indian River Lagoon Graduate Research Fellowship to Gabrielle Pantoni.

Samples within St. Lucie Inlet Preserve State Park were collected under permits 01221915, 012102115, 0602215, and 08222325 to Joshua Voss. Coral samples collected outside of the park in the Kristin Jacobs Coral Aquatic Preserve were collected under Special Activity License SAL-23-1702-SRP and SAL-24-1702-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 National Marine Sanctuary (FKNMS). 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 Aquatic Preserve (KJCAP) 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 better protect and restore our coral reef resources.

2. PROJECT DESCRIPTION

This project includes multiple complementary approaches to understand, reduce, and mitigate coral reef ecosystem declines in SE Florida. Our major effort was to extend ongoing monitoring of coral reef health and status in the northern section of Florida's Coral Reef. In addition, this project was designed to improve understanding of the current spatial extent of the disease outbreak, prevalence, and species affected. Our objectives included:

- Coral monitoring and reconnaissance 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.
- Building coral population genetics and connectivity data in Palm Beach, Martin, and northern Broward counties to support restoration planning for *Montastraea cavernosa, Porites astreoides, Stephanocoenia intersepta,* and *Xestospongia muta*.
- Experimental assessment of coral salinity thresholds to inform freshwater management in Florida, including impacts of hyposalinity stress on coral wound healing and tissue growth.
- Continued collaborative effort in the SCTLD Resilience Research Consortium including analyses of genetic data from *Orbicella faveolata* corals and algal symbionts.
- Coral restoration efforts in St. Lucie Inlet Preserve State Park including monitoring transplanted corals from Broward County tire reefs in conjunction with Dave Gilliam at NSU.
- Molecular analyses of algal symbionts from outplanted corals in the Restoration Team Trials to evaluate potential changes in symbiont profiles over time.

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 coral disease

outbreaks, help 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.

3. METHODOLOGY

Tables 1-3 below summarize the operational activities at each of our project sites in this period of performance. Project sites, as shown in Figure 1, were chosen in concert with DEP management input 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 and to complement annual Southeast Florida Coral Reef Evaluation and Monitoring Program surveys.

Table 1. FAU Harbor Branch Project Quarterly Monitoring Sites

Location	Site Name	Lat	Long	County
St. Lucie Reef	SLR Central	27.13166667	-80.1340333	Martin
St. Lucie Reef	SLR Ledge	27.12143333	-80.1275	Martin
St. Lucie Reef	SLR South	27.11186667	-80.12551667	Martin
Jupiter	SEFL-4	26.94370833	-80.02197167	Palm Beach
Jupiter	SEFL-5	26.927445	-80.0301	Palm beach
Jupiter	SEFL-6	26.897735	-80.01638333	Palm Beach
Breakers/WPB	SEFL-8	26.71043333	-80.01581667	Palm Beach
Breakers/WPB	SEFL-11	26.6785	-80.01825	Palm Beach
Breakers/WPB	SEFL-12	26.65238667	-80.02068167	Palm Beach
Pompano	T328	26.17611	-80.09388	Broward
Pompano	BC1	26.14758	-80.0961	Broward
Pompano	FTL4	26.13661	-80.09738	Broward

Table 2. FAU Harbor Branch Restoration Sites

Restoration Team Trials with FWC et al.

Location	RTT Site Name	Established	Lat	Long	County
St. Lucie Reef	1-1 Array	4/26/21	27.13121	-80.13388	Martin
St. Lucie Reef	1-1 Control	5/7/21	27.13167	-80.13403	Martin
St. Lucie Reef	1-2 Array	4/21/21	27.1116	-80.12532	Martin
St. Lucie Reef	1-2 Control	5/7/21	27.111867	-80.125517	Martin
Breakers/WPB	1-3 Array	4/16/21	26.71082	-80.01603	Palm Beach
Breakers/WPB	1-3 Control	5/12/21	26.71043	-80.015817	Palm Beach
Breakers/WPB	1-4 Array	4/16/21	26.67856	-80.01797	Palm Beach
Breakers/WPB	1-4 Control	4/16/21	26.67881	-80.01807	Palm Beach

SLR Tire Coral Transplant Site

Location	Site Name	Lat	Long	County
St. Lucie Reef	SLR Central	27.13074	-80.13347	Martin

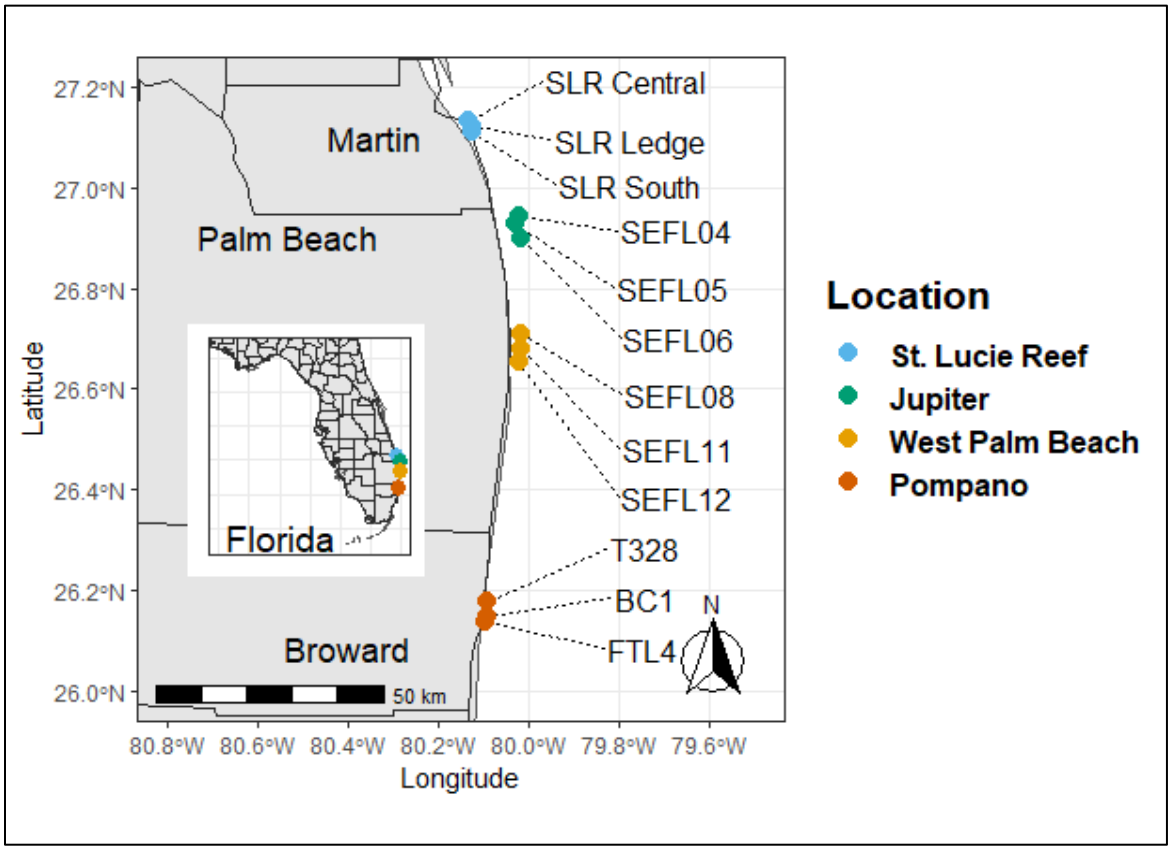

Figure 1. Map of study locations throughout Florida's Northern Coral Reef.

3.1. SCTLD Surveys and Reconnaissance

Quarterly roving diver disease surveys (see Table 2) similar to Disease Response Monitoring (DRM) surveys were conducted to assess the greatest reef area possible, quantifying disease prevalence over an estimated range of 1000–2000 m^2 per survey based on conditions, principally underwater visibility. Scientific divers swam for 20 min and recorded the coral species and disease status of every living coral colony ≥10 cm in diameter. From the data, SCTLD incidence and prevalence, species diversity, and species richness were calculated. To assess multivariate variation in disease prevalence among sites and survey times, a permutational analysis of variance was conducted in the R package *vegan*. Sites include those continuously surveyed by the Voss lab since 2017.

Table 3. Operational Activities by Site

	Date	Central	Ledge	South
St. Lucie Reef	8/22/2024	RD Survey & Transplant Monitoring	RD Survey	RD Survey
St. Lucie Reef	11/21/2024	RD Survey & Transplant Monitoring	RD Survey	RD Survey
St. Lucie Reef	2/11/2025	Transplanting Monitoring	-	-
St. Lucie Reef	3/14/2025	RD Survey	RD Survey	RD Survey
St. Lucie Reef	5/14/2025	RD Survey & Transplant Monitoring	RD Survey	RD Survey
	Date	SEFL-04	SEFL-05	SEFL-06
Jupiter	7/16/2024	-	RD Survey	RD Survey
Jupiter	7/31/2024	RD Survey	-	-
Jupiter	10/16/2024	RD Survey	RD Survey	RD Survey
Jupiter	1/13/2025	RD Survey	RD Survey	RD Survey
Jupiter	4/17/2025	RD Survey	RD Survey	RD Survey
	Date	SEFL-08	SEFL-11	SEFL-12
Palm Beach	7/15/2024	RD Survey	RD Survey	RD Survey
Palm Beach	12/6/2024	RD Survey	RD Survey	RD Survey
Palm Beach	2/4/2025	RD Survey	RD Survey	RD Survey
Palm Beach	5/8/2025	RD Survey	RD Survey	RD Survey
	Date	FTL4	BC1	T328
Lauderdale	8/22/2024	RD Survey	RD Survey	RD Survey
Lauderdale	11/20/2024	RD Survey	RD Survey	RD Survey
Lauderdale	2/11/2025	RD Survey	RD Survey	RD Survey
Lauderdale	5/14/2025	RD Survey	RD Survey	RD Survey

3.2. Coral Population Genetics to Inform Restoration Activities and Management

During this period of performance, we focused on analyses of *Stephanocoenia intersepta* and *Xestospongia muta* across the entire Florida Coral Reef and in comparison to sites in across the Gulf of Mexico. For all the *S. intersepta* and *X. muta* samples, ~2-5 cm^2 tissue biopsies 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.

Returned sequencing reads were demultiplexed, deduplicated, filtered and trimmed with custom perl scripts and CUTADAPT v3.4. Sample reads were aligned to the *X. muta* reference genome with BOWTIE2 v1.2.2. As there was no *S. intersepta* genome available we employed a de novo reference analysis pipeline. Reads from each sample were first aligned to a concatenated Symbiodiniaceae reference consisting of representative genomes for four genera (*Symbiodinium, Breviolum, Cladocopium, and Durusdinium*) using BOWTIE2 v1.2.2. After excluding the Symbiodiniaceae aligned reads, resulting high-quality reads were used to create a de novo RAD tag reference assembly for *S. intersepta* consisting of clustered (91% similarity) unique sequences found in at least 10% of all samples using custom perl scripts and CD-HIT v4.8.1 (Fu et al., 2012). Potential contamination sequences were removed from the clustered sequences with KRAKEN2 (standard KRAKENdatabase with added Symbiodiniaceae genomes) and remaining RAD tags were concatenated into a de novo reference and formatted as 30 equally-sized pseudo-chromosomes to be used for mapping. Sample reads were aligned to the de novo reference with BOWTIE2 v1.2.2. The program ANGSD v0.933 was used to determine SNP loci from sequencing reads, generate genotype likelihoods, and create an identity-by-state (IBS) matrix. SNP loci underwent stringent filtering, requiring a minimum mapping quality of 20, minimum base quality score of 30, minimum allele frequency of 0.05, minimum p-value of 10-5 for deviation from Hardy-Weinberg equilibrium, minimum p-value of 10-5 for strand bias, p-value of 10-5 for heterozygosity bias, remove triallelic SNPs, p-value that a locus is variable of 10-6, and had to be present in ≥ 75% of samples. No clonal multi-locus genotypes were identified through hierarchical clustering using an IBS threshold determined by the technical triplicate samples. Two of each of the three technical replicates were removed and ANGSD was run on the clone-free sample set with the previously described filters.

Principal component analysis based on IBS distances was conducted using pcangsd. Then ngsadmix was used to calculate population structure models for cluster values from K = 1–

11. In addition to calculating clusters with pcangsd, the most likely value of K was evaluated using the Evanno method through Clumpak.

Genetic connectivity between sampled sites was measured through recent migration rate estimates (immigrant individuals from the previous 2–3 generations) with the BA3SNP function of the program BAYESASS v3.0.4.2. Ten independent runs were conducted with random start seeds for 30 million Markov-Chain Monte Carlo (MCMC) model simulations with a 10 million burn-in and 1000 run sampling frequency. Mixing parameters were adjusted to allow adequate mixing within model runs (acceptance rates = 0.2–0.6). Trace files from independent runs were visualized with TRACER v1.7 to ensure model convergence and consistency between independent model runs. Bayesian deviance was calculated for each independent run and the run with the lowest deviance was used for further analysis. Migration rate estimates (m) were calculated as the mean of the posterior distribution and their uncertainty as 95% high posterior density (HPD) intervals.

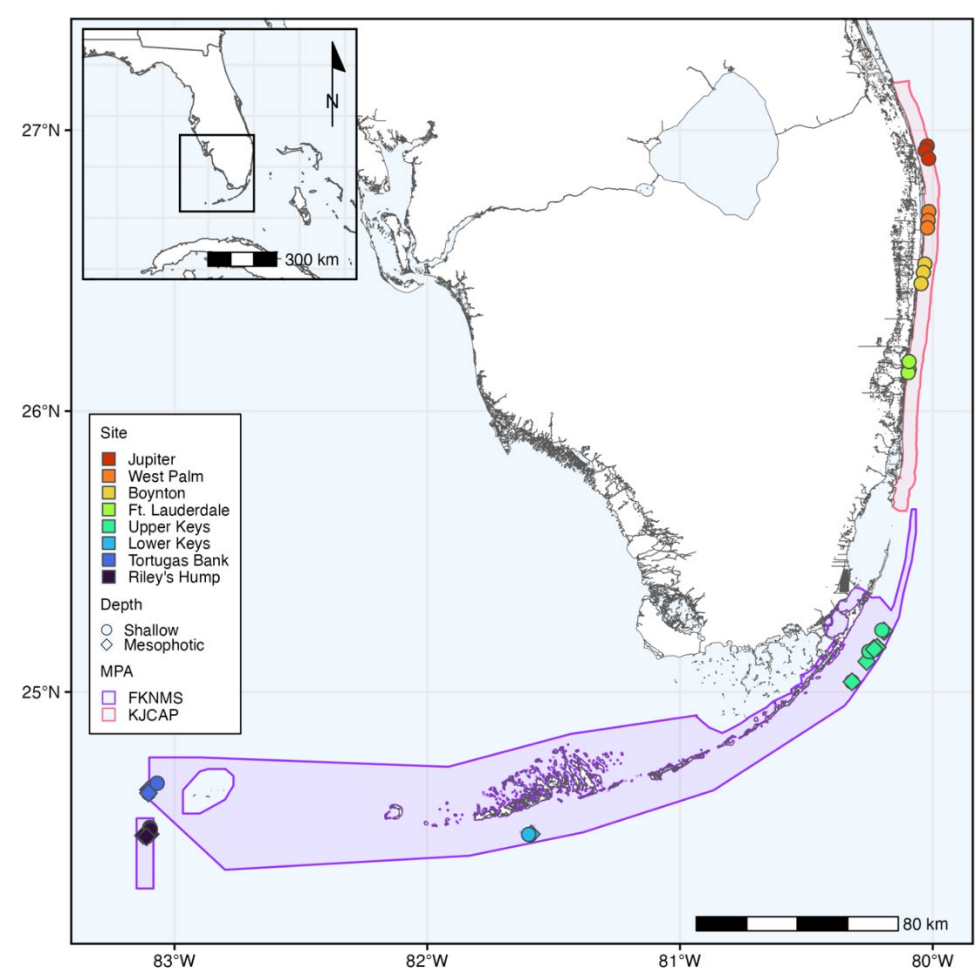

Figure 2. Collection locations for *Stephanocoenia intersepta* and *Xestospongia muta* population genetics and connectivity assessment in the KJCAP and FKNMS.

3.3. Coral Salinity Threshold Experiments

To evaluate the effects of hyposalinity stress on the wound healing responses of *Montastraea cavernosa*, we completed 3 experimental trials: acute hyposalinity stress, chronic hyposalinity stress, and chronic hyposalinity with wounding. A fourth trial, chronic hyposalinity plus SCTLD, was not successful as the donor SCTLD colonies appeared not infectious.

Coral collections for all experiments took place over a series of three dives in West Palm Beach where 10 colonies of *Montastraea cavernosa* were collected. 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.

At the initiation of the experiment, 6 fragments from each of the 8 *M. cavernosa* genets were visually ranked by size and haphazardly sorted into one of 6 tanks, with one representative of each genotype in each tank. To prevent confounding variables, the positions of the fragments within the tank were randomized, ensuring that orientation did not introduce any bias.

Experiment 1: Acute Hyposalinity Tolerance Threshold

Each experiment was initiated with an acclimation period in which all six aquaria were held at a control salinity of 35 PSU for five days (day -5 to -1, Figure 3). The salinity in experimental aquaria was then reduced by 2 PSU/day continuously. Each day, 20% water changes took place in each tank, maintaining 35 PSU in control tanks and reducing the salinity of experimental tanks daily until the termination of the experiment (Figure 2).

Experiment 2: Intermediate-Hyposalinity Tolerance Duration (Chronic Exposure)

Based on mortality and health scores in the acute hyposalinity experiment, 25 PSU was selected as the intermediately stressful salinity for the following experiments. After the same acclimation phase, the salinity in three experimental tanks was reduced to and maintained at an intermediately stressful salinity of 25 PSU utilizing 20% water changes. During the “drop” phase, the treatment tanks underwent a continuous drop in salinity by 2 PSU (days 1–5), until reaching and being maintained at 25 PSU (days 6–26).

Experiment 3: Chronic Hyposalinity + Wound Healing

This study utilized 8 remaining colonies of *M. cavernosa* in an effort to better understand hyposalinity impacts on recovery rates of damaged corals. The same procedures outlined above in Experiment 2 were repeated, with the addition of inflicting artificial tissue wounds on the day that treatment tanks reached 25 PSU. Every fragment in both the control and intermediate hyposalinity treatment received a single wound in the center of the fragment

using a ¼" diameter, cylinder end, tungsten carbide burr bit in a drill press set to a ¼" depth stop into the coral tissue.

Each day, images were taken of the fragments, and additional photos were taken directly following manual agitation to cause polyps retraction and create better visibility of the wound. At ten time points (days 1, 5, 10, 15, 20, 25, 30, 35, 40, and 45) retracted-polyp photos were lens-corrected using Photoshop (2022), and then traced in Image J to calculate wound size in cm^2. A small plastic ruler was placed at the bottom of the tank to scale each image. This experiment ended after 45 days, allowing for several control fragments to reach 100% healed. Fragments were given a qualitative health assessment each day by adopting a physical scoring system from Woodley and Downs (2014). These "physio-scores" were compiled by examining the following attributes: coloration, tissue integrity, and polyp activity. Each frag received three scores, one for each category; scores ranged from 5 (best possible score) to 1 (worst possible score). Coloration ranking was based on tissue bleaching, where normal color received a 5 and completely bleached tissue received a 1. The scoring system for tissue integrity was adapted slightly to fit the parameters of this study; a fully healed fragment received a 5 and all wounded fragments received a 4. Additionally, any tissue loss observed after drilling was scored accordingly. A perfect activity score of 5 was given to polyps that were fully extended, while the lowest score of 1 was given to fragments with sunken and retracted polyp bodies.

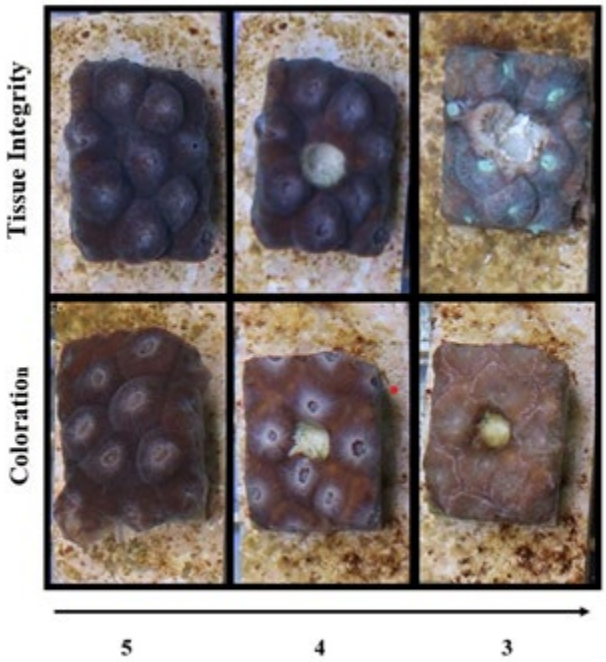

Figure 3. Coloration and tissue integrity ranges observed throughout the experiment are represented above.

Scaled images were taken daily and all photos taken were lens-corrected to eliminate underwater distortion caused by optical refraction. New tissue growth (cm^2) following artificial wounds was calculated using ImageJ based on the protocol developed by the

Restoration Team Trials project (Pantoni 2023). Each fragment was traced at 10 different time points, beginning at injury infliction (day 1) and concluding on day 45 after 41.6% of fragments from control tanks were healed.

Each day after health scoring and images were taken, all aquarium temperatures and salinities were recorded to ensure stable parameters were maintained. After data collection had concluded, every tank received a 20% water change (18 L) daily maintaining either 25 PSU for the treatment tanks or 35 PSU for the control tanks. Transparent lids covered each tank to reduce evaporation and maintain temperature.

All statistical analysis was completed in RStudio using the package ggplot2 for data visualization (v3.3.3; Wickham, 2016). Area values of coral wounds obtained from Image J tracing represented the raw surface area of the lesion, with 0 cm^2 given to fragments that had completely healed. A repeated measures analysis of variance (RM ANOVA) was run on area tissue growth comparing tank and treatment over ten time points at 5-day intervals (days 1-45). RM ANOVAs were used to analyze health score data individually with each parameter (color, tissue integrity, and polyp activity) over time. In order to determine which group means showed significance, post-hoc pairwise comparisons were performed using the Bonferroni correction method.

3.4. SCTLD Research Resilience Consortium

The threatened hermatypic coral, *Orbicella faveolata*, demonstrates intraspecific variation in SCTLD affectedness with some colonies experiencing chronic disease lesions, while other nearby *O. faveolata* colonies appear unaffected with no disease signs over long monitoring periods. This study evaluated potential genotypic underpinnings of variable disease responses to SCTLD by monitoring and sampling 90 *O. faveolata* colonies from southeast Florida and the lower Florida Keys. High resolution analyses of >11,000 single nucleotide polymorphisms (SNPs) generated from 2bRAD sequencing indicated there were no SNP loci or genetic lineages significantly associated with *O. faveolata* SCTLD affectedness. Algal symbiont community structure characterized from 2bRAD data revealed that the presence of *Durusdinium* spp. corresponded with SCTLD-affected colonies as compared to unaffected colonies, suggesting that algal symbiont community make-up may play some role in SCTLD resistance (Klein et al. 2024).

DNA extracts and data generated by our initial study were shared with University of Miami (Baker Lab) for comparisons between 2bRAD, ITS2, and qPCR methods of evaluating algal symbiont assemblages. Ongoing complementary molecular and physiological approaches to further investigate the complex drivers of intraspecific SCTLD susceptibility and resilience.

For 2bRAD analyses that we completed, high-quality reads that aligned uniquely to the concatenated Symbiodiniaceae metagenome were used to determine the dominant algal

symbiont type for each sample. Relative alignment rates to each of the four symbiont genomic references were used as a proxy for the relative abundance of the four algal symbiont genera associated with each colony. A permutational multivariate analysis of variance (PERMANOVA, 999 permutations) was run using a beta diversity metric to assess differences in the population structure across differing disease susceptibility status as well as across sites. Abundances of each symbiont genera were square root transformed for this PERMANOVA to minimize influence of the most abundant symbiont group. An Indicator Species Analysis (999 permutations) was conducted using the package 'indiscpecies' in R to identify potential taxa associated with SCTLD affectedness.

3.5. Expanding Coral Restoration Efforts on St. Lucie Reef

St. Lucie Reef was identified as a target for additional coral restoration based on the success of previous outplants in the Restoration Team Trials (RTT) study. During the previous fiscal year, we identified a site for this transplantation study that is in close proximity to the RTT outplant site at SLR Central, which had strong outplant survival and growth. The depth at this site is about 3.5m and the bottom composition is mainly worm rock. Corals of three target species, *Montastraea cavernosa*, *Siderastrea siderea*, and *Stephanocoenia intersepta* were collected from the relic tire artificial reef off the coast of Ft. Lauderdale, FL by David Gilliam and his lab at NSU. They held the corals in their *in situ* nursery until we retrieved all 64 colonies and transplanted approximately 10 small (4-7 cm diameter) and 10 large (>10 cm diameter) individuals of each species. The corals were transplanted onto St. Lucie Reef on 26 February 2024. We used coral anchor cement in piping bags to attach each colony to the reef and assigned a numbered (701-764) cattle tag nailed into the reef. After transplanting, each colony was photographed using a Olympus TG-6 camera mounted on a fixed and scaled PVC stand so that each photo is the same distance above each colony (Figure 4).

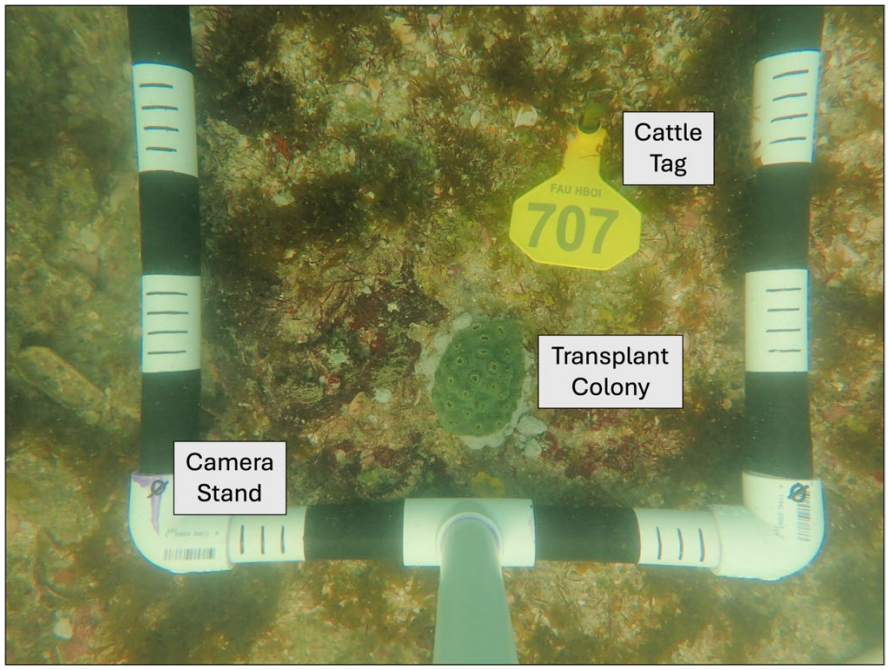

Figure 4. Example image of a transplant colony of *Montastraea cavernosa* #707 taken on the day of transplanting with the colony, cattle tag, and camera stand labeled accordingly.

Monitoring of the transplanted colonies continued quarterly through this fiscal year, and our 1-year post transplantation monitoring was completed in February 2024. During each monitoring interval, we continued to photograph and take notes on the status of each colony. The notes include any signs of disease, bleaching, predation, algal overgrowth, and sediment smothering on the transplants. The percent of surviving colonies of each species within each size class was calculated for each monitoring interval (initial outplanting to 15-months). To compare probability of survival between size classes for each species, we created Kaplan-Meier survival curves and then used log-rank tests to identify any significant differences between the curves.

Using the photos taken at each monitoring interval, we monitored growth of the transplants by calculating changes in live tissue surface area over time. Images were uploaded to ImageJ, scaled, and two-dimensional surface area was traced using a Wacom CTL472K1A One tablet with stylus. Percent change in surface area was calculated from starting colony size to the size at each traced monitoring interval. A mixed analysis of variance (ANOVA) was used to identify significant differences in percent change in surface area between species, size classes, and across time.

In addition to continued monitoring, we also completed additional site maintenance this year. We initially marked each colony used a numbered cattle tag (Figure 4), however these tags proved to not be suitable due to a combination of high wave action at the site causing the tags to become detached from the bottom and difficulty hammering nails used to attach the tags into the hardbottom at this site. As a result, tags were regularly detaching from the bottom making it difficult for divers to conduct quarterly monitoring and increasing the amount of time spent at this site on a given dive day as additional maintenance was required to retag colonies. To solve this issue, we created small cement tag anchors using quick-dry cement with an eyebolt in the center that we then attached small, low profile numbered tags to (Figure 5). We removed our previous cattle tags and retagged each transplant colony using the new tag anchors attached to the bottom with the same Coral Anchor cement used for the transplant colonies. The tag anchors were deployed in February 2025 for 1-year monitoring and after returning for quarterly monitoring in May 2025, all tag anchors except one, which was located and recemented, are still secured in place.

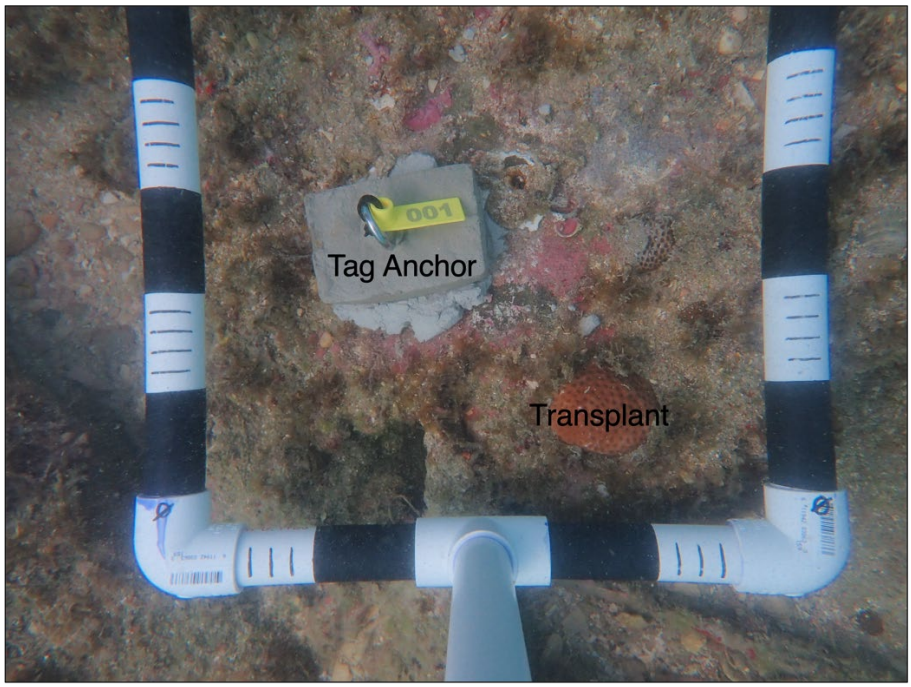

Figure 5. Image of *Siderastrea siderea* transplant colony #001 (previously #701) with new tag anchor and tag number at 1-year monitoring on 11 February 2025.

3.6. Restoration Team Trials Algal Symbiont Analyses

A sampling protocol for evaluating algal symbiont structure within the RTT outplants was created by HBOI and shared with all partners to ensure sampling was conducted consistently across the 6 regions. Biopsies were taken using a hammer and 3/8" diameter round leather punch from the center of the colony or the center of the largest fragments if not all fragments were fused (Figure 6). Each biopsy was transferred into a labeled plastic bag underwater and leather punches were cleaned with a wire brush between colonies. The colony number and corresponding sample bag number were recorded on a datasheet. Once a leather punch became chipped after several uses, a new punch was used. After each dive, samples were transferred from the labeled plastic bags into labeled 15 mL falcon tubes filled with Zymo DNA/RNAshield. The sample tube number and corresponding colony number were also recorded onto the datasheet used during underwater sampling.

Tissue biopsy samples were collected from all living coral outplants by RTT partners in their respective regions for Timepoint 2 between 15 May 2023 and 14 June 2023 at the end of the project's 2-year monitoring period, and then for Timepoint 3 between 11 Jan 2024 and 27 Feb 2024, following the 2023 bleaching event that impacted areas in Florida from Miami south. Once the samples were collected, they were delivered to HBOI, categorized by region, and put into the –80° C freezer for storage until extraction.

DNA was extracted from preserved tissue samples with a modified dispersion buffer/phenol-chloroform-isoamyl alcohol extraction (Sturm, 2020). Extracted DNA was

cleaned with a Zymo DNA Clean and Concentrator Kit following the manufacturer's instructions. DNA quality was determined with a NanoDrop 2000 (Thermo Fisher) and dsDNA quantity was measured with a broad-range assay kit on a Qubit 4.0 fluorometer (Thermo Fisher). Extracted DNA was used for symbiont community typing through high-throughput sequencing of the internal transcribed sp*acer* 2 (ITS2) region of ribosomal DNA operon (Arif et al., 2014; Baker, 2003; Correa & Baker, 2009; LaJeunesse, 2001; LaJeunesse et al., 2018). The Symbiodiniaceae specific primer pair, SYM_VAR_5.8S2/SYM_VAR_REV, was used to target the ITS2 region of Symbiodiniaceae ribosomal DNA operon for sequencing (Hume et al., 2018). Each sample was run in an initial 30 µL PCR containing 20 ng of the template genomic holobiont DNA (Eckert et al., 2020; Klepac et al., 2015). Products from the first PCR were cleaned using a Zymo DNA Clean and Concentrator kit following the manufacturer's instructions. Initial PCR products were quantified with Qubit (Invitrogen) and diluted for a second PCR which incorporated a unique combination of indexed forward and reverse Illumina adapter primers producing a unique dual index or barcode for each sample (Klepac et al., 2015). Each sample was then visualized on a gel to ensure quality before sending out to the sequencing facility. The samples are then pulled and sent for sequencing at The Herbert Wertheim UF Scripps Institute for Biomedical Innovation and Technology. The pulled libraries will be sequenced across three runs on the Illumina MiSeq platform (v3 chemistry) with paired-end 250 bp reads.

Sequencing of algal symbiont DNA from 1,462 coral tissue samples returned an average of 43,800 reads per sample. Raw sequences were submitted to SymPortal for initial filtering and alignment using standard sequence quality control protocols implemented with MOTHUR 1.39.5 (Schloss et al. 2009), the BLAST+ suite of executables (Camacho et al. 2009), and minimum entropy decomposition (Eren et al. 2015; Hume et al. 2019). Within R Studio, ITS2 type profiles generated by SymPortal were further subsetted by respective coral species and low-abundance ITS2 types were subsequently purged from the dataset. ITS2 types were normalized and sorted based on relative abundances to generate stacked bar plots illustrating Symbiodiniaceae community compositions in each outplanted coral species across restoration region and time point. Symbiodiniaceae genera were further color coded based on within-genus descending abundance (*Symbiodinium* = purple; *Breviolum* = blue; *Cladocopium* = green; *Durusdinium* = red).

The full Symbiodiniaceae *ITS2* sequencing library preparation can be found here: https://ryaneckert.github.io/website/ITS2_protocol/

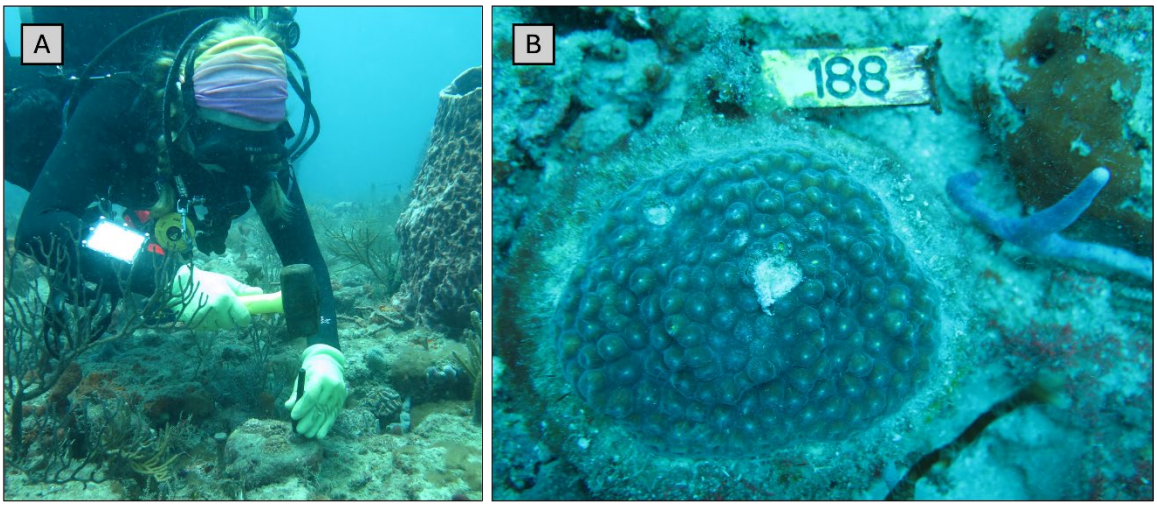

Figure 6. A diver using a 3/8" diameter round leather punch and hammer to collect a tissue biopsy (A), and a colony post-sampling with the tissue biopsy removed (B).

3.7. QA/QC

All roving diver, fate tracking, 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

From June 2024 to May 2025 SCTLD prevalence was very low at all survey regions, with a total of 17 colonies with SCTLD across all survey sites and dates. There were no observations of SCTLD on St. Lucie reefs and Jupiter reefs, we only observed SCTLD on one colony on Palm Beach reefs and the remaining colonies with SCTLD were observed on Ft. Lauderdale reefs. Across all the monitoring regions Ft. Lauderdale reefs continued to have the highest SCTLD prevalence, but still very low, ≤ 2% (Figure 7). From 2017 SCTLD prevalence has significantly decreased between locations and years (PERMANOVA: $P < 0001$; Figure 8). In 2025, the only species with observed SCTLD at any of our monitoring sites was *Montastraea cavernosa* (Figure 9).

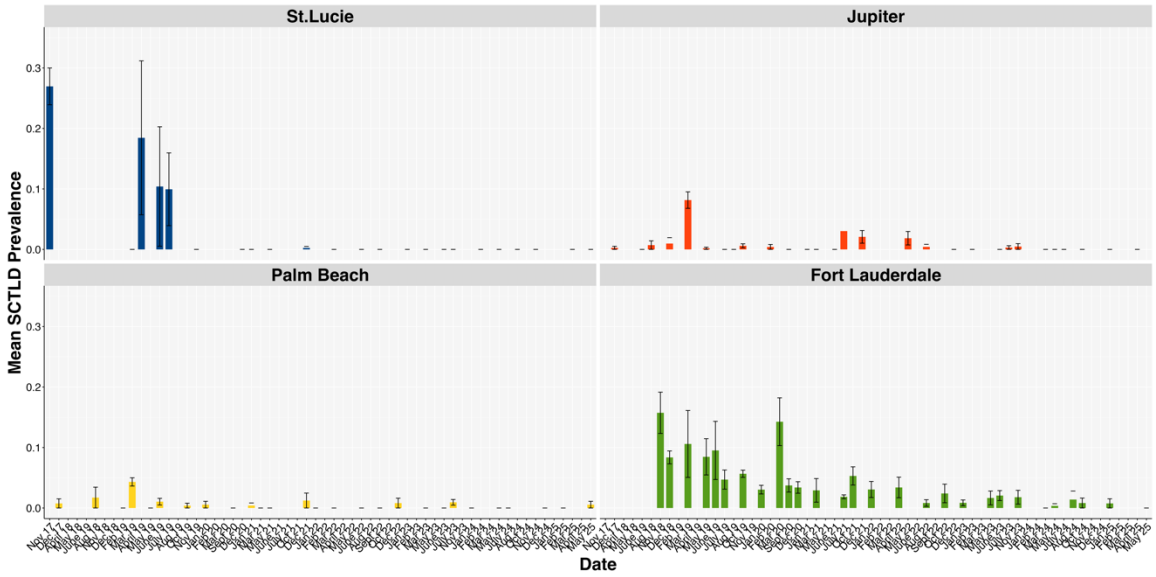

Figure 7. Mean SCTLD prevalence ± SD from roving diver surveys within our four sites from 2017 to present.

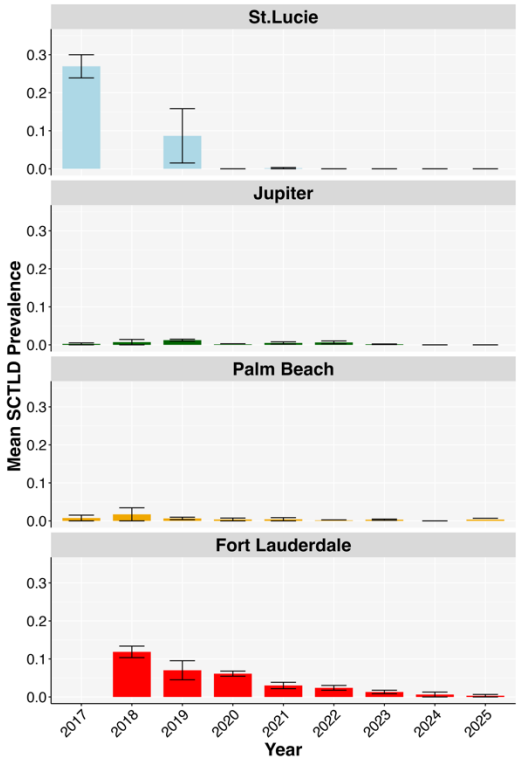

Figure 8. Mean SCTLD prevalence ± SD from roving diver surveys across survey year within each survey region from 2017 to 2025.

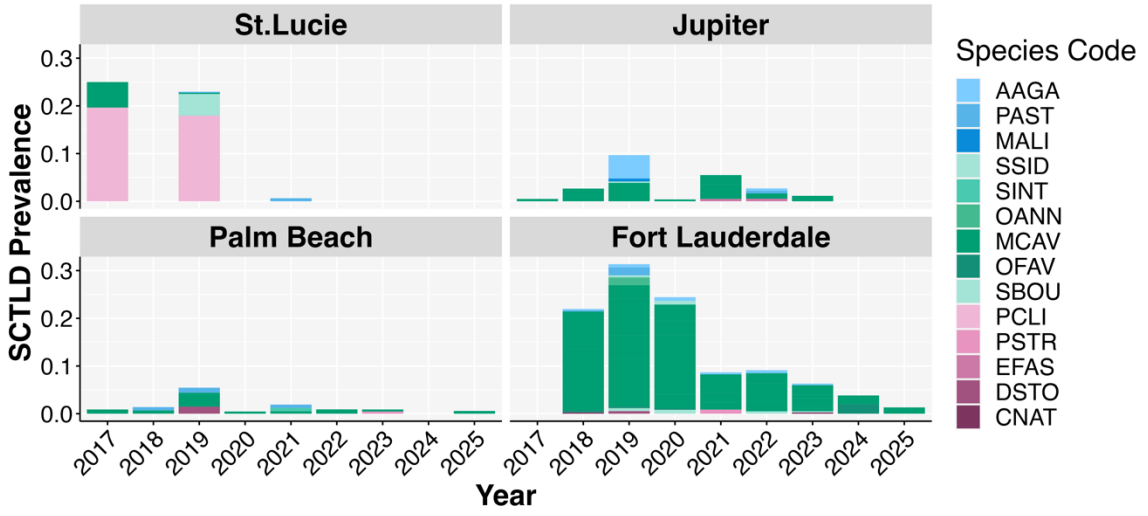

Figure 9. Mean SCTLD prevalence of species affected by SCTLD at each monitoring region across survey years, coral species are color coded by SCTLD susceptibility group. Blue shades represent low susceptible species, green shades represent intermediately susceptible species, and pink shades represent highly susceptible species

4.2. Coral Population Genetics to Inform Restoration Activities and Management

Stephanocoenia intersepta

STRUCTURESELECTOR suggested *K* values of 4 and 5. Based on admixture, dendrogram, and principal components analyses we continued analyzing five cryptic lineages of *S. intersepta* (Figure 10). Very few *S. intersepta* samples were considered admixed for further analyses (3 samples; Figure 10D). Samples from FGBNMS were distinct from most Florida samples (Figure 10A, B). Within Florida, FKNMS and KJCAP samples were fairly distinct from one another, but less so than from FGBNMS samples (Figure 10B, C). Flower Garden Banks samples were exclusively assigned to a single lineage, Si1, while Florida samples were comprised of four cryptic lineages (Figure 10A, E, 11D). Within Florida MPAs, FKNMS sites were dominated by the lineages Si2 and Si4, while the majority of samples from KJCAP belonged to the Si3 and Si5 lineages (Figure 10E, 11D).

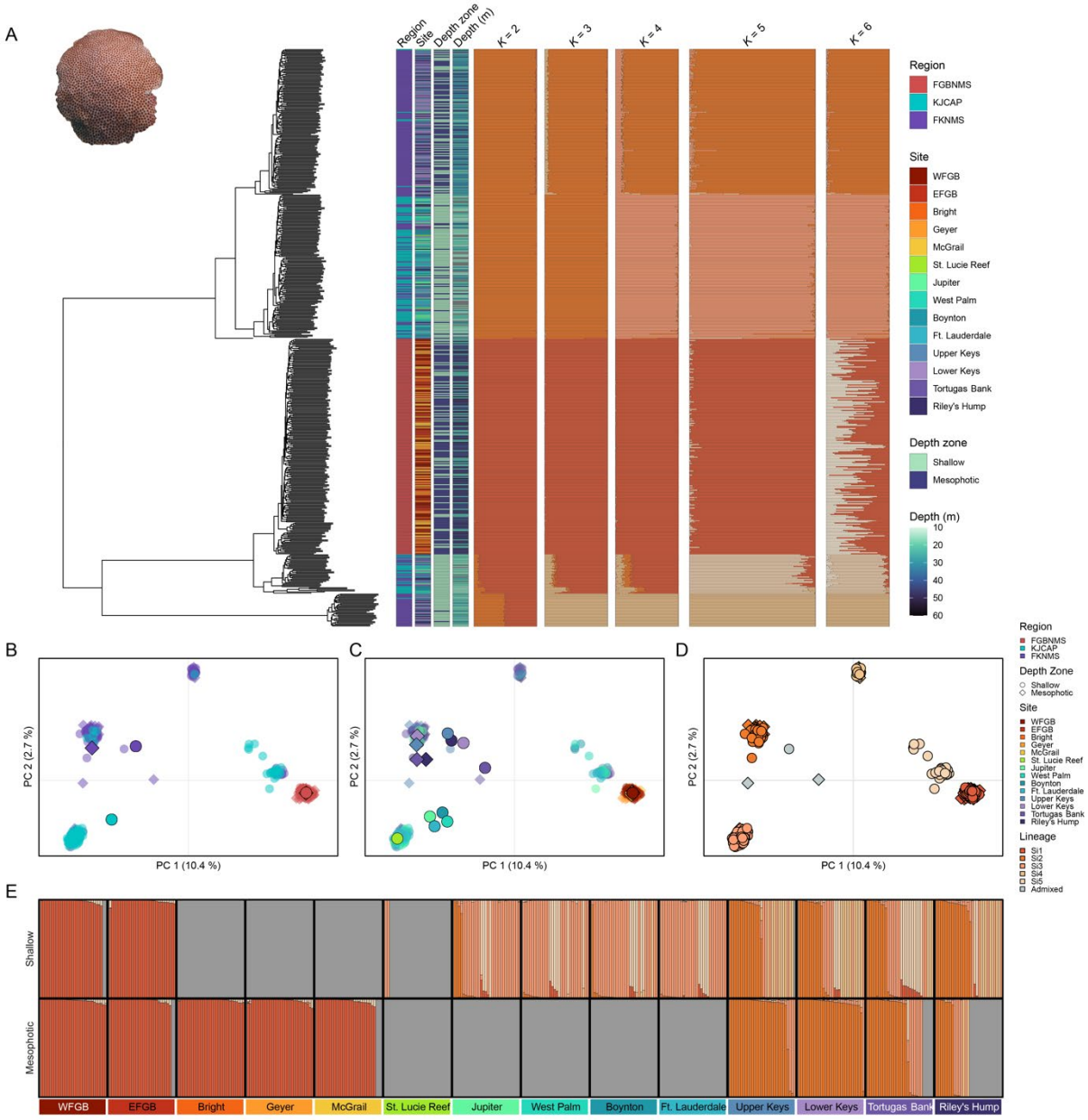

Figure 10. *Stephanocoenia intersepta* population genetic structure. **A** Dendrogram of samples based on IBS matrix from ANGSD with matching region, site, depth zone, and depth (m) data and structure plots for K =2–6 for each S. intersepta sample. **B** Principal component analysis from PCANGSD, where color represents sampling region and shape represents sampling depth zone. Larger, opaque shapes are sample population centroids and smaller, transparent shapes individual samples. **C** Same plot as **B** and with samples colored by sampling site. **D** Same plot as **B** and **C** with samples colored by lineage assignment from K = 5 data set. **E** Structure plot from NGSADMIX analysis with samples grouped by site and depth zone. Each bar represents an individual sample and the proportion of each color is the probability of membership to that genetic lineage.

There was distinct distribution of lineages across depth (F = 109.41, p < 0.001; **Fig. 11A**) The first three *S. intersepta* lineages (Si1, Si2, and Si3) were found across a broad depth range, from shallow to mesophotic reefs. The Si4 and Si5 lineages were almost exclusively found in shallow depths (**Fig. 11A**). Genetic differentiation varied widely among lineages (F_{ST} = 0.046–0.23; **Fig. 11F**). The FGBNMS lineage, Si1 was least differentiated from the Si5 lineage (F_{ST} = 0.05). The Si2 and Si3 lineages were also among some of the least differentiated lineages (F_{ST} = 0.046).

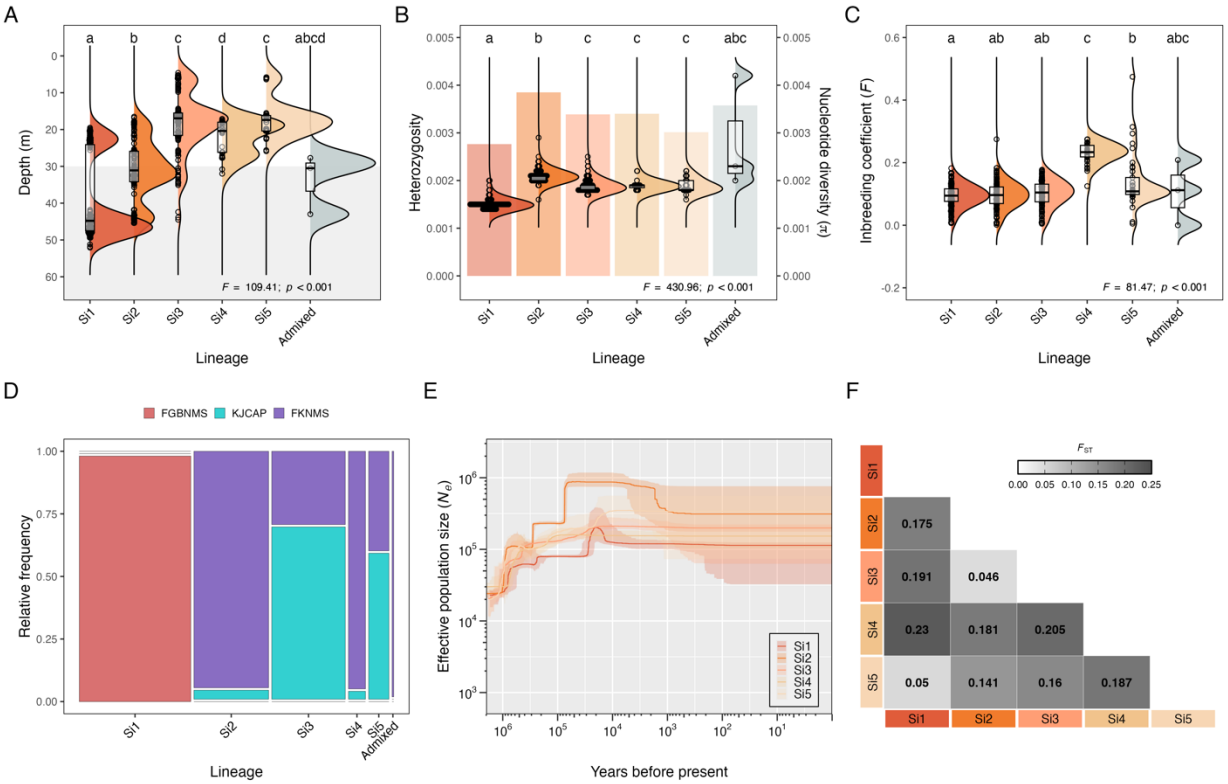

Figure 11. *Stephanocoenia intersepta* lineage diversity and demographics. **A** S. intersepta lineage depth distribution. Shaded portion of graph indicates mesophotic depths. **B** Lineage heterozygosity across variant and invariant loci. Transparent bars behind violins represent nucleotide diversity (π) for each lineage. **C** Mean inbreeding coefficient by lineage. Letters indicate significant differences among lineages and Welch's ANOVA results are listed in **A**, **B**, & **C**. **D** Distribution of lineages across regions. Color of bars corresponds to sampling region. Width of bars is indicative of the relative proportion of samples assigned to that lineage. **E** Lineage effective population size through time. X-axis is thousands of years ago (KYA). Bounding ribbons are 75% confidence intervals. Note log scale for axes. **F** Pairwise fixation index (F_{ST}) heatmap among lineages. Darker coloration indicates higher F_{ST}.

Genetic diversity also varied among *S. intersepta* lineages (Figure 11 B, C) Heterozygosity was lower in the FGBNMS Si1 lineage, on average and highest, on average, in Si2, the major depth-generalist lineage in FKNMS (Figure 11B). These same patterns are reflected in the nucleotide diversity (π) of these lineages (Figure 11B). On average, inbreeding was greater in the shallowest Florida lineages (Si4, Si5) with the greatest inbreeding in Si4 (Figure 11C). All other lineages exhibited fairly low inbreeding levels on average (Figure 11C). Effective population size varied widely among *S. intersepta* lineages and throughout time (Figure 11E). The FGBNMS lineage (Si1) has historically had one of the lowest effective population sizes, consistent with the constrained genetic diversity estimates we found in FGBNMS. Conversely the predominant, depth-generalist lineage in FKNMS (Si2) has remained one of the largest effective population sizes throughout time (Figure 11E). All lineages experienced large population expansions followed by population contractions, though the scale of this flux was greatest in the FKNMS depth-generalist lineage, Si2 (Figure 11E).

Xestospongia muta

STRUCTURESELECTOR selected *K* values of 7–9, with the majority of selection methods suggesting *K* = 7. Based on STRUCTURESELECTOR results, dendrogram, and admixture analyses we continued to analyze *X. muta* as seven cryptic lineages (Figure 12A). Within FGBNMS and Florida there were multiple cryptic lineages found (Figure 12A, B, E). In FGBNMS samples predominantly assigned to one of three lineages and were distinct from most Florida samples, as evidenced by PCA (Figure 12A, B, C, D). Within Florida, *X. muta* populations were largely similar across MPAs and sampling sites, with the exception of Ft. Lauderdale which hosts a small population of genetically distinct *X. muta* not found in our other sampling sites. Population structure differed more across depth in FGBNMS than in FKNMS (Figure 12C, E).

Xestospongia muta lineages exhibited distinct distribution across depth (Figure 12A). There were several lineages which were predominantly found on either mesophotic or shallow reefs, as well as several depth-generalist lineages. Admixed samples were found with a wide distribution across all depths (Figure 12A). Lineages were also distinctly distributed across regions, with FGBNMS comprised of three lineages (Figure 13D) not found in either FKNMS of KJCAP (Figure 13D). The four lineages found across Florida (Figure 13D) were found across both MPAs and the majority of admixed *X. muta* were found in Florida (Figure 13D). Differentiation among lineages varied (F_{ST} = 0.013–0.186; Figure 13F). The lineage at Ft. Lauderdale (Xm5) was among the most differentiated lineages from all other lineages (F_{ST} = 0.128–0.186; Figure 13F). The least differentiated lineages were Xm3:Xm7 and Xm1:Xm6 (F_{ST} = 0.013 and 0.028, respectively; Figure 13F), which were both comparisons of lineages found within a region (Florida and FGBNMS, respectively; Figure 13D).

Heterozygosity also varied among lineages, though overall was lower than in *S. intersepta* lineages. The Ft. Lauderdale lineage, Xm5 had significantly higher heterozygosity and nucleotide diversity than any other lineage or admixed samples (Figure 13B). There was a significant difference in inbreeding among lineages, though there were no strong trends between FGBNMS and Florida lineages, with most lineages containing a large distribution of inbreeding (Figure 13C). Throughout history, X. muta lineages have fluctuated in effective population size (Figure 13E). The FGBNMS lineages (Xm1, Xm4, Xm7) had some of the lowest effective population sizes in the recent past, with only the relatively small Xm5 lineage from Ft. Lauderdale having a smaller effective population size than Xm4 (Figure 13E). While the relative population sizes vary among lineages, most lineages experienced a period of growth and stasis followed by a decline to recent effective population sizes (Figure 13E). However, two lineages in Florida, Xm2 and Xm7 have remained relatively stable following past periods of expansion (Figure 13E).

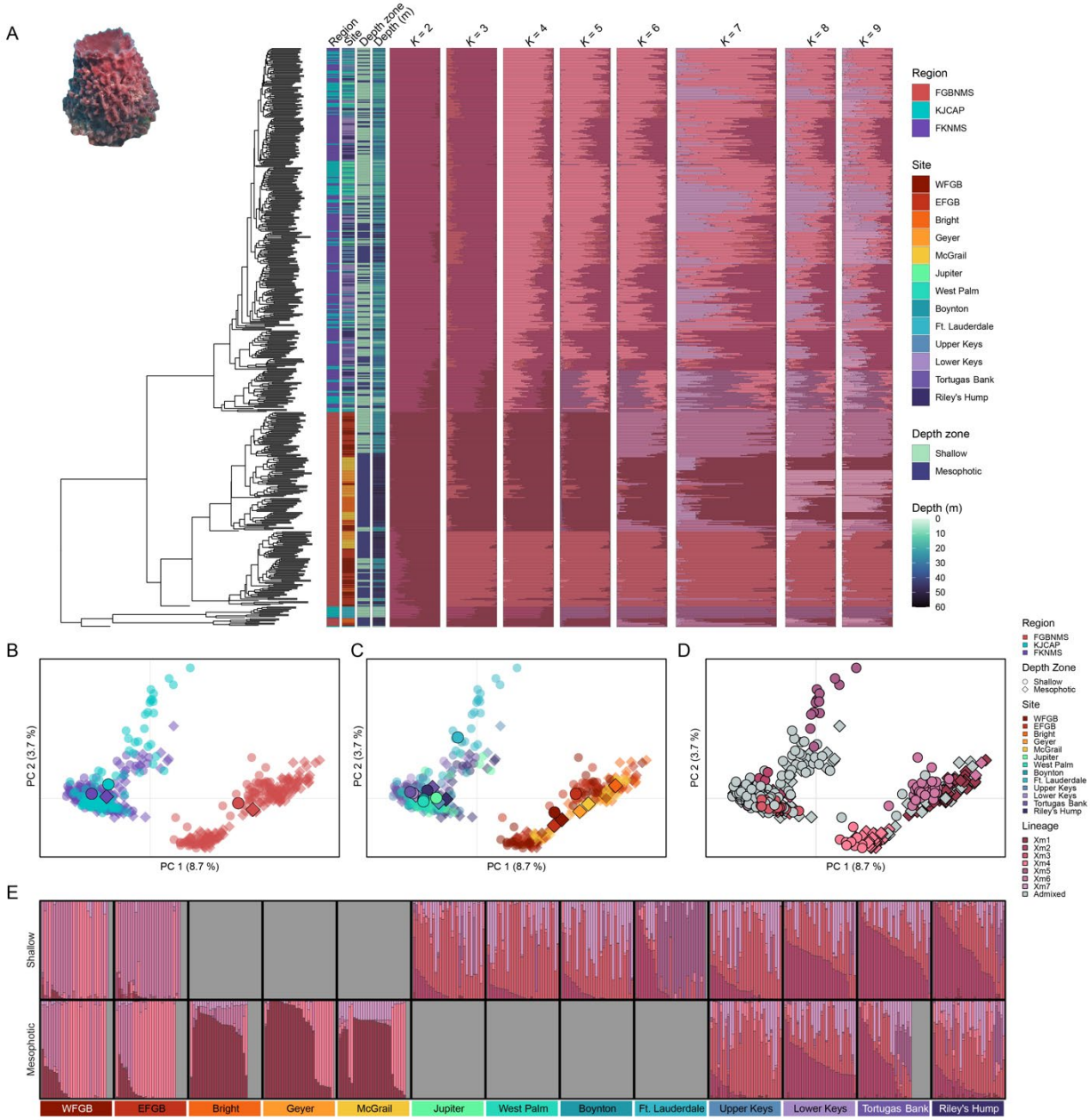

Figure 12. *Xestospongia muta* population genetic structure. **A** Dendrogram of samples based on IBS matrix from ANGSD with matching region, site, depth zone, and depth (m) data and structure plots for K =2–9 for each *X. muta* sample. **B** Principal component analysis from PCANGSD, where color represents sampling region and shape represents sampling depth zone. Larger, opaque shapes are sample population centroids and smaller, transparent shapes individual samples. **C** Same plot as **B** and with samples colored by sampling site. **D** Same plot as **B** and **C** with samples colored by lineage assignment from K = 7 data set. **E** Structure plot from NGSADMIX analysis with samples grouped by site and

depth zone. Each bar represents an individual sample and the proportion of each color is the probability of membership to that genetic lineage.

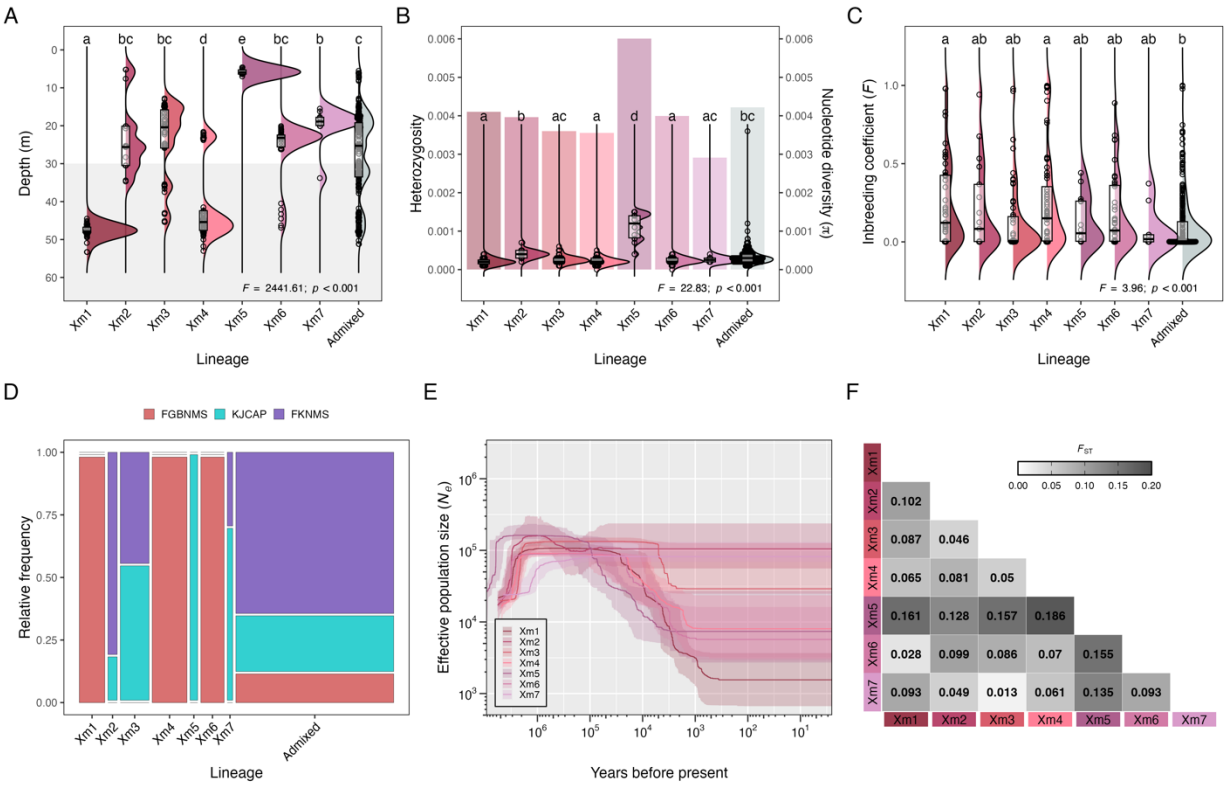

Figure 13. *Xestospongia muta* diversity and demographics. **A** X. muta lineage depth distribution. Shaded portion of graph indicates mesophotic depths. **B** Lineage heterozygosity across variant and invariant loci. Transparent bars behind violins represent nucleotide diversity (π) for each lineage. **C** Mean inbreeding coefficient by lineage. Letters indicate significant differences among lineages and Welch's ANOVA results are listed in **A**, **B**, & **C**. **D** Distribution of lineages across regions. Color of bars corresponds to sampling region. Width of bars is indicative of the relative proportion of samples assigned to that lineage. **E** Lineage effective population size through time. X-axis is thousands of years ago (KYA). Bounding ribbons are 75% confidence intervals. Note log scale for axes. **F** Pairwise fixation index (F_{ST}) heatmap among lineages. Darker coloration indicates higher F_{ST}.

4.3. Coral Salinity Threshold Experiments

Acute Hyposalinity Stress

Under acute hyposalinity stress, corals showed decreased health through reduced polyp activity as early as 27 PSU, and paling or bleaching by about 25 PSU (Figure 14, 15). This suggests that corals are undergoing physiological stress prior to mortality, which occurred at 19 PSU or 9 days of experimentation for both species. This study provides evidence that acute low salinity (e.g. <20 PSU) is more devastating than chronic exposure to intermediate hyposaline conditions (e.g. 25 PSU) for *M. cavernosa* and *P. astreoides* in Southeast Florida.

Hyposalinity events can pose significant risks to coral survival in regions heavily influenced by freshwater discharge. At St. Lucie Reef, one of the northernmost coral reefs in Florida, the lowest recorded salinity between 2015 and 2016 was 18 PSU (Shatters 2017; Studivan et al. 2021, Whitall 2019), suggesting corals in this area may have been negatively impacted by hyposalinity. Both *M. cavernosa* and *P. astreoides* are less tolerant to acute hyposalinity than prominent backreef corals in the Caribbean and South Florida, *Siderastrea* spp., which can tolerate salinities as low as 10–15 PSU (Table 4, Muthiga & Szmant 1987; Chartrand et al. 2009). However, there are differential hyposalinity tolerances associated with coral life stages (Hédouin et al. 2015), and future larval studies of study species *M. cavernosa* and *P. astreoides* may yield further important data regarding the ecological impacts of late-summer freshwater events.

Chronic Hyposalinity Stress

While both species had a similar response to acute hyposalinity exposure, these two species showed differential responses to chronic exposure at 25 PSU, with *M. cavernosa* being more tolerant to extended moderate hyposalinity than *P. astreoides*. The results of this study were confirmed by an additional trial with similar conditions, which yielded extremely similar results and thus is largely not reported here. Mortality of 50% occurred for *P. astreoides* by day 18, yet *M. cavernosa* had only 10% overall mortality by day 21 (Figure 16).

Furthermore, the data from these trials suggest that corals experiencing either acute or chronic hyposaline stress may suffer from impacts on physiological function in both autotrophic (via changes in color/pigmentation) and heterotrophic (via changes in polyp activity and associated feeding) activity. While the relationship between bleaching and autotrophic activity is well established, studies also show that corals under stress alter their trophic activity which may be closely linked to bleaching events and available feeding mechanisms (Hughes and Grottoli 2013). Further data from Rossi and colleagues (2019)

suggests a strong connection between polyp activity and feeding activity, providing further evidence for this connection. This could be useful for future reef monitoring, as reefs with universally low *M. cavernosa* polyp activity may be an indicator of increased stress. Corals can require months to years to recover from acute stress events that do not end in mortality (Hughes & Grottoli 2013; Osborne et al. 2017). Even if hyposaline stress does not lead to mortality directly, over-stretched coral energy budgets may come at the cost of certain non-vital functions, such as reproduction, impacting coral recruitment and ecological function (Beal et al 2012).

Chronic Hyposalinity + Wound Stress

We found that M. cavernosa colonies exposed to this same intermediately stressful salinity survived more than 45 days in the chronic hyposlinity + wound experiment (Figure 17). This study provided strong evidence that while this coral is clearly resilient to extended periods of low salinity, it is less capable of combating additional stressors under these conditions.

This idea is in fact reinforced by our final study which showed that *M. cavernosa* colonies under long-term hyposaline stress are showing compromises in other physiological functions. These results may suggest a down-regulation of wound healing functions in order to devote more of its energetic budget to osmotic functions, although gene expression work would need to be done to confirm this. This study helps draw attention to the impact of the multiple stressors we may see during Florida's hurricane season, where storm damage may cause wounds or damage to corals, and hyposaline conditions may persist for weeks as the watersheds drain stormwater back into coastlines.

Montastraea cavernosa

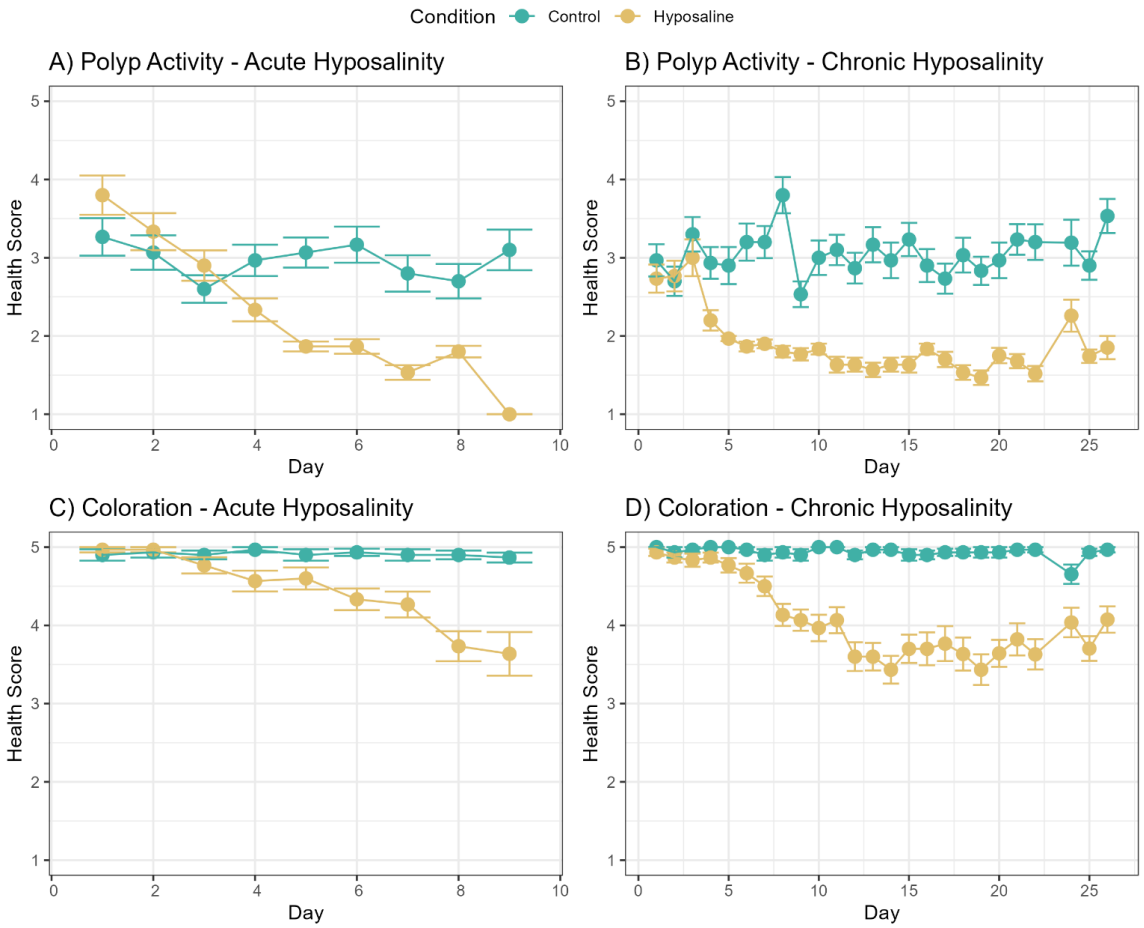

Figure 14. *Montastraea cavernosa* polyp activity and coloration scores throughout each experiment where points represent averages, bars represent standard error, and color denotes control (teal) vs treatment (tan). Each column represents a single experiment, and each row represents a different parameter. While polyp activity has more variation than coloration, over the course of each trial, scores for both parameters declined in the treatment condition.

Porites astreoides

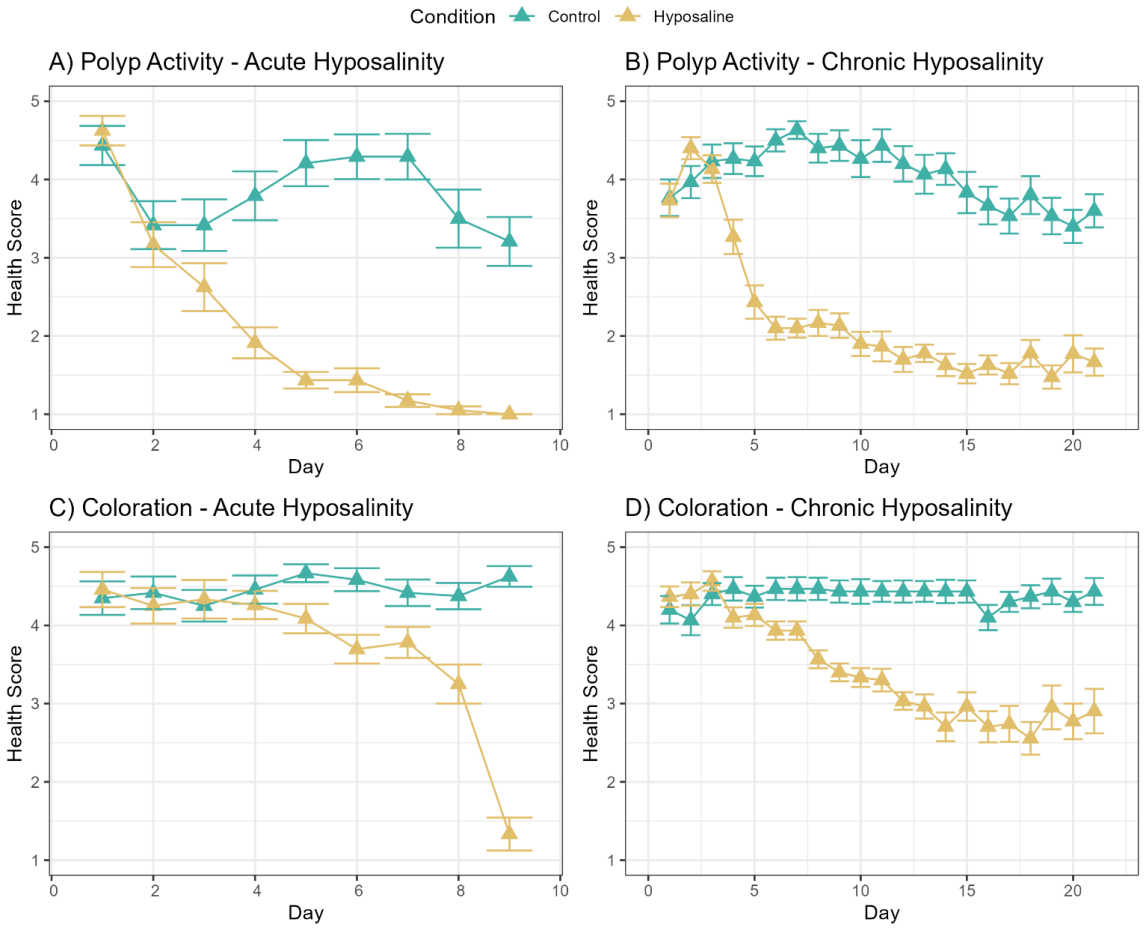

Figure 15. *Porites astreoides* polyp activity and coloration throughout each experiment where points represent averages, bars represent standard error, and color denotes control (teal) vs treatment (tan). Each column represents a single experiment, and each row represents a different parameter. Polyp activity is a strong and early indicator of reduced health in response to hyposalinity, with coloration declining more gradually, yet still significantly.

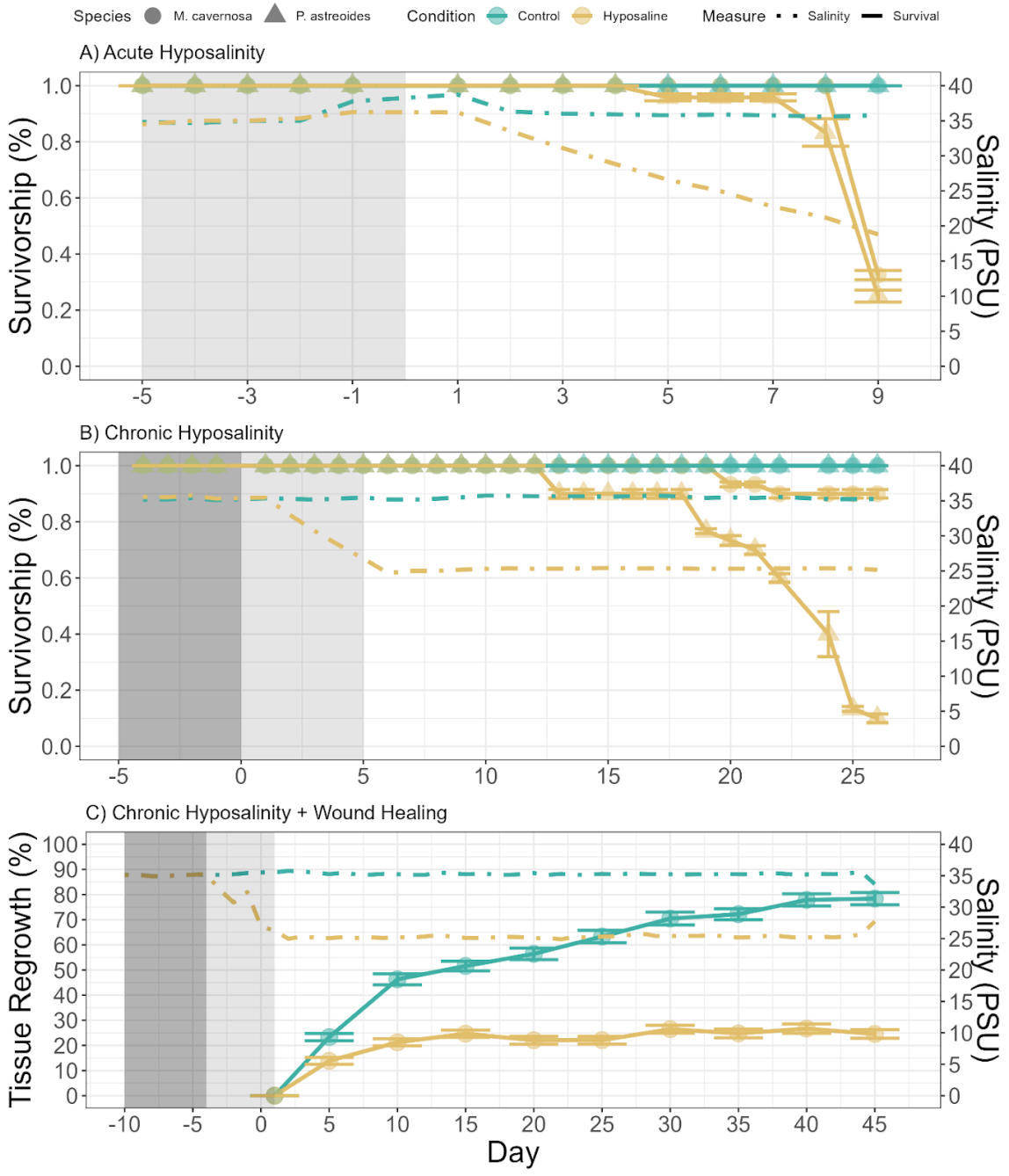

Figure 16. Mortality data for each experiment where salinity can be seen decreasing over time for treatment conditions (tan dashed) and holding steady in controls (teal dashed). In coordination, survivorship decreases for both *M. cavernosa* (circles) and *P. astreoides* (triangles) over time. The grey boxes represent the acclimation period preceding experimentation.

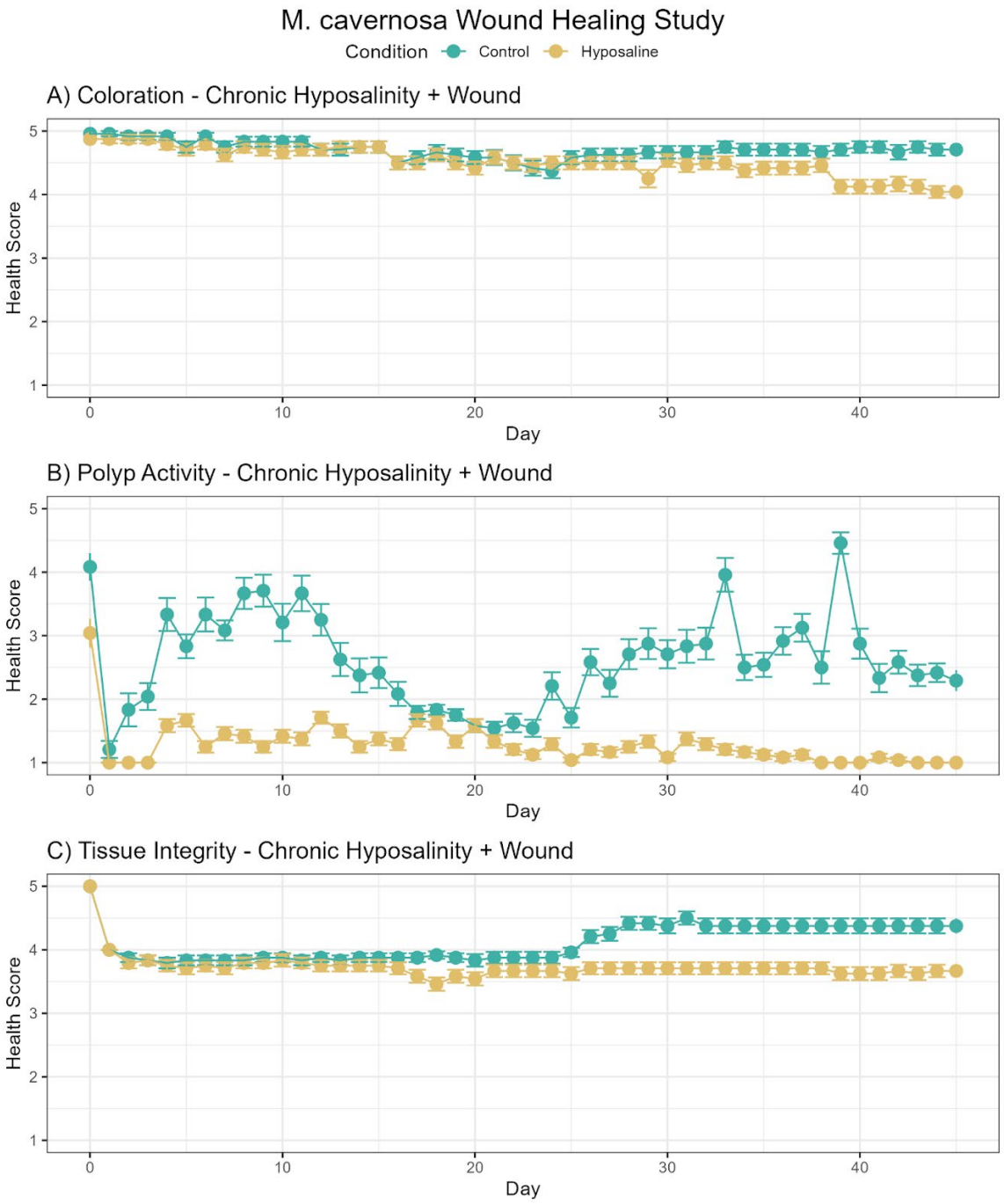

Figure 17. *Montastraea cavernosa* Polyp Activity, Coloration, and Tissue Integrity across exposure to Hyposalinity and Wounding for 45 days where points represent averages, bars represent standard error, and color denotes control (teal) vs treatment (tan). Each row represents a different parameter. While polyp activity has more variation than coloration or tissue integrity, over the course of each trial, scores for both parameters declined in the treatment condition.

4.4. SCTLD Research Resilience Consortium

We have evaluated patterns in the RRC microbial sequencing data between healthy-looking *Orbicella faveolata* samples collected in May/ June 2021, Aug/Sept 2021, and Feb/March 2022 on the same colonies across Florida's Coral Reef. Microbial community structure was significantly influenced by the time of sample collection, sample location, and genetic lineage, along with their interactions. There were 37 Bacterial Orders observed across all samples, with no bacterial order unique to Timepoint, Lineage, or Disease status. Figure 18 illustrates the variation in microbial orders across sample periods (T1, T2, T3).

74 orders exhibited significant differential abundance; 21 had increased abundance with SCTLD affected, 53 had decreased abundance with SCTLD affected (Figure 19). Two orders had both positive and negative log fold change suggesting that taxa within these orders have differing interactions with disease status. Research continues on this extensive dataset including finding associations between these data and the data gathered by the other teams (e.g. transcriptome, proteome, metabolome, chemical defenses, environmental factors, tissue recovery rates, etc).

These preliminary results suggest that shifts in the corals' microbiomes could serve as early indicators of disease susceptibility or resilience in *O. faveolata*. The significant effects of time, space, and genetic lineage on microbial community structure highlights the dynamic nature of coral-associated microbial communities and their potential role in coral health. Overall, 74 bacterial orders exhibited differential abundance based on disease status, with some orders increasing and others decreasing in conjunction with SCTLD activity. It is possible that specific microbial signatures may be predictive of disease onset or progression, however a larger data set or discrete experimental SCTLD infections would be needed to further evaluate this hypothesis. Further integration of microbial data with other omics datasets (e.g., transcriptomics, metabolomics) and coral recovery metrics may allow for the development of diagnostic tools to identify corals at greater risk of disease before visible symptoms appear, offering new avenues for proactive reef management and intervention strategies.

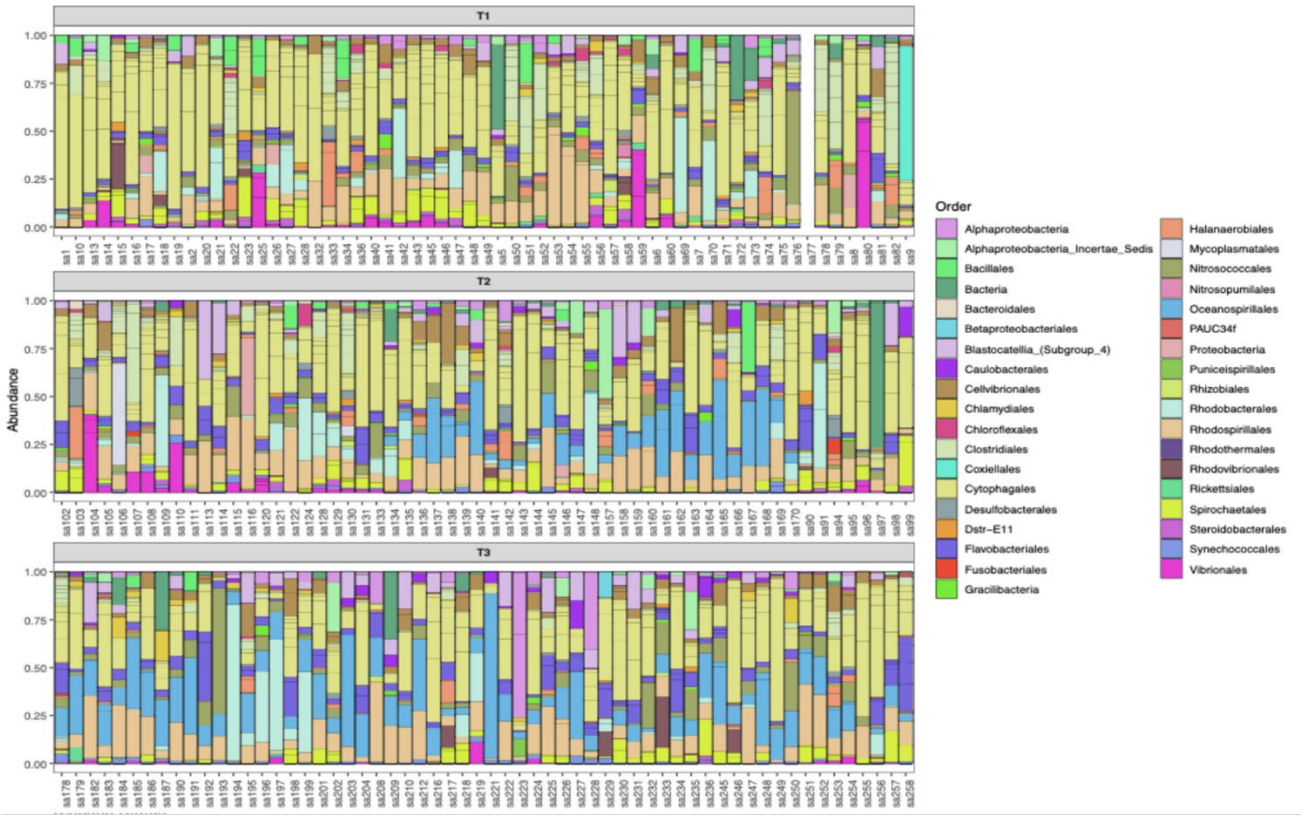

Figure 18. Bar plots depicting the relative abundance of microbial orders in SCTLD Affected samples. The plot is faceted by Timepoint, showing variations in microbial community composition. The x-axis represents different samples, and the y-axis shows the relative abundance of orders, stacked to represent the total abundance for each sample. The plot uses a custom color palette for order differentiation, with sample names on the x-axis rotated for clarity. There were no orders unique to a specific timepoint, disease status, or the intersection of the two.

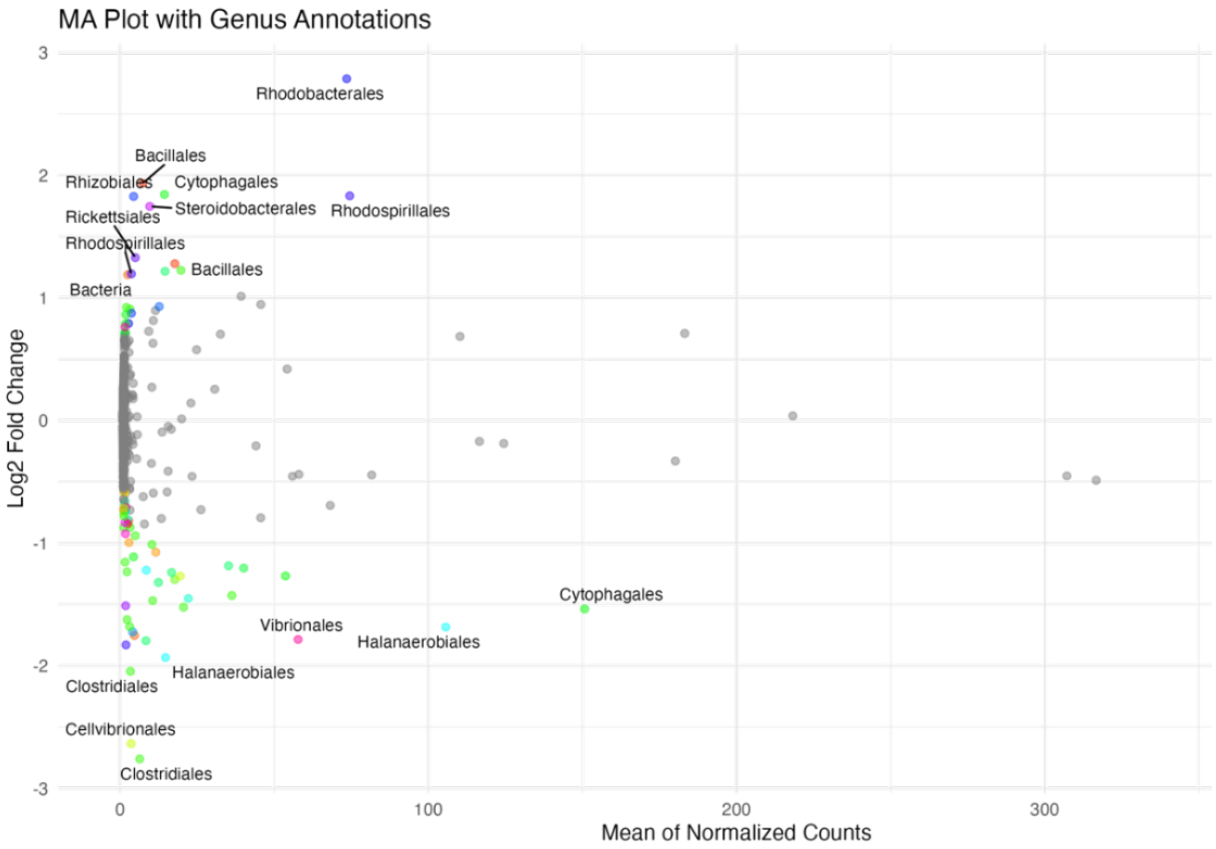

Figure 19. MA plot illustrates the differential abundance of microbial taxa with annotations for each order. The x-axis shows the mean of normalized counts, and the y-axis displays the log2 fold change between conditions. Points are colored by taxonomic order, with some orders labeled. Due to overlapping, not all orders are labeled. The plot is zoomed to visualize taxa with mean counts between 0 and 400.

The PERMANOVA analysis of 2bRAD generated Symbiodiniaceae revealed that dominant symbiont type had a significant effect on disease affectedness (PERMANOVA $F_{(1,89)}= 3.687$, $p < 0.01$). Overall, the majority of reads that aligned to the algal symbiont genomes aligned to *Breviolum* (Figure 20). However, all coral samples dominated by *Durusdinium* (n=7) fell under the SCTLD-affected category (Figure 20). The Indicator Species Analysis found that *Durusdinium* was a notable indicator species in SCTLD-affected colonies as compared to SCTLD-unaffected colonies (Indicator value: 0.281, $p < 0.001$). Clonal groups also had variation in dominant symbiont taxa. Clonal group 'a' had three individuals dominated by *Breviolum* and two samples dominated by *Cladocopium*, in which one sample dominated by *Cladocopium* was SCTLD-affected while all other clonal members were unaffected. Clonal group 'e' had one individual dominated by *Breviolum* and one individual dominated by *Durusdinium*. Both clones were affected by SCTLD.

Across the samples and algal symbiont identification and quantification methodologies *Symbiodinium, Breviolum*, *Cladocopium*, *Durusdinium*, and *Gerakladium* were detected (Figure). Primer/probed based qPCR assays and ITS2 did not detect any amount of *Symbiodinium* in any of the samples, while 2bRAD sequencing detected background amounts of *Symbiodinium* (<20%) in all the samples. Additionally, ITS2 sequencing detected <2% *Gerakladium* in one sample. There were significant differences in the abundance of Symbiodiniaceae across the various methodologies (Figure 20). 2bRAD sequencing yielded a higher abundance of both *Symbiodinium* and *Cladocopium* relative to that detected in qPCR assays and ITS2 sequencing. However, the detection of *Breviolum* using 2bRAD was less than that of qPCR and ITS2. Finally, the abundance of *Durusdinium* detected using 2bRAD was significantly more than that detected using qPCR assays but not statistically different than that detected using ITS2. It is believed that the detection of *Symbiodinium* and inflated presence of *Cladocopium* using 2bRAD sequencing is likely an artifact of the approach and qPCR and ITS2 sequencing are more accurate means of estimating Symbiodiniaceae genera. To test this, comparative analyses of known combinations of algal symbiont assemblages would be ideal, e.g. 100% cultures of each, equally mixed with 25% each. Even starting with known assemblages, gene copy number variance among and within algal endosymbiont genera may influence the resultant data.

Given the method comparison results, additional ITS2 sequencing was conducted of the RRC unified core samples. SP1 samples were extracted, amplified, and sequenced using ITS2 primers to better understand the specific algal symbiont species present within the 90 corals selected. Generally, across the corals selected for this study we identified 14 unique *Breviolum* type profiles, six *Cladocopium* type profiles, and eight *Durusdinium* type profiles, with a couple samples having ITS2 profile reads mapping to the genus *Gerakladium* (likely an artifact of low read numbers associated with a couple samples). Patterns between SCTLD affected and unaffected colonies remain elusive (**Error! Reference source not found.**), but there are interesting patterns associated with region and dominant contributing water inlet (**Error! Reference source not found.**) that may be inhibiting detection of SCTLD effects. Noteably, corals located north of Port Everglades inlet do not contain any detectable amounts of *Cladocopium* and/or *Durusdinium*.

The second plate of ITS2 sequencing has been sent to our sequencing facility and we are awaiting our results. This plate includes the remaining samples for the RRC unified corals for sample periods 2 and 3, as well as the 90 samples selected to compare 2bRAD, qPCR, and ITS2 algal symbiont identification.

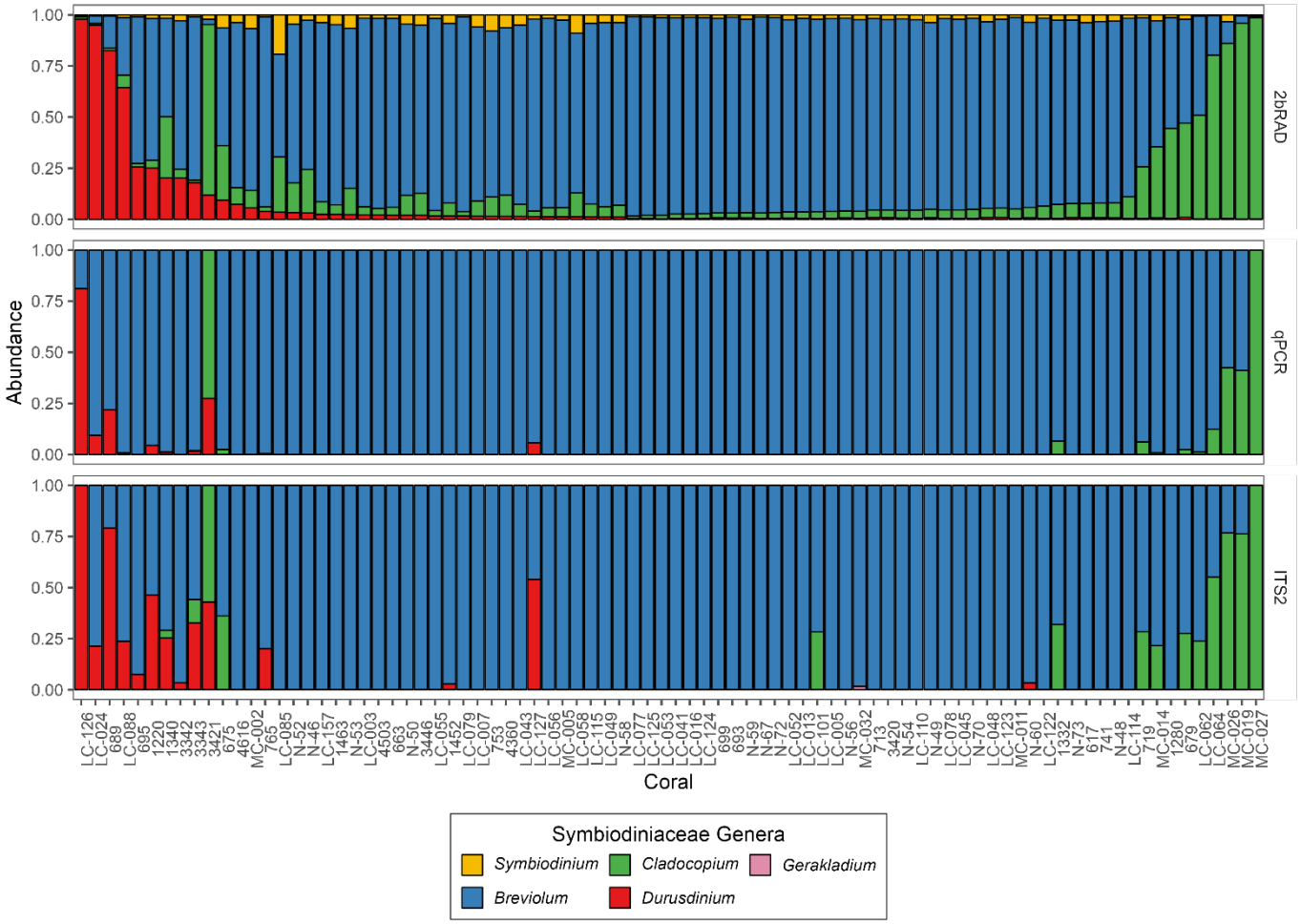

Figure 20. Relative abundance of Symbiodiniaceae genera detected across the same samples using different identification and quantification approaches.

4.5. Expanding Coral Restoration Efforts on St. Lucie Reef

After 1-year post transplanting in February 2025, 89% (57 out of 64) transplants were alive and after an additional quarterly monitoring period in May 2025, 87.5% (56 out of 64) transplants were alive. After 15 months, all large colonies of all three species are alive and 90% of small *M. cavernosa*, 72.7% of small *S. intersepta*, and 66.6% of small *S. siderea* colonies are alive (Figure 21). Additionally, after 15 months, larger colonies of *S. intersepta* and *S. siderea* have a significantly higher survival probability than small colonies and there is no difference in survival probabilities between size classes of *M. cavernosa* (Figure 22). At this site, it is difficult to determine whether a colony is missing or is dead and covered in sediment or algae. Because of this, missing and dead have been combined into one category for these analyses. We have observed no disease or predation

on the transplants over the entirety of the project so far, but we have noticed algae overgrowth and sedimentation on the transplants, especially the small size class individuals. While we did observe a few instances of bleaching, it seemed to be independent of size class and occurred only during the warmer months of the year that we monitored (May, August).

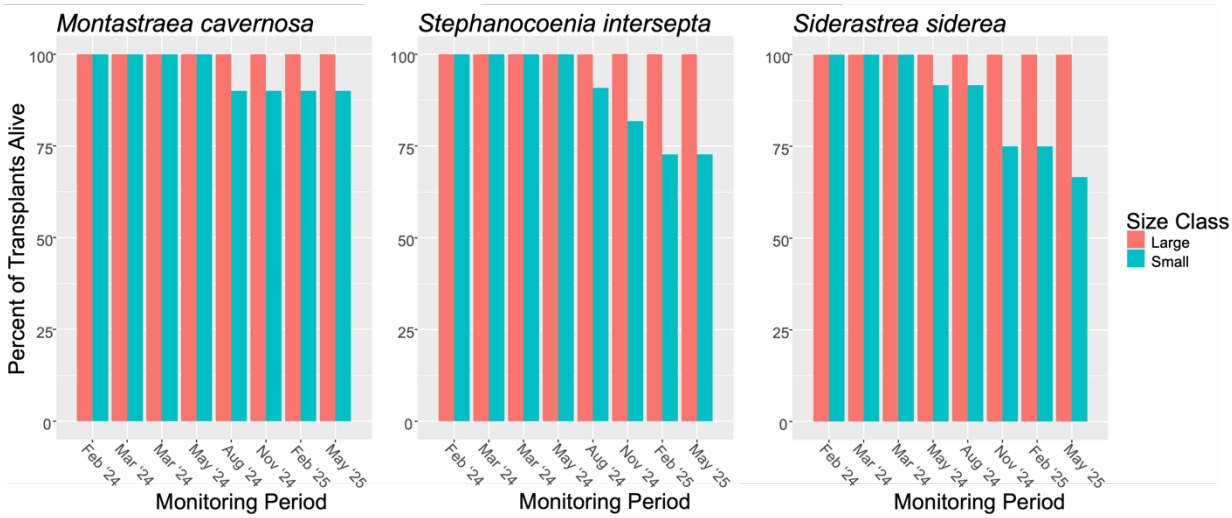

Figure 21. Total percent of transplanted colonies of the three species noted as alive during all monitoring intervals to date and separated into a small and a large size class.

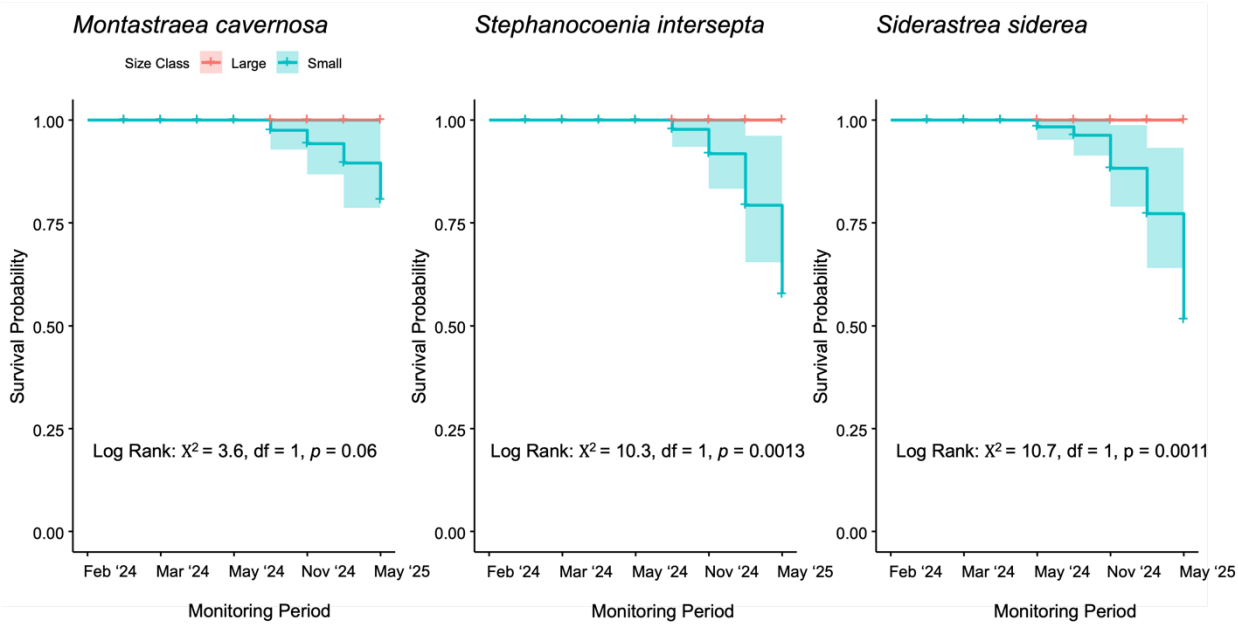

Figure 22. Kaplan-Meier survival curves show the survival probabilities between size classes for each species over all quarterly monitoring intervals from initial transplanting to the 15-month interval in May 2025. Shaded regions surrounding each curve represent 95% confidence intervals. Test statistics from log-rank tests comparing differences in survival

curves between size classes of each species are included, and *p*-values less than 0.05 indicate significant differences in survival probability.

In addition to overall survival, we also assessed changes in live tissue surface area of the outplanted colonies over the first year using the scaled photos taken during monitoring. Initial colony size did not differ among species but was significantly different between the two size classes (ANOVA: $F_{1,46}$ = 54.975, p = 2.18e-9, η^2_G = 0.539). There was no significant change in surface area or percent change in surface area among species or between size classes after 1 year (Figure 23).

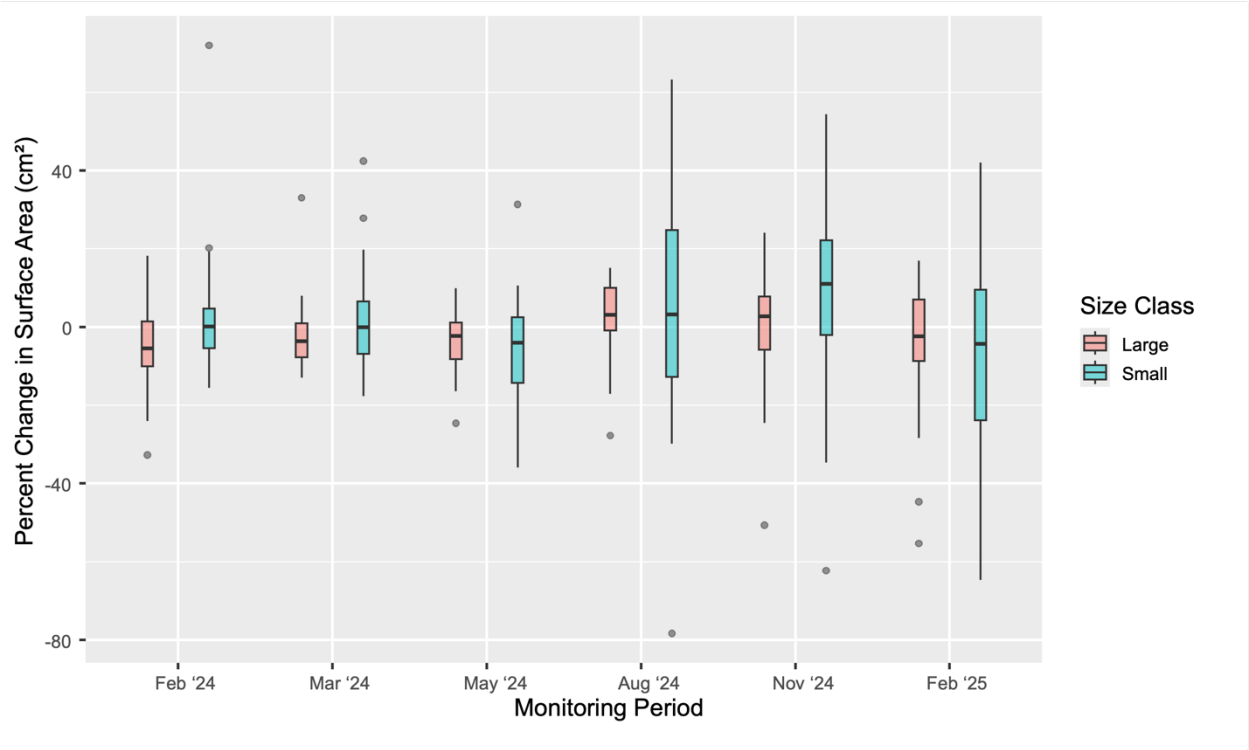

Figure 23. Box plots show the percent change in surface area (cm²) between initial colony size and size at each traced month for each size class. All species are combined because there was no significant difference between them.

While diving this site continues to pose a challenge due to its shallow depth (3.5m), typically poor visibility, and strong swell even at high tide, we were able to complete all quarterly monitoring intervals on time this year. This site was chosen as a target for restoration based on the success of the RTT outplants, and if these additional transplants are successful, it may be advisable to ramp up restoration efforts on St. Lucie Reef. However, as this site creates a difficult environment for diving, future efforts to increase restoration here will likely need to adjust expectations for frequency of monitoring.

4.6. Restoration Team Trials Algal Symbiont Analyses

We found that *Pseudodiploria clivosa* widely maintained *Breviolum* across both time points (Figure 24), whereas both *Montastraea cavernosa* (Figure 25) and *Orbicella faveolata* (Figure 26) exhibited stark shifts to the thermotolerant *Durusdinium* following the 2023 bleaching event. Furthermore, a clear spatial trend was observed in both *Orbicella* and *Montastraea*, with *Durusdinium* increasingly dominating the symbiont community in more southern restoration regions, from Miami-Dade through the Florida Keys.

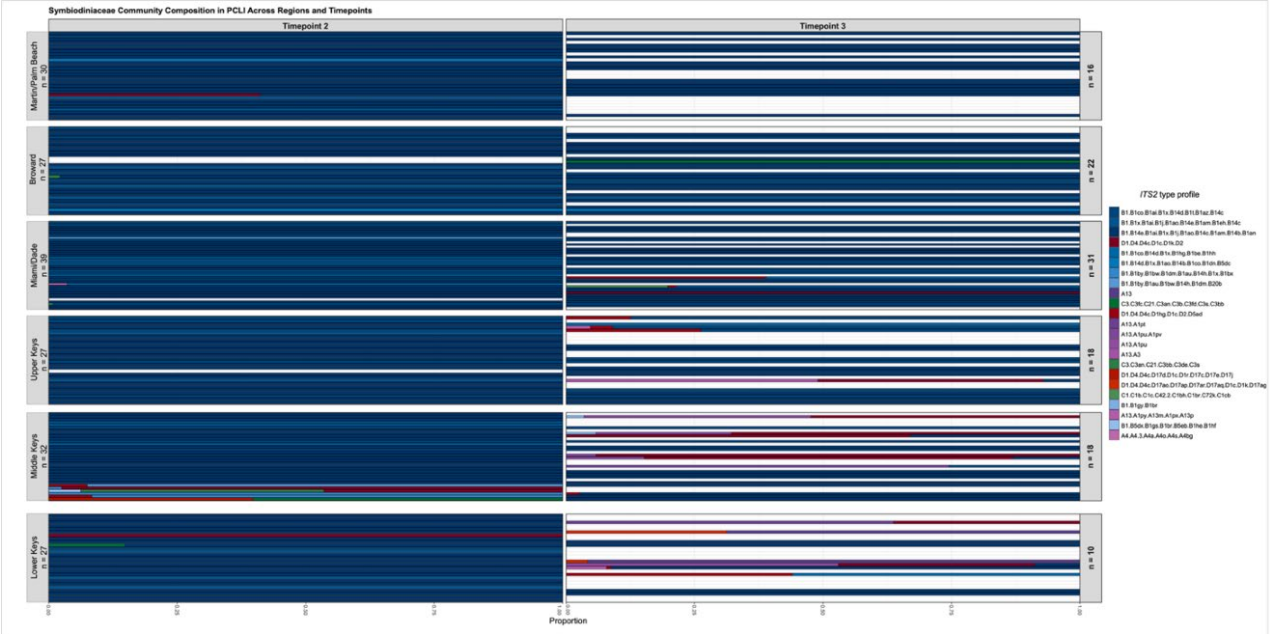

Figure 24. Bar plot visualizing ITS2 type proportions within each *P. clivosa* sample (n = 295) per region and time point. Bars are aligned for direct comparison of samples between time points. Blank spaces in either time point indicate the sample was not collected, either due to death or insufficient living tissue.

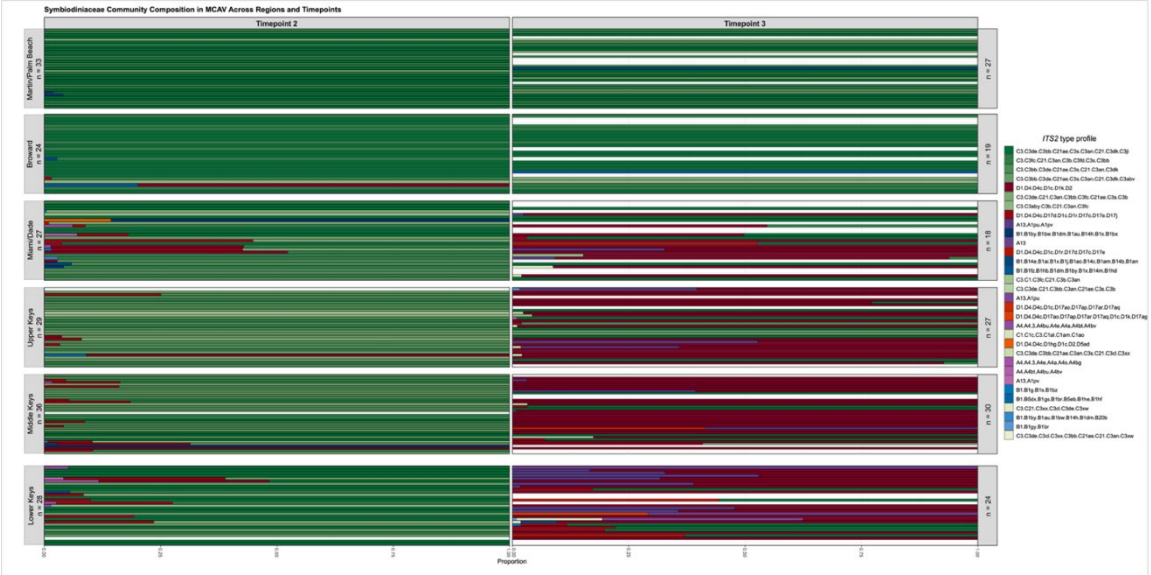

Figure 25. Stacked bar plot visualizing ITS2 type proportions within each *M. cavernosa* sample (n = 321) per region and time point. Bars are aligned for direct comparison of samples between time points. Blank spaces in either time point indicate the sample was not collected, either due to death or insufficient living tissue.

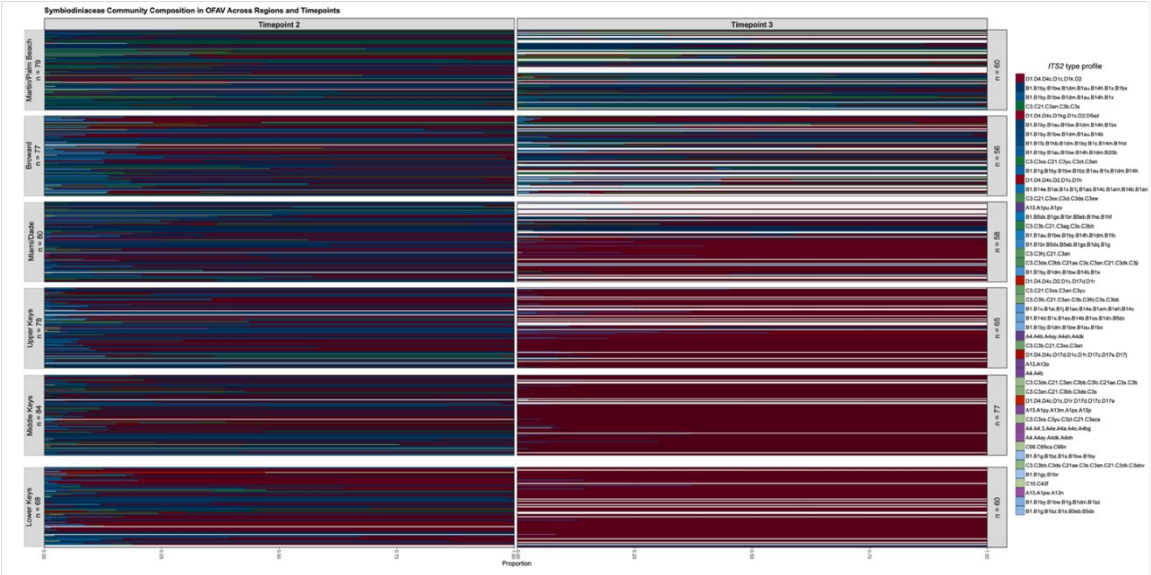

Figure 26. Stacked bar plot visualizing ITS2 type proportions within each *O. faveolata* sample (n = 839) per region and time point. Bars are aligned for direct comparison of samples between time points. Blank spaces in either time point indicate the sample was not collected, either due to death or insufficient living tissue.

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 most likely due to shifts in coral community composition and less environmental impacts. For example, on St. Lucie Reef, which is mostly dominated by low SCTLD susceptible species, SCTLD was essentially absent. Overall, our regional surveys demonstrate that KJCAP is well into the endemic phase of SCTLD and perhaps phase to a low SCTLD state, with extremely few observations of SCTLD during the past year. Indeed, we hypothesize that the low levels of SCTLD across the region are attributable largely to the reductions in susceptible species abundance in this area.

We found four distinct cryptic lineages of *Stephanocoenia intersepta* throughout southeast Florida and the Florida Keys, two being depth generalists across both shallow and mesophotic sites, and two being exclusively observed in shallow reef locations. This newly discovered cryptic biodiversity within Florida's corals suggests that restoration planning would be improved by accounting and planning for lineage diversity across species and restoring appropriate lineages that have a highly likelihood of survival. 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. When combined, data from *S. intersepta* and *X. muta* allow us to understand how populations of these species are structured across multiple MPAs on a large geographic scale. Based on previously evidence of connectivity between FGBNMS and Florida in *M. cavernosa* (Sturm et al., 2023), we hypothesized that we would find a similar trend within *S. intersepta.* However, given the reproductive characteristics of *X. muta*, more isolation across the same regions was anticipated. The data indicate a complete lack of connectivity among FGBNMS and Florida for both species. *S. intersepta* across all of FGBNMS sample sites was comprised of a single, panmictic lineage. While *X. muta* was more structured, with three distinct lineages. In Florida we also found results that were different from what we would have expected. *Stephanocoenia intersepta* lineages were found throughout the reef tract, however there were clear differences in the distribution between FKNMS and KJCAP. *Xestospongia muta*, exhibited less distinct zonation of lineages across Florida MPAs and was much less structured than we would assume, given its reproductive and larval characteristics.

This project provides critical data regarding the effects of low salinity stress on corals in southeast Florida. In addition to our previously reported impacts on coral mortality, we found that exposure to intermediate salinity stress (25 PSU) significantly reduced *M. cavernosa* corals' ability to heal from tissue damage injuries. Indeed, control corals at 35 PSU healed 3.5 times faster on average than the hyposalinity stressed corals. This suggests that recovery of damaged corals after tropical storms and hurricanes will be impeded by low salinity stress from freshwater discharges, and that recently fragmented corals for restoration should be outplanted during times that avoid low salinity stress.

Based on the relative success observed in our collaborative Restoration Team Trials (RTT) experiment, we expanded our coral restoration efforts at St. Lucie Reef by transplanting corals from tire reefs in Ft. Lauderdale. This year we completed 15 months of monitoring these transplanted corals and observed an 87.5% overall survival rate. For two of the three species, *Stephanocoenia intersepta* and *Siderastrea siderea*, large colonies had a significantly greater probability of survival than small individuals. In fact, all large transplant colonies are still alive. This is not surprising, as this site regularly experiences large blooms of turf algae which can smother smaller coral colonies. This project also evaluates the impacts of species and colony size on growth and survival. After one year post transplantation, we found no difference in percent change in colony size between size classes or among species. As these three species are typically slow growing, it is not surprising that we have not seen any significant changes after one year. Continued monitoring will be needed to better understand the relationship between outplant size and growth and survival among these three species and we plan to extend monitoring through the next year.

Through our collaborative efforts with the Restoration Research Consortium, we assessed microbial communities associates with *O. faveolata* colonies of varying SCTLD susceptibility or resistance. Microbial communities vary through time, space, and lineage. Shifts in the corals' microbiomes may serve as early indicators of disease susceptibility or resilience in *O. faveolata*. Bacterial functions varied by region, Symbiodiniaceae composition, and by disease history. Corals with active disease had more variable bacterial functions, indicating a disruption of the function of the microbiome in the whole colony as disease lesions were not sampled. In the Lower Keys where SCTLD was more active, unaffected colonies harbored bacteria with more abundant genes for the production of antibiotics, which may play a protective role for the coral. These results suggest that while SCTLD disrupts bacterial functions in apparently healthy tissue on actively diseased colonies, this effect is temporary and is outweighed by the influence of the Symbiodiniaceae composition or local environmental conditions.

Finally, we collaborated with our RTT partners to resample outplanted corals after ~ 2 years and again following the 2023 bleaching event to assess potential changes in their associated algal endosymbiont types. Both *M. cavernosa* and *O. faveolata* outplants faired well after the 2023 bleaching event, but *P. clivosa* appears to have been move severely impact with survivorship now below 50% in 5 of the 6 regions. Preliminary algal symbionts suggest significant changes among outplants in the southern regions following bleaching in 2023.

6. RECOMMENDATIONS

Recommendation 1: *Continue quarterly monitoring in Kristin Jacobs Coral Aquatic Preserve and St. Lucie Inlet Preserve State Park to capture trends in coral ecosystems, impacts of episodic events, and evidence of resilience/recovery.* More frequent monitoring efforts (e.g. quarterly) can better link events to impacts than annual monitoring surveys. Having both will provide complementary data sets that best capture the changes and trends in the Southeast Florida region. For enhanced efficiency, we recommend DEP or another partner take on monitoring at the Broward county sites, >2 hours drive from FAU Harbor Branch.

Recommendation 2: *Advance coral conservation initiatives with support from Magnuson-Stevens Act and implement actions/regulations for Kristin Jacobs Coral Aquatic Preserve.* 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 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's intraspecies genetic diversity. These assessments need sampling from the greatest number of locations and depths feasible. Expanding our efforts has led to the discovery of cryptic lineages within at least two coral species thus far, which impact our management and restoration strategies. Successful coral restoration approaches will require knowledge of genetic stocks among various coral populations to design and implement effective restoration plans across regions and depths.

Recommendation 4: *Update 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 that when exposed to reduced salinity levels, corals are both more likely to perish and significantly impeded in their recovery from physical damage.

Recommendation 5: *Continue restoration efforts with hypothesis driven designs to continue improving and optimizing restoration success.* The RTT experiment demonstrated that outplants of SCTLD susceptible species can survive, grow, and fuse. However, we saw significant differences among species, outplant regions, and coral sources. Further experiments to access the impacts of these and other factors on outplant survivorship, growth, and reproduction need to be coupled to the genomic efforts discussed above.