Large-scale Outplanting Designed to Assess Coral Restoration Feasibility in Response to Stony Coral Tissue Loss Disease

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William C. Sharp, Erinn M. Muller, Amanda Bourque, David S. Giliam, Dustin W. Kemp, Diego Lirman, Kristene T. Parsons, Colin P. Shea, and Joshua D. Voss

> Florida Fish and Wildlife Conservation Commission

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Project Title:

Large-Scale Outplanting Designed to Assess Coral Restoration Feasibility in Response to Stony Coral Tissue Loss Disease

Principal Investigators:

William C Sharp Florida Fish and Wildlife Conservation Commission Fish and Wildlife Research Institute 2796 Overseas Hwy, Suite 119 Marathon, FL 33050

Erinn Muller Co-Lead: Disease Advisory Committee Restoration Team Mote Marine Laboratory & Aquarium 1600 Ken Thomson Parkway Sarasota, FL 34236

Co-Applicants:

Joshua Voss Florida Atlantic University Harbor Brach Oceanographic Institute 5600 US Highway 1 North Fort Pierce, FL 34949

David Gilliam Nova Southeastern University 3301 College Ave. Fort Lauderdale, FL 33314

Diego Lirman Rosenstiel School of Marine & Oceanographic Sciences 4600 Rickenbacker Causeway Miami, FL 33149 Amelia Moura Coral Restoration Foundation 5 Seagate Blvd Key Largo, FL 33037

Sarah Hamlyn Mote Marine Laboratory 24244 Overseas Highway Summerland Key, FL 33042

Project Collaborators:

Kerry Maxwell FWC/FWRI 2796 Overseas Hwy, Suite 119 Marathon, FL 33050

Rene Baumstark FWC/FWRI 100 Eighth Ave SE St. Petersburg, FL 33701 Ken Nedimyer, Reef Renewal 212 Silver Palm Ave. Tavernier, FL 33070

Amanda Bourque Biscayne National Park U.S. National Park Service Homestead, FL 33033

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Executive Summary/Management Recommendations:

The survival rate of outplanted coral over the two-year study was 77%. Survival rates ranged from 71.4% at lower Keys sites to 85.9% at middle Keys sites. Species-specific survival rates were ~ 86% for *Montastraea cavernosa*, ~ 78% for *Orbicella faveolata*, and ~ 68% for *Pseudodiploria clivosa*.

The mean SCTLD rate of living colonies across all survey periods was 1.4%. A total of 194 (16.6%) of the outplanted colonies were recorded as exhibiting signs of SCTLD infection during at least one survey period. Of those colonies, 76 (39%) had died by the end of the survey, which represents 6.5% of the total outplanted colonies.

Species-specific probabilistic modeling did not identify statistically significant differences in the probability of colonies exhibiting external signs of SCTLD infection across survey regions, reef strata, or colony source. Moreover, the prevalence of SCTLD on natural coral communities at the outplant sites was not different than the control sites and consequently yielded no support for the hypothesis that outplanting SCTLD-susceptible coral species will increase the prevalence of the disease in the natural coral community in the immediate area. Therefore, we recommend that Florida's resource managers continue efforts to develop a coral reef restoration strategy that includes outplanting SCTLD susceptible coral species in SCTLD endemic regions in Florida.

Neither host genetic lineages nor algal symbiont types of the genotypes tested significantly affected SCTLD susceptibility, negating the hypothesis of SCTLD-resistant coral lineages. Future research should target specific gene regions known to correspond to coral immunity to further the chances of identifying genomic variations that may affect SCTLD resistance or survival. Furthermore, based on the results from this study and previous work, research into the drivers of SCTLD should combine genomic approaches with microbial, transcriptomic, metabolomic, or environmental conditions.

Probabilistic modeling identified differences in each of the three species across the survey area. In general, survival of all three species was higher on sites located comparatively closer to shore than those on the offshore sites. Coral abundance of the natural coral communities along the FCR is typically comparatively higher on nearshore habitats to those offshore, so it is reasonable to suspect that there were additional stressors acting on corals outplanted at the offshore sites compared to those outplanted closer to shore. We also note that *P. clivosa* is typically confined to shallower habitats than *M. cavernosa* or *O. faveolata*, so lower survival at offshore sites is in part likely depth related. *P. clivosa* was included in this project as it was, apart from *M. cavernosa* and *O. faveolata*, the only other SCTLD-susceptible species being maintained in coral propagation facilities in sufficient numbers to accommodate this study's experimental design and is the only one of the three species considered by the SCTLD case definition as being highly susceptible to SCTLD.

Colony source was also a predictor in the probability of survival to varying degrees among the three species and was a particularly important predictor of *P. clivosa* survival. Differences in colony source, coupled with clear geographic differences in survival, underscore the need for a coral reef restoration strategy that continues to prioritize the use of genetically diverse coral

assemblages in its coral outplanting efforts. We recommend that genotyping should be used within nurseries and before coral outplanting projects specifically to maximize genetic variation to increase coral reef resilience in response to the stressors threatening coral reefs.

A protocol using ImageJ software was developed and used to examine species-specific growth information. As with colony survival, the net growth of the outplanted colonies that survived to the end of the study varied by species and across geographic regions and colony sources.

As with earlier experimental outplant efforts, predation by corallivorous finfish was common on all three of the outplanted species during the first-month post-outplant then declined. Predation was particularly intensive at sites located off Miami-Dade and Broward Counties. The probability of whole colony survival decreased as predation intensity – measured as the proportion of coral tissue damaged or removed – increased. However, the survival rate of less severely damaged colonies was similar to colonies that had not exhibited evidence of finfish predation. Yet, we note that we cannot discount that many of the coral fragments that went missing early in the study before they fused with one another may have been the result of unrecorded finfish predation. We further note that there was evidence that net growth of *M. cavernosa* colonies measured at the end of the study was negatively affected by finfish predation. We had hoped to confirm the role of coral lipid content as a driver of predation on newly-outplanted colonies and had opportunistically retained some coral fragments to test that hypothesis. However, lipid data were ultimately quite limited, and we did not find an association between predation rates of coral genotypes and their pre-outplant lipid content.

Background:

Florida's Coral Reef (FCR) is an incredibly diverse ecosystem that supports thousands of organisms including reef-building corals, gorgonians, sponges, fish, algae, and invertebrates. Spanning more than 350 miles from the Dry Tortugas to St. Lucie Inlet in Martin County, it is the only coral reef that you can drive to in the continental U.S., making it a national treasure. Florida's Coral Reef not only protects our coastline and supports the economy, but also provides opportunities for recreation, education, and medical research. This ecosystem is uniquely positioned next to Southeast Florida's densely populated and highly urbanized coastal community. One third of Florida's population (approximately 6 million people) lives in this region with more than 38 million visitors annually.

Florida's Coral Reef is currently experiencing a multi-year disease-related mortality event, that has resulted in massive die-offs in multiple coral species. This disease, termed stony coral tissue loss disease (SCTLD), affects approximately 21 species of coral, including both Endangered Species Act-listed and the primary reef-building species. First observed near Virginia Key in late 2014, the disease has since spread to the northernmost extent of Florida's Coral Reef, and south to the Dry Tortugas in the Lower Florida Keys. The best available information indicates that the disease outbreak is continuing to spread southwest and throughout the Caribbean. The severity of this disease epidemic has resulted in an unprecedented concerted, collaborative, and organized effort among management agencies, researchers, conservation practitioners, nongovernmental organizations, veterinarians, and engaged citizens. This response network is led by the Florida Department of Environmental Protection (DEP), Florida Fish and Wildlife Conservation Commission (FWC), National Oceanic and Atmospheric Administration (NOAA), and National Park Service (NPS). Within this group, extensive research is being directed towards the identification and accurate diagnosis of the causative etiological agent(s) and its modes and rate of transmission, documenting its distribution and prevalence, identifying potentially contributory environmental factors, developing novel intervention approaches to mitigate infections at the colony level (Neely and Hower 2018; Walker and Brunelle 2018), preserving the genetic diversity of FCR, and testing the efficacy of outplanting new coral colonies to restore areas affected by SCTLD.

During the past decade, such active coral restoration efforts have expanded. These efforts entail propagating via asexual fragmentation and growing coral colonies within either in situ nurseries or in ex situ facilities and outplanting them onto degraded reefs along FCR, resulting in tens of thousands of coral colonies being outplanted annually (Schopmeyer et al. 2017). Although the vast majority of coral outplanting has focused on Acropora cervicornis and A. palmata, two species that are not susceptible to SCTLD, recent advances have been made allowing the successful propagation of several species of massive corals and restoration practitioners have begun incorporating these species into restoration efforts. However, many massive coral species are susceptible to SCTLD, and in response to the epizootic, restoration practitioners have either ceased or scaled down the restoration of these susceptible coral species until a better understanding of the disease dynamics emerges. Resuming coral restoration in a direct and concerted effort to mitigate the impacts caused by this epizootic event was identified as a high priority by resource managers involved in the collective SCTLD response. Accordingly, the Restoration Trials Team (RTT) was assembled and tasked to develop, coordinate, and conduct an experimental coral outplanting effort to determine when and where these future coral restoration efforts are most likely to be successful under potentially chronic persistence of SCTLD.

Project Goals and Objectives:

This study was developed and agreed upon by the RTT and addresses the SCTLD response team's priority of developing a coral restoration plan given the chronic persistence of SCTLD. Through a series of experimental-scale coral outplantings at sites using SCTLD-susceptible nursery-propagated coral colonies, we sought to evaluate the consequences and outcomes of outplanting these SCTLD- susceptible species. This study was designed to provide key information to resource managers charged with managing coral restoration activities by 1) determining geographic and species-specific SCTLD incidence rates of susceptible coral species outplanted along FCR, 2) assessing whether the outplanting effort affects the SCTLD prevalence in the neighboring wild coral communities, 3) determine if coral outplant performance can be correlated with particular genotypes, 4) determine if outplant performance can be correlated with particular endosymbionts, 5) evaluate the lipid content of representative coral outplants to determine the role of lipid content in coral predation, and 6) use computer image analysis to estimate coral colony growth.

<u>Coral outplant and community monitoring</u> – Monthly surveys of the outplanted coral colonies and the surrounding coral communities were conducted through April 2023, resulting in a 24-month long time series from which to evaluate geographic and species-specific SCTLD incidence rates and the effects of the outplanting on SCTLD prevalence in the nearby wild coral communities.

<u>Coral Genotyping</u> – The only relatively static, intransient factor among the corals used in this study was coral colony genotype. Previous studies have demonstrated variable success among different conspecific coral genotypes in controlled experiments and advances in sequencing technology allow for rapid, cost-effective measures of the corals' genetic diversity. The overall goals of this effort were to 1) to quantify the inherent diversity among the outplanted coral genotypes across the three coral species used in this project, 2) or even particular suites of SNPs, and 3) to provide raw sequence files and SNP data in publicly accessible databases to inform future coral restoration efforts in Florida.

<u>Algal Symbiont typing</u> – Coral's algal endosymbionts are important for coral's overall physiology, energy production, and growth. Although corals are capable of heterotrophy, they are particularly reliant upon the photosynthate produced by algal symbionts to fully meet their energetic requirements. Using coral fragments that had been opportunistically reserved from the outplanting work, the goals of this effort were to 1) to quantify the inherent diversity among the algal symbionts across the three coral species used in this project, and 2) to determine if outplant performance can be correlated with corals with particular endosymbionts.

<u>Coral Imaging</u> – After reviewing many of the photographs taken of the coral colonies during outplanting and the initial two post-outplant surveys (May-June 2021), it was determined that to accurately assess size-specific susceptibility of coral colonies to SCTLD and to better estimate colony growth, computer assisted image analyses of the survey photographs would be required. We used the computer software package ImageJ to produce an estimate of coral colony size for each of the first six survey periods and survey periods 18 and 25.

<u>Coral Lipid Analysis</u> – As with the algal symbiont typing, coral fragments were opportunistically reserved for lipid analysis. Prior limited research has suggested that the lipid content of corals may influence their palatability to fish predators. Though the coral fragments were not

specifically preserved for lipid analysis, the project afforded the opportunity to examine the role of lipid content and finfish predation on the outplanted coral.

The outcomes of this project will inform the on-going coral reef disturbance response effort which seeks, in part, to improve understanding about the scale and severity of the coral disease outbreak on Florida's Coral Reef, to identify primary and secondary causes, to identify management actions to remediate disease impacts, to restore affected resources and, ultimately, to prevent future outbreaks.

Description of Tasks Associated with Deliverables:

Task 1: Coral community monitoring. Conduct monthly monitoring of the outplanted coral colonies and the natural coral community in accordance with the experimental design from May 2021 through April 2023. Conduct quarterly monitoring of the control sites in accordance with the experimental design from May 2021 through May 2023.

Task 2: Coral genotyping. Conduct 2bRAD-Seq SNP pipeline for representative fragments of *M. cavernosa*, *O. faveolata*, and *P. clivosa* for each genet used in the restoration team trial project. Deliverables: 1) Provide summary report that includes detailed protocols and methodologies for 2bRAD-Seq pipeline for RTT corals, and 2) submission of sample metadata and submitted sequencing libraries, followed by posting of raw sequence files, SNP loci, and gene counts (once available) on open-access data repositories.

Task 3: Algal symbiont typing. Conduct ITS2 analysis pipeline for representative fragments of *M. cavernosa*, *O. faveolata*, and *P. clivosa* for each genet used in the restoration team trial project. Deliverables: 1) Summary report of the detailed protocols and methodologies for ITS2 pipeline for RTT corals, and 2) submission of sample metadata and submitted sequencing libraries, followed by posting of raw sequence files, species/clade identities, and relative abundances (once available) on open-access data repositories.

Task 4: Coral image analysis. To better estimate coral colony size and growth rates, use Image J analysis software to estimate the size of each coral fragment that composes each coral colony.

Task 5: Coral lipid analysis. To better evaluate potential differences in predation rates observed on outplanted coral colonies, conduct an analysis of total lipid content on representative subset of coral fragments of *M. cavernosa*, *O. faveolata*, and *P. clivosa* used in the restoration team trial project.

Methodology

Task 1: Coral community monitoring.

Experimental Coral Outplant Design – Three species of coral, each susceptible to varying degrees to SCTLD were used in the experimental outplanting study, *Montastraea cavernosa*, *Pseudodiploria clivosa*, and *Orbicella faveolata*. *M. cavernosa* and *O. faveolata* are considered intermediately susceptible species, and *P. clivosa* is considered highly susceptible to disease (FKNMS, 2018). The inclusion of these three species was ultimately driven by their availability within various *in situ* coral nurseries and land-based coral facilities. All corals were sourced from the in-water nurseries managed by the FWC, Coral Restoration Foundation, and Reef Renewal.

Mote Marine Laboratory and the University of Miami supplied coral from their respective landbased facilities.

The coral outplanting effort spanned the FCR from Martin County in the north to the lower Florida Keys. The survey area was divided into six regions: Region 1) Martin and Palm Beach Counties, Region 2) Broward County, Region 3) Miami-Dade County, 4) the upper Florida Keys, 5) the middle Florida Keys, and 6) the lower Florida Keys (Figure 1).

At each of the six regions, four coral outplant sites were established. To the extent possible, these were stratified by reef type. Within the four southern regions (Miami-Dade, upper Keys, middle Keys, and lower Keys) two sites were selected on offshore bank reef habitat and two on nearshore patch reef habitat. Because of differences in the Reef Tract off the two most northern regions (West Palm Beach and Broward) sites were established on the available suitable habitat, with two offshore and two inshore sites. Site locations are summarized in Table 1.

Each outplant site consisted of 48 colonies comprising three SCTLD-susceptible coral species. Figure 2 presents a conceptual diagram of an outplant site. Each colony was composed of five individual coral fragments, and colonies were placed approximately 1 m apart from one another such that the completed site is an approximate 8 x 6 m rectangle. The project contracted Reef Cells LLC to fabricate a cement base to hold five coral fragments. These bases were designed to standardize outplant structure across regions, elevate corals to increase survival, and decrease the time it takes for the corals to fuse into one colony (Figure 3). Reef Cells fabricated these bases specifically to fit the various coral pucks used by each of the partners that contributed coral. Each base was fitted with a nylon pin with a unique numerical sheep ear identification tag. The bases were secured to flat reef substrate at least one week prior to coral outplanting using a mortar substance provided by Reef Cells.

Experimental Coral Outplant Survey Methodology – Corals were outplanted across the six regions in the design described above during May 2021. Each site was scheduled to be surveyed twice during May 2021, then monthly thereafter until April 2023. During each survey, divers using SCUBA took photographs of each coral colony using a standardized camera mount with a ruler visible for scale. Using a standardized field data sheet, divers then recorded the general status (*i.e.*, live, dead, missing) of each colony, as well as any external signs of SCTLD. SCTLD was defined as exhibiting small circular or irregular lesions of white, newly exposed skeleton indicative of rapid tissue mortality (FKNMS 2018). If SCTLD is observed, divers recorded the presence of bleaching on each colony and evidence of fish bites or the number of corallivorous snails. If detected, the percentage of damaged tissue was estimated as a proportion of the total colony. If any loose or detached fragment with live tissue is encountered and can be positively associated with the cluster from which it originated, it was epoxied back to the base.

Natural Coral Community Survey Methodology – During the coral outplant site set up and each subsequent outplant site survey, a roving diver survey of the natural coral community at the outplant site was conducted. This survey was conducted at the outplant sites to establish a baseline for coral species diversity and abundance, size classes of live corals present and disease prevalence. Two divers conducted a non-overlapping 10-minute roving diver survey to assess the presence of active SCTLD. Each diver noted the condition of the SCTLD-susceptible colonies they encountered (*i.e.*, healthy, active diseased, dead). Using the same methodology, an additional

survey was conducted quarterly at a site at least 500 m from the outplant site that mirrors its coral community. These surveys served as 'control sites' to assess the potential relationship between the outplanted corals and the prevalence of SCTLD within the community in the immediate vicinity of the outplant site and sites without outplanting activity.

As predation on new bouldering-coral outplants has been a topic of interest, the partners also decided to include counts of selected finfish that have been observed to forage on new coral transplants on the outplant sites. These included all species of adult-sized parrotfish (>10 cm Total length) and all species and sizes of butterflyfish. These surveys followed the Reef Visual Census (RVC) protocol to the extent possible. In brief this method entailed a diver envisioning a 7.5 m radius when centered over the outplant site. If visibility was less than 7.5 m, the diver estimated the visibility and used that measurement as the radius of the cylinder. One diver used this cylinder to count fish for 5 minutes before any additional divers entered the water. These surveys were completed when the bases were installed, on outplant day, and at each monitoring period.

To evaluate the role of temperature as a potential driver of SCLTD prevalence (*see* Sharp *et al.* 2019), a HOBO pendant temperature logger supplied by the FWC was also deployed at each of the control sites.

DRM-Style Survey Methodology – Although not originally part of this project, all research partners agreed prior to establishing the outplant sites that conducting benthic surveys to characterize the outplant sites would provide valuable baseline information on disease prevalence. The survey was based upon Florida Reef Resilience Program's (FRRP) Disturbance Response Monitoring (DRM) program (<u>https://myfwc.com/research/habitat/coral/drm/</u>). The DRM protocol, adjusted for this project, was summarized in the first interim report submitted to the DEP (Sharp and Smith, 2021). These surveys included site depth, reef habitat type, presence / absence of *Diadema antillarum*, and assessing the condition of each coral along four 10×1 m transects. The presence of disease, bleaching, predation, abrasion, and other potential sources of mortality was recorded for each colony. Apart from one site in Region 1 and two sites in Region 3, all DRM surveys were completed either prior to coral outplanting or on outplant day. The site in Region 1 was conducted on the day coral was outplanted. The two sites in Region 3 were surveyed the day after outplanting.

Data Analysis of Outplanted Coral -

Outplant Plots

We evaluated the probability of coral colony survival, the probability of coral colonies exhibiting signs of SCTLD infection, and probability of colonies exhibiting signs of finfish predation by fitting mixed effects generalized linear regression models to the coral outplant data. Due to the number of predictor variables and their interactions, we evaluated each coral species used in the outplant study separately to ensure the inclusion of interactions and convergence of logistic regression models. For each of the three species, we fitted a mixed effects logistic regression model to the binary survival (1 = alive; 0 = dead), SCTLD (1 = evidence of SCTLD present; 0 = evidence of SCTLD absent) and finfish predation (1 = yes, 0 = no) metrics (*i.e.*, response variables) recorded for each colony during each of the 25 survey periods. Predictor variables were: 1) Region (*i.e.*, a categorical variable representing geographic Regions 1-6), 2) Reef Stratum (*i.e.*, a categorical variable representing "Inshore Sites" and "Offshore Sites"), 3) Colony

Source (*i.e.*, a categorical variable representing corals from in-water nurseries sourced from FWC, Reef Renewal L.L.C., and the Coral Restoration Foundation, and those from land-based facilities sourced from Mote Marine Laboratory and University of Miami), and 4) a continuous variable representing each of 25 survey periods (hereafter, survey intervals). Because many of the temperature sensors either failed or went missing over the course of the project, resulting in gaps in the water temperature time series, we did not include water temperature as a predictor. To account for spatial and temporal dependence (*e.g.*, autocorrelation and other instances of non-independence), we included a random intercept representing unique combinations of tudy site, species, geographic region, reef stratum, and survey intervals.

We then fitted a suite of (up to) eight candidate logistic regression models, starting with the global model that included all predictor variables, including several interaction terms. The number of models fitted varied due to convergence issues owing to quasi- or complete separation (i.e., instances where all individuals in a group exhibited all, or almost all, 1 or 0 responses in the binary logistic regression). Each of the candidate models represented a unique combination of the predictors listed above, and all were subsets of the global model. We used Akaike's Information Criterion corrected for small sample size (AICc; Hurvich and Tsai 1989) to rank the relative support for each candidate model. We identified the best approximating logistic regression model as the one with the lowest AICc.

Following model fitting, we assessed goodness-of-fit for each species' best-approximating model using a simulation-based approach to residual analysis, implemented in the R package `DHARMa` (Hartig 2022), to look for evidence of unexplained patterns in model residuals. Additionally, we assessed in- and out-of-sample predictive performance for each model by calculating two fit statistics, Receiving-Operator Characteristic Curve (AUC) and Brier scores, both of which range from 0 to 1, where values closer to 1 and 0 indicate a well-predicting mode, respectively (*i.e.*, 0 = perfect prediction for Brier scores and 1 = perfect prediction for AUC scores).

Coral Community Monitoring

We used a logistic regression model to compare SCTLD prevalence on the natural coral community between outplant sites and adjacent control sites, with the response variable being the mean proportion of SCTLD within the naturally occurring coral colonies. The fixed predictors were Region, Reef Strata ("Offshore" and "Inshore") and Treatment (Outplant Site and Control Site). To account for temporal autocorrelation and other instances of non-independence, a random variable was incorporated into the models. This variable was formed by a unique combination of the variables Site, Region, Species, and Survey Interval into its own group. Pairwise comparisons (False Detection Rate-adjusted) were then examined to detect differences in SCTLD prevalence between outplant and control sites at each Region and Reef Strata combination.

Task 2: Coral genotyping.

The coral genotyping task was conducted at FAU by Sydney Bell under the direction of J. Voss and the methodology and results presented herein has been taken from S. Bell's thesis document (Bell 2023).

Coral tissue samples were preserved from 75 fragments of the source colonies used in the outplanting and retained before outplanting in May 2021. The original coral fragments (3–5 cm²) were further fragmented to be preserved in duplicate 2 mL cryovials filled with 1 mL of Zymo

DNA/RNA shield and stored at -20°C until transport to Harbor Branch Oceanographic Institute where they were stored at -80°C until DNA extraction. Of the original 99 source colonies used in the outplanting, 24 source colonies lacked sufficient biomass for preservation and were therefore excluded from sampling and analyses.

Coral holobiont 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). Concentrations of the purified DNA were equalized prior to undergoing 2bRAD library preparation protocols following Wang et al. (2012) including modifications described in the protocol's GitHub repository (https://github.com/z0on/2bRAD denovo). Genomic DNA was digested by BcgI endonuclease, indexed adapters were ligated to the sticky ends of digested DNA fragments, and indexed ligations were pooled and amplified via PCR. Triplicate libraries were prepared for three samples which were used as a sequencing quality check and to identify naturally occurring clones (Manzello et al. 2019). Individual sample libraries had a combination of a 3' in-line indexed adaptor, and a set of unique i5 and i7 25 indices allowing for the pooling of all 75 sample libraries into a single sequencing pool. Each sample has a unique combination of i5 and i7 indices (dual indexing) which allows for distinguishing between pooled libraries and demultiplexing the pooled samples. An additional degenerate adaptor-ligated onto the 5' end allowed for downstream identification and removal of PCR duplicates. The final pool underwent automated size selection with Pippin Prep (Sage Science) before 100-bp single-end sequencing on an Illumina NovaSeq S1 SR100 single lane with 20% phiX. The phiX was added to provide the balanced fluorescent signals that low diversity sample libraries lack during each sequencing cycle and in turn, assists with discriminating clusters and improves overall run performance (Bourlat et al. 2016). Sequences were de-multiplexed based on their unique index combinations, then qualityfiltered and trimmed by custom Perl scripts (trims.sh, sampleRename.py, trimse.sh, readCounts.sh, https://github.com/z0on/2bRAD denovo,

https://github.com/RyanEckert/Stephanocoenia_FKNMS_PopGen/tree/main/scripts). To discriminate between the coral host and Symbiodiniaceae sequences, high-quality reads were first mapped to a concatenated Symbiodiniaceae metagenome consisting of the genomes *Symbiodinium microadriacticum* (Aranda *et al.* 2016), *Breviolum m*inutum (Shoguchi *et al.* 2013), *Cladocopium goreaui* (Liu *et al.* 2018), and *Durusdinium trenchii* (Shoguchi *et al.* 2013) using the sequence aligner Bowtie2 (Langmead *et al.* 2009). High-quality 2bRAD reads putatively from their respective coral host were analyzed as follows for the three study species. Reads from *M. cavernosa* were aligned to the *M. cavernosa* genome (version July 2018,

<u>https://matzlab.weebly.com/data</u> code.html), *O. faveolata* to the *O. faveolata* genome (Prada *et al.* 2016), and *P. clivosa* to a constructed de novo reference from this study since no published genome was available. Sequence reads that mapped to both the Symbiodiniaceae metagenome and coral host reference were discarded from subsequent analyses since their origin could not be determined. The remaining reads that mapped with high levels of uniqueness (*i.e.*, they did not map to the coral host reference or multiple Symbiodiniaceae genomes) to each of the Symbiodiniaceae genomes were retained as a proxy for the relative abundance of these four algal symbiont genera associated with each source colony. High-quality reads that only aligned to a coral host genome were used for downstream genetic analyses.

The program ANGSD v0.933 was used to generate genotype likelihoods across SNP loci from sequencing reads and to subsequently construct an identity-by-state (IBS) genetic distance matrix among samples for each study species (Korneliussen et al. 2014). ANGSD was run with the following parameters: minimum mapping quality scores of 20, minimum base quality scores of 25, p-value of 10^{-5} that a locus is variable, at least 75% of non-missing genotypes across samples, minimum p-value for deviation from Hardy– Weinberg equilibrium of 10–5, minimum p-value for strand bias of 10-5, minimum allele frequency of 0.05, and a filter that removed any tri-allelic SNPs. Population structure analyses were conducted with the program NGSadmix (Skotte et al. 2013), and the programs Clumpak, StructureSelector, and PCAngsd were used to assess for K = 1-11. These programs hypothesize the number of populations of origin or lineages represented by K, which can be anywhere from one to eleven. Clumpak uses the Evanno method, StructureSelector uses the Puechmaille method, and PCAngsd uses a singular value decomposition model to determine the most likely number of populations/lineages (Kopelman et al. 2015; Li and Liu 2018; Meisner and Albrechtsen 2018; Puechmaille 2016). PCAngsd was then used to generate principal component analyses (PCAs) by population genetic clusters. Lastly, the survival v3.5.3 package was used to create survival and disease Kaplan-Meier curves for each unique genet as well as run Cox Regressions to determine the effect of genet and/or outplanting region on survival and disease over time. Within the survival v3.5.3 package, the function survdiff was utilized to test for significant differences among two or more survival curves using the Gp family of tests, or for a single curve (observed) against a known alternative (expected). Bonferroni post-hoc tests were conducted for significant Cox Regressions (stats v3.6.2).

Task 3: Algal symbiont typing.

The algal symbiont typing task was conducted at FAU by Sydney Bell under the direction of J. Voss and the methodology and results presented herein has been taken from S. Bell's thesis document (Bell 2023).

DNA extracted for 2bRAD sequencing was used for symbiont community typing. Highthroughput sequencing of the internal transcribed spacer 2 (ITS2) region of ribosomal DNA operon has been implemented in several studies and is considered the standard for identifying Symbiodiniaceae within and among coral colonies (Arif et al. 2014; Baker 2003; Correa and 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). These primers are modified to include adapter regions for the incorporation of indexed forward and reverse Illumina adapters. 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 with the Thermo Scientific GeneJET PCR Purification Kit following the manufacturer's protocols. Products were quantified with Qubit (Invitrogen) and diluted for a second PCR which incorporated a unique combination of indexed forward and reverse Illumina adapter 28 primers producing a unique dual index or barcode for each sample (Klepac et al. 2015). Each sample was run in a 20 µL PCR with 5 ng initial PCR product and 0.15 µM of each indexed Illumina forward and reverse adapter primer (Eckert et al. 2020; Klepac et al. 2015). The libraries were sequenced with 20% phiX on the Illumina MiSeq platform (v3 chemistry) with paired-end 250 bp reads. Bioinformatic analyses of the ITS2 sequences were conducted on the SymPortal platform where sequences were grouped by genera after non-Symbiodiniaceae and sequencing artifacts were filtered from the dataset using standard sequence quality control protocols implemented with

MOTHUR 1.39.5, the BLAST+ suite of executables, and minimum entropy decomposition (Camacho *et al.* 2009; Eren *et al.* 2015; Hume *et al.* 2019; Schloss *et al.* 2009). Sequences were organized by genera; within genera, groups with over 200 sequences were algorithmically searched to identify defining intragenomic variants (DIVs) which were used to generate ITS2 type profiles representative of putative Symbiodiniaceae taxa (Hume *et al.* 2019). ITS2 profiles are specific combinations of intragenomic sequences and represent the taxonomic unit used by SymPortal (Hume *et al.* 2019). Statistical analyses of Symbiodiniaceae diversity were conducted from SymPortal outputs within the R statistical environment (R Core Team, 2019). Permutational multivariate analysis of variance (PERMANOVA) was used to test differences in Symbiodiniaceae ITS2 type profiles between disease status groups as well as source nurseries (9,999 permutations; vegan 2.6.4). Finally, Procrustes analyses were run for each species to determine any correlations between host genetic distance and Symbiodiniaceae Bray-Curtis distance.

Task 4: Coral image analysis.

Each of the photographs taken of the outplanted coral colonies during the first six survey intervals (May 2021-September 2021), the 18th survey interval (September 2022), and the final survey interval (April 2023) were analyzed using Image J, a commonly used computer software product to estimate coral colony size. A detailed protocol was developed by Gabby Pantoni of FAU-HBO and is attached as Appendix 1. In brief, for each of the photographs selected, the 2D surface area of living coral tissue was estimated by first defining the scale of the image using either a ruler attached to the camera housing developed for the project or, in the absence of a visible ruler, measuring the identification tag attached to the coral base. Once a known distance was defined, the coral colony was traced. The operator was careful to include only living coral tissues. Once complete the ImageJ software estimated the 2D surface area of the colony.

We estimated the net growth for each of the three coral species as the final surface area (cm²) in April 2023 minus the initial surface area in May 2021.We then fit linear regression models to mean net growth data. As with the logistic regression models described above, we fitted a suite of candidate regression models, starting with the global model that included all predictor variables, including several interaction terms. Each of the candidate models represented a unique combination of the predictors listed above, and all were subsets of the global model. We used Akaike's Information Criterion corrected for small sample size (AICc; Hurvich and Tsai 1989) to rank the relative support for each candidate model. We identified the best approximating regression model as the one with the lowest AICc.

Following model fitting, we assessed goodness-of-fit for each species' best-approximating model using a simulation-based approach to residual analysis, implemented in the R package `DHARMa` (Hartig 2022), to look for evidence of unexplained patterns in model residuals. Additionally, we assessed in- and out-of-sample predictive performance for each model by calculating two fit statistics, Receiving-Operator Characteristic Curve (AUC) and Brier scores, both of which range from 0 to 1, where values closer to 1 and 0 indicate a well-predicting mode, respectively (*i.e.*, 0 = perfect prediction for Brier scores and 1 = perfect prediction for AUC scores).

Task 5: Coral lipid analysis.

Total lipids were extracted from coral samples using a modified version of the procedure outlined by Folch *et al.* (1957) to extract total lipids from the provided coral specimens. Because the coral samples were opportunistically preserved using 100% ethanol, ethanol was substituted for methanol in the extraction process. This adjustment was based on the closely matched polarities of the two solvents. The volumetric ratio of the solvents was maintained as 8:4:3 (v/v/v) for chloroform:ethanol:NaCl solution (0.88% weight to volume), ensuring consistency.

To guarantee the complete separation of coral tissue from the skeletal material, the samples underwent a gentle sonication for 30 seconds in the ethanol solution they were initially preserved in. The ethanol solution, along with any coral tissue, was then carefully transferred to an acid-washed graduated cylinder, and the volume was recorded. Subsequently, the mixture was transferred to a 500 ml separatory funnel, into which two portions of 100% chloroform were introduced. The separatory funnel was sealed and placed on an orbital shaker for a duration of 2 hours to facilitate the partitioning of components.

Following this incubation period, the aqueous solution was subjected to filtration using Whatman filter paper, and sodium chloride (NaCl) solution was added to achieve the intended 8:4:3 proportion. The solution was then returned to the separatory funnel, thoroughly mixed, and allowed to stand vertically on a ring stand. Over the course of an hour, the mixture separated into three distinct phases.

The lower phase, which contained the soluble lipids, was meticulously collected from the separatory funnel and introduced into a pre-weighed glass tube that had been previously combusted at 500 °C. Subsequently, the samples were dried under a constant stream of nitrogen gas at a temperature of 30 °C. The mass of total lipid content was determined through gravimetric measurement, accurate to the nearest tenth decimal place in milligrams. To account for variations in coral fragment size, the quantified total lipid content was normalized to the surface area of the coral fragment. This normalization was carried out using the aluminum foil method described by Marsh (1970).

Results

Task 1: Coral community monitoring.

Pre-Outplant DRM-Style Surveys

Few diseased coral colonies were encountered during the initial DRM surveys (Figure 4). SCTLD was recorded on two colonies, both on *Siderastraea siderea*: one in the upper Keys region and one in the middle Keys region. Only the colony in the middle Keys surveys was estimated to have recent tissue mortality. The Dark Spot Disease recorded in those same two regions was largely observed on *Stephanocoenia intersepta* and *S. siderea*.

Summary of Coral Outplant Monitoring

Corals were outplanted across the study area from May 4 through May 6, 2021. Coral fragments were distributed across the sites to maximize the representation of each species, source, and genotype across all regions. The species, source, and genotype, if known, for each coral cluster was carefully tracked, distributed, and attached to a specific base depending on the source at each site. Given the complexity of dispersing corals, reported difficulties were exceedingly minimal.

These entailed a few instances of coral source/base mismatches. However, these were recorded and tracked accordingly and posed no difficulties in evaluating the monitoring data. There were also additional coral fragments available at the coral swap, and these were distributed to the partners in case any corals were damaged or misplaced during transport. NSU and MML chose to use these fragments to assemble additional colonies and incorporate them into their outplant sites. NSU and MML surveyed six and seven additional colonies, respectively, in addition to the 192 dictated by the experimental design. In all, 1,165 colonies were outplanted. In total, 223 colonies of *M. cavernosa*, 652 of *O. faveolata*, and 290 of *P. clivosa* were outplanted.

Of the potential 1,200 surveys of the outplant sites scheduled from May 2021 through April 2023, 1,196 were completed. Due to weather and logistical considerations, four sites were not completed (Region 3, Site 1 and Site 2 during the second May 2021 survey period; Region 1, Site 1 during February 2022, and Region 1, Site 1 during November 2022).

By the final surveys conducted during April 2023, two-years post-outplant, 901 colonies (77.3%) were alive, 260 (22.3%) had completely died (or were never again observed after being recorded as missing and were consequently presumed dead). The status of the remaining four colonies (0.3%) was not recorded (Figure 5).

The percentage of surviving colonies across the survey regions by the final survey interval ranged from 71.4% (Region 6; lower Keys) to 85.9% (Region 4; upper Keys) (Figure 6). The overall proportion of surviving colonies across the survey area was higher at inshore sites (91.2%) compared to the offshore sites (84.7%). The percentage of surviving *M. cavernosa* colonies across the regions at the final survey period was 86.1%, and the surviving percentage of *O. faveolata* and *P. clivosa* was 78.4% and 68.3%, respectively.

Species-specific frequencies of SCTLD Infection – Outplanted Colonies

The proportion of living coral colonies recorded as exhibiting signs of SCTLD infection during the study period ranged from 0% (May 2021) to 4.2% (July 2022) ($\bar{x} \pm 1SE = 1.14\% \pm 0.20\%$) (Figure 7). A total of 194 colonies were recorded as exhibiting SCTLD (Figure 8). Of the 223 *M. cavernosa* colonies outplanted, 32 (14.0%) were recorded as exhibiting SCTLD during at least one survey interval. Of the 652 *O. faveolata*, 120 (18.4%) were recorded as exhibiting SCTLD. Of the 290 *P. clivosa* colonies, 42 (21.0%) were recorded as exhibiting SCTLD. Of the total 194 colonies, 76 (39.2%) were dead by the end of the survey period. The whole colony mortality rate of *M. cavernosa* was 25.0%, the whole colony mortality rate of *O. faveolata* was 36.7%, and the whole colony mortality rate of *P. clivosa* was 57.1%. Of those colonies that died, 33 (43.4%) were dead by the next survey period after initially being observed with SCTLD and a total of 45 colonies (59.2%) were dead by two survey periods after the initial observation.

Probability of SCTLD Infection—Outplanted Colonies

The logistic regression model indicated that the probability of SCTLD infection across regions, reef stratum, and colony source for *M. cavernosa* was $< \sim 1\%$ across all treatment levels (Figure 9). Pairwise comparisons of the Estimated Marginal Means detected no differences in the probability of SCTLD infection across regions or reef strata (Table 2). There was a marginally non-significant difference in SCTLD infection probability between the FWC and UM-sourced coral colonies. Given the low probability of SCTLD infection overall, we consider this difference between coral sources to be of no consideration in the broader context of coral restoration efforts.

The logistic regression model describing the probability of SCTLD infection in *O. faveolata* revealed a greater degree of uncertainty describing SCTLD infection rates, resulting in wider confidence intervals around the mean predictions than *M. cavernosa* (Figure 10), but mean probabilities did not exceed ~ 2% across treatment levels. Pairwise comparisons of the Estimated Marginal Means did not detect differences between regions or between colony sources apart from the comparison between FWC-sourced and RR-sourced colonies (Table 3). However, as with *M. cavernosa*, given the low probability of SCTLD infection and cognizant of the number of interacting factors in these analyses, we note that individual comparisons should be interpreted conservatively, and given this we conclude that colony source was not an important factor describing SCTLD susceptibility of *O. faveolata* in this study.

As with the other two species logistic regression model describing the probability of SCTLD infection in *P. clivosa* described SCTLD infection rates $\sim 1\%$ across the survey period (Figure 11). Pairwise comparisons did not detect significant differences between the treatment levels (Table 4).

Prevalence of SCTLD of the natural coral communities

The timed roving diver surveys conducted during monthly surveys of the outplanted coral colonies indicated that the prevalence of SCTLD on the natural coral communities was generally low (Figure 12). Similar prevalence of SCTLD was observed during surveys of each outplant site's control site (Figure 13). Pairwise comparisons of mean SCTLD prevalence across regions and reef stratum did reveal significant differences between the control and outplant sites at the inshore reef stratum in Region 5 and the Offshore reef stratum in Region 6 (Figure 14, Table 5). However, in both instances the prevalence of SCTLD was higher at the control sites compared to the outplant sites, a result contrary to our hypothesis that outplanting SCTLD-susceptible coral colonies would increase the disease on the immediate natural coral community. Consequently, we conclude that there is no support for this hypothesis and that a restoration strategy that includes outplanting SCTLD-susceptible species should continue to be developed and refined.

Post-Outplant DRM-Style Surveys

The post-outplant surveys encountered only one colony with SCTLD, an *M. cavernosa* colony in Region 5 (Figure 15). No coral diseases or discolored colonies were observed in Region 1, Region 2, or Region 3. Dark Spot Disease (DSD) was present on approximately 4% of the *Siderastrea siderea* colonies in Region 4. DSD was also observed on *S.siderea* and *Stephanocoenia intercepta* colonies in Region 5 and on *S. intercepta* in Region 6.

Probability of Colony Survival – Outplanted Colonies

The logistic regression model indicated that the probability of survival of *M. cavernosa* across regions, reef stratum, and colony source differed significantly across all treatment levels (Figure 16). Pairwise comparisons of the Estimated Marginal Means confirmed differences between reef strata. The probability of survival was higher on sites within the inshore reef strata compared to the offshore reef strata (Table 6). Region differences in colony survival were also identified. The probability of survival of colonies in Region 5 was significantly higher compared to all of the other regions, and the probability of survival was higher in Region 1 compared to Region 2 and Region 6. Finally, the probability of survival of three colony source facilities that provided *M. cavernosa* for the study all differed from one another.

The logistic regression model also detected significant differences in the probability of survival of *O. faveolata* across regions, reef stratum, and colony source (Figure 17). Pairwise comparisons of

the Estimated Marginal Means confirmed that probability of survival was significantly higher at inshore sites compared to the offshore sites (Table 7). Region differences in colony survival were also identified. The probability of survival of colonies in Region 2 was significantly lower compared to Region 3, Region 4, and Region 5. The probability of survival of colonies in Region 6 was significantly lower than those in Region 3, Region 4 and Region 5. Finally, the probability of survival of three colony source facilities that provided *O. faveolata* for the study all differed from one another. The probability of survival was lower for colonies sourced from CRF compared to those sourced from MML or RR. No other differences in the probability of survival were identified between all other pairwise comparisons of colony sources.

Finally, the logistic regression model also detected differences in the probability of survival of P. clivosa across regions, reef stratum, and colony source (Figure 18). However, compared to the other two species, the probability of survival across regions was similar. The only differences in survival identified by the pairwise comparisons of the Estimated Marginal Means occurred between Region 6 and Region 3 and Region 4 (Table 8). The probability of survival in Region 6 was lower compared to the other two Regions. There were significant differences in the probability of survival between inshore and offshore sites, as well as between colony sources. As with M. cavernosa and O. faveolata, the probability of survival of P. clivosa was higher at inshore sites compared to offshore sites, though the difference was more pronounced (Figure 18). This difference could have been anticipated, since P. clivosa is typically confined to more shallow water habitats relative to *M. cavernosa* and *O. faveolata*. The species was included in the present study as it was the only SCTLD-susceptible coral species other than M. cavernosa and O. faveolata being maintained by coral restoration practitioners and researchers in numbers sufficient for the experimental design. The probability of survival between source colonies was also more pronounced in P. clivosa compared to M. cavernosa and O. faveolata, with lower probability of survival in colonies sourced from the two land-based facilities MML and UM compared to the other sources.

Finfish Predation – Outplanted Colonies

A time series summarizing the surveys for parrotfishes and butterflyfishes is presented in Figure 19. Counts of parrotfishes differed across the regions but did not differ across survey interval (Table 5). Highest counts of parrotfishes occurred in Region 3. Observations of the targeted finfishes were uncommon in Region 1.

Finfish predation was common on the newly-outplanted coral across the study area with the exception of Region 1. Of the 1,165 colonies outplanted, 612 (~52%) exhibited signs of tissue loss consistent with finfish predation within one-month of outplanting. By the end of the study, 788 (68%) showed evidence of finfish predation.

Figure 20 and Tables 9-11 present species-specific logistic regressions of the probability of finfish predation across survey intervals by region, reef strata, and colony source. Region 1 has been excluded as predation was so infrequently observed in this region it was necessary to exclude from the analysis to allow the logistic regression models to reach full convergence. In general, predation was highest in the initial survey intervals across all regions and decreased as the study continued (Figure 20). Finfish predation was highest on *M. cavernosa*. The probability of predation were also detected between colony sources (Tables 10-11). Colonies of *O. faveolata* and *P. clivosa*

colonies sourced from Mote Marine Laboratory's land-based facility had higher incidence of predation than others sourced from in-water nurseries.

A detailed report of the effects of finfish predation data collected in this study is summarized by McAnally et al. 2023. They evaluated the effect of coral survival as a function of early predation intensity (defined in that report as the number of days where finfish predation accounted for at least 10% of tissue loss observed in each of the regions). They noted that predation intensity was greatest on *M. cavernosa*, and that the probability of coral colony survival decreased as a function of increasing predation intensity (Figure 21). We note, however, that approximately 74% of the colonies with evidence of finfish predation exhibited tissue loss estimated to be 10% or less (Figure 22), and those that exhibited < 50% tissue damage had survival rates in the last survey interval that were comparable to colonies that were never observed with signs of finfish predation (Figure 23). Furthermore, there were losses of coral fragments from bases early in the study, and these could have potentially been the result of finfish predation and consequently the acute mortality of colonies as the direct result of finfish predation may be higher than captured by the surveys. McAnally (2023) notes that the effects of finfish predation could be mitigated by using larger coral fragments than those used in the present study. Further, early finfish predation potentially results in longer term negative effects on colony growth (see Task 4 Coral image analysis below).

Task 2: Coral genotyping.

All 75 colony samples were extracted and prepped for both the 2bRAD and ITS2 analysis pipelines. Four samples failed during quality filtering (one *M. cavernosa*, one *O. faveolata*, and two *P. clivosa*), leaving a total of 71 samples for host genetic analyses. 2bRAD sequencing generated 499 million total reads for an average of 6.65 million reads per sample. Species were evaluated separately from this point on due to differences in SCTLD-susceptibility, genomes, dominant ITS2 type profiles, and source nurseries.

Through the *M. cavernosa* clustered dendrogram and relatedness coefficients, three clonal groups were identified (Figure 24). All clonal groups contained source colonies from the same source nursery. Varying SCTLD resistance levels were observed in the monthly monitoring within one clonal group; however, within the other two clonal groups, source colonies were either all SCTLD affected or SCTLD unaffected (Figure 24A). One member of each clonal group was randomly selected and retained for subsequent analyses. After clones and technical replicates were removed, ANGSD was re- run, and the total number of 19,246 SNPs for M. cavernosa was identified. All three methods and estimators selected K = 2 as the best value of K, represented by the colors black and white (Figure 24C). The admixed population includes genets that did not have dominant membership to either of the genetic clusters (\geq 75%). PCAs generated by the program PCAngsd showed clustering of proposed genetic clusters (Figure 24B). All three of the genetic clusters for *M. cavernosa* had both SCTLD-affected and unaffected genotypes (Figure 3B).

From the *O. faveolata* clustered dendrogram and relatedness coefficients, three clonal groups were identified (Figure 25). One clonal group showed varying resistance levels (Figure 25). Within the dendrogram there is an outgroup that is indicative of a separate lineage or potentially a separate species within the Orbicella species complex (Figure 25). One member of each clonal group was randomly selected and retained for subsequent analyses. After clones and technical replicates were removed, ANGSD was re- run, and the total number of SNPs was identified as 19,463 SNPs for O. faveolata. All three methods and estimators selected K = 2 as the best value

of K, represented by the colors black and white (Figure 25C). The admixed population includes genets that did not have dominant membership to either of the genetic clusters and is denoted in gray (\geq 75%). PCAs generated by the program PCAngsd showed tight clustering of proposed genetic clusters for all three study species (Figure 25B). All three of the genetic clusters for *O*. *faveolata* had both SCTLD-affected and unaffected genotypes. The previously identified outgroup within the *O*. *faveolata* samples was identified as a separate, smaller lineage denoted in white (Figure 25B). The genets within this outgroup were similar on axis 1 but were very different on axis 2 (Figure 25B).

Lastly, two clonal groups were identified from the P. clivosa clustered dendrogram and relatedness coefficients (Figure 26). Similar to the other study species, one clonal group, all colonies sourced from the same nursery, had varying levels of resistance among samples (Figure 26). Aside from clonal groups, one outgroup consisting of two samples was found in the P. clivosa clustered dendrogram (Figure 26). These samples, one from Mote Marine Laboratory and one from the University of Miami, had a high genetic distance from the rest of the samples and were subsequently identified as *P. strigosa* colonies from *in situ* monitoring photographs (Figure 27). These P. strigosa samples were removed from the dataset for all subsequent analyses. One member of each clonal group was randomly selected and retained for subsequent analyses. After clones, technical replicates, and P. strigosa samples were removed, ANGSD was re-run and the total number of SNPs was identified as 21,800 for P. clivosa. All three methods and estimators selected K = 2 as the best value of K, represented by the colors purple and blue (Figure 26C). The admixed population included genets that did not have dominant membership to either of the genetic clusters and is denoted in yellow ($\geq 75\%$). PCAs generated by the program PCAngsd showed tight clustering of proposed genetic clusters (Figure 26B). The most dominant genetic cluster (denoted in purple) had the least number of diseased colonies compared to the less dominant genetic cluster in blue and the admixed population (Figure 26B). The less dominant, blue genetic cluster was solely made up of source colonies from FWC and there was no variation in SCTLD-susceptibility, all the genotypes were SCTLD-affected (Figure 26B).

Task 3: Algal symbiont typing.

Algal symbiont communities were classified through both 2bRAD and ITS2 methods (Figure 28). A Procrustes analysis identified a significant correlation (82.12%) between the Symbiodiniaceae genera profiles produced by both 2bRAD and ITS2 (t $\theta = 0.8212$, p < 0.0001). ITS2 type profiles differ significantly between all three study species (PERMANOVA; Pseudo-F = 25.209 2,78, p = 0.0001). A pairwise PERMANOVA identified highly significant differences in ITS2 type profiles between *M. cavernosa* and *O. faveolata* (df = 1, F = 10.631, p = 0.0001), *M. cavernosa* and *P. clivosa* (df = 1, F = 68.994, p = 0.0001), and *O. faveolata* and *P. clivosa* (df = 1, F = 42.645, p = 0.0001). Most *M. cavernosa* samples contained the *Cladocopium* genus with a few samples hosting *Durusdinium* (Figure 28). *Orbicella faveolata* samples primarily contained the genus *Durusdinium* but exhibited the most variation among the three species (Figure 28). Source colonies hosted all four genera of Symbiodiniaceae (Figure 28). Lastly, *P. clivosa* samples were almost entirely dominated by the genus *Breviolum* (Figure 28).

A PERMANOVA determined significant variation in the ITS2 type profiles within species and among source nurseries for *M. cavernosa*, *O. faveolata*, and *P. clivosa* respectively (Pseudo-F = $5.6734\ 2.18$, p < 0.0027; Pseudo-F = $2.8479\ 4.32$, p < 0.0027; Pseudo-F = $3.4827\ 3.29$, p < 0.0267). A pairwise PERMANOVA for *M. cavernosa* identified significant differences in ITS2

type profiles based on the source nurseries Florida Fish and Wildlife Conservation Commission (FWC) and the University of Miami (df = 1, F = 7.3581, p = 0.0094). Colonies sourced from FWC were the only *M. cavernosa* colonies harboring *Durusdinium* while colonies sourced from the University of Miami (UM) were the only *M. cavernosa* colonies harboring the *Cladocopium* type profile C3.C3fc.C21.C3an.C3b.C3bb.C3fd.C3s (Figure 29). A pairwise PERMANOVA for *O. faveolata* identified significant differences in ITS2 type profiles based on the source nurseries Mote Marine Laboratory (MML) and Reef Renewal (df = 1, F = 6.6844, p = 0.004). Colonies sourced from Reef Renewal (RR) were a mixture of *Breviolum* and *Cladocopium* (Figure 29). There were no significant differences in ITS2 type profiles among source nurseries for *P. clivosa*; these colonies ubiquitously harbored *Breviolum*.

PERMANOVA results indicated no significant differences in algal symbiont type profiles between SCTLD-affected and unaffected genotypes (Figure 30). Algal symbiont type profiles also did not significantly differ between survivorship groups, except for within *M. cavernosa* where genets with no mortality contained *Durusdinium* (Pseudo-F = 6.0982 1, 17, p = 0.0057). Lastly, host genetic distance did not significantly correlate with Symbiodiniaceae Bray-Curtis distance for any of the study species after a Procrustes analysis.

Task 4: Coral image analysis.

The mean net growth rates for each of the three coral species partitioned by region, reef strata, and colony source are summarized in Figures 31-33. *Montastraea cavernosa* exhibited largely negative net growth throughout the study area (Figure 31). Pairwise comparison of estimated marginal means from the analysis did not detect overall differences in net growth between regions or colony source, but contrasting differences between reef strata were identified (Table 12). In Region 1, colonies in the offshore reef strata had higher net growth than those in the inshore sites, whereas colonies in the inshore strata in Region 4, Region 5, and Region 6 had higher net growth compared with those offshore.

In contrast to *M. cavernosa*, the net growth of *O. faveolata* was generally positive across the study area, particularly at the three northernmost regions (Regions 1-3) (Figure 32). Pairwise comparison of estimated marginal means identified significant differences in net growth across reef strata, differing in regions apart from Region 1 and Region 3 (Table 13). Differences in mean growth were also identified between colonies sourced from MML and RR.

P. clivosa exhibited differences in net growth between reef strata, with higher net growth predicted for sites on the inshore strata than the offshore strata (Figure 33; Table 14). Highest net growth was predicted for colonies outplanted on the inshore reef stratum in Region 1 whereas lowest growth rates were predicted for colonies on the offshore stratum in Region 1. Significantly higher net growth was predicted for the FWC-sourced *P. clivosa* colonies relative to all the other sources of coral.

To assess the effect of finfish predation that was so common after the corals were outplanted, we added a predictor to the original suite of species-specific growth models. A binary predictor was developed for each coral colony based on if that colony exhibited evidence of finfish predation within the first month of outplanting (0 = no evidence of finfish predation; 1 = evidence of finfish predation). Those models indicated that early predation (*i.e.*, within one month of outplanting), significantly reduced the predicted net growth rate of *M. cavernosa* relative to colonies that had

not been bitten by finfish (Figure 34; Table 15). Colony source was also identified as a significant predictor. Colonies sourced from MML and UM, both land-based facilities, exhibited lower predicted net growth than those sourced from the FWC in-water nursery. No differences in net growth were identified with either *O. faveolata* (Figure 43; Table 16) or *P. clivosa* (Figure 34, Table 17).

Task 5: Coral lipid analysis.

The total lipid content per unit area (cm⁻²) exhibited a range of 1.91 to 13.57 mg cm⁻² across all examined coral fragments (Figure 35). Notably, *Orbicella faveolata* samples obtained from the University of Miami displayed the highest mean lipid content of 6.40 ± 5.09 mg cm⁻² (mean \pm standard deviation). This observation, however, was tempered by substantial variability within this small sample size (n=3).

The collective mean lipid content across different coral species and research groups spanned from 2.4 ± 0.60 to 6.40 ± 5.09 mg cm⁻² (Figure 35). It is noteworthy that this variation in means was predominantly influenced by instances with limited replication (*i.e.*, n=2 or n=3). In a broader context, the general trend indicated that the total lipid content ranged approximately from 3.5 to 4.5 mg cm⁻² across various species and source groups.

In comparison, the observed concentrations exhibited values greater than those reported by Fitt *et al.* (1993) for bleached and recovering *O. faveolata* samples in the Florida Keys, which had mean lipid concentrations of less than 2 mg cm⁻². Nonetheless, the measured concentrations were comparatively lower than the comprehensive lipid values documented by Teece *et al.* (2011) for *O. faveolata*, indicating a range of approximately 15 to 50 mg cm⁻² for naturally thriving corals within the non-thermally stressed environment of the Florida Keys. These findings are consistent with the conclusions drawn by Gantt *et al.* (2023), who noted that land nursery corals exhibited significantly lower biomass when contrasted with conspecifics from wild populations.

Considering these findings, it is plausible to infer that the accumulation of critical energy reserves such as lipids might necessitate several years when a coral is transplanted to a field location.

As the primary impetus for examining the role lipid content may have on finfish predation on newly outplanted coral, we compared the number of colonies for which we had lipid content information and that showed evidence of finfish predation through the first month post-outplant to those that did not show evidence of predation (Figure 36). We found no difference in the lipid content of those colonies that showed evidence of predation and those that did not (t = 1.299; df 564.783; p = 0.195; Independent Samples T-test).

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			D (Outplant	Outplant	Control	Control
Dogion	Sito	Nama	Reef Stratum	Site Latitudo	Site	Site Lotitudo	Site Longitudo
	1_1	SLR	Nearshore	27 1312	80 1339	27 1317	80.1340
1	1 2		Nearshore	27.1312	80.1253	27.1317	80.1255
1	1-2	SER	Offshore	26.7108	80.1255	26.7104	80.1255
1	1-3		Offshore	26.7108	80.0100	26.7104	80.0138
2	2 1	SEFL Stachorn City	Nearshore	26.0780	80.0180	26.0788	80.0181
2	2-1	N. Snowning Hub	Offshore	20.2003	80.0805	26.2043	80.0002
2	2-2	N. Spawning Hub	Nagashaga	20.1458	80.0890	20.1392	80.0902
2	2-3		Nearshore	26.9909	80.1088	25.9804	80.1088
2	2-4	S. Spawning Hub	Offshore	25.9771	80.0998	25.9811	80.9983
3	3-1	Yungs Reef	Nearshore	25.5647	80.1047	25.6596	80.0974
3	3-2	Fowey	Offshore	25.5718	80.0995	25.5660	80.0990
3	3-3	Isa's Reef	Nearshore	25.3320	80.1979	25.3407	80.1896
3	3-4	Ball Buoy North	Offshore	25.3182	80.1847	25.3262	80.1802
4	4-1	No Name Patch Reef	Nearshore	25.1097	80.3387	25.1024	80.3439
4	4-2	North Dry Rocks	Offshore	25.1230	80.2936	25.1360	80.2899
4	4-3	Pickles Patch Reef	Mid Channel	25.0084	80.4587	25.0039	80.4555
4	4-4	Pickles Reef	Offshore	24.9849	80.4160	24.9925	80.4085
5	5-1	West Turtle Shoal	Mid Channel	24.7018	81.9636	24.6994	80.9669
5	5-2	Smanatha's Ledge	Offshore	24.6587	81.0042	24.6569	81.0092
5	5-3	Washerwoman Shoal	Mid Channel	24.6640	81.0771	24.6646	81.0726
5	5-4	Sombrero Reef	Offshore	24.6254	81.1124	24.6268	81.1081
6	6-1	Inshore of Looe Key	Mid Channel	24.5782	81.4411	24.5773	81.4441
6	6-2	Looe Key	Offshore	24.5466	81.4015	24.5450	81.4103
6	6-3	Inshore American Shoal	Mid Channel	24.5487	81.5274	24.5475	81.5331
6	6-4	American Shoal	Offshore	24.5231	81.5160	24.5219	81.5219

Table 1. Location of each coral outplant site and its corresponding control site.

Table 2. Pairwise comparisons of the estimated marginal means (False Discovery Rate-adjusted) comparing the probability of SCTLD infection in *Montastraea cavernosa* between regions, reef stratum and colony sources.

	Probability of SCTLD Infection							
	Odds							
Contrasts	Ratios	SE	df	Ζ	р			
Region Averaged over the levels: Reef Strata, Colony Source								
Region1/Region 2	0.0834	0.258	inf	-0.803	0.9671			
Region 1/Region 3	0.0483	0.148	inf	-0.991	0.9210			
Region 1/Region 4	0.0951	0.299	inf	-0.748	0.9758			
Region 1/Region 5	0.2560	0.829	inf	-0.421	0.9983			
Region 1/Region 6	0.6813	2.369	inf	-0.110	1.0000			
Region 2/Region 3	0.5795	1.027	inf	-0.308	0.9944			
Region 2/Region 4	1.1409	2.189	inf	0.069	0.9547			
Region 2/Region 5	3.0706	6.346	inf	0.543	0.9992			
Region 2/Region 6	8.1734	19.841	inf	0.865	0.9625			
Region 3/Region 4	1.9689	3.664	inf	0.364	0.8771			
Region 3/Region 5	5.2991	10.675	inf	0.828	0.9974			
Region 3/Region 6	14.1053	33.604	inf	1.111	0.8771			
Region 4/Region 5	2.6913	5.768	inf	0.462	0.9974			
Region 4/Region 6	7.1640	17.847	inf	0.790	0.9692			
Region 5/Region 6	2.6619	6.943	inf	0.375	0.9990			
Reef Stratum Average	ed over the l	evels: Regi	<u>on, Colon</u>	<u>y Source</u>				
Inshore/Offshore	0.642	0.422	inf	-0.674	0.5006			
Colony Source Avera	ged over the	e levels: Re	gion, Reef	Strata				
FWC/MML	1.30	0.540	inf	0.622	0.8078			
FWC/UM	2.24	0.832	inf	2.182	0.0742			
MML/UM	1.73	0.768	inf	1.239	0.4303			

Table 3. Pairwise comparisons of the estimated marginal means (False Discovery Rate-adjusted) comparing the probability of SCTLD infection of *Orbicella faveolata* between regions, reef strum, and colony sources.

		Probability	/ of SCTLE) Infection	. <u> </u>
	Odds	-			
Contrasts	Ratios	SE	df	Ζ	р
Region Averaged over	er the levels:	Reef Strata	a, Colony S	Source	
Pagian1/Pagian 2	0 0024	0.259	inf	0 002	0.0671
Region 1/Region 3	0.0034	0.230	inf	-0.803	0.9071
Pogion 1/Pogion 1	0.0403	0.140	inf	0.331	0.921
Region 1/Region 5	0.0951	0.299	inf	-0.740	0.9756
Region 1/Region 5	0.0013	2.309	 inf	-0.421	0.9903
Region 1/Region 6	0.0813	2.309	 :nf	-0.11	1.0000
Region 2/Region 3	0.5/95	1.027	Int	-0.308	0.9996
Region 2/Region 4	1.1409	2.189	Int	0.069	1.0000
Region 2/Region 5	3.0706	6.346	inf	0.543	0.9944
Region 2/Region 6	8.1734	19.841	inf	0.865	0.9547
Region 3/Region 4	1.9689	3.664	inf	0.364	0.9992
Region 3/Region 5	5.2991	10.675	inf	0.828	0.9625
Region 3/Region 6	14.1053	33.604	inf	1.111	0.8771
Region 4/Region 5	2.6913	5.768	inf	0.462	0.9974
Region 4/Region 6	7.164	17.847	inf	0.79	0.9692
Region 5/Region 6	2.6619	6.943	inf	0.375	0.9990
Reef Stratum Averag	ed over the l	evels: Regi	on, Colon	<u>y Source</u>	
Inshore/Offshore	1.04	0.26	inf	0.161	0.8722
Colony Source Avera	ged over the	e levels: Re	g <u>ion, Reef</u>	<u>Strata</u>	
CRF/FWC	0.475	0.148	inf	-2.386	0.1190
CRF/MML	0.967	0.177	inf	-0.184	0.9997
CRF/RR	1.304	0.314	inf	1.101	0.8063
CRF/UM	0.614	0.200	inf	-1.495	0.5657
FWC/MML	2.034	0.653	inf	2.214	0.1745
FWC/RR	2.743	0.98	inf	2.826	0.0380
FWC/UM	1.292	0.542	inf	0.612	0.9732
MML/RR	1.348	0.341	inf	1.182	0.7619
MML/UM	0.635	0.213	inf	-1.355	0.6564
RR/UM	0.471	0.174	inf	-2.035	0.2492

Table 4. Pairwise comparisons of the estimated marginal means (False Discovery Rate-adjusted) comparing the probability of SCTLD infection of *Pseudodiploria clivosa* between regions, reef stratum, and colony sources.

		Probability	of SCTL	D Infection	<u> </u>
	Odds	-			
Contrasts	Ratios	SE	df	Ζ	р
Region Averaged o	over the lev	vels: Reef Sti	rata, Colo	ony Source	
Region1/Region 2	0.0547	0.0777	inf	-2.046	0.3162
Region 1/Region 3	0.1834	0.2779	inf	-1.119	0.8737
Region 1/Region 4	0.1250	0.1844	inf	-1.409	0.7216
Region 1/Region 5	0.2915	0.4482	inf	-0.802	0.9673
Region 1/Region 6	0.4841	0.8500	inf	-0.439	0.9980
Region 2/Region 3	3.3535	3.6654	inf	1.107	0.8787
Region 2/Region 4	2.2854	2.3664	inf	0.798	0.9679
Region 2/Region 5	5.3314	6.0024	inf	1.487	0.6730
Region 2/Region 6	8.8547	11.3930	inf	1.695	0.5350
Region 3/Region 4	0.6815	0.7968	inf	-0.328	0.9995
Region 3/Region 5	1.5898	1.9845	inf	0.371	0.9991
Region 3/Region 6	2.6404	3.6752	inf	0.698	0.9823
Region 4/Region 5	2.3328	2.7959	inf	0.707	0.9812
Region 4/Region 6	3.8744	5.2250	inf	1.004	0.9167
Region 5/Region 6	1.6608	2.3509	inf	0.358	0.9992
Reef Stratum Avera	aged over	the levels: R	egion, Co	olony Source	<u>e</u>
Inshore/Offshore	0.561	0.222	inf	-1.458	0.1449
Colony Source Ave	eraged ove	r the levels:	Region,	<u>Reef Strata</u>	
FWC/MML	2.994	1.652	inf	1.988	0.1924
FWC/RR	1.899	0.687	inf	1.773	0.2865
FWC/UM	2.31	0.908	inf	2.13	0.1437
MML/RR	0.634	0.387	inf	-0.746	0.8782
MML/UM	0.771	0.484	inf	-0.414	0.9761
MML/UM	1.216	0.571	inf	0.417	0.9755

Constrast	Reef Stratum	Region	Estimate	SE	df	Ζ	Р
Control Sites - Outplant Sites	Inshore	1	-0.07998	0.68	Inf	-0.118	0.9821
Control Sites - Outplant Sites	Offshore	1	0.04697	0.525	Inf	0.089	0.9821
Control Sites - Outplant Sites	Inshore	2	-0.91653	1.128	Inf	-0.813	0.6244
Control Sites - Outplant Sites	Offshore	2	0.90766	0.435	Inf	2.088	0.1103
Control Sites - Outplant Sites	Inshore	3	0.57999	1.034	Inf	0.561	0.7667
Control Sites - Outplant Sites	Offshore	3	1.31173	1.169	Inf	1.122	0.5132
Control Sites - Outplant Sites	Inshore	4	0.00574	0.255	Inf	0.022	0.9821
Control Sites - Outplant Sites	Offshore	4	-0.84724	0.449	Inf	-1.887	0.1419
Control Sites - Outplant Sites	Inshore	5	1.58202	0.47	Inf	3.363	0.0092
Control Sites - Outplant Sites	Offshore	5	-1.15355	1.111	Inf	-1.038	0.5132
Control Sites - Outplant Sites	Inshore	6	0.52559	0.226	Inf	2.324	0.0804
Control Sites - Outplant Sites	Offshore	6	1.11995	0.358	Inf	3.131	0.0104

Table 5. Estimated Marginal Means (False Discovery Rate adjusted) comparing SCTLD prevalence between Control Sites and Outplant Sites across Regions and Reef Strata.

	Parrotfish Count				
Predictors	Estimates	CI	р		
(Intercept)	0.26	-1.33 – 1.85	0.75		
Survey Period	0.03	-0.05-0.10	0.476		
<u>Region</u>					
Region 1					
Region 2	2.62	0.74 – 4.50	0.006		
Region 3	14.25	12.38 - 16.12	<0.001		
Region 4	12.43	10.57 – 14.28	<0.001		
Region 5	10.71	8.87 – 12.55	<0.001		
Region 6	6.41	4.68 - 8.15	<0.001		
Observations	638				
R ²	0.361				

Table 5. Odds ratios, Confidence Intervals, p values, and random effects statistics from a fixed General Linear Model assessing parrotfish counts across survey periods and regions recorded during per-outplant site surveys.

Table 6. Pairwise comparisons of the estimated marginal means (False Discovery Rate-adjusted) comparing the probability of survival of *Montastraea cavernosa* between regions, reef strata, and colony sources.

		Proba	oility of S	urvival	
	Odds				
Contrasts	Ratios	SE	df	Ζ	р
Region Averaged ov	er the levels:	Reef Strata	, Colony	<u>Source</u>	
Region1/Region 2	5.01	1.89	inf	4.269	0.0003
Region 1/Region 3	2.78	1.10	inf	2.596	0.0981
Region 1/Region 4	1.72	7.08	inf	1.318	0.7752
Region 1/Region 5	0.03	0.03	inf	-3.860	0.0016
Region 1/Region 6	3.38	1.35	inf	3.042	0.0284
Region 2/Region 3	0.56	0.02	inf	-1.839	0.4403
Region 2/Region 4	0.34	0.12	inf	-3.130	0.0216
Region 2/Region 5	0.01	0.01	inf	-5.791	<0.0001
Region 2/Region 6	0.67	0.22	inf	-1.208	0.8332
Region 3/Region 4	0.62	0.22	inf	-1.337	0.7646
Region 3/Region 5	0.01	0.01	inf	-5.085	<0.0001
Region 3/Region 6	1.21	0.42	inf	0.558	0.9936
Region 4/Region 5	0.02	0.02	inf	-4.508	0.0001
Region 4/Region 6	1.96	0.72	inf	1.841	0.4393
Region 5/Region 6	114.00	102.00	inf	5.286	<0.0001
Reef Stratum Averag	ed over the l	evels: Regio	on, Color	y Source	
Inshore/Offshore	4.37	0.635	inf	10.172	<0.0001
Colony Source Avera	aged over the	e levels: Reg	gion, Ree	f Strata	
FWC/MML	1.55	0.251	inf	2.732	0.0173
FWC/UM	2.66	0.351	inf	7.412	<0.0001
MML/UM	1.71	0.239	inf	3.848	0.0004

Table 7. Pairwise comparisons of the estimated marginal means (False Discovery Rate-adjusted) comparing the probability of survival of *Orbicella faveolata* between regions, reef strata, and colony sources.

	Probability of Survival								
	Odds		-						
Contrasts	Ratios	SE	df	Ζ	р				
Region Averaged over the levels: Reef Strata, Colony Source									
Region1/Region 2	1.550	0.2890	inf	2.350	0.1744				
Region 1/Region 3	0.574	0.1628	inf	-1.956	0.3682				
Region 1/Region 4	0.699	0.1511	inf	-1.657	0.5602				
Region 1/Region 5	0.740	0.1625	inf	-1.373	0.7431				
Region 1/Region 6	1.582	0.2975	inf	2.439	0.1428				
Region 2/Region 3	0.371	0.0989	inf	-3.721	0.0027				
Region 2/Region 4	0.451	0.0875	inf	-4.107	0.0006				
Region 2/Region 5	0.477	0.0942	inf	-3.750	0.0024				
Region 2/Region 6	1.021	0.1645	inf	0.127	1.0000				
Region 3/Region 4	1.217	0.3510	inf	0.680	0.9842				
Region 3/Region 5	1.288	0.3747	inf	0.868	0.9540				
Region 3/Region 6	2.754	0.7377	inf	3.783	0.0021				
Region 4/Region 5	1.058	0.2393	inf	0.250	0.9999				
Region 4/Region 6	2.264	0.4424	inf	4.180	0.0004				
Region 5/Region 6	2.139	0.4250	inf	3.826	0.0018				
Reef Stratum Averaged	l over the	levels: Regio	on, Colon	<u>y Source</u>					
Inshore/Offshore	2.78	0.368	inf	7.734	<0.0001				
Colony Source Average	ed over th	<u>e levels: Reg</u>	<u>(ion, Reef</u>	<u>Strata</u>					
CRF/FWC	0.820	0.1897	inf	-0.857	0.9126				
CRF/MML	0.627	0.0686	inf	-4.270	0.0002				
CRF/RR	0.545	0.0811	inf	-4.079	0.0004				
CRF/UM	1.023	0.2249	inf	0.103	1.0000				
FWC/MML	0.764	0.1830	inf	-1.122	0.7948				
FWC/RR	0.665	0.1724	inf	-1.574	0.5143				
FWC/UM	1.247	0.3820	inf	0.721	0.9518				
MML/RR	0.870	0.1399	inf	-0.867	0.9088				
MML/UM	1.631	0.3726	inf	2.143	0.2018				
RR/UM	1.876	0.4679	inf	2.521	0.0859				

Table 8. Pairwise comparisons of the estimated marginal means (False Discovery Rate-adjusted) comparing the probability of survival of *Pseudodiploria clivosa* between regions, reef strata, and colony sources.

	Probability of Survival								
	Odds		-						
Contrasts	Ratios	SE	df	Ζ	р				
Region Averaged over the levels: Reef Strata, Colony Source									
Region1/Region 2	0.887	0.249	inf	-0.464	0.9973				
Region 1/Region 3	0.638	0.191	inf	-1.502	0.6628				
Region 1/Region 4	0.921	0.284	inf	-0.226	0.9998				
Region 1/Region 5	0.953	0.332	inf	-0.140	1.0000				
Region 1/Region 6	2.004	0.578	inf	2.409	0.1529				
Region 2/Region 3	0.728	0.187	inf	-1.235	0.8197				
Region 2/Region 4	1.051	0.282	inf	0.185	1.0000				
Region 2/Region 5	1.087	0.340	inf	0.265	0.9998				
Region 2/Region 6	2.286	0.560	inf	3.378	0.0095				
Region 3/Region 4	1.444	0.410	inf	1.295	0.7877				
Region 3/Region 5	1.493	0.487	inf	1.230	0.8225				
Region 3/Region 6	3.142	0.821	inf	4.383	0.0002				
Region 4/Region 5	1.034	0.346	inf	0.100	1.0000				
Region 4/Region 6	2.175	0.592	inf	2.856	0.0491				
Region 5/Region 6	2.104	0.664	inf	2.357	0.1715				
Reef Stratum Averag	ged over the	levels: Regio	on, Colon	<u>y Source</u>					
Inshore/Offshore	2.42	0.385	inf	5.567	<0.0001				
Colony Source Avera	aged over the	e levels: Reg	ion, Reel	<u>Strata</u>					
FWC/MML	10.542	2.1041	inf	11.800	<0.0001				
FWC/RR	2.643	0.5618	inf	4.573	<0.0001				
FWC/UM	3.201	0.7162	inf	5.199	<0.0001				
MML/RR	0.251	0.0499	inf	-6.957	<0.0001				
MML/UM	0.304	0.0637	inf	-5.682	<0.0001				
RR/UM	1.211	0.2675	inf	0.867	0.8220				

Table 9. Pairwise comparisons of the estimated marginal means (False Discovery Rate-adjusted) comparing the probability of finfish predation on *Montastraea cavernosa* between regions, reef strata, and colony sources.

	Probability of Predation								
	Odds								
Contrasts	Ratios	SE	df	Ζ	р				
Region Averaged over the levels: Reef Strata, Colony Source									
Region 2/Region 3	1650.00	7697.10	inf	1.051	0.5444				
Region 2/Region 4	0.0001	0.0000	inf	-4.053	0.0005				
Region 2/Region 5	0.0002	0.0010	inf	-3.231	0.0108				
Region 2/Region 6	0.0008	0.0002	inf	-2.701	0.0538				
Region 3/Region 4	0.0000	0.0000	inf	-4.227	0.0002				
Region 3/Region 5	0.0000	0.0000	inf	-3.770	0.0015				
Region 3/Region 6	0.0000	0.0000	inf	-3.452	0.0050				
Region 4/Region 5	4.6800	5.6760	inf	1.273	0.7077				
Region 4/Region 6	15.2000	20.8510	inf	1.986	0.2727				
Region 5/Region 6	3.25	5.21	inf	0.735	0.9485				
Reef Stratum Average	<u>d over the l</u>	evels: Regio	o <mark>n, Colo</mark> ny	<u>y Source</u>					
Inshore/Offshore	0.001	0.00197	inf	-3.62	0.0003				
Colony Source Averag	ged over the	e levels: Reg	<u>ion, Reef</u>	<u>Strata</u>					
FWC/MML	4.694	6.903	inf	1.051	0.5444				
FWC/UM	0.881	0.854	inf	-0.131	0.9906				
MML/UM	0.188	0.263	inf	-1.192	0.4576				

Table 10. Pairwise comparisons of the estimated marginal means (False Discovery Rate-adjusted) comparing the probability of finfish predation on *Orbicella faveolata* between regions, reef strata, and colony sources.

	Probability of Predation								
	Odds								
Contrasts	Ratios	SE	df	Ζ	р				
Region Averaged over the levels: Reef Strata, Colony Source									
Region 2/Region 3	2.000	3.0000	inf	-3.721	0.9140				
Region 2/Region 4	0.000	0.0000	inf	-4.107	0.0008				
Region 2/Region 5	0.000	0.0000	inf	-3.750	0.0002				
Region 2/Region 6	4.91E+35	3.96E+39	inf	0.127	1.0000				
Region 3/Region 4	0.000	0.0000	inf	0.680	0.0011				
Region 3/Region 5	0.000	0.0000	inf	0.868	0.0004				
Region 3/Region 6	1.98E+35	1.60E+39	inf	3.783	1.0000				
Region 4/Region 5	1.000	0.0000	inf	0.250	0.9934				
Region 4/Region 6	7.83E+36	6.32E+40	inf	4.180	1.0000				
Region 5/Region 6	9.76E+36	7.88E+40	inf	3.826	1.0000				
Reef Stratum Average	ed over the l	evels: Regi	on, Colony	<u>/ Source</u>					
Inshore/Offshore	0.416	0.174	inf	-0.3449	0.0360				
Colony Source Avera	ged over the	e levels: Reg	gion, Reef	<u>Strata</u>					
CRF/FWC	1.000	1.0000	inf	-0.349	0.9968				
CRF/MML	11.000	0.0000	inf	4.685	<0.0001				
CRF/RR	1.000	0.0000	inf	0.313	0.9979				
CRF/UM	2.95E+35	2.37E+35	inf	0.010	1.0000				
FWC/MML	14.000	13.0000	inf	2.905	0.0302				
FWC/RR	20.000	1.0000	inf	0.477	0.9895				
FWC/UM	3.91E+35	3.16E+39	inf	0.010	1.0000				
MML/RR	0.000	0.0000	inf	-3.902	0.0009				
MML/UM	2.70E+34	2.18E+38	inf	0.010	1.0000				
RR/UM	2.59E+35	2.09E+39	inf	0.010	1.0000				
Table 11. Pairwise comparisons of the estimated marginal means (False Discovery Rate-adjusted) comparing the probability of finfish predation on *Pseudodiploria clivosa* between regions, reef strata, and colony sources.

	Probability of Predation						
	Odds						
Contrasts	Ratios	SE	df	Ζ	р		
Region Averaged over the levels: Reef Strata, Colony Source							
Region 2/Region 3	68.240	180.000	inf	1.598	0.4987		
Region 2/Region 4	104.800	377.000	inf	1.293	0.6954		
Region 2/Region 5	0.020	0.030	inf	-2.874	0.0330		
Region 2/Region 6	1555.120	614000.00	inf	1.861	0.3386		
Region 3/Region 4	1.540	6.310	inf	0.105	1.0000		
Region 3/Region 5	0.000	0.000	inf	-3.339	0.0075		
Region 3/Region 6	22.790	10200.00	inf	0.700	0.9565		
Region 4/Region 5	0.000	0.000	inf	-2.489	0.0930		
Region 4/Region 6	14.840	75.500	inf	0.530	0.9984		
Region 5/Region 6	81270.700	3.11E+05	inf	2.950	0.0263		
Reef Stratum Averaged over the levels: Region, Colony Source							
Inshore/Offshore	0.277	0.253	inf	-1.404	0.1603		
Colony Source Averaged over the levels: Region, Reef Strata							
FWC/MML	9539.641	2.8900	inf	3.024	0.0133		
FWC/RR	7.257	1.8100	inf	0.795	0.8569		
FWC/UM	80.150	1.8500	inf	1.897	0.2293		
MML/RR	0.001	0.0030	inf	-2.054	0.1684		
MML/UM	0.008	0.0280	inf	-1.429	0.4811		
RR/UM	11.045	29.4000	inf	0.901	0.8043		

·		N	let Growt	h	·
	Odds				
Contrasts	Ratios	SE	df	t ratio	р
Region Averaged over the l	levels: Reef	Strata, Col	lony Sourc	<u>e</u>	
Region1/Region 2	14.7070	4.29	170	-0.803	0.9671
Region 1/Region 3	18.3650	4.26	170	-0.991	0.9210
Region 1/Region 4	15.3480	4.06	170	-0.748	0.9758
Region 1/Region 5	19.8370	3.81	170	-0.421	0.9983
Region 1/Region 6	12.8390	4.03	170	-0.110	1.0000
Region 2/Region 3	3.6580	4.56	170	-0.308	0.9944
Region 2/Region 4	0.6410	4.38	170	0.069	0.9547
Region 2/Region 5	5.1300	4.15	170	0.543	0.9992
Region 2/Region 6	-1.8680	4.37	170	0.865	0.9625
Region 3/Region 4	-3.0170	4.34	170	0.364	0.8771
Region 3/Region 5	1.4720	4.1	170	0.828	0.9974
Region 3/Region 6	-5.5260	4.33	170	1.111	0.8771
Region 4/Region 5	4.4890	3.9	170	0.462	0.9974
Region 4/Region 6	-2.5090	4.13	170	0.790	0.9692
Region 5/Region 6	-6.9980	3.88	170	0.375	0.9990
Reef Stratum Averaged over	er the levels	: Region, C	<u>Colony Sou</u>	irce	
Inshore/Offshore	5.53	2.41	170	2.296	0.0229
<u>Stratum/Region</u>					
Region 1 Inshore/Offshore	-12.58	5.61	170	-2.187	0.0301
Region 2 Inshore/Offshore	-2.54	6.49	170	-0.392	0.6955
Region 3 Inshore/Offshore	-3.3	6.39	170	-0.517	0.6061
Region 4 Inshore/Offshore	15.96	5.87	170	2.719	0.0072
Region 5 Inshore/Offshore	14.2	5.15	170	2.755	0.0065
Region 6 Inshore/Offshore	21.13	5.8	170	3.642	0.0004
Colony Source Averaged over the levels: Region, Reef Strata					
FWC/MML	7.52	3.240	170	2.341	0.0555
FWC/UM	5.22	2.7	170	1.931	0.1332
MML/UM	-2.30	3.14	107	-0.733	0.7444

Table 12. Pairwise comparisons of the estimated marginal means (False Discovery Rate-adjusted) comparing net growth of *Montastraea cavernosa* between regions, reef strata, and colony sources.

Table 13. Pairwise comparisons of the estimated marginal means (False Discovery Rate-adjusted) comparing net growth of *Orbicella faveolata* between regions, reef strata, and colony sources.

	Net Growth					
	Odds					
Contrasts	Ratios	SE	df	t ratio	р	
Region Averaged over the levels: Reef Strata, Colony Source						
Region1/Region 2	-4.712	2.6400	483	-1.788	0.4745	
Region 1/Region 3	-3.156	2.6200	483	-1.204	0.8348	
Region 1/Region 4	-0.133	2.6100	483	-0.051	1.0000	
Region 1/Region 5	-0.336	2.5400	483	-0.132	1.0000	
Region 1/Region 6	-2.403	2.6100	483	-0.921	0.9412	
Region 2/Region 3	1.559	2.6600	483	0.585	0.9920	
Region 2/Region 4	4.582	2.6500	483	1.727	0.5146	
Region 2/Region 5	4.379	2.5900	483	1.694	0.5364	
Region 2/Region 6	2.312	2.6500	483	0.871	0.9532	
Region 3/Region 4	3.023	2.6400	483	1.147	0.8615	
Region 3/Region 5	2.820	2.5700	483	1.099	0.8815	
Region 3/Region 6	0.753	2.6400	483	0.286	0.9997	
Region 4/Region 5	-0.203	2.5600	483	-0.079	1.0000	
Region 4/Region 6	-2.270	2.6300	483	-0.864	0.9547	
Region 5/Region 6	-2.067	2.5600	400	-0.808	0.9660	
Reef Stratum Averaged ove	r the leve	ls: Region, (483 <u>Colony So</u>	urce		
Inshore/Offshore	3.39	1.51	483	2.254	0.0246	
Stratum/Region						
Region 1 Inshore/Offshore	-4.44	3.67	483	-1.21	0.2269	
Region 2 Inshore/Offshore	-8.99	3.78	483	-2.378	0.0178	
Region 3 Inshore/Offshore	-3.32	3.74	483	-0.887	0.3757	
Region 4 Inshore/Offshore	10.77	3.72	483	2.898	0.0039	
Region 5 Inshore/Offshore	13.87	3.51	483	3.947	0.0001	
Region 6 Inshore/Offshore	12.47	3.72	483	3.357	0.0009	
Colony Source Averaged ov	ver the lev	els: Region	, Reef Stra	<u>ta</u>		
CRF/FWC	-6.200	3.8300	483	-1.622	0.4842	
CRF/MML	3.240	1.7700	483	1.829	0.3580	
CRF/RR	-3.820	2.1300	483	-1.788	0.3815	
CRF/UM	0.250	3.6000	483	0.070	1.0000	
FWC/MML	9.440	3.8900	483	2.427	0.1100	
FWC/RR	2.390	4.0800	483	0.586	0.9772	
FWC/UM	6.450	5.0100	483	1.289	0.6983	
MML/RR	-7.050	2.2500	483	-3.130	0.0159	
MML/UM	-2.990	3.6800	483	-0.812	0.9268	
RR/UM	4.070	3.8600	483	1.053	0.8302	

	Probability of Survival				
	Odds				
Contrasts	Ratios	SE	df	t ratio	р
Region Averaged over the levels: Reef Strata, Colony Source					
Region1/Region 2	21.779	5.5500	483	3.921	0.0017
Region 1/Region 3	7.712	5.4200	483	1.422	0.7136
Region 1/Region 4	9.486	5.5600	483	1.706	0.5298
Region 1/Region 5	9.771	5.5200	483	1.770	0.4878
Region 1/Region 6	10.204	5.7400	483	1.778	0.4827
Region 2/Region 3	-14.066	4.9600	483	-2.834	0.0568
Region 2/Region 4	-12.293	5.1200	483	-2.403	0.1609
Region 2/Region 5	-12.008	5.0500	483	-2.376	0.1702
Region 2/Region 6	-11.575	5.3000	483	-2.183	0.2511
Region 3/Region 4	1.774	4.9600	483	0.358	0.9992
Region 3/Region 5	2.059	4.8900	483	0.421	0.9983
Region 3/Region 6	2.491	5.1500	483	0.484	0.9967
Region 4/Region 5	0.285	5.0500	483	0.056	1.0000
Region 4/Region 6	0.718	5.3100	483	0.135	1.0000
Region 5/Region 6	0.433	5.2300	483	0.083	1.0000
Reef Stratum Averaged over	er the levels	s: Region, C	<u>Colony Sou</u>	irce	
Inshore/Offshore	23.3	3.06	177	7.623	<0.0001
Stratum/Region					
Region 1 Inshore/Offshore	64.4	8.45	177	7.623	<0.0001
Region 2 Inshore/Offshore	3.5	7.23	177	0.483	0.6294
Region 3 Inshore/Offshore	13.7	6.8	177	2.014	0.0455
Region 4 Inshore/Offshore	15.7	7.3	177	2.154	0.0326
Region 5 Inshore/Offshore	13.9	7.02	177	1.974	0.0499
Region 6 Inshore/Offshore	28.8	7.82	177	3.68	0.0003
<u>Colony Source Averaged over the levels: Region, Reef Strata</u>					
FWC/MML	21.710	4.9400	177	4.397	0.0001
FWC/RR	23.650	3.8400	177	6.154	<0.0001
FWC/UM	25.440	3.9300	177	6.477	<0.0001
MML/RR	1.950	5.4200	177	0.359	0.9841
MML/UM	3.730	5.4900	177	0.679	0.9048
RR/UM	1.790	4.5000	177	0.397	0.9788

Table 14. Pairwise comparisons of the estimated marginal means (False Discovery Rate-adjusted) comparing net growth of *Pseudodiploria clivosa* between regions, reef strata, and colony sources.

	Net Growth		
Predictors	Estimates	CI	р
(Intercept)	9.71	1.44-17.97	0.021
Region			
Region 1			
Region 2	-5.74	-17.12-5.64	0.323
Region 3	-7.98	-20.10-4.14	0.197
Region 4	4.19	-7.77-16.15	0.493
Region 5	-3.56	-14.26-7.14	0.514
Region 6	5.57	-5.20-16.35	0.311
Reef Stratum			
Inshore			
Offshore	11.83	0.98-22.69	0.033
<u>ColonySource</u>			
FWC			
MML	-6.67	-12.980.37	0.038
UM	-5.25	-10.47 0.02	0.049
Early Finfish Predation			
No			
Yes	-7.26	-13.511.02	0.023
Region/Reef Stratum			
Region 1/Inshore			
Region 2/Offshore	-6.61	-23.43-10.21	0.441
Region 3/Offshore	-7.47	-23.96-9.03	0.375
Region 4/Offshore	-26.83	-42.5911.06	0.001
Region 5/Offshore	-24.02	-38.889.16	0.002
Region 6/Offshore	-30.93	-46.6815.19	<0.001
Observations	185		
R^2/R^2 adjusted	0.302/0.24	40	

Table 15. Linear regression table of early predation on *Montastraea cavernosa*.

		Net Growth	
Predictors	Estimates	CI	р
(Intercept)	3.44	-2.22-9.11	0.233
<u>Region</u>			
Region 1			
Region 2	2.3	-5.90-10.51	0.582
Region 3	3.59	-4.54-11.72	0.386
Region 4	7.68	0.51-14.85	0.036
Region 5	9.44	2.22-16.67	0.01
Region 6	10.83	3.57-18.09	0.003
Reef Stratum			
Inshore			
Offshore	4.43	-2.76-11.62	0.227
<u>Colony Source</u>			
CRF			
FWC	6.19	-1.32-13.69	0.106
MML	-3.27	-6.86-0.32	0.074
RR	3.82	-0.36-8.00	0.073
UM	-0.27	-7.34-6.80	0.941
Early Finfish Predation			
No			
Yes	0.16	-3.75-4.07	0.935
Region/Reef Stratum			
Region 1/Inshore			
Region 2/Offshore	4.54	-5.79-14.87	0.389
Region 3/Offshore	-1.13	-11.41-9.15	0.83
Region 4/Offshore	-15.25	-25.514.98	0.004
Region 5/Offshore	-18.36	-28.398.32	<0.001
Region 6/Offshore	-16.93	-27.176.69	0.001
Observations	500		
R^2 / R^2 adjusted	0.109/0.0	77	

Table 16. Linear regression table of early predation on Orbicella faveolata.

		Net Growth	
Predictors	Estimates	Cl	р
(Intercept)	65.15	55.90-74.41	<0.001
Region			
Region 1			
Region 2	-51 81	-66 1837 44	< 0 001
Region 2	-01.01	-46 72 - 18 48	<0.001
Region 3	-32.0	47.40 10.40	<0.001
Region 4	-33.48	-47.49 19.48	<0.001
Region 5	-34.65	-48.9520.35	< 0.001
Region 6	-27.85	-41.5214.18	<0.001
Reef Stratum			
<u>Inshore</u>			
Offshore	-64.43	-81.0047.86	<0.001
<u>Colony Source</u>			
<u>FWC</u>			
MML	-21.59	-31.4211.75	<0.001
RR	-23.61	-31.1716.05	<0.001
UM	-25.39	-33.1217.66	<0.001
Early Einfich Drodation			
<u>No</u>	0 56	-8 51 - 7 30	0 90
Region /Reaf Stratum	-0.50	-0.51-7.55	0.09
Region 1/Inchara			
Region 2/Offeboro	60.09	20 15 - 82 81	<0.001
Region 3/Offshore	50 91	29 50 - 72 12	~0.001
Region 4/Offebare	10.70	20.00 - 72.12	<0.001
Region 4/Onshore	48.73	20.94 - 70.53	<0.001
Region 5/ UTISNOR	50.59 25 56	29.03 - 72.15 12.08 - 59.12	<0.001
Observations	102	12.00-00.10	0.002
	192	5.6	
K ⁺ / R ⁺ adjusted	0.500/0.4	55	

Table 17. Linear regression table of early predation on *Pseudodiploria clivosa*.



Figure 1. Map of south Florida delineating the six survey regions. Acronyms indicate the research group responsible for conducting the field activities within each region. FAU = Florida Atlantic University, NSU = Nova Southeastern University, UM/BISC = University of Miami and Biscayne National Park, FWC = Florida Fish and Wildlife Conservation Commission, MOTE = Mote Marine Laboratory. The colored circles withing each region indicate outplant sites.



Figure 2. A conceptual diagram of a coral outplant site displaying the randomized placement of coral species and genotypes in a 6 x 8 grid pattern. "Genotype Known" colonies were propagated from source colonies with a known history (source location, length of time in nursery, etc.). "Genotype Unknown" are colonies that had not been tracked from source colony with a known history and had not been subject to genetic sequencing.



Figure 3. Examples of the standardized cement base designed by Reef Cells, LLC. Bases were fabricated to fit the various coral pucks used by each of the project's research partners that contributed coral for the experimental coral outplanting effort. The bases were designed to standardize the outplanted coral across the study area. A) An example base into which five wells have been routed to accept the coral fragments that had been mounted on cement pucks. Note the attached tag. Each coral colony was identified by a uniquely numbered tag. The visible raised lettering identified the source of the coral mounted to the base. B) A base photographed shortly after outplanting to which five fragments of *Montastraea cavernosa* had been attached.



Figure 4. Disease status of coral colonies recorded during the pre-outplant DRM-style surveys conducted at each of the four outplant sites in each region.



Figure 5. Coral colony status (alive, dead, or not surveyed) at each survey period.



Figure 6. Coral colony status (alive, dead, or not surveyed) at each sampling region, reef stratum (*i.e.*, the inshore and offshore sites), and survey period.



Figure 7. Proportion of colonies observed Live-no disease, dead, exhibiting active SCTLD infection, other diseases, and unidentified disease/condition.



Figure 8. Species-specific status of colonies at the last survey period (April 2023) that exhibited signs of SCTLD infection during at least one survey period.



Figure 9. Results of the binomial logistic regression estimating the probability of outplanted *Montastraea cavernosa* being observed with evidence of SCTLD infection partitioned by survey region, reef stratum, and coral colony source. Coral colonies source codes are: MML = Mote Marine Laboratory, FWC = Florida Fish and Wildlife Conservation Commission, UM = University of Miami.



Figure 10. Results of the binomial logistic regression estimating the probability of outplanted *Orbicella faveolata* being observed with evidence of SCTLD infection partitioned by survey region, reef stratum, and coral colony source. Coral colonies source codes are: UM = University of Miami, CRF = Coral Restoration Foundation, FWC = Florida Fish and Wildlife Conservation Commission, MML = Mote Marine Laboratory, and RR= Reef Renewal, L.L.C.



Figure 10. Results of the binomial logistic regression estimating the probability of outplanted *Pseudodiploria clivosa* being observed with evidence of SCTLD infection partitioned by survey region, reef stratum, and coral colony source. Coral colonies source codes are: FWC = Florida Fish and Wildlife Conservation Commission UM = University of Miami, RR= Reef Renewal, L.L.C., and MML = Mote Marine Laboratory.



Figure 12. Frequency histogram summarizing the proportion of heathy, SCTLD-infected, and colonies with other diseases observed during roving diver surveys of the coral outplant sites by survey period and across regions and reef strata.



Figure 13. Frequency histogram summarizing the proportion of heathy, SCTLD-infected, and colonies with other diseases observed during roving diver surveys of the control sites located \sim 500m from coral outplant sites.



Figure 14. Mean $(1 \pm SE)$ percentage of the natural coral colony exhibiting SCTLD infection observed during roving diver surveys of the outplant sites and associated control sites. March 2021 – April 2023.



Figure 15. DRM-style surveys of the natural coral communities in the outplant area conducted after the conclusion of monthly outplant surveys, April/May 2023.



Figure 16. Results of the binomial logistic regression estimating the probability of survival of outplanted *Montastraea cavernosa* partitioned by survey region, reef stratum, and coral colony source. Coral colonies source codes are: MML = Mote Marine Laboratory, FWC = Florida Fish and Wildlife Conservation Commission, UM = University of Miami.



Figure 17. Results of the binomial logistic regression estimating the probability of survival of outplanted *Orbicella faveolata* partitioned by survey region, reef stratum, and coral colony source. Coral colonies source codes are: UM = University of Miami, CRF = Coral Restoration Foundation, FWC = Florida Fish and Wildlife Conservation Commission, MML = Mote Marine Laboratory, and RR= Reef Renewal, L.L.C.



Figure 18. Results of the binomial logistic regression estimating the probability of survival of outplanted *Pseudodiploria clivosa* partitioned by survey region, reef stratum, and coral colony source. Coral colonies source codes are: FWC = Florida Fish and Wildlife Conservation Commission UM = University of Miami, RR= Reef Renewal, L.L.C., and MML = Mote Marine Laboratory.



Figure 19. Mean (± 1 SE) counts of Parrotfishes and Butterflyfishes observed during the coral outplant surveys by region and survey period.

Figure 20. Results of the binomial logistic regression estimating the probability of predation of outplanted A) *Montastraea caverosa*, B) *Orbicella faveolata*, and C) *Pseudodiploria clivosa* partitioned by survey region, reef stratum, and coral colony source. UM = University of Miami, CRF = Coral Restoration Foundation, FWC = Florida Fish and Wildlife Conservation Commission, MML = Mote Marine Laboratory, and RR= Reef Renewal, L.L.C.





Figure 21. After McAnally *et al.* 2023. Binomial logistic regression comparing the probability of survival as a function of the percentage of tissue removed in the respective early period of the given coral base's outplanting region. The influence of early predation intensity was statistically significant for survivorship, both by the end of the early predation period (left panel), and by the end of the study period (right panel) (p values = 7.86 e^{-8} , and 5.95 e^{-4} , respectively).



Figure 22. Frequency histogram summarizing the number of coral colonies by their maximum observed tissue loss due to finfish predation.



Figure 23. The proportion of colonies observed live or dead at the last survey of coral outplants in April 2023. Shown are colonies that did not exhibit signs of finfish predation during the first month post-outplant, those that did show signs of predation with < 50 tissue loss, and those that showed evidence of finfish predation and ≥ 50 % tissue loss.



Figure 24. (A) Dendrogram identifying clusters of *Montastraea cavernosa* samples based on Identity-by-State matrix calculations. Source nursery is denoted by shape and disease status is denoted by color. The dashed red line indicates the minimum genetic distance for clonal groups and was determined by the lowest level at which the technical replicate groups were present and relatedness coefficients. Sample numbers are indicated by the numbers at the end of each branch. (B) Principal component analysis based on PCAngsd population genetic clusters. Unique genets are represented by points. The percentage of variation explained by each axis is indicated. (C) Population structure models. Each bar indicates an individual genotype and the relative proportion of the two colors represent the relative likelihood of membership to each of the two proposed genetic clusters.



Figure 25. (A) Dendrogram identifying clusters of *Orbicella faveolata* samples based on Identity-by-State matrix calculations. Source nursery is denoted by shape and disease status is denoted by color. The dashed red line indicates the minimum genetic distance for clonal groups and was determined by the lowest level at which the technical replicate groups were present and relatedness coefficients. Sample numbers are indicated by the numbers at the end of each branch. (B) Principal component analysis based on PCAngsd population genetic clusters. Unique genets are represented by points. The percentage of variation explained by each axis is indicated. (C) Population structure models. Each bar indicates an individual genotype and the relative proportion of the two colors represent the relative likelihood of membership to each of the two proposed genetic clusters.



Figure 26. (A) Dendrogram identifying clusters of *Pseudodiploria clivosa* samples based on Identityby-State matrix calculations. Source nursery is denoted by shape and disease status is denoted by color. The dashed red line indicates the minimum genetic distance for clonal groups and was determined by the lowest level at which the technical replicate groups were present and relatedness coefficients. Sample numbers are indicated by the numbers at the end of each branch. (B) Principal component analysis based on PCAngsd population genetic clusters. Unique genets are represented by points. The percentage of variation explained by each axis is indicated. (C) Population structure models. Each bar indicates an individual genotype and the relative proportion of the two colors represent the relative likelihood of membership to each of the two proposed genetic clusters.



Figure 27. *Pseudodiploria clivosa* initial dendrogram showing an outgroup with high genetic distance from the rest of the source colonies.



Figure 28. Algal symbiont communities were classified at the genus level through both 2bRAD and *ITS2* methods. Communities are organized by source nursery on the x-axis and by species on the y-axis. MCAV = *Montastraea cavernosa*, OFAV = *Orbicella faveolata*, and PCLI = *Pseudodiploria clivosa*.



Figure 29. The relative proportion of *ITS2* type profiles of the Symbiodiniaceae communities hosted by source colonies across species and source nursery. *ITS2* type profiles are named for the defining intragenomic variants (DIVs) used to characterize them. *Montastraea cavernosa* harbors predominantly *Cladocopium*, *Orbicella faveolata* harbors predominantly *Durusdinium*, and *Pseudodiploria clivosa* harbors a majority of *Breviolum*.


Figure 30. The relative proportion of ITS2 type profiles of the Symbiodiniaceae communities hosted by source colonies across species and disease status. ITS2 type profiles are named for the defining intragenomic variants (DIVs) used to characterize them.



Figure 31. Mean net growth of living *Montastraea cavernosa* colonies by region, reef stratum and colony source at the last survey period, April 2023.



Figure 32. Mean net growth of living *Orbicella faveolata* colonies by region, reef stratum and colony source at the last survey period, April 2023.



Figure 33. Mean net growth of living *Pseudodiploria clivosa* colonies by region, reef stratum and colony source at the last survey period, April 2023.



Figure 34. Comparison of net growth of colonies that exhibited evidence of finfish predation within onemonth post-outplant with those that did not. A = *Montastraea cavernosa*, B= *Orbicella faveolata*, and C) *Pseudodiploria clivosa*. Note differences in y-axis scales.



Figure 35. Total lipid content of the corals *Orbicella faveolata* (OF), *Montastraea cavernosa* (MC) and *Pseudodiploria clivosa* (PC). Samples were obtained from the University of Miami (UM), Reef Relief (RR), Coral Restoration Foundation, Florida Wildlife Commission (FWC) and Mote Marine Lab (MOTE). Bars are means \pm standard deviation error bars, with all raw data included as individual points (n=2-15).



Figure 36. Boxplots comparing the number of coral colonies that showed evidence of finfish predation within by 1-month post-outplant and those that did not as a function of lipid content.

Appendix 1. Image analysis protocol developed for the RT project by Gabby Pantoni of FAU to standardize the project's coral imaging effort.

RTT Image Tracing Protocol ImageJ (version 1.53a)

Developed by Gabby Pantoni

Last updated April 25, 2022, by Marina Garmendia

- 1. To open an image file using ImageJ:
 - a. "File, Open," then import the desired photo.
- 2. The scale must be reset for EACH photo:
 - a. If ruler is visible, use the "line tool" to measure 1 cm, if ruler is NOT visible measure:
 - i. The PVC T joint at the edge closes to the base, length is 6.6 cm.
 - ii. Or if joint not visible use the yellow ID tag closest to the base, height is 2 cm.
 - b. Select "analyze" then "set scale".
 - c. Enter the distance measured ("1" if 1 cm was measured) into "known distance" and set the "unit of length" as "cm".
 - d. Check the box next to "Global"- this will save your unit as cm
 - e. Now your scale is set for this image
 - f. REMEMBER: You need to scale each image individually; it will not carry the measured scale over between images
- 3. Use the "freehand tool" if you have a tablet to trace each fragment, or "Polygon tool" if tracing with mouse or trace pad, one at a time. <u>Only trace the visible living coral tissue</u>.
 - a. Trace pad, Wacom DTK1660K0A Cintiq 16
 - b. Do not use built in 'track pad' on laptop to trace images.
 - c. Trace only the living SA of each fragment (check protocol for when fragments are fused).
 - i. Bleached or pale tissue is live tissue
 - ii. Missing or 100% dead fragments are not traced
 - iii. Dead tissue in the middle of the colony is traced and subtracted to get living tissue area.
 - d. You can use the "+" and "-" buttons on your keyboard to zoom in and out
 - e. NOTE: if a portion of the fragment is covered by algae or sediment, check the previous/future photos and use best judgment to determine colony edge
- 4. To measure surface area
 - a. Click "analyze", "measure". (Shortcut: Control or Command+M).
 - b. A popup screen will appear with the values measured from the shape you traced. The value given as "area" is the 2D surface area measured for that fragment.
 - c. You can choose which values are given in the table by selecting "analyze" and "select measurements". Then select "area" and deselect all other values.
- 5. Record all 5 fragment surface areas individually.
 - a. ImageJ will save the analyzed measurements for each photo in the popup table, so you can repeat steps 3 and 4 for each fragment in a photo
 - b. Once you have the surface area values for each fragment in a photo, copy and paste your values into an excel spreadsheet where each value corresponds to a fragment. (See example sheet below)
 - c. To get the total surface area for the colony, use excel to compute the sum of the living tissue surface areas of each fragment.

If the fragments are fused:

d. Measure the surface area of the fused fragments as one large fragment.

- e. In the data sheet, if fragments F1 and F2 are fused, enter the SA value for the fused tissue into F1 and enter 0 into F2. Make a note that these fragments are fused.
- 6. For the next photo:
 - a. You don't need to save changes to the files.
 - b. If you opened an image in a folder with the rest of the images to be analyzed, you can open the next photo in the folder by clicking "Import, Open Next."

QAQC - RTT Outplanting Project (due May 9th 2022)																
Colony #	Photo name	F1	F2	F3	F4	F5	Substracted area	Living SA Total	Bleached Y/N	Fusing Y/N	Predation Y/N	Diseased Tissue Y/N	Discrepancy with data (description)	Scale used	Measured by	Notes

Note: For consistency, F1-F5 corresponds to the fragment number.



1. If there are any empty or dead spaces in the fused colony subtract trace that area and record sum of all dead/empty area in the "subtract area" column and subtract value from the sum of the area traced to get SA living total.

Data entry

- 1. Enter colony name
- 2. Enter photo name
- 3. F1-F5: Fragment measurements
 - a. If missing or dead value is "0"
- 4. Subtracted area: any holes or old dead in the fragments
 - a. Sum of all individual areas within an image are totaled here
- 5. Living SA total: add all the fragment measurements (F1 to F5)
- 6. QAQC ONLY: Bleached, predation, or diseased tissue (Y or N)
- 7. Fusing: (Y or N) put Y if any of the corals are fused
- 8. Discrepancy with data: observer needs to check that recorded *in situ* data matches image, if there is an obvious discrepancy with the *in situ* data, observer needs to describe issue in this column.
 - a. Ex. Bleaching was recorded *in situ*, however obvious bite mark is visible in image.

9. Scaled used: if the ruler was used leave it blank, if T Joint or tag was used, please note which was used.

Metadata

Make sure to complete the metadata when tracing, to track for effort spent during tracing.
a. Method used is either tablet, tracing pad or a mouse.