Final report on Florida DEP project C2020B: Drivers of resistance to stony coral tissue loss disease (SCTLD), infrastructural improvements, and 2023 bleaching response

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Final Report

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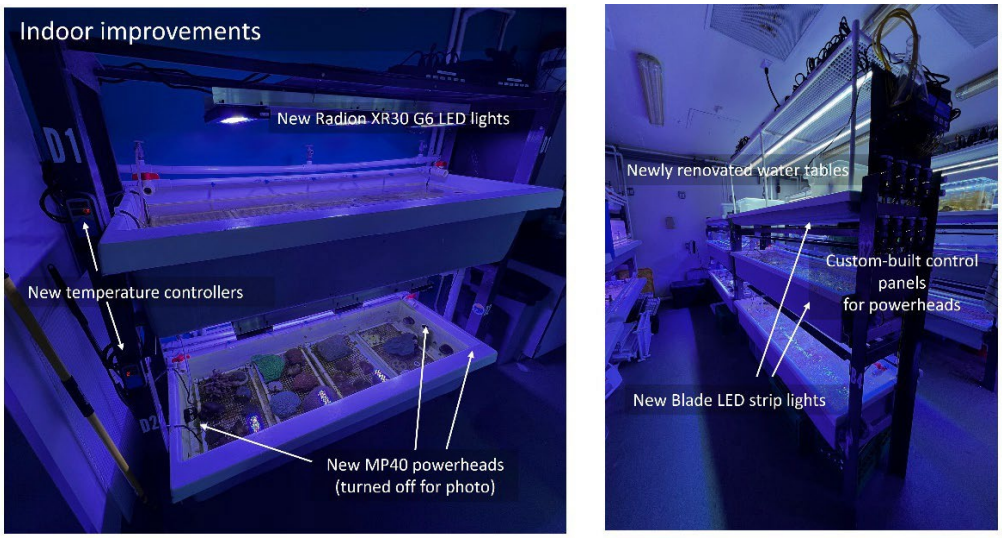
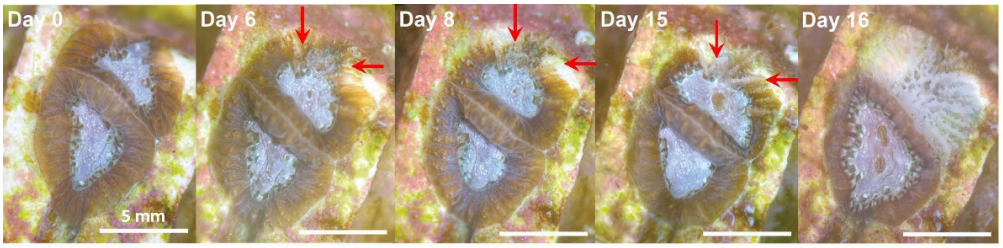
Management Summary (300 words or less)

This project improved indoor and outdoor land-based facilities at the University of Miami, and provided upgrades that significantly improved our experimental and propagation capacity. Specifically, it helped prepare existing facilities to hold corals as part of the 2023 Florida bleaching response and funded the care of over 2,000 corals that were removed from nurseries during the onset of heat stress, helping avoid any mortality of these corals during this event. This served as an important proof-of-concept showing that this approach can be employed in future, and that this approach can be scaled-up as needed to help protect important coral stocks.

This project also helped fund genetic (SNP genotyping and microbiome analyses) of coral parents and offspring from an experiment supported by DEP in FY23. This experiment was designed to study potential heritability in corals' resistance to stony coral tissue loss disease (SCTLD). Although we are experiencing a delay obtaining data due to outside factors (the discovery by FWRI that there may be more than one species of *Colpophyllia* in Florida, which is complicating the SNP assays), these data will be critical in helping assess whether heritability is due to genetic factors or maternal factors (i.e., bacterial communities) that are passed from parents to offspring. We are excited to bring these datasets together and plan to publish and disseminate our findings as soon they become available.



Infrastructure improvements to indoor coral systems to support coral propagation and emergency response



Progression of stony coral tissue loss disease (SCTLD) in early-stage recruit of the grooved brain coral, *Diploria labyrinthiformis*

Executive Summary (max 1 page)

This project supported three different projects at the University of Miami. The first project undertook genetic analyses of samples that were collected from an experiment run with DEP support in FY23, which assessed how early-stage recruits of three different coral species responded to exposure to stony coral tissue loss disease (SCLTD). With FY24 funding, we initiated genetic analyses to determine the parentage of different recruits to assess whether certain parents contributed disproportionately to the recruits that died from SCTLD compared to those that survived. We also undertook microbiome (16S) analyses of the bacterial communities in these recruits to determine whether components of the microbiome were heritable from parents to offspring, and whether there are correlations between certain microbes and susceptibility/resistance of recruits.

To genotype our recruits and parents, we collaborated with FWRI to apply new genetic assays they had developed for the Florida Coral Rescue project. However, recently FWRI, using these new assays, found evidence for a cryptic species of *Colpophyllia* in Florida. This has complicated the analysis of our samples and led to a delay obtaining final data in the project timeframe (although the samples are still in the pipeline). However, our microbiome analyses are complete and although we need the genetic data to fully interpret them, we have already found that recruit microbiomes vary by coral species and SCTLD exposure, and that SCTLD induces microbiome changes in favor of particular bacterial taxa that are associated with diseased adult corals, even when lesions have not yet been observed. Once the genetic data become available from FWRI, we will be able to relate microbiome composition to parentage and assess whether maternal transmission of the microbiome might help explain patterns of susceptibility in our experimental trials.

In the second project, we converted flow-through coral systems in our indoor labs into recirculating systems to improve water quality and decrease the impacts of variation in the local seawater supply on coral health. This involved a complete tear-down and reconfiguration of the lab, refinishing of tanks, addition of sumps, and multi-purpose plumbing to allow different systems to operate either together or independently.

In the third project, we improved indoor and outdoor coral propagation systems in order to support the 2023 bleaching response in Florida, and cared for >2,000 corals that were rescued from nurseries during that event. These corals have since been successfully returned to the nurseries with no loss of genotypes.

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Task 1 - SNP Chip genotyping of parent colonies and offspring from 2021 and 2022 spawning

Methods

Sample Collection and DNA Extraction

A total of 317 adult parents (n=18) and recruits (n=299) were sampled for DNA (Table 1). Sterile razor blades were used to collect 0.5 cm^2 of live tissue from each sexually produced offspring. Adult broodstock from the Florida Aquarium were previously sampled as a part of the Coral Rescue and samples are held at the Florida Fish & Wildlife Research Institute as a part of the Coral Rescue Project. Total DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, California) under a modified protocol (Baums & Kitchen 2020) and total genomic DNA was quantified (ng/µL) using a Nanodrop. All samples have been extracted, DNA normalized, and are ready for sequencing.

Genetic samples are currently being genotyped using species-specific KASP™ genotyping assays for DLAB, PSTR, and CNAT that were created for the Coral Rescue Project. Each genotyping assay has 192 species-specific single nucleotide polymorphism (SNP) markers that are suitable for downstream analysis.

The CNAT assay has found more than one species of *Colpophyllia* in the Florida population (potentially *C. breviserialis*). Consequently, two genotyping assays have been created, one for each of the two assays. Samples that fail the first assay are assumed to belong to the other putative species and are run on the second assay.

Table 1: Number of genetic samples taken per species, includes both adult broodstock and sexually produced offspring.

Species Name	Number of Adult Broodstock	Number of Offspring
Colpophyllia natans	4	80
Diploria labyrinthiformis	10	137
Pseudodiploria strigosa	4	82

Parentage Analysis

Analysis of genetic data will be conducted using a parental contribution software program designed by staff from the Florida Fish & Wildlife Research Institute for the Coral Rescue Project. This program determines parental contribution to offspring using exclusion methods.

Results

Genetic samples are currently being genotyped using the species-specific KASP™ genotyping assays. Delays with the sequencing vendor have resulted in limited available data.

The first 80 *Colpophyllia natans* samples have been processed on the first CNAT genotyping assay. However, all samples are suspected to belong to the second CNAT

species because they failed to meet minimum SNP call rates. They are therefore being run on the second genotyping assay. The need to develop a second CNAT assay and re-run samples has led to delays in the DLAB and PSTR being submitted for processing

Of the 400 samples we originally estimated, only 317 were able to be sampled (18 parents and 299 offspring). Of these, a total of 97 have been sequenced to date (17 parents and 80 offspring). These data are included in the "SNP data" folder on the Google Drive.

The 80 CNAT offspring will have to be re-run (as well as one parent that was omitted from first run in error) as well as the remaining 219 offspring. We anticipate we will still run a total of at least 400 sequencing runs (including re-runs), as originally planned. However, extended delays in the production of sequencing data, for the reasons outlined above, mean we do not yet have the data in hand. Once data is received, all raw genetic data will be imported into the FWC Coral Genet Registry and parental analysis will be completed.

Task 2 - Investigations into microbial-signature of SCTLD-resistance using prokaryotic 16S microbiome analysis

Note: Figures and tables for Task 2 can be found in the "Task 2 Figures" and "Task 2 Tables" files on the Google Drive.

Background

Stony Coral Tissue Loss Disease (SCTLD) has severely affected Caribbean reef corals over the past decade (Sharp et al., 2020). While its impact on adult corals is well-documented (Precht et al., 2016), its effects on early life stages are less understood (Williamson et al., 2022). Coral recruits are vital for the recovery of reefs damaged by disease or bleaching. Gaining insights into the susceptibility and survivorship of early life stages to SCTLD will improve our understanding of the long-term impacts of this disease on reef ecosystems.

The coral microbiome, which plays a critical role in defending against pathogens, likely influences disease susceptibility (reviewed in Vega Thurber et al., 2020). Traits of the microbiome, such as specific taxa, community composition, and stability, may be related to coral resilience against SCTLD. Understanding these traits is essential for assessing juvenile coral resistance and susceptibility to the disease. Disease susceptibility varies within adult colonies, and this variability is likely present in juveniles as well (Guzmán-Urieta and Jordán-Dahlgren, 2021). Identifying factors that contribute to disease resistance could help in developing intervention strategies, such as probiotic treatments.

In our study, we examined the microbial communities of juvenile corals (6–9 months old) from three highly susceptible brain coral species—*Diploria labyrinthiformis*, *Colpophyllia natans*, and *Pseudodiploria strigosa*—spawned in captivity. We sampled recruits that showed no visible SCTLD lesions after 16 days of exposure to SCTLD and performed 16S amplicon sequencing to identify microbiome traits associated with disease resistance.

Methods

Experimental design

Details of the experimental design are provided in Williamson et al. (2022). In brief, colonies of *Diploria labyrinthiformis* (n=7), *Colpophyllia natans* (n=4), and *Pseudodiploria strigosa* (n=2) were spawned at the Florida Aquarium's Center for Conservation in May 2022. These spawning colonies were not believed to have been exposed to SCTLD prior to spawning, based on the collection site and time of initial removal from the reef.

Larvae were settled on ceramic tiles at the University of Miami's Rosenstiel School of Marine, Atmospheric, and Earth Science and were provided with UV-sterilized, one-micron filtered seawater (FSW). Tissue samples (< 0.25 cm^2) from juvenile *Colpophyllia natans* (n = 32), *Diploria labyrinthiformis* (n = 52), and *Pseudodiploria strigosa* (n=30) were collected at an initial pre-exposure time point, placed individually in DNA/RNA Shield (ZYMO), and stored at -80ºC for 16S amplicon sequencing.

Following pre-experiment sampling, half of the remaining juveniles in each species group were assigned to either a disease-exposed or a control treatment. The disease treatment involved a water bath containing adult corals with visible lesions from stony coral tissue loss disease (SCTLD), while the control bath contained adult corals free of lesions. The experiment concluded and corals were sampled after approximately 50% of juveniles had died in each species group in the disease-exposed treatment. Tissue biopsies were again collected from both the control and disease-exposed corals, stored in DNA/RNA Shield (ZYMO), and kept at -80ºC.

DNA was extracted using the Quick-DNA Fecal/Soil Microbe 96 Kit (ZYMO), and library preparation was performed following standard protocols for the 16S V3 region. Sequencing was conducted using the C-MAIKI pipeline (Cleveland et al., 2022), and data analysis was carried out in R. Data and scripts are available at: https://github.com/shaylematsuda/SCTLD.

Results and Discussion

Microbiomes differ between juvenile brain coral species: juvenile microbiome overview

The microbiomes of juvenile brain corals under 1 year of age differed significantly by species (PERMANOVA p = 0.001; Table 1, Figure 1). Pairwise comparisons showed that each species pair was significantly different from one another (post-hoc tests, p = 0.001; Table 2). There was also a significant difference in the homogeneity of variance within groups between species pairs (p = 0.001; Table 3), with post-hoc comparisons indicating differences (all p < 0.001). Among the species, *P. strigosa* juveniles exhibited the greatest variation in microbial communities, while *C. natans* showed the least variation.

There are differences in the relative abundances of the top ten phyla across coral species. *Diploria labyrinthiformis* does not host *Nitrospirae*, whereas all coral species host unclassified Bacteria. The most dominant phylum in *D. labyrinthiformis* is *Epsilonbacteraeota*, with a relative abundance of 46% across samples, compared to 13% in *C. natans* and 10% in *P. strigosa*. The most dominant phylum in *Colpophyllia natans* is *Cyanobacteria*, with a relative abundance of 18% across samples.

At the family level, *D. labyrinthiformis* is dominated by *Arcobacteraceae*, *Desulfobacteraceae*, and *Family_XII*. *Colpophyllia natans* is dominated by unclassified *Alteromonadales* and *Oxyphotobacteria*, while *Blattabacteriaceae* is the most dominant family in *P. strigosa*. Among the top ten most abundant taxa in each of the three host species, only four ASVs were found in all three species: *Ruegeria*, an uncultured bacterium, and an uncultured *Oxyphotobacteria*.

Alpha diversity metrics (richness, evenness, Shannon diversity) varied by host species (p-values <0.001; Table 4). *Colpophyllia natans* juveniles exhibited the highest microbial community diversity and evenness, with the highest Shannon Index value (Figure 2). *Diploria labyrinthiformis* had the lowest richness but high evenness, while *P. strigosa* had the lowest evenness (Figure 2).

We also sequenced the adult corals that were in the control (healthy, no lesions) and disease (active lesions) baths. While there was not high replication, both diseased and "healthy" adult coral microbial communities were different than the three target species here (Figure 3).

Colpophyllia natans

No taxa were present in all initial *C. natans* juveniles. At the initial time point, 39 ASVs were found in 95% of *C. natans* samples (threshold ≥0.0001). The most dominant genus was *Ruegeria*, present in 100% of the samples (Figure 4). *Ruegeria* is a common coral-associated bacterium known to inhibit *Vibrio coralliilyticus* (Miura et al., 2019). Additionally, there were higher abundances of *Alkalimarinus* (Gammaproteobacteria), *Thalassotalea* (Gammaproteobacteria), and an unclassified *Rhodobacteraceae* (Alphaproteobacteria).

Diploria labyrinthiformis

Juvenile *D. labyrinthiformis* microbial taxa represented 642 genera across 373 families. No taxa were present in all initial juvenile *D. labyrinthiformis* samples. Eight ASVs were found in 95% of the samples with a relative abundance >0.01%:

- *Ruegeria* (Alphaproteobacteria)
- *Hyphomonas* (Alphaproteobacteria)
- *Lentilitoribacter* (Alphaproteobacteria)
- *Anderseniella* (Alphaproteobacteria)
- An uncultured *Thalassobaculales* (Alphaproteobacteria)
- An uncultured bacterium
- An unclassified *Flavobacteriaceae* (Bacteroidia)
- *D90_ge* (Gammaproteobacteria)

The most abundant genus in juvenile *D. labyrinthiformis* was *Ruegeria*, present in ≥99% of juveniles with a relative abundance >0.01%. Other core taxa included *Hyphomonas*, *Lentilitoribacter*, *Anderseniella*, the uncultured *Thalassobaculales*, an uncultured bacterium, an unclassified *Flavobacteriaceae*, and *D90_ge* (Figure 5). *Anderseniella* has been associated with disease in *Platygyra carnosus* (Ng et al., 2015).

Pseudodiploria strigosa

Pseudodiploria strigosa exhibited the greatest dispersion from the mean among juveniles. The microbial taxa found in *P. strigosa* represented 642 genera across 373

families. Six core ASVs were present in ≥95% of samples with a relative abundance ≥0.0001% (Figure 6):

- *Hyphomonas* (Alphaproteobacteria)
- *Ruegeria* (Alphaproteobacteria)
- *Anderseniella* (Alphaproteobacteria)
- An uncultured ASV (Family: Kiloniellaceae; Alphaproteobacteria)
- An unclassified Alphaproteobacteria
- D90_ge (Gammaproteobacteria)

Hyphomonas is known to be associated with dinoflagellates (Sekar et al., 2006) and coral exudates (Nelson et al., 2013), and is not considered a pathogen (Nelson et al., 2013). *Kiloniellaceae* is a common coral-associated bacterial family typically found in the coral skeleton (Ricci et al., 2022) and plays a role in nitrogen cycling (Imhoff and Wiese, 2014). Similar to *C. natans* and *D. labyrinthiformis*, *Ruegeria* is the most abundant taxon in *P. strigosa*.

The impact of exposure to SCTLD on the juvenile brain coral microbial community

We assessed the microbial communities in *C. natans, D. labyrinthiformis*, and *P. strigosa* after exposure to SCLTD after 16 days (Figure 7) and found significant effects species ($p = 0.001$; Table 5) on microbial community structure as well as significant effects of the interaction of species over time ($p = 0.001$; Table 5), and the treatments over time ($p = 0.001$; Table 5).

Colpophyllia natans

There was a significant difference between the SCTLD-exposed and SCTLD non-exposed microbial communities after 16 days (PERMANOVA $p = 0.001$; Table 6, Figure 8). However, there was no difference in the variance between samples in each treatment (homogeneity of dispersion test, $p = 0.247$). At the end of the experiment, nine core ASVs were present in ≥ 95% of control samples, and 13 core ASVs were present in treatment samples (detection rate ≥ 0.0001%). The following taxa were unique to the core disease treatment group:

- *Pseudohongiella* (Gammaproteobacteria)
- KI89A_clade_ge (Gammaproteobacteria)
- *Hyphomonas* (Alphaproteobacteria)
- An uncultured and an unclassified *Kiloniellaceae* (Alphaproteobacteria)
- *Pelagibius* (Alphaproteobacteria)
- An uncultured Alphaproteobacteria
- SAR324_clade (Marine_group_B)_ge (Deltaproteobacteria)
- An unclassified *Saprospiraceae* (Bacteroidia)

Taxa found in the controls but not in the disease treatment included:

- *Pelagibius* (Alphaproteobacteria)
- An unclassified Proteobacteria
- *Planctomicrobium* (Planctomycetacia)
- *Candidatus Amoebophilus* (Bacteroidetes)
- D90_ge (Gammaproteobacteria)

There was no effect of SCTLD exposure on microbial community richness (p = 0.081; Table 7) or Shannon diversity (p = 0.829; Table 7; Figure 9). However, microbial community evenness in SCTLD-exposed juveniles was significantly lower than in controls (p = 0.022; Table 7; Figure 9), suggesting that disease exposure may lead to a loss of microbial diversity.

We conducted an indicator analysis using the R package `indicspecies` to identify taxa significantly associated with either control or disease-treated corals (stat = specificity × fidelity). This analysis identified 101 ASVs associated with disease-treated corals (p-values = 0.001–0.044) and 94 ASVs associated with controls (p-values = 0.001–0.039; Figure 10). Further investigation is needed to determine which of these taxa might be correlated with or driving the lesion-free phenotype.

The highest potential indicator taxa with the greatest abundance in disease-treated corals are *Acanthopleuribacter* and *Alteromonas* (Figure 11). *Acanthopleuribacter* has been previously identified in sediments associated with corals exhibiting SCTLD tissue loss (Studivan et al., 2022). *Alteromonas* is a common nitrogen-translocating bacterium found in both adult and juvenile corals (Ceh et al., 2013) and has been proposed as a potential probiotic against SCTLD (Paul et al., 2021). The taxa with the highest indicator values include an unclassified *Flavobacteriaceae*, *SAR324_clade (Marine_group_B)_ge* (Deltaproteobacteria), a *Saprospiraceae*, and *Halodesulfovibrio*.

C. natans adults are highly susceptible to SCTLD, with 100% of fragments showing lesions in a disease-exposed experiment. The disease state significantly impacts both the tissue and mucus microbiome community structure (Huntley et al., 2022). In our study, after only 16 days of exposure, we observed differential appearance of lesions on juvenile *C. natans*. *Saprospiraceae* is a known bioindicator of SCTLD in adult *C. natans* (Huntley et al., 2022), which could suggest that disease-exposed individuals without lesions yet might be in the early stages of lesion development.

Comparing only the controls from the initial to the final time point, we observed a significant shift in community structure (PERMANOVA, p = 0.001; Table 8), but no difference in variance between samples (p = 0.804). Changes in the microbiome during early life stages are common, so differences between treatments at the final time point might reflect natural shifts or tank effects.

Diploria labyrinthiformis

Exposure to SCTLD significantly affected the microbial community structure of *Diploria labyrinthiformis* over time (p = 0.001; Table 9, Figure 12). Pairwise comparisons reveal significant differences between:

- Disease-exposed initial and final time points (p = 0.001)
- Control and disease-exposed at the final time point (p = 0.001)

These results suggest an effect of disease exposure. No significant difference was observed between treatments at the initial time point (p = 0.050). However, a significant change was detected in the control treatment between the initial and final time points (p = 0.001), likely due to expected microbiome changes during early life stages.

There was no difference in community dispersion within groups (homogeneity of dispersion test, p = 0.466). Given that repeat sampling was only performed on *D.*

labyrinthiformis due to size constraints, genotype was included in the model. This analysis showed that microbial communities differ by genotype ($p = 0.005$; Table 9) and that the interaction between genotype, exposure treatment, and time affects microbial community composition ($p = 0.002$; Table 9). A strong genotype effect on adult coral microbial community structure is common.

The microbial community structure in the controls shows a clear pattern from the initial to the final time point (Figure 12), likely reflecting a natural shift in the microbial community over time. Interestingly, this pattern is disrupted in disease-exposed juveniles (Figure 12).

Nine ASVs were identified as core taxa, present in 95% of control samples with a detection level of 0.001%. These core ASVs include (Figure 13):

- *Hyphomonas* (Alphaproteobacteria)
- *Ruegeria* (Alphaproteobacteria)
- *Lentilitoribacter* (Alphaproteobacteria)
- *Anderseniella* (Alphaproteobacteria)
- *Pelagibius* (Alphaproteobacteria)
- Two uncultured *Thalassobaculales* (Alphaproteobacteria)
- An unclassified Oxyphotobacteria
- D90_ge (Gammaproteobacteria)
- An unclassified Alphaproteobacteria
- An unclassified Bacteria

At the final time point, the relative abundance of *Pelagibius* was higher in control samples compared to disease-treated samples. Conversely, disease-treated juveniles exhibited higher average abundances of *Ruegeria* (Figure 13) and lower abundances of an uncultured bacterium.

The indicator analysis returned a greater number of putative taxa in the disease treated juveniles than the controls at the end of the experiment (Figures 14–15. The putative taxa with the five highest stat values for the diseased treated juveniles include Saprospiraceae, Halieaceae, Lewinella (Saprospiraceae), unclassified Rhodobacteraceae, and a DEV007_ge (Verrucomicrobiales: Verrucomicrobiae); the highest for the controls include Lewinella, unclassified Devosiaceae, Dokdonia, Portibacter, and an unclassified Cytophagales (Figure 15). Interestingly, in the controls, the two indicator taxa with the highest abundance are two uncultured ASVs. Saprospiraceae was previously identified as a disease bioindicator bacteria of SCTLD in adult coral mucus (but not tissue), including *C. natans and Siderastrea siderea* (Huntley et al. 2022). Huntley et al. (2022) observed a shift in the microbiome of mucus in disease exposed adult corals that hadn't yet developed lesions. Here, for our disease-exposed individuals that did not exhibit lesions, the higher abundance of these disease indicators could indicate that these individuals will develop lesions. Further work would need to be undertaken to understand if the same bioindicators in adult coral mucus are also indicative of disease in juveniles. Lastly, in an indicator analysis of control juveniles over time, 44 putative taxa were indicative of the older juvenile microbiomes (Figure 16), however, only unclassified Cytophagales was indicative of the later life stage and the control state vs. the disease state.

The microbial communities of SCTLD-exposed corals exhibited higher microbial richness, evenness, and Shannon diversity compared to control corals at the final time point ($p < 0.001$; Table 10; Figure 17). Unlike in *C. natans*, *D. labyrinthiformis* juveniles exposed to SCTLD maintained higher species richness and high evenness, suggesting that if additional taxa were opportunistic, they did not dominate the community by day 16. Since the sampled SCTLD-exposed colonies were the survivors in the treatment, this might indicate that a diverse and even microbial community is a trait associated with SCTLD resistance in *D. labyrinthiformis* juveniles—a characteristic often linked to ecological stability. Bacterial indicators associated with the controls could be further investigated to assess potential functional trait loss in SCTLD-exposed juveniles during disease exposure.

However, when examining potential shifts in the microbiome of the control group from the initial to the final time point, a significant shift in community structure was observed (PERMANOVA $p = 0.001$; Table 11), along with a difference in variance between samples ($p = 0.02$). While changes in the microbiome over early life stages are commonly observed, the differences between treatments at the final time point could also reflect natural shifts or tank effects.

Pseudodiploria strigosa

Juveniles of *P. strigosa* exposed to SCTLD exhibited differences in microbial communities (PERMANOVA, $p = 0.001$; Table 12, Figure 18). Similar to *D. labyrinthiformis* and *C. natans*, SCTLD-exposure did not impact dispersion within treatment groups (homogeneity of dispersion test, $p = 0.716$).

Among the top 10 most abundant families in both the control and disease-exposed treatments, Alteromonadaceae, Arenicellaceae, Haliangiaceae, and Oxyphotobacteria showed higher relative abundance in the disease treatment. In contrast, the control group exhibited higher relative abundances of D90_fa, Devosiaceae, Hahellaceae, Kiloniellaceae, Rhodovibrionales, and an uncultured family (Figure 19). There are 9 ASVs found in >95% of samples at > 0.001% detection at the final time point (Figure 19). These core taxa include an unclassified Oxyphotobacteria that is enriched in some of the control samples compared to the disease treatment, but not all.

The indicator species analysis identified 108 putative taxa of interest ($p < 0.05$), with the highest stat values observed for Lentilitoribacter (Rhizobiaceae), Aquimarina (Flavobacteriaceae), an unclassified Gammaproteobacteria, an uncultured Colwelliaceae, and Haloferula (Rubritaleaceae). The top indicator taxa for disease-exposed juveniles (102 total) included Flavobacteriaceae, an unclassified Saprospiraceae, Rubritalea (Rubritaleaceae), Arenicella (Arenicellaceae), and Alteromonas (Alteromonadaceae). Notably, two species of Saprospiraceae and Rubritalea were also enriched in the mucus of *P. strigosa* adults and identified as indicator taxa of SCTLD (Huntley et al. 2022), suggesting that the juveniles enriched with these taxa could potentially develop lesions later. Rhotobacteraceae, also identified in the adult mucus (Huntley et al. 2022), were significantly associated with the SCTLD-exposed juveniles in this study.

SCTLD exposure had no effect on microbial species richness, evenness, or Shannon diversity ($p > 0.05$; Figure 20, Table 13).

P. strigosa adults are highly susceptible to SCTLD (Huntley et al. 2022), but some individuals did not develop lesions upon exposure, a pattern also observed in the

juveniles here. The microbial communities in *P. strigosa* shifted over time (controls: initial to final; PERMANOVA $p = 0.023$; Table 14), with increased dispersion in the final treatment ($p < 0.001$). Due to the small size of the juveniles, repeat sampling was not possible. While we can recommend these taxa for further investigation, we cannot definitively designate them as indicators of juvenile corals more resistant to SCTLD without additional research.

Significance and Management Recommendations

We characterized the microbiomes of three juvenile brain coral species that are highly susceptible to SCTLD but did not develop visible lesions after 16 days of exposure. This study was part of a larger experiment examining the impacts of SCTLD on early life stages of corals. In all three coral species, *Ruegeria* was one of the most abundant taxa across juveniles in both the SCTLD-exposed and control treatments. However, after disease exposure, the mean relative abundances of *Ruegeria* changed differently in each species: increasing in *C. natans*, decreasing in *P. strigosa*, and remaining stable in *D. labyrinthiformis*.

We observed a directional shift in the microbiomes of all three species when exposed to SCTLD compared to the controls at day 16. However, the variance between samples did not increase (indicating no greater dispersion that could suggest dysbiosis), suggesting that stability might be a microbiome trait in juveniles that show resistance to SCTLD after 16 days of exposure. Nonetheless, microbial communities are known to change during early life stages. In *C. natans* and *P. strigosa*, destructive sampling due to the small size of juveniles prevented us from fully accounting for the impact of the natural shift in microbial communities.

In *D. labyrinthiformis*, where repeat sampling was possible, we observed that while the overall community shifted directionally when exposed to disease-treated water, this directional shift pattern was lost when accounting for genotype in the controls. This suggests that the disease treatment impacted the microbiome of juveniles, potentially leading to disease or dysbiosis if tracked longer, and possibly resulting in lesions.

We identified putative indicator taxa in both the controls and disease-treated juveniles, which are candidates for further exploration. These taxa or functional traits could be inhibited by disease exposure and might be useful in probiotics or as indicators of disease before visible lesions appear. Interestingly, in *C. natans* and *D. labyrinthiformis*, the microbiomes of disease-exposed juveniles at the end of the experiment were more similar to those of the initial controls than to the controls at the end of the experiment. This suggests that disease exposure may delay a natural temporal shift in the juvenile microbiome, potentially leading to the loss of key traits or services provided by these important microbial communities during early life stages.

Adult *C. natans*, *P. strigosa*, and *D. labyrinthiformis* are all highly susceptible to SCTLD. We observed that juveniles of these species also develop lesions when exposed to water containing adult corals with active SCTLD lesions, but many do not. The microbiomes of these lesion-free juveniles differ from those not exposed to SCTLD. Some putative indicator taxa in the disease-exposed juveniles without lesions are similar to taxa found in adults of the same species with active SCTLD lesions (Huntley et al., 2022). With further investigation, these taxa could be indicators of early onset disease before visible lesions in juveniles as well. However, if the lesion-free corals in the disease

treatment remain lesion-free, these microbial community traits may indicate that SCTLD resistance in juveniles still results in shifts in the microbial community, which could have implications for other physiological traits such as growth, immune response, or nutrient cycling. At this stage, it is crucial to consider the impact of SCTLD on juveniles, both in terms of disease-induced mortality and potential impacts on the robustness of the microbial consortia in those exposed to disease but without active lesions.

Limitations and Future Steps

Due to the nature of the experimental design, we could only examine the microbiomes of juveniles exposed to SCTLD but did not develop lesions (not juveniles with active lesions). This allowed us to identify putative taxa that may indicate SCTLD exposure and/or resistance. However, without samples from juveniles that developed lesions, we cannot confirm whether these taxa or communities differ significantly from those that succumbed to the disease.

In *C. natans* and *P. strigosa*, the inability to repeatedly sample the same individual made it impossible to account for genotype effects, which we observed in *D. labyrinthiformis*. Additionally, while we expect a natural shift in the microbiomes of the control group from the initial sampling point, this limitation restricts our analysis to comparing treatment and control at the final time point for coral species without repeat sampling. Depending on the hardening time for the juvenile microbiome, an earlier or later final time point could have shown more or less of an impact on community structure.

Further testing of juvenile corals with active SCTLD lesions would help identify microbial taxa and traits directly affected by the disease. Future research would benefit from a longer-term experiment to observe if resistant juveniles remain resistant to SCTLD in both the short and long term. If they do, using disease exposure at early life stages could become a scalable screening method to select SCTLD-resistant genotypes for propagation and outplanting. Next steps also include testing if taxa identified as probiotics for adults have the same effect on juveniles.

All raw sequences can be found in the "16S sequences" folder on the Google Drive as a zip of the fasta files. All analysis was done in R and can be found at the following link: https://github.com/shaylematsuda/SCTLD.

Task 3 - Convert indoor experimental facilities to recirculating systems to improve coral health and reduce variation in quality of incoming seawater

The Coral Reef Futures Laboratory under this DEP agreement has renovated the indoor, coral laboratory used for research on Florida-native coral and their algal symbionts. This laboratory space, named the "Baker Wetlab" is used for the rearing of larval coral and coral recruits, the fragmentation of mature adult colonies, and the conducting of temperature- and disease-related experiments via sixteen seawater aquariums. This overhaul had been long overdue, and sought to remove, replace and/or refurbish aging systems and technology. The new aquarium systems have been designed to be fully recirculating, with a multifunctional layout that allows for interconnected or independent tank systems, as well as flow-through processes to be used if needed.

This project involved an almost full deconstruction of existing flow-through aquarium systems. All plumbing was extricated, beginning with the existing seawater supply lines from the building infrastructure. The supply lines were rebuilt in a design that accommodates both efficiency and allows for each individual aquarium to have its own controllable spigot for filtered seawater. The drainage plumbing was also removed and reconstructed in a more efficient manner. These PVC pipes had accumulated a large amount of detritus, and various encrusting filter-feeding organisms to such an extent that water flow was constricted. Now, the newly installed, unobstructed drains are all connected to an exotic well, allowing for the holding of foreign corals and the drainage of diseased water without any biocontamination of local ocean and waterways. Flow valves were fitted to standpipes to ensure prevention of biocontamination.

Each of the sixteen aquariums was then repositioned in a more space-efficient layout and modified to provide ample space underneath each aquarium. This space was necessary for the installation of exterior filtration systems (sumps). Sumps provide the addition of an aquarium recirculating loop, and a separate space in which filtration equipment can be maintained. These sumps allow for the use of in-line U.V. sterilizers, protein skimmers, bag filters, and live rock on each individual aquarium. The worn, previously flow-through aquariums have been gutted, re-plumbed and converted to multipurpose recirculating systems. Improvements to incoming seawater quality via installation of mechanical filtration at 5- and 1-micron filter bags, as well as fitting of an upgraded UV sterilizer have helped raise the quality of incoming aquarium water. A schematic (side-view) of each of the eight tank rack layouts is included in Fig. 1, and a plan view of the wetlab showing the position of all eight tank racks is shown in Fig. 2,

Removal of the buildup of pipe blockages over time was conducted. Refurbishing of aquarium interiors using muriatic acid for cleansing, and epoxy for resurfacing has restored the quality, rigidity, watertightness and functionality of the sixteen coral aquariums. Aquarium lighting was upgraded from AI hydra lighting to generation six Radion XR30 LED lights. Additionally, a new domestic water line was implemented and fitted around the ceiling perimeter, and expandable hoses installed at various points for easy access to tap water for cleaning of aquariums and gear related to fieldwork and scuba dive operations. A reverse osmosis filter was fitted on the freshwater line to provide filtered freshwater to make up for evaporative water loss.

During April and May of 2024, all renovations were completed. Students are now setting up their experiments and are caring for captive bred corals recently spawned on site, as well as coral colonies collected from offshore locations. Increases to incoming seawater filtration have resulted in better water quality for use in recirculating aquarium systems. As such, the need for maintenance and upkeep in the form of cleaning of aquariums has been reduced. Lighting upgrades also allow for better control of the solar and lunar schedules that corals are innately dependent on for sexual reproduction.

Recirculating aquarium systems grant tighter control over the water quality in coral aquariums. They also allow for the use of aquarium equipment that increases the quality of life, survivorship and growth rates of coral in these systems. Nutrients need to be accounted

for as they are depleted in the water column, but by supplementing minerals such as calcium, alkalinity and magnesium one can ensure that ideal parameters are in check to accommodate skeletal growth as well as subsequent tissue development and symbiont uptake. Recirculating systems ensure that aquariums and the corals within are not at the mercy of incoming water quality fluctuations, and via regularly scheduled water changes of 20% water volume weekly, nutrients are kept within suitable ranges.

All orders were placed and processed. All shipments have been received. All projects and installations are complete, as follows:

- Room and aquarium breakdown: All electrical and plumbing stripped from existing aquarium systems. 100% complete.
- Room restructure: Repositioning of 8x aquarium racks into designed layout. 100% complete.
- Installation of aquarium supports: Aluminum stands for aquarium height increase were designed, outsourced, delivered, and then installed. 100% complete.
- Installation of domestic water line: PVC lines installed around the lab ceiling for easy access from multiple locations. 100% complete.
- Installation of hose lines: 2x 25ft expandable hoses installed on opposite ends of the room. 100% complete.
- Restructure of room drain: Old drainage system broken down, cleaned out, and restructured. New drainage system installed and drain cover grates modified to allow for ease of access to drains. 100% complete.
- Connection of aquariums to drain: Each aquarium system drain needed to be cut into to realign to the new water drainage arrangement. All aquariums now connect seamlessly to the exotic drainage system. 100% complete.
- Installation of seawater supply: Pipes plumbed to connect seawater supply to each individual aquarium. Allows for easy filling of each individual tank via central supply lines. 100% complete.
- Retrofitting of aquarium sumps: All aquarium sumps needed to be drilled and connected so that two sumps and respective aquariums can be connected and separated at will. Stainless steel ball bearing wheels were also installed to allow for easy movement of sumps when installed. 100% complete.
- Fabrication and installation of sump rail struts: Rails were designed and constructed to secure the movement of aquarium sumps. 100% complete.
- Reverse osmosis filtration system: Received, plumbed, and installed. 100% complete.
- Plumbing of recirculating aquariums: All parts received, plumbed, and installed. 100% complete.
- Installation of UV sterilizer: Aqualogic ALSV-3 UV sterilizer, previously used for treatment of seawater entering lab, reinstalled on incoming water supply. 100% complete.
- Installation of mechanical filtration: 5-micron and 1-micron filter bags received and installed. 100% complete.
- Installation of electrical equipment: All electrical equipment including pumps, protein skimmers, in-line UV sterilizers have been ordered, received, and installed. 100% complete.

- Sterilization of aquariums: All aquariums have been sterilized using muriatic acid prior to commissioning. 100% complete.
- Installation of LED lighting over aquariums: All lights have been installed. 100% complete

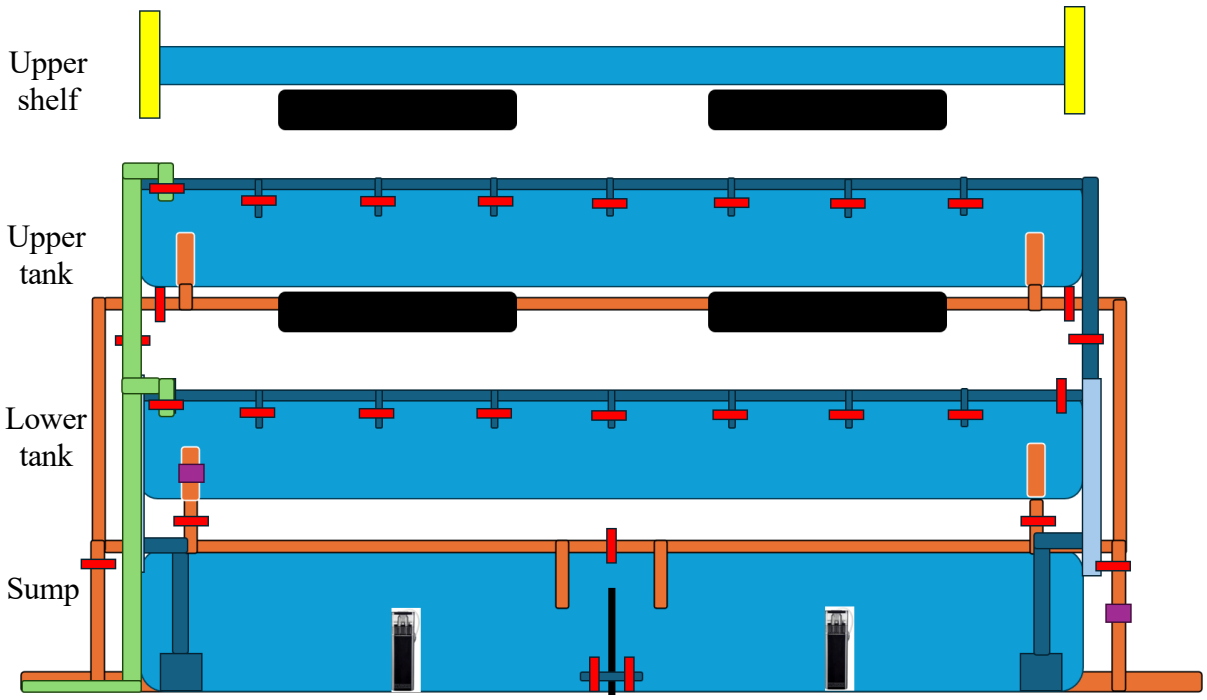

Figure 1: Schematic of tank layout/design for each of the eight (8) racks Upper shelves are for electricals/storage. Sumps are plumbed with flexible tubing and mmounted on sliding tracks for ready access and maintenance.

Color legend:
Yellow: Electrical outlet multi-switch in yellow,
Black: Radion XR30 Gen 6 lights
Orange: Drain line, connected to drainage via exotic well
Purple: Check valves for prevention of biocontamination
Dark blue: Recirculating seawater line
Light blue: In-line Aqua Ultraviolet UV sterilizer
Red: Ball valve for flow control

Photos and schematics of the improvements to coral systems can be found in the "Infrastructure improvements" folder on the Google Drive.

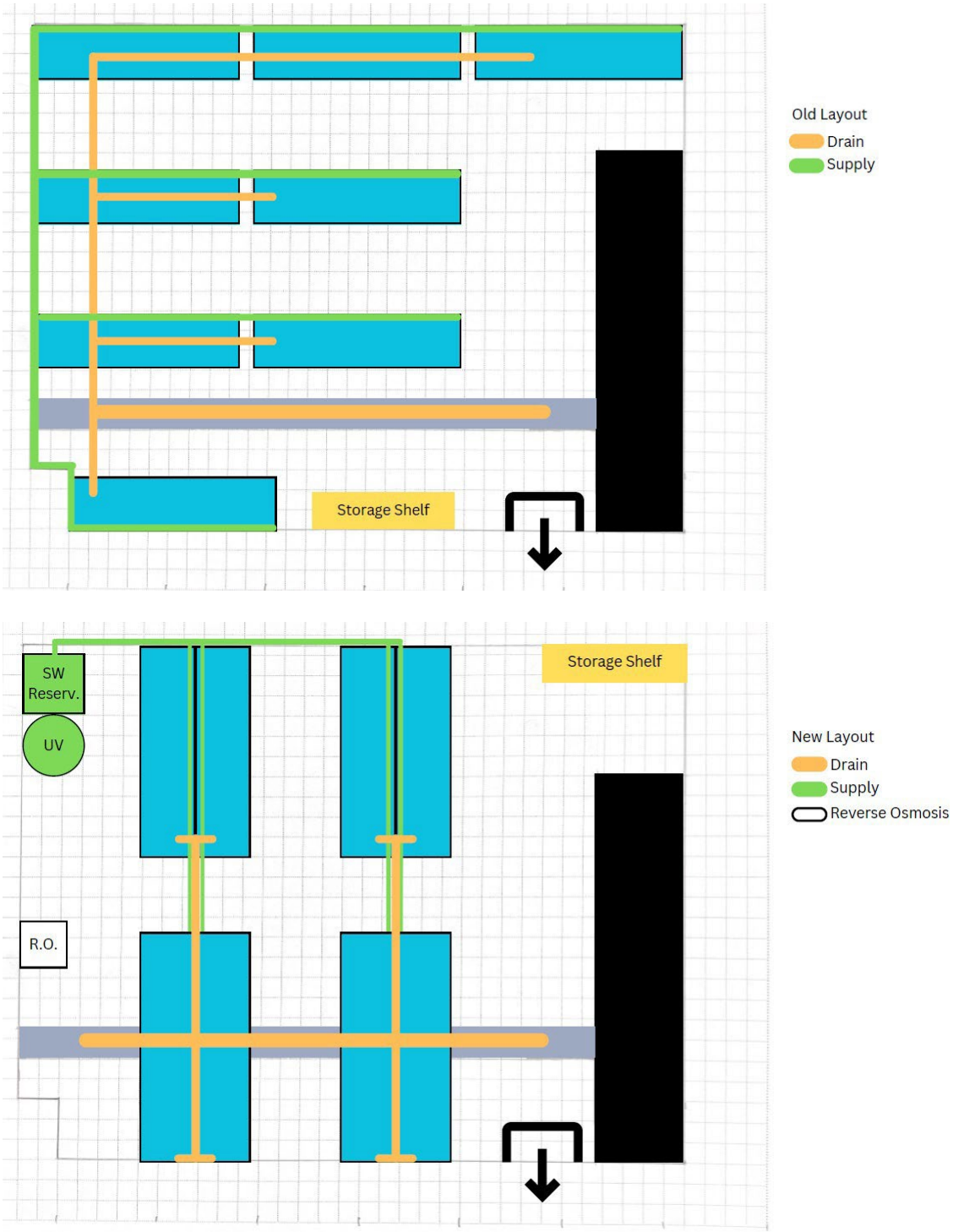

Figure 2: Plan view of wetlab showing position of all eight (8) tank racks before renovation (upper panel) and after renovation ((lower panel).

Task 4 - Expand and maintain infrastructure to support the 2023 Florida bleaching response emergency efforts

The following items were installed in the Baker indoor lab to further improve water quality for incoming corals from the 2023 Florida bleaching response:

- UV sterilization: Incoming seawater is already filtered and sterilized via UV. In addition, in-line Pentair 120 W sterilizers were installed on the sumps of each of six "flex" aquarium systems, and four 1,200-gallon raceways, to aid in preventing the spread of pathogens or other undesirable organisms. Pumps were ordered, received and installed to maintain flow through these UV sterilizers.
- Live rock: Dry rock was received and cultured within aquarium sumps to develop live nitrifying bacteria to aid in the biological filtration of these tanks. These live rocks act as an extra layer of filtration, and a safety measure should any nitrogenous waste compounds to begin to be released. If this were to happen, these bacteria break down any harmful ammonia or nitrite into a safe form, nitrate.
- Reverse osmosis system: A 200GPD reverse osmosis filter from Bulk Reef Supply was installed and connected to a 200-gallon reservoir. Domestic tapwater is then run through this filter to provide R.O. water with TDS < 1, which can then be used to make up for evaporative water loss, or also for the mixing of artificial seawater.
- Water flow: Each of the four 1,200-gallon raceways was originally equipped with one or two 2400gph Danner Aquamag pumps. After installation, each raceway now runs with three 3600 gph Danner Aquamag pumps, providing ample water movement within the aquariums for better homogenization of aquarium water and flow over coral surfaces. This further improves coral health for those species that depend on high flow environments to thrive.
- Indoor irradiance: We replaced remaining AI LED lights with with Radion XR30 Gen 6 lights. These LED lights are less prone to connection and/or equipment failure and are able to provide are broader spectrum of light wavelengths to improve coral health.

At the outdoor hatchery, the following improvements were made:

- Outdoor irradiance (hatchery): Shade cloths are used over outdoor tanks to control the amount of being received by corals. Light levels were measured and deemed insufficient for more delicate acroporid colonies. As such, layers of shade cloth were removed or replaced with preferential percentages to give the coral a range of PAR between 150-250.
- Recirculation of outdoor tank systems had only been possible for short periods of time prior to this however now can be ran in recirculating fashion. The tanks are filled via ozonated seawater and maintained with 20% water changes occurring weekly to account for nutrient depletion and/or accumulation. Powdered mineral supplements are used to account for calcium and alkalinity uptake as needed, supporting accelerated coral calcification, and feedings of protein rich powdered food and liquid amino acids are given twice weekly to increase heterotrophic nutrient uptake.

In the indoor Lirman wet lab, water quality was improved significantly with the addition of three sequential bag filters (25µm, 5µm, 1µm) and a second 150W high-output UV

sterilizer. When used in combination, this system removes fine sediments and neutralizes potentially harmful microbes from the incoming, flow-through seawater supplying the lab's raceways. A Neptune Apex system was installed to provide round-the-clock monitoring of the temperature, salinity, pH, and oxidative reduction potential of the incoming seawater. These data will be used to evaluate the need for any future water quality improvement systems in order to provide a high standard of care for the corals and other organisms in our care.

Additionally, we focused on two key pillars of corals' health in ex situ systems: light and water movement. We replaced 10-year-old T5 fluorescent light fixtures with LED strip lights. Several of the old fixtures were rusted and malfunctioning from years of use in proximity to salt water. The new LED lights allow improved access to the tanks facilitating regular care and maintenance tasks, produce significantly less heat than T5 fluorescent lights providing more stable water temperatures, and allow for much finer control of the timing, intensity, and spectrum of light provided to each tank. These programmable LEDs also have an acclimation mode that allows practitioners to dim the lights to a set amount and slowly raise the intensity over a period of days to months. This feature is very useful for treating bleached and stressed corals.

Water movement was greatly improved with the installation of programmable, variable-speed powerheads on all tanks, replacing the old single-speed circulation pumps used previously. By running at variable speeds, the new pumps more closely mimic typical in situ water movement characteristics and reduce "dead zones" where detritus can collect and lead to blooms of opportunistic organisms. The new powerheads are also significantly easier to remove and clean, facilitating routine maintenance tasks and are connected to battery backups that will keep water circulating in the tanks (at a reduced rate) in the event of a power failure.

Finally, we renovated the top row of shallow water tables (previously used to house cables) to accommodate up to 12 replicate 3-gallon aquaria for experiments and for quarantining potentially diseased corals. Canopies were constructed over the tables to house cables and LED strip lights were installed above the water tables.

Photos and schematics of the improvements to coral systems can be found in the "Infrastructure improvements" folder on the Google Drive.

Task 5 - Provide coral care to support the 2023 Florida bleaching response emergency efforts

Approximately 1,700 colonies of massive species and 350 acroporid colonies were collected from Miami's in situ nurseries and brought to holding tanks at the University of Miami's Rosenstiel School of Marine, Atmospheric, and Earth Science and Experimental Hatchery facilities. To house large acroporid colonies, a custom frame system was designed and built using upcycled nursery structures to suspend the corals in holding tanks and achieve water movement on all sides of each colony. For massive species, each plug was individually cleaned to remove biofouling organisms. Corals were kept at temperatures equal to those being measured at the in-situ nurseries (below bleaching temperatures) and were monitored multiple times per day to ensure any problems were responded to rapidly. In the event of tissue loss, the diseased portion of the colony was resected, and the remaining portion(s) were treated with Lugol's iodine

solution and held in separate raceways where treatments with Lugol's and/or amino acids continued as needed. Water quality parameters were monitored and can be found in the "ICP analyses" folder on the Google Drive.

Rescued corals remained relatively stable in land-based systems. Cases of tissue loss were minor and typically related to competition with other nearby colonies and excessive flow from adjacent water pumps after routine cleaning and repositioning. These issues were generally remedied quickly. However, some persistent cases of tissue loss continued to occur in some acroporids. In these cases, colonies were divided into two pieces and the unaffected fragment transferred to a separate raceway. Consequently, although there was some partial mortality among some fragments, there was no loss of genotypes during their time in holding.

Fortunately, UM's offshore coral nurseries did not experience mass bleaching and mortality as was observed further south in Florida. The prevalence of complete bleaching in corals at the offshore nurseries remained below 10% for the duration of the summer despite their accumulating 12 degree heating weeks (DHW) by late September (see Fig. 3). However, certain *Acropora* genotypes (two *A. cervicornis* and one *A. palmata*) were

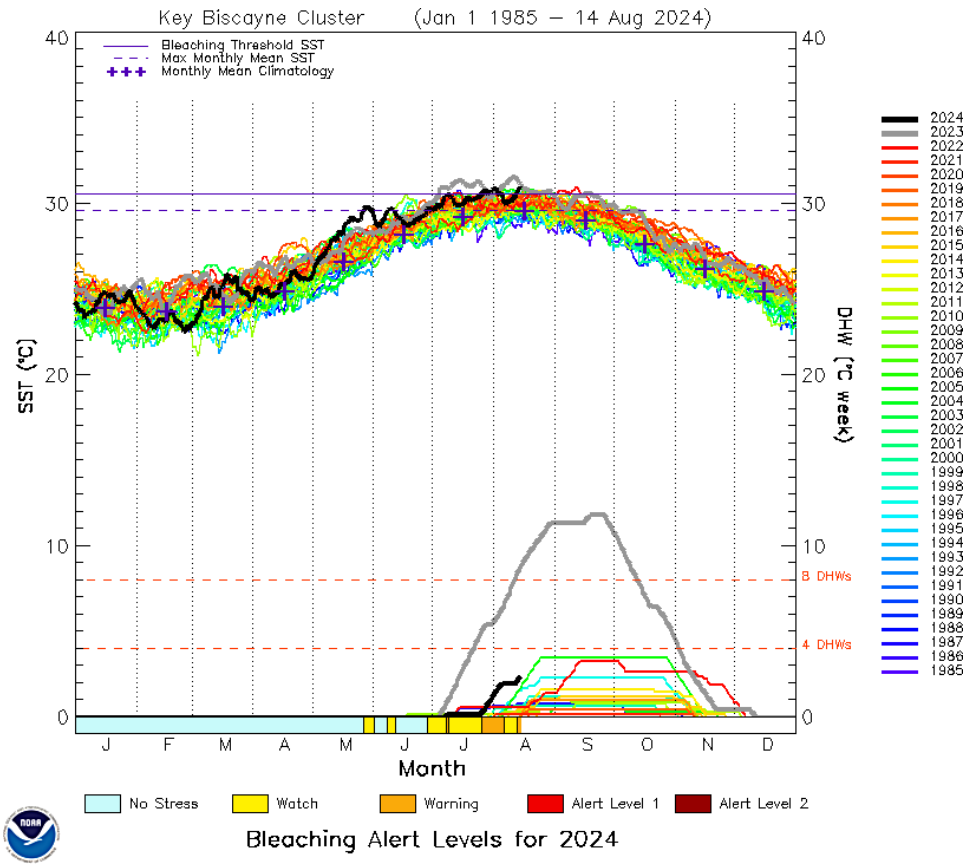

Figure 3: Heat stress accumulation (measured in degree heating weeks, DHWs) at Key Biscayne nursery sites during the marine heatwave of 2023 (lower panel, grey line). Peak heat stress of 12 DHWs was reached by late September 2023. Image from: https://coralreefwatch.noaa.gov/product/vs_single_pixel_exp/florida_keys.php

observed early in the summer to be bleaching more severely than other genotypes. These genotypes experienced 100% mortality at the offshore nurseries by the end of the summer, indicating that the land-based rescue effort indeed led to the preservation of this genetic diversity within our subregion. Samples of these genotypes, recovered from the land-based facility, are currently being grown and propagated in both in- and ex-situ nurseries. No genotypes of any massive species were lost from either nursery (in- or ex-situ) during the summer.

These corals were kept on land for a period of 2.5 months until seawater temperatures returned to levels deemed safe for return to the local UM coral nurseries. Prior to their return to the nurseries, fragments were taken of individual genotypes to provide redundant backups, which are now being propagated in the land-based facility.

Social media photos and captions

Figure 4: As part of the 2023 Florida bleaching response emergency efforts, the University of Miami evacuated over 2,000 coral colonies from coral nurseries in Miami-Dade County and maintained them in land-based facilities for 2.5 months.

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