Investigating stony coral tissue loss disease (SCTLD) susceptibility in brain coral recruits

[Alt text: Progression of stony coral tissue loss disease (SCTLD) lesion across a *Colpophyllia natans* recruit over time.]

Investigating stony coral tissue loss disease (SCTLD) susceptibility in brain coral recruits

Final Report

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June 15, 2023

Completed in Fulfillment of Award No. C05F10 for

Florida Department of Environmental Protection
Coral Protection and Restoration Program
8000 N Ocean Dr.
Dania Beach, FL 33004

This report should be cited as follows:
Williamson OM, Baker AC, O'Neil KL. 2023. Investigating stony coral tissue loss disease (SCTLD) susceptibility in brain coral recruits. Florida DEP. Miami FL. 1-16.

This report was prepared for the Florida Department of Environmental Protection's (DEP) Coral Protection and Restoration Program by the University of Miami. Funding was provided by the DEP Award No. C05F10. The views, statements, findings, conclusions, and recommendations expressed herein are those of the authors and do not necessarily reflect the views of the State of Florida or any of its sub-agencies.

UNIVERSITY OF MIAMI
ROSENSTIEL SCHOOL of MARINE, ATMOSPHERIC, & EARTH SCIENCE

FLORIDA'S CORAL REEF

CORAL REEF FUTURES LAB MIAMI

Executive Summary

Although stony coral tissue loss disease (SCTLD) has been well documented in adult colonies, little is known regarding disease susceptibility and dynamics in coral juveniles. Such information is particularly relevant to restoration efforts using sexually produced recruits, which may be compromised by unknown levels of SCTLD-related mortality. Here, we exposed recruits of three "highly-susceptible" coral species – *Diploria labyrinthiformis, Colpophyllia natans,* and *Pseudodiploria strigosa* – to SCTLD to investigate differential susceptibility. Parents of these recruits included both "pre-invasion" colonies (that are naïve to SCTLD and putatively susceptible to it) and "endemic" colonies (that are presumed to have been exposed to SCTLD and are putatively more resistant). Rates of infection and mortality were remarkably similar across species, but some recruits of all three species remained apparently healthy throughout exposure. In all species, chlorophyll fluorescence of algal symbionts in coral tissue along lesion margins declined sharply, indicating significant impairment. Our results indicate that SCTLD is likely to be a major risk for coral recruits outplanted in the endemic and emergent zones, but provide evidence for differential susceptibility within species and cohorts. Next steps involve processing samples for SNP genotyping and 16S microbiome analysis to investigate whether elements of SCTLD resistance (through genetics or microbiome constituents) may be heritable and help explain any of the differential susceptibility found in this experiment.

Acknowledgements

This research was funded by the Florida Department of Environmental Protection's Office of Resilience and Coastal Protection. The authors thank the Florida Fish and Wildlife Commission and The Florida Aquarium for providing the parent colonies that spawned at The Florida Aquarium through the Coral Rescue Project. Parent corals were collected under FKNMS Superintendent's permit FKNMS-2017-100, and larvae were transferred to the University of Miami under Animal Transaction Agreements (ATTN: 22CFC025, ATTN: 22CFC037) with the Florida Aquarium (USDA LIC# 58-C-0977, AZA# 2305000). The authors also thank those who helped facilitate the collection of diseased colonies, in particular Dr. Karen Neely and Dr. Andrew Bruckner. Finally, we would like to acknowledge the help of Victoria Barker, Kristi Kerrigan, Joanna Walczak, and Maurizio Martinelli for their assistance throughout this project.

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List of Acronyms

SCTLD: Stony coral tissue loss disease

GLM: Generalized linear model

1. BACKGROUND

1.1. Overview

Stony coral tissue loss disease (SCTLD) has devastated coral reefs throughout the Florida and the Caribbean, causing widespread mortality in nearly two dozen scleractinian species (Precht et al., 2016). Despite well-documented impacts on adult colonies (Precht et al., 2016; Walton et al., 2018; Álvarez-Filip et al., 2019; Sharp et al., 2020; Kolodziej et al., 2021; Neely et al., 2021; Spadafore et al., 2021), there is comparatively little information on how the disease has impacted coral early life stages (Williamson et al., 2022). Common methods used to survey and quantify disease prevalence typically omit colonies ≥4 cm in diameter, thus excluding recruits (Precht et al., 2016; Gilliam et al., 2019; Kramer et al., 2019), and only one study has experimentally exposed juveniles to SCTLD to test their susceptibility (Williamson et al., 2022). Without documenting SCTLD dynamics during coral early life stages, we have likely underestimated the true scale of the disease's impact on Caribbean reefs. In addition, reef restoration efforts using sexually-produced juveniles may be undermined if juvenile health and/or survival are indeed compromised by SCTLD. If populations of susceptible species are to recover, it will be important to investigate the extent to which new generations of corals are susceptible or resistant to disease, especially those produced through assisted reproduction for reef restoration purposes.

Despite the staggering magnitude of losses triggered by this SCTLD, not all colonies appear to be equally susceptible. Numerous observations in the field have reported how some colonies have remained apparently healthy despite high disease prevalence and rapid tissue loss in adjacent conspecifics (Sharp et al. 2020; Guzmán-Urieta and Jordán-Dahlgren, 2021). Such patterns suggest the existence of differentially resilient colonies or communities that fail to succumb even in the midst of severe disease outbreaks (Sharp et al. 2020). Many different factors could help explain this variable proliferation, including fine-scale genetic variation that confers some degree of relative resistance in some corals. Since many traits, such as heat tolerance, can be passed onto the next generation in corals (Quigley et al., 2020; Howells et al., 2021; Weeriyanun et al., 2022), there may also be heritable components to disease resistance.

Here, we investigated the potential for differential SCTLD susceptibility in six-month-old *Colpophyllia natans* and *Pseudodiploria strigosa* and nine-month-old *Diploria labyrinthiformis* from a variety of known parent colonies spawned in captivity. By supplying them with water from around infected adult colonies and tracking their survival and lesion progression, we tracked the fate of each recruit throughout exposure and characterized susceptibility by lesion progression, chlorophyll fluorometry, and recruit mortality. This report covers Phase 1, which examines the physiological results of this experiment. In Phase 2, we will investigate the heritability of SCTLD resistance, either through genetics or inherited microbiome components, in these recruits.

1.2. Project Goals

This research (Phase 1) aimed to further elucidate dynamics of SCTLD in coral recruits by (1) observing responses in additional species (i.e., *P. strigosa*), (2) measuring

chlorophyll fluorescence throughout exposure in addition to quantifying survival and lesion progression, and (3) generate genetic and microbiome samples in recruits that survived exposure in order to conduct future investigations of the potential heritability of disease resistance. Phase 2 of the project, to be completed in FY24 (under renewed DEP funding), will investigate whether genetics or microbiome constituents help explain any of the differential susceptibility found in the current (FY23) experiment.

2. METHODS

2.1. Pre-experiment

2.1.1. Gamete collection and fertilization

This study utilized coral recruits collected as gametes from *ex-situ* induced spawning facilities at the Florida Aquarium's Center for Conservation. Parent colonies had been collected by the Florida Fish and Wildlife Conservation Commission as part of the Coral Rescue Project (under FKNMS Superintendent permit FKNMS-2017-100), after which they were loaned to The Florida Aquarium for long-term care.

On May 28, 2022, seven *Diploria labyrinthiformis* colonies from the Florida Keys spawned, and their gametes were pooled in approximately equal quantities to make a cohort of offspring (Table 1). All parent colonies had been removed from the reef prior to SCTLD arriving, thus they are putatively "naïve" to the disease having never been exposed to it.

On August 19, 2022, four *Colpophyllia natans* colonies spawned, and their gametes were pooled in approximately equal quantities to make a cohort of offspring (Table 1). These included two "pre-invasion" colonies (putatively naïve to disease) and two colonies from the endemic zone (which presumably had been exposed to SCTLD in the wild).

On August 19, 2022, two *Pseudodiploria strigosa* colonies spawned, and their gametes were pooled in approximately equal quantities to make a cohort of offspring. These included one "pre-invasion" colony (putatively naïve to disease) and one colony from the endemic zone (which presumably had been exposed to SCTLD in the wild). On August 20, 2022, three *P. strigosa* pre-invasion colonies spawned, and their gametes were pooled in approximately equal quantities to make a cohort of offspring (Table 1). Upon transport to the University of Miami, these two *P. strigosa* cohorts were combined and reared as one batch.

Table 1: Parent information for each cohort of recruits used in the experiment. All parent corals spawned in ex-situ induced spawning tanks at the Florida Aquarium's Center for Conservation in Apollo Beach, FL. Parent colonies were originally collected from various locations of Florida's Coral Reef by the Florida Fish and Wildlife Commission as part of the Coral Rescue Project.

Species	Date	Collection zone	Parent ID	Relative contribution	No. of recruits in experiment
Diploria labyrinthiformis	05/28/22	Pre-invasion Pre-invasion Pre-invasion Pre-invasion Pre-invasion Pre-invasion Pre-invasion	10_DLAB_006 22_DLAB_003 39_DLAB_001 BW16_DLAB_002 BW24B_DLAB_001 BW5_DLAB_007A IRMA 109_DLAB_003	High Medium Medium Low Low High Low	237
Colpophyllia natans	08/19/22	Endemic Pre-invasion Pre-invasion Endemic	V1164_CNAT_017 IRMA 109_CNAT_003 5753_CNAT_004 1160_CNAT_015	Medium Medium Medium Medium	450
Pseudodiploria strigosa	08/19/22 08/20/22	Pre-invasion Endemic Pre-invasion Pre-invasion Pre-invasion	5830_PSTR_001 T1081_PSTR_005 IRMA 109_PSTR_002 5830_PSTR_001 IRMA 109_PSTR_001	Medium Medium Medium Medium Medium	493

[Alt text: Table showing parent information for three species of coral recruits used in the SCTLD exposure experiment.]

2.1.2. Recruit rearing

In all species, swimming larvae were transferred to an indoor laboratory at the University of Miami's Rosenstiel School of Marine, Atmospheric, and Earth Science, where they were reared in UV-sterilized, one-micron filtered seawater (FSW). Each batch of larvae was settled on CCA-conditioned ceramic tiles (Boston AquaFarms, USA). After settlement, recruits were provisioned with Symbiodiniaceae by inoculating them with approximately one million cells per liter of cultured *Breviolum* or *D. trenchii* twice per week until all recruits were visibly infected (approximately three weeks). Recruits were fed twice per week with 5-50 micron Golden Pearls (Aquatic Foods and Blackworm Co.). Tiles with recruits were regularly cleaned by hand to minimize algal growth, until recruits were several months old, when juvenile *Lithopoma* snails were introduced as grazers.

2.1.3 Pre-experiment sampling

One week before the experiment began in late February 2023, each plug with recruits was individually tagged for tracking. At this point, six recruits from each species were sampled to characterize their Symbiodiniaceae communities. Briefly, small tissue biopsies (<0.25 cm^2) were taken from each recruit using a razor blade and preserved in

1% SDS, genomic DNA was extracted following modified organic extraction methods (Rowan and Powers, 1991; Baker & Cunning, 2016), and quantitative PCR (qPCR) assays were used to identify Symbiodiniaceae to genus level following reactions described in Cunning and Baker (2013) using a QuantStudio 3 Real-Time PCR Instrument (Applied Biosystems, USA).

In addition, small tissue biopsies were also taken from recruits for SNP genotyping (preserved in molecular-grade ethanol) and 16S microbiome analysis (preserved in DNA/RNA shield). In larger recruits (mostly *D. labyrinthiformis*), non-lethal samples were taken. In smaller recruits (mostly *C. natans* and *P. strigosa*), recruits were sacrificed to obtain enough tissue (~1 mm^2) for each sample. Recruits sampled non-lethally were allowed to recover for one week after sampling before the experiment began.

2.2. SCTLD exposure experiment

Plugs were then distributed randomly into eight 2.5-gallon aquaria, so that each contained approximately equal numbers of each species. As they were transferred, recruits were counted and photographed using a Zeiss Stemi 305 LAB microscope (ZEISS International, Jena, Germany) to obtain baseline data for the initial time point. All experimental aquaria were immersed in a larger 50-gallon seawater tank to maintain temperature, which was held at 27 +/- 0.5°C using heaters. Light (70–90 µmol quanta m−2 s−1, measured by an Apogee Underwater Quantum PAR Meter MQ‑210) was maintained on a 12h:12h light:dark cycle using AI Hydra lights (AI Inc., USA).

Following methods described in Williamson et al. (2022), four aquaria were supplied with seawater from another tank containing colonies of multiple species with active SCTLD lesions (approximately ~60 cm^2 of tissue in total infected at the start of the experiment), which were sloughing off tissue and mucus that were presumed to contain the disease causative agent (Dobbelaere et al., 2020). Species present in this "disease bath" included *Colpophyllia natans, Montastraea cavernosa*, and *Pseudodiploria strigosa*, that had just been collected from the Florida Keys by Dr. Karen Neely, each of which presented with varying degrees of SCTLD severity (FKNMS, 2018; Landsberg et al., 2021; Meiling et al., 2021). The disease bath received an exchange rate of 200 L per hour of UV-sterilized seawater. Water was dripped from the disease bath at 2L per hour into four of the aquaria to create the "disease" treatment. The other four control aquaria were supplied with were supplied with seawater from another tank containing apparently healthy colonies of the same species represented in the disease bath, plus *D. labyrinthiformis* and *Orbicella faveolata* (approximately ~60 cm^2 of apparently healthy tissue present). Water was dripped from the healthy bath at 2L per hour into each control aquarium. In all aquaria, one 4W submersible pump (VicTsing CAAA3-HG16) was placed into each aquarium to circulate water evenly.

Recruits in both control and disease aquaria were fed twice per week, and algae were manually removed from plugs to prevent overgrowth and/or competition. Recruits and diseased colonies were rotated within their respective tanks daily to avoid proximity bias, and aquaria were shuffled every other day to minimize small differences in light. Every other day, the Zeiss Stemi 305 LAB microscope was used to count the number of surviving and dead recruits, as well as score each recruit into one of the following

condition categories: apparently healthy (normal appearance, no lesions visible); septa showing (no visible lesion but tissue appearing thin, with septa protruding in some places); lesioned (visible lesion and tissue loss); and dead (all tissue lost). Pictures of each recruit and plug were taken under the microscope to document condition and lesion progression. In addition, recruits were measured for photosynthetic efficiency (Fv/fm) using a MINI-PAM (Walz, Germany).

Disease exposure ended when all three species reached approximately 50% mortality (which we will refer to as the lethal dose at which half of the recruits died [LD50]). The drip of diseased water was stopped, and all surviving recruits that had not developed lesions were sampled (as relatively "resistant" or "less susceptible" recruits) for analysis of symbiont identity and density as described above. They were also sampled for SNP genotyping and 16S microbiome analyses as described above, which will be processed in Phase 2 of this project (with FY24 DEP funding).

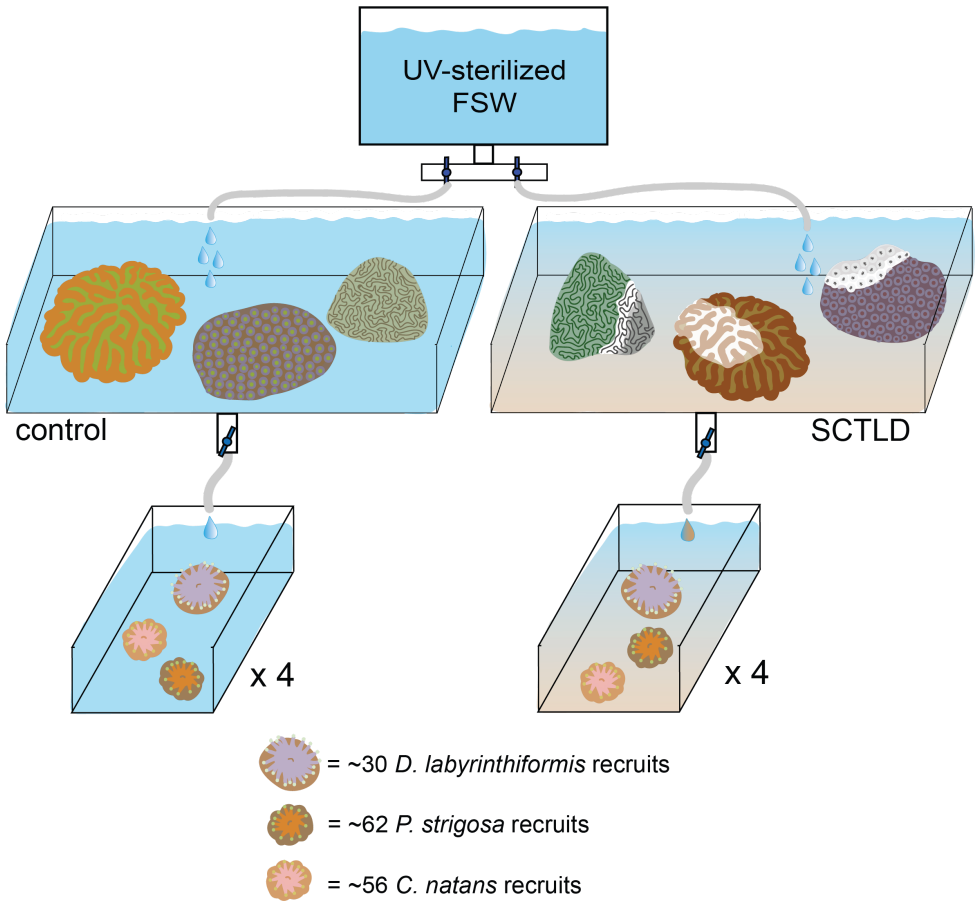

Figure 1: Experimental design. Nine-month-old *Diploria labyrinthiformis* recruits (n=237) and six-month-old *Colpophyllia natans* (n=450) and *Pseudodiploria strigosa* recruits (n=493) were exposed to either control or SCTLD treatments for 16 days. Each experimental aquarium contained an equal number of plugs of all three species, which were regularly rotated to minimize differences in light.
[Alt text: Illustration showing experimental setup, with three species of brain coral recruits exposed to control and disease treatments.]

2.3. Data analysis

Photographs from each time point were utilized to confirm recruit condition and lesion progression. In the Fv/Fm data, areas of interests (AOIs) were assigned condition categories based on the health of the tissue below them; apparently healthy, recruit with septa showing, apparently healthy tissue on a recruit with lesion(s) elsewhere, and along the lesion margin (Fig. 2). Since control recruits did not develop lesions or show septa, they were not similarly categorized for analysis. R was used to run logistic regression models using the glm (generalized linear model) function comparing recruit survival and Fv/Fm over time by species and treatment. Models were fitted with logit links to account for binomial (survivorship) and quasibinomial (Fv/Fm) distributions in the data.

Quantitative PCR (qPCR) assays were used to identify Symbiodiniaceae to genus level and quantify symbiont-to-host (S:H) cell ratios for each recruit sampled. Assays targeting specific actin loci for *Breviolum, Durusdinium* and coral were performed using a QuantStudio 3 Real-Time PCR Instrument (Applied Biosystems, USA) and followed reactions described in Cunning and Baker (2013). The StepOneR software package in R was used to quality-filter assay results, calculate relative abundance of each symbiont, and compute S:H cell ratios. GLMs were created to compare the effects of experimental treatments and species on algal community composition and S:H cell ratios.

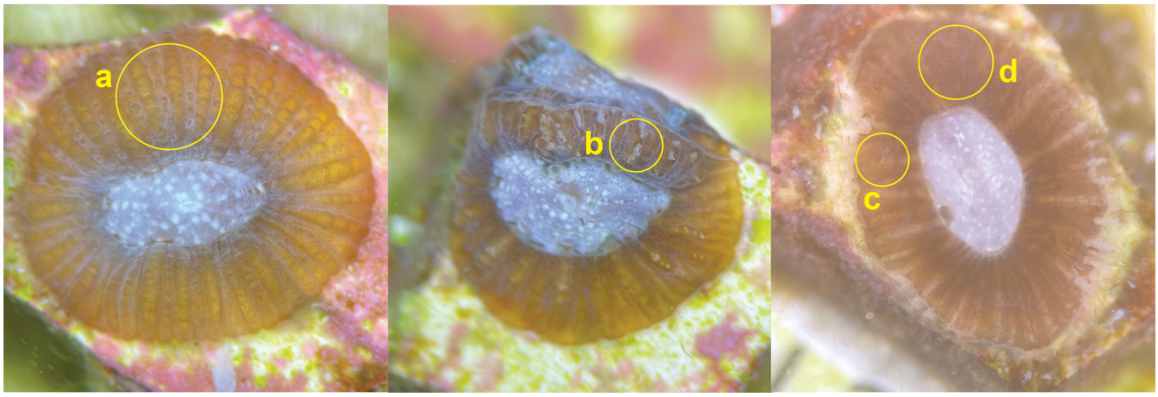

Figure 2: Examples of how areas of interest (AOIs) on Fv/fm measurements of disease-exposed recruits were categorized for data analysis. (a) Apparently healthy recruit, (b) recruit with septa showing; (c) lesion margin; (d) lesioned recruit, apparently healthy tissue.
[Alt text: Images showing differing responses to SCTLD exposure in *D. labyrinthiformis* recruits. Some show no signs of stress or disease, while others show protruding septa, lesions, and/or tissue loss.]

3. RESULTS

3.1. Lesion progression

In all three species, some recruits began to show signs of distress (such as thinning tissue that showed septa) and develop lesions immediately (Fig. 3, 4, 5), while others remained apparently healthy. Indeed, some recruits of all three species continued to appear healthy, with normal appearance, normal coloration, and thick tissue, throughout

all 16 days of exposure, despite considerable infection and mortality occurring in adjacent recruits in the same treatment. In some cases, recruits on a single plug showed variation in their responses, with some losing all tissue while others appeared normal and healthy.

Lesions resembled those described in surveys of adult conspecifics infected with SCTLD (FKNMS, 2018; Landsberg et al., 2021), as well as those documented in Williamson et al. (2022) in *C. natans* and *D. labyrinthiformis* recruits. In general, lesions appeared on a polyp's outer edge(s) before progressing towards the center and across the recruit. Although most tissue loss was focal in nature, some recruits exhibited multifocal tissue loss whereby lesions progressed from more than one area. In some recruits, lesion progression stalled for many days before tissue loss began again (Fig. 5), reflecting the "chronic necrosis" described by Landsberg et al. (2021) in adult colonies.

Figure 3: Representative time-series photos of lesion progression across a six-month-old *Colpophyllia natans* recruit experimentally exposed to SCTLD. The recruit progressed from apparently healthy to dead in five days. Scale bar represents 1 mm.
[Alt text: Images of lesion progression across a six-month-old *Colpophyllia natans* recruit experimentally exposed to SCTLD.]

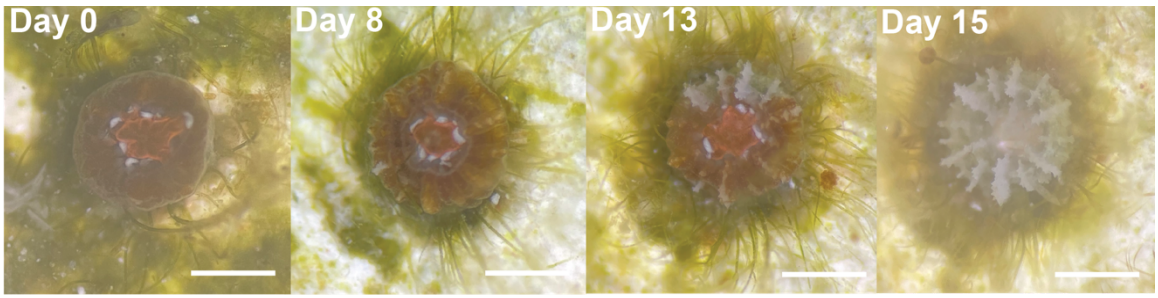

Figure 4: Representative time-series photos of lesion progression across a six-month-old *Pseudodiplora strigosa* recruit experimentally exposed to SCTLD. The recruit first developed the beginning of a visible lesion on day 8, but tissue was not fully lost until day 15. Scale bar represents 1 mm.
[Alt text: Images of lesion progression across a six-month-old *Pseudodiplora strigosa* recruit experimentally exposed to SCTLD.]

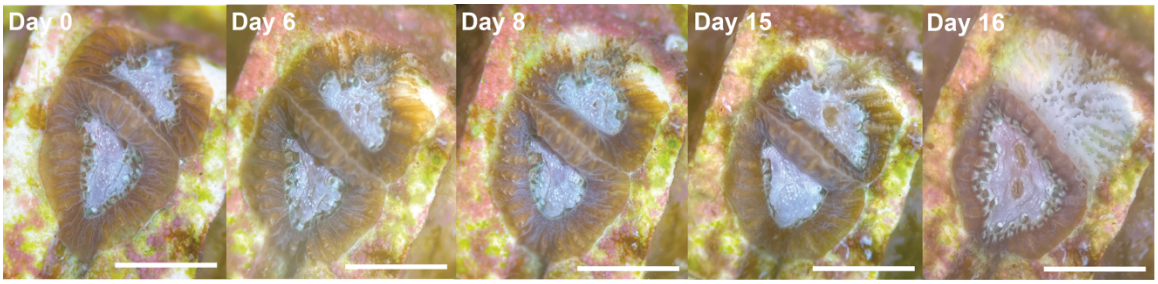

Figure 5: Representative time-series photos of lesion progression across a nine-month-old *Diploria labyrinthiformis* recruit experimentally exposed to SCTLD. The recruit first showed lesions on day 6, which then progressed relatively slowly until progressing more quickly between days 15 and 16. On day 16, the recruit had fully lost all tissue (died). Interestingly, another recruit bordering the diseased one did not show signs of lesions by day 16. Scale bar represents 5 mm.
[Alt text: Images of lesion progression across a nine-month-old *Diploria labyrinthiformis* recruit experimentally exposed to SCTLD.]

3.2. Survivorship

Recruits of all three species in the SCTLD exposure treatment showed strikingly similar rates of mortality (Fig. 6). On day 16, all three species were very close to 50% mortality, so this was identified as the LD50; *C. natans* had exactly 50% survivorship, while *D. labyrinthiformis* had 48.3% and *P. strigosa* had 56.2%. No recruits in the control treatment died during the experiment.

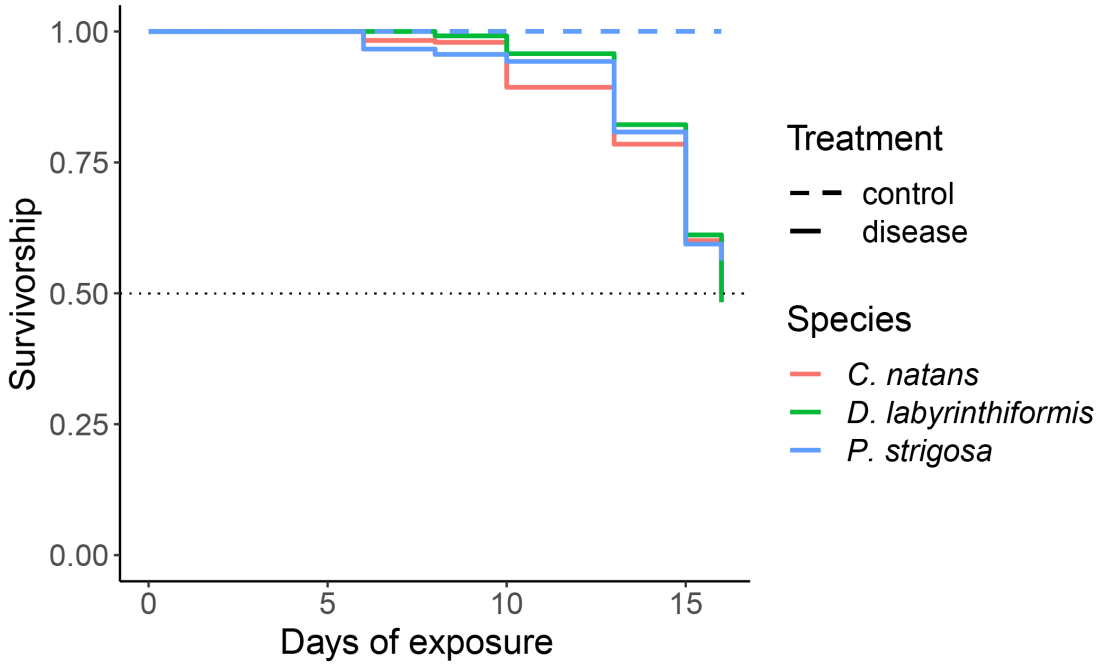

Figure 6: Survivorship of three species of recruits exposed to SCTLD. Dotted line indicates 50% survivorship (LD50), which was reached in all three species around 16 days of exposure.
[Alt text: Plot showing survivorship coral recruits experimentally exposed to SCTLD.]

3.3. Chlorophyll fluorescence

Patterns of Fv/Fm were largely consistent across all three species. The only significant declines in Fv/Fm were found in AOIs along the lesion margins (Fig. 7, 8). However, all other areas of recruits exposed to disease – including healthy tissue and recruits with septa showing – did not significantly differ from controls. These patterns proved to be fairly consistent across all time points (Fig. 8).

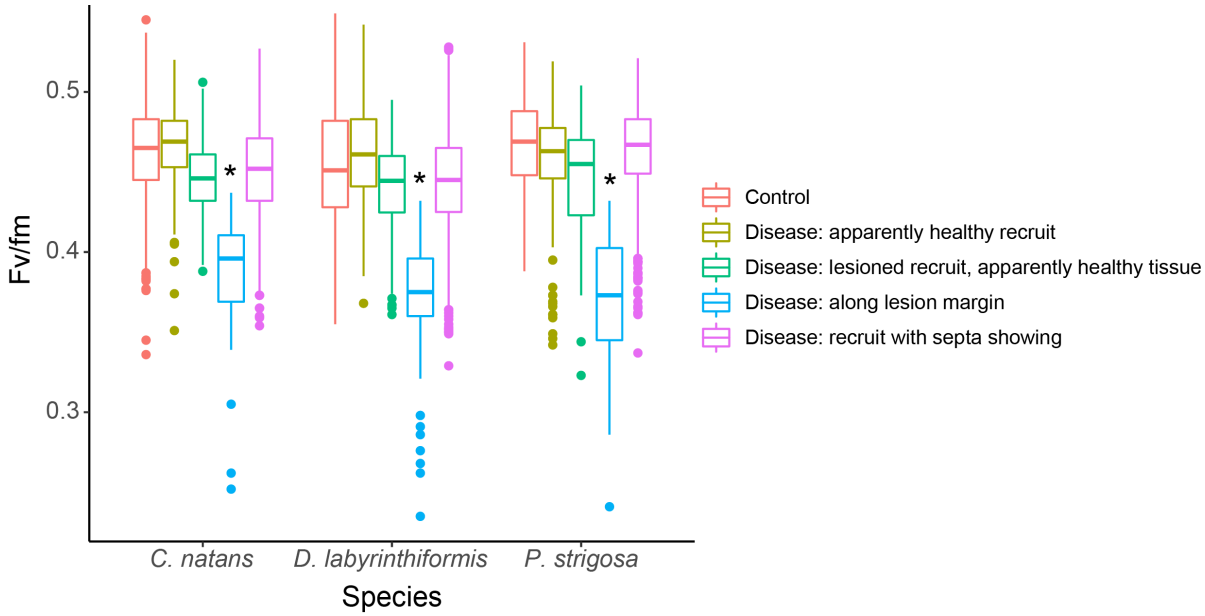

Figure 7: Chlorophyll fluorometry (Fv/Fm) measurements in three species of coral recruits exposed to SCTLD for 16 days. Data from all time points are combined. Asterisk denotes statistical significance.
[Alt text: Plot showing chlorophyll fluorometry (Fv/Fm) measurements in three species of coral recruits exposed to SCTLD for 16 days.]

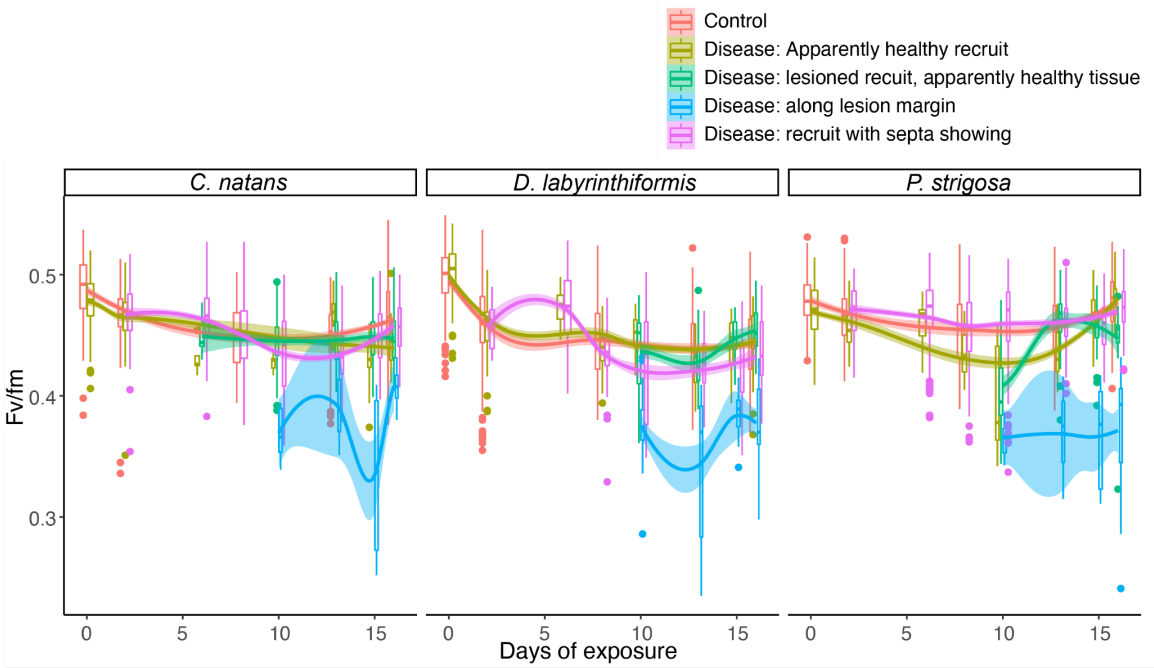

Figure 8: Chlorophyll fluorometry (Fv/Fm) measurements over time in three species of coral recruits exposed to SCTLD. Lines represent data smoothed using the "loess" method, and shaded area represents the 95% confidence interval.
[Alt text: Plot showing chlorophyll fluorometry (Fv/Fm) measurements over time in three species of coral recruits exposed to SCTLD.]

3.4. Symbiodiniaceae communities

Symbiont load (expressed as S:H cell ratios) varied between species, but not within them (Fig. 9). In all treatments, each species had significantly different S:H cell ratios than each of the other species ($p < 0.05$); *C. natans* exhibited the highest mean S:H cell ratios, followed by *D. labyrinthiformis* and *P. strigosa*. In all species, S:H declined slightly throughout the experiment, with slightly more pronounced declines in disease-exposed recruits compared to controls, but these declines were not statistically significant.

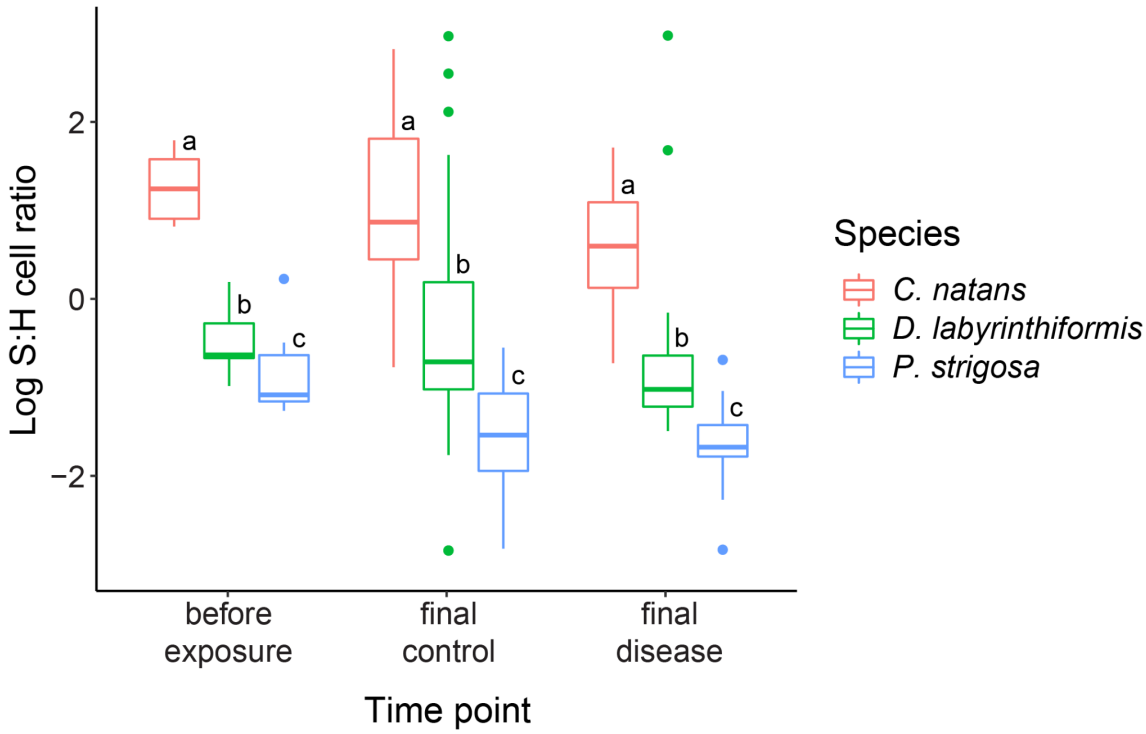

Figure 9: Symbiont load in coral recruits before and after SCTLD exposure. Symbiont-to-host (S:H) cell ratios have been log-transformed. Letters indicate significant differences between species.
[Alt text: Plot showing symbiont load in three species of coral recruits before and after SCTLD exposure.]

Symbiodiniaceae community composition was not found to change significantly with treatment. The majority of recruits prior to exposure hosted primarily *Durusdinium*, and in both control and disease-exposed treatments after exposure hosted primarily *Durusdinium* (Fig. 10). In *C. natans* and *P. strigosa*, recruits in both control and disease-exposed treatments shifted towards *Durusdinium* throughout the experiment, though this shift was only statistically significant between the before-exposure and final-control recruits (Fig. 10). However, *D. labyrinthiformis* exhibited a slight increase in *Breviolum* prevalence in disease-exposed corals throughout the experiment. There were no significant differences in the proportion of *Durusdinium* or *Breviolum* between species under any treatment.

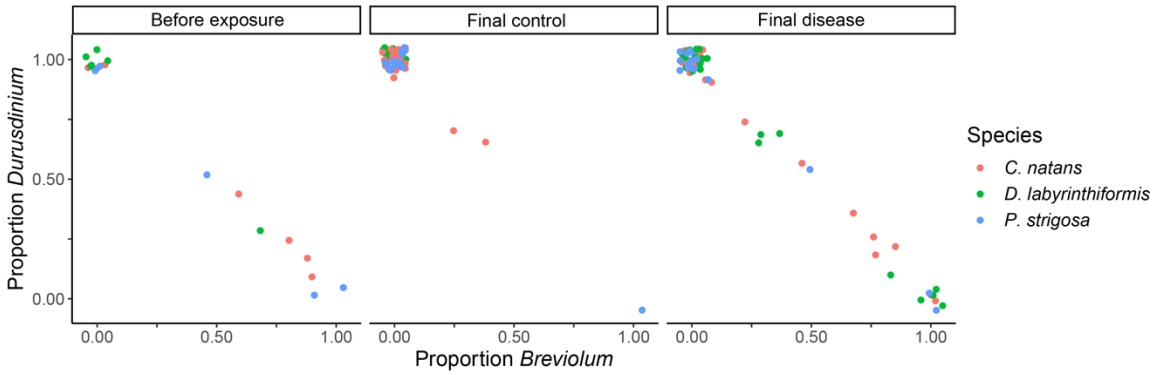

Figure 10: Proportion of *Durusdinium* versus *Breviolum* hosted by recruits before and after SCTLD exposure. Each point represents one recruit.
[Alt text: Plot showing proportion of *Durusdinium* versus *Breviolum* hosted by coral recruits before and after SCTLD exposure.]

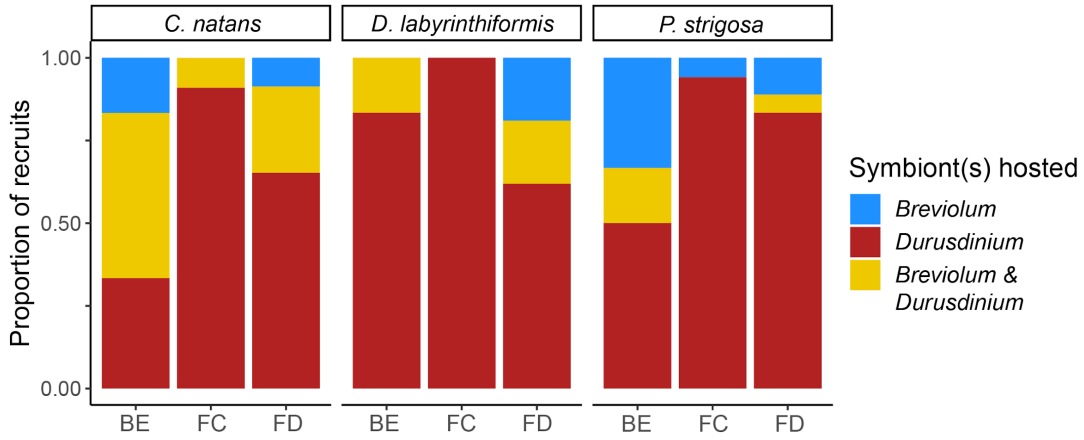

Figure 11: Proportion of recruits hosting different genera of Symbiodiniaceae. "BE" = before exposure (initial time point, a few days prior to the experiment beginning and prior to recruits being assigned to control or disease treatments); "FC" = final control; "FD" = final disease.
[Alt text: Bar graph showing proportion of coral recruits hosting different genera of Symbiodiniaceae before and after an experiment.]

4. DISCUSSION

4.1. Recruit susceptibility and response to SCTLD

4.1.1. Lesion progression and mortality

The three species in this experiment – *Colpophyllia natans, Diploria labyrinthiformis,* and *Pseudodiploria strigosa* – are considered "highly susceptible" to SCTLD (FKNMS, 2018). In all three species, recruits showed a variety of responses to SCTLD exposure. Lesion development appeared very similar to that reported in adult corals of the same species (FKNMS 2018; Landsberg et al., 2021) and in a previous study

with recruits (Williamson et al., 2022); lesions mainly appeared on the margin of a polyp before spreading across it, with instances of both focal and multifocal tissue loss.

Rather than developing lesions, some recruits in the disease treatment were observed to have their septa showing visibly. This condition was not observed to be a precursor to lesion development. Protruding septa through thinned tissue has been observed as a generalized stress response in other experiments with recruits exposed to high temperatures and other stressful conditions.

All three species showed remarkably similar rates of mortality throughout the experiment, with the first mortality observed after six days of SCTLD exposure. The LD50 was determined to be day 16, when ~50% of recruits of each species had died. Because the pathogen remains unknown, we cannot quantify the dose delivered. However, because of the relatively small volumes of seawater used in this experiment (both tank size and turnover rate) it is likely that our pathogen dose was higher than recruits would naturally experience on the reef, where pathogens are likely to be far more diluted.

4.1.2. SCTLD and Symbiodiniaceae

All three species showed very similar Fv/Fm to one another across treatments and time, with significant declines only in AOIs adjacent to active lesions. It is not surprising that lesions might trigger declines in Fv/Fm, given that a coral's algal symbionts have been implicated in SCTLD progression (Landsberg et al., 2020; Dennison et al., 2021; Rubin et al., 2021; Work et al., 2021). However, it is noteworthy that only areas immediately adjacent to lesions show responses in symbionts, not areas further away from these lesions. This suggests that the symbiont response may be very localized to the area near active lesions, rather than spread throughout a coral colony ahead of the disease margin.

Symbiont load (S:H cell ratio) was found to be significantly different between species across time and treatments, but did not appear to be impacted by SCTLD exposure. This may be due to our samples mostly originating from apparently healthy recruits, which had survived 16 days of disease exposure and still appeared relatively normal without lesions. Perhaps in very localized areas of diseased recruits (i.e., immediately adjacent to the lesion) declines in S:H cell ratio may be detectable, but this would require much smaller samples to be taken at the time of lesion progression as well as more sensitive detection protocols.

In addition, our data do not associate specific Symbiodiniaceae genera with survival or mortality, since the majority of recruits hosted primarily *Durusdinium* from before exposure and these communities did not change during the course of the experiment, although slight shifts towards hosting a higher proportion of *Durusdinium* were observed in *C. natans* and *P. strigosa*. However, these shifts occurred in both control and disease-exposed recruits, so this is likely due to the experimental conditions (e.g., temperature, light), rather than the disease exposure itself. Additional research will be necessary to determine whether hosting different types of algal symbionts can impact a coral recruit's relative susceptibility and/or resistance to SCTLD (Dennison et al., 2021).

4.2. Implications for management

This study has implications for coral reef management practices in regions where SCTLD is endemic, emergent, or may emerge. In particular, our results add to a growing body of evidence that coral recruits and juveniles may be just as susceptible to SCTLD as their adult counterparts (Williamson et al., 2022), which has the potential to impact restoration projects that rear and outplant sexually-produced juveniles.

High rates of mortality are common in outplanted coral juveniles, often attributed primarily to predation. However, our results indicate that SCTLD may also contribute to recruit mortality in areas where the disease is present. As such, we suggest that restoration practitioners working with coral recruits consider the risk of SCTLD as they breed and outplant, perhaps with special attention to whether local outbreaks are occurring at the time of outplanting. In order to avoid losses of precious recruits, we suggest that juveniles of highly-susceptible species, such as the ones we tested here, not be outplanted on reefs where active outbreaks are underway.

In addition, our finding that photochemical efficiency (Fv/Fm) declines significantly near the SCTLD lesion margin provides further evidence that Symbiodiniaceae are implicated in disease proliferation, warranting future studies into how the identity of these algal partners can influence relative susceptibility (Dennison et al. 2021).

4.3. Next steps

In order to further elucidate drivers of differential susceptibility, Phase 2 of this project (to be continued with FY24 DEP funding) will involve genetic and microbiome analyses of recruits and their parents. We will SNP-genotype parents and subsets of 2021 and 2022 recruits to validate parentage of recruits and investigate whether certain parents contributed disproportionately to recruits that survived SCTLD exposure. We will also conduct 16S microbiome analysis on parents and recruits to test the hypothesis that maternally-transferred microbial communities may also contribute to heritable resistance to SCTLD, in addition to host genetics.

5. REFERENCES

Alvarez-Filip, L., Estrada-Saldívar, N., Pérez-Cervantes, E., Molina-Hernández, A., & González-Barrios, F. J. (2019). A rapid spread of the stony coral tissue loss disease outbreak in the Mexican Caribbean. *PeerJ*, *2019*(11), e8069. https://doi.org/10.7717/peerj.8069

Baker, A.C., Cunning, R. (2016) Bulk gDNA extraction from coral samples. protocols.io

Cunning, R., Baker, A.C. (2013) Excess algal symbionts increase the susceptibility of reef corals to bleaching. *Nature Climate Change, 3*, 259–262.

Dennison, C. E., Karp, R. F., Weiler, B. A., Goncalves, A., del Campo, J., Rosales, S. M., Traylor-Knowles, N., Baker, A. C. (2021). *The role of algal symbionts (genus Breviolum) in the susceptibility of corals to Stony Coral Tissue Loss Disease in South Florida*. Florida DEP, 1-23.

Eaton, K. R., Landsberg, J. H., Kiryu, Y., Peters, E. C., & Muller, E. M. (2021). Measuring Stony Coral Tissue Loss Disease Induction and Lesion Progression within Two Intermediately Susceptible Species, *Montastraea cavernosa* and

Orbicella faveolata. *Frontiers in Marine Science*. https://doi.org/10.3389/FMARS.2021.717265

FKNMS (Florida Keys National Marine Sanctuary) (2018). *Case Definition: Stony Coral Tissue Loss Disease (SCTLD)*. (Silver Spring, MD.:National Ocean Service) 1–29

Gilliam, D. S., Hayes, N. K., Ruzicka, R., & Colella, M. A. (2019). *Southeast Florida Coral Reef Evaluation and Monitoring Project*. Retrieved from https://floridadep.gov/sites/default/files/SECREMP Year 17 Executive Summary_Final_0.pdf

Guzmán-Urieta, E. O., & Jordán-Dahlgren, E. (2021). Spatial Patterns of a Lethal White Syndrome Outbreak in *Pseudodiploria strigosa*. *Frontiers in Marine Science*, *8*(669171). https://doi.org/doi: 10.3389/fmars.2021.669171

Howells, E. J., Abrego, D., Liew, Y. J., Burt, J. A., Meyer, E., & Aranda, M. (2021). Enhancing the heat tolerance of reef-building corals to future warming. *Science Advances*, *7*(34). https://doi.org/10.1126/SCIADV.ABG6070

Kramer P. R., Roth L., Lang J. (2019). Map of Stony Coral Tissue Loss Disease Outbreak in the Caribbean. Available at: www.agrra.org (Accessed August 26, 2021).

Kolodziej, G., Studivan, M. S., Gleason, A. C. R., Langdon, C., Enochs, I. C., & Manzello, D. P. (2021). Impacts of Stony Coral Tissue Loss Disease (SCTLD) on Coral Community Structure at an Inshore Patch Reef of the Upper Florida Keys Using Photomosaics. *Frontiers in Marine Science*, *8*, 682163. https://doi.org/10.3389/FMARS.2021.682163

Landsberg, J. H., Kiryu, Y., Peters, E. C., Wilson, P. W., Perry, N., Waters, Y., … Work, T. M. (2020). Stony Coral Tissue Loss Disease in Florida Is Associated With Disruption of Host–Zooxanthellae Physiology. *Frontiers in Marine Science*, *7*, 1090. https://doi.org/10.3389/FMARS.2020.576013

Meiling, S. S., Muller, E. M., Lasseigne, D., Rossin, A., Veglia, A. J., MacKnight, N., … Brandt, M. E. (2021). Variable Species Responses to Experimental Stony Coral Tissue Loss Disease (SCTLD) Exposure. *Frontiers in Marine Science*, *8*, 464. https://doi.org/10.3389/FMARS.2021.670829

Neely, K. L., Lewis, C. L., Lunz, K. S., & Kabay, L. (2021). Rapid Population Decline of the Pillar Coral *Dendrogyra cylindrus* Along the Florida Reef Tract. *Frontiers in Marine Science*, *8*, 656515. https://doi.org/10.3389/FMARS.2021.656515

Precht, W. F., Gintert, B. E., Robbart, M. L., Fura, R., & Van Woesik, R. (2016). Unprecedented Disease-Related Coral Mortality in Southeastern Florida. *Scientific Reports*, *6*(1), 1–11. https://doi.org/10.1038/srep31374

Quigley, K. M., Bay, L. K., & Oppen, M. J. H. (2020). Genome‐wide SNP analysis reveals an increase in adaptive genetic variation through selective breeding of coral. *Molecular Ecology*, *29*(12), 2176–2188. https://doi.org/10.1111/mec.15482

Rowan, R., Powers, D.A. (1991). Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae). *Marine Ecology Progress Series, 71*, 65–73

Rubin, E. T., Enochs, I. C., Foord, C., Mayfield, A. B., Kolodziej, G., Basden, I., & Manzello, D. P. (2021). Molecular Mechanisms of Coral Persistence Within Highly Urbanized Locations in the Port of Miami, Florida. *Frontiers in Marine Science*, *8*, 695236. https://doi.org/10.3389/FMARS.2021.695236

Sharp, W. C., Shea, C. P., Maxwell, K. E., Muller, E. M., & Hunt, J. H. (2020). Evaluating the small-scale epidemiology of the stony-coral -tissue-loss-disease in the middle Florida Keys. *PLOS ONE*, *15*(11), e0241871. https://doi.org/10.1371/JOURNAL.PONE.0241871

Spadafore, R., Fura, R., Precht, W. F., & Vollmer, S. V. (2021). Multi-Variate Analyses of Coral Mortality From the 2014–2015 Stony Coral Tissue Loss Disease Outbreak Off Miami-Dade County, Florida. *Frontiers in Marine Science*, *8*, 723998. https://doi.org/10.3389/FMARS.2021.723998

Walton, C. J., Hayes, N. K., & Gilliam, D. S. (2018). Impacts of a regional, multi-year, multi-species coral disease outbreak in Southeast Florida. *Frontiers in Marine Science*, *5*(SEP), 323. https://doi.org/10.3389/fmars.2018.00323

Weeriyanun, P., Collins, R. B., Macadam, A., Kiff, H., Randle, J. L., & Quigley, K. M. (2022). Predicting selection–response gradients of heat tolerance in a widespread reef-building coral. *Journal of Experimental Biology*, *225*(Suppl_1). https://doi.org/10.1242/JEB.243344

Williamson, O. M., Dennison, C. E., O'Neil, K. L., & Baker, A. C. (2022). Susceptibility of Caribbean Brain Coral Recruits to Stony Coral Tissue Loss Disease (SCTLD). *Frontiers in Marine Science*, *9*, 821165. https://doi.org/10.3389/fmars.2022.821165

Work, T. M., Weatherby, T. M., Landsberg, J. H., Kiryu, Y., Cook, S. M., & Peters, E. C. (2021). Viral-Like Particles Are Associated With Endosymbiont Pathology in Florida Corals Affected by Stony Coral Tissue Loss Disease. *Frontiers in Marine Science*, *8*, 750658. https://doi.org/10.3389/FMARS.2021.750658/BIBTEX