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and

Investigating changes in algal symbiont load and community structure in response to *in situ* shading

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Final Report

Prepared By:

Caroline Dennison, Ph.D.
Andrew Baker, Ph.D.

University of Miami
Department of Marine Biology and Ecology
Rosenstiel School of Marine, Atmospheric, and Earth Science
4600 Rickenbacker Causeway
Miami, FL 33149

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8000 N Ocean Dr.
Dania Beach, FL 33004

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Management Summary

As part of ongoing efforts to identify effective interventions against stony coral tissue loss disease (SCTLD), we conducted a laboratory-based trial using polyinosinic-polycytidylic acid (poly I:C), a synthetic analog of viral double-stranded RNA that has been shown to stimulate coral immune responses in laboratory settings. Due to difficulties sourcing sufficient coral colonies with active SCTLD lesions in South Florida, we collected corals from the Cayman Islands that showed active SCTLD lesions and imported them, under permit, to our experimental laboratory in Miami to conduct our experimental SCTLD trials. Due to the scarcity of diseased colonies, we ran multiple transmission assays in parallel, which ultimately helped us interpret the results of our study. Overall, we saw very little SCTLD transmission in our disease trials, with only one species (*Meandrina meandrites*, one of the most SCTLD-susceptible species) developing lesions over the 65-day assay. Although the low experimental transmission observed in the study limited our ability to evaluate the efficacy of poly I:C to prevent SCTLD, it raises questions regarding the relative lack of transmission, with potential implications for the epidemiology of SCTLD in Florida.

In collaboration with Dr. Karen Neely (NSU), we also tested the temporary deployment of *in situ* coral shades to help mitigate the impacts of coral bleaching during the summer. Across the experimental coral colonies, shaded corals exhibited higher symbiont to host cell ratios, a metric of coral bleaching, compared to the unshaded controls especially once temperatures surpassed 8-degree heating weeks. Our results, combined with Dr. Neely's results of visual bleaching, support the deployment of temporary shades to reduce stress on critically important coral species and/or colonies. We recommend that managers consider the strategic deployment of shading structures in high-value or high-risk reef areas, or on targeted coral colonies, as part of the response to summer thermal anomalies.

Executive Summary

Stony coral tissue loss disease (SCTLD), which first appeared in South Florida in 2014, has spread throughout Florida and the wider Caribbean effectively altering coral reef community structure and diminishing stony coral populations which are vital for promoting biodiverse ecosystems, protecting coastlines, and contributing to local economies. Here we tested the use of polyinosinic-polycytidylic acid (poly I:C) as a prophylactic treatment against SCTLD in a laboratory-based disease assay. Coral colonies with active SCTLD lesions were sourced from the Cayman Islands, as sourcing multiple colonies of various species with SCTLD proved to be difficult. Experimental fragments of *Pseudodiploria clivosa* (sourced from Miami-Dade County in 2021 and kept in running seawater facilities on Virginia Key since collection) were exposed to poly I:C via injections and water bath methodologies and then exposed to SCTLD. We found no transmission to either the poly I:C treated, or the control fragments during a 60-day exposure. Despite long exposure times and significant disease lesions in the source colonies, we found extremely low rates of SCTLD transmission to the experimental colonies, even after resorting to direct contact of

healthy colonies to SCTLD lesions. We hypothesize the low experimental transmission is the result of reduced susceptibility of the remaining corals on reefs in Miami-Dade County Florida corals after a decade of chronic exposure, which we elaborate on in the first portion of this report.

Coral bleaching is widely recognized as a major factor contributing to the global declines of scleractinian corals. While often attributed primarily to elevated ocean temperatures, light also plays a critical role in influencing the severity of coral bleaching. Both thermal and light stress can damage the photosynthetic machinery of the algal symbionts (Family Symbiodiniaceae), initiating the bleaching response and leading to a positive feedback loop. Shading, which reduces light exposure during heat stress events, presents a practical management tool that may help lessen the impacts during summer bleaching. In collaboration with Dr. Karen Neely, we found the temporary deployment of *in situ* shade structures reduced bleaching severity in colonies of *Colpophyllia natans* and *Pseudodiploria clivosa* at Newfound Harbor, FL. This was evident both visually, through bleaching index scores, and at the molecular level, based on symbiont to host cell ratios – an indicator of algal symbiont "load" on the coral host – compared to nearby unshaded coral colonies. This effect is likely due to reduced light-driven stress on the Symbiodiniaceae, leading to the lower production of reactive oxygen species and reducing the need for the coral to expel associated algal symbionts as a protective response. These findings highlight shading as a promising short-term strategy to alleviate stress on vulnerable coral populations during marine heatwaves.

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1. USING POLY I:C AS A PROPHYLACTIC TREATMENT AGAINST SCTLD

1.1. Introduction

For over a decade, stony coral tissue loss disease has spread throughout Florida and into the wider Caribbean altering coral community structure (Dobbelaere et al. 2024; Kramer, P.R., Roth, L. Lang, J 2024; Precht et al. 2016). To date, research has largely focused on identifying effective treatments to treat SCTLD, understanding the etiology of this, and investigating the involvement of members of the coral holobiont including the coral host, bacteria, viruses, and the associated Symbiodiniaceae (Rosales et al. 2023; Traylor-Knowles et al. 2022; Work et al. 2021; Traylor-Knowles et al. 2021). Though the causative agent of SCTLD has yet to be definitively described, various studies have suggested bacterial and/or viral agent(s) affecting the coral host and/or associated Symbiodiniaceae.

Unlike vertebrates, corals lack an adaptive immune system and thus cannot generate classical immunological memory. Instead, corals rely entirely on innate immunity, including physical barriers like mucus, cellular responses, and molecular mechanisms such as the production of antimicrobial peptides and melanin (Mydlarz, Jones, and Harvell 2006). Central to innate recognition are pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), which detect conserved microbial-associated molecular patterns (MAMPs) (Miller et al. 2007). While this system lacks specificity, there is growing evidence that corals may exhibit a form of immune priming, whereby prior exposure to a pathogen or environmental stressor enhances the response to subsequent challenges (Ferrara et al. 2025; Brown and Barott 2022; Hackerott, Martell, and Eirin-Lopez 2021). Polyinosinic-polycytidylic acid (poly I:C), a synthetic analog of viral double-stranded RNA, has been shown to induce sustained upregulation of innate immune genes in invertebrates including corals (Fuess et al. 2020). This immune stimulation provides a controlled approach to investigating immune priming in the absence of adaptive immunity and offers a conceptual basis for developing immune-based disease mitigation strategies in marine invertebrates, including corals.

Previous efforts to manage SCTLD have demonstrated that topically applied antibiotics can effectively slow lesion progression in both laboratory and field (Neely et al. 2020; Aeby et al. 2019). More recently, probiotic interventions have emerged as a complementary strategy to slow disease progression (Ushijima et al. 2023). Building on these proactive efforts, poly I:C "pseudo-vaccination" could serve as a prophylactic immunostimulant – conditioning corals innate immunity in advance of infection. The concept draws from stress-hardening frameworks in coral thermal tolerance research, where pre-expose to sublethal heat stress has been shown to enhance resilience to subsequent thermal stress (DeMerlis et al. 2022). Here, we investigate the potential use of poly I:C as a prophylactic treatment against SCTLD using a laboratory-based transmission assay, directly testing whether immune priming can mitigate disease incidence and progression in fragments of *Pseudodiploria clivosa* and exploring its potential applicability to other coral diseases.

1.2. Methods

1.2.1. Experimental design overview

Following the 2023 bleaching event in South Florida, sourcing coral colonies with active SCTLD lesions became increasingly difficult due to the patch distribution of the disease and the need to maintain species diversity and a high disease load in donor tanks, necessitating multiple colonies needed. As a result, we initiated two parallel SCTLD transmission experiments once viable disease material became available. In March 2025, three colonies of *Pseudodiploria strigosa* and one colony of *Diploria labyrinthiformis* with active SCTLD lesions were collected from the Cayman Islands by collaborators and transported to the University of Miami. Colonies ranged from ~23 cm to ~40 cm in diameter and retained over 70% live tissue. Lesions were either focal or multifocal and progressed rapidly (Figure 1).

The two parallel experiments consisted of: (1) a poly I:C prophylaxis study using *P. clivosa*, and (2) a multispecies SCTLD susceptibility assay. The latter included fragments of *Acropora cervicornis*, *D. labyrinthiformis*, *Meandrina meandrites*, *Orbicella faveolata*, *P. strigosa*, to investigate SCTLD susceptibility across taxa associated with different *Breviolum* strains. The results of both studies are presented here.

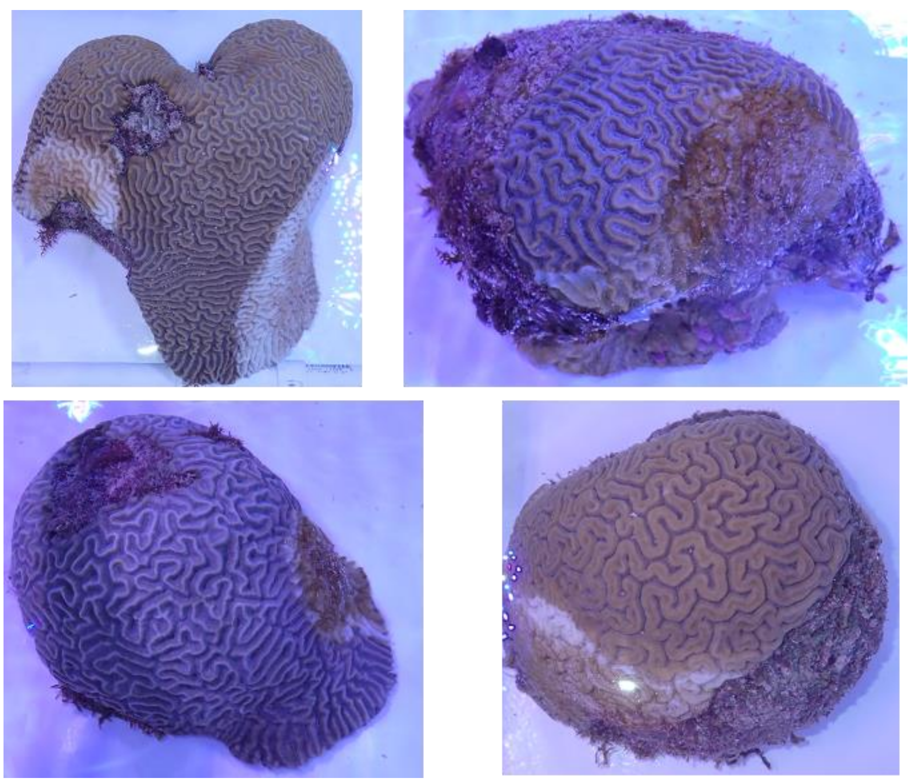

Figure 1: *Photos of coral colonies collected from the Cayman Islands with active SCTLD lesions. Three colonies of* Pseudodiploria strigosa *were collected (top left, top right, bottom left) and one colony of* Diploria labyrinthiformis *was collected (bottom right) for this experiment.*

1.2.2. Species selection and fragmentation

Pseudodiploria clivosa was selected for the poly I:C study due to its high susceptibility to SCTLD, its associations with algal symbionts in the genus *Breviolum*, and the availability of existing colonies, eliminating the need for additional wild harvests. Two colonies of *P. clivosa* were collected in 2021 from the Port of Miami as corals of opportunity (COOs) due to a cruise terminal expansion project. These colonies were maintained at the University of Miami's Experimental Hatchery for three years in temperature-controlled systems.

The poly I:C experiment was run in parallel with a multispecies SCTLD susceptibility experiment testing the susceptibility of different Breviolum species found in marine organisms to SCTLD. Coral colonies for this study were selected based on known algal symbiont associations from fragments remaining after a previous study. Two colonies of each of four scleractinian coral species – *Diploria labyrinthiformis*, *Meandrina meandrites*, *Orbicella faveolata*, and *Pseudodiploria strigosa* – were selected based on their dominant associations with *Breviolum* and recollected from the field for this study. Four colonies of *Acropora cervicornis* were collected from the University of Miami's Key Biscayne Nursery.

Prior to the start of the experiment, the two *P. clivosa* colonies were fragmented into thirty-six replicate ~5 cm^2 fragments using an Aquasaw. Each colony of *D. labyrinthiformis, M. meandrites, O. faveolata*, and *P. strigosa* was fragmented into ten replicate ~ 5 cm^2 pieces. All fragments were allowed to recover for one month in the Coral Reef Future Lab's experimental wetlab prior to the start of SCTLD exposure. During this period, fragments were acclimated to experimental conditions in two 75-gallon flow-through systems supplied with UV-sterilized seawater sourced from Bear Cut. Tanks were equipped with two circulation pumps and a heater to maintain a constant temperature of 27°C and light levels at 150 µEinsteins. Fragments were fed twice weekly for 30 minutes with ReefRoids.

1.2.3. Poly I:C immune priming assay

Seventy-two fragments of *P. clivosa* were randomly assigned to one of four treatment groups (n = 18 per group): (1) poly I:C injection, (2) poly I:C bath, (3) control injection, and (4) control bath. Injected fragments received 20 µL of either poly I:C (20 µg/mL) or UV-sterilized, autoclaved seawater via microinjection into a single polyp. Following injection, fragments recovered for 48 hours in 9-liter aquaria containing UV-sterilized, autoclaved seawater and a circulation pump. Fragments assigned to the water bath treatment were exposed for 48 hours in 9-liter aquaria to either poly I:C (10 µg/mL, high molecular weight) in UV-sterilized, autoclaved seawater or to UV-sterilized, autoclaved seawater alone (control). Aquaria were maintained at 27°C using a surrounding water bath and equipped with a pump for flow.

Following treatment, fragments were placed in 100 mL Tri-Pour cups in pairs (two fragments per cup) (Figure 2). Water changes were conducted three times daily alternating which experimental source tank supplied the water for each water change. Fragments were monitored daily for 60 days for signs of SCTLD.

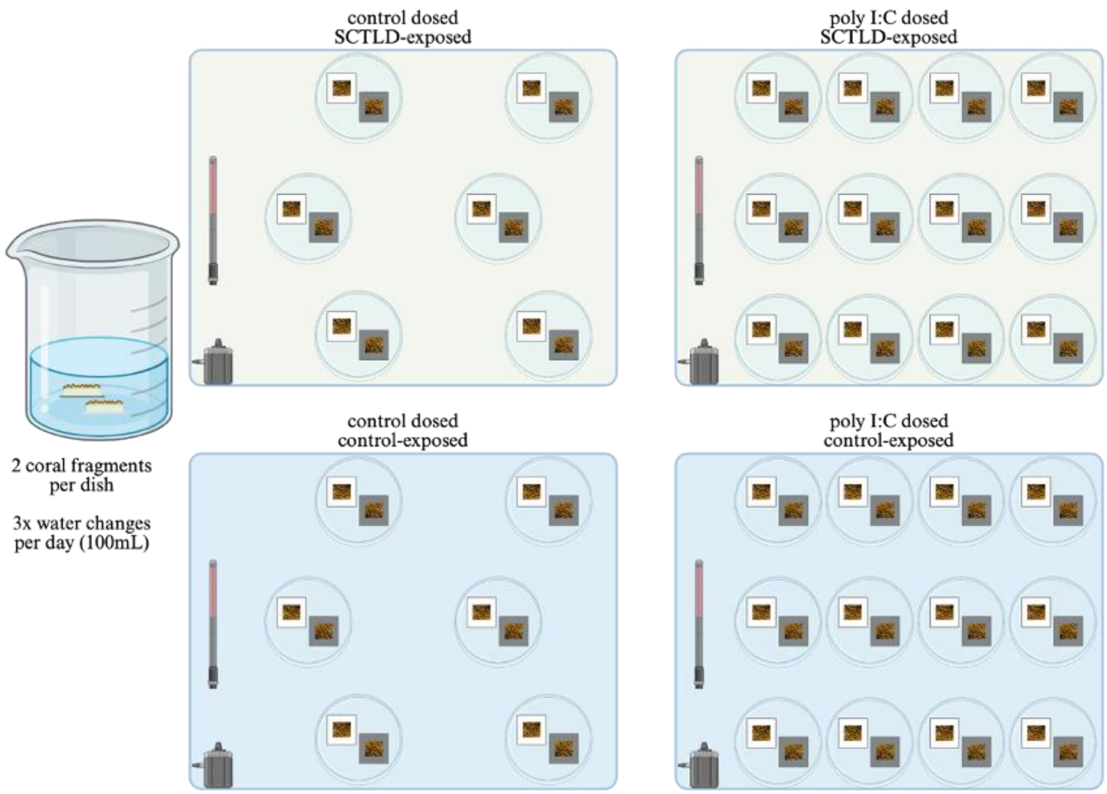

Figure 2: *Experimental set up for the poly I:C study where replicate fragments of Pseudodiploria clivosa generated from two colonies were exposed to SCTLD following an immune priming period in a subset of fragments. For disease exposures, two fragments were placed in each 100mL Tri-pour cup. Cups were maintained in their respective treatment tanks with temperature and circulation. Water was changed thrice daily in each cup.*

1.2.4. Multispecies SCTLD susceptibility assay

In the parallel multispecies experiment, each coral species was assigned to a dedicated aquarium. Water from either the SCTLD donor tank or the control tank was continuously dripped into each experimental aquarium each containing one circulation pump (Figure 3). Fragments were monitored daily for 60 days for lesion development and tissue loss.

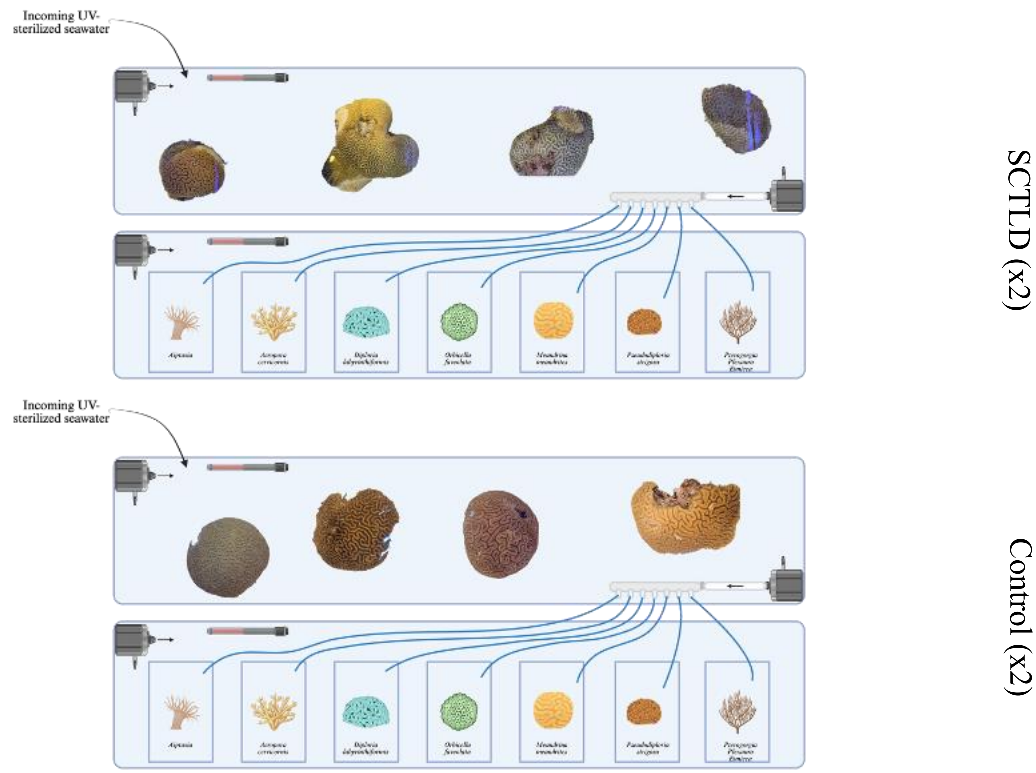

Figure 3: *Experimental design of the multispecies SCTLD study where there was one species per aquarium that was constantly supplied with either water from a tank containing SCTLD presenting donor colonies it apparently healthy donor colonies collected from the Cayman Islands.*

1.2.5. Sourcing donor colonies for waterborne assays

Both SCTLD-infected and control donor colonies were collected from the Cayman Islands and exported under CITES permit 2025/KY/001003. The SCTLD donor tank housed three colonies of *P. strigosa* and one colony of *D. labyrinthiformis*, each exhibiting active lesions. The control donor tank contained one colony of *P. strigosa* and three colonies of *D. labyrinthiformis* with no visible signs of disease.

Replicate SCTLD and control source tanks were established in 75-gallon aquaria, each equipped with two circulation pumps, one heater to maintain 27°C, and a continuous supply of fresh incoming seawater at a rate of 283 L/day. Water from these tanks was used both for the drip exposure system in the multispecies experiment and for the thrice-daily water changes in the poly I:C experiment, ensuring consistency across exposure protocols.

1.3. Results

Across the 60-day experimental SCTLD transmission assay, very little disease transmission was recorded on the experimental fragments. Indeed, SCTLD-like lesions only presented on *M. meandrites* between 15-days and 25-days post SCTLD exposure. Lesions typically appeared in the center of the fragments typically beginning around the mouth and proceeding outwards until fragment death. All the SCTLD-exposed fragments of *M. meandrites* presented with lesions which resulted in mortality. No lesions were observed in any treatment of the SCTLD exposed *P. clivosa* including coral fragments not prophylactically treated with poly I:C (Figure 4).

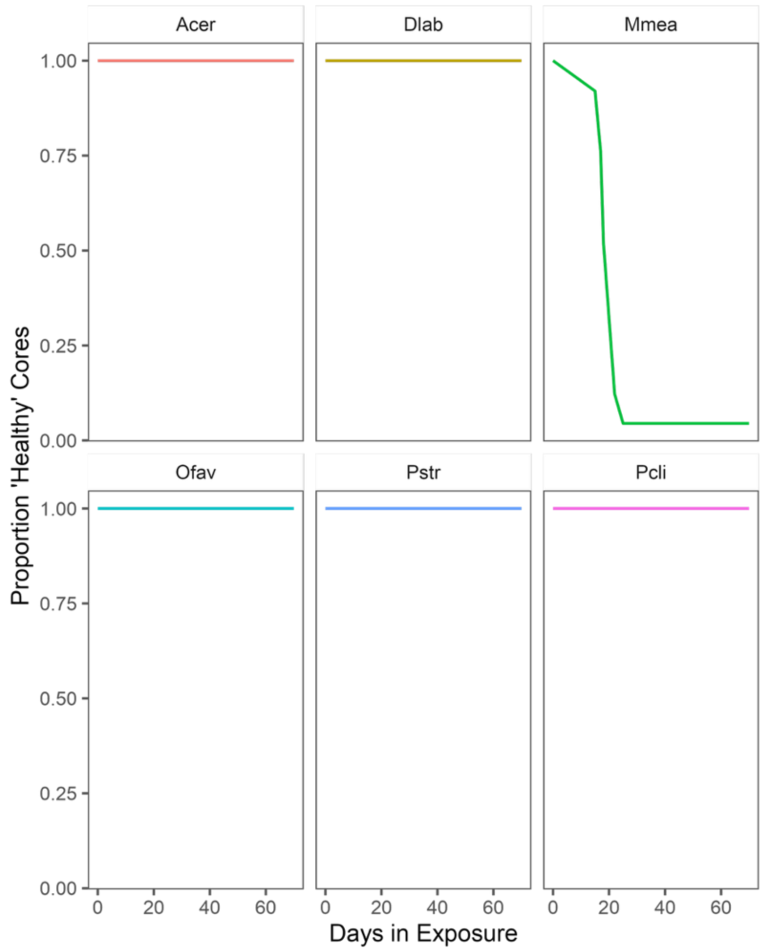

Figure 4: *Cox proportional hazard curves generated for each scleractinian coral species present in the two parallel SCTLD studies (Acropora cervicornis = Acer, Diploria labyrinthiformis = Dlab, Meandrina meandrites = Mmea, Orbicella faveolata = Ofav, Pseudodiploria strigosa/clivosa = Pstr, Pcli). P. clivosa was the study species exposed to poly I:C while the other coral species present were maintained under ambient conditions and not exposed to poly I:C.*

Notably, no visual signs of disruption and/or mortality was observed in any of the poly I:C treatment fragments suggesting the methodologies may be applicable for future studies.

1.4. Discussion and Management Recommendations

All experimental colonies for both studies presented here were selected based on their algal symbiont associations with *Breviolum*, as previous studies have highlighted increased SCTLD susceptibility in corals associating with *Breviolum* (Dennison et al. 2021). Unfortunately, very little disease transmission was observed across this 65-day experiment with the appearance of gross lesions only appearing on *M. meandrites* fragments. In an attempt to extend the study timeline and generate results, we added additional corals to both disease source tanks however we were unsuccessful at transmission. Additionally, following 50-days of SCTLD exposure, we attempted to infect the experimental fragments using direct contact with SCTLD-donor colonies where we were again unsuccessful in transmission.

Although there are many hypotheses that may explain the low transmission to experimental fragments in the presented studies, including a low experimental disease dose and a change/mutation in the disease agent in the Cayman Islands (relative to Florida), we hypothesize this is due to increased resistance in the coral hosts as a result of long-term chronic exposure to SCTLD. The coral colonies selected for these experiments were originally collected from Miami-Dade County in 2021, almost seven years after the initial appearance of the disease in the area. Furthermore, they have been kept in our running seawater land-based facilities for an additional four years, indicating that they have likely been exposed to SCTLD for a decade or more in total. Increased resistance might be due to fixed genetic factors (with susceptible colonies being lost from the population due to differential mortality) or due to compensatory response on the part of exposed corals (with resistant colonies successfully upregulating immune response pathways and/or shuffling components of their microbiome, including algal symbionts and other eukaryotic and prokaryotic microbes). Since the coral colonies were selected on their dominant associations with *Breviolum*, it does not appear that these colonies resisted disease transmission due to having shuffled their symbionts to favor *Durusdinium* (which have been shown to be more SCTLD-resistant than associations with *Breviolum*), although they may have shifted in favor of disease-resistant *Breviolum* species. Of particular interest is the *Breviolum* B1 subclade which comprises *B. faviinorum*, *B. meandrinium*, *B. dendrogyrum*, *B. endomadrasis*, and *B. minutum* (Lewis et al. 2019). Corals associating with these *Breviolum* species vary in their susceptibility to SCTLD. For example, *Dendrogyra cylindrus*, one of the most SCTLD-susceptible coral species, associates predominantly with *B. dendrogyrum*, while *P. strigosa* and *D. labyrinthiformis* associate with *B. faviinorum* and are slightly less susceptible to SCTLD. Furthermore, *B. psygmophilum*, part of the *Breviolum* B2 subclade, which is typically found in facultatively symbiotic *Cladocora* and *Oculina* has shown low SCTLD susceptibility in culture (Karp et al. 2023). We will be testing these corals to identify the specific types of *Breviolum* they host in order to rule out shifts in *Breviolum* as explaining the increase in resistance to SCTLD. For the time being, we suggest the most likely hypothesis for the lack of SCTLD transmission in these studies is that differential mortality has decreased the population of susceptible individuals, possibly helping explain the much lower incidence of SCTLD on Miami's reefs in recent years.

Sourcing coral colonies with active SCTLD has become increasingly challenging over the last two years, and while isolated diseased colonies can still be found (particularly in the Dry Tortugas, for example) our findings suggest that remaining colonies, at least Miami-Dade County, are much more resistant to SCTLD compared to when the disease first appeared over a decade ago. This increase in resistance could be due to differential mortality of susceptible individuals and/or reduced susceptibility of surviving individuals (potentially due to priming of the coral host's immune responses and/or changes in their associated algal symbiont communities). Declines in SCTLD incidence as a result of increased resistance of remaining corals has significant implications for coral reef management and restoration strategies in Florida now that the initial outbreak has passed and the causative agent(s) of SCTLD can now be considered endemic to the region (much like other coral diseases). In particular, it paves the way to prioritize the propagation and/or production of resilient genotypes to rebuild Florida's Coral Reef while simultaneously helping inform future intervention planning. Such strategies may include managed selection of disease tolerant/resistant individuals, and/or managed breeding, and we suggest that rescue corals collected prior to the outbreak should be preferentially crossed with corals that survived the outbreak in order to potentially restore offspring that are more resistant, yet which also have traits from the original rescue population.

Finally, although experimental SCTLD transmission was low in this study, we suggest the use of poly I:C in scleractinian corals may still be a valuable tool to protect coral colonies against SCTLD. From our studies, we did not observe any visual signs of disruption associated with the administration of poly I:C on the experimental fragments. When taken together with other research showing the stimulation of coral host immune pathways (Fuess et al. 2020), the use of poly I:C may help alleviate stress on the coral host and bolster a stronger more targeted response to the SCTLD causative agent(s).

2. INVESTIGATING CHANGES IN ALGAL SYMBIONT LOAD AND COMMUNITY STRUCTURE IN RESPONSE TO *IN SITU* SHADING

2.1. Introduction

Coral bleaching driven by thermal stress – particularly prolonger or severe heat exposure – is widely recognized as one of the most significant threats to coral reef persistence worldwide, and its frequency and intensity are projected to increase under future climate scenarios (Mellin et al. 2024; Hughes et al. 2018). Bleaching occurs when the coral host expels its endosymbiotic dinoflagellates (Family Symbiodiniaceae), which normally provide up to 90% of the host's energy requirements through photosynthesis (Muscatine and Porter 1977). Elevated temperatures impair photosynthetic machinery of the associated algal symbionts, leading to the overproduction and leakage of reactive oxygen species (ROS) that cause oxidative stress in host tissues (Lesser 1997; Weis 2008). If thermal stress is brief, corals may survive by relying on stored energy reserved and heterotrophic feeding while gradually re-establishing their Symbiodiniaceae communities (Grottoli, Rodrigues, and Palardy 2006). However, if elevated temperatures persist and the is unable to re-establish a functional algal symbiosis, prolonged bleaching can result in starvation and eventual mortality.

Not all algal symbionts within the Family Symbiodiniaceae confer equal benefits to their coral hosts. Members of the genus *Durusdinium*, particularly *Durusdinium trenchii,* are known for their relatively high thermal tolerance enabling corals associating with these algal symbionts to better withstand elevated temperatures compared to those associating with *Cladocopium* or *Breviolum* (Howells et al. 2012; Berkelmans and van Oppen 2006). However, this thermal tolerance comes at a potential cost as *D. trenchii* has also been shown to translocate less fixed carbon to the coral host under non-stressful conditions leading to decreased host growth (Cunning, Silverstein, and Baker 2015). Indeed, a study published by Claar et al. (2020) found that corals associating with *Durusdinium* at the onset of a heatwave were less likely to survive, while those that began with *Cladocopium* and transitioned to *Durusdinium* during thermal stress exhibited higher survival—highlighting the importance of symbiont flexibility in coping with prolonged heat events and further emphasizing ecological tradeoffs associated with Symbiodiniaceae identity.

In the summer of 2023, Florida's Coral Reef experienced one of the most extreme and early-onset bleaching events recorded in the region to date. Sustained sea surface temperatures exceeding 32°C resulted in more than 11-degree heating week (DHWs) accumulating at Newfound Harbor, in the Florida Keys, levels historically associated with severe bleaching and widespread coral mortality (Neely et al. 2024). The combination of prolonged high temperatures and intense solar irradiance compounds damage to Symbiodiniaceae photosynthetic machinery resulting in the production of ROS. In situ coral shading has been proposed as a mitigation strategy to reduce photodamage during such events. Studies conducted outside of the Caribbean have found that shaded corals have delayed bleaching than unshaded controls (Butcherine et al. 2023).

In this project, we worked with Dr. Karen Neely (NSU) to assess the efficacy of in situ shading on bleaching in two species of corals – *Colpophyllia natans* and *Pseudodiploria clivosa* – in Newfound Harbor, a patch reef off the Lower Florida Keys prone to annual bleaching. In the field Dr. Neely monitored changes in visual bleaching using bleaching index scores, while we analyzed algal symbiont identity and symbiont to host cell ratios in these corals during the Summer of 2024.

2.2. Methods

For field methodologies including coral colony selection, shade deployment, and visual bleaching monitoring methodologies please refer to Dr. Neely's final DEP report entitled "Mitigating high-temperature bleaching impacts on high-value corals using low-cost shading approaches, and assessments of a potentially novel coral disease affecting key reef building corals (phase 2)".

Twenty colonies of each species were selected and divided between unshaded controls and shaded treatments. Initial tissue biopsies were collected when in mid-July 2024 when approximately two-degree heating weeks had accrued at Newfound Harbor. Shades were deployed when 4 DHWs had accrued at Newfound Harbor which occurred in late-July, however due to delays in permitting shades were not deployed until mid-August when ~7 DHWs had accrued (Figure 5). Small tissue biopsies were collected at each monitoring period, occurring at least every two-weeks from the edge of each colony (per permitting requirements). Samples were placed in DNA/RNA Shield and transported to the University of Miami for DNA extraction and subsequent Symbiodiniaceae identification and quantification.

Coral host and associated algal symbiont DNA was extracted using a modified organic DNA extraction protocol to isolate gDNA (Cunning and Baker 2013). Symbiont to host cell ratios (S:H) was used as a proxy to quantify differences in algal symbiont abundance relative to the coral host cells in response to in situ shading. S:H ratios were estimated using real-time PCR (qPCR) assays that targeted the actin gene in *Symbiodinium*, *Breviolum*, *Cladocopium,* and *Durusdinium,* and the Pax C gene in *Colpophyllia* and *Pseudodiploria*. S:H were calculated using the StepOneR package. Differences in S:H were analyzed using linear mixed effects models using the lme4 package.

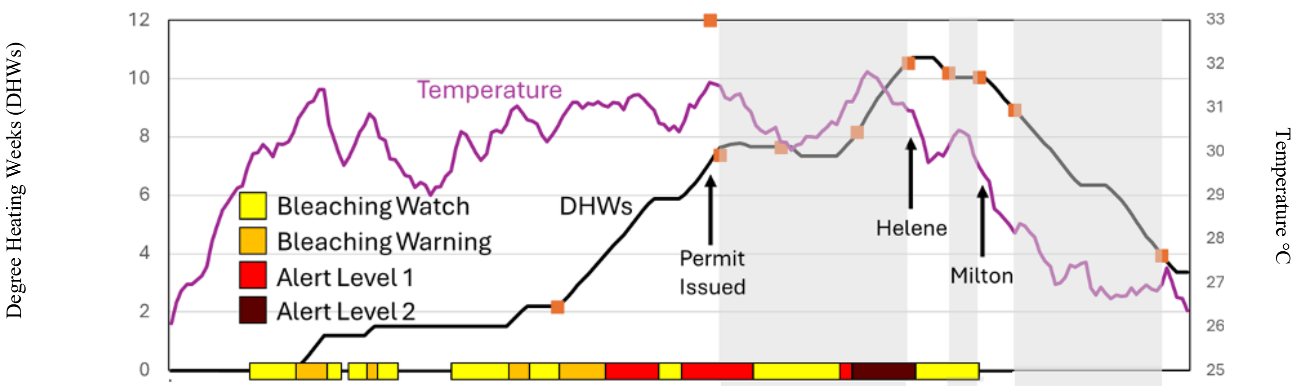

Figure 5: *Summer 2024 temperature monitoring at Newfound Harbor. Gray shaded bars indicate timepoints when the shades were deployed (including the removal during two tropical storms), while orange squares indicate monitoring timepoints. Temperature, degree heating weeks (DHWs) and bleaching alert level data are from the NOAA Coral Reef Watch single-pixel virtual station. Figure courtesy of Dr. Neely.*

2.3. Results

Dominant algal symbiont associations with *Durusdinium* were found in all forty coral colonies selected for this study regardless of species. Notably, background amounts of *Symbiodinium* were detected in only *C. natans* with one colony (888) predominantly associating with *Symbiodinium* throughout the monitoring period (Figure 6).

Figure 6: *Symbiodiniaceae community structure of the experimental coral colonies selected for this studying using qPCR. Algal symbionts were identified to the genus level. Each bar represents the algal symbiont community at each monitoring point where the color of the bar indicates the relative abundance of each Symbiodiniaceae genera,* Symbiodinium *(orange),* Breviolum *(yellow),* Cladocopium *(blue), and* Durusdinium *(purple). White bars indicate no data associated with the sample at that timepoint.*

A linear mixed effects model did not find significant differences in the log-transformed symbiont to host cell ratios (logSH) between shaded corals and unshaded control corals in this study. However, generally shaded corals tended to have higher symbiont to host cell ratios compared to unshaded controls when 8 DHWs were surpassed (SP5-SP8) in this study (Figure 7). There were differences between the symbiont to host cell ratios between species with *C. natans* typically having higher symbiont to host cell ratios when compared to *P. clivosa.*

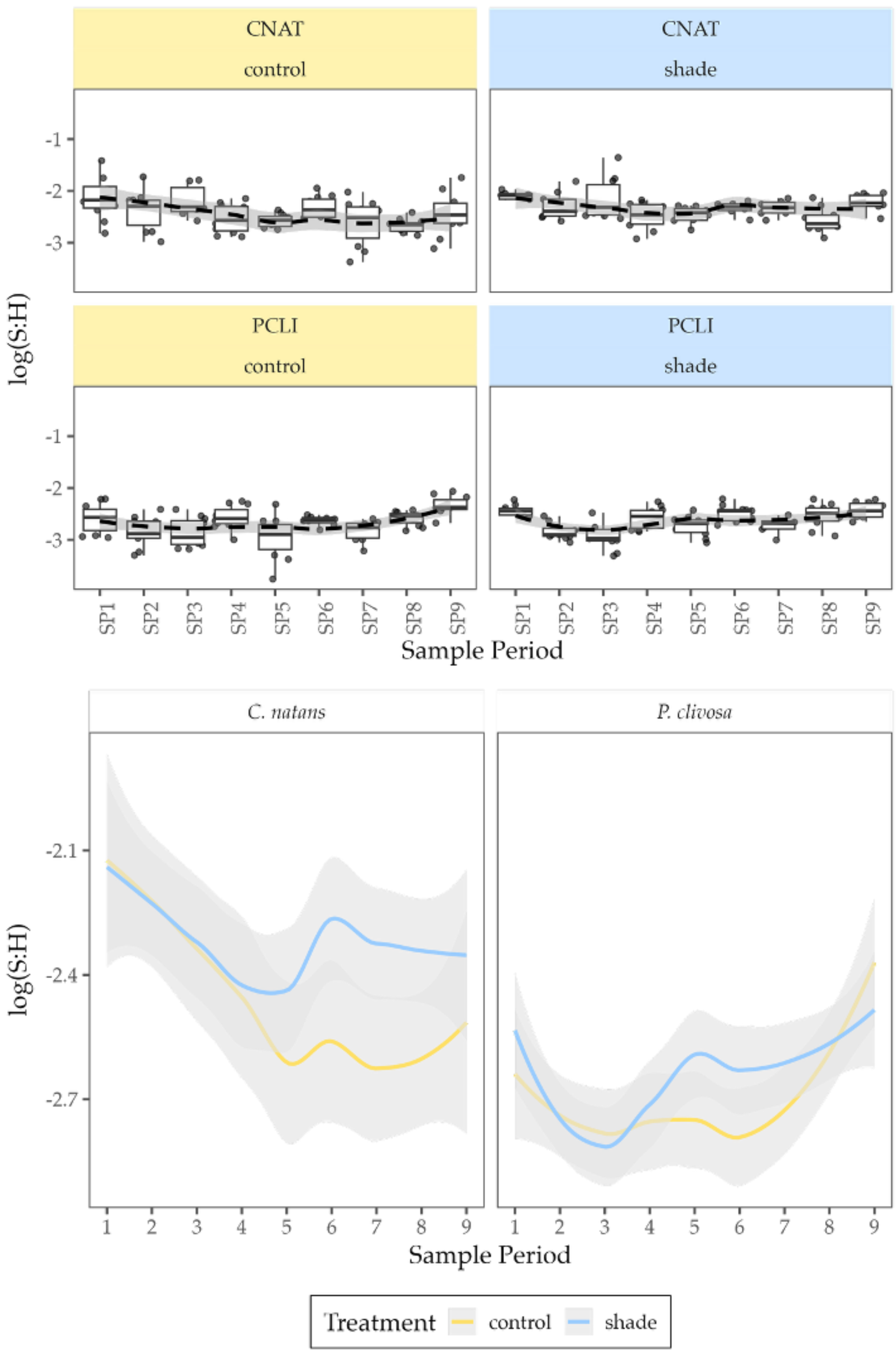

Figure 7: *Differences in log-transformed symbiont to host cell ratios between unshaded (yellow) and shaded (blue)* Colpophyllia natans *(CNAT) and* Pseudodiploria clivosa *(PCLI) across the nine monitoring periods in this study.*

2.4. Discussion and Management Recommendations

Generally, colonies of *C. natans* and *P. clivosa* selected for this study at Newfound Harbor in the Lower Keys showed strong associations with algal symbionts in the genus *Durusdinium*. Background amounts of *Symbiodinium* were detected in *C. natans* but the abundance varied through time. Notably, one colony of *C. natans* was found associating predominantly with *Symbiodinium* across all monitoring periods in this study. Given the annual bleaching of coral colonies in Newfound Harbor, it is not surprising the coral colonies in this study predominantly associated with *Durusdinium* especially given the heat stress that occurred during the summer of 2023 which may have selected for colonies associating with *Durusdinium* (Neely et al. 2024). It is important to note that the presented algal symbiont community structure is based off a single sample taken from one spot, on the edge, on each coral colony. This approach may oversimplify the algal symbiont community structure making it hard to distinguish temporal shifts in symbiont community structure and spatial mosaicking of algal symbionts within colonies (Kemp et al. 2015, 2014). Regardless, it is unlikely the differences in bleaching response resulted from the algal symbiont community structure as very little change was detected.

Although no significant differences in log-transformed symbiont to host cell ratios were detected between the shaded and unshaded coral colonies in this study, we do generally see higher symbiont to host cell ratios in the shaded corals compared to the unshaded corals especially once 8 DHWs are surpassed. This also corresponds with a decreasing in bleaching index as reported in Neely (2025). Changes in light fields have been shown to affect algal symbiont pigments more than algal symbiont cell densities in *Stylophora pistillata* where the cellular densities between shade-adapted and light-adapted fragments were very similar but there was an ~4-fold decrease in pigment (Chl *a*) found in the light adapted corals resulting in stark differences in color (Falkowski and Dubinsky 1981). Therefore, the changes in bleaching index scores, reported in Neely (2025), may be due to changes in Chl *a* content and less associated with the expulsion of algal symbionts. Additionally, given that all but one coral colony in this study were associating predominantly with *Durusdinium*, the symbionts may not be under 'severe' stress, especially given the corals may be primed due to the annual bleaching that is reported at Newfound Harbor.

Regardless, in situ shading of corals likely does mitigate bleaching stress because less pigments and/or few algal symbiont cells result in a reduction in the production of reactive oxygen species under heat and/or light stress and therefore the temporary deployment of shade structures may alleviate some of the stress on targeted corals.

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