Are acroporid corals a potential vector of stony coral tissue loss disease?

Restoration Trials Team
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In April 2019, the Restoration Trials Team created an Action Plan that identified priority research questions to be addressed within the context of conducting restoration within the stony coral tissue loss disease (SCTLD) outbreak. The first question was "Are acroporid corals a potential vector of stony coral tissue loss disease?". Indeed, most coral restoration along the Florida Reef Tract (FRT) has used acroporids, including both *Acropora palmata* and *A. cervicornis*. Acroporid corals have not been observed to exhibit SCTLD despite being subjected to the unknown infectious agents of SCTLD throughout the FRT. These observations, as well as other anecdotal information, suggest that acroporids are not susceptible to SCTLD. However, directed experiments to test susceptibility of acroporids to SCTLD should be conducted. Additionally, concerns have been expressed that acroporid corals could serve as a vector for SCTLD. To address these concerns, the Restoration Trials Team suggested that both field and laboratory experiments should be conducted to determine whether acroporids are indeed resistant to SCTLD and to identify if acroporids may be a vector of SCTLD. In response, two opportunistic experiments were conducted. One included a field experiment conducted by Andrew Bruckner with the Florida Keys National Marine Sanctuary and Erich Bartels at Mote Marine Laboratory. The second included a laboratory exposure experiment conducted by Erinn Muller with the Coral Health and Disease Program at Mote Marine Laboratory. Results from these two opportunistic experiments are included within this report.

Conclusions

The field experiment showed that plugs of *Acropora palmata* outplanted near or on SCTLD colonies did not show signs of disease, further supporting the conclusion that acroporids are not susceptible to SCTLD. The laboratory experiment indicated that plugs of *A. palmata* did not show signs of disease after exposed to SCTLD and did not transmit the disease to more susceptible species after this exposure occurred. However, subsequent studies should focus on longer exposure times and complementary field studies to ensure that this short-term laboratory experiment is consistent with other research conducted over longer periods of time within appropriate environmental settings. In addition, the integration of complementary positive controls, to show that transmission from the diseased corals to susceptible species occurs, is highly recommended to increase the confidence within the conclusions of the present report.

Field Study: Susceptibility of *Acropora palmata* outplants to Stony Coral Tissue Loss Disease (SCTLD)

Andrew Bruckner, FKNMS
Erich Bartels, Mote Marine Laboratory

Summary

Thirty land-based nursery grown microfragments of *Acropora palmata* were transplanted around or on six massive/boulder corals representing species susceptible to SCTLD, three with active disease and three apparently "healthy" controls. All *A. palmata* microfragments, and the massive/boulder corals they were outplanted adjacent to or on, were reexamined multiple times during the first month, and again after approximately seven months. All of the *A. palmata* microfragments survived over the duration of the study with the exception of one fragment placed next to a control colony that was detached from the epoxy and lost during the initial outplanting due to fish predation. The apparently "healthy" control corals (*Siderastrea siderea, Colpophyllia natans* and *Diploria labyrinthiformis*) still showed no signs of SCTLD after 30 days, but by seven months two of the control corals (*C. natans* and *D. labyrinthiformis*) succumbed to SCTLD. All outplanted *A. palmata* remained unaffected by SCTLD over the duration of this study, confirming that ex-situ nursery grown *A. palmata* colonies that had never been exposed to SCTLD are not susceptible to this disease.

Background

Coral restoration practitioners have been outplanting nursery-reared corals within the Florida Keys since 2007. To date, most outplants are staghorn coral (*A. cervicornis*), with the addition of *A. palmata* and several boulder coral species in the last few years. At a coral disease workshop (Key Largo, July 2018), scientists and managers highlighted the need to evaluate the potential risks of outplanting corals into locations that have been or are being affected by SCTLD. There were concerns raised that 1) the outplants could succumb to the disease and die; 2) they could "add fuel to the fire" by providing more tissue for the disease, potentially causing the outbreak to flare up or possibly increasing pathogen load; and 3) the corals could serve as a vector/carrier for the pathogen responsible for the disease.

To date, acroporid corals are thought to be resistant to SCTLD. Nevertheless, they are susceptible to other diseases (e.g. white band disease, rapid tissue necrosis, white patch), and it is currently unknown whether they are susceptible to SCTLD and succumb when placed near or in direct contact with a diseased coral. Furthermore, if acroporids are resistant to the disease, they could serve as carriers and potentially increase disease prevalence and further promote its spread.

Both Mote Marine Laboratory and the Coral Restoration Foundation are actively expanding efforts involving the outplanting of nursery grown acroporids, and there has been movement of colonies between disease zones, as well as from land-based facilities into endemic, epidemic, invasion and pre-invasion zones. Due to these ongoing restoration activities, the need for preliminary information that could be used to help identify best practices for outplanting of acroporids from land-based and in situ nurseries into different disease "zones" has become critical. As a result, in November 2018, a small pilot experiment was undertaken in the field to further evaluate disease susceptibility of acroporids in a natural setting.

Methods

Thirty microfragments representing five genotypes of *Acropora palmata* were transported from the land-based coral nursery at Mote, Summerland Key to Looe Key Reef on November 14th, 2018. Five microfragments (1 replicate each of 5 genotypes) were secured using two-part epoxy either on the substrate directly adjacent to (within 10 cm) or directly onto recently denuded skeleton adjacent to the active disease margin on each of 3 diseased corals (*Colpophyllia natans, Psuedodiploria strigosa, Siderastrea siderea*; Fig. 1). Five microfragments (1 replicate each of the same 5 genotypes) were also placed around the perimeter of each of three control corals (100% live, susceptible corals; *S. siderea, C. natans, Diploria labyrinthiformis*) in an identical manner (Fig. 1). All corals were located near the seaward edge of a single spur (24.54641 N, -81.40331 W) nearing mooring 5, at an average depth of 16-20 feet. Corals were visually monitored and photographed at 6, 14, and 32 days post-outplant, followed by one final examination approximately 7 months later on June 10, 2019.

Results

A. palmata microfragments planted adjacent to and on top of wild massive corals of three species exhibiting signs of SCTLD, and adjacent to three wild colonies of SCTLD susceptible species that were not visibly affected, did not succumb to SCTLD over the duration of the study. Tissue loss remained active on the disease colonies throughout the duration of the study. Initial observations a few days after outplanting showed that all *A. palmata* outplants were lighter in color than when first transplanted, but appeared to regain normal coloration within 30 days (Fig. 2). Seven of the fragments had fish bites affecting 2-40% of their surface, but the tissue had regenerated over these lesions by the end of the study. In total, 23% experienced partial tissue loss due to fish predation that affected up to 40% of their surface area, and one fragment was partially killed due to smothering by sediment/turf algae. One outplant placed next to a control colony was also lost within 30 minutes of transplanting due to removal by a fish. Over the next six months, outplants began to resheet over the base and started to grow upward. None of the *A. palmata* outplants succumbed to SCTLD over the duration of the study.

Fig. 1. Setup of *Acropora palmata* susceptibility study. **A**. Thirty fragments of five genotypes used in experiment. **B**. Diver securing a coral plug to the substrate with epoxy. **C**. *Pseudodiploria strigosa,* tag # 377, with SCTLD and five *A. palmata* outplants. **D**. Control *Colpophyllia natans*, tag #377, with five *A. palmata* outplants **E.** *C. natans* with SCTLD, tag #380, with five *A. palmata* outplants **F.** Control *Siderastrea siderea*, tag #383, with five *A. palmata* outplants. **G**. *S. siderea* with SCTLD, tag # 379, with five *A. palmata* outplants **H**. Control (presumed healthy) *D. labyrinthiformis*, tag # 375, with five *A. palmata* outplants.

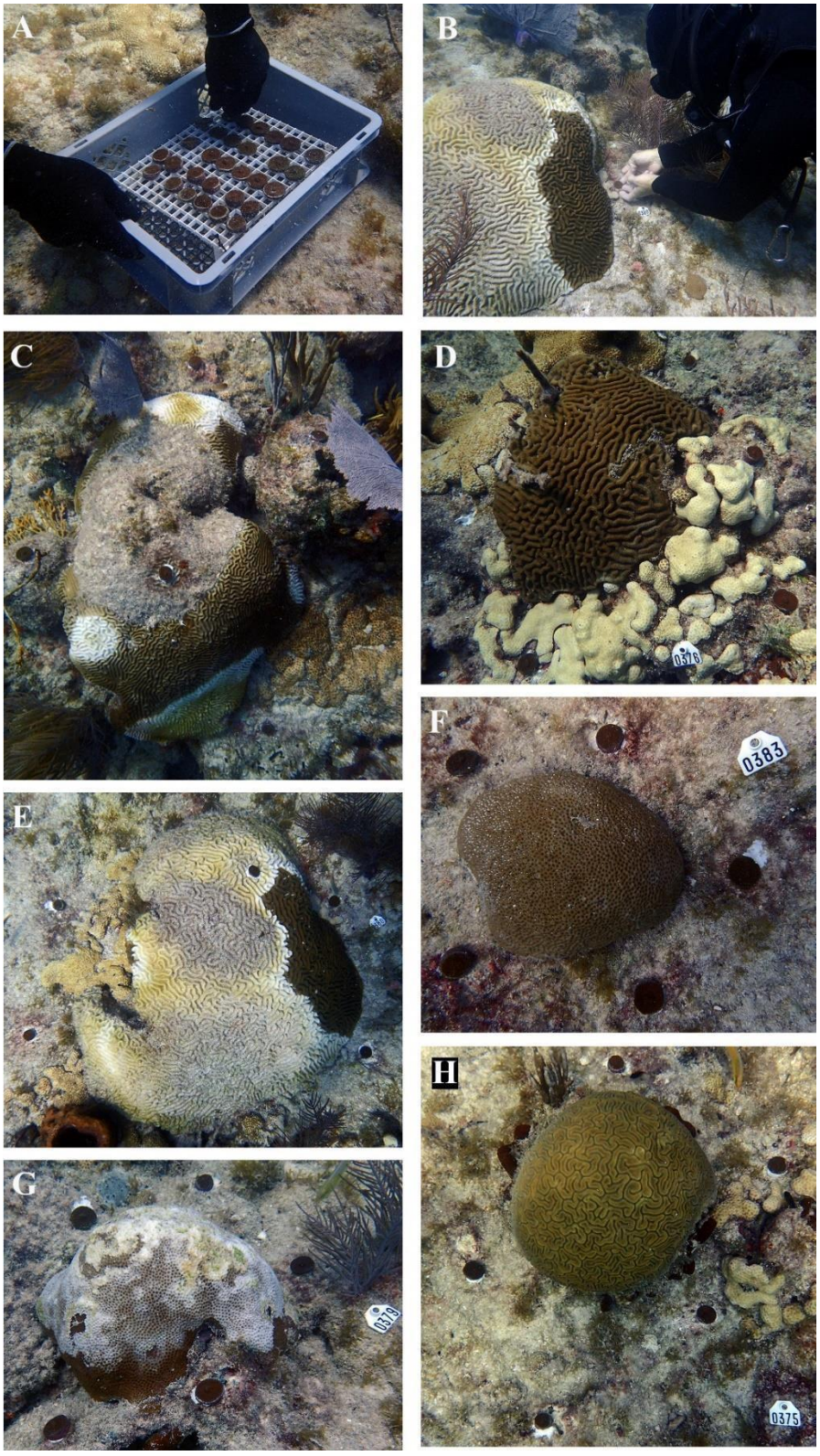
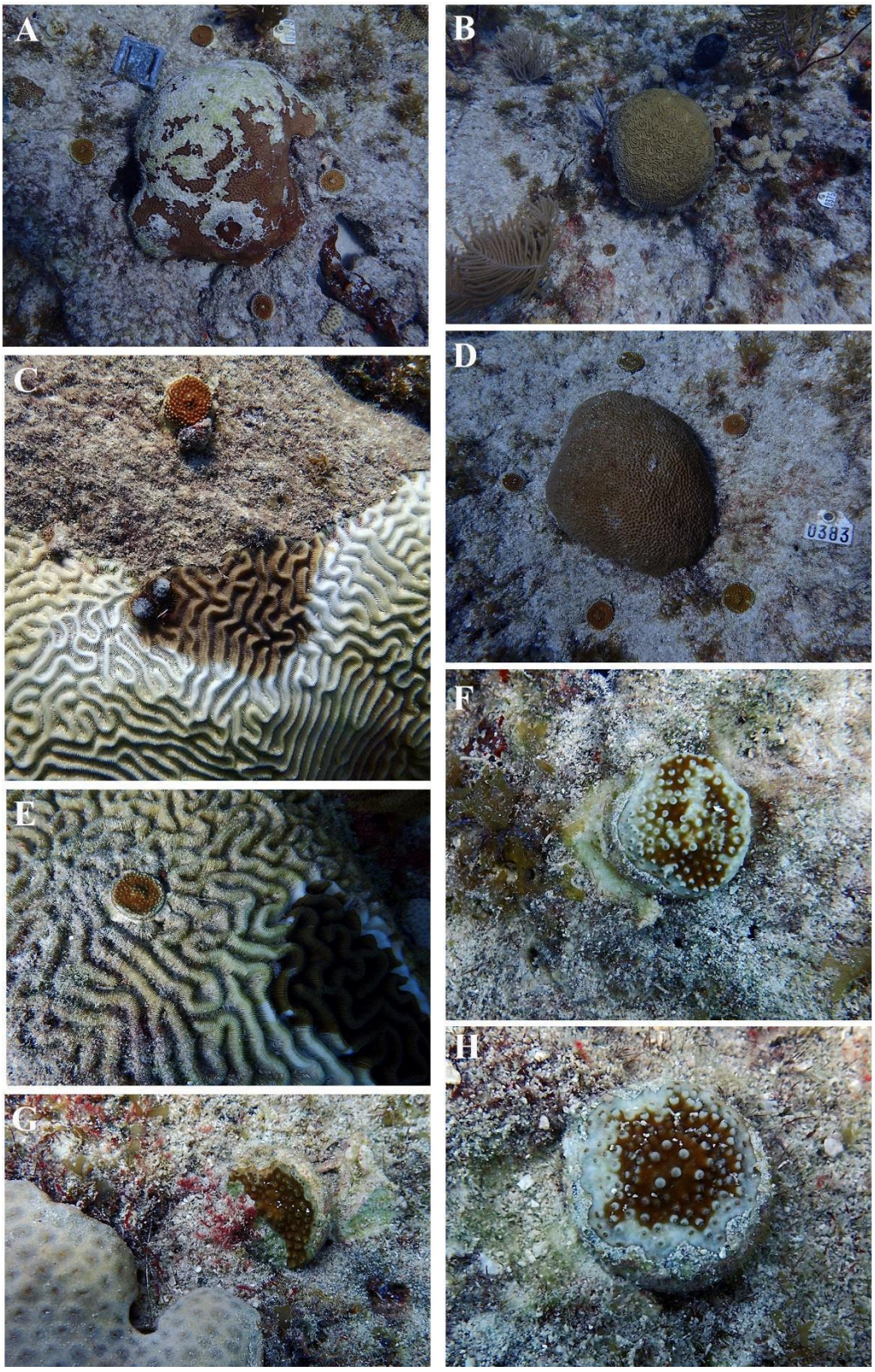


Fig. 2. *Acropora palmata* outplants after ~30 days. **A**. Five outplants next to colony #379. **B**. Five outplants next to colony #375. **C**. One outplant on colony #377. **D**. Five outplants next to colony #383. **E**. One outplant on colony #380. **F**. One outplant that sustained fish predation and is regrowing. **G**. An outplant that sustained tissue loss from algal/sediment interactions but is still surviving. **H**. An outplant that was bitten by parrotfish and has resheeted, but is still pale.



Laboratory Study: Is *Acropora palmata* susceptible and/or a potential vector of Stony Coral Tissue Loss Disease (SCTLD)?

Erinn Muller; Coral Health and Disease Program Manager
Ray Banister, Coral Health and Disease Technician
Mote Marine Laboratory

Objective 1: Determine whether lab-raised *Acropora palmata* fragments are susceptible to Stony Coral Tissue Loss Disease

Objective 2: Determine whether lab-raised *Acropora palmata* fragments can transmit Stony Coral Tissue Loss Disease

Methods

On September 14th, 2018, fifteen *Acropora palmata* fragments from Mote Marine Laboratory's Elizabeth Moore International Center for Coral Reef Research and Restoration on Summerland Key, FL were transported to Mote's Coral Health and Disease Laboratory in Sarasota. The 15 fragments of *A. palmata* consisted of single replicates of a random assortment of genotypes from the land-based nursery, which had been in cultivation on land for at least the previous two years. Fragments were approximately 2 cm in diameter.

Corals were divided into two 5 gallon control tanks and two 5 gallon disease exposure tanks (3 - 4 *A. palmata* fragments per tank). Within the control tanks, three *A. palmata* fragments were placed surrounding a ~10 cm x 10 cm healthy *Pseudodiploria clivosa* colony (Control 1) and four *A. palmata* fragments were placed surrounding a ~10 cm x 10 cm healthy *Oribicella faveolata* colony (Control 2), which were previously collected for another study and opportunistically used for the present research. Fragments were placed approximately 1 cm away from the edge of the large colony (Fig. 3). A previous study attempted to hold corals in direct contact between the *A. palmata* fragments and the large control colony, but intercolony aggression occurred, which resulted in rapid complete mortality of the small fragments within hours. In the present study, each tank also contained a stand and two powerheads to ensure appropriate water circulation.

The diseased colonies were collected on August 26th, 2018 from Looe Key buoy #2 (24.54653 N, 81.40194 W) and were held within the Coral Health and Disease wetlab until the beginning of this experiment. The diseased fragments consisted of one *P. strigosa* (Disease Tank 1) and one *Dichoceonia stokesii* (Disease Tank 2), which were the two species found displaying disease signs within permitted collection areas. Both of these colonies showed active disease progression in the field and subsequently within the laboratory. Within each tank, four *A. palmata* fragments

were placed within 1 cm of the diseased fragments, similar to the control tanks (Fig. 3). Diseased tanks also contained a stand and two powerheads for water circulation. Under both conditions, water was held static within the tanks. Fifty percent water changes were preformed every day and contaminated water was sterilized using bleach and UV exposure and then depositing into a land-based swale to percolate through the substrate prior to any interaction with the nearshore environment.

The health of the corals was visually assessed daily over a one week period. After one week, the large diseased colonies were replaced with healthy colonies of susceptible species (*O. faveolata* and *P. strigosa*) to determine whether the *A. palmata* fragments previously exposed to the diseased coral could transmit the disease to a healthy coral of a highly susceptible species.

To determine the bacterial load within the water of each of the treatment tanks, after one week of exposure, a total of 100 µl of tank water was plated onto marine agar in triplicate. Samples were spread using sterile glass plating beads, the plates were inverted, and then incubated overnight at 28°C. Bacterial colonies were counted on each plate after 24 hours and morphologies were compared among treatments. Logistical constraints prohibited documenting the bacterial load during the second week of the experiment.

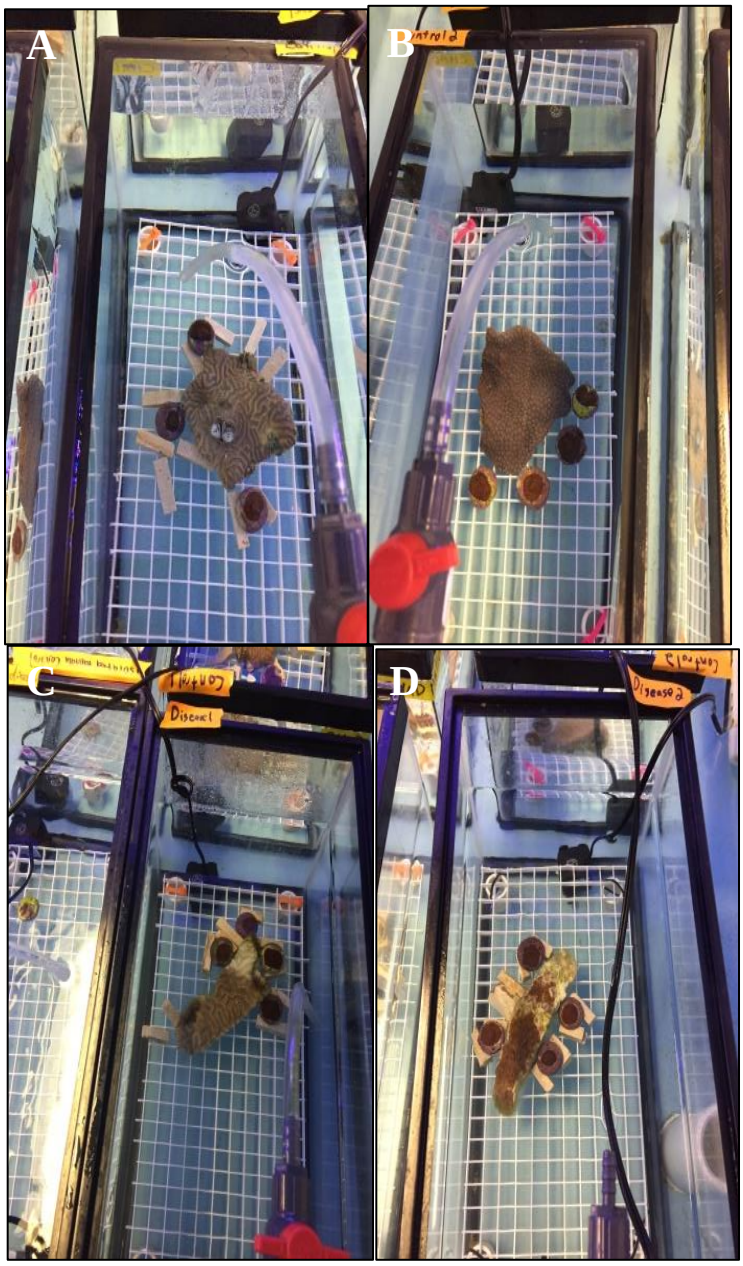


Fig. 3. Overhead view of tank setup for A) Control Tank 1, B) Control Tank 2, C) Disease Tank 1, D) Disease Tank 2.

Results

There was no mortality observed within the *A. palmata* fragments during the first week of exposure to the diseased coral fragment (Table 1). Disease progression was documented visually within the large, originally disease, colony suggesting that disease-containing flocculent was within the water column of the tank. This also indicates that the *A. palmata* fragments were likely exposed to the disease flocculent that persisted within the static water of the tank each day.

Similarly, there was no mortality observed within the *A. palmata* fragments during the second week of exposure when the diseased corals were replaced with healthy corals of the same species (Table 1). Additionally, there was no mortality observed within the healthy coral fragments of the susceptible coral species after being held next to the *A. palmata* fragments previously exposed to SCTLD. There was also no mortality within the control tanks for either the *A. palmata* fragments or the large fragments of the susceptible coral species (Table 1).

Although there were no positive controls within the study presented here, the coral colonies were collected primarily for another transmission study that occurred simultaneously. Fragments of diseased corals collected from the same colonies within the present study indeed transmitted SCTLD to fragments of susceptible coral species (*P. clivosa* and *O. faveolata*) using similar methodologies.

Table 1: Summary of results of the *Acropora palmata* fragments tested within the present study. The study lasted for a total of 14 days. All acroporid corals survived and appeared disease free at the end of the study.

Genotype	Treatment (C: control, D: diseased)	Survival (Y/N)	Days Survived
AP13-4	C	Y	14
AP13-X10	C	Y	14
AP13-X10	D	Y	14
AP13-X4	D	Y	14
AP13-X4X	C	Y	14
AP13-X5	C	Y	14
AP13-X5	D	Y	14
AP13-X8	D	Y	14
AP13-XA	C	Y	14
AP13-XK	D	Y	14
AP13-XL	C	Y	14
AP14-3	D	Y	14
AP14-4	C	Y	14
AP14-4	D	Y	14
AP14-4	D	Y	14

The total bacterial load, as cultured using marine agar, showed fewer bacteria within the control tanks (339.5 ± 20.7 colony forming units (CFUs)/100 µl) compared with disease tanks (434.7 ± 47.7 CFUs/100 µl; Fig. 4). These results indicate that the *A. palmata* fragments within the diseased tanks were exposed to higher bacterial concentrations that those in the control tanks. Whether the SCTLD pathogenic load was higher, similar to the general bacterial load, is unknown.

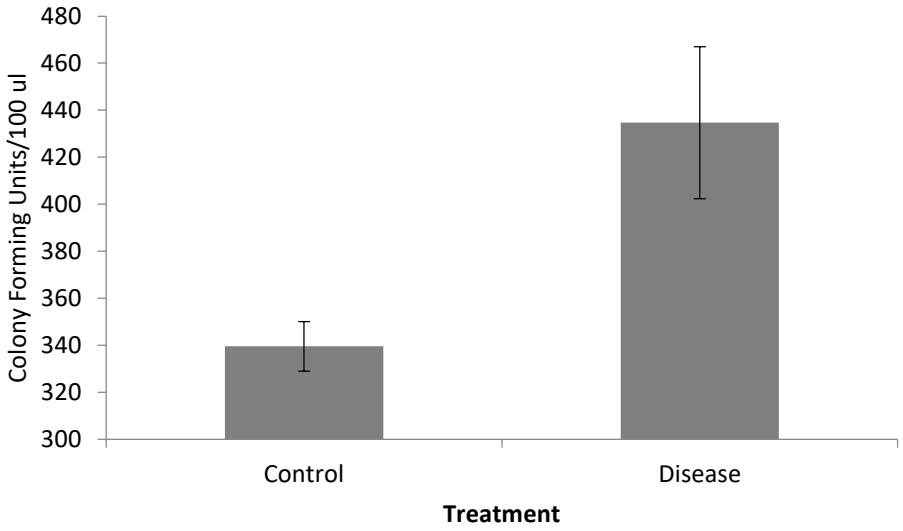


Fig. 4. Average bacterial colony forming units per 100 µl of seawater collected from control and disease tanks and plated on marine agar at 1 week post exposure. Error bars indicate standard error of the mean.

Conclusion: The results of the this preliminary study indicate that lab-reared *A. palmata* fragments do not appear highly susceptible to Stony Coral Tissue Loss Disease, nor do they appear to be a vector for transmission. However, subsequent studies should focus on longer exposure times and complementary field studies to ensure that this short-term laboratory experiment is consistent with other studies conducted over longer periods of time, within appropriate environmental settings. In addition, the integration of positive controls, to show that transmission from the diseased corals to susceptible species occurs, is highly recommended to increase the confidence within the conclusions of the present report.