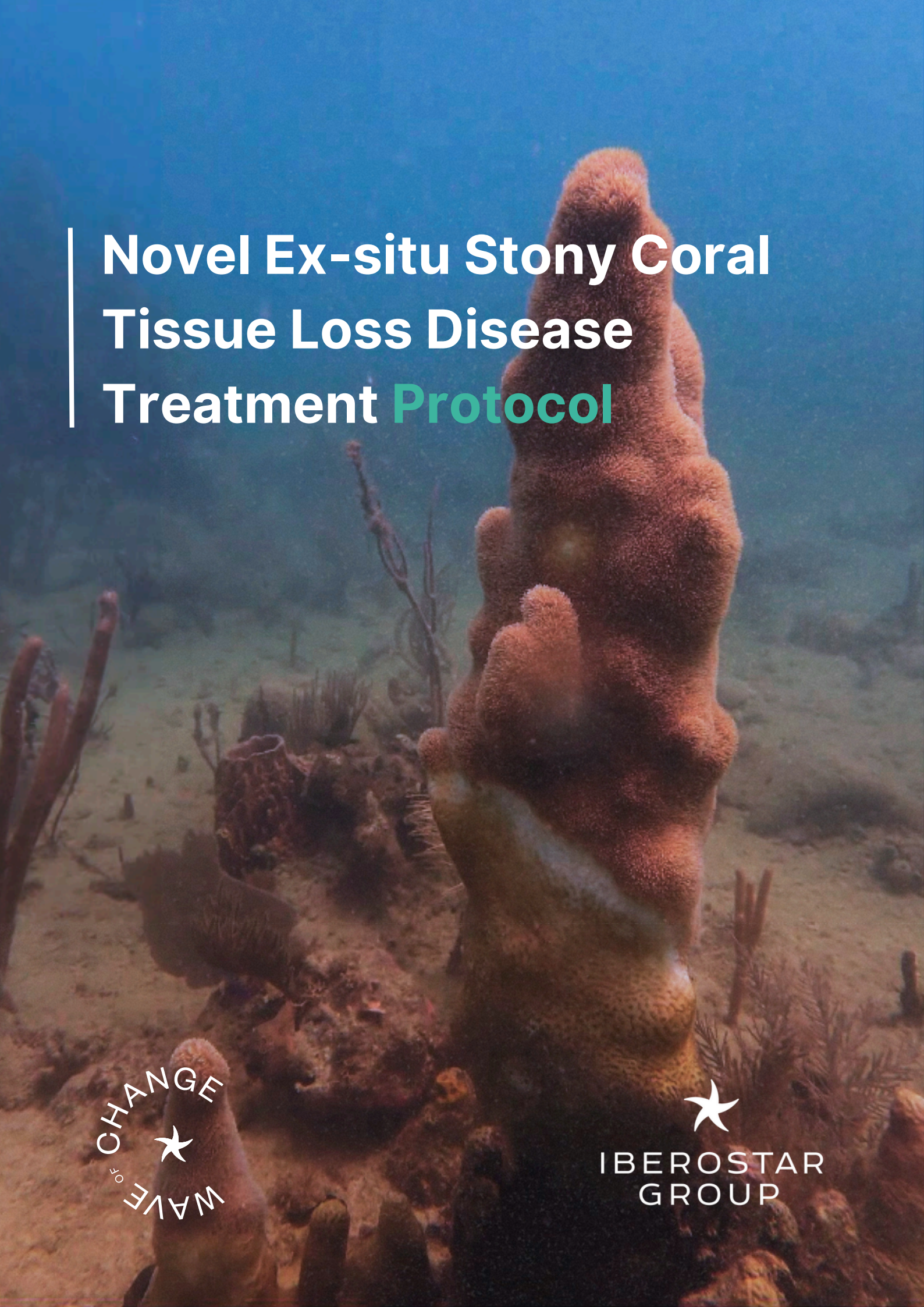
Novel Ex-situ Stony Coral Tissue Loss Disease Treatment Protocol



Novel Ex-situ Stony Coral Tissue Loss Disease Treatment Protocol

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Table of Contents

Abstract

Stony Coral Tissue Loss Disease (SCTLD) is a highly lethal coral tissue loss disease that has shown to cause catastrophic loss of coral cover and biodiversity in Caribbean coral reef ecosystems. The unprecedented loss to SCTLD has demonstrated significant harm to reef functionality, making intervention and mitigation strategies essential. Current *in-situ* and *ex-situ* treatment methods have shown varying levels of success. Here, we present a 10-day *ex-situ* treatment protocol demonstrating a 94% success at treating coral colonies across four genera with SCTLD-like lesions (n=34 with *Dendrogyra cylindrus, Montastraea cavernosa, Pseudodiploria strigosa, Orbicella faveolata, O. annularis,* and *O. franksi*) with a 0% reinfection rate after treatment (*ex-situ*) (Pelose et al., *In prep*). The three main components of the treatment include a pre-cleaning procedure to remove necrotic tissue, hydrogen peroxide (H_2O_2) dips to oxidize potential pathogens on exposed skeleton and the coral's outer tissue layer, and a multicomponent antibiotic treatment dip to eliminate any remaining potential pathogens. This protocol explains how to conduct this novel treatment and provides recommendations for coral collection and preparation methods, coral husbandry, and post-treatment care.

Keywords: Stony coral tissue loss disease, SCTLD, Treatment, Intervention, Amoxicillin, Ciprofloxacin, Ex-situ, *Dendrogyra cylindrus*, Caribbean, Coral Restoration.

Reasoning for Protocol

Stony Coral Tissue Loss Disease (SCTLD) was first observed in 2014 near Miami, Florida and has rapidly spread across the Caribbean (Precht et al. 2016; Walton et al., 2018; Estrada-Saldívar et al., 2020). SCTLD affects at least 22 species of Caribbean stony corals and has resulted in the loss of up to 60% of coral cover in many Caribbean regions following initial exposure (Brandt et al., 2021; Heres et al., 2021; Papke et al., 2024). The opportunistic behavior of SCTLD is exacerbated when in contact with highly susceptible species, with as high as 90-100% reduction in local abundance reported within months of exposure (Walton et al., 2018; Estrada-Saldívar et al., 2020; Papke et al., 2024). This unprecedented loss of coral cover has been shown to reshape reef functionality (Alvarez-Filip et al., 2022) and has resulted in an urgent need for effective intervention and mitigation strategies to combat SCTLD and preserve genetic diversity of susceptible coral species. Current *in-situ* and *ex-situ* treatments have shown varying levels of success at halting the progression of SCTLD-like lesions in coral colonies (Miller et al., 2020; Walker et al., 2021; Forrester et al., 2022; Studivan et al., 2023; Papke et al., 2024). There are currently no published protocols available to effectively treat affected coral tissue in *ex-situ* facilities. The authors here seek to address this gap by presenting a 10-day treatment method that has shown to treat Caribbean coral colonies displaying signs of SCTLD with a 94% success rate (Pelose et al., *In prep*). The methods for this protocol were derived from treatments and resources within the reef-keeping hobbyist community and have been tested with 34 coral colonies from 6 Caribbean coral species (*Dendrogyra cylindrus* (n= 14), *Montastraea cavernosa* (n= 8), *Pseudodiploria strigosa* (n= 4), *Orbicella faveolata* (n= 3), *Orbicella annularis* (n= 2), and *Orbicella franksi*) (n= 3)).

Coral Collection and Preparation

Coral collection, transport, and labeling

This protocol suggests that when collecting Caribbean coral showing similar conditions to those of SCTLD, tissue near active lesions should be prioritized for collection as opposed to areas of 100% apparently healthy tissue (see Figure 1 for examples). Prioritizing active lesions and their surrounding live tissue, and thus removing active disease from the environment, may reduce potential pathogen load and support the ecosystem (Prentice et al., 2019; Papke et al., 2024). Once donor colonies are located, fragments showing signs of SCTLD can be removed and immediately placed and sealed in ziplock bags. When possible, epoxy is applied to open lesions left on the donor colonies after collection. Bags should remain sealed to minimize potential contamination during transport to a boat or shore-based location where they can then be placed in a transport vessel filled with seawater. Once the sealed bags have been placed in their transport vessel and contamination risk is no longer present, the bags should be opened during transport. Fragments should then be brought to a temporary quarantine holding system within a land-based holding facility in a timely manner.

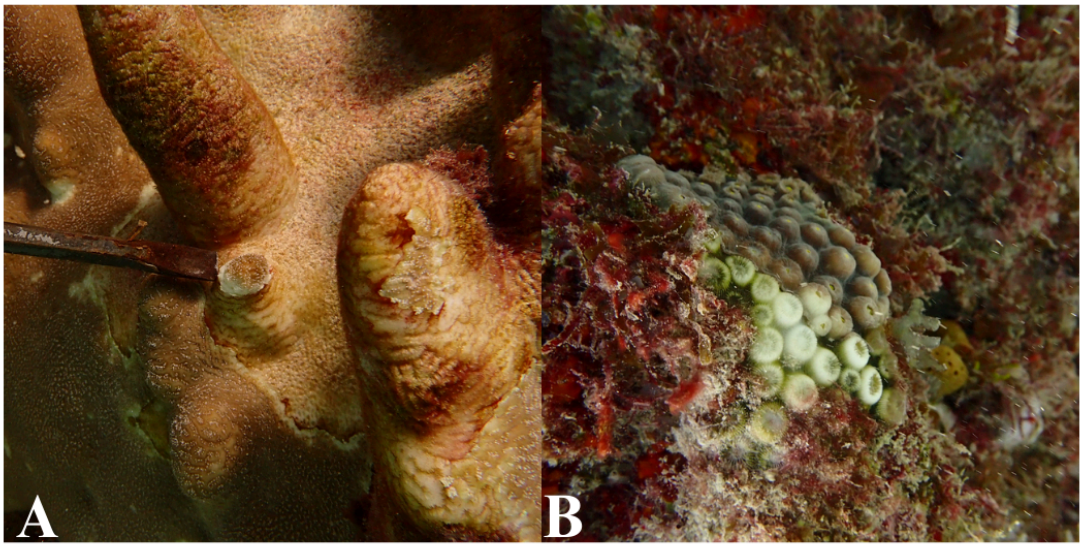

Figure 1. *A) Dendrogyra cylindrus* colony from the Dominican Republic showing SCTLD-like lesions. The chisel in the image points to the collection location within the donor colony. *B) Montastraea cavernosa* colony from the Dominican Republic showing SCTLD-like lesions. This whole colony was removed for treatment. Both *(A,B)* collections removed active disease lesions from the environment and did not damage apparently healthy tissue from the donor colony.

Prior to starting the treatment protocol, also referred to as the Treatment, colonies should be trimmed of excess exposed skeleton, wherever possible, and attached to a labeled base for easier transport and identification throughout the Treatment (Figure 2A). The labeled base should allow for the colony to be picked up and moved into containers without physically touching living tissue (Figure 2B). While attaching the corals to their respective base, using epoxy or super glue, it is important to minimize covering up exposed skeleton. Covering the skeleton may create a reservoir of pathogens or nuisance organisms that will not be mitigated by the Treatment. These reservoirs may allow continued exposure to potential pathogens or nuisance organisms during treatment or post-treatment and negatively impact coral health. Once the colonies are attached to their bases, they can remain in their quarantine tank until commencing the Treatment. To prevent further advancement of tissue loss, the Treatment should begin the day after collection, but can begin later if necessary. Trials have shown the Treatment to still be successful up to 26 days after collection (Pelose et al. *In Prep*).

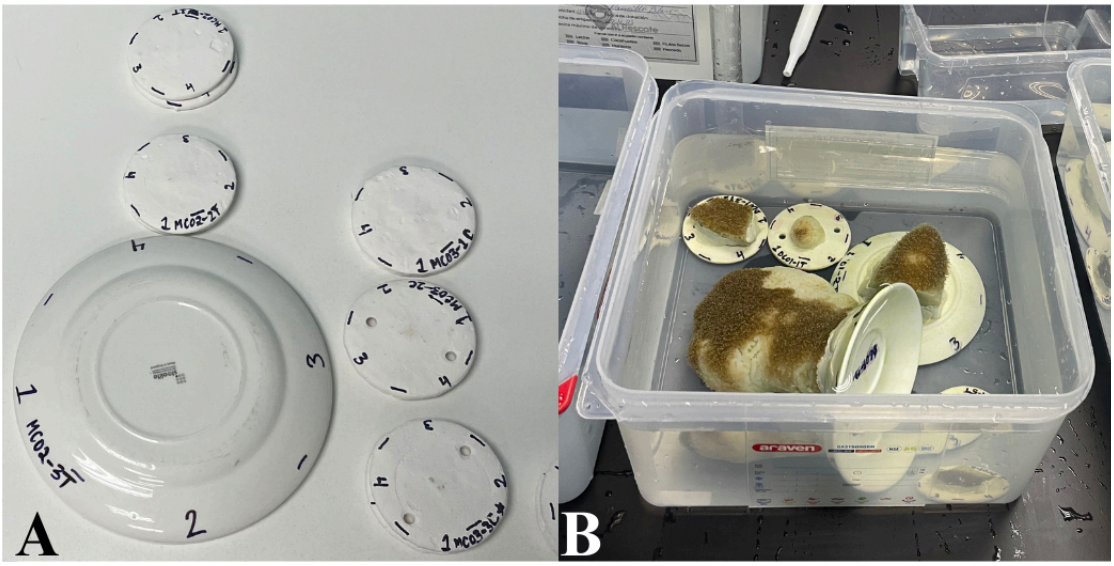

Figure 2. *A)* Examples of labeled bases, made from cement and ceramic plates respectively, used to prevent from having to make contact with living tissue during the Treatment. Colony identification labels were written on the top and bottom of the bases using a permanent marker and were coated in clear coat nail polish. Additional numbers and scale bars were written on the top of the bases to aid in image analysis. *B)* Representative image of the bases in use with *D. cylindrus* colonies. These colonies were added into the container without making any contact with their living tissue. The larger *D.*

cylindrus colony was placed onto a side that had no living tissue so that it could be fully submerged in the container.

Quarantine and post-treatment holding system set-up

Corals need to be held in a holding system that has shown the capability to support long term coral growth and recovery prior to the potential stress of the Treatment. If the holding system has not shown the capability to successfully hold and grow corals, it may result in a decreased functionality of the Treatment. Water quality parameters should stay stable and ideally be maintained within the "Ideal Parameters for Reef Aquariums" reference chart (https://www.bulkreefsupply.com/content/post/md-2021-03-reef-tank-parameters). It is best to replicate PAR values recorded at the collection site or this protocol recommends providing your corals with an average of 200 PAR during the 10 day treatment. Antibiotic treatments should be started at a time that minimally affects the normal photic period of the colonies being treated (approximately 2 P.M. or later is recommended).

Corals should be held within a temporary quarantine holding system until the treatment starts. After day one of the Treatment, colonies will be moved into a post-treatment holding system where they will reside for the remaining 9 days of treatment and thereafter. There are two options for a post-treatment holding system (Figure 3). The first, and preferred option, includes moving the colonies into a separate, clean system that does not have other coral colonies in it. If a separate system is not available, it is still possible to conduct the treatment by modifying the original quarantine tank (Figure 3, Option Two) during day one of treatment. Modifications include using a siphon hose to clean detritus from the tank until 50% or more of the water is removed. This modification needs to occur during the first four-hour antibiotic treatment dip conducted on Day 1. Experimental trials for this protocol were conducted in a flow-through, open system utilizing Option Two (Figure 3), where the original quarantine holding system was modified during day one of treatment and then utilized as the post-treatment holding system.

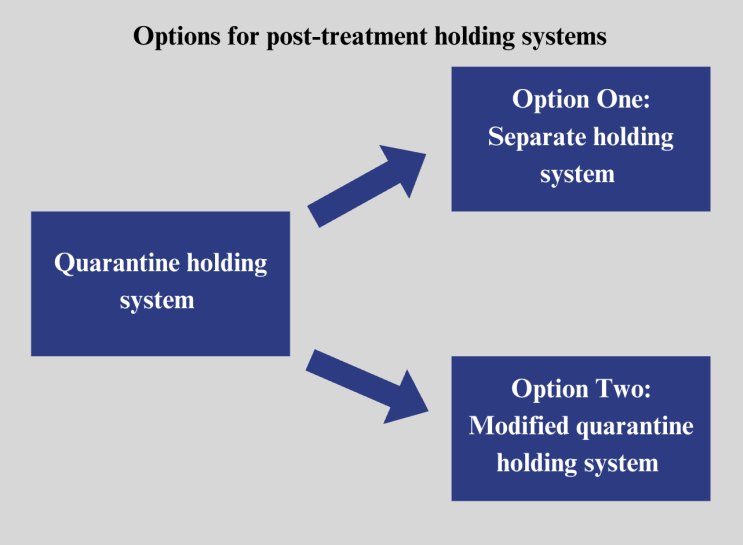

Figure 3. Two post-treatment holding system options for colonies after day one of the Treatment. Option One includes moving colonies into a separate holding system that does not have any other colonies. Option two includes adding colonies back into the original quarantine holding system after it has been modified during the first four-hour antibiotic dip by siphoning detritus from the tank until 50% or more of the water is removed.

Coral Feeding

While in holding and during the treatment schedule, colonies should receive a consistent feeding schedule to support tissue growth and overall coral health. This protocol suggests feeding once a day with one and a half teaspoons (tsp) of Reef-Roids (https://www.polyplab.com/) coral food mixed in approximately 300 mL of saltwater coming directly from the holding system the corals are held in (Figure 4).

Figure 4. Materials needed for the daily feeding protocol: 1) Reef-Roids coral food, 2) measuring spoons, 3) 300 mL container, 4) kitchen baster used to mix and administer the food.

The total amount of this mixture is dependent on the quantity of corals being fed and should be calculated accordingly. For reference, 300 mL of mixture can feed approximately 60 colonies of similar size to the *Montastraea cavernosa* colony in Figure 11 at day 349 (approximately 33 cm^2). The Reef-Roid solution should be mixed thoroughly with a baster and allowed to hydrate for a minimum of 15 minutes prior to feeding. While waiting for the coral food mixture to hydrate, all internal flows of the holding tank should be turned off including any form of internal pump, wavemaker, and flow that may inhibit coral feeding. At the end of the hydration process, thoroughly mix the food again with the baster and target feed each coral. All corals in a tank should be fed this mixture and carefully monitored to ensure the majority of corals are eating. Once all corals have been target-fed, they should be allowed to digest for 45 to 60 minutes. If any coral is not observed to feed during the initial feeding, those individuals should be target-fed again after all corals are fed. All colonies should be periodically monitored throughout the digestion period. The feeding preparation should be started at least 1.5 hours prior to treatment and all water flow should remain off during this period. Since treatment is suggested to start at or after 2:00 P.M. to minimize changes to the coral's normal photic period, feeding should start at 12:00 to 12:30 P.M. or later.

This concentration of coral food has successfully supported corals undergoing the Treatment, but at this time, has only been conducted in an open system with a

constant influx of new seawater. The constant incoming flux of seawater likely aids in the system's ability to manage this concentration of nutrient load. As such, conducting this feeding regime within a closed system may result in a significant change to water quality and subsequent water quality monitoring, water changes, and tank maintenance should be increased to reduce the impacts of high nutrient loads.

Treatment Materials

The 10-day Treatment includes a pre-treatment hydrogen peroxide (H_2O_2) dip, an antibiotic/antiseptic treatment dip, and a post-treatment hydrogen peroxide dip each day. For hydrogen peroxide dips, three percent hydrogen peroxide and a 50 to 100 mL graduated cylinder are required (Figure 5). The antibiotic/antiseptic treatment requires three dry antibiotic reagents and two liquid reagents (Figure 6). The three dry reagents are amoxicillin, ciprofloxacin, and an aquarium treatment product called Chemiclean (https://chemi-pure.com/products/chemiclean/). Local procurement procedures should be followed to obtain antibiotics (i.e., may require a permit and/or prescription). The two liquid reagents are Lugol's solution (iodine antiseptic) and Restor (coral tissue regrowth supplement), both made by Brightwell Aquatics (https://brightwellaquatics.com/products/index.php). The concentrations of each reagent used for a four gallon treatment can be found in Figure 6, but can be adjusted as needed for larger or smaller treatments to accommodate the quantity or size of colonies being treated (Table 1). Five containers with the same dimensions will be needed to conduct this protocol (Figure 7). It is also important to note that a four gallon treatment will utilize up to 20 gallons (75.7 Liters) of seawater during each day of the Treatment. If a closed system is being utilized, it is recommended that seawater is pre-mixed and prepared to refill the holding system after each day of treatment.

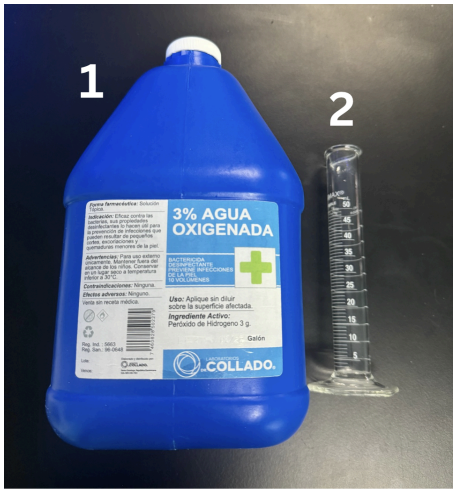

Figure 5. Materials needed to prepare the hydrogen peroxide dips. 1) One gallon of 3% Agua Oxigenada (Hydrogen Peroxide) and 2) 50 mL graduated cylinder used to measure the quantity of Hydrogen Peroxide.

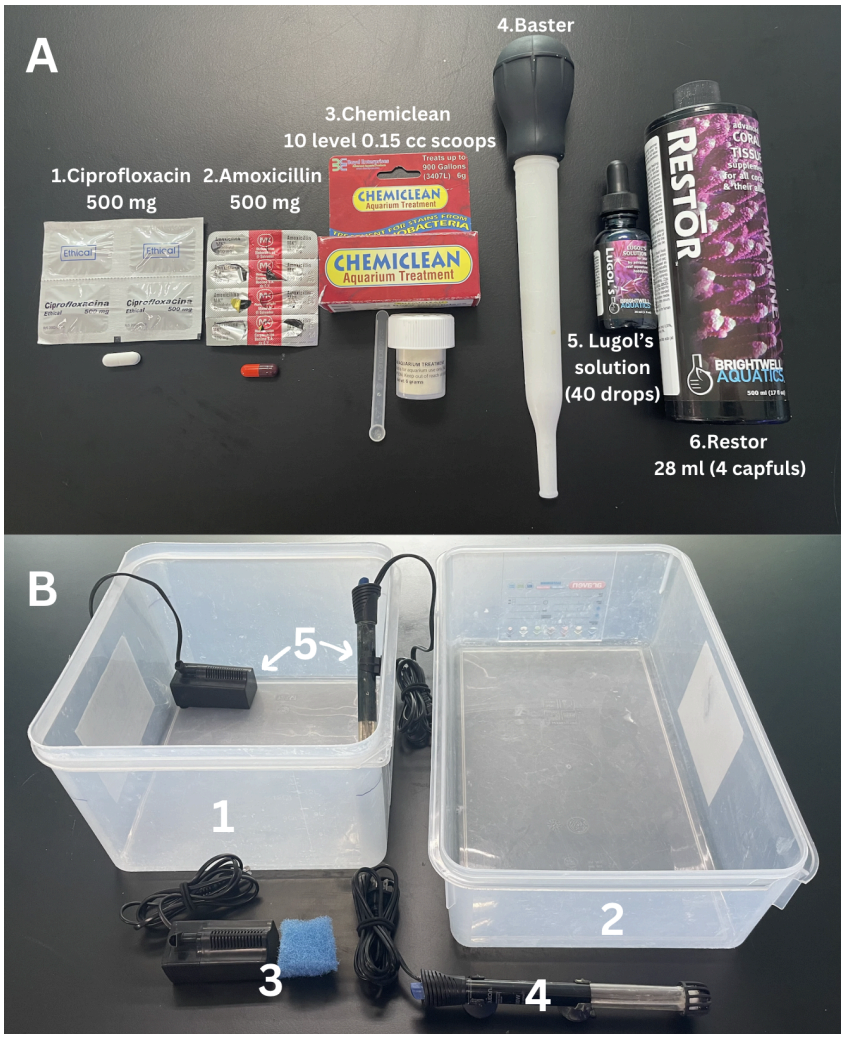

Figure 6. Materials needed to conduct the 10-day antibiotic treatment. *A)* Reagents should be added in the order displayed, 1 through 6: 1) Ciprofloxacin (500 mg), 2) Amoxicillin (500 mg), 3) Chemiclean (10 level scoops (0.15 cc) = 1.5 cc total), 4) Kitchen baster used to ensure dry reagents are not floating on the surface before adding the liquid reagents, 5) Brighwell Lugol's iodine solution (40 drops), 6) Restor reagent (coral tissue supplement) (4 capfuls = 28 mL). *B)* Containers and other equipment/supplies: 1 and 2) Treatment containers, 3) Internal filter (Dophin Internal Filter KF-350) with sponge, 4) Optional 100-watt internal tank heater if ambient water temperature is below 23.3 degrees Celsius, and 5) Placement of filter and heater. Different types of treatment containers may be used to accommodate taller branching colonies (B1) and flatter bouldering colonies (B2).

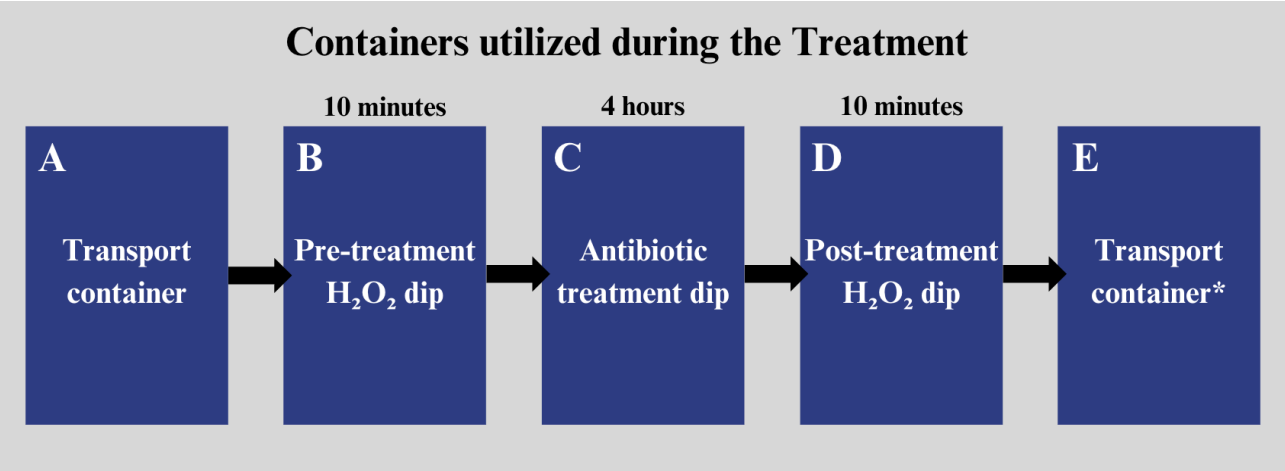

Figure 7. Up to five treatment containers will be utilized during each day of the protocol (*A-E*). All containers used as part of the Treatment should be relatively the same size to ensure all corals will fit for each phase of the treatment. *A*) Transport container used to remove the corals from their holding system and conduct the pre-cleaning procedure. Colonies are soaked in the pre-treatment H_2O_2 dip container (*B*) for 10 minutes, an antibiotic treatment dip container for four hours (*C*), and a post post-treatment H_2O_2 dip container for 10 minutes (*D*). Colonies are then brought back to their post-treatment holding system utilizing a transport container (*E*).

∗If the post-treatment holding system is near the treatment location (approximately 5 seconds or less to transfer corals between systems), the colonies can be brought directly into the holding system making the second transport container (*E*) not necessary.

Reagant Amount

	1 gallon (3.875 L)	4 gallons (15.142 L)	8 gallons (30.284 L)
Ciprofloxacin (mg)	125.0*	500	1000
Amoxicillin (mg)	125.0*	500	1000
Chemiclean Aquarium Treatment (scoops)	2.5**	10	20
Lugol's Advanced Iodine Solution (drops)	10.0	40	80
Brightwell's Restor (mL)	7.0	28	56
Hydrogen Peroxide (mL)	40.0	160	320

Table 1. Concentrations of reagents for one gallon, four gallon and eight gallon treatment solutions.
* Ciprofloxacin and Amoxicillin are regularly supplied as 500 mg per capsule. To utilize the entire capsule, 500 mg of each antibiotic reagent can be dissolved into four gallons and then one gallon may be transferred into a smaller container. The other reagents can then be added into this smaller one gallon container.
** The 0.5 scoop can be estimated for one gallon treatments.

Treatment Preparation

Antibiotic treatment dip and pre-treatment H_2O_2 dip preparation

The antibiotic treatment dip container should be prepared first to allow time for all reagents to dissolve. Collect four gallons (15.1 L) of seawater in a treatment container (Figure 6B) from the same tank or water source where the corals are held. Do not fill the treatment container completely as it is important to allow volume to add the corals. The small internal filter (Dophin Internal Filter KF-350 with sponge) is added next to start circulating the water and remove particulates that may be floating throughout the antibiotic treatment dip. A 100-watt heater can also be added into the antibiotic treatment dip container to maintain the water temperature above 23.3 degrees Celsius (74 degrees Fahrenheit).

In the following order, mix the dry materials, Ciprofoxacin (500 mg), Amoxicillin (500 mg), and Chemiclean (10 level scoops (0.15 cc) = 1.5 cc total) into the antibiotic treatment dip container. Adding the ciprofloxacin first allows the hard 500 mg tablet to dissolve over time. Next, the internal powder contents of the 500 mg amoxicillin capsule is added to the antibiotic treatment dip container being careful to not allow water to come into contact with the capsule. Add 10 level scoops of Chemiclean

(use a flat utensil to level off the powder inside the scoop to reduce variability). After adding the Chemiclean, use the kitchen baster to mix the surface of the water until all floating dry reagents are inundated to ensure that liquid reagents do not bind with them on the surface. Once there are no longer dry materials visibly floating on the surface of the water, add the liquid reagents in the following order: 1) Lugol's iodine solution (40 drops) and 2) four capfuls (7 mL each for a total of 28 ml) of Brightwell's Restor. Once all reagents are added, the solution should be left to continue dissolving for 20-30 minutes. The solution should be mixed with the kitchen baster roughly every 5 minutes to assist the dissolving process. Note that it is not uncommon to have some small undissolved particles present in the bottom of the container at the end of the wait time.

While the antibiotic dip is dissolving, prepare the pre-treatment H_2O_2 dip by collecting three gallons (11.4L) or four gallons (15.1 L) of tank water into another treatment container. The water level should be high enough that all the corals will be fully immersed when added into the container. Next, add 40 mL of 3% H_2O_2 per gallon (3.8 L) of seawater. This prepared pre-treatment H_2O_2 dip container can be placed next to the dissolving antibiotic treatment dip container.

Start of Protocol

Pre-cleaning

Fully submerge the transport container into the holding system that the corals are in and carefully place all treatment colonies into the submerged container without exposing them to air. Attempt to arrange the colonies evenly and in an order that can be duplicated within the H_2O_2 dip container and antibiotic treatment container. Carefully remove the transport container from the holding system with the colonies inside and set it next to the pre-treatment H_2O_2 dip container and antibiotic treatment dip container. If the holding system does not have ample space to submerge the transport container inside, then the transport container may be filled with tank water using a siphon hose. Colonies should then be placed into the filled transport container one by one, ensuring that each colony is first lightly shaken in the tank to promote tentacle retraction prior to being temporarily removed from the water.

Within the transport container, all corals should receive a pre-cleaning with water pressure from a kitchen baster to remove any necrotic or dead tissue near or on

active SCTLD-like lesions. It is important to only apply enough water pressure to remove the tissue that has become detached and avoid damaging the living tissue still attached to the skeleton. Depending upon the progression of the lesion, up to an inch of decaying tissue may come off from active disease lesions. The transport container may become clouded, and the decaying tissue and potential pathogens may produce a foul smell. In many cases, it will not be possible to remove all necrotic or dead tissue detached from the skeleton.

Pre-treatment hydrogen peroxide (H_2O_2) dip

After pre-cleaning, use a clean kitchen baster to stir the contents of the prepared pre-treatment H_2O_2 dip container immediately prior to adding the corals to prevent layers from forming in the container. In addition, the antibiotic treatment dip should also be stirred one last time to allow any undissolved reagents time to settle to the bottom of the container. Coral colonies should be shaken underwater while still in the transport container to remove any particulates that rest on top of the colony post pre-cleaning and also to promote the retraction of polyp tentacles before being removed from the water. Place each coral into the pre-treatment H_2O_2 dip container for 10 minutes and record the order that the colonies are added so they can be removed in the same order. It is important to let the corals sit for the full 10 minutes to allow for the H_2O_2 to oxidize and disinfect the exterior of the coral tissue, exposed skeleton and labeled ceramic base. During this process, micro-bubbles may form on the colonies and may be concentrated on the exposed skeleton created during cleaning (Figure 8A). It is common for colonies to expel waste (Figure 8A) or temporarily expose mesentery digestive filaments (Figure 8B) during the pre-treatment H_2O_2 dip. At the 10-minute mark, colonies should be lightly shaken under the water to remove microbubbles and exposed skeleton should be cleaned using water pressure from the kitchen baster. Colonies should then be removed from the pre-treatment H_2O_2 dip in the order that they were originally added.

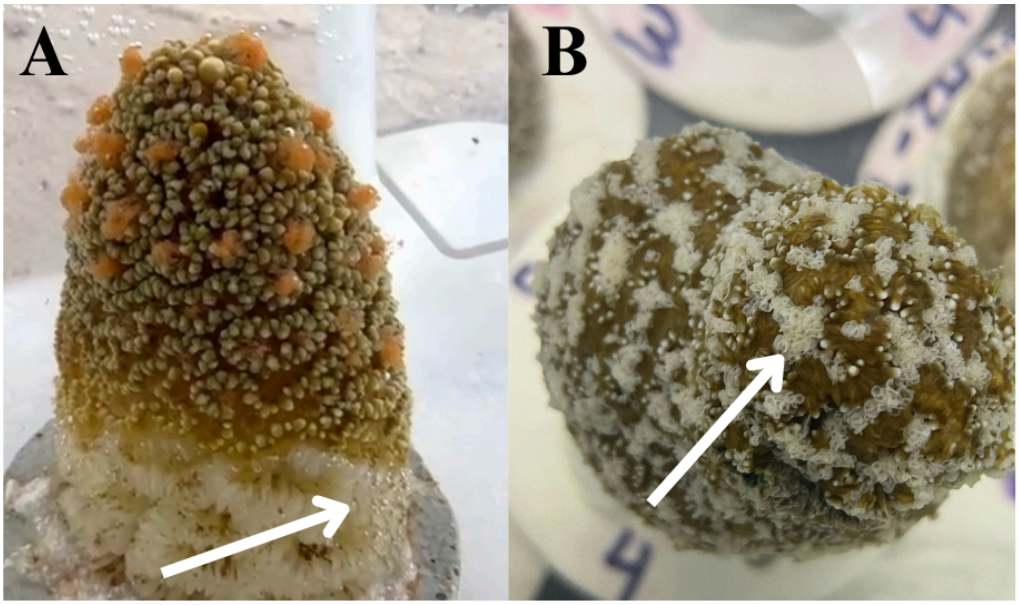

Figure 8. *A) Dendrogyra cylindrus* colony in a pre-treatment H_2O_2 dip. The coral is actively expelling waste which appears as small red-orange bundles sitting near the polyps' mouths. The white arrow points to bubbles that may form on the skeleton of the corals. *B)* Exposed mesentery filaments of a *D. cylindrus* colony during a pre-treatment H_2O_2 dip (indicated by the white arrow).

Antibiotic treatment dip

Corals should be placed directly from the pre-treatment H_2O_2 dip container into the antibiotic treatment dip container in the same order that each colony was added to the pre-treatment H_2O_2 dip. Ensure that the flow coming from the internal filter in the antibiotic treatment dip container is not pointed directly at any coral tissue. Corals will soak in the antibiotic treatment dip for four hours and should be temporarily monitored, ensuring that the internal filter does not become dislodged and pointed directly at living tissue. After day one of the antibiotic treatment dip, colonies will utilize the post-treatment holding system (see *Quarantine and post-treatment holding system set-up)*.

Post-treatment hydrogen peroxide (H_2O_2) dip and transfer to post-treatment tank

Prepare the post-treatment H_2O_2 dip near the three-and-a-half-hour mark of the antibiotic treatment dip. Fill the post-treatment H_2O_2 Dip container with three gallons (11.4L) or four gallons (15.1 L) of tank water from the post-treatment holding system and add 40 mL of 3% H_2O_2 per gallon (3.8 L). Prior to adding the corals, the

post-treatment H_2O_2 dip solution should be mixed with a baster to disrupt any layers that may have formed in the mixture. Post mixing, fragments can then be moved in the same order that the colonies entered the antibiotic treatment dip into the post-treatment H_2O_2 dip container. Colonies will remain in the post-treatment H_2O_2 dip for 10 minutes. If a coral has significant polyp extension while in the antibiotic treatment dip container, they should be lightly shaken so that they retract their tentacles prior to being taken out of the water and transferred into the post-treatment H_2O_2 dip container. While the corals are in the post-treatment H_2O_2 dip container, a clean transport container should be used to collect water from the post-treatment holding system where the corals will be held post-treatment. If the treatment is being conducted close to the post-treatment holding system (i.e., 5 seconds or less to transfer the corals), then a transport container is not necessary. After 10 minutes in the post-treatment H_2O_2 dip, colonies should be lightly shaken under the water to remove microbubbles and exposed skeleton should be cleaned using water pressure from the kitchen baster. Next, transfer the corals from the post-treatment H_2O_2 dip container in the order that they originally were placed into their post-treatment holding system via the transport container or directly into the tank. If a transport container is being used, it can be submerged into the post-treatment holding system if possible to avoid taking the colonies out of the water again. If it does not fit, then the colonies can be removed from the water and placed into the post-treatment holding system one by one.

Cleanup and prep

The antibiotic infused water from the antibiotic treatment container may be stored in large and labeled containers for professional disposal or it may be disposed via local regulations. The contents coming from the pre- and post-treatment H_2O_2 dips and the pre-cleaning may be discharged down drains that lead to sewage treatment plants or as required by local regulations. All containers should be rinsed with freshwater and set to fully dry overnight to be used again the following day. All basters and coral feeding containers can be placed in a mild bleach solution (20 ml bleach to one liter of H_2O) overnight and then set to air dry the following morning to be utilized in the afternoon.

Following 9 days of treatment

The same procedure is to be repeated for a total of 10 days. All saltwater used for the rest of the treatment should come from the post-treatment holding system.

Figure 9 below provides a timeline that can be followed to aid in completing each day of the Treatment.

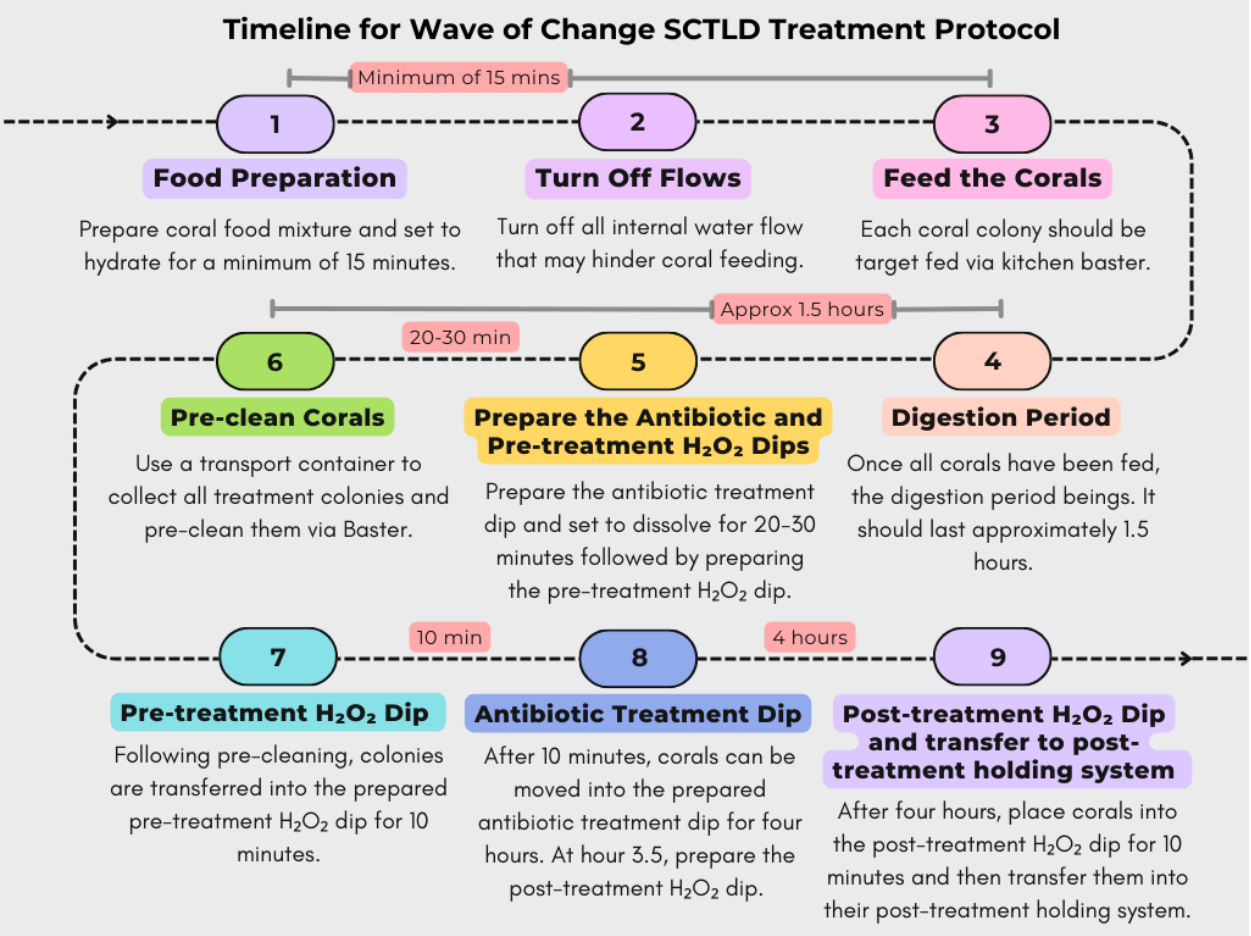

Figure 9. Timeline for the Treatment. Step 1: Prepare a Reef-Roids coral food mixture. Step 2: Turn off all internal tank flow that may hinder coral feeding. Step 3: After 15 minutes of the coral food mixture hydrating, each coral colony can be target fed with a kitchen baster. Step 4: Allow corals to digest food over approximately 1.5-hours. Step 5: Prepare the antibiotic treatment dip container and pre-cleaning H_2O_2 dip container. Step 6: Use a transport container to collect all treatment colonies and pre-clean them with a kitchen baster. Step 7: Conduct pre-treatment H_2O_2 dip. Step 8: Conduct antibiotic treatment dip for four hours and prepare the post-treatment H_2O_2 dip at the three-and-a-half-hour mark. Step 9: Conduct post-treatment H_2O_2 dip for 10 mins followed by transporting colonies into their post-treatment holding system. This is to be repeated for 10 days.

Post Treatment

After the final day of Treatment, the colonies can either stay in the post-treatment holding system or be moved to a different holding system. It is recommended that feeding is conducted in the same way post-treatment to help enhance regrowth over existing lesions.

Protocol Trial Results

Using the protocol described above, 32 of the 34 treated colonies (94% success) survived the treatment and had no additional tissue recession post-treatment (Pelose et al. *In prep*). Figures 10 and 11 show examples of coral colonies that were treated with this protocol. These colonies, along with many others, demonstrated the capability to regrow over previously exposed skeleton.

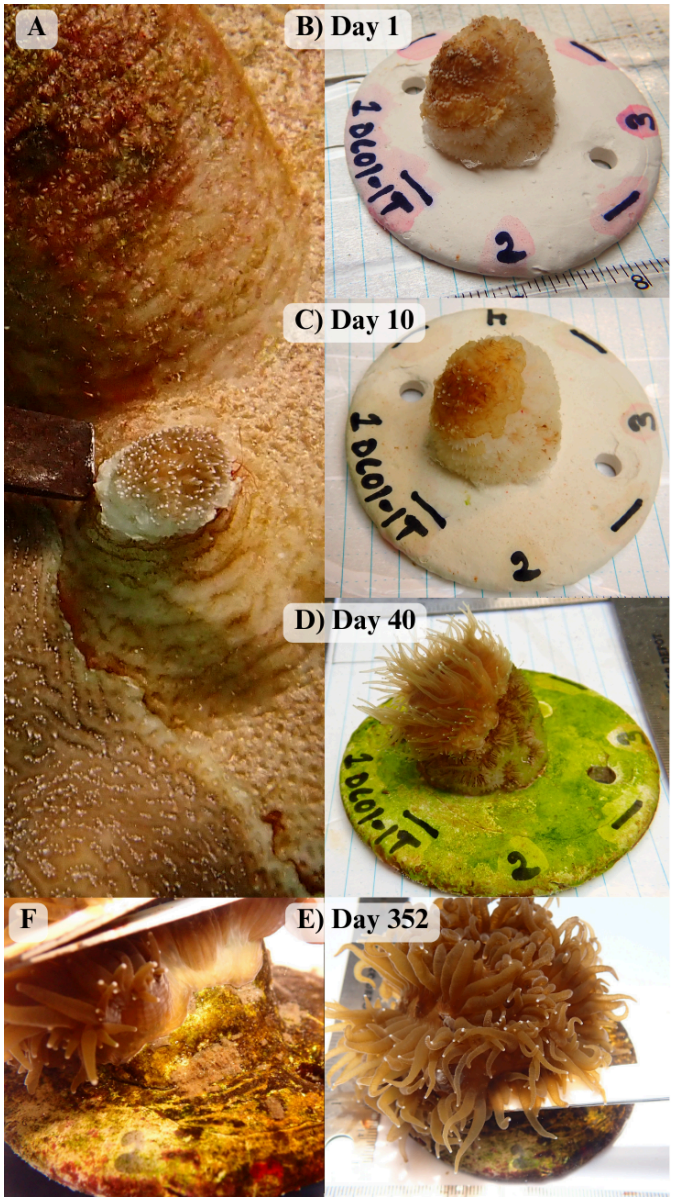

Figure 10. *D. cylindrus* colony before and after the Treatment. Image A on the top left shows the fragment prior to collection from its donor colony. The four images on the right show the colony on day 1 before pre-cleaning of necrotic tissue (B), 10 (C), 40 (D), and 352 (E). On day 352 (F), note that the colony's tissue has resheeted down previously exposed skeleton.

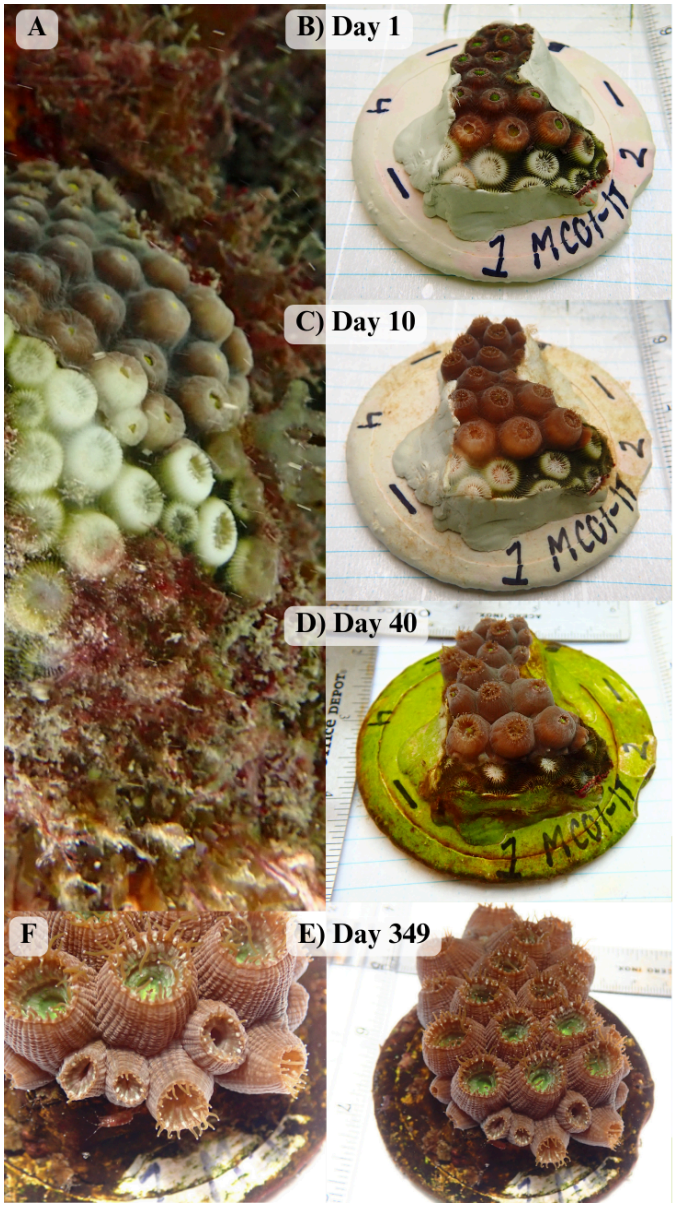

Figure 11. *Montastraea cavernosa* colony before and after the Treatment. Image A on the left shows the fragment prior to collection from its donor colony. The four images on the right show the colony on day 1 after pre-cleaning of necrotic tissue (B), 10 (C), 40 (D), and 349 (E). On day 349 (F), note that new polyps have formed over previously exposed skeleton.

A variety of colony sizes were treated using this protocol, one of the smallest being approximately 5.10 cm^2 (± 0.05 cm^2) of size (Figure 12). This colony had nearly an undetectable amount of tissue remaining on the skeleton after pre-cleaning (Figure 12, Day 1).

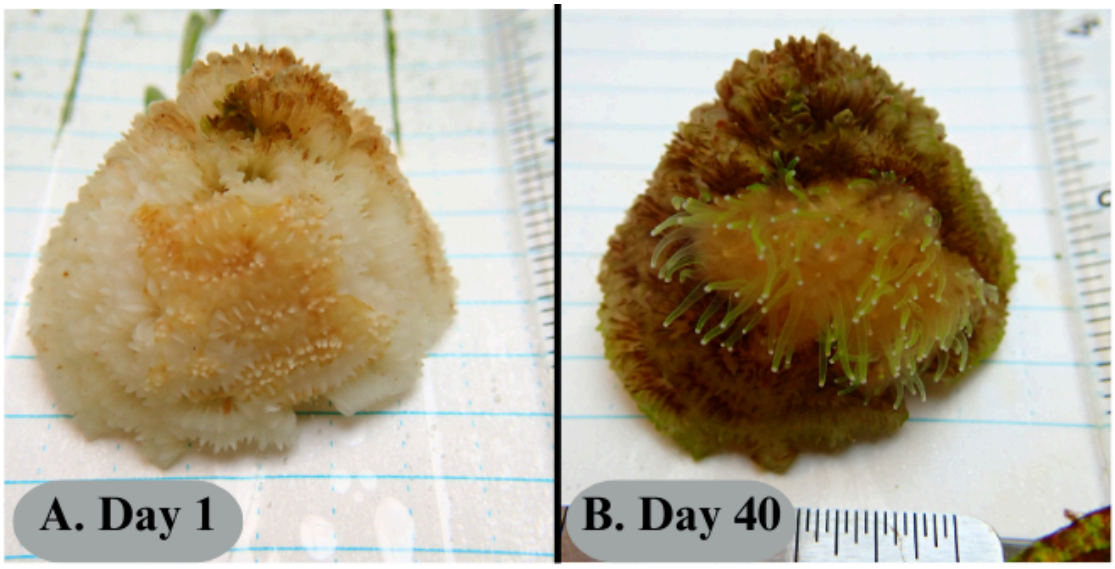

Figure 12. A 5.1 cm^2 (± 0.05 cm^2) *D. cylindrus* colony that recovered from SCTLD-like symptoms after treatment. *A)* Day 1 of treatment after pre-cleaning of necrotic tissue. *B)* Day 40 (10-days of treatment and 30-days in-lab).

Therefore, the use of this protocol demonstrates that colonies containing even small portions of healthy tissue (approximately 5.10 cm^2 (± 0.05 cm^2)) may survive and recover. Some colonies showing signs of SCTLD were also partially bleached before going through the treatment protocol. These colonies were able to survive the treatment and also increase symbiont density post-treatment (Figure 13).

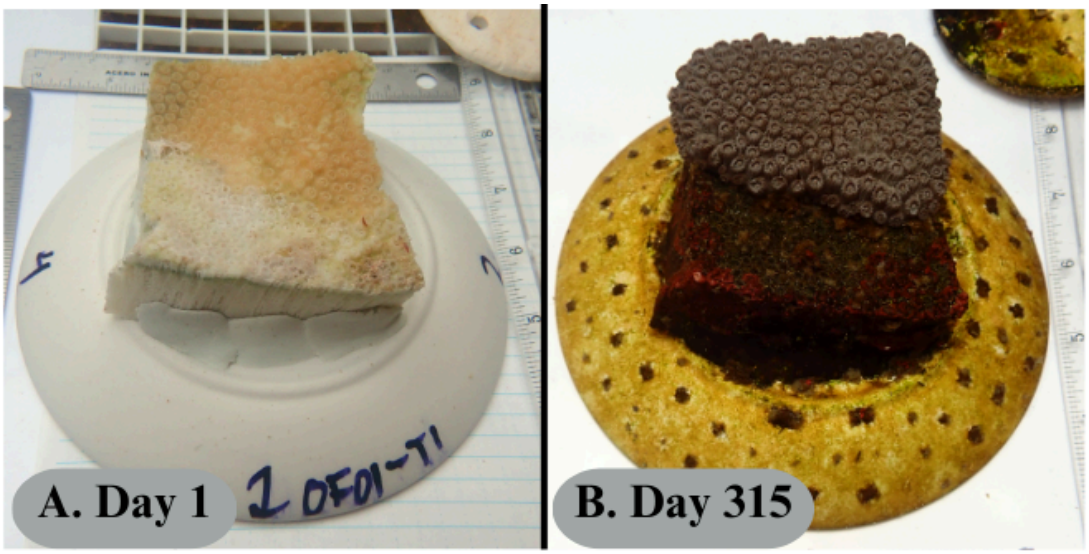

Figure 13. A partially bleached *Orbicella faveolata* colony showing SCTLD-like symptoms that was put through the Treatment. This colony was able to recover and is now available for future restoration opportunities.

All treated colonies have been placed in Iberostar's land-based restoration facility located in the Dominican Republic. These recovered colonies represent valuable genetic material as many of the mother colonies that the treated fragments came from have since fully perished. It is the hope of this publication that this treatment, if scaled, can play an important role in Caribbean coral genetic preservation while also helping other organizations keep SCTLD out of their land-based restoration and genetic banking facilities.

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