JNCC – UK Overseas Territories Report Series

Stony Coral Tissue Loss Disease: Treatment and Management Strategy for the Caribbean UK Overseas

Territories 2023/2024





Report Number: 5 Authors: Dr Greta Aeby, Bryony Meakins, Sabrina Weber, Abbie Dosell, Chloe Hatton, and Sara De Giorgio Publication date: August 2024 ISSN: 2753-6270 Front cover photo: © Amanda Nicholls – Pura Vida Photography

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Summary

The Collaborative Stony Coral Tissue Loss Disease (SCTLD) Treatment and Management Strategy 2023/2024 provides an update to the previously written, jointly developed strategy for the Coral Conservation in the UK Overseas Territories (C-COT) group, funded through the Darwin Plus project "Collaborative approach to managing coral disease in UK Overseas Territories" (DPLUS 147). The strategy has been developed to optimise SCTLD response in the UK Overseas Territories (OTs), minimise impacts on biodiversity, and share OT and wider regional experiences, scientific expertise, and resources. This report presents a summary of research up to December 2023 into treatment methodologies for SCTLD alongside recommendations and guidelines for future SCTLD treatment trials, and robust design and data collection to inform future management strategies.

This Darwin Plus project is a partnership between the governments of the British Virgin Islands, Cayman Islands, and the Turks and Caicos Islands, alongside Kalli de Meyer (C-COT chairperson and Director at Nature2) and coral disease expert, Dr Greta Aeby. Recognising the broad scale of threats to coral reef ecosystems, the project shares knowledge and resources across the OTs, including Anguilla, Bermuda, and Montserrat, working with government departments, Non-Governmental Organisations and other key stakeholders.

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1. Introduction

1.1. Stony Coral Tissue Loss Disease (SCTLD)

Stony coral tissue loss disease (SCTLD) was first reported off the coast of Miami, Florida in the USA in 2014 (Precht *et al.* 2016). Emergence of the disease coincided with summer bleaching events across the Florida Reef Tract (Manzello 2015; Walton *et al.* 2018). In addition, dredging operations between 2013 and 2015 in the channel at Port of Miami, Florida, resulted in massive sedimentation near the initial site of the outbreak (Miller *et al.* 2016). SCTLD has since spread throughout the Caribbean (Alvarez-Filip *et al.* 2019; Estrada-Saldívar *et al.* 2020; Croquer *et al.* 2021) and into the Gulf of Mexico (Johnston *et al.* 2023). SCTLD affects more than 30 scleractinian coral species in 32 countries/territories (AGRRA, 2023) and has resulted in massive die-offs in multiple coral species (Alvarez-Filip *et al.* 2023). The coral species most impacted include brain, pillar, star, and starlet corals with other species suffering less mortality from the disease (AGRRA 2023). Figure 1 below shows countries that have identified or suspect to have SCTLD on their reefs, as reported by AGRRA in December 2023.



Figure 1. Map showing location of SCTLD-affected areas (red dots), suspected SCTLD (purple dots) and unaffected areas (green dots) (from Kramer *et al.* 2019; Updated as of December 2023).

SCTLD is an infectious, waterborne disease (Aeby *et al.* 2019), which can be spread by natural ocean currents (Dobbelaere *et al.* 2020; Muller *et al.* 2020). Within the wider Caribbean, it has been suggested that the cross-regional dispersion of SCTLD has been facilitated by commercial shipping via contaminated ballast water, biofouling, and sediments (Dahlgren *et al.* 2021; Rosenau *et al.* 2021; Evans *et al.* 2022; Studivan *et al.* 2022). Although the cause of SCTLD is still unknown, it is thought that bacterial infections of the coral tissue are involved (Aeby *et al.* 2019; Neeley *et al.* 2020; Walker *et al.* 2021a), along with possible co-infections (Ushijima *et al.* 2020). Viral infection of the endosymbiotic zooxanthellae has also been proposed as a causal agent (Work *et al.* 2021). SCTLD

prevalence and disease mortality varies across reefs and regions, suggesting that environmental factors contribute to disease patterns and characteristics (Sharp *et al.* 2020; Aeby *et al.* 2021). A recent study using spatial and temporal statistical models found SCTLD prevalence in tagged *Orbicella faveolata* colonies in South East Florida was linked to anthropogenically impacted water flowing out of inlets adjacent to colonies (Walker *et al.* 2021b). Alvarez-Filip *et al.* (2022) also found a link between coastal development and SCTLD prevalence. Evidence continues to build coupling increased water temperatures, thermal stress, and coral bleaching with disease outbreak prevalence (Croquer & Weil 2009; Walton *et al.* 2018), making this issue increasingly important to monitor and respond to in the face of climate change. Interestingly however, maintained heat stress has in some cases also lead to an abatement of SCTLD on affected colonies (Meiling *et al.* 2020; Williams *et al.* 2021). A review of SCTLD is provided by Papke *et al.* (2024).

Most outbreaks of tissue loss diseases on coral reefs have a limited spatial range and are short-lived in nature (e.g. Williams and Miller 2005; Brandt *et al.* 2012; Aeby *et al.* 2016). However, SCTLD is continuing to spread across the Caribbean ten years after its onset in Florida, and has recently emerged in Bonaire, Aruba, Curacao, and Cuba (AGGRA, October 2023). This is producing extreme coral mortality along with reduced species richness and shifts in coral assemblages across these reefs (Hayes *et al.* 2022), indicating the urgent need to develop unique strategies for coral disease management and mitigation. Disease management guides, such as this one, were developed and currently 12 countries/territories across the Caribbean are engaged in a form of SCLTD intervention (Table 1).

Government	Disease present	Staff trained	Interventions	Monitoring	SCTLD Rescue
Antigua & Barbuda	Х	-	-	Х	-
Aruba	-	Х	-	Х	-
The Bahamas	Х	Х	-	Х	-
Belize	Х	Х	Х	Х	Х
Bonaire	Х	Х	-	Х	-
British Virgin islands	Х	Х	Х	Х	-
Cayman Islands	Х	Х	Х	Х	Х
Colombia	Х	-	-	Х	-
Curacao	-	-	-	Х	-
Dominica	Х	Х	Х	Х	Х
Dominican Republic	Х	Х	Х	Х	Х
Grenada	Х	Х	-	Х	-
Guadeloupe	Х	-	-	Х	-
Guatemala	-	Х	-	Х	-
Honduras	Х	Х	Х	Х	Х

Table 1. SCTLD response efforts of islands/territories across the Caribbean (from AGGRA, October 2023).

Government	Disease present	Staff trained	Interventions	Monitoring	SCTLD Rescue
Jamaica	Х	Х	-	Х	-
Martinique	Х	Х	-	Х	-
Mexico	Х	Х	Х	Х	Х
Puerto Rico of the United States	Х	Х	Х	Х	Х
Saba	Х	Х	-	Х	-
Saint Barthelemy	Х	-	-	Х	-
St Kitts & Nevis	Х	-	-	Х	-
Saint Lucia	Х	Х	-	Х	-
Saint-Martin	Х	-	-	Х	-
Saint Vincent and the Grenadines	Х	Х	-	Х	-
Sint Eustasius	Х	Х	Х	Х	-
Snt Maarten	Х	-	-	Х	-
Turks and Caicos Islands	Х	Х	Х	Х	Х
United States	Х	Х	Х	Х	Х
Venezuela	-	-	-	Х	-
Virgin Islands of the United States	Х	Х	Х	Х	Х

Many of the UK's inhabited Overseas Territories (OTs) are small, remote islands containing a combined coral reef area of around 4,700 km² (Sheppard 2013; Hamylton & Andréfouët 2013). These islands provide a home to 220,000 people who are reliant on their natural environment and the benefits that it provides for their economic welfare, security, and culture. Reef habitats are particularly important as they provide fish nursery grounds, coastal protection, and opportunities to develop tourism (Petit & Prudent 2008; Forster *et al.* 2011). Additionally, coral reefs are a habitat known to contribute to cultural identity and social norms (Cinner 2014), particularly in island nations or territories.

The JNCC-led Darwin Plus (DPLUS147) project, *Collaborative approach to managing coral disease in UK Overseas Territories*, is supporting the response of UK Overseas Territories to SCTLD. The project funds the running of the 'Coral Conservation in the UK Overseas Territories' (C-COT) working group and fosters partnerships between governments, Non-Governmental Organisations (NGOs), academic institutions, and wider stakeholders to coordinate effective coral reef management decisions. As part of the project, the focus of this report is to develop and implement a joint treatment and management strategy to optimise SCTLD response efforts, minimise impacts on biodiversity, and share UK OT and wider regional experiences, scientific expertise, and resources. This strategy can also be used to inform the development of OT-specific response plans to SCTLD. In the Cayman Islands, Montserrat and the Turks and Caicos Islands, OT-specific disease and bleaching response plans have already been taken forward.

2. Overview of SCTLD Strategy

The overall strategic plan is divided into actions required during the three recognised stages of SCTLD infection on a coral reef: pre-invasion, initial invasion, and outbreak.

2.1. Stage 1: Pre-invasion (no SCTLD present)

- Legal preparation.
- Training.
- Monitoring (baseline survey data on coral communities and diseases present).
- Identify vulnerable sites.
- Develop decision criteria for assessing risk:
 - Biological risk (species present and percentage coral cover).
 - Anthropogenic risk (near ports, popular dive sites, near coastal construction).
- Communication (education for water-users, divers, fishers, resorts) develop an "early warning network".

2.2. Initial invasion (initial SCTLD sightings)

- Community engagement (community members, share location of SCTLD infected areas with ocean-users, divers, fishers, and resorts).
- Biosecurity (best management practices such as disinfecting dive gear, awareness
 of which sites are affected in order to encourage divers and fishers to go from
 'clean' to 'dirty' sites).
- Monitoring (Spatial distribution and spread, coral species/genera susceptibility, site differences in response).
- Management actions (area closures, treatments ->decision tree, develop capacity for follow-up monitoring to evaluate management action effectiveness, communication-agencies, NGOs, dive operators, stakeholders).

2.3. SCTLD outbreak (SCTLD spreading within and between reefs)

- Intervention protocol: treatment strategy refinement, parallel alternative treatment trials.
- Monitoring to evaluate spread and treatment effectiveness (spatial and temporal decisions).
- Long term management reduce other stressors, rescue, restoration, reduce fishing pressure on herbivores.

3. Treatment method trials and study locations

Below (Table 2) is a list of treatments used to treat SCTLD, and the regions in which trials have been run.

Treatment Method	Brief Treatment Description (for more detailed description, see Appendix 2)	Study Location
Epoxy-chlorine Chlorinated clay plasters	Antiseptics, such as chlorine, kill the infected coral tissue and pathogens, where applied. Its use also reduces the pathogen load in the environment by killing the decaying disease tissue before it sloughs off and is carried to other colonies.	Florida Turks and Caicos Islands, Montserrat, Anguilla, and British Virgin Islands
Probiotics	Probiotics are live bacterial cultures that can provide lasting protection when applied to corals, with little to no requirement for retreatment. Probiotics can also be used preventatively on healthy corals to prevent disease transmission. They provide a more complex treatment than antibiotics and release a variety of antibacterial compounds, increasing the likelihood of disease resistance.	Florida and, at the time of writing, was under development in Montserrat, San Andres, Columbia and Punta Cana, Dominican Republic
Antibiotics (e.g. Amoxicillin) + Base2b	Antibiotics have shown to be 67%– 90% effective in stopping disease progression depending on what coral species is treated (<u>Neely <i>et al.</i> 2020</u>) and in what region of the Caribbean. Base2b is an ointment designed to be mixed with antibiotics and applied to the coral disease margin, adhering and releasing the antibiotics into the coral at a fixed dosage rate over approximately 3 days (<u>Coral Ointment</u> <u>Information Ocean Alchemists LLC</u>).	Caymans, Florida, Turks and Caicos Islands, British Virgin Islands
Non-antibiotic Base2b	An antiseptic based ointment was developed by Ocean Alchemists LLC.	Turks and Caicos Islands and US Virgin Islands
Coral Cure D rope method	Natural ingredients-based antiseptic developed by Ocean Alchemists LLC.	Turks and Caicos Islands

Table 2. Treatment methods used to treat SCTLD.

Treatment Method	Brief Treatment Description (for more detailed description, see Appendix 2)	Study Location
Natural products	Treatment of the tissue adjacent to the lesion with natural substances that have antimicrobial properties such as honey, garlic, onion, neem, bonnet pepper, Mexican tea, oregano, thyme, sodium bicarbonate, methyl chloride, potassium iodide, aspirin.	Mexico
Lesion removal	By manually removing infected colonies (small colonies only) the source of an infection can be eliminated, potentially decreasing disease spread on the reef and reducing 'pathogen load' in the environment. This intervention can be particularly beneficial during the earlier stages of SCTLD progression and decrease the rate of transmission to new sites.	US Virgin Islands
Firebreak/trenching	Creating reef-scale firebreaks or trenches, involve the removal of susceptible coral species across an extended area between a diseased area and unaffected area of reef or cutting a trench to isolate a diseased area in a colony. The goal is to reduce transmission and contain the affected area of reef.	Cayman Islands, Florida
	SCTLD is a water-borne disease and as such has the potential to transmit through large areas in the water column. In some cases, this has rendered the firebreak technique unsuccessful.	

For potential longer-term management options, less focused on direct treatment of SCTLD symptoms, please see Appendix 4.

4. Development of treatment trials across the regions

To make best use of treatment efforts, decisions must be made based on the best available science. Whilst numerous studies have been conducted to look at different treatment methods, they were not necessarily designed to answer questions regarding treatment efficiency between species, colony sizes, treatment methods, etc. In addition, most of the studies were conducted in Florida where coral condition, disease severity and management capacity may differ greatly from in the UK Overseas Territories.

It is recommended that each territory conduct initial trials of the treatment methods of their choice, using similar methods among OTs. Then, based on the initial findings, larger-scale treatments and management options can be defined.

To maximise information gathering, the following data should be collected for each trial (or as many is logistically possible):

4.1. Basic site information

- Site location (GPS) and Depth (m).
- Site survey which includes a measure of substrate cover, belt transects with colony counts (by size class if possible) to calculate coral community structure and disease prevalence, full disease assessment (all diseases).

4.2. Marked colonies for testing treatment efficiency

- Tagged or mapped colonies (for relocation at subsequent monitoring periods) for treatments and controls.
- Tagging and following SCTLD progression in control colonies gives you information on which coral species suffer the highest or lowest mortality from SCTLD. This allows you to compare treatment efficiency and allows you to make decisions on which species to target for future efforts (e.g. targeting coral species with higher mortality rates). It will also allow for cross regional comparisons in response to SCTLD among coral species.
- Information for each tagged colony should include: species (if possible), colony size, number of lesions and lesion morphologies, and photographs showing the entire colony as well as close ups of the affected region and lesions. Treated colonies should be photographed before and after treatments. Further detail on how to record signs of disease can be found in Appendix 1.
- Each colony should be assessed for what proportion of the colony is old dead (unsure if due to SCTLD), percentage of SCTLD visible (active lesions and recent tissue loss) and percentage still visually healthy. This can be done later from whole colony pictures. At the lesion level, treatments should be monitored by recording the progression of the disease margin past the treatment line.
- Based on other studies, it is known that most treatment methods require follow-up treatments and monthly monitoring. Initially, monitoring and re-treatment should occur weekly or bi-weekly, and then in the time between monitoring (can be extended based upon your findings).

• At each re-monitoring period photographs should be taken, recording the number and morphology of all new lesions, condition of treated lesions (e.g. has there been more tissue loss or healing on the colony) and condition assessed (old dead, SCTLD or healthy). Condition can be assessed from photographs but estimating the number of new lesions can be difficult, so it is recommended they are recorded in-situ.

Note: any in-situ monitoring should be undertaken adhering to strict biosecurity practices to avoid accidental spread of SCTLD on equipment or in ballast water. Please see Appendix 3 for more information.

4.3. Data analysis and reporting

- Response variables should include percentage tissue loss, as well as treated lesion response and new lesion development.
- In addition to colony and lesion monitoring, ecological reef health surveys are key to understanding the overall impact of management interventions on the health of the coral reef ecosystem. Where possible this should also be reported on to give managers a broader understanding of the ecological outcomes of management action.

5. Development of decision tree for treatment

The decision tree is a visualisation of the systematic approach used by managers. This can be used as a valuable tool to decide which SCTLD treatment method is most appropriate for a coral. An example of a decision tree for SCTLD treatment has been developed for the <u>USVI response plan</u> and can be seen below (Figure 2).

Based upon outcomes of fieldwork and treatment trials, UK OTs may wish to develop a similar tool to guide managers when identifying the appropriate method of intervention for various diseased coral colonies.

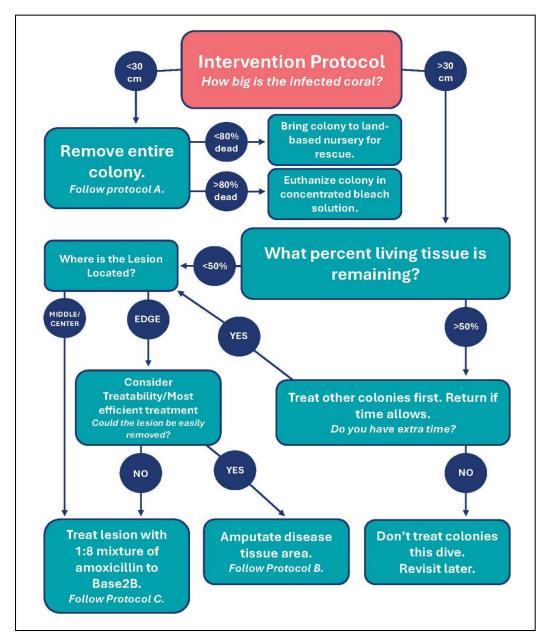


Figure 2. Decision Tree outlining the systematic approach used by managers to decide which method of intervention is most appropriate for a coral, taken and adapted from the USVI SCTLD response plan (adapted from Meiling *et al.* 2020).

6. Studies on the treatment effectiveness for SCTLD on various coral species

The effectiveness of the different treatment methods varied by coral species and by region, and the studies were often limited in scope (small sample sizes, limited coral species treated and duration of study, etc.). Hence, we have reported these critical study variables which managers will need when evaluating which treatment method might work best for their respective coral reefs.

6.1. Wider Region

6.1.1. Shilling *et al.* 2021: Assessing the effectiveness of two intervention methods (Base2b plus amoxicillin, and chlorinated epoxy) for SCTLD on *Montastraea cavernosa*

Location: Florida

Species: Montastraea cavernosa (MCAV)

Treatments:

- Antibiotic paste (amoxicillin & Base2b) and trenching (n = 11)
- Chlorinated epoxy and trenching (n = 11)
- Untreated controls (n = 10)
- Healthy controls (n = 9)

Monitoring:

- Weeks 3, 5, 9, 14, 23, 46
- No re-treatment

Response variable: Percentage of colonies with no active lesions

Outcome: See Figure 3, which illustrates the effectiveness of two treatment methods and an untreated control on the status of SCTLD-infected *M. cavernosa* corals. Over a span of 46 weeks, trenching supported antibiotic-paste-treated lesions demonstrated the highest quiescence rates, with consistently high levels of lesion inactivity after the initial three-week period, making this the most effective treatment. In contrast, trenching supported chlorine-treated colonies demonstrated remained largely diseased for most of the trial, showing some quiescence only in the later stages. Untreated colonies exhibited both coral death due to SCTLD and limited quiescence in the trial's later stages.



Figure 3. SCTLD status of colonies by treatment group over time, shown in proportion of total (from Shilling *et al.* 2021).

Conclusions:

- Amoxicillin treatment success at 3 weeks was > 75% and at week 46 was > 65%.
- Amoxicillin was the most effective treatment in the first 9 weeks of monitoring. At 14 weeks onwards there was no difference with untreated controls however, there was higher mortality in controls. At 46 weeks there was no difference between treatments.
- Chlorinated epoxy had some minimal success at 3 and 5 weeks.
- Trenching was not found to improve the effectiveness of the chlorinated epoxy treatment.
- The initial number of lesions on a colony or colony size was not found to affect treatment outcome at 46 weeks.
- A positive relationship was observed between colony size and initial number of lesions, and between colony size and number of new lesions.
- Amoxicillin treatment had 95% success rate at healing individual lesions but did not prevent treated colonies from developing new lesions.
- There was no influence of treatment on new lesion development

6.1.2. Walker *et al.* 2021a: Optimising SCTLD intervention treatments on *Montastraea cavernosa* in an endemic zone

Location: Florida

Species: Montastraea cavernosa (MCAV)

Treatments:

- Antibiotic paste (amoxicillin & Base2b) with and without trenching (n = 21).
- Chlorinated epoxy with and without trenching (n = 20).

Monitoring:

- Days 9, 15, 23, 30, 44, 52, 65, 85, 99, 157, 270, 351
- Retreatment at every visit

Response variables:

- Percentage of colonies with inactive lesions on margin
- Percentage of colonies with inactive lesion at firebreak
- Total percentage of colonies with inactive lesions (lesion and firebreak)

Outcome: See Table 3, which presents the results of antibiotic paste treatments with and without trenching, and chlorinated epoxy treatments with and without trenching. Initially the antibiotic and chlorinated epoxy treatments were administered to infected colonies at the disease margins, with efficacy determined by the number of lesions that successfully quiesced. Lesions where SCTLD persisted underwent trenching at the disease break and were reassessed. The "combination" category in the table refers to the overall success of lesions in achieving quiescence either from margin treatments alone or from margin treatments followed by disease-break trenching.

The lesions treated with antibiotic paste at the disease margin were the most successful, with 20 out of 34 achieving quiescence. In contrast, chlorinated epoxy treatment at the disease margins was less effective, with only 2 out of 34 lesions quiescing. Similarly, the antibiotic paste treatment group showed higher rates of success after the disease-break and in the combination approach, with a total of 31 out of 34 lesions quiesced following both margin and disease-break treatments. The chlorinated epoxy treatment group had a lower combination success rate, with only 8 out of 34 lesions quiescing. The chlorinated epoxy group also required more retreatments and had a slightly lower success rate in achieving lesion quiescence after retreatment compared to the antibiotic paste treatment group.

Table 3. Treatment success metrics by treatment group, material and application (Table adapted from Walker *et al.* 2021a) (* = retreatments to previously treated and failed lesions) (? = no values available).

Treatment Group	Treatment material	Treatment application	Lesions				Corals		
Croup			Number tested (Lesions)	Number failed (Lesions)	Number quiesced (Lesions)	Proportion quiesced (Lesions)	Number treated (Corals)	Number quiesced (Corals)	Proportion quiesced (Corals)
Original	Antibiotic paste	Margin	34	14	20	0.5882	21	10	0.4762
combination		Disease-break	14	3	11	0.7857	?	8	0.3810
		Combination	34	3	31	0.9118	?	18	0.8571
		Retreatments *	6	1	5	0.833	2	1	0.5000
Original	Chlorinated	Margin	34	32	2	0.0588	20	1	0.0500
combination	ероху	Disease-break	32	26	6	0.1875	?	3	0.1500
		Combination	34	26	8	0.2353	?	4	0.2000
	Mixed	Retreatments *	26	5	21	0.8077	16	11	0.6875
Original margin only	Antibiotic paste	Margin	17	0	17	1.00	5	5	1.00
New lesions on	Antibiotic paste	Margin	33	1	32	0.9697	13	12	0.9231
previously treated corals	Mixed	Margin	23	1	22	0.9565	11	10	0.9091
New lesions on previously untreated corals	Antibiotic paste	Margin	18	6	12	0.667	8	4	0.5000

Conclusions:

- Treatment success at the colony level after 351 days:
 - Antibiotic paste = 47.6%.
 - Antibiotic paste and trenching = 85.7%.
 - Chlorinated epoxy = 5%.
 - Chlorinated epoxy and trenching = 20%.
- By day 351, 50.6% of the total antibiotic paste disease-break tissue was fully healed compared to 2.2% of the chlorinated epoxy disease-break area.
- Most margin treatments failed within the first 9 days, however most disease-breaks failed before 44 days.
- New lesions appeared on previously treated colonies indicating revisitation is necessary to eliminate the disease.
- New lesions occurred on healthy colonies meaning when the pathogen was still present in the environment healthy corals are not immune.
- After the original treatments, colonies in both treatment groups had a similar proportion of new lesions.
- The new infection rate peaked on day 23, indicating a temporal component to infections.
- Tissue healing over disease-breaks treated with antibiotic paste was more frequent (90.5%) than to those treated with chlorinated epoxy (15%).

6.1.3. Neeley *et al.* 2020: Effectiveness of topical antibiotics in treating corals affected by SCTLD

Location: Florida

Species:

- Colpophyllia natans (CNAT)
- *Montastraea cavernosa* (MCAV)
- Orbicella faveolata (OFAV)
- Diploria labyrinthiformes (DLAB)
- *Pseudodiploria strigosa* (PSTR)

Treatments:

- Amoxicillin and Base2b paste (n = 6).
- Amoxicillin and New Base (n = 5).
- Base2b paste placebo (n = 4).
- New Base paste placebo (n = 5).
- Untreated controls (n = 1).

This study evaluates the effectiveness of amoxicillin combined with two different paste types in treating SCTLD-infected coral lesions across five coral species. Placebo pastes and untreated controls are included to determine the true efficacy of amoxicillin when used with the two paste formulations. Table 4 provides details on the sample sizes for each treatment, including the number of colonies and lesions treated, along with the specific treatment types applied within each coral species group.

Treatment Area	Species Code	Control	New Base Placebo	New Base + Amoxi	Base2b Placebo	Base2b + Amoxi
Colonies	CNAT	2	2	2	2	1
	MCAV	2	3	2	3	5
	OFAV	2	3	3	5	5
	DLAB	-	-	-	5	4
	PSTR	-	-	-	4	6
Lesions	CNAT	8	2	7	8	3
	MCAV	4	6	4	9	9
	OFAV	4	19	12	14	23
	DLAB	-	-	-	8	8
	PSTR	-	-	-	8	25

Table 4. Number of colonies and lesions receiving each treatment type across 5 coralspecies. "Amoxi" = addition of powdered amoxicillin (Table adapted from Neeley *et al.* 2020).

Monitoring:

- 1-month post-treatment.
- Retreatment of new or failed lesions at 1 month and re-evaluated 2 months later.

Response variable: Percentage of inactive lesions on margin.

Outcome: (After 1 month)

- **Base2b and amoxicillin:** CNAT = 67% (n = 1 colony: n = 3 lesions), MCAV = 89% (n=5 colonies: n=9 lesions), OFAV = 91% (n = 5 colonies: n = 23 lesions), DLAB = 88% (n=4 colonies: n=8 lesions), PSTR = 73% (n = 6 colonies: n = 15 lesions).
- New Base and amoxicillin: CNAT = 29% (n = 2 colonies: n = 5 lesions), MCAV = 100% (n = 2 colonies: n = 4 lesions), OFAV = 83% (n = 3 colonies: n = 12 lesions).
- Controls: 0% except:
 - Base2b paste: CNAT = 38% (n = 2 colonies; n = 8 lesions), DLAB= 13% (n = 5 colonies, n = 8 lesions).
 - New B paste: OFAV = 5% (n = 3 colonies; n = 19 lesions).

Figure 4 displays the average percentage of effective lesion treatments per coral species, categorised by treatment type. The figure shows that across all species, the percentage of halted lesions was higher for the New Base + Amoxicillin and Base2b + Amoxicillin treatments compared to placebo-treated colonies and controls. Among the tested coral species, Base2b + Amoxicillin was the most effective in halting disease lesions per colony, except for *M. cavernosa*, where the New Base + Amoxicillin treatment proved to be more effective.

For *D. labyrinthiformes* and *P. strigosa* corals, only the Base2b + Amoxicillin and Base2b placebo treatments were assessed. Both species showed better outcomes with the Base2b + Amoxicillin treatment, as indicated by a higher percentage of halted lesions per colony.

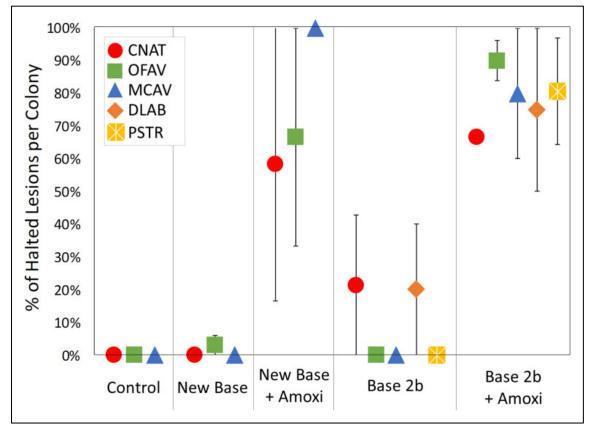


Figure 4. Percentage of effective lesion treatments per colony, separated by species and treatment type. "Amoxi" = addition of powdered amoxicillin (From Neeley *et al.* 2020).

Conclusions:

- Base2b and amoxicillin treatment found to be more effective than the New Base and amoxicillin treatment, except for *M. cavernosa*.
- Base2b and amoxicillin effectiveness (% lesions) ranged from 67% to 91%.
- The effectiveness of the pastes without antibiotics (placebo treatments) was 4% and 9%, making them no different from untreated controls.
- OFAV coral species responded best to Base2b and amoxicillin treatment.
- New lesions appeared on colonies across all treatment groups.

6.1.4. Walker *et al.* 2020: South East Florida Reef-building-coral Response to Amoxicillin Intervention and Broader-scale Coral Disease Intervention

Location: Florida

Species:

- *Montastraea cavernosa* (MCAV)
- Orbicella spp. (ORBI)

- Pseudodiploria strigosa (PSTR)
- Siderastrea siderea (SSID)

Treatment: Amoxicillin and Base2b

Sample sizes:

- n = 40 colonies total:
- MCAV. = 25 lesions,
- ORBI. = 271 lesions,
- PSTR = 1 lesion,
- SSID = 3 lesions.

Monitoring:

- Monthly
- Retreated as needed

Response variable: Percentage of inactive lesions

Outcome:

Table 5 outlines the percentage of successes and failures relative to the total number of treatments administered across four coral species, as well as across all species combined. The results indicate that the amoxicillin and Base2b treatment was most successful for *P. strigosa* and *S. siderea* colonies, though the small sample sizes (1 and 3, respectively) may influence this finding. *Orbicella* spp. also showed better treatment responses compared to *M. cavernosa*. Across all species, the success rate was significantly higher than the failure rate. Out of 300 total treatments, the amoxicillin and Base2b treatment achieved an overall success rate of 81.2%, highlighting its effectiveness.

Table 5. Total treatment failure and success rates for margin-only treatments using antibiotic ointment from August 2019 to October 2020, shown by species and cumulative results (Table adapted from Walker *et al.* 2020).

Species Code	Total Treatments	Total failures (percent)	Total successes (in percent)
MCAV	25	26.1%	73.9%
ORBI	271	18.4%	81.6%
PSTR	1	0%	100%
SSID	3	0%	100%
All species	300	18.8%	81.2%

Conclusions:

- Amoxicillin and Base2b success on lesions ranged from 73.9 to 100%.
- Retreated needed on 44.4% of the colonies.

- More retreatments (e.g. appearance of new lesions) required from June through October, potentially due to warmer and wetter conditions in Florida.
- Monthly treatments found to be required to keep the disease at bay.
- Base2b adheres better to ORBI spp. than *M. cavernosa*.
- Research focused on larger colonies and percentage tissue loss was not reported.
- In a prior study (Walker & Pitts, 2019), there was higher success in *M. cavernosa* using the Amoxicillin treatment and a firebreak.

6.1.5. Ushijima *et al.* 2023: Chemical and genomic characterisation of a potential probiotic treatment for stony coral tissue loss disease

Location: Florida

Species: Montastraea cavernosa (MCAV)

Treatment:

- Probiotic bacteria: *Pseudoalteromonas* sp. strain McH1-7
- Control: filtered seawater (FSW)

Sample sizes:

- n = 22 paired fragments tested for disease treatment
- n = 12 paired fragments tested for disease transmission

Response variable: progression of tissue loss or evidence of disease transmission (development of tissue loss lesion)

Experiment duration: 21 days

Disease Treatment Outcome: Figures 5 and 6 illustrate tissue loss in *M. cavernosa* fragments over 11 and 21 days under two treatments: a probiotic bacteria (McH1-7) and filtered seawater (FSW) as a control. In Figure 5, images over the 11-day period show that tissue loss is much more pronounced in the FSW-treated corals compared to those treated with McH1-7, where minimal to no tissue loss is observed throughout the trial.

Figure 6 presents the average percentage of total tissue remaining on *M. cavernosa* colonies treated with either FSW or McH1-7 over 21 days. The graph demonstrates that, overall and throughout the study, the McH1-7 treated colonies retained a higher percentage of total tissue compared to those treated with FSW.

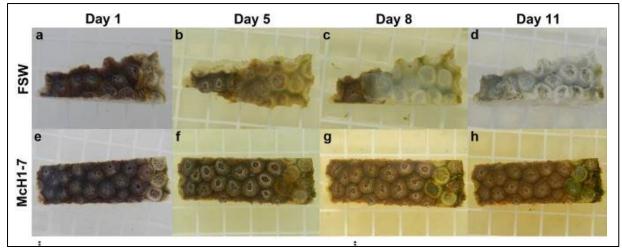


Figure 5. Images depicting tissue loss in *M. cavernosa* coral fragments at four time points over an 11-day trial, comparing the treatment of SCTLD using probiotic bacteria (strain McH1-7) versus a filtered seawater control (FSW) (Figure adapted from Ushijima *et al.* 2023).

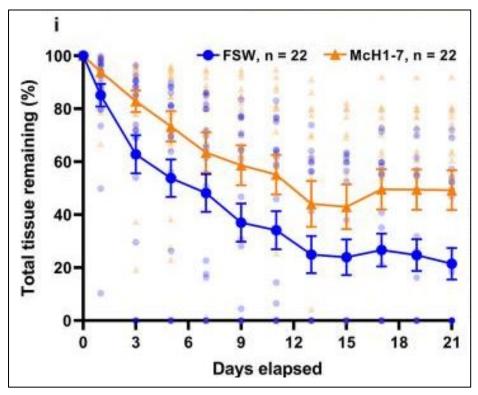


Figure 6. Percentage of total tissue remaining on *M. cavernosa* coral fragments at seven time points over a 21-day trial, comparing the treatment of SCTLD using probiotic bacteria (strain McH1-7) versus a filtered seawater control (FSW) (Figure adapted from Ushijima *et al.* 2023).

Disease Transmission Outcome: Figures 7 and 8 demonstrate the effectiveness of the probiotic bacteria treatment (McH1-7) in protecting *M. cavernosa* fragments from SCTLD transmission. Figure 7 illustrates how McH1-7 treated and untreated corals responded when placed in contact with SCTLD-infected corals over a 7-day period. The figure also highlights

the varying disease responses in the SCTLD-infected corals themselves. As a control, healthy untreated corals were paired with other healthy corals.

Figure 7 shows that McH1-7 treated corals remained healthy throughout the 7-day trial and appeared to influence the SCTLD-infected corals, slowing the progression of the disease compared to SCTLD-infected corals in contact with untreated fragments. Untreated healthy corals in contact with SCTLD-infected fragments had a 33.3% transmission rate within 4-6 days, as shown in Figure 7e-h, where previously healthy corals began showing signs of SCTLD infection. Healthy control corals placed in contact with other healthy corals remained disease-free throughout the study.

Figure 8 presents the average percentage of total tissue remaining on *M. cavernosa* fragments that were placed in contact with either untreated healthy fragments or McH1-7 treated healthy fragments over a 21-day period. The figure highlights the effectiveness of McH1-7 treated corals in slowing the progression of SCTLD when exposed to infected coral fragments. Over the 21 days, diseased corals in contact with McH1-7 treated fragments exhibited a slower decline in remaining tissue percentage compared to diseased corals in contact with untreated healthy fragments.

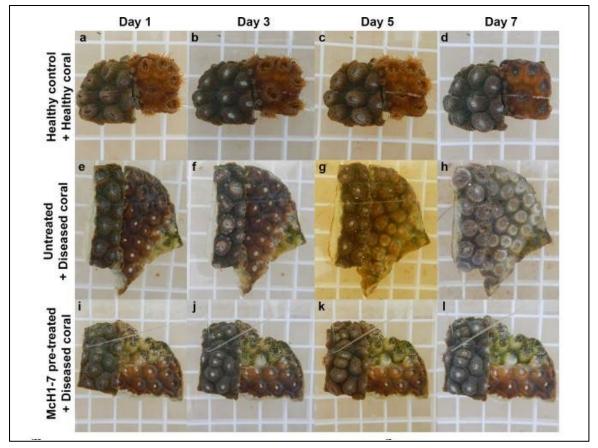


Figure 7. Images showing SCTLD transmission impacts: healthy, McH1-7 pre-treated corals (left) in contact with SCTLD-infected corals (right) (i-l) versus healthy, untreated corals (left) in contact with SCTLD-infected corals (right) (e-h). Healthy corals paired with healthy controls (a-d) are also shown. Results reflect a 7-day period (Figure adapted from Ushijima *et al.* 2023).

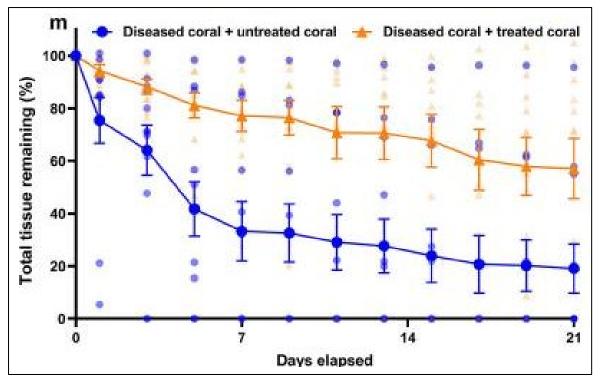


Figure 8. Percentage of total tissue remaining on *M. cavernosa* coral fragments at four time points over a 21-day trial period, comparing the tissue loss impact of SCTLD diseased corals put in contact with healthy McH1-7 treated corals (yellow) and healthy untreated corals (blue) (Figure adapted from Ushijima *et al.* 2023).

Conclusions:

- *Pseudoalteromonas* sp. strain McH1-7 exhibits broad-spectrum antibacterial activity against SCTLD-associated bacterial isolates.
- Chemical analyses indicated McH1-7 produces at least two potential antibacterials, korormicin and tetrabromopyrrole.
- Treatment with *Pseudoalteromonas* sp. strain McH1-7 significantly reduced SCTLDrelated tissue loss compared to untreated controls.
- Treatment with *Pseudoalteromonas* sp. strain McH1-7 prevented SCTLD transmission between healthy & diseased fragments.
- *Pseudoalteromonas* sp. strain McH1-7 is an effective prophylactic with possible implications for use with restoration activities and direct treatment for SCTLD providing a potential alternative to antibiotic use.
- Probiotic strains must be developed within each region and tested in aquaria before being trialled in the field.
- The Ushijima Lab has received samples, for probiotic development, from 8 areas within the Caribbean and Gulf of Mexico (<u>https://www.ushijima-lab.com/research</u>) but probiotic strains must first be tested in aquaria within each region.
- The Ushijima lab has conducted aquaria and field trials in Florida and numerous bacterial strains are currently being tested in aquaria trials in Montserrat.
- The Ushijima lab is involved in developing aquaria trials (planned for 2024) for San Andres, Columbia and Punta Cana, Dominican Republic.

6.1.6. Pitts *et al.* (In Prep): In-situ treatment of SCTLD with probiotics.

Location: Florida

Species: Montastraea cavernosa (MCAV)

Treatments:

- Probiotic paste
- Control paste
- Untreated controls
- Colonies bagged with probiotics injected (2-hour exposure)
- Control bag

Sample sizes:

- probiotic paste (n = 10),
- control paste (n = 7),
- untreated controls (n = 7),
- Colonies bagged with probiotics injected (n = 8),
- control bag (n = 7)

Monitoring: 3 treatment periods: day 0, day 44, day 134 (9/1/20, 10/14/20, 1/15/21)

Response variable: lesion response (active, stopped, healing)

Outcome: See Figure 9, which illustrates the effectiveness of two treatment types and their respective controls in stopping and/or healing SCTLD lesions on *M. cavernosa* coral colonies. The figure shows that probiotic bag treatments were more effective at both stopping and healing lesions compared to the probiotic paste and control treatments. While the probiotic paste was quite effective at stopping lesion spread, its performance in healing the lesions was comparable to, and slightly lower than, the control paste. The probiotic bag control was the least successful in both stopping and healing lesions.

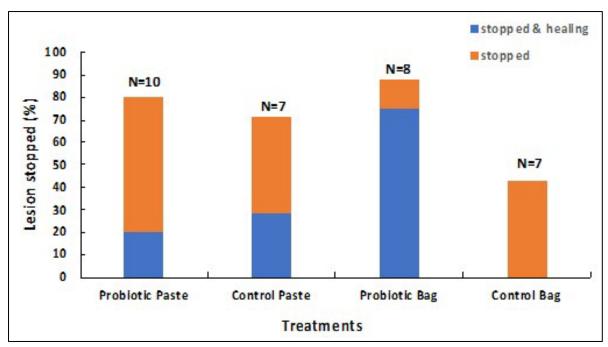


Figure 9. Percentage of SCTLD lesions stopped, and stopped and healing, across two probiotic treatment groups and two control treatment groups in *M. cavernosa* colonies (Figure from Pitts *et al.* (In Prep)).

Conclusions:

- Probiotic treatments found to be over 80% effective at stopping lesions.
- Probiotic bag had higher proportion of healing lesions compared to the paste treatment.
- Treatments using probiotic found to be time consuming but effective.
- Effective for preventing transmission in aquaria trials so should be considered in out-planting trials.
- Only tested on *M. cavernosa* with subacute tissue loss lesions with bleached edges in endemic zone.
- Probiotic bacterium occurs on healthy corals in Florida.
- Regional probiotics are required and need to be developed.
- Recommends method to be trialled on other coral species and lesion types.

6.2. UK OT SCTLD Treatment trials

6.2.1. Forrester *et al.* (In Prep): Effectiveness of different treatments on SCTLD in the BVI

Location:

• Anegada, British Virgin Islands

Species:

• Diploria labyrinthiformes (DLAB)

- Orbicella annularis (OANN)
- Orbicella faveolata (OFAV)
- Siderastrea siderea (SSID)
- *Pseudodiploria strigosa* (PSTR)
- Agaricia agaricites (AAGA)
- Montastraea cavernosa (MCAV)
- Colpophyllia natans (CNAT)

Treatment:

- Antibiotics in Base2b (n = 28)
- Chlorinated clay band (n = 29)
- Controls left untreated (n = 26)

Response variable: Colony mortality and progression of tissue loss

Experiment duration: 5 months (Jan-May 2023)

Monitoring: approximately every 2 weeks with retreatment of new or failed lesions

Colony mortality Outcome:

See Figure 10, which shows the survival probability of antibiotic and chlorine treatment types, along with an untreated control group. According to the figure, over the course of 120 days, the untreated control group exhibited the lowest survival probability, with samples declining rapidly and reaching near-zero survival around 90 days. The chlorine treatment group showed slightly better survival probability, but it steadily decreased throughout the study to about 0.25 by day 120. In contrast, the antibiotic treatment group maintained a steady 1.00 survival rate throughout the entire study, indicating its effectiveness.

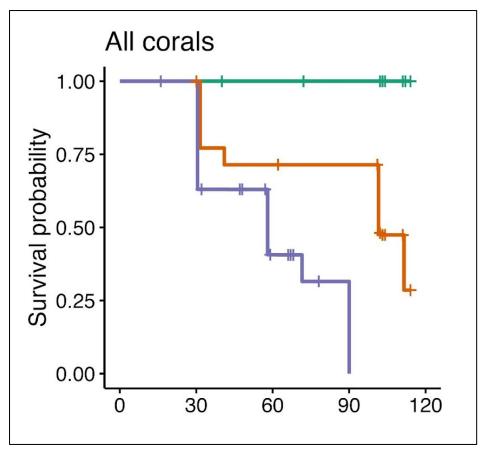


Figure 10. Survival probability of various coral species in two treatment groups and an untreated control group over a 120-day period (Figure adapted from Forrester *et al.* (In Prep)).

Average Tissue Loss Outcome: Figure 11 illustrates the daily tissue loss proportion across antibiotic, chlorine treatment, and untreated control groups, based on a cumulative assessment of various coral species. The figure shows that the antibiotic-treated group had the lowest and most stable daily tissue loss proportion. The chlorine-treated group exhibited slightly higher and more varied tissue loss rates. In contrast, the control group experienced the highest and most variable tissue loss per day across the coral species tested.

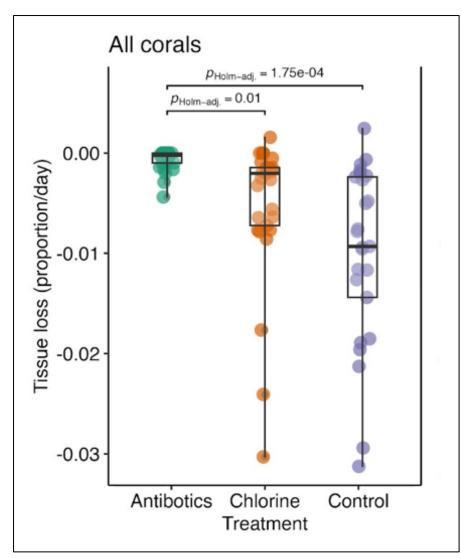


Figure 11. Proportional tissue loss per day of corals treated with antibiotics and chlorine in comparison to a control treatment group. (Figure adapted from Forrester *et al.* (In Prep)).

Conclusions:

- Antibiotics and chlorinated clay bands both significantly reduced colony mortality and tissue loss compared to controls.
- Antibiotics were the most successful at disease treatment with 0% colony mortality after 3 months and an avg. tissue loss of 0.00007% per day.
- Chlorinated clay bands had 48% colony mortality and an average tissue loss of 0.005% per day.
- Controls had 46% colony mortality with an average of 0.01% tissue loss per day.
- There were differences among coral species in treatment success.
- Of the three species with adequate sample sizes in each group (*D. labyrinthformes*, *O. annularis*, *P.strigosa*), there was significantly less tissue loss in *D. labyrinthformes*, and *O. annularis* colonies treated with antibiotics, significantly less tissue loss in *O. annularis* treated with chlorinated clay bands and no significant effect of either treatment in *P. strigosa*. *P. strigosa* showed limited tissue loss in controls suggesting that some species may not need treatment.
- Regardless of treatment, new lesions developed on colonies through time.

6.2.2. Zimmermann *et al.* (In Prep): Stony Coral Tissue Loss Disease in the Turks & Caicos Islands

Location: Turks & Caicos Islands

Species:

- *Montastraea cavernosa* (MCAV)
- Orbicella faveolata (OFAV)
- Orbicella annularis (OANN)
- Orbicella franksi (OFRA)
- Dichocoenia stokesi (DSTO)
- Eusmilia fastigiata (EFAS)
- Stephanocoenia intersepta (SINT)
- Colpophyllia natans (CNAT)
- Diploria labyrinthiformes (DLAB)
- *Pseudodiploria strigosa* (PSTR)
- Meandrina meandrites (MMEA)
- *Mycetophyllia* ferox (MFER)
- Agaricia agaricites (AAGA)
- Siderastrea siderea (SSID)
- *Mussa angulosa* (MANG)
- Dendrogyra cylindrus (DCYL)

Treatment: Amoxicillin and Base2b - tagged colonies at 3 sites

Sample sizes: Total of 241 samples

Monitoring:

- Variable (between 1 and 8 months)
- Re-treated as needed.
- 7 sites (1 to 3 transects per site) in 3 geographical locations. 3 sites at Northwest Point (NWP), 1 site at Grace Bay and 3 sites on the Turks Bank.
 - NWP S1 3 revisits in 8 months.
 - NWP S2 2 revisits in 3 months.
 - NWP S4 2 revisits in 2.5 months.
 - Grace Bay S3 3 revisits in 6 months.
 - Turks Bank S5 3 revisits in 6 months.
 - Turks Bank S6 3 revisits in 6 months.
 - Turks Bank S7 2 revisits in 6 months.

Response variable: Percentage of inactive lesions & new lesions

Overall treatment success outcome: See Table 6 and Figure 12, which detail the success and treatment efficacy of amoxicillin and Base2b treatments on various coral species. Table 6 summarizes the number of corals treated per species, the number of successfully treated corals (where amoxicillin and Base2b halted lesion spread), and the overall success rate per

species. According to the table, treatment was most successful on the species *E. fastigiata, M. angulosa* and *S. intersepta*, each with 100% success rates, though these high rates may be due to small sample sizes (1 and 2 corals, respectively). Treatment was least successful on *M. meandrites, M. ferox* and *O. franksi.* Overall, the treatments achieved a 58.5% success rate across all tested species.

Figure 12 shows the proportion of colonies within each species group that experienced lesion cessation (or quiescence) after treatment with amoxicillin and Base2b. *E. fastigiata, S. intercepta*, and *M. angulosa* showed the highest success rates (likely influenced by small sample sizes), followed by *M. cavernosa* and *D. stokesi* colonies. The lowest success rates were observed in *O. faveolata, M. meandrites*, and *M. ferox* colonies.

Coral species code	Total treated	Total success	Total % success
MCAV	36	25	69.4
OFAV	76	49	64.5
OANN	47	27	57.4
OFRA	1	0	0.0
DSTO	3	2	66.7
EFAS	2	2	100.0
SINT	1	1	100.0
CNAT	7	2	28.6
DLAB	29	17	58.6
PSTR	4	2	50.0
MMEA	2	0	0.0
MFER	2	0	0.0
AAGA	14	9	64.3
SSID	4	2	50.0
MANG	1	1	100.0
DCYL	12	2	16.7
Total	241	141	58.5

Table 6. Total success and percentage success of treated corals by species (from Zimmerman *et al.* (In Prep)).

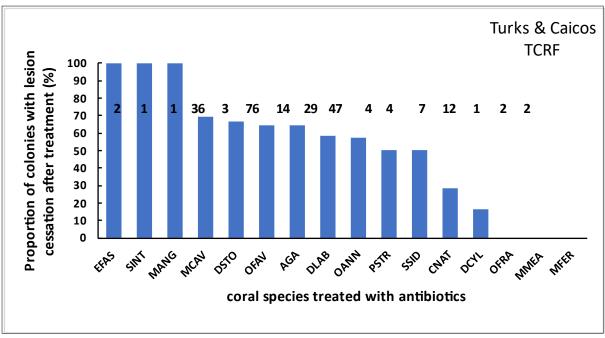


Figure 12. Treatment efficiency of amoxicillin and Base2b treatments (percentage of colonies with lesion cessation after treatment) across different coral species. Sample sizes are indicated by the numbers above each bar (Figure adapted from Zimmerman *et al.* (In Prep)).

Figure 13 shows the average lesion treatment success across several coral species treated with amoxicillin and Base2b. Success is defined as the quiescence of the diseased lesion. The highest treatment success was observed in *D. stokesi* colonies, while the lowest success was seen in *D. cylindrus* colonies.

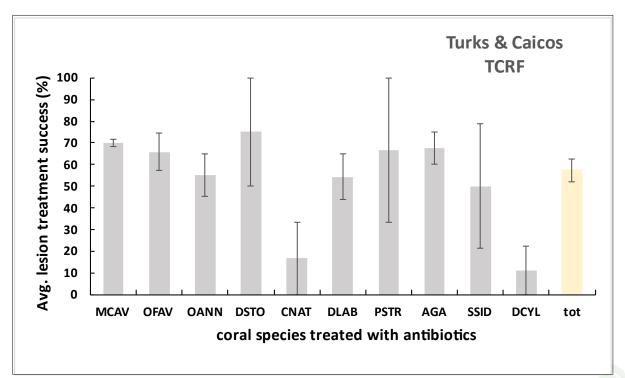


Figure 13. Average lesion treatment success (quiescence) for each coral species treated with antibiotics (Figure adapted from Zimmerman *et al.* (In Prep)).

Conclusions:

- In general, treatment success was similar among sites (low geographic variability) but differed among species.
- Amoxicillin and Base2b success on treating lesions ranged from 0 to 100% depending on the coral species.
- 13 of the 16 coral species tested responded well (+50% success) to amoxicillin and Base2b.
- Amoxicillin treatment found to be less than 20% effective on *C. natans* and *D. cylindrus* colonies.
- The sample sizes were small for some of the coral species (*E. fastigiata, S. intersepta, M. angulosa, D. stokesi, P. strigosa, S. siderea, O. favelata, M. meandrites, M.ferox*).
- Lesion failures were scored the same as new lesions.

6.2.3. McLeod & Aeby (In Prep): Field trial treating SCTLD with chlorinated cocoa butter covered with a clay band

Location: Turks and Caicos

Species:

- Pseudodiploria strigosa (PSTR)
- Montastraea cavernosa (MCAV)
- Eusmilia fastigiata (EFAS)
- Orbicella franksi (OFRA)
- Diploria labyrinthiformes (DLAB)
- Meandrina meandrites (MMEA)
- Orbicella annularis (OANN)
- Siderastrea siderea (SSID)
- *Mycetophyllia* sp. (MYCE)
- Agaricia spp. (AGAR)
- Porites astreoides (PAST)
- Dichocoenia stokesii (DSTO)

Treatment:

- Lesion covered with a chlorinated clay band
- Untreated controls

Monitoring:

- Day 20, 36, 48
- Re-treatment at subsequent monitoring periods

Response variable: Percentage tissue loss/colony

Sample sizes: See Table 7

Table 7. Sample sizes for treated and untreated control groups at each test site, categorised by coral species (Table adapted from McLeod & Aeby (In Prep)).

Coral species code	Group	Sites	J (. ,,		
		S3T1 (n = 12)	S3T2 (n = 14)	S4T1 (n = 14)	S5T1 (n = 14)	Total (n = 54)
PSTR	Treated	1	2	-	-	3
	Control	-	1	-	-	1
MCAV	Treated	2	3	3	3	11
	Control	3	1	1	3	8
EFAS	Treated	-	-	-	-	0
	Control	1	1	-	-	2
OFRA	Treated	-	-	2	1	3
	Control	2	1	1	1	5
DLAB	Treated	1	1	2	2	6
	Control	1	-	1	2	4
MMEA	Treated	1	-	-	-	1
	Control	-	-	-	-	0
OANN	Treated	-	-	-	-	0
	Control	-	2	-	-	2
SSID	Treated	-	1	-	-	1
	Control	-	1	-	-	1
MYCE	Treated	-	-	1	-	1
	Control	-	-	-	-	0
AGAR	Treated	-	-	1	-	1
	Control	-	-	1	-	1
PAST	Treated	-	-	-	-	0
	Control	-	-	1	-	1
DSTO	Treated	-	-	-	1	1
	Control	-	-	-	1	1

Outcome: See Figures 14 and 15, which depict the average percentage of total coral tissue loss in control and treated groups across selected coral species affected by SCTLD.

Figure 14 shows the average percentage of tissue loss in control and treatment groups for selected coral species with SCTLD. This figure indicates that, overall, control groups experienced significantly higher tissue loss in all tested corals except *O. faveolata* and *D. labyrinthiformes*. *S. siderea* had the lowest tissue loss in the treatment group and the greatest difference between control and treatment tissue loss, though this may be influenced by sample size.

Figure 15 illustrates the average percentage of tissue loss in coral species affected by SCTLD without treatment. The highest tissue loss averages were observed in *S. siderea* and *D. cylindrus* colonies, possibly due to small sample sizes, while *O. annularis* and *E. fastigiata* corals had the lowest average tissue loss percentages.

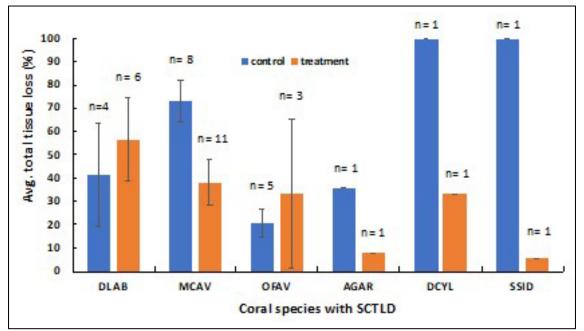


Figure 14. Average percentage total tissue loss in control and treatment groups for coral species with SCTLD (from McLeod & Aeby (In Prep)).

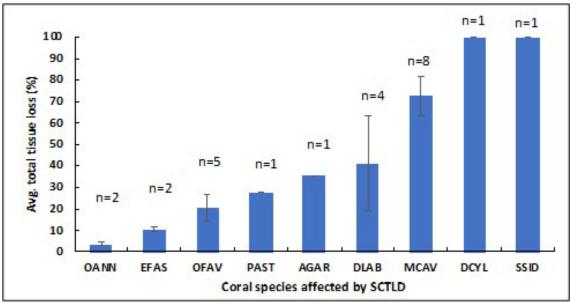


Figure 15. Average percentage total tissue loss in untreated coral species affected by SCTLD (from McLeod & Aeby (In Prep)).

Conclusions:

- Treatment saved a significant amount of tissue on *M. cavernosa* colonies.
- *Agaricia* spp., *D. cylindrius* and *S. siderea* were also found to respond well to treatment, but sample sizes are small.

- O. faveolata and D. labyrinthiformes did not respond well to the clay band treatment.
- Percentage of tissue loss included treatment areas lost.
- 68% of the colonies developed new lesions, with the number of new lesions ranging from 1–4 per colony.
- Positive relationship found between percentage initially affected and total tissue loss for controls.
- Weak positive relationship found between percentage initially affected and total tissue loss for treated.
- Amount of total tissue loss in the control treatments differed among species, potentially impacting future treatment decisions.
- Colony sizes were not reported in study.

7. Strategy outcome reporting and communication within C-COT

Project partners and members of C-COT provide brief updates on the progress of their fieldwork at monthly C-COT meetings. More comprehensive reporting will take place verbally at quarterly C-COT meetings in addition to any written updates required by project funding (typically bi-annually).

8. Conclusions

SCTLD is the most devastating coral disease in recent history, unprecedented in its spatial spread across the Caribbean and the long temporal duration of the outbreak (here described from 2014 to 2023). SCTLD has resulted in a significant reduction in coral cover on affected reefs and in shifts in coral community composition and size class structure. Colony mortality also leads to an overall decrease in carbonate production hampering reef accretion and thus the ability of coral reefs to continue to provide essential ecosystem services (Toth *et al.* 2023). This outbreak event has brought a new awareness of the threats to coral reef health and the need for pro-active strategies and long-term monitoring programmes. Countries/territories without response plans in place or active monitoring programmes were limited in their ability to quickly respond to SCTLD.

In response to this outbreak, novel management strategies and in-situ disease treatments were developed to reduce colony mortality. Of all the different treatments that were trialled, direct application of antibiotics (amoxicillin) proved successful in stopping lesion progression on colonies. However, it comes with the risk of the development of antibioticresistant bacteria in the nearshore waters (Shilling et al. 2021). There have already been two outbreaks of tissue loss disease in Florida that appeared grossly to be SCTLD but did not respond to antibiotic treatment, one of which was presented to Caribbean marine practitioners during a disturbance advisory committee meeting (Chaparro et al. 2024). This could be due to the SCTLD pathogen developing antibiotic resistance or a novel pathogen causing the disease. Amoxicillin treatment is also specific to SCTLD and may or may not be useful for future disease outbreaks. Another novel treatment, chlorinated clay bands, were less effective than antibiotics, but successfully reduced mortality in several coral species tested. It comes with the benefit of being a general treatment for any infectious disease not just SCTLD. The use of probiotics for treating SCTLD and potentially enhancing restoration success is another promising tool for helping maintain coral reef health. However, it does require identification and testing of probiotic bacterial strains within each region. This is currently being actively explored in TCI and Montserrat.

None of the treatment methods prevented re-infection of colonies and so saving corals required long-term investment in repetitive monitoring and treatment. Modelling of short-term treatment success using antibiotics in Florida suggested that SCTLD prevalence should continue to decrease through time (Neeley *et al.* 2021). However, the one study that has followed treatment success through time (2019 to 2023) found SCTLD prevalence in tagged colonies (*O. faveolata*) continues unabated despite persistent treatment (Walker *et al.* 2023).

Each OT must conduct their own cost-benefit analysis considering whether to treat corals on the reef (coral saved) vs. the amount of money and labour required. Currently, three options would be:

- 1. constant treatment and monitoring;
- 2. one-time treatment;
- 3. no treatment.

It is anticipated that coral disease outbreaks will continue to increase through time and all coral reef dependent regions should further develop the capacity and strategies to protect their critical coral reefs. This document helps to address this need by developing a strategic plan to inform response to any future disease outbreaks and highlights studies which trial different treatment options for SCTLD and potentially other tissue loss diseases.

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Appendix 1: Coral species and coral disease ID

Stony coral tissue loss disease has been reported to affect more than 20 species of coral however some species are more susceptible to the disease and are typically the first species on a reef to show signs of infection. Careful attention should be paid to identifying and monitoring these species to rapidly identify any initial signs of the disease on a reef.

Highly susceptible species

Early onset (the species first affected during an outbreak), rapid progression, and total mortality ranging from one week for smaller colonies to complete mortality over 1–2 months for larger colonies. Typically, M. *meandrites* and *D. stokesi* are the first to become affected at a site, followed by *C. natans*, and then the others show disease signs shortly thereafter.

- Colpophyllia natans (CNAT)
- Dendrogyra cylindrus (DCYL)
- Dichocoenia stokesi (DSTO)
- Diploria labyrinthiformes (DLAB)
- Eusmilia fastigiata (EFAS)
- Meandrina meandrites (MMEA)
- Pseudodiploria strigosa (PSTR)
- Pseudodiploria clivosa (PCLI)

Source: Case definition: SCTLD (2018) Florida Disease Advisory Committee.

Intermediately susceptible species

Onset of tissue loss typically occurs about a month after onset in highly susceptible species, but lower numbers may also show disease signs before or as those species are affected. Smaller colonies die out over months, and larger colonies may show new lesions continuing with possible mortality occurring over years.

- Orbicella annularis (OANN)
- Orbicella faveolata (OFAV)
- Orbicella franksi (OFRA)
- Montastraea cavernosa (MCAV)
- Solenastrea bournoni (SBOU)
- Stephanocoenia intersepta (SINT)
- Siderastrea siderea (SSID)

Note *S. siderea* may show disease signs before highly susceptible species, during outbreaks, and after the outbreak has progressed through a reef system. The presentation of disease may be similar to SCTLD in some but not all cases, and the epidemiology, for example the patterns of lesion spread within and among colonies and duration of tissue loss, does not always match those of other species. This raises some uncertainty about inclusion of *S. siderea* in this case definition.

Source: Case definition: SCTLD (2018) Florida Disease Advisory Committee.

Presumed susceptible but insufficient data to categorise onset.

- Agaricia agaricites (AAGA)
- Agaricia spp. (AGAR)
- Mycetophyllia spp. (MYCE)
- Madracis auretenra (MAUR)
- Favia fragum (FFRA)
- *Helioseris cucullata* (HCUC)
- Mussa angulosa (MANG)
- Scolymia spp. (SCOL)
- *Isophyllia* spp. (ISOP)

Source: Case definition: SCTLD (2018) Florida Disease Advisory Committee.

Low susceptible species

During outbreaks, the following corals are rarely or not affected.

- Porites astreoides (PAST)
- Porites porites (PPOR)
- *Porites divaricata* (PDIV)
- Porites furcata (PFUR)
- Acropora palmata (APAL)
- Acropora cervicornis (ACER)
- Oculina spp. (OCUL)
- Cladocora arbuscula (CARB)

Source: Case definition: SCTLD (2018) Florida Disease Advisory Committee.

Stony coral tissue loss disease identification

Stony coral tissue loss disease can be difficult to identify and distinguish between other diseases. Coral colonies will display loss of tissue (not just loss of colour), and lesions can be either focal (one spot) or multi-focal (many spots). Soft coral tissue will die, while a band of disease or the 'disease front' progresses across the colony. Progression patterns vary between species.



Figure 16. Images depicting SCTLD lesions on Caribbean coral species, *D. stokesi* (left), *M. meandrites* middle) and *D. labyrinthiformes* (right) (Images taken from Neely, 2019).

ID Guides

- <u>A visual SCTLD ID guide by Andy Bruckner</u>
- Perry Institute: How to identify SCTLD
- Dr Karen Neely: Appearance of SCTLD on susceptible species
- USVI: Susceptible coral species ID Flashcards

Training videos

- Dr Greta Aeby:
 - Part 1 Insights into investigation approach and disease ecology
 - Part 2 In-situ disease treatment and identification
- Virgin Islands Disease Advisory Committee: <u>BleachWatch VI and the hunt for coral</u> <u>disease</u>
- Turks and Caicos Reef Fund
 - SCTLD Observer training Section 1
 - SCTLD Observer training Section 2
 - SCTLD Observer training Section 3
 - SCTLD Observer training Section 4
- Andy Bruckner: <u>How to recognise and describe SCTLD lesions</u>
- Perry Institute: <u>Protecting Bahamian Reefs: Identifying and Preventing SCTLD</u>

Diagnostic descriptions of lesions

An important component of disease ID and assessment is the use of standard nomenclature to describe coral lesions in the field. It can be challenging to assign a specific name to a disease in the water, as there is limited information on coral disease etiologies, ecologies and pathogen specifies (<u>Obura *et al.* 2019</u>). Disease lesions encountered during surveys should be described according to national standards. Information should be recorded on the affected taxa, its size, and condition. The lesion should be described in terms of its physical characteristics with avoidance, or minimal interpretation, of processes producing the features (<u>Work & Aeby 2006</u>).

<u>Work & Aeby 2006</u> provides a detailed framework to systematically describe diseases in corals in a consistent and standardised manner to avoid subjective interpretation.

The morphologic diagnosis has 6 components including extent, time, distribution, lesion, location, and structure affected, each (ideally) consisting of 1 word (see figure 17). Table 8 summarises the terminology suggested for describing lesions and morphologic diagnosis.

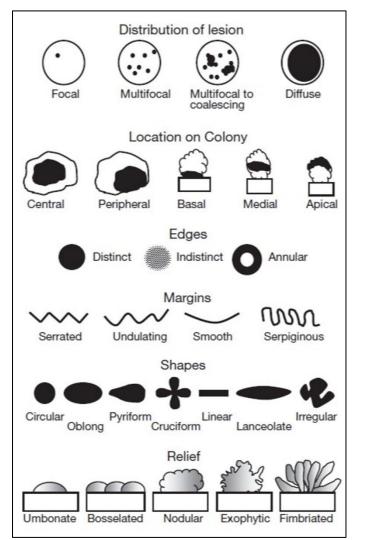


Figure 17. Illustration of terms to describe distribution, location, edges, margins, shapes and relief of lesions in corals (Image taken from Work & Aeby, 2006).

Table 8. Table depicting categories and terms used to describe a lesion (D) and to make a
morphologic diagnosis (M). Some terms apply to both lesion description and morphologic
diagnosis (D, M) (Table adapted from Work & Aeby, 2006).

Category	Term	Application
Distribution (Di)	Focal, multifocal, multifocal to coalescing, diffuse	D, M
Location (Lo)	Basal, medial, apical, peripheral, central, colony-wide	D, M
Edges (Ed)	Distinct, indistinct, annular	D
Margins (Ma)	Serrated, undulating, smooth, serpiginous,	D

Category	Term	Application
Shapes (Sh)	Circular, oblong, pyriform, cruciform, linear, lanceolate, irregular	D
Relief (Re)	Umbonate, bosselated, nodular, exophytic,	D
Size (Si)	Small, medium, large, actual measurement	D
Number (Nu)	Small, medium, large, actual count	D
Colour (Co)	White, black, tan, brown, red, green, orange, pink, purple, blue, yellow,	D
Texture (Te)	Rugose, smooth	D
Extent (Ex)	Mild (1 – 20%), moderate (21 – 50%), severe (51 – 100%)	М
Time (Ti)	Acute, subacute, chronic	Μ
Lesion (Le)	Tissue loss, discoloration, growth anomaly	D, M
Structures altered (St)	Polyp, coenosarc, skeleton	D, M

Appendix 2: Management interventions

Coral disease outbreaks are often more virulent at the start of an outbreak and then prevalence and virulence decrease through time (<u>Brandt *et al.* 2012</u>; <u>Aeby *et al.* 2016</u>) and some early research on <u>environmental co-factors potentially influencing the disease</u> <u>dynamics of Florida's coral tissue loss diseases</u> indicates that SCTLD may also follow a similar trend. However, the severity and mortality of SCTLD has led to managers across the region treating their corals in a bid to save their reefs.

When considering whether to begin treatment, and deciding upon the appropriate methods, managers need to weigh up to potential impacts of no active coral treatment, the cost and resources required for treatment intervention, the spatial and temporal scale that treatment will be implemented in and the pros and cons of each method. The biggest issue with lesion-level disease interventions is that they do not appear to keep the colony from being re-infected (Florida DEP, 2019). Corals with previous disease lesions have new lesions more frequently than those that have never shown a lesion (Walker and Brunelle, 2019).

Antibiotics

Pros

Shown to be 67%–90% effective in stopping disease progression depending on what coral species is treated (<u>Neely *et al.* 2020</u>) and in what region of the Caribbean. As an example, treatment of diseased corals in the U.S. Virgin Islands showed a much-reduced success rate when using the standard amoxicillin in <u>coral ointment Base2b</u>.

Cons

The antibiotic treatment requires numerous applications to re-treat lesions, and does not prevent the colony from developing new SCTLD lesions in other locations over time (<u>Voss *et al.* 2020</u>) Antibiotics disrupt the host's natural microbiome and microbial communities in the environment. They can also be toxic to higher animals and facilitate the development of antibiotic-resistant microbes (<u>Kraemer *et al.* 2019</u>). Antibiotic resistant bacteria pose a risk to both the environment and human health, where possible, antibiotics should be kept from entering the environment. This risk, alongside the efficacy of the treatment, is something that should be considered carefully by managers when deciding upon a treatment approach

Resources

- <u>How-to video, applying amoxicillin and Base2b (Gulf & Caribbean Fisheries</u> <u>Institute)</u>.
- <u>Assessing the effectiveness of two intervention methods for stony coral tissue loss</u> <u>disease on *Montastraea cavernosa*</u>. Shilling *et al.* (2021).
- <u>Effectiveness of topical antibiotics in treating corals affected by Stony Coral Tissue</u> <u>Loss Disease</u>. Neeley *et al.* (2020).

Antiseptics

Antiseptics, such as chlorine, kill the coral tissue and pathogens where applied. It is not as effective as antibiotics in stopping disease lesions, however, it does reduce the pathogen load in the environment by killing the decaying disease tissue before it sloughs off and is carried to other colonies. Many groups are experimenting with different treatment methods including combining methods of using a chlorine mixture to kill the lesion tissue combined with mechanical (coral colony scale) firebreaks and treatment of the tissue adjacent to the lesion with natural substances that have antimicrobial activity such as honey. Ointments that can be mixed with potential anti-microbials (honey, etc.) and that will adhere to live tissue, for a day, are being trailed in Turks and Caicos Islands.

Pros

Medium efficacy in stopping disease lesions and reduces pathogen load in the environment.

Cons

The use of antiseptics has not been as successful as antibiotics, but they pose no known further risk to the environment nor in the development of antibiotic-resistant bacteria. The clay band-aid method of applying chlorine has shown some success, but this method is unlikely to kill the bacteria already in the tissue adjacent to the lesion, as research suggests that the bacteria may be present within tissue before accumulating at high enough levels to form lesions.

Resources

- Intervention and fate tracking for corals affected by stony coral tissue loss disease in the northern Florida Reef Tract. Voss et al. (2020).
- <u>SE FL Reef-building-coral Response to Amoxicillin Intervention and Broader-scale</u> <u>Coral Disease Intervention</u>. Florida DEP (2019).
- <u>Assessing the effectiveness of two intervention methods for stony coral tissue loss</u> <u>disease on *Montastraea cavernosa*</u>. Shilling *et al.* (2021).

Probiotics

Pros

Probiotics can provide lasting protection with little to no requirement for retreatment. Probiotics can also be used preventatively on healthy corals to prevent disease transmission. They provide a more complex treatment than antibiotics and release a variety of antibacterial compounds, reducing the likelihood of resistance. They also are often more economical to produce than antibiotics. Research has suggested that some probiotics can be used as not only a preventative measure on the specific colony they are applied to, but they can also stop disease progression in neighbouring infected coral colonies (<u>Coral health</u> <u>and marine probiotic lab – Smithsonian Marine Station</u>). Studies on probiotics identified to treat SCTLD in Florida found the probiotic McH17 to effectively arrest disease in 63% of corals treated, and slow disease progression in a further 31% (<u>Smithsonian Marine Station</u>).

Cons

Probiotics can potentially harm the host and testing with healthy corals and genome sequencing is required before field application. Specific probiotics are regionally applicable only and testing will be required before using probiotics in a new region. Probiotics may also be host specific, which can be problematic due to the level of research and testing required to implement on a large scale but can also be beneficial as the treatment is less likely to interfere with other animals in the environment. Most of the research and testing undertaken so far has focused on the Florida Reef Tract, and additional testing is required before this can be implemented at a larger scale across the Caribbean

Resources

- <u>Development of probiotics and alternative treatments for stony coral tissue loss</u> <u>disease</u>. Paul *et al.* (2020).
- <u>Gluing It Back Together: Probiotic Treatments for Stony Coral Tissue Loss Disease</u>. Smithsonian Marine Station (2020).
- <u>Coral Health Series: Treating corals with probiotics</u>. Smithsonian Marine Station.

Infected coral removal

Pros

By completely removing infected colonies (small colonies only) you eliminate that colony as a source of infection potentially decreasing disease spread on the reef and reducing 'pathogen load' in the environment. This intervention can be particularly beneficial during the earlier stages of SCTLD progression; by reducing the pathogen load, managers can potentially decrease the rates of transmission to new sites. Care would need to be taken to "bag" the colony prior to removal to prevent pathogen shedding during the stress of the removal.

Cons

By removing the colony, you are also removing the 3-dimensional structure on the reef which would have remained, at least for some time period, even if the colony died from disease. Alternatively, the infected colony could be "bagged" in-situ and an antiseptic, such as bleach, injected in the bag to kill the colony and pathogen but leave the skeleton in-tact.

Firebreak (coral colony-scale)

Pros

Creating a firebreak on a treated coral colony increases the success of the treatment especially for the less successful non-antibiotic treatments (Walker et al. 2021a). Florida scientists are now suggesting that intra-polyp pathogen transfer may be through the gastrovascular canal (E. Peters, pers. comm). The pathogen can be in the tissue adjacent to the lesion but not yet showing disease signs. The firebreak provides a mechanical

disruption, typically using underwater angle-grinders, from one polyp to another, preventing the existing pathogens from spreading further within the colony.

Cons

Most effective when used in conjunction with other treatments (e.g. chlorinated epoxy or antibiotics).

Firebreak (reef-scale)

Reef-scale firebreaks involve the removal of susceptible species across an extended area between a diseased area and unaffected area with the goal of reducing transmission as there are no susceptible species near the disease to be transmitted to.

Pros

Does not require the introduction of foreign substances into the marine environment and can be less resource intensive once completed than other methods. Believed to have slowed transmission and 'bought time' when used in the Cayman Islands after initial discovery of SCTLD, although no analysis has been done at this stage. The disease has recently crossed the 300m firebreak.

Cons

SCTLD is water-borne and as such has the potential to transmit through large areas in the water column. Firebreak on the larger scale, such as reefs, would not be effective based on lessons learnt from Florida's response. In Florida, the disease jumped around between reefs with unaffected reefs found in between affected ones, e.g. the downstream and upstream reefs got the disease, but the reef in between did not. Removing coral colonies from the reef can be detrimental to the structural integrity of a reef with further consequences on storm protection and provision of habitat. Consideration should be given to where to move the removed corals to (in water nursery or aquaria).

Appendix 3: Biosecurity for in-situ treatments or reef surveys

Once SCTLD is identified, biosecurity practices are essential to minimise transmission between reefs and control the spread. Biosecurity should be considered in fieldwork, lab work and general reef visits. Below are some suggested actions available for managers controlling cross contamination in a disease outbreak.

- Visit sites with no signs of disease first.
- Sample healthy coral first, then affected/diseased coral.
- Use one set of sampling gear for healthy colonies and a separate set of gear for diseased colonies.
- On the boat, decontaminate collection equipment by soaking in dilute hypochlorite (5–10% bleach) solution for at least 10 minutes and rinsing in fresh water.
- Clean dive gear by soaking in decontaminating solution and rinsing in fresh water at the end of each dive: <u>see dive gear decontamination.</u>
- Ballast water disposal

Appendix 4: Longer-term management interventions

Preservation of genetic material

Pros

The establishment of a genetic bank for live tissue culture and cryopreservation (e.g. microfragments, germinated cells, sperm, ovules, and fertilised larvae) has been suggested as an ex situ strategy to preserve the genetic diversity of coral species most susceptible to SCTLD (<u>Shearer *et al.* 2009</u>; <u>Afiq-Rolsi *et al.* 2019</u>). For instance, the US has established a genetic bank in collaboration with aquaria for 50 genotypes of each of the 20+ species of stony coral that are susceptible to SCTLD.

Cons

Human and financial resource limitations mean that this may not be appropriate and/or not cost-effective for some nations, such as Belize and possibly Honduras.

Resources

- <u>Stony Coral Tissue Loss Disease (SCTLD) In The Mesoamerican Reef Region:</u> <u>Recommendations For Addressing SCTLD</u>.
- <u>Maximising genetic diversity during coral transplantation from a highly impacted</u> <u>source reef</u>. Afiq-Rosli *et al.* (2019).
- <u>Repeated ex Situ Spawning in Two Highly Disease Susceptible Corals in the Family</u> <u>Meandrinidae</u>. O'Neil *et al.* (2021).
- <u>The use of DNA banks</u>.
- Healthy reefs cryopreservation and rescue webinar.

Coral rescue – nurseries

'Rescuing' corals from diseases and moving them into a nursery, either land or field based, is an important tool to ensure that sufficient individuals and species are preserved for future re-introduction into the wild.

Pros

Removal of susceptible species, or fragments of species, to a coral nursery (in-water or land-based) has successfully been used as a means of protecting genetic diversity and providing sources of coral for re-introduction.

Cons

In-water nurseries are the most cost effective and there is a lot of information on successful ways of developing them. However, as the SCTLD is waterborne there is always the risk of

infection of nursery corals. Land-based nurseries can provide protection from infection but require a large amount of infrastructure, money, and manpower to develop and maintain healthy corals through time. One strategy is to build partnerships with already established aquaria facilities to handle and house rescued corals.

Resources

- <u>Reef Resilience: Field-based nurseries</u>.
- <u>Florida Reef Tract: Nursery Management and treatment of pillar coral (DEP)</u> (landbased nursery).
- Florida Coral Rescue Team & Dashboard.

Reef repopulation / restoration

Another key step in combating SCTLD is to strategically restore the most biologically and economically valuable reef areas to change the trajectory of the coral reefs at risk of the disease, as well as to protect the economy that depends on it. This involves involving multiple stakeholder groups in the preparation and management of restoration sites to reduce invasive species, marine debris, poor water quality and/or sedimentation, which should provide a greater chance of recovery for corals restored to the reefs (Graham *et al.* 2015). A key example of a restoration programme is "<u>Mission: Iconic Reefs</u>", which is currently underway in the Florida Keys.

Resources

- <u>Reef Resilience: Coral Reef Restoration course</u>.
- Restoration in the age of disease.
- <u>A Decision Framework for Interventions to Increase the Persistence and Resilience</u> of Coral Reefs. The National Academies of Sciences (2019).

Reduction of anthropogenic stressors

By controlling the input of anthropogenic stressors on reefs, we can optimise conditions favourable for reef health and coral growth. Ultimately, this might be the most powerful and successful management strategy, one with multiple positive consequences on all coastal ecosystems, and one whereby local management agencies can exert some control (<u>Harvey et al. 2018</u>). For instance, strong links have been made between poor water quality and the occurrence and prevalence of several coral diseases (<u>Bruno et al. 2003</u>; <u>Vega Thurber 2013</u>; <u>Wiedenmann et al. 2013</u>; <u>Zaneveld et al. 2016</u>), so improving water quality management (e.g. sewage management) may prevent this local stressor from exacerbating the severity and prevalence of SCTLD.

Resources

- Land-Based Sources of Pollution Focus Area.
- <u>Fate tracking, molecular investigation, and amputation assessment of SCTLD in the northern Florida Reef Tract</u>.

Ballast water management

Another factor that may be contributing to the spread of SCTLD includes the potential transfer of pathogens in ballast water (<u>Strudivan *et al.* 2022</u>). Current federal regulations (USCG and EPA) are encouraging ships exchanging ballast water to follow the best practices to avoid more drastic measures, such as no discharge zones, being enforced by NOAA. Some of the voluntary best management practices include:

- 1) If the ship has not yet reached its ballast water (BW) compliance date and is legally still permitted to conduct BW exchange (BWE), but has a US type approved ballast water management system (BWMS) or an alternate management system installed, these should be used, as they are potentially more effective than BWE;
- 2) Any BW discharge should be conducted as far from shore and known coral reef locations as possible, even if regulations permit it;
- 3) If planned passage does not go 200 nm from shore, voluntarily divert to 200 nm and conduct BWE, or at least in waters that are at least 50 nm from shore and 200 m deep; and
- 4) Minimise BW uptake when within 50 nm from shore and discharge all unmanaged and partially exchanged BW beyond 50 nm from shore.

Resources

- <u>Ballast Water Best Management Practices</u>.
- <u>Transmission of stony coral tissue loss disease (SCTLD) in simulated ballast water</u> <u>confirms the potential for ship-born spread.</u> Strudivan *et al.* (2022).
- Report: <u>https://nbic.si.edu/wp-content/uploads/2017/09/</u> <u>MSIB.07.19_SCTLD_analysis.pdf</u>. Everett *et al.* (2021).

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