Puerto Rico Stony Coral Tissue Loss Disease Intervention Plan

September 2021

This document is a final draft under revision by the Department of Natural and Environmental Resources.

Cover photo courtesy of Miguel G. Figuerola Hernández.
Background photo courtesy of Nilda Jiménez.

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Introduction

Stony Coral Tissue Loss Disease (SCTLD) affects more than 30 species of corals in Florida and the Caribbean (AGRRA, 2020). SCTLD appears to be different from previously described coral diseases because of its rapid spread in coral species with high or intermediate susceptibility and the high number of coral species that it affects (*Appendix I*). In addition, it is characterized by a high prevalence and virulence in sites where the disease is established. In the field, this disease can be difficult to distinguish from other "white plague" or "white band" coral diseases, as they may exhibit similar signs such as a bleached front, sloughing and fast tissue mortality, multi-focal lesions, and final exposure of the clean white skeleton.

On the reef, many of these different diseases could be occurring at the same time and affecting several species, such as dark spot disease, white plague, black band disease, and Caribbean yellow band disease, among others (Weil and Rogers, 2011). Stony Coral Tissue Loss Disease could be observed in a coral colony as a single or multi-focal fast growing lesions, which continue to advance killing tissues across the colony. The following references contain more information on the characteristics of SCTLD: SCTLD Case Definition (2018), SCTLD identification guide (Bruckner, 2018), and on the Atlantic and Gulf Rapid Reef Assessment (AGRRA) website. In addition, Croquer et al. (2021) released a manuscript comparing SCTLD and white plague disease. The photos in *Figure 1* and *Figure 2* show the progression of the disease in two coral species, *Pseudodiploria strigosa* and *Dichocoenia stokesii*, respectively.

Although scientists and researchers continue to study the disease, the putative pathogen(s) causing SCTLD have not been clearly identified. Therefore, SCTLD is only suspected at a given reef site if the disease characteristics are observed in SCTLD susceptible coral species with multiple colonies affected at the same time, if the disease spreads more rapidly than other diseases, and if there is high coral mortality on the reef.

The way in which SCTLD spreads locally, regionally, and over great distances (from country to country) is unknown. The disease was first documented in Florida in 2014, and has since spread throughout much of the Caribbean (Kramer et al., 2019). One hypothesis is that potential pathogens that cause SCTLD can be carried by untreated ballast water in ships and discharged near reefs (Dahlgreen et al., 2021; Everett et al., 2021). This hypothesis helps to explain the appearance of the disease in countries distant from Florida that do not follow an evident directional pattern of the dominant ocean currents. For example, four years after documentation in Florida, SCTLD was documented in Jamaica, then Mexico, and then St. Maarten (Kramer et al., 2019). In the Bahamas, researchers found that the mortality and infection rate of many SCTLD susceptible species were greater at sites closer to international commercial shipping ports (Dahlgreen et al., 2021). Notwithstanding, the link to shipping ports is not a pattern that explains the SCTLD spread in other areas.

Figure 1. Rapid progression of Stony Coral Tissue Loss Disease in a Pseudodiploria strigosa colony on Punta Arenas reef in Vieques. Photo credit: Sarah Elise P. Field

Figure 2. Rapid progression of Stony Coral Tissue Loss Disease in a Dichocoenia stokesii colony on Rompeolas reef in Vieques. Photo Credit: Sarah Elise P. Field

SCTLD in Puerto Rico

In Puerto Rico, the first documented case of SCTLD was off of the west coast of Culebra in November 2019, reported by Dr. Edwin Hernández. As of September 2021, the disease continues to spread westward from reef to reef in the north and south of mainland Puerto Rico. The disease has been documented in shallow sites as well as in mesophotic sites. *Figure 3* shows the sites where SCTLD has been documented up until September 2021. Coral cover has declined significantly at sites where SCTLD is already established in Puerto Rico; for example, up to an estimated 50% mortality has been observed in sites affected by SCTLD since 2019 *(Nilda Jiménez, personal communication)*.

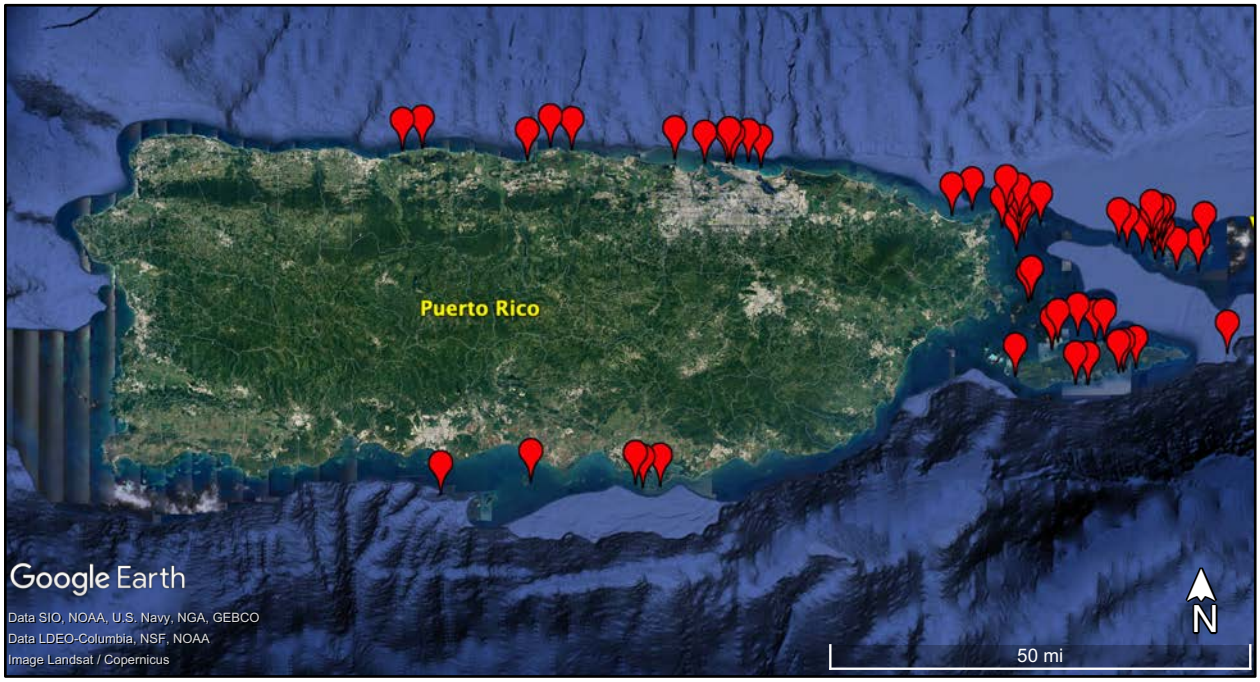

Figure 3. A map of the coral reef sites in Puerto Rico documented with Stony Coral Tissue Loss Disease as of August 2021, marked with a red pin.

The first treatment of coral colonies with SCTLD in Puerto Rico was by Sociedad Ambiente Marino (SAM), where treatment was applied to 27 colonies, representing seven coral species. The treatment used was an ointment from the company Ocean Alchemists called CoralCure Base2B, mixed with the antibiotic amoxicillin. This specific mixture has shown effectiveness in treating SCTLD lesions in both laboratory and field studies (Voss & Combs, 2018; Shilling et al., 2021; Neely et al., 2020; Walker et al., 2021). In June 2020, the Puerto Rico Department of Natural and Environmental Resources (DNER) began applying this same treatment in colonies at various sites with SCTLD, and in November 2020, the company Sea Ventures joined these efforts. The coral reef sites treated in Puerto Rico as of August 2021 are shown in *Figure 4*. The effectiveness of the treatment has been estimated from a subset of colonies treated in some sites which have been numerically tagged and monitored over periods of several months to a year, depending on the

locality. Data collected in Puerto Rico has shown that the success in slowing disease progression or stopping lesion growth with treatment is highly variable, with an approximate range of 30% to 95% effectiveness at the colony level. Success appears to increase when colonies are retreated at bimonthly or monthly frequencies to treat progressing and new lesions on the colony, which is an observation consistent with those from Florida (Neely et al., 2020; Walker et al., 2021).

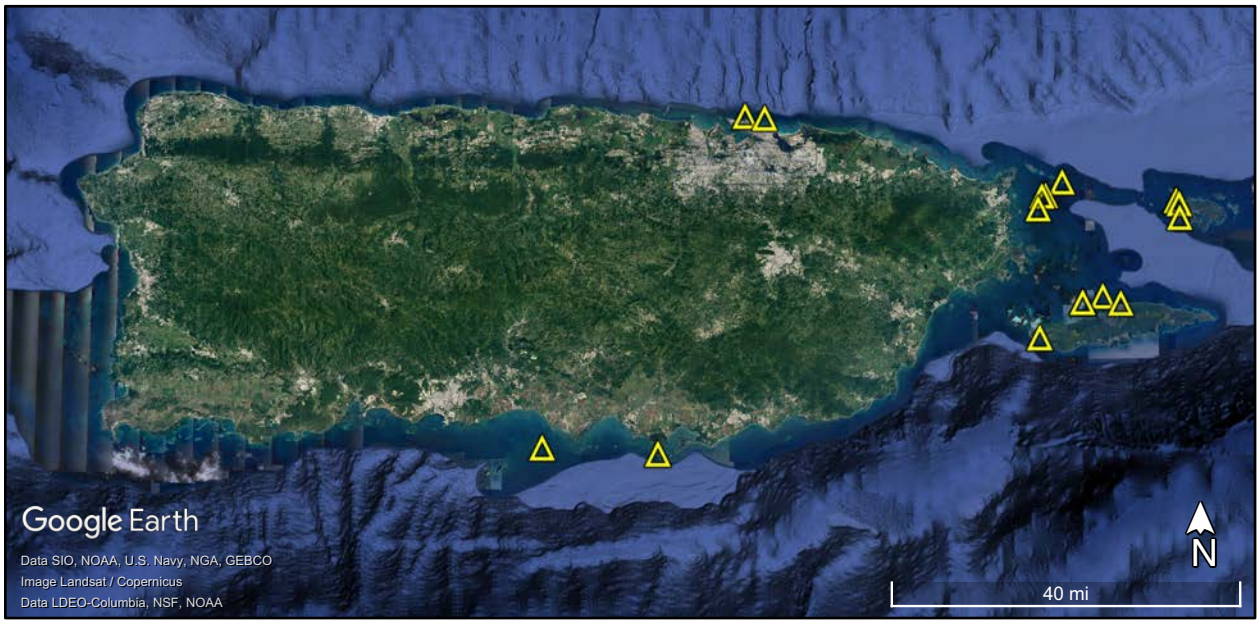

Figure 4. A map of the 17 coral reef sites in Puerto Rico treated for Stony Coral Tissue Loss Disease as of August 2021, marked with a yellow triangle.

Intervention Plan Goals and Objectives

Given the threat of SCTLD to coral reefs in Puerto Rico, the DNER Coral Reef Conservation and Management Program (Coral Program) recognizes the importance of coordinating early intervention to reduce the mortality occurring across the affected reefs. The goal in developing this intervention plan is to standardize the intervention methodology and determine where to focus intervention efforts in Puerto Rico. The objectives under this goal are to:

1. Establish SCTLD intervention methodologies in Puerto Rico
2. Provide guidance on the most recent best practices and recommendations for SCTLD intervention
3. Present coral reef sites selected to focus intervention efforts

This intervention plan was developed using local and regional experiences, lessons learned, and literature, including those from Florida, USVI, and other Caribbean partners. Meetings coordinated by Florida and USVI partners, as well as by the Caribbean Cooperation Team, have been vital in

this process to develop and implement disease response in Puerto Rico. This plan is meant to be updated annually to adapt to changing conditions and to integrate new scientific findings.

Intervention

Throughout this document, the term intervention is defined as applying any type of treatment to coral colonies possibly infected with SCTLD. The purpose of intervention is to improve the chance of survival of the treated colony. In addition to applying Base2B and antibiotic ointment, this may include practices that are not currently being utilized in Puerto Rico but may be used in the future, such as amputation, firebreaks, and probiotics, among others. The plan does not include options like coral rescue, for example, which involves removing live, apparently healthy coral colonies from a reef to move them to in-water nurseries or land-based nursery facilities. The following sections describe the current practices used in Puerto Rico to conduct assessments and intervene at coral reef sites with possible or confirmed cases of SCTLD. As a precautionary approach, it is recommended to disinfect equipment before and/or after each dive, especially when diving in a site that may not be affected by SCTLD after diving a site with SCTLD. To disinfect equipment and avoid potentially transferring pathogens between reefs, first check snorkeling or diving equipment and the tools used in the water, and remove any debris stuck to it. Afterwards, soak all equipment for 10 minutes in a 1% chlorine solution, rinse thoroughly with fresh water, and air dry. For more information, check out the Guidelines for Disinfection of Dive Gear created by the NOAA Florida Keys National Marine Sanctuary.

Site Assessments

In order to determine if a site may be affected by SCTLD, an assessment is conducted targeting SCTLD susceptible species. In Puerto Rico, the methodology detailed in *Appendix II* is used along with the datasheet in *Appendix III*. The data collected can be reported to the Coral Health Report platform, created by the USVI and adapted to include Puerto Rico, and / or to the AGRRA platform. This information, which may include photos of susceptible colonies and data collected by the observer, can usually be used to determine if SCTLD could be affecting the reef and, if so, the prevalence of the disease. The DNER Coral Program and stakeholders can use this information to monitor the spread of the disease throughout the archipelago and for reef management decision making.

SCTLD Site Intervention

The intervention practices used at coral reef sites with SCTLD are based on the lessons learned from intervention in Puerto Rico as well as the experiences from other jurisdictions and countries. The intervention method currently used in Puerto Rico to treat SCTLD infected coral colonies is the application of the ointment composed of amoxicillin and Base2B on the active lesion, covering the border of the newly exposed skeleton and the living tissue. Of all of the treatment methods tested on colonies with SCTLD, although effectiveness is variable, the treatment using amoxicillin

and Base2B applied directly to the edge of the lesion is one of the most effective in halting the advance of lesions caused by the disease (Neely et al., 2020; Walker et al., 2021). Based on preliminary data managed by the DNER Coral Program, the groups that are applying treatment locally have had success halting lesion progression with this treatment. These groups are also continually searching for ways to maximize treatment effectiveness.

Treatment

Once confirmed that the suspected colonies in a site are likely infected with SCTLD, the authorized collaborator will coordinate the response with the DNER staff. At the reef site, colonies chosen as high priority for treatment are identified based on their ecological value and treatability. In the case of SCTLD treatment, the colonies deemed to have the highest ecological value are those that meet the following criteria:

- Large colony sizes (> 50cm in height and / or maximum diameter)
- listed under the Endangered Species Act (ESA)
- reef framework builder
- close proximity to other SCTLD susceptible coral colonies

Likewise, the treatability criterion is defined by colonies that:

- have 60% or more live coral tissue
- have five (5) or less active and treatable lesions
- are susceptible and treatable species[1]
  Some of the priority species for treatment include:
  - *Colpophyllia natans*
  - *Pseudodiploria strigosa*
  - *Meandrina meandrites*
  - *Diploria labyrinthiformis*
  - *Orbicella faveolata, O. franksi, and O. annularis*
  - *Montastraea cavernosa**
  - *Dendrogyra cylindrus**

**These species can be difficult to treat because the amoxicillin and Base2B paste may not stick to the colony for long, especially in areas subject to turbulence caused by currents and waves.*

Note that this process of selecting colonies for treatment is a recommendation; different factors at each site may affect which colonies can be successfully treated.

[1] Research in the state of Florida and work carried out in the USVI suggest that the treatment is more effective on some species than in others (Neely et al. 2020).

The materials recommended for applying treatment are presented in *Appendix IV*. *Appendix V*The steps to apply the mixture of amoxicillin and CoralCure Base2B are found in *Appendix VI*.

Data management and monitoring treatment success

To monitor colony level treatment success, the colony must be monitored after the first treatment to determine the status of the treated lesions and if new lesions have developed. The datasheet used to collect data underwater is found in *Appendix V*, which has a list of data that must be collected each time a colony is treated. The datasheets and photos can be organized in a standardized way to compare and analyze them. The methodology of how to organize these data and how to determine the status of a colony is found in *Appendix VII*. It is recommended to visit and re-treat a colony, if needed, two weeks after the first treatment, and at least monthly afterwards.

Permitting

All intervention activities must be coordinated with the DNER. All participants conducting intervention activities must be trained to do so and have the applicable permit from the DNER. The training and workshops are provided by DNER, following a curriculum designed to prepare volunteers on the activities and skills needed for intervention. For the permit to intervene on a coral reef with SCTLD, the participant must fill out the Application for Emergency Authorization for Coral Management Activities against SCTLD, or the Solicitud para autorización de emergencia para actividades de manejo de corales contra la condición SCTLD. This document must be submitted directly to the DNER. Permits are issued for a one-year period; therefore, permits should be renewed annually if intervention activities are expected to be continued. Note that permits can take weeks to months before they are reviewed and issued, therefore both new permits and permit extension requests should be submitted a few months ahead of time. It is also important to note that this permit does not apply to scientific research. For SCTLD research related activities, the permitting process and the required documents are separate.

Federal authorization for coral disease response activities is covered under the Programmatic Biological Opinion on Research, Restoration, and Relocation of Threatened Caribbean Corals (2016) and the DNER does not need to coordinate with the NOAA Protected Resources Division (PRD) unless the actions are funded, authorized, or carried out by a federal agency. However, groups collaborating in intervention activities have the responsibility to inform and coordinate the response with the DNER.

Communications

Communications are necessary to expand awareness of the importance of coral reefs, help to change public behavior that can be harmful to reefs, and increase protection and conservation activities. Communications pertaining to SCTLD intervention help to raise awareness about the threat of the disease and promote support with intervention efforts, for example, documenting the

presence of the disease in sites where intervention could be conducted, identifying volunteers to join intervention efforts, and gaining public support in these efforts.

In the countries and territories where SCTLD appeared, education and outreach to stakeholders and the public has been of great importance. In 2019, various stakeholders in Puerto Rico began to meet and collaborate in planning the response to SCTLD, including how best to communicate these efforts. Collaborators have worked together to translate and create educational materials to inform scientists and the public about the disease outbreak. This information is shared on social media, in presentations, and through email groups to the coral reef community. In addition, virtual and practical trainings are provided to train stakeholders on how to evaluate, report, and apply treatment to colonies with SCTLD, some of which have been recorded and shared on the Coral PR YouTube channel.

To inform scientists of the coral reef sites where intervention has been conducted, increase collaborations in treatment areas, and avoid duplicating assessment and treatment efforts, an email group was created to share information on coral disease response and monthly meetings are held with key stakeholders. Infographics and additional webpages providing information on SCTLD and intervention activities in Puerto Rico are being developed and updated for greater accessibility to the public and scientists.

Coral Reef Intervention Site Selection

Methodology

In order to rank coral reef sites to help determine where to focus SCTLD intervention efforts with the available resources, the DNER Coral Program met with state and federal government stakeholders to determine the list of criteria used to prioritize intervention sites. During this meeting, a list of possible criteria for ranking each site was discussed. *Table* 1 shows the list of the selected criteria.

Using these criteria, coral reef sites were assessed using five datasets where quantitative and/or qualitative data was collected. The dataset sources were the Puerto Rico Coral Reef Monitoring Program (PRCRMP) from 2018 to 2020, the National Coral Reef Monitoring Program (NCRMP) benthic assessment dataset from 2019, the NCRMP coral demographic dataset from 2019, data collected by NOAA from surveys around the island after Hurricane Maria in 2018, and information from a participatory mapping exercise conducted in 2021 by Emma Korein, National Coral Reef Management Fellow. The first four datasets contained quantitative and qualitative data, while the participatory mapping exercise contained qualitative data. The participatory mapping exercise was conducted with 30 coral research, conservation, and management experts representing 20 organizations across Puerto Rico. Using the program SeaSketch, participants were asked to select sites which they believed should hold priority in coral disease monitoring and intervention efforts based on a range of criteria, including ecological value, economic value, cultural value, disease

prevalence, chance of recovery from disease, risk of disease outbreak, previous monitoring effort, and logistical considerations.

Table 1. A list of criteria selected by stakeholders to use in the SCTLD intervention site ranking process.

Ecological Criteria	**Definition**
High abundance of susceptible, treatable coral colonies	Includes the sites with the highest density, coverage and/or number of susceptible, treatable colonies in comparison to other sites
Structure	Sites with large, reproductively active, reef-building corals that are susceptible, treatable colonies
Epidemiology	**Definition**
Status of site pertaining to SCTLD	The site's SCTLD status as of August 2021. With SCTLD, Without SCTLD, or presumed to have SCTLD.
Treatability	**Definition**
Site accessibility	General consideration of accessibility, such as the distance from boat ramps or land, general conditions, depth, and wave energy
Previous treatment for SCTLD	The site has or has not been previously treated for SCTLD as of August 2021
Regularly visited sites	If the site is regularly visited by a group, defined as at least once a month
Availability of low cost or free resources	Low cost or free transportation and availability of volunteers
Economic Contribution	**Definition**
Economic value	If the area is economically important, including if the site is found within a Marine Protected Area

Data representing each of the selected criteria were extracted and consolidated into an Excel spreadsheet. Each dataset was reviewed separately since data cannot be compared directly due to the use of different methodologies. Within each dataset, data was standardized with values from 0 to 1, while applying a weighted value determined by the state and federal stakeholders. All final standardized and weighted values were averaged to provide a ranking of each site per dataset. The top three sites per region of Puerto Rico (north, south, east, west, Vieques, and Culebra) were then used as a starting point to select sites for SCTLD intervention efforts.

The links to publicly available data are:

- PRCRMP reports: https://www.drna.pr.gov/coralpr/monitoreo/
- PRCRMP data: https://www.ncei.noaa.gov/access/metadata/landing-page/bin/iso?id=gov.noaa.nodc:0204647
- NCRMP datasets: https://www.ncei.noaa.gov/archive/accession/0217139
- NOAA Hurricane Maria Assessment: https://www.ncei.noaa.gov/access/metadata/landing-page/bin/iso?id=gov.noaa.nodc:0221189

Top sites for SCTLD Intervention in Puerto Rico

Six coral reef sites, one per region, were selected as the top sites for intervention efforts using the results of the ranking exercise described above and validation by stakeholders. The list of the top sites selected for SCTLD intervention are listed in *Table 2* and mapped in *Figure 5*.

Table 2. Using the top three sites per region per dataset determined by the ranking exercise and stakeholder validation, one coral reef site was selected per region to focus SCTLD intervention efforts per region in Puerto Rico.

Region	Site	Coordinates
North	Peñon de Mera	18.48900, -66.67630
West	Tres Palmas	18.34766, 67.26411
South	Pinaculos	17.93263, -67.0117
East	Palominos	18.3373, -65.5654
Vieques	Mosquito	18.16185, -65.49912
Culebra	Carlos Rosario	18.3307, -65.3331

Figure 5. A map of the coral reef sites in Puerto Rico that were selected as potential sites to focus intervention efforts.

Before applying treatment at a site, the site will need to be assessed and verified to ensure that intervention is possible or needed. Some of these sites do not have SCTLD as of September 2021, and others will require ideal weather conditions for at least three days in order for the applied treatment to be effective. Although an attempt was made to take into account weather conditions while selecting sites for intervention, it is difficult to know how the weather will affect the site or will change throughout the year, especially in areas that have not previously been treated for SCTLD. In the case that SCTLD responders are not able to intervene at one or more of the above sites for any reason, or responders are ready to move on to a new site, the top three sites per region

per dataset were reviewed by stakeholders with local knowledge of each region, and three additional backup sites considering the ranking exercise and/or local knowledge for each region were also included (*Table 3*). Maps of each region are found in Figure 6, Figure 7, Figure 8, and Figure 9.

Table 3. Using the top three sites per region per dataset from the ranking exercise and stakeholder knowledge, three backup coral reef sites were selected per region for SCTLD intervention efforts in Puerto Rico.

Region	Site	Coordinates
North	La Ocho	18.46835, -66.09176
North	Arrecife Roncador	18.342962, -65.61379
North	Arrecifes Isla Verde	18.448965, -66.01670
West	El Ron	18.102967, -67.28540
West	El Negro	18.15425, -67.24183
West	Buye	18.044856, -67.20308
South	Turrumote II	17.9165737, -66.97248
South	Cayo Coral	17.93626, -66.88836
South	Media Luna Channel	17.935351, -67.05061
East	Cayo Largo	18.31604, -65.57680
East	Cayo Lobo	18.373081, -65.56984
East	Seven Seas	18.373854, -65.63744
Vieques	Punta Arenas	18.10098, -65.57663
Vieques	Cayo Blanco	18.17115, -65.46693
Vieques	Coral reef in northern Vieques (no name)	18.15412, -65.47327
Culebra	La Raja	18.336817, -65.336097
Culebra	Carlos Rosario S	18.3271008, -65.33139801
Culebra	Coral reef between Punta Tamarindo Grande y Punta Tamarindo (no name)	18.32265, -65.32595

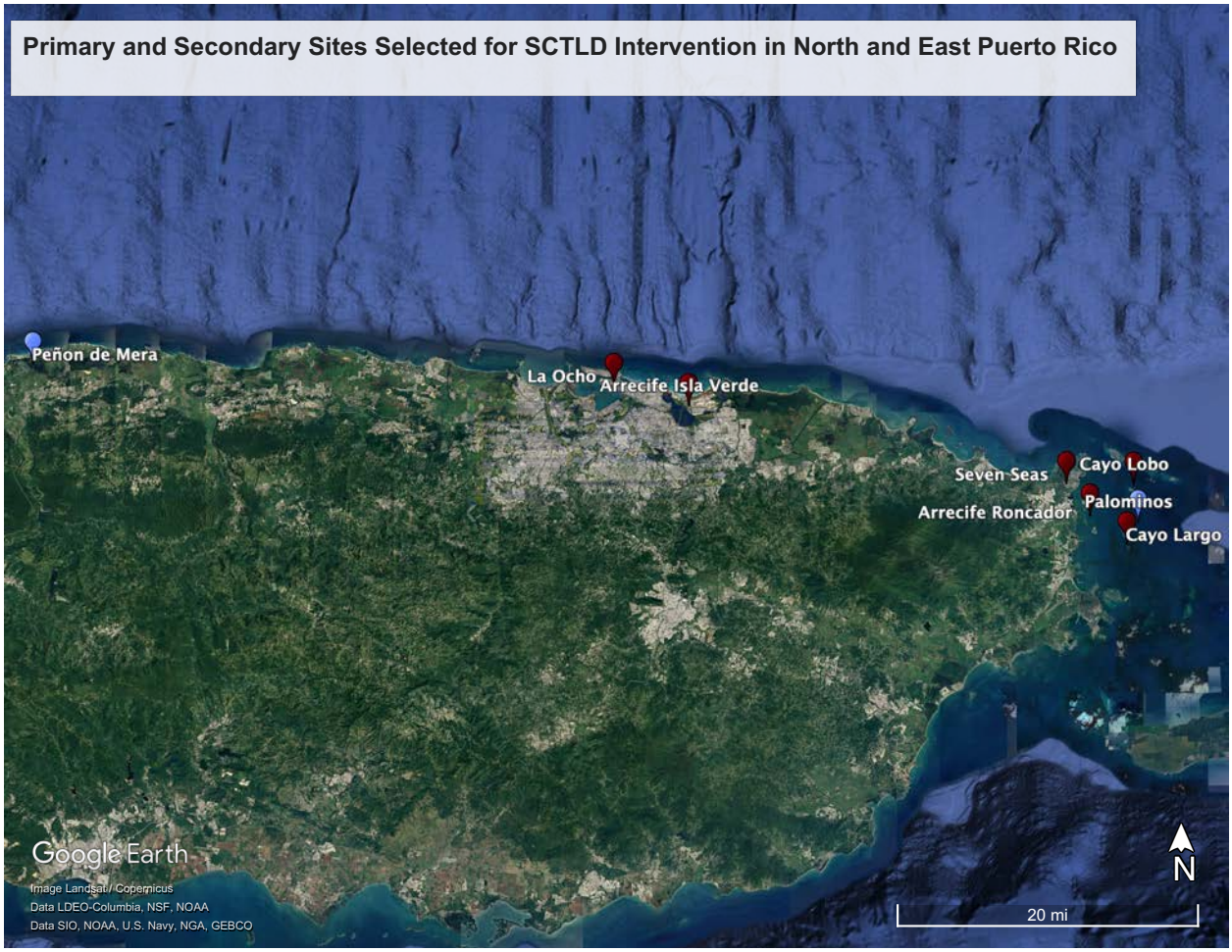

Figure 6. A map of the selected SCTLD intervention sites in the north and east of Puerto Rico. Blue pins are the primary sites (Peñon de Mera in the north and Palominos in the east), while red pins are secondary coral reef sites.

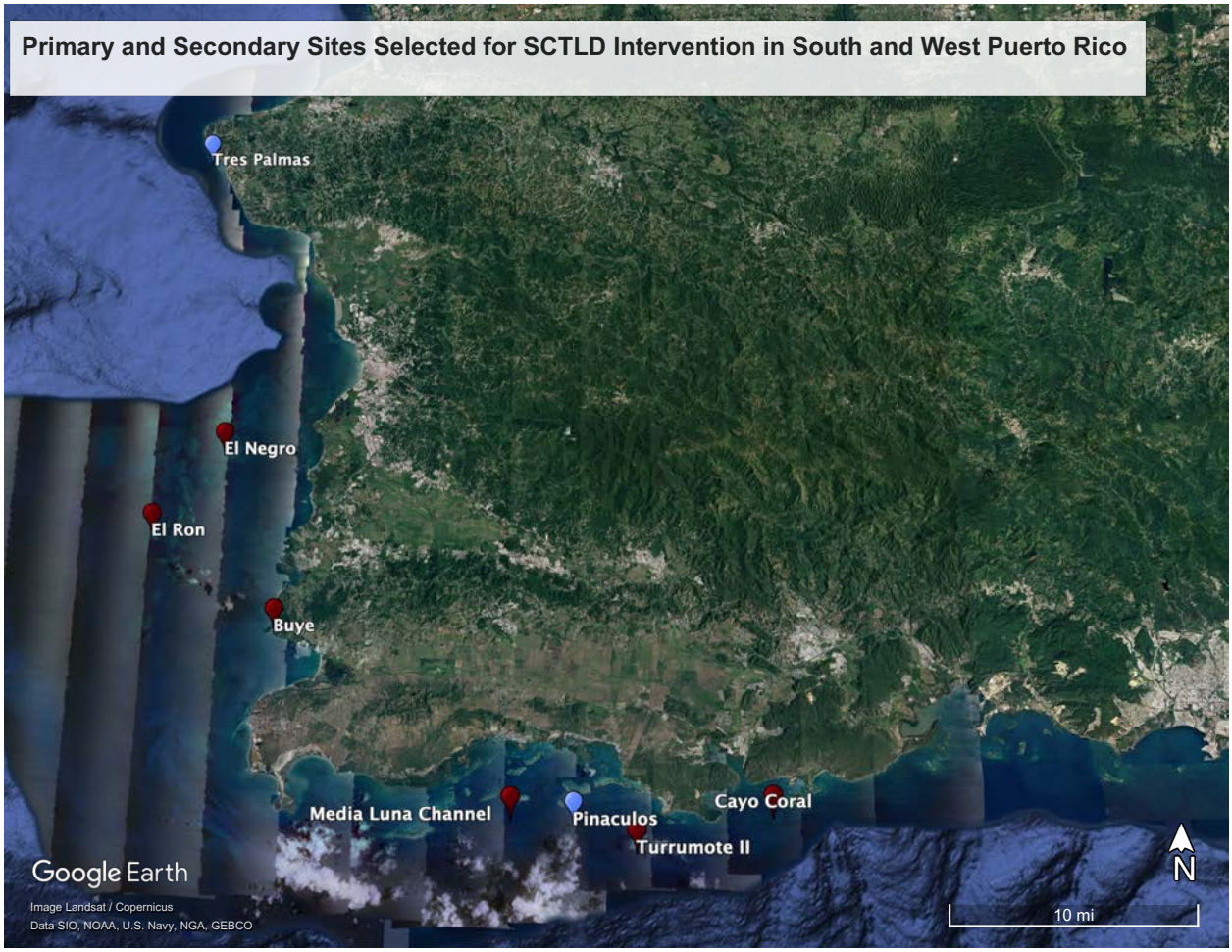

Figure 7. A map of the selected SCTLD intervention sites in the south and west of Puerto Rico. Blue pins are the primary sites (Pinaculos in the south and Tres Palmas in the west), while red pins are secondary coral reef sites.

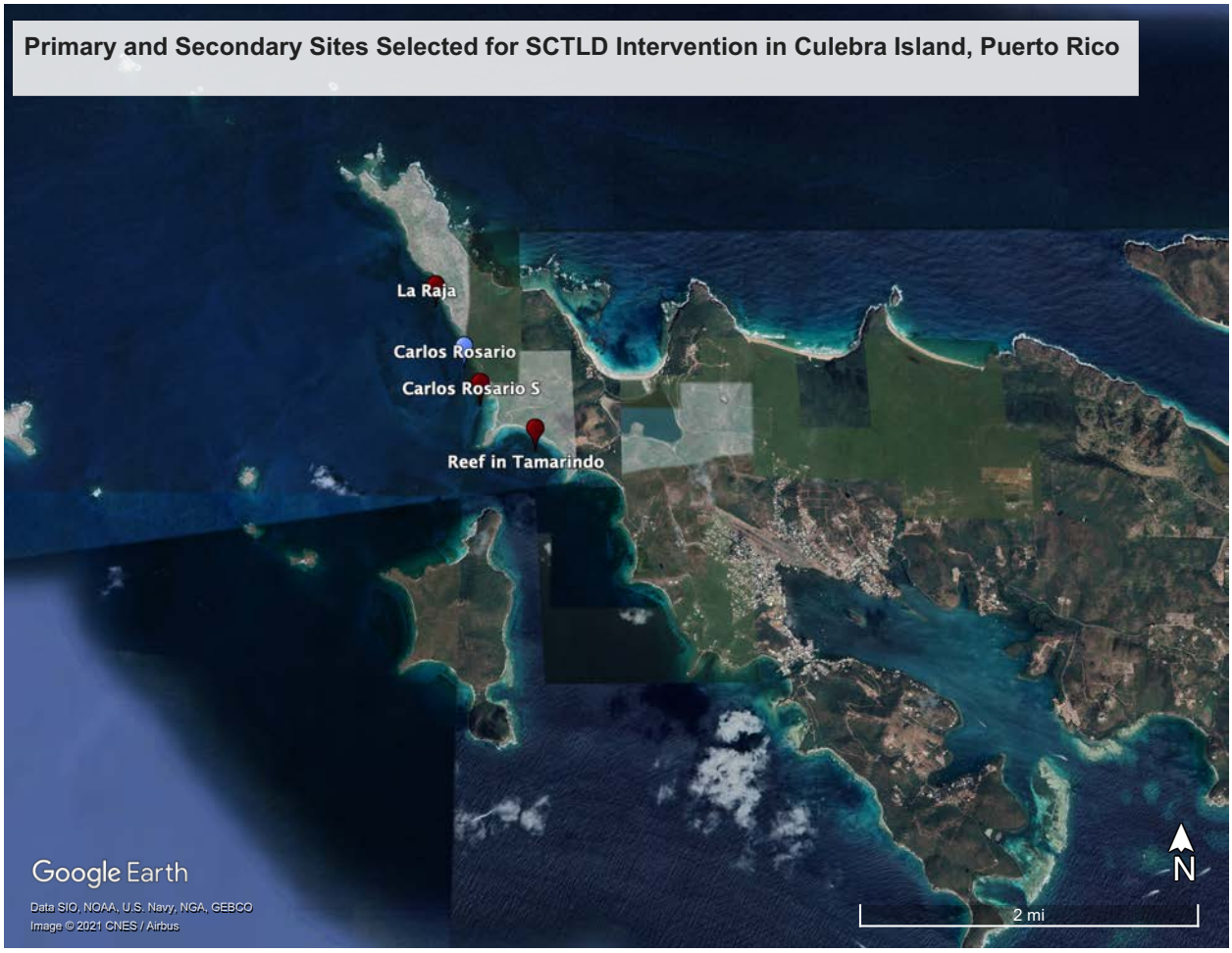

Figure 8. A map of the selected SCTLD intervention sites in Culebra Island, Puerto Rico. The blue pin is the primary site (Carlos Rosario), while red pins are secondary coral reef sites.

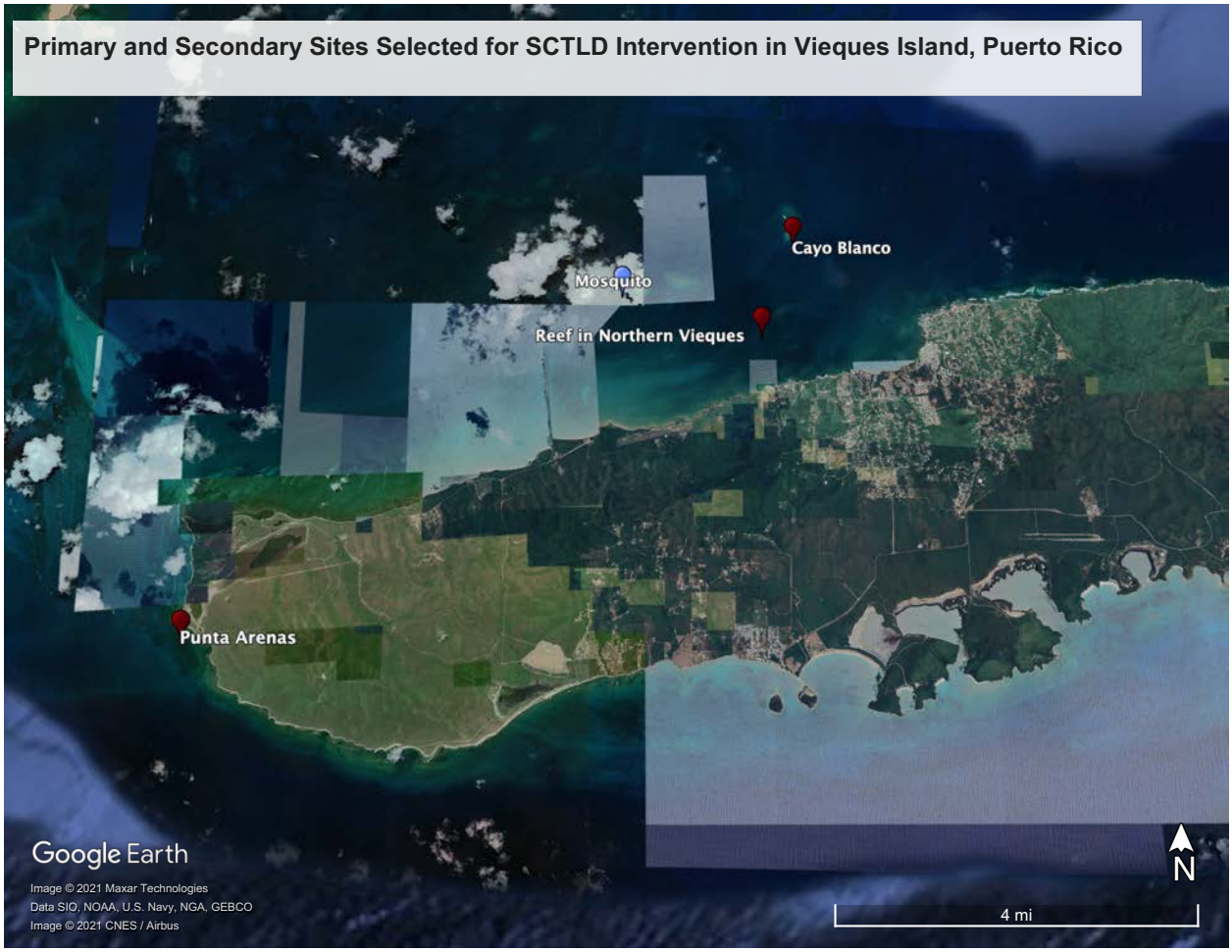

Figure 9. A map of the selected SCTLD intervention sites in Vieques Island, Puerto Rico. The blue pin is the primary site (Mosquito), while red pins are secondary coral reef sites.

Recommendations

The following recommendations related to SCTLD intervention can help to efficiently invest resources in efforts that are beneficial to the health of coral reefs.

1. Monitor colonies treated for SCTLD and treat as needed to increase success in halting lesions and likely increase colony survival.
2. Improve and increase communications about SCTLD intervention, while adapting the message to ensure that the target audience is reached in the way intended.
3. Use a consistent message among local SCTLD stakeholders to communicate intervention efforts to legislators, scientists, and the general public.
4. Expand capacity in the response to SCTLD by training different groups around the island in coral reef assessment and SCTLD intervention. In addition, expand research capacity locally. For example, identify possible collaborations with professors, undergraduate, master's and/or doctoral students that are interested in focusing their research on SCTLD intervention.

5. Develop a broad citizen scientist program where citizens can help assess coral reef health and track disease progression by providing a platform for communicating reef condition in a central location. Citizens who spend time in the water regularly, for example snorkelers, divers, fishermen, surfers, among others, are found throughout the island and have and can continue to provide important information about coral reef conditions.
6. Conduct a statistically sound assessment to determine the changes in the structure and composition of coral communities associated with the disease outbreak and the effectiveness of treating coral colonies with SCTLD locally. Miguel G. Figuerola Hernández, Coral Reef Specialist under the DNER Coral Reef Conservation and Management Program, developed experimental designs that could be used to answer the following questions:
   - What are the changes in the structure and composition of the coral reef benthic community (abundance and diversity) that occur due to SCTLD impact/outbreaks?
   - What are the long-term changes in colony mortality in treated and untreated corals between depths and SCTLD sites?

The experimental designs are accessible by the link below:

https://www.ncei.noaa.gov/data/oceans/coris/library/NOAA/CRCP/other/grants/Hernandez2021_ExperimentalDesign_SCTLD_PR.pdf

Next Steps

The next step for Puerto Rico to improve its response to SCTLD is to continue expanding and strengthening SCTLD response efforts. This includes identifying groups of interested scientists and citizens around Puerto Rico that can dedicate their time to SCTLD intervention efforts, and providing those groups SCTLD intervention training and the resources they need for these efforts. This way, intervention efforts can be expanded across the archipelago. In addition, high level and public communications should be tailored to the audience and continue to expand to gain support in these efforts. Interorganizational collaborations and grant opportunities for intervention efforts exist and can be used to strengthen capacity for intervention.

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Photo: Miguel G. Figuerola Hernández

Appendices

Appendix I. SCTLD Susceptible Coral Species

This list is adapted from the 2018 SCTLD Case Definition.

Highly susceptible species

- *Colpophyllia natans* (boulder brain coral)
- *Dendrogyra cylindrus* (pillar coral) – Threatened species
- *Dichocoenia stokesii* (elliptical star coral)
- *Diploria labyrinthiformis* (grooved brain coral)
- *Eusmilia fastigiata* (smooth flower coral)
- *Meandrina meandrites* (maze coral)
- *Meandrina jacksoni* (whitevally maze coral)
- *Pseudodiploria strigosa* (symmetrical brain coral)
- *Pseudodiploria clivosa* (knobby brain coral)*
- *Montastraea cavernosa* (large-cup star coral)**

  * In Vieques Island, it does not appear that *P. clivosa* is highly susceptible, as in one local reef surveyed in its entirety 41% of *P. strigosa* colonies were infected with SCTLD, while only 2% of the *P. clivosa* colonies were infected (*Sarah Elise P. Field, pers. comm.*). However, in Cayo Diablo, a higher percentage of *P. strigosa* colonies were seen to be infected with SCTLD (*Stacey Williams, pers. comm.*), suggesting that susceptibility may be site specific.

  ** *M. cavernosa* was identified as a highly susceptible species in some areas, including Puerto Rico, and as intermediately susceptible species in Florida.

Intermediately susceptible species

- *Orbicella annularis* (lobed star coral) - Threatened species
- *Orbicella faveolata* (mountainous star coral) - Threatened species
- *Orbicella franksi* (boulder star coral) - Threatened species
- *Solenastrea bournoni* (smooth star coral)
- *Stephanocoenia intersepta* (blushing star coral)
- *Siderastrea siderea* (starlet coral)**

  ** As mentioned in the Case Definition (2018), there is uncertainty as to whether *S. siderea* shows signs of SCTLD or of a different disease. This species may show signs of disease earlier than other highly susceptible species during outbreaks. However, anecdotal evidence suggests that due to the pattern of lesions and the difference of duration of tissue loss in comparison to other susceptible species, the disease affecting *S. siderea* may be different from SCTLD.

Appendix II. Roving Diver Survey Methodology

To conduct a roving diver survey in search of SCTLD, the diver should swim through the coral reef site, while recording information on SCTLD susceptible species. Several divers can conduct the survey at one site, but they must avoid overlapping each other and should submit data separately.

Non-susceptible species, should not be included in this survey, including *Millepora* species. *Siderastrea siderea* (SSID) can be very common on some coral reef sites, and it may be difficult to count all of the colonies. This species can be included, however, if there are too many colonies and it makes it difficult to record all of them, then a description of the observation can be included under "Additional Notes" on the datasheet.

The roving diver survey should be conducted as follows:

1. Swim around the area, staying within approximately 50m of a buoy line for at least 10 minutes. If conducting a survey with other divers, avoid overlapping the area surveyed with one another.
2. For each survey, collect the following data using the designated datasheet.
    a. Name
    b. Date
    c. Site
    d. Latitude and Longitude (in decimal degrees at the survey starting point [ex. 17.970115, - 67.040006])
    e. Start and end time of the survey (10 minutes or more)
    f. Average survey depth
    g. Type of coral reef (*Reef crest, patch reef, forereef,* or others)
3. Record the code of the coral species identified during the survey, excluding species not susceptible to the SCTLD. Use the code legend in the data sheet. Focus your observations on colonies larger than 10 cm. For each species, count and record the number of colonies displaying one or more of the following conditions:
    a. Recently dead colonies that are presumed to be due to SCTLD; colonies dead from other causes (physical damage, overturned) must be excluded.
    b. Sick colonies that still have living tissue and colonies showing any signs of SCTLD.
    c. Colonies that do NOT show signs of SCTLD, but show pale, white, or dark spots.
    d. Healthy colonies that are not actively sick and don't have any abnormal signs.

    Photos of unusual sightings or diseases of interest can be taken but are not required.

Note: This methodology can be used for quick assessments of reefs, however, for a more accurate estimate of SCTLD prevalence or to monitor reef recovery over the long-term, permanent transects, randomly stratified across a reef site, will provide a better understanding of prevalence and recovery.

Appendix III. Roving Diver Survey Datasheet

Name:	Site:	Start time:
Date:	Type of Coral Reef:	Final time:
Longitude:	Depth:	*Excluding species that are not susceptible to SCTLD*
Latitude:	Temperature:	

Species Code	# of colonies with recent mortality (probably due to SCTLD)	# of colonies actively sick	# of corals with abnormal signs (pale, white, or dark spots)	# of healthy corals

Appendix IV. Materials used to apply treatment

Examples of products used to apply treatment
Antibiotic (amoxicillin) (50g)
CoralCure Base2B (Ocean Alchemists) (400g)
Syringe (60mL) *(instead of a caulking gun with empty caulk tubes)*
Caulking gun (*instead of syringes)*
Empty caulk tubes
Bowl to mix antibiotic and Base2B
Spatulas, metal spoons and/or forks to mix and pack antibiotic and Base2B
Nitrile gloves
Steel drilling hammer (1-48 oz or 1-32 oz.)
Cattle tags, numbered
Nails, 2 or 2.5 inches
Bleach (disinfectant)
Bin to disinfect gear (38 gal)
Mesh bag
Small cooler
Underwater Slate (or clipboard and waterproof paper)
Pencils
Clips
Camera

Appendix V. SCTLD Treatment Datasheet

Site	**Date**		**Diver/Snorkeler Names**				
Coordinates	**Tag #**	**Species**	**% of coral that is dead or sick**	**Max. Length**	**Max. Height**	**# of treated lesions**	**Notes** (ex. Tag color, if the colony is deal, if another treatment was used

Codes for species susceptible or possibly susceptible to SCTLD

Códigos para las especies de corales		
CNAT[1]	*Colpophyllia natans*	Boulder brain coral
DCYL[1]	*Dendrogyra cylindrus*	Pillar coral
DSTO[1]	*Dichocoenia stokesii*	Elliptical star coral
DLAB[1]	*Diploria labyrinthiformis*	Grooved brain coral
EFAS[1]	*Eusmilia fastigiata*	Smooth flower coral
MMEA[1]	*Meandrina meandrites*	Maze coral
MJAC[1]	*Meandrina jacksoni*	Whitevalley Maze Coral
PSTR[1]	*Pseudodiploria strigosa*	Symmetrical brain coral
PCLI[1]	*Pseudodiploria clivosa*	Knobby brain coral
OANN[2]	*Orbicella annularis*	Lobed star coral
OFAV[2]	*Orbicella faveolata*	Mountainous star coral
OFRA[2]	*Orbicella franksi*	Boulder star coral
MCAV[2]	*Montastraea cavernosa*	Great star coral
SBOU[2]	*Solenastrea bournoni*	Smooth star coral
SINT[2]	*Stephanocoenia intersepta*	Blushing star coral
SSID[2]	*Siderastrea siderea*	Massive starlet coral
AAGA[3]	*Agaricia agaricites*	Lettuce coral
ALAM[3]	*Agaricia lamarcki*	Lamarck's sheet coral
AGAR[3]	*Agaricia* spp.	-
MFER[3]	*Mycetophyllia ferox*	Rough cactus coral
MALI[3]	*Mycetophyllia aliciae*	Knobby cactus coral
MAUR[3]	*Madracis aurentenra*	Yellow pencil coral
FFRA[3]	*Favia fragum*	Golfball coral
HCUC[3]	*Helioseris cucullata*	Sunray lettuce coral
MANG[3]	*Mussa angulosa*	Large flower coral
SCOL[3]	*Scolymia* spp.	-
ISOP[3]	*Isophyllia* spp.	-
1 = Alta susceptibilidad, 2 = susceptibilidad intermedia, 3 = susceptibilidad baja/desconocida		

Appendix VI. Treatment application

When treating corals, prioritize large colonies with small, new lesions.

Preparation of the amoxicillin and Base2B mixture

IMPORTANT:

- Those allergic to penicillin, cephalosporin, or any antibiotic among beta-lactams (β-lactam), must consult their doctor before working with amoxicillin.
- Follow the precautionary measures required to work with chemical / pharmaceutical products. Nitrile gloves are recommended to avoid skin contact and cross contamination. It is important to wear a mask to avoid inhaling powdered amoxicillin.
- Store amoxicillin and CoralCure Base2B in the refrigerator and transport the ointment in a cooler with ice.

1. Thoroughly mix a 1:8 ratio of amoxicillin to CoralCure Base2B to create the ointment for coral treatment (For example: 400 g of CoralCure Base2B and 50 g of amoxicillin).
   - A sample of 100 g amoxicillin and 800 g of CoralCure Base2B can yield enough product to treat up to 50 corals in one day of field work (~ 2 dives) between two divers using between 11-13 syringes. A 50-60 cc syringe is sufficient to apply between 180-200 linear cm of treatment. However, the number of colonies that can be treated per treatment quantity will depend on the number of lesions, the size of the lesions, the skeletal structure of the coral species being treated, among other factors including the experience of the diver. Syringes are the best option for working in hard-to-reach areas and when a group of divers will be applying treatment at the same time, as the treatment could be divided in more syringes. For open areas and a reduced number of divers applying treatment, a caulking gun should be considered to simplify field work by reducing the number of items to carry and clean.

> ***Tip***
> Mixing the paste and the antibiotic between 12 to 18 hours before its application in the field, keeping it refrigerated, helps the treatment adhere better to the surface of the coral colonies.

2. Pack the ointment into syringes or an empty tube for the caulking gun (pull out the plunger and use a spatula or spoon to pack ointment from the back). To use catheter syringes, you may need to cut the tip of the syringe to improve flow.
3. Store the syringes in a refrigerator or cooler with ice until use. Treatment must be mixed as close as possible to the moment of application.

Applying treatment ointment to coral colonies

Use the syringe to apply the amoxicillin and Base2B ointment along the lesion and the immediate surrounding area (~1 cm). When applying, spread and press down with your finger. If possible, use gloves to avoid skin contact.

The mixture adheres best to the colony skeleton rather than to the tissue. Note that the coral tissue under the ointment will die, so try to place only a small amount over live tissue. Small amounts of the mixture may float but can usually be caught and added to the application.

When the coral colony has its tentacles exposed, which is commonly seen in the pillar coral (*D. cylindrus*), the ointment does not adhere well to the lesion. If this happens, you can use a small amount of modeling clay to anchor the ointment and hold it in place. It is important to make sure that you do not cover the ointment completely with the modeling clay, as water must flow through the ointment for it to work.

Tips:

- Syringes are positively buoyant. If you tape multiple nails around a syringe, it helps to maintain neutral buoyancy.
- The caulking gun tubes are also positively buoyant, so they should already be in the caulking gun when taking underwater.
- Cleaning the exposed skeleton of algae and sediment can help the treatment stick.

The process of treating a colony can take an average of approximately 5-16 minutes per coral, depending on factors such as the length of the lesions, the underwater conditions, among others. This includes the time it takes to search for the colony, tagging, and treatment.

Tagging colonies

It is recommended to tag about 10 colonies or 10% of treated colonies per site, or more, with numbered cattle tags to use these colonies in determining treatment success over time, as well as increase the probability of finding the same treated colony in future treatments. If any tagged colony dies, the tag should be removed and can be used for another treated colony. To tag a colony, nail a numbered tag close to a colony or within the dead area of the colony. Always make sure that the tag is nailed into dead substrate, and that it does not break or touch a live organism, including the live tissue of the coral colony. Attempts should be made to revisit these colonies regularly, for example, two weeks after the first treatment and monthly thereafter, treating the colony whenever one or more disease lesions are noted to be progressing.

When placing a tag on the dead part of a coral colony, care should be taken as not to break the coral colony, thus it is not recommended to place the tag directly on the coral colony treated, unless it is large, solid, and stable. Tags can be used as a reference to draw a map of the treatment site so that treated colonies can be relocated during additional visits. This will decrease the number of tags needed while being able to identify and monitor more treated colonies. Treated colonies can also be monitored without a tag by using a time series of photos. This will require the divers to be familiarized with the reef and remember the corals that were treated so that they can return and take photos during every visit.

Appendix VII. Methodology for data management

This methodology is based on the data management methodology implemented by the United States Virgin Islands (USVI) SCTLD response group.

All colony treatment and monitoring data in Puerto Rico are organized in an Excel data sheet. The SCTLD treatment data sheet should be used to collect data on the colonies that are treated in the field. Treatment data to collect in the field includes:

- Region
- Site name
- Coordinates (latitude and longitude of the colony or site)
- Date
- Tag number (if tagged)
- Maximum colony length
- Maximum colony height
- Species (Use the four-letter species code made up of the first letter of the genus and the first three letters of the species)
- Number of treated lesions
- Additional notes, such as the tag color, the type of treatment used, if the colony is dead, etc.

Additional data can be collected either in the field or through photos that are taken of each colony by the divers. It is important that you keep this in mind when taking the photo, as it may be used as a reference to collect other data, such as:

- Percent of the colony that is sick or dead
- The current status of the colony
    - This information is obtained using previous photos of the colony and comparing them with current photos to determine how it has changed.

The status assigned to each coral colony are:

- **Treated:** Treated for the first time
- **Halted:** If after being treated one or more times, all previously treated lesions have not progressed beyond the treatment line (appear to have halted)
- **Declining:** If one or more lesions have progressed beyond the line of previously applied treatment
- **New appeared:** If one or more new lesions have appeared on the colony since the last visit to the colony
- **Untreated:** Not treated
- **Dead:** Completely dead
- **No photo:** The colony status could not be determined because of missing photos.

Photos

All intervention photos are organized by the DNER Coral Reef Program. Photos are organized and named using the following structure below.

Complete photo name: tag number or X if untagged, species code, number pertaining to the number of the same species visited in that site on that day (if untagged), -, number pertaining to the number of photos of the same colony

Tag number: Full number of the tag marking the colony. If the colony is not marked, X is used. *No space is used between the tag number and the species code.*

Species code: The first letter of the genus and the first three letters of the species. If the species is unknown, use the first letter of the genus and SPP. If the species and genus are unknown, UNKN is used.

Number pertaining to the number of the same species visited in that site on that day: All photos taken at a site on the same day will be saved in the same folder. Many photos of the same species will likely be taken, so to differentiate these different colonies a number is used, starting with 1, for colonies that are not tagged. This number may be omitted if the colony is tagged.

Dash: Include the dash (-) after the previous number

Number pertaining to the set of photos of the same colony: All photos of the same colony will be named by including a number starting at 1 for the first photo of that colony, 2 for the second photos of that colony, and so on. This is to differentiate different photos of the same colony.

<u>Examples</u>

001MCAV-2 is an *Montastraea cavernosa* (MCAV) colony treated and tagged with the tag number 001 and it is the second photo in a set of photos of the same colony.

XPSTR3-5 is a *Pseudodiploria strigosa* (PSTR) colony treated but not tagged (X), it is the third (3) *P. strigosa* not tagged in the site visited that day and it is the fifth (5) photo in a set of photos of the same colony.

When taking photos, please:

- Take a maximum of 6 representative photos of the colony
- The first photo should be of the TAG ONLY, unless the colony has not been tagged.
- The following photo should be of the colony with the tag, if possible. Photos thereafter should be of the colony as a whole.
- Take at least one photo before and one after treatment, if possible. If concerned that the camera battery will run out and you must choose, it is preferable to take the photo after treatment has been applied.