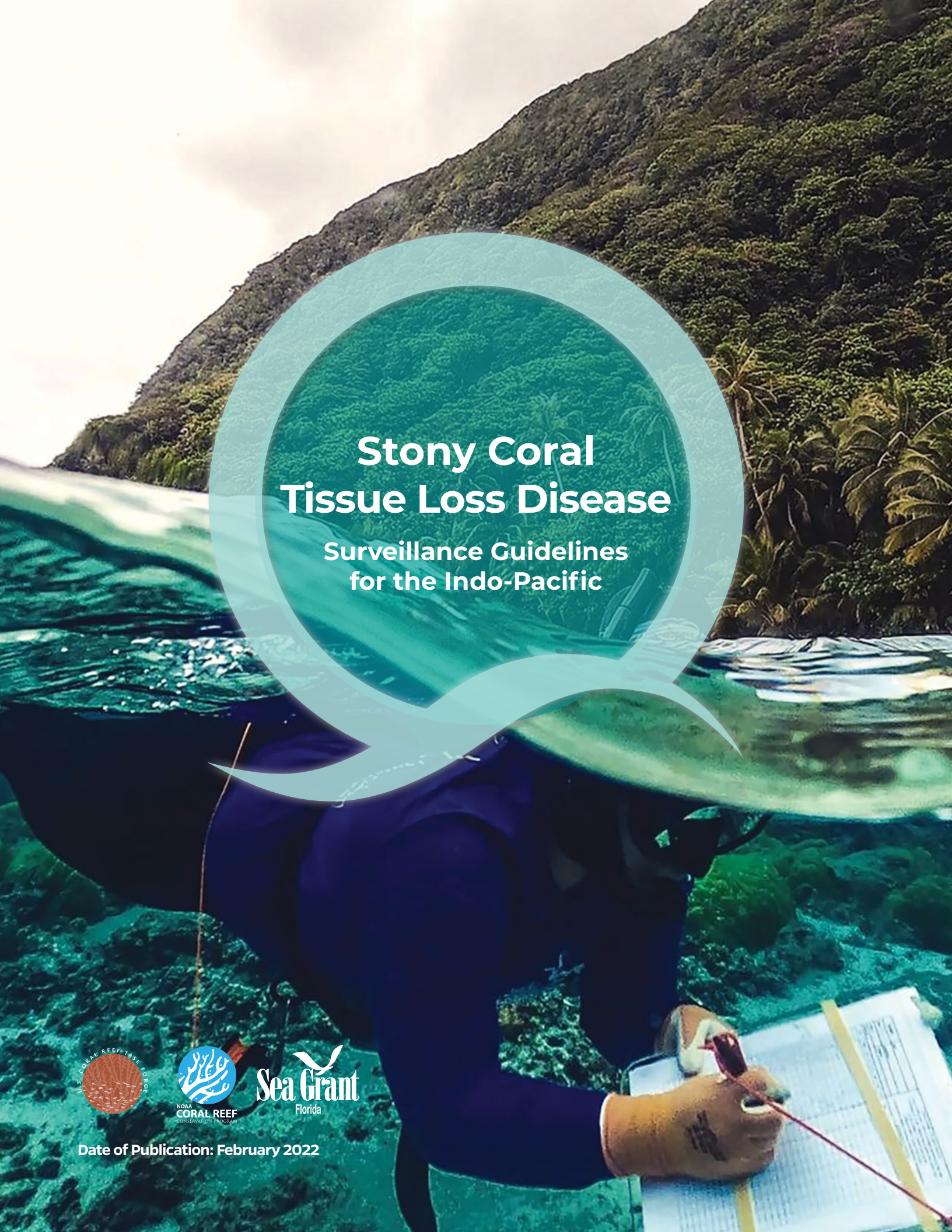

Stony Coral Tissue Loss Disease

Surveillance Guidelines for the Indo-Pacific

U.S. Coral Reef Task Force

NOAA Coral Reef Conservation Program

Sea Grant Florida

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PURPOSE

This document was developed in an effort to provide guidelines for coral reef managers and field conservation practitioners and researchers in the Indo-Pacific region regarding surveillance and detection of stony coral tissue loss disease (SCTLD). SCTLD is a highly lethal and fast spreading coral disease impacting the Atlantic and Caribbean but not yet found in the Pacific. This guide is not intended to replicate the more technical guidance in existing Pacific coral disease response plans.

This document includes numerous photos of Atlantic and Caribbean corals affected by SCTLD. While we are aware these species are not found in the Pacific, at this time not much is known about how SCTLD may affect Pacific corals. Therefore, we thought it would be helpful to include images showing the many different ways in which SCTLD presents on different morphologies and species that are closely related to those in the Indo-Pacific. Guidelines in this document are based on current knowledge of how SCTLD presents in Atlantic and Caribbean coral reef ecosystems.

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PHOTOGRAPHY:
Cover: American Samoa Coral Reef Advisory Group's Ofu Project Manager, Motusaga Vaeoso, surveys reefs on Ofu (PHOTO BY VALENTINE VAEOSO)

Inside Front Cover/Pg. 11: A diver conducts a survey for the Deep Coral Reef Monitoring Program on a mesophotic reef offshore of St. Thomas (PHOTO BY S. MEILING)

INTRODUCTION

STONY CORAL TISSUE LOSS DISEASE (SCTLD) is a highly lethal and potentially infectious disease that has devastated coral reefs throughout Florida and the wider Caribbean in recent years. First discovered in September 2014 off the coast of Miami, SCTLD spread rapidly along Florida's Coral Reef and starting in 2017, throughout the wider Caribbean. As of November 2021, SCTLD has been detected in 20 Caribbean territories and countries.[1] The disease affects more than 30 reef-building coral species in the Atlantic and Caribbean and has high rates of mortality.[2,3,4,5] Indeed, once a coral begins to lose living tissue, it will likely die within weeks to months without active intervention. Experts believe this may be the most lethal coral disease ever recorded.[3,6,7] While many different coral diseases have been documented across the globe, the damage wrought by SCTLD has truly been unprecedented. Some reefs in Florida and the US Virgin Islands have lost between 40-50% of their coral cover in just a handful of years and reefs throughout the Atlantic and Caribbean are losing biodiversity and reef function at a rapid pace.[3,8,9,10,11]

In a short amount of time, scientists have made great strides in understanding how SCTLD spreads, with experiments supporting transmission directly through contact with sick corals and indirectly through ocean currents and neutrally buoyant materials, such as resuspended sediment.[12,13,14,15] While these methods of transmission can explain the movement of the disease locally, these methods of transmission *cannot* explain how the disease has moved across greater distances, such as from Florida to Jamaica, where the disease was first detected in the Caribbean.[1] The prevailing theory is that the disease is being spread via some form of human activity, likely the movement of vessels via ballast water or biofilms.[16,17] If this is the case, the potential exists for the disease to move west from the Caribbean, through the Panama Canal, and into the Pacific. While SCLTD has not been detected in the Indo-Pacific, the possibility remains.

Despite promising research, the causative agent of SCTLD remains unknown. Thus, verifying the presence of this disease is based largely on visual confirmation in the field. Disease surveillance involves collecting data that is then analyzed for the purpose of action. It includes the gathering, recording, and analysis of data and the dissemination of this information to interested parties so that action can be taken to control disease. This differs from monitoring in that monitoring consists of periodic observations with no action taken beyond data collection. Proactive surveillance and response planning can help ensure that if SCTLD is ever transmitted to the Pacific, coral managers and practitioners will be able to detect it early and can launch a rapid, strategic response that may help save corals and prevent the disease from continuing to spread throughout the region.

"This document was developed in partnership by the U.S. Coral Disease Task Force Coral Disease Working Group, Florida Sea Grant, and NOAA's Coral Reef Conservation Program. It directly supports Goal 2 of the NOAA Strategy for SCTLD Response and Prevention "Build capacity for coral disease detection, prevention, and intervention," by promoting the implementation of surveillance protocols to provide early warning and track disease progression. This guide is intended to provide high-level guidance regarding where to focus surveillance, signs and symptoms of SCTLD, guidance for managers if SCTLD is observed, and tips and tricks for successful surveillance.

WHERE TO FOCUS SURVEILLANCE EFFORTS

RESEARCHERS suspect that SCTLD is being transmitted throughout the wider Caribbean region through shipping activity via ballast water or biofilms.[16,17] In some locations, SCTLD mortality and infection rates have corresponded with shipping traffic and major port locations.[18] Based on this theory, if SCTLD is transmitted to the Indo-Pacific, it will likely be through a vessel. Therefore, surveillance efforts to detect the first appearance of SCTLD should focus on areas of high shipping activity, including:

- Commercial ports
- Cruise ship terminals
- Anchorage areas and marinas
- Reefs that are connected to areas of significant shipping activity by oceanographic currents

Reefs with high coral cover should also be prioritized, as it will be more likely to detect SCTLD in areas of dense coral cover, regardless of depth or size of coral, if the disease is present.

Once SCTLD is established in an area, the focus of surveillance may shift to implementing effective intervention strategies. Coral reef managers should determine which reefs are a priority for SCTLD intervention, considering both **ecological** and **regulatory principles, likelihood of successful treatment,** and **existing management goals.**[19] Priority areas for surveillance may include:

- Iconic reefs with high coral coverage and diversity, particularly if colonies are of reproductive size
- Reefs that have a high number of critical species (i.e. foundation-building species or endangered species)
- Reefs that have high connectivity to other habitat types and support a range of human activities
- Reefs in priority managed and/or protected areas
- Reefs that are frequently visited or experience heavy human impacts

Regulatory Considerations

- **Within a marine protected area:** Protected areas may potentially reduce stressors caused by fishing pressure or other activities, and thus may respond more positively to treatment.
- **Within a recreational area:** Treating corals within a recreational area may increase project visibility and allow for some community involvement. However, there may be increased stressors from recreational activities and potential concerns about human safety during or after treatments

Treatment Considerations

- **Coral density:** While high density sites may allow for more corals to be located, treated, and monitored in a smaller amount of time, there will be a higher effort needed to survey every colony.
- **Size of site:** There is no ideal size site, but projects should consider potential visitation and treatment rate, availability of supplies, and ability to obtain permits when selecting a site.
- **Number of sites:** Projects should consider whether efforts will be highly focused at a limited number of sites to maximize treatment success or treat a larger number of sites with a potentially lower success rate.
- **Location of sites:** Projects may consider factors such as transportation time, accessibility, and general visibility at the site in selecting treatment sites.

SIGNS OF SCTLD

SURVEILLANCE EFFORTS should focus primarily on stony corals. Soft corals in the Atlantic and Caribbean have not been affected by SCTLD, yet about half of Caribbean stony, or scleractinian, species appear to have some degree of susceptibility. However, signs and symptoms can vary within and among species and the disease may present differently in the Indo-Pacific. Appendix I includes SCTLD cards from the Atlantic/Caribbean region to help depict the ways in which SCTLD may present differently among different species and morphologies. Therefore, this list should not be considered exhaustive but rather a starting point for surveillance efforts.

In the initial stage of the disease, signs of SCTLD may include:

- **Rapid tissue loss**
  - Areas of recently denuded skeleton will have a stark white appearance.
  - Signs of SCTLD are different from recent predation (Fig. 1B-C). Corals with SCTLD will have a stark white area of exposed skeleton where tissue has recently died but the skeleton is fully intact (Fig. 2). Lesions may have some sloughing tissue present in the disease margin (Fig. 1D). Behind the stark white leading edge of the disease, the coral skeleton may be covered with turf algae (Fig. 1E). Predation may also have a stark white appearance, however, it can appear less uniform and have some tissue still present.
  - SCTLD is also different from bleaching (Fig. 1A). Corals that have recently bleached may have a stark white appearance, but their polyps are still visible. With SCTLD there will generally be no live tissue (i.e. polyps) remaining in the area of denuded skeleton. However, bleaching margins can be accompanied by SCTLD in certain species, particularly in the later stages of site infections (Fig.9C).

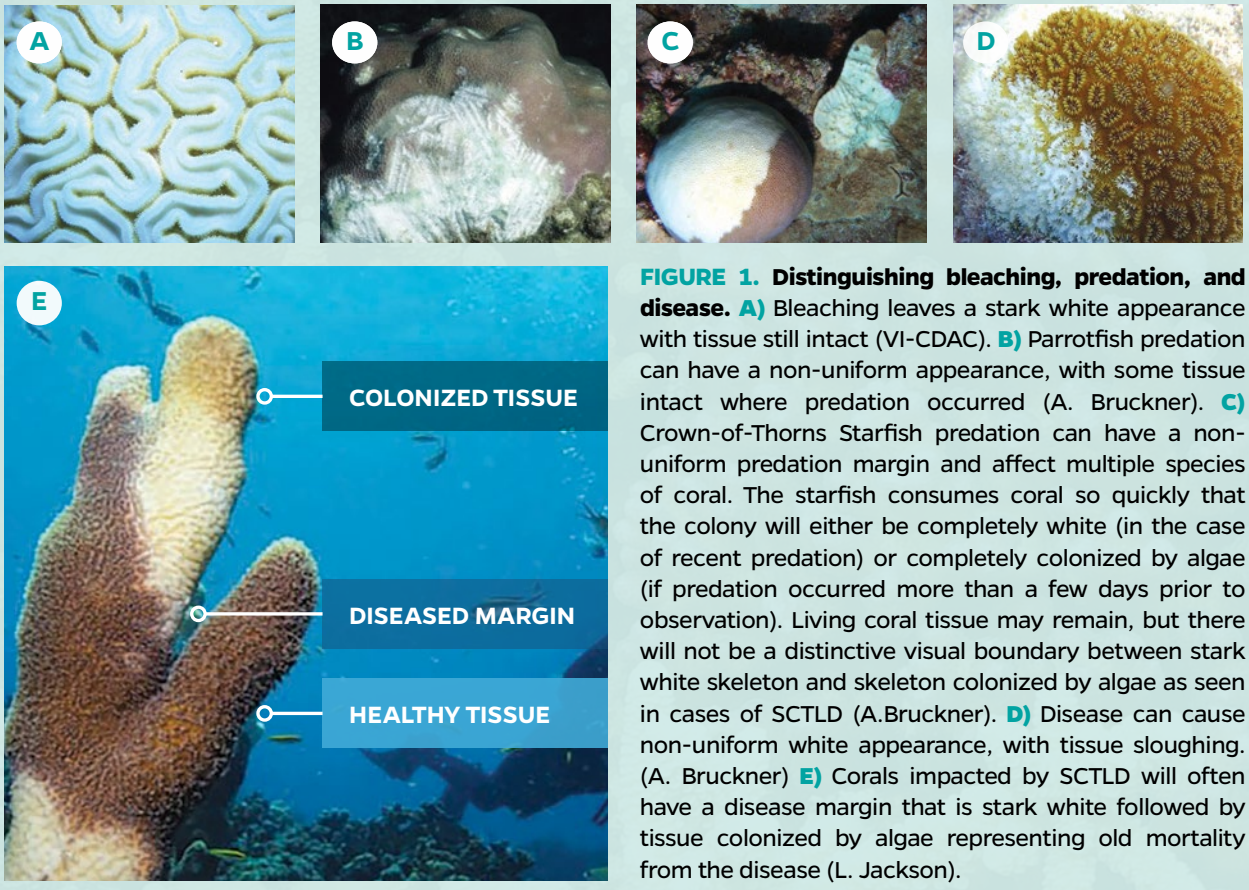

FIGURE 1. Distinguishing bleaching, predation, and disease. A) Bleaching leaves a stark white appearance with tissue still intact (VI-CDAC). **B)** Parrotfish predation can have a non-uniform appearance, with some tissue intact where predation occurred (A. Bruckner). **C)** Crown-of-Thorns Starfish predation can have a non-uniform predation margin and affect multiple species of coral. The starfish consumes coral so quickly that the colony will either be completely white (in the case of recent predation) or completely colonized by algae (if predation occurred more than a few days prior to observation). Living coral tissue may remain, but there will not be a distinctive visual boundary between stark white skeleton and skeleton colonized by algae as seen in cases of SCTLD (A.Bruckner). **D)** Disease can cause non-uniform white appearance, with tissue sloughing. (A. Bruckner) **E)** Corals impacted by SCTLD will often have a disease margin that is stark white followed by tissue colonized by algae representing old mortality from the disease (L. Jackson).

Lesion formation

- Lesions can appear anywhere on the colony but often initiate at margins. Colonies may experience a singular lesion or multifocal lesions that radiate into one large lesion, though this can vary (Fig 2).

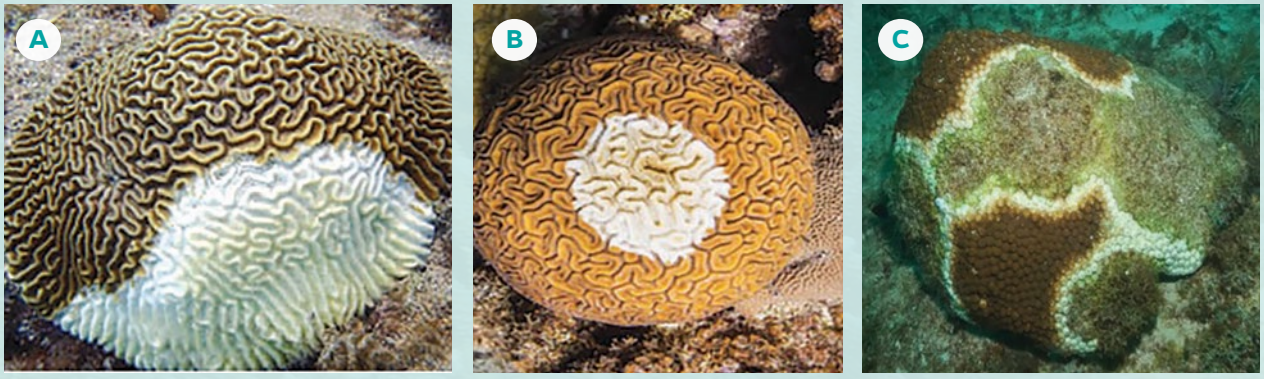

FIGURE 2. SCTLD Lesion Morphology. **A)** SCTLD can have linear (A. Bruckner), **B)** focal (L. Jackson), **C)** multifocal, or diffuse disease margins (FL DEP).

Multiple coral colonies showing signs of SCTLD lesions (Fig. 3)

- SCTLD has a high prevalence on reefs, with 66-100% of corals showing signs of the disease and rapid mortality.[20]
- Species that prove highly susceptible to the disease will likely experience rapid total mortality, dying within 1-4 weeks.

FIGURE 3. SCTLD Prevalence. During the initial stage of disease outbreak, highly susceptible species exhibit significant mortality while intermediate species begin to display signs of SCTLD (K. Neely).

- **Rapid change**
  - While SCTLD initially appears similar to other coral diseases, it progresses much faster. Corals showing tissue loss lesions should be tagged and revisited within days to one week. If the disease margin has spread rapidly (> 1 cm), it may be SCTLD (Fig. 4).

FIGURE 4. SCTLD Progression. SCTLD can cause total mortality in as little as one month (S. Meiling, University of the Virgin Islands).

POTENTIALLY SUSCEPTIBLE CORALS

CORALS in the Caribbean region show various degrees of susceptibility to SCTLD. Some taxonomic groups are seemingly immune, some take time to show signs of infection, and some are early harbingers of SCTLD, being the first to show symptoms. Even within taxonomic groups, some species and even individual colonies show different ranges of susceptibility. One active line of research indicates that it may be the species of zooxanthellae rather than the coral hosts themselves that represent different levels of susceptibility. If this is the case, the impacts on Pacific corals may differ substantially from those in the Caribbean. Whether because of host or symbiont susceptibility, taxonomic groupings of corals within the Caribbean are discussed here as a potential guide for the appearance of SCTLD in the Pacific.

While more research is needed to determine the potential susceptibility of Indo-Pacific corals, we may be able to glean some useful parallels by looking at corals that are susceptible in the Atlantic and Caribbean. *Meandrinidae* is a highly susceptible family of coral species found in the Caribbean but not present in the Indo-Pacific. While several Caribbean species are highly susceptible in the *Faviidae* family, the single genus found in the Indo-Pacific (*Favia*) is presumed to have low susceptibility in the Caribbean. However, there are coral families that are intermediately susceptible in the Caribbean that are present in the Indo-Pacific: *Merulinidae, Agariciidae, Astrocoeniidae,* and *Siderastreidae* (Fig. 5-8). Below are photos showing the way SCTLD affects different families of corals in the Atlantic and Caribbean, which may prove useful to practitioners looking to identify SCTLD in the Pacific.

FIGURE 5. SCTLD in Family *Merulinidae*. **A)** Healthy (E. Weil), **B)** diseased (FWC-CREMP), and **C)** diseased with bleaching margin (K. Neely). Note: bleaching reflects a reduction in zooxanthellae in response to a stressor. While it is typically associated with thermal extremes, bleaching can also be present due to stress from the presence of a disease.

FIGURE 6. SCTLD in Family *Agariciidae*. **A)** Healthy (M. Vermeij), **B)** diseased (FWC-CREMP), and **C)** diseased with mottled margins (FWC-CREMP).

FIGURE 7. SCTLD in Family *Astrocoeniidae*. **A)** Healthy (M. Vermeij), **B)** diseased (S. Schopmeyer), and **C)** diseased with dark spots (S. Schopmeyer).

FIGURE 8. SCTLD in Family *Siderastreidae*. **A)** Healthy (A. Bruckner), **B)** diseased (K. Neely), and **C)** diseased with pale margins (J. Bartoszek).

STEPS TO TAKE IF SCTLD IS SUSPECTED

IF YOU SEE extensive tissue loss on multiple colonies that is fast moving with high rates of mortality, act immediately to determine whether it may be SCTLD (Fig. 9):

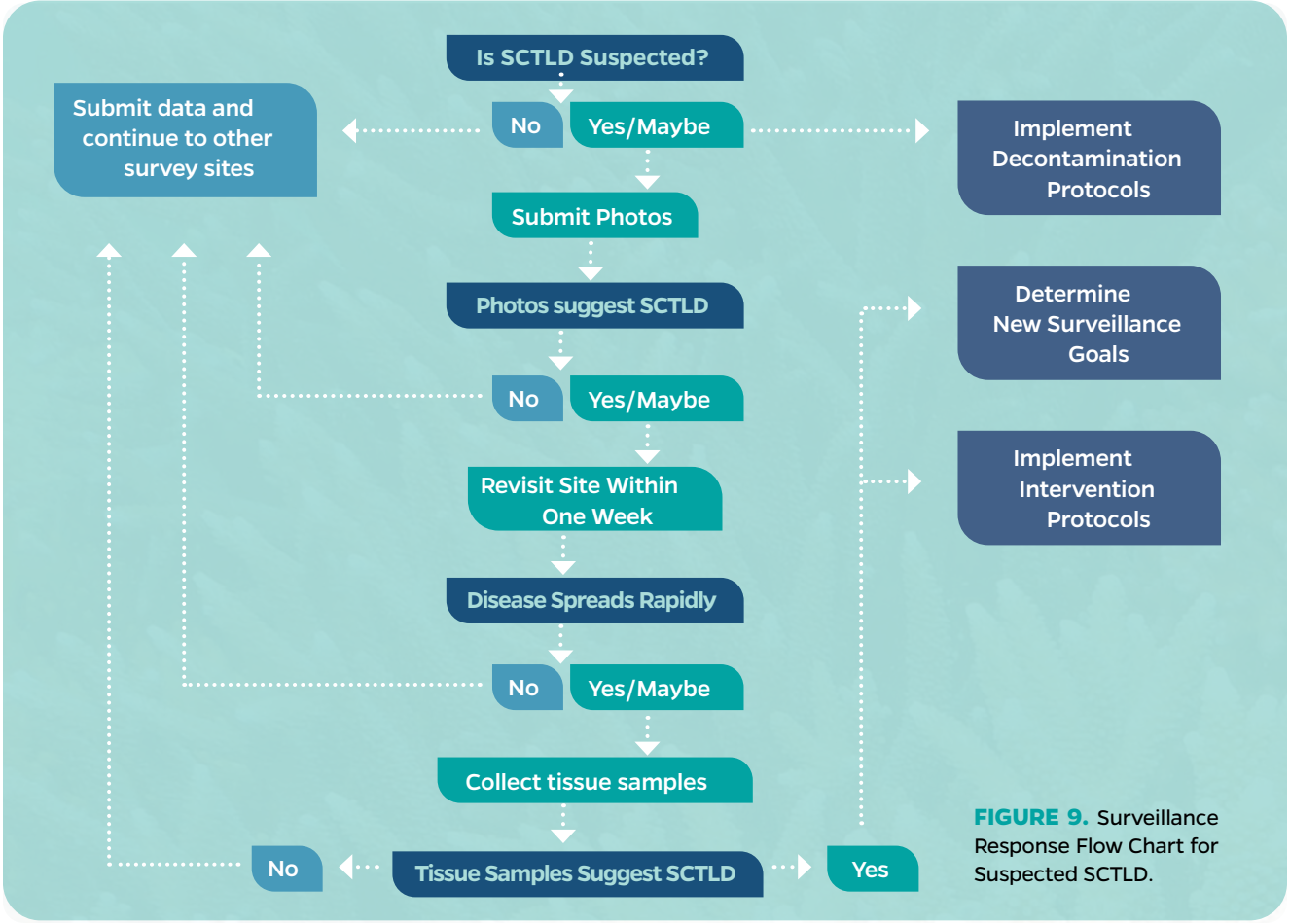

FIGURE 9. Surveillance Response Flow Chart for Suspected SCTLD.

1 **Take photos and submit them, along with dive site information, to your jurisdiction's point of contact for photo collection and review. Implement decontamination protocols.**

- Multiple in-focus and white-balanced photographs should be taken for each submission, consisting of a landscape view of the reef (including the coral of concern along with other colonies), a photo of the whole colony of concern, and a close-up photo of the lesion border (Fig. 10).
- Implement decontamination protocols for dive gear and sampling equipment. Work from nondiseased areas towards diseased areas decontaminating equipment between each site.

FIGURE 10. Photographs for reporting SCTLD. A) A landscape view can show if other corals show signs of disease. **B)** A whole colony view can show the extent of the disease. **C)** A close up of the lesion border can help determine if a coral has SCTLD (J. Townsend/VI-CDAC).

2 If photos are reviewed and SCTLD is suspected:

- Review surveillance and monitoring procedures, activate local response teams, and designate a central repository for data management.
- Revisit diseased corals days to one week after initial sighting to determine if it is SCTLD or a different white disease/syndrome based on rapid spread of the disease margin (>1 cm).

3 If corals are revisited and SCTLD is suspected:

- Collect tissue samples for disease verification and etiology assessment, following tissue sampling protocols established by your jurisdiction (Appendix V).[21] Protocols for collecting tissue samples should consider permitting, supply availability, shipping restrictions, and collaborators for sample analyses.
- Once samples have been collected, slides should be prepared for histopathology and reviewed by an expert coral epidemiologist

4 If SCTLD is suspected due to surveillance and tissue sampling:

- Determine goals for future surveillance. Goals may include determining the extent of the outbreak, understanding mortality rates, or predicting future patterns of disease spread. Once goals are established, implement surveillance strategies, which may include:
  - Monitor nearby areas based on major current patterns, as SCTLD is waterborne and will likely spread the fastest along with currents.
  - Monitor diseased corals bi-monthly to track the fate of infected colonies and rate of transmission.
  - Establish the spatial extent of the outbreak via belt transects (if in the initial stage) or by roving diver surveys, manta tows, or underwater scooters to cover a larger area if SCTLD is already widespread.

TIPS AND TRICKS FOR SUCCESSFUL SURVEILLANCE

- **Develop species and disease signs field guides** that are in full color and laminated that include photos of different presentations of SCTLD on Caribbean corals. The guides should also show the difference between tissue loss disease, predation, and bleaching.
- **Develop standardized surveillance and reconnaissance monitoring procedures** (i.e. tagging procedures) and reporting methods (i.e. data sheets; Appendix II).
- **Develop gear and equipment disinfection protocols** for monitoring an impacted area (Appendix III).
- **Develop a reliable network of partners** (managers, biologists, citizen scientists, dive shops, fishers, etc.) who have local knowledge of reefs and may be able to contribute to surveillance efforts.
- **Establish a system of collecting and housing data and photos** generated from community based monitoring (i.e AGGRA, USVI-CDAC, BleachWatch or SeaFan) and identify designated experts to review data and photos (Appendix IV).
- **Identify a strategy for tissue sampling.** Incorporate strategies for tissue sampling into existing response plan frameworks as appropriate. Strategies should consider:
  - Permitting processes for tissue sampling, which should occur before an SCTLD outbreak is suspected. Permits may take 30-90 days or more to acquire and this delay could impede early intervention efforts and result in significant coral mortality and rapid spread of the disease.
  - Funding for surveillance, sampling, and shipping. Consider obtaining sampling materials in advance to avoid shipping delays.

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APPENDICES

DISEASE IDENTIFICATION CARDS

(Florida Department of Environmental Protection, Florida Fish and Wildlife Conservation Commission, National Oceanic and Atmospheric Administration, National Park Service)

These identification cards depict Atlantic and Caribbean corals affected by SCTLD. While we are aware these species are not found in the Pacific, at this time not much is known about how SCTLD may affect Pacific corals. Therefore, we thought it would be helpful to include images showing the many different ways in which SCTLD presents on different species and different morphologies

Symmetrical Brain Coral *Pseudodiploria strigosa* **PSTR**

Knobby Brain Coral *Pseudodiploria clivosa* **PCLI**

Grooved Brain Coral *Diploria labyrinthiformis* **DLAB**

Boulder Brain Coral *Colpophyllia natans* **CNAT**

Maze Coral *Meandrina meandrites* **MMEM**

Florida Department of Environmental Protection, Florida Fish and Wildlife Conservation Commission, National Oceanic and Atmospheric Administration, National Park Service

DISEASE IDENTIFICATION CARDS

(Florida Department of Environmental Protection, Florida Fish and Wildlife Conservation Commission, National Oceanic and Atmospheric Administration, National Park Service)

Andrew Bruckner, FKNMS

Florida Department of Environmental Protection, Florida Fish and Wildlife Conservation Commission, National Oceanic and Atmospheric Administration, National Park Service

DISEASE IDENTIFICATION CARDS

(Florida Department of Environmental Protection, Florida Fish and Wildlife Conservation Commission, National Oceanic and Atmospheric Administration, National Park Service)

Ellipitical Star Coral *Dichocoenia stokesii* **DSTO**

Lobed Star Coral *Orbicella annularis* **OANN**

Mountainous Star Coral *Orbicella faveolata* **OFAV**

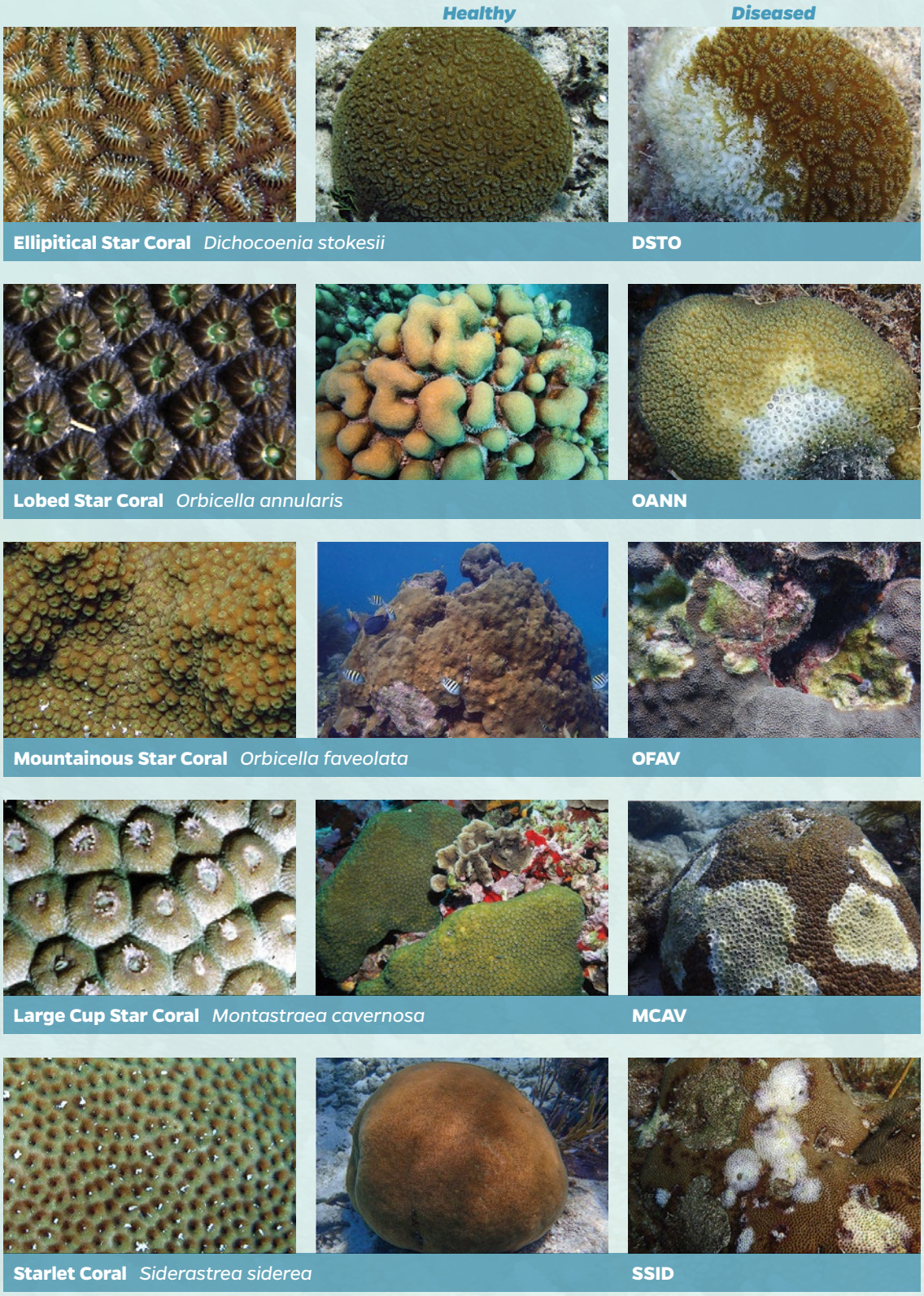
Large Cup Star Coral *Montastraea cavernosa* **MCAV**

Starlet Coral *Siderastrea siderea* **SSID**

Florida Department of Environmental Protection, Florida Fish and Wildlife Conservation Commission, National Oceanic and Atmospheric Administration, National Park Service

DISEASE IDENTIFICATION CARDS

(Florida Department of Environmental Protection, Florida Fish and Wildlife Conservation Commission, National Oceanic and Atmospheric Administration, National Park Service)



Florida Department of Environmental Protection, Florida Fish and Wildlife Conservation Commission, National Oceanic and Atmospheric Administration, National Park Service

SAMPLE DATASHEET

(Atlantic and Gulf Rapid Reef Assessment)

AGRRA SCTLD-Bleaching Survey Datasheet

Surveyor Name:	Date: Time:	Latitude: Longitude: (or Location):	Reef Name *(if known)*:
Detailed Surveys: AGRRA Site Code if any:	*Detailed Surveys:* MPA Status: Yes? No? Unsure?	*Detailed Surveys:* If a Restoration Site: Outplant? Nursery?	Reef Type: Backreef? Reef Crest? Patch Reef? Fore Reef? Other *(Describe)*:
Average Depth: m? or ft?	Bottom Temp.: °C or °F?	Site Comments *(e.g.*, major organisms):	

Tally all corals (including clumps) of species known to be susceptible to SCTLD.

Species	# Healthy Corals	# SCTLD Corals	# Corals with SCTLD &/or Fully Bleached (BL), Partially Bleached (PB), or Pale (P)			# Corals Fully Bleached (BL), Partially Bleached (PB), or Pale (P)			# Corals with other Diseases(s)	# Recently Fully Dead Corals	Notes; any Photos?
			BL	PB	P	BL	PB	P			
OFTEN SEEN *Colpophyllia natans:* CNAT (Boulder Brain)***											
Dendrogyra cylindrus: DCYL (Pillar)***											
Dichocoenia stokesii: DSTO (Elliptical Star)***											
Diploria labyrinthiformis: DLAB (Grooved Brain)***											
Eusmilia fastigiata: EFAS (Smooth Flower)***											
Meandrina jacksoni: MJAC (White-valley Maze)***											
Meandrina meandrites: MMEA (Maze)***											
Montastraea cavernosa: MCAV (Great Star)**											
Orbicella annularis: OANN (Lobed Star)**											
Orbicella faveolata: OFAV (Mountainous Star)**											
Orbicella franksi: OFRA (Boulder Star)**											
Pseudodiploria clivosa: PCLI (Knobby Brain)***											
Pseudodiploria strigosa: PSTR (Symmetrical Brain)***											
Siderastrea siderea: SSID (Massive Starlet)**											
Stephanocoenia intersepta: SINT (Blushing Star)**											
SEEN LESS OFTEN *Agaricia agaricites:* AAGA (Lettuce)*											
Agaricia lamarcki: ALAM (Whitestar Sheet)											
Agaricia tenuifolia: ATEN (Thin Leaf Lettuce)											
Space for other species, e.g., *Porites astreoides?:* PAST (Mustard Hill)?											

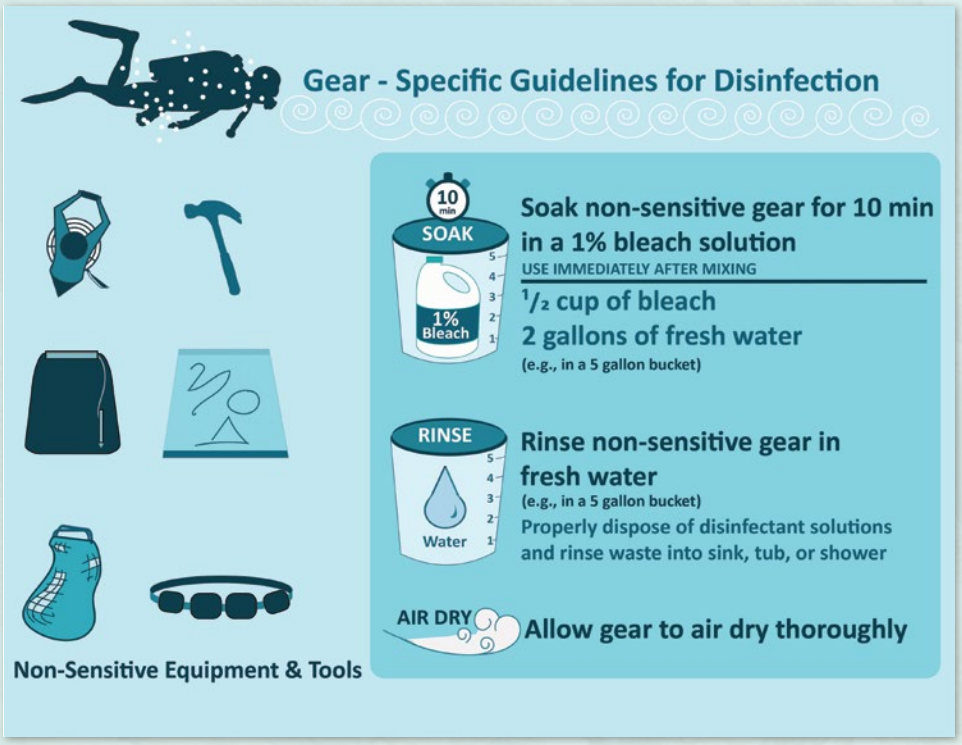
Describe the survey protocol used:	*Detailed Surveys:* What % of all corals at the site have each kind of partial mortality? New? Trans? Old?	*Detailed Surveys:* How were the data collected? Qualitatively? Quantitatively?	Other Comments

* numbers represent **usual** order of contracting SCTLD, from first (***) to last (*), if known.

Revision 2021-02-16

SAMPLE GEAR DECONTAMINATION PROTOCOLS

(Florida Keys National Marine Sanctuary)



(CONTINUED ON NEXT PAGE)

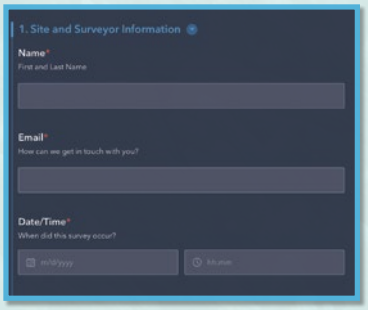
SAMPLE GEAR DECONTAMINATION PROTOCOLS

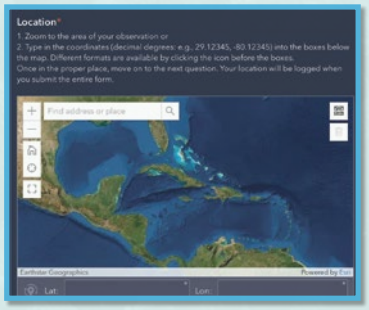
(Florida Keys National Marine Sanctuary)

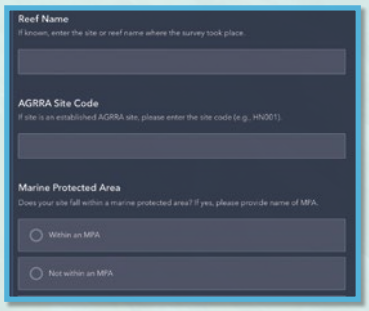
REPORTING DATABASE

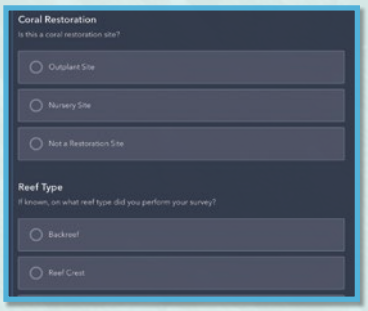
(Atlantic and Gulf Rapid Reef Assessment)

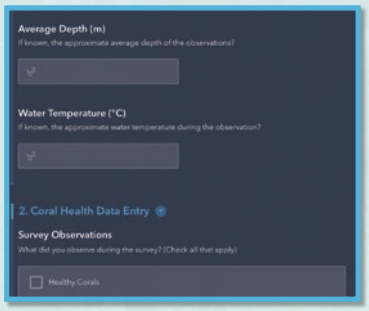
DETAILED SURVEY FORM

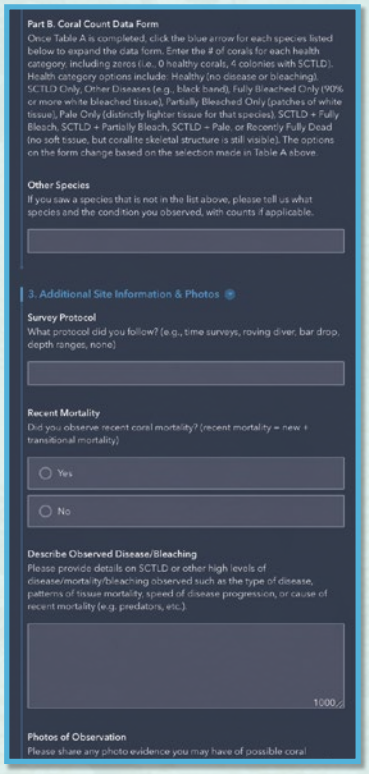












NOAA CORAL TISSUE SAMPLING PROTOCOL

(NOAA National Centers for Coastal Ocean Science/Coral Reef Conservation Program)[1]

Please note that this is only one example of tissue sampling and analyses for light microscopy. Protocols may vary by jurisdiction and by type of analyses being conducted. Please also note that a minimum core size of 2.2 cm is required for SCTLD histopathology.

TISSUE SAMPLE PROTOCOL (page 1 of 2)

Labeling Scheme Guideline

- Letter or number designation of the collection site
- Four letter abbreviation for coral species (first letter of genus, first three of species)
- Colony number within site
- Two letter sample type abbreviation

Colony Type	Analyses/Collection Method		Example
Reference	Water	Protein	R-P
Healthy	Sediment	Fixative	H-F
Unaffected	Mucus	Bacteria	U-M
Diseased	Applicator (Swab)		D-S

Colony Type Analyses/Collection Method Example

Reference Water Protein R-P Healthy Sediment Fixative H-F Unaffected Mucus Bacteria U-M Diseased Applicator (Swab) D-S

ex. *Reference Site A= A.Dstr.1.R-P Diseased Site B= B.Apal.4.D-F and B.Apal.2.U-M*

DEFINITIONS

- "Reference" - uninfected or 'healthy-looking' colonies from areas where no corals exhibit signs of the disease
- "Healthy" - apparently healthy corals in affected sites
- "Unaffected" - areas of diseased colonies with normal appearance, distant from the lesion
- "Diseased" - margin of the lesion

Due to time sensitivity of some samples, such as the tissue for protein analyses, sampling should adhere to a specific order. Within each site, samples should include:

- Water
- Sediment
- Applicator/Swab
- Syringe/Mucus
- Core or Clipped Tissue Samples for each analysis planned

Sediment

- Scoop sediment with sterile pre-labeled 15mL conical or similar container.

This type of sample is used solely for microbiological sample analyses as a reference for microbes situated in the sediments that may be mobilized from disturbances such as storms.

DNA Swab

- Wipe across the area to be sampled three times.

This is currently experimental and may provide less invasive sampling. The swab samples are limited to DNA analyses of surface tissue and mucus.

Water

- Collect one reference volume for each colony
- Should be equal in volume to mucus sample
- Collect in a 3cc or larger syringe

This sample is used as a reference for microbiological analyses to allow analyst to account for possible water contamination of mucus and tissue samples as well as a comparison for microbes that may be found in surrounding waters, but not primary colonizers of corals.

Mucus

A sterile syringe without the needle is used to aspirate (draw in) mucus from the surface of the coral. For diseased samples, mucus is collected along the disease margins and unaffected samples across the surface of unaffected areas. If swab samples are collected, this should be done first which should provide the irritation required to obtain mucus. It is important to collect mucus already present on the colony. The diver should avoid initially irritating the colony, as mucus subsequently released by the coral will have a depauperate microbial flora community.

Mucus samples have been one of the primary types of specimens used in culture dependent and independent microbiological analyses. It seems to provide consistency across temporal and spatial sampling for microbial diversity studies. Recent work however has shown different microbial profiles are obtained depending on whether liveground tissue or mucus is being analyzed. It appears that these two micro-environments contain different microbial communities, with tissue samples having a more diverse and robust community than mucus.

Tissue Biopsy

FRAGMENT/TISSUE

- Coring technique- 1- 2.2cm diameter uniform disk samples of tissue + skeleton for larger colonies, using two punch sizes. *clay should be inserted after coring to minimize further damage (Roma Plastalina, no 2-from Rex Art, Miami, FL)
- Clippers/Pliers/Garden Shears- can be used for clipping from branching specimens

Tissue samples are collected for a variety of clinical analyses. Currently available analyses include histology/histopathology, microbiology, cellular diagnostics (primarily protein chemistry based and includes a suite of various biochemical and cell-based parameters that can be measured for building a diagnosis) and genetic or functional genomic assays.

NOAA CORAL TISSUE SAMPLING PROTOCOL

(NOAA National Centers for Coastal Ocean Science/Coral Reef Conservation Program)[21]

TISSUE SAMPLE PROTOCOL (page 2 of 2)

Sample Processing for Biological Analyses

Each sample has a predetermined experimental or analytical role, which determines how each will be processed on the boat and back on land. The Sample Technician of the Support Team will do most processing.

SUPPORT TEAM

This team will consist of at least 2 members who will provide topside and field-lab support. The primary job of the Sample Technician is to ensure the proper handling, documentation and stabilization of each sample collected. The Logistics Chief is responsible for all dive gear and collection equipment and assists the Sample Technician.

PROCESS TIME SENSITIVE SAMPLES FIRST

- **Tissue for Protein (H-P, U-P, D-P)** samples should come to the surface in dark bags or covered (e.g., glove) to protect them from light for light sensitive assays. They are time sensitive and need to be processed in a dark or shaded area. Mucus should be rinsed by swishing in seawater, dabbing on Bounty™ paper towel (or lint-free paper towel), and placing in a new, prelabeled Whirlpak™. Since Whirlpak™ bags are prone to shattering at liquid nitrogen vapor temperatures, the bags are wrapped in aluminum foil with an identifying label on the outside and placed immediately into a dry shipper. Do not write on aluminum foil as it is not permanent, use labeling tape or cryotags and waterproof marker. The time interval between collection and freezing should be approximately 15 minutes, longer than this will exclude certain cellular diagnostic assays due to creating artifact by changes in the sample.
- **Tissue for Histology** - The tissue biopsies collected from Healthy, Unaffected portion of diseased colony and the Disease margin (H-F, U-F, D-F) are placed in bags or tubes underwater and on reaching the boat, if transport of fixative is logistically sound, the samples are immediately placed in a 50cc polypropylene tube containing approximately 25 mL of an appropriate fixative for a 2cm punch biopsy or an approximately 2-3 cm branch (if larger, the fixative volume should be increased in proportion). When fixative transport is precluded, histological samples should be stored in bags or a container containing seawater and securely stored to minimize stress until a destination for fixation is reached. We routinely use Z-fix (Anatech Ltd.) diluted 1:4 in sterile artificial sea water (ASW; 35ppt) and held at ~25°C, because of the ability to retrieve intact DNA from the samples for subsequent molecular and immuno-staining. The ratio of tissue to fixative should be at minimum 1:10 (1:20, preferred). DO NOT FREEZE THESE SAMPLES.
  - Alternatively seawater-buffered formalin can be used for fixation of corals for light microscopy and formalin is generally available at marine labs, hospitals and veterinary clinics. This is prepared by filtering either natural or artificial seawater and diluting formalin stock (37.5% formaldehyde) 1 part formalin to 9 parts filtered seawater. The samples are fixed from 4 hrs to overnight then rinsed in tapwater and stored in 70% ethanol or alternatively can be stored in the 3.75% formalin-seawater.
  - For shipping Kim-wipes™ or other lint-free paper is saturated with the preservative (e.g., 70% ethanol) and stuffed into the tube. This stabilizes the samples and keeps them moist, while avoiding shipping tubes filled with a hazardous material.
- **Tissue for Microbiology (H-B, U-B, D-B)** should be kept in a Whirlpak™ with sterile 35 ppt artificial sea water added if needed, keep at ambient temperature in a cooler with local seawater. Upon return to shore, homogenize tissue and skeleton with sterile mortar and pestle (with its own mucus), flash freeze half of homogenate, and culture bacteria on marine agar or other desired media, with other half of homogenate.
- **Swabs or Applicators (H-A, U-A, D-A),** if they are Whatman FTA™ type swabs, should be wiped on the card, and then the tip should be broken off and stored in a 15 cc tube or cryovial. Other types of swabs (Epicenter, Madison WI) simply need to be broken off and the tip stored. Cards can be stored in a reclosable food storage bag (e.g., ziploc™) and shipped at ambient temperature; swab tips should go in the cryovial. **Alternative storage to freezing is being investigated using sodium chloride saturated dimethylsulfoxide (DMSO).
- **Mucus samples** which were collected in a syringe without needle need to be split: Half should be placed in a cryogenic vial and immediately flash frozen in a liquid nitrogen dry shipper for molecular analyses. The other half should be kept in screw top vials at ambient seawater temperature and cultured on marine agar media as soon as possible for microbiology.
- **Surface sediment (H-S, D-S)** - loosen cap and attempt to remove as much water as possible. Leave about a 2 cm gap between sample and cap, cap tightly and freeze in dry shipper. Water (H-W, D-W) should be split into two samples. Half can be transferred from the syringe to a 2.0 mL cryogenic vial and placed in the dry shipper. The other half should also go in a 2.0 mL vial, but be kept at ambient temperature for culture dependent methods.

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