

SCTLD: Re-sampling an established site



Goals:

1. Re-photograph tagged coral colonies

- A picture is worth a thousand words! See the gross changes and assess the health of an individual colony over time.
- Provides a second record and ability to assess healthy status independent from written datasheets.



CBC30N PSTR 6 at three time points

2. Re-sample tagged coral colonies

- Assess molecular changes in one individual over time and connect those changes to its visible health. The samples we take can be used to look at all parts of the holobiont (the coral animal, photosymbionts and microbiome).

3. Download EnvLogger

- Gives us temperature data over time that can be linked to bleaching and disease.

4. Conduct coral survey

- Documents scleractinian community composition over the same 30 x 1m transect.
- Gives us data on overall reef health and a picture of what is happening on a community wide level. Is there bleaching, paling, or a disease spreading? More predation?
- We survey every time we sample. These demographic and condition data allow us to document the reef at the moment of sampling.
- Take photo quadrats. One photo every meter for first 30m of the transect. Allows for analysis of benthic composition including non target species capturing a broader picture of phenomena like bleaching, storm damage, disease, and phase shifts. Allows us to measure changes in percent cover.

Mock Schedule for SCTLD Re-sampling Site

Dive 1: 60-90 mins

- lay transect
- retrieve ENVlogger
- relocate, photograph and sample tagged colonies*
- photograph datasheets underwater

Surface: 20-30 mins (or for determined surface interval depending on site depth)

- process coral samples ASAP
- download ENVlogger
- photograph datasheet with tube labels
- water+snack

*if not finished sampling you will finish sampling then surface and process again before continuing

Dive 2: 45-90 mins

- redeploy ENVlogger
- coral survey
- photograph datasheets underwater
- photoquadrats
- roll up transect/ clean up site



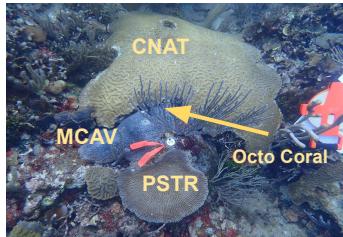
Goal 1: Re-Photographing Tagged Coral Colonies Protocol

*Refer to [SCTLD Packing List: Established Site](#) for materials checklist

Finding and photographing tagged colonies

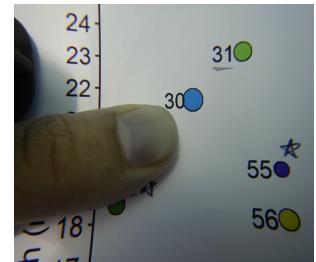


Need a white balance refresher?
Here it is!



PAN_BDT_T4_PSTR_20_102023

- **White balance camera** against slate or sand as soon as you are at the bottom
- Use map and photo books to relocate a tagged colony.
 - The transect + landmarks in photos can help
 - ex: PSTR to the left has a water droplet shape and a big purple octocoral coming out of it. Also in a fairly distinct grouping of corals!
 - Photobooks typically have one more zoomed out photo and one close up photo of each colony



PSTR 12 at CBC30N photographed in August 2024. Even though the number of the tag is not visible, it is marked in the photos with flagging tape. The photographer took a photo of the photobook to let us know what colony was being photographed. All angles of the coral are captured and there is a nice wide angle shot that orients us next to the transect and a sand patch. We can also see where the coral was sampled in the thirds photo from the right.



What if the tagged colony is dead?

Still take photos and fill out the first three columns in the Molecular Sampling Datasheet "Tag", "Species", "Health Status". You just won't take any samples! This helps us keep track of if/ when corals die.



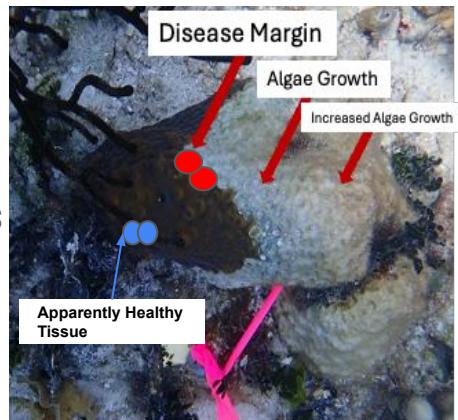
Need help with assessing coral health?

We got you. [Protocol for Assessing Health Status](#)

Goal 2: Re-Sampling Tagged Coral Colonies Protocol

Resampling the colony

- You will use the Coral Molecular Sampling Sheet to document this process.
- Write down the Tag # and Species
- Then note if the colony is Healthy, Diseased, Other Disease (Disease other than SCTLD), Dead, CLP= Color Loss Paling, CLB=Color Loss Bleaching or DC=Discoloration
 - for help or more details read the [Assessing Health Status](#) protocol
 - If a colony has more than one condition document each and the %
 - ex: CLP, CLB, 5%, 15%
- To sample the coral - take two $\frac{1}{4}$ " leather punch samples from the side of the colony (slightly overlapping so there is only one scar)
 - taking a sample from the side allows the coral to heal more rapidly because the scar doesn't fill with sediment
- Place both samples from an individual coral in the same compartment of a numbered pill box and make note of the compartment # on the datasheet
- Note the time on the datasheet in 24hr format - ex: 3:00pm = 15:00
- If a colony is diseased you will take **two samples from the apparently healthy (●)** tissue and **two from the disease margin (●)**. Make note of sample type/ location in the "notes" section on the Molecular Sampling Datasheet
 - **USE A DIFFERENT PUNCH AND PILL BOX FOR DISEASE SAMPLES**
 - We also use this same methodology for corals which have
 - Other Disease
 - Partial CLP/CLB (ie colony has < 100% CL)
- Regardless of sample type, wipe the leather punch on your wetsuit or in the sand between samples



Need help with assessing coral health?

We got you.

[Protocol for Assessing Health Status](#)



See something? Heavy predation by snails?
Damsel farming? Knocked over the colony
while sampling?
Put it in the "Notes" column for that tagged
colony



What if there is almost no tissue left?



BEL_CBC_T1_SSID_11_122022

Use your judgement, if you decide there is not enough to sample make a note on the Molecular Sampling Sheet about what's going on and take photos



This coral doesn't look like it has SCTLD but it looks terrible
What do I write? Where do I sample?

Other Disease, DC 10%

Sample 1&2: Apparently Healthy tissue

Sample 3&4: Disease margin

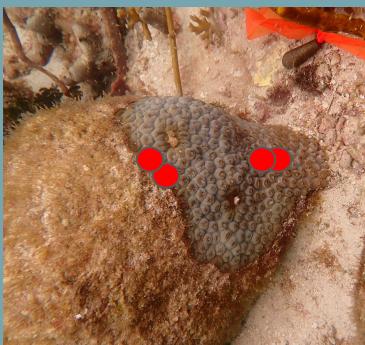
*all these samples go into Disease pillbox

Other Disease, DC 75%

Sample 1&2: Disease sample from the edge of a slough area. In notes say make observations where sample was taken ex: "sampled sloughy edge with dark webbing"

- if no apparently Healthy tissue - no need to sample for that

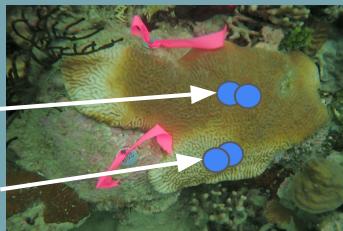
*these samples go into Disease pillbox



What if the colony is partially or entirely bleached?

CLP, CLB, 5%, 35%

Sample 1&2: Apparently Healthy tissue



Sample 3&4: CLP/CLB margin

*all these samples into H pillbox

PAN_BDT_T6_PSTR_32_102023

BEL_CBC_T3_MCAV_2_062024

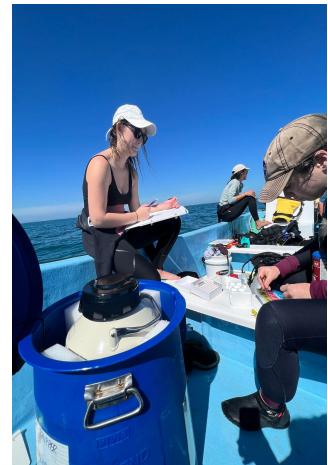
BEL_CBC_T1_SSID_17_122022

Sample Processing Protocol

*Refer to [SCTLD Packing List: Established Site](#) for materials checklist

If less than 20 minutes from station: can return to station to process if you like

- Always store coral samples (the entire pill box) in a shaded bucket of sea water
 - (especially if transporting to station)
 - **KEEP DISEASE AND HEALTHY SAMPLES IN SEPARATE BUCKETS**
- The most important things about sample processing:
 - **Sterilization - AN ABSOLUTE MUST**
 - **Accurate labeling / keeping track of samples**



- Once in the lab/ or processing on the boat you will need a 50 ml tube of alcohol and a propane blow torch. You will also need cryovials pre-labelled for the site.
- Best to work in a team of two- one person processing and one person writing the tube labels on the "Molecular Sampling Sheet"
- Sterilize forceps by dipping in alcohol then flaming. Wait a moment for the tweezers to cool. Remove sample from labeled pill box with forceps and place in pre-labeled cryovial . Repeat sterilization between each pill box compartment/individual coral.
- ** if using Dry Shipper : Wrap each dry tube with tinfoil and put into dry shipper.
- RNALater samples must be stored at room temperature overnight and may sit out unfrozen for up to one week, after which they must be frozen at -20C.



Goal 3: Downloading EnvLogger

- This works best with a phone with data, but not necessary.
- Get logger on 1st sampling dive. Always located at the beginning of the transect on the site marker.
- Wipe any CCA/ fouling off
- Open EnvLogger App
- Hold logger against the top left corner of an Iphone or middle of an Android
- Once the logger had registered, hold the logger in the sample place and press "Download Data". Hold still until top blue bar is full and graph of temperature data is showing on screen
- Send file in text message to Leah, Sarah and Felicia OR Save file on phone and send once you are back on wifi
- Redeploy on second/ survey dive



OH NO... blow torch isn't working?

Look for back up flames! There should be at least two flame sources on the boat at all times. **Always keep an extra lighter in a dry bag!** Worse comes to worse, dip the tweezers/ forceps in 10% bleach and then rinse in DI H2O. Have 2 falcon tubes of each solution as backups.



Pro tip: keep 2 x 50mL falcon tubes of EtOH/alcohol because they spill!



Mr. Shipper Dry

Need to top him up?

Do not fill more than $\frac{1}{3}$ of the way up the canes.



Need to dump out the extra liquid before flying?

Take the full/ partially full canes out and then pull the inner container out of the blue shipping container and invert the inner container, dumping excess liquid onto the ground. ** watch your toes**

Worried about space?

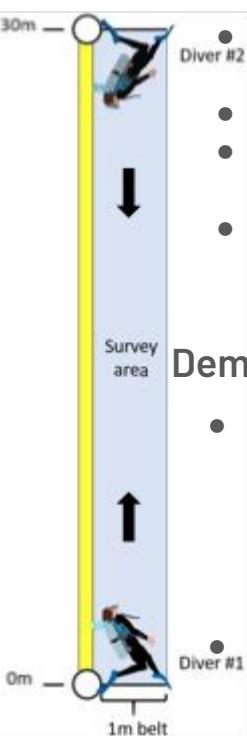
Use some canes just for cryovials. Make sure there is not a lot of air in whirlpacks and use forceps or another tool to jam things down to the bottom of the canes. If there is still not enough space can remove canes and toss aluminum foil bundles into the abyss or use pantyhose, just make sure you don't fill them too much and can still pull through the opening . can put some tubes in, then tie a section off and continue filling. Repeat.

Goal 4: Coral Survey Protocol

*Refer to [SCTLD Packing List: Established Site](#) for materials checklist

For more detailed information go to this protocol's source: MarineGEO Coral Reef Habitat Monitoring (2021) Harper, Leah, Tennenbaum Marine Observatories Network, MarineGEO, Smithsonian Institution. <https://doi.org/10.25573/sekc.14714175.v1>

Overview & Set Up



Transect will be laid along a pre-existing path. We will survey a 1m belt of the first 30m of the transect on the right hand side (if you are hovering at 0m and looking towards 30m) If two divers are doing the survey they will start at opposite ends and work towards each other and communicate to ensure that corals aren't counted twice or skipped. Divers should tally all corals within the belt on their demographics datasheet. When they encounter a coral with a condition, they should tally that coral on the demographics datasheet, then conduct the detailed survey on the conditions datasheet.

Demographic Survey

- Count, measure (with your PVC "coral stick") then tally all scleractinian corals with live tissue that fall within the 1m belt (Use your meter stick as a reference to measure 1m). Include corals with live tissue directly under the tape.
- Search crevices for cryptic corals, and include any corals found on overhangs that are situated above the belt. If a coral has live tissue that falls within the belt transect, measure all of that colony's live tissue with your marked coral stick, even if portions lie outside the transect.
- Tally each coral by species under the appropriate size class. Size classes are (in cm): 1-4cm juvenile ; 1-4cm isolate ; 5-10 ; 11-20 ; 21-40 ; 41-80 ; >80 ; 0 (recent total mortality)
- Measure coral across the longest axis of the live tissue. If an individual has several tissue isolates separated by dead skeleton, estimate the size of the total tissue area if isolates were combined (to prevent overestimating a colony's contribution of live coral



Do not tally/include:

- dead skeleton in the measurement. (Measuring only live tissue prevents misrepresenting colonies as large if only a small tissue isolate is remaining.)
- encrusting hydrozoans (ex: *Millepora alcicornis*) in the survey.

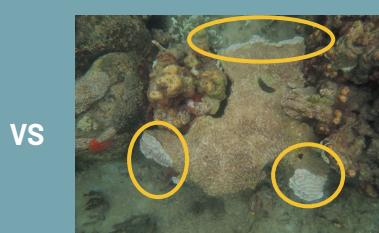


What if a once large colony has only patches left?

When the closest distance between the two tissue isolates that are >4cm in diameter exceeds the maximum diameter of the larger isolate, count and measure as separate corals.



Tissue isolate



VS

Separate corals



There is A LOT of one type of coral all together...

Monospecific, continuous thickets can be measured as one large colony when individuals are difficult to distinguish. (e.g., in the Caribbean, this strategy may be employed with *Acropora cervicornis*, *Porites porites*, *Agaricia tenuifolia* etc.)



Acropora cervicornis



Porites porites

Single colonies

Goal 4: Coral Survey cont.

Coral Conditions Sheet

- Does a coral in the belt have one of the following?
 - Tissue Loss
 - Color Loss
 - Discoloration
 - Growth Anomaly
- If YES, perform an assessment.



Need help with assessing coral health?

We got you.

Protocol for Assessing Health Status

You do not need to document a coral on the Conditions datasheet which has any of these conditions (ex tissue loss or discoloration) because of an "incidental" cause for the condition from biotic and abiotic factors such as:

- tissue loss from sedimentation, corallivory, or boring sponges
- discoloration from sponge or octocoral interactions
- growth anomalies from damsel fish predation or gall crabs

- If the tissue loss, color loss, or discoloration does not have an obvious external cause (like the things listed above), tally the coral on the Demographics datasheet, then record the following on the Conditions datasheet (each category is a column)

Need help figuring out how to make all these assessments?

Check out the **decision tree** on the back of this sheet

A coral can have more than one condition.

(Ex: colony has a tissue loss lesion, but remaining live tissue is bleached.) Record all conditions in the same row of your conditions datasheet. Separate conditions using a comma. Fill out the subsequent relevant columns (e.g. percent affected, distribution) for each condition you listed in the same order. Separate with commas.



DLAB	CLP,CLB	20,80
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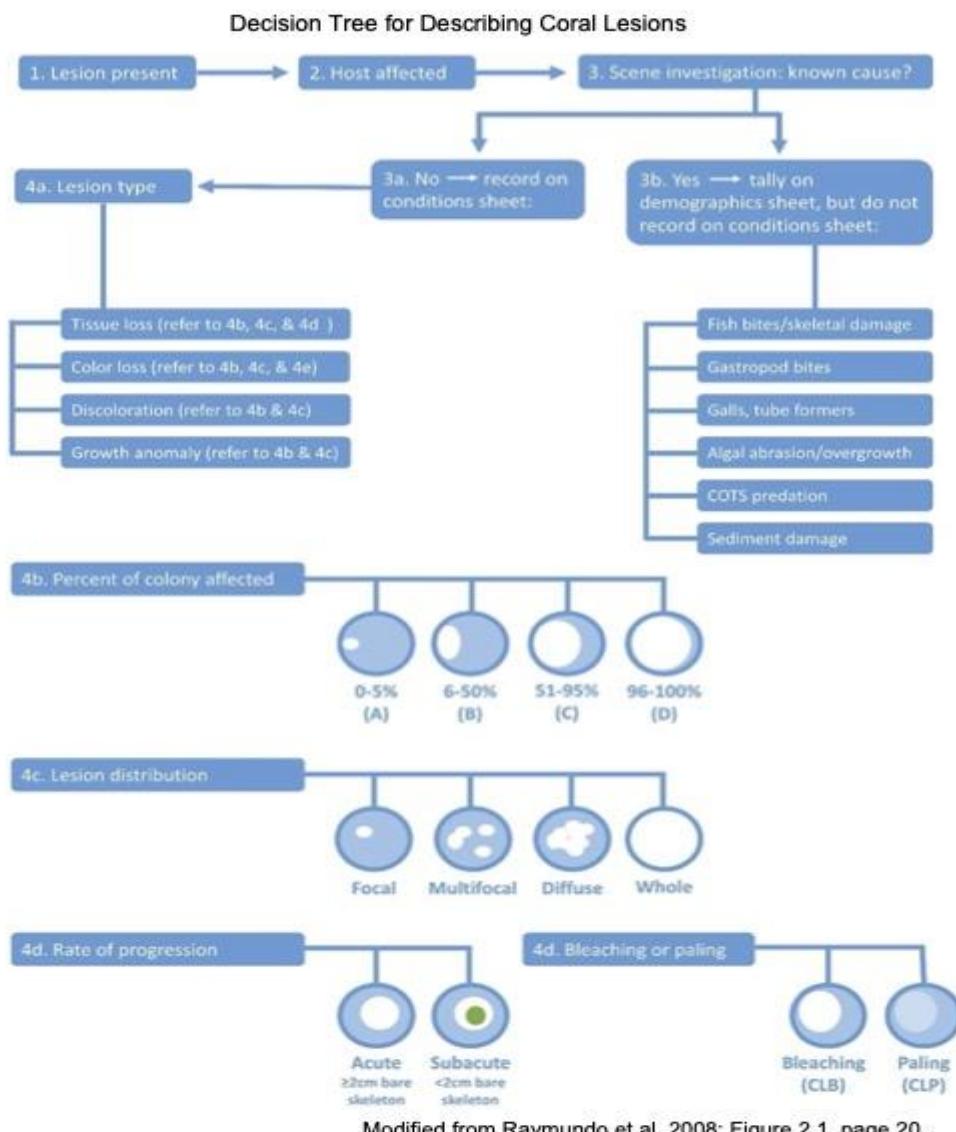
SSID	TL,DC	15,15
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- **Coral species ID**
- **Maximum diameter (cm)** across longest axis of the entire colony, including dead skeleton
- **Maximum height (cm)** of coral measured perpendicular to substrate
- Estimated **percent mortality**, including old mortality unrelated to the observed condition
- **Condition code:**
 - **TL**:tissue loss
 - **CLP**:colorloss-paling
 - **CLB**:colorloss-bleaching
 - **DC**:discoloration
 - **GA**:growth anomaly
- **Distribution** of the condition on the individual: focal "F", multifocal "MF"
- For tissue loss only, describe as acute "A" (clean skeleton >2cm) or subacute "SA" (clean skeleton <2cm)
- In "**Notes**" section, put the **JPEG # of the photo of the condition** and any other observations

Goal 4: Coral Survey cont.



Coral Demographics



What if SO MANY corals are bleaching it would take a whole Conditions Sheet (or more!) to document?

We will always decide if we are going to alter a protocol as a group. If underwater, confer with your dive buddy.

If there is a mass paling/ bleaching event we can always draw a line down the size class columns up to 20cm, splitting each size category in to two sections. The left of your split column will become the "Bleached" section and the right "Healthy" section. Tally corals on your survey as you normally would but instead of recording a CLB coral on the Demographics AND Conditions sheet, you will ONLY tally it on the left part of the split column on the Demographics sheet.

Species	CBC BLC		CURLWNT		E SHILLING		F. MURISON	
	1-4cm Juvenile	1-4cm Isolate	1-4cm	5-10cm	11-20cm	21-40cm	41-80	>80
AAGA								
ATEN								
ACER								
CNAT								
DSTO								
DLAB								

If a coral is >20 cm record CLP/CLB on the Demographics AND Conditions

If a lot of individuals from specific species are bleaching ex: ATEN, AAGA, PAST, PPOR just split the columns for these specific species, not all species

*put a little "B" at the top of the side for bleached just in case! Just split the first 4 size categories!

Goal 4: Coral Survey cont.

Photoquadrats

- These allow us to analyze the benthic composition including non target species capturing a broader picture of phenomena like bleaching, storm damage, disease, and phase shifts. Also allows us to measure changes in percent cover.
- One person should swim from 0m-30m and take one photo every meter with the transect numbers visible and centered in the photo. Stay about 1m above the transect. Camera should be white balanced.

