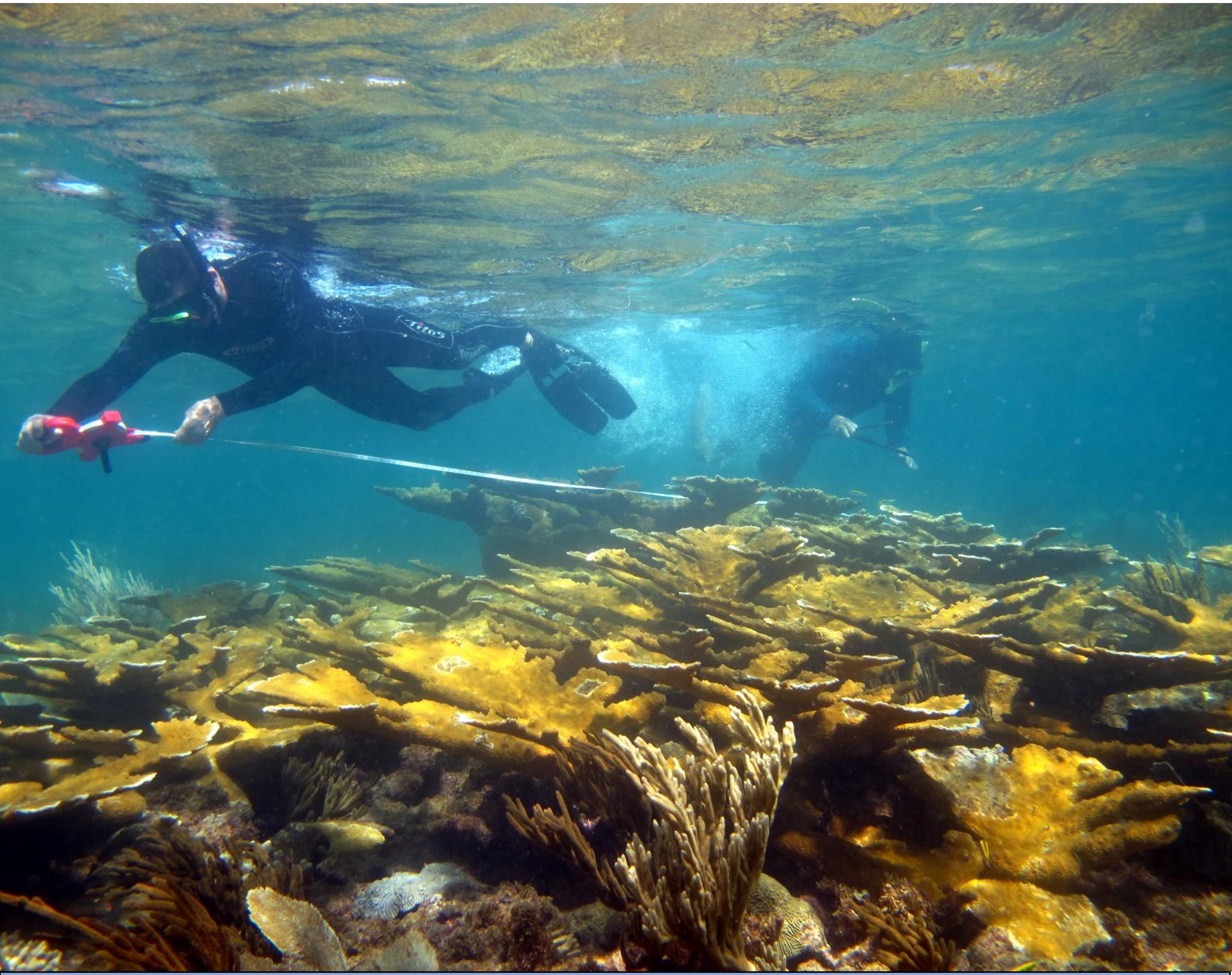

Field Methodology of the Puerto Rico Coral Reef Monitoring Program

Contents

The following descriptions of the PRCMP field methodologies are extracted from the most recent PRCRMP reports available at http://drna.pr.gov/programas-y-proyectos/arrecifes-monitoreo/. Additional descriptions of changes in methodologies are provided.

Site Selection

The PRCRMP follows a depth, distance from shore and geographical (east-west; north-south) sampling design that includes some of the main oceanographic gradients that appear to drive the ecological health and community structure of neritic coral reefs in Puerto Rico. Neritic coral reef systems included in this monitoring program are all shallower than 40m, and thus lie within the Caribbean Surface Mixed Layer water mass with pycnocline at depths that vary seasonally between 45 – 70 m. Due to the permanent stratification forces acting on this water mass, oceanic waters around Puerto Rico remain highly oligotrophic, and the coastal estuarine influence of river discharge, watershed runoff and resuspension/remineralization processes from the insular shelf produce marked inshore-offshore gradients of water turbidity associated with both organic (phytoplankton) and inorganic (sediments) sources. Coral reefs located to the east of the mainland, such as those in the Cordillera de Fajardo (Palomino, Palominito, Diablo), and the islands of Vieques (Canjilones, Boya Esperanza and El Seco) and Culebra (Dakity, Carlos Rosario, Luis Pena) are at the head of the current and receive minor estuarine influence from landmasses. Likewise, reefs located in the oceanic Isla Desecheo are also far from estuarine influences. Shelf-edge reefs associated with the mainland are intermediate across this inshore-offshore gradient and their estuarine influence is geographically variable, being higher in the west and north coasts, and lower in the south coast due to the presence/absence of major rivers.

The natural exponential decline of light penetration with increasing depth creates another relevant gradient for coral reef ecology that needs to be addressed in the understanding of potential causes of reef degradation and management options. Thus, the coral monitoring program includes reefs located across inshore-offshore gradients, vertically (depth) stratified sampling stations on several Puerto Rico reef sites, and at similar depths on the east, west and south coasts to enable comparative analyses between depths and across natural turbidity gradients associated with riverine influences and island mass effects.

Site characteristics for each monitoring station are provided in the PRCRMP Site Classification Database file, including site coordinates (Figure 1).

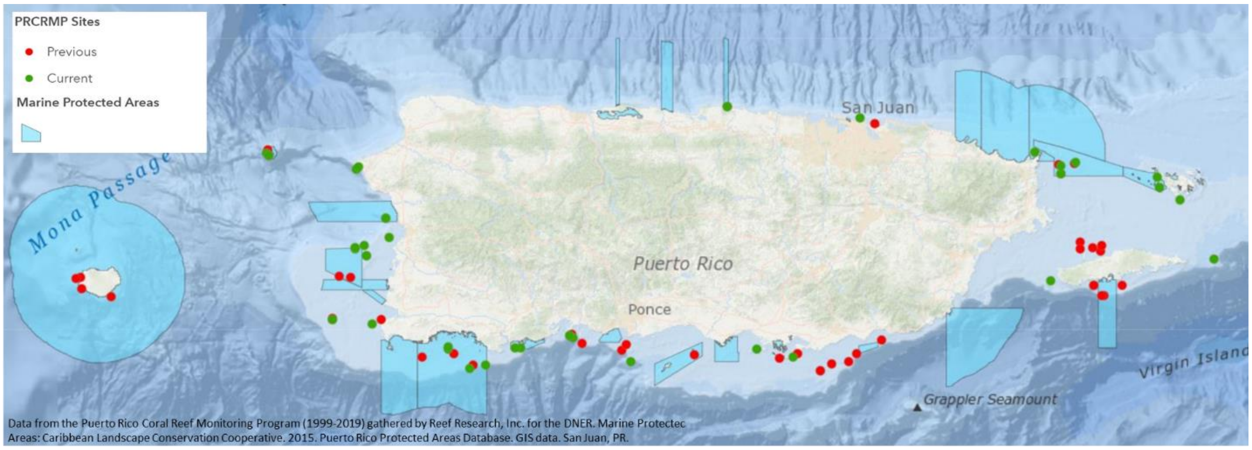

Figure 1. Distribution of PRCRMP monitoring stations (sites) around the Puerto Rican archipelago.

Sessile-benthic Reef Communities

At each reef station, a set of five 10 m long transects is surveyed. Transects are positioned non- randomly (fixed) in areas visually considered to be of optimal coral growth within similar depths (± 3 m) and reef physiographic zones. This allows for better detection of coral cover changes through time. Transect mean depths are determined from the five depth measurements taken at the start rebar marker, but depths vary along transect paths. All transects are permanently marked with steel rebars set on naturally occurring crevices or holes in abiotic sections of the reef substrate at both ends. A tag with the transect number has not been installed in all transects. Plastic zip-ties are attached to the beginning of the transect to identify the transect number and are replaced when lost due to heavy fouling or material oxidation/degradation. Wherever possible, the starting point of the transect was marked with rebar on a reef structure of high topographic relief to facilitate visual recognition during future surveys. Kitchen twine is used as a reference line to delimit the two end-markers to identify the transect paths during reef monitoring activities and then removed upon survey completion (Figure 2).

Figure 2. Sessile-benthic transect layout at Cayo Caribe monitoring site (2016). Both ends of the transect are marked with rebar on a high relief substrate. The transect numerical identity is provided by one zip tie in the start rebar, identifying transect #1 in this case. The kitchen twine delimits the chain path and the length of the transect (10 meters) between the two rebars.

Determinations of percent substrate cover by sessile-benthic categories at mesophotic depths (≥ 30m) are obtained from Coral Point Count (CPC) analyses of digital photographic images due to the reduced bottom-times associated with SCUBA diving at mesophotic depths (≥ 25 m). A total of 10 non-overlapping photos of the reef substrate are photographed over the permanent transect reference line. A set of 25 random points is overlaid on each photo frame and sessile-benthic categories under each point are classified following the same criteria used with the chain-link method. The total number of points over each substrate category is divided by the total number of points applied to the images analyzed for each transect to obtain the data on percent cover by each substrate category.

Octocorals, except for encrusting forms (e.g. *Erythropodium caribaeorum*, *Briareum asbestinum*) are counted as the number of colonies intercepted per transect, whenever any of their branches crossed the transect reference line. Hard live coral colonies under the transect line are counted and examined visually for the prevalence of apparent infectious diseases. Colonies of similar coral species growing close together and sharing attachment surfaces are counted as individual colonies if separated by a distance of 15 cm or more. Diseased colonies on each transect are identified and counted. Preliminary field identifications of potential diseases were made whenever possible following the photographic guidelines by Raymundo et al. (2008) Coral Disease Handbook. The percent coral disease prevalence is calculated based on the total number of diseased colonies divided by the total number of colonies intercepted by the five transect array at each reef station.

Sessile-benthic reef communities are characterized by the continuous intercept chain-link method (as modified from Porter, 1972), following the CARICOMP (1994) protocol. This method provides information on the percent linear cover by sessile-benthic biota and other substrate categories along transects. It allows the construction of reef community profiles by assignment of metric units to each substrate transition, which serves as a high precision baseline for monitoring. The chain has links of 1.4 cm long (0.014m), marked every 10 links for the facilitation of counting underwater. The exact position of the chain was guided by a series of steel nails set into available hard (abiotic) substrates along transects. Individual measurements of substrate categories, as recorded from the number of chain links are sorted, added and divided by the total distance (in chain links) on each transect to calculate the cumulative percent linear cover by each substrate species and category. Rugosity is estimated as the difference between the 10m linear transect distance and total number of links overlayed in the benthos multiplied by 0.014m.

Substrate cover percentage by sessile-benthic categories at El Seco Reef at 30 meters depth in Isla de Vieques was estimated using Coral Point Count (CPC) random point count analyses of digital photographic images due to the reduced bottom-times associated with SCUBA diving at mesophotic depths (>30 m). A total of 10 non-overlapping photos of the reef substrate were photographed over the permanent transect reference line. A set of 25 random points was overlaid on each photo frame and sessile-benthic categories under each point classified following the same criteria used with chain-link method. The total number of points over each substrate category was divided by the total number of points applied to the images analyzed for each transect to obtain the data on percent cover by each substrate category.

During conditions of extreme wave and surge action, such as those occurring during hurricanes and/or exceptionally high North Atlantic swells, rebar transect markers may become detached from the reef structure. In such cases, the protocol is to re-install the marker in the same substrate position that it was before without any alteration of the transect path. In cases where the reef structure supporting the rebar was physically displaced, overturned, or collapsed, then the transect path was identified using the remaining marker and the sequence of existing nails and continued until a 10 m linear path was reached. A new rebar marker was installed at the transect endpoint whenever the original rebar was lost in the sand or could not be found.

Reef Fishes and Motile Megabenthic Invertebrates

Demersal diurnal non-cryptic reef fish populations and motile megabenthic invertebrates are surveyed by sets of five 10 m long by 3 m wide (30 m^2) belt-transects centered along the reference line of transects used for sessile-benthic characterizations at each reef station. Transect width was marked with flagging tape stretched and tied to weights on each side of the transect. Each transect is surveyed for 12 - 15 minutes depending on the complexity of the fish community on each transect. The initial one or two minutes are dedicated to the detection of elusive and/or transitory species that swim away from the "belt-transect" area as soon as they detect a diver (e.g. snappers, jacks, mackerels, groupers, hogfish, large parrotfishes, etc.). During the next three to four minutes, the diver swam over both sides of the transect area counting fishes that form schooling aggregations over the reef (e.g. *Chromis* spp., *Clepticus* spp., etc.) and other transitory species as they enter the survey area, including the wrasses (e.g. *Thalassoma*, *Halichoeres* spp.) which tend to be attracted to divers and thereby, may increase in density during the survey. A second run over both sides of transects was performed during the next four to six minutes in order to count demersal and territorial fishes (e.g. *Stegastes* spp., *Gramma loreto*, squirrelfishes, etc.) that remain within the transect area. The last two or three minutes are dedicated to counting the small gobies, echinoderms, mollusks, and crustaceans associated with coral heads and crevices on both sides of transects.

Since 2015, upon completion of the 10-meter belt-transect survey the diver swims along the same depth and physiographic reef zone for an extra 10 meters to identify fishes and megabenthic invertebrates of commercial value (snappers, groupers, hogfishes, barracuda, mackerels, sharks, lobsters and queen conch) and/or fish species that are considered important reef herbivores (parrotfishes, doctorfishes). This provides a total of 60 m^2 where a visual total length (TL) estimate (in cm) was recorded for each individual. The cephalothorax length (measurement from the tip of the rostrum to end of the thorax), also known as carapace length (CL) in cm was used to report the size of lobsters (*Panulirus* spp., *Scyllarides* spp.) within belt-transects. Queen Conch (*Strombus gigas*) length was reported as the total (diagonal) shell length in cm. The precision of length estimates allowed discrimination between new recruits, small juveniles, juveniles, adult and large adult size classes.

From 2004-2013, the size-frequency observations were surveyed using an Active Search Census (ASEC) technique. This is a non-random, fixed-time method designed to optimize information on the numbers of fish individuals present at each of the main reef habitats, providing simultaneous information on size-frequency distributions. At each reef station, the total number of individuals of each species observed within a fixed time frame of 30 minutes was registered. Individuals were actively searched for in the water column and within crevices, ledges and potentially important hiding places. For each individual sighted, a length estimate was recorded. One ASEC survey was performed at each reef station. The change in methodology from ASEC to 60 m^2 band transects in 2015 was done to align the DNER PRCRMP with the NOAA National Coral Reef Monitoring Program (NCRMP) methodology.

Protocol for reestablishment of lost transects and time series data management

To maintain the quality of the data and information derived, it is paramount to establish standardized protocols for transect maintenance and time-series data management. The loss of transect rebar markers often occurs under various circumstances. In shallow water (<6 m depth), transect rebar detachment and burial are common due to the impact of swells generated by tropical systems in the summer months or cold fronts and low-pressure systems during the winter months. In deeper habitats, between 20 and 30 meters, the effects of currents while descending, available bottom time, and visibility often lead to transect loss at homogeneous seascapes. Furthermore, independently of depth, if stations are not surveyed for more than five years transects rebars can be difficult to find due to corrosion and overgrowth by benthic organisms. Other human errors can lead to the loss of transects such as problems with GPS data, collection of wrong coordinates, and failure of GPS unit.

In almost a fourth of currently monitored stations (10 out of 45) transect rebar markers in the reef substrate have been lost and reinstalled at variable points in time, introducing potential confounding effects with profound implications to time-series analyses. The list of sites where the reestablishment of transects has occurred is provided in **table 1**. The reestablishment of transects is evidenced by a change in the station central coordinates. After transect reestablishment, the distance between the previous and updated central coordinates of stations within the same reef site has ranged from approximately 40 meters in Gallardo to 1,400 meters in Cayo Caribes, for example. Coral reef benthic assemblages are typically highly heterogeneous at less-than-one-meter scales. Thus, the reestablishment of transect markers can result in a significant change in benthic community structure and composition that is not related to temporal change, but to the relocation of the central line that connects two transect rebar markers that lead the chain overlay (see PRCRMP field methodology). This is known as a confounding effect. If this is not accounted for, time series analyses might lead to erroneous conclusions about the relationship between time and benthic assemblages (ex. false sense of coral cover recovery or loss). Thus, it is important to identify whether permanent transects within a station have been reestablished to determine if a site time series is to be continued or a new baseline must be set.

The protocol for the reestablishment of lost transects is provided to define the steps and criteria to continue the monitoring of stations where the loss of transect rebar markers leads to the installation of new rebar markers while avoiding confounding effects in time-series analyses.

First Step: Locating transects

The waypoint to navigate to the monitoring station is dictated by the **central coordinate** (in decimal degrees) at transect #3 (T-3). This coordinate is essential to relocating transects and reestablishment in case they cannot be found.

After arrival at each monitoring station, a reconnaissance diver and buddy go in to **locate the rebar markers** and tie kitchen twine between the two rebar markers for each transect, remove fouling from each rebar, and add new numeric tags to transects if needed. This will facilitate the work of the benthic and fish survey divers and maximize their bottom time. At shallow sites (<5m depth), the reconnaissance diver might do this by snorkeling with the help of a GPS unit (with individual transect coordinates) attached to a dive buoy.

To facilitate the location of transects a **diagram of the transect arrangement** within the monitoring station should be prepared, printed in waterproof paper, and taken in an underwater slate during the surveys. These diagrams are prepared during the baseline survey of a station. Geomorphological features (drop-offs, mounds, sand channels, reef trenches, faults, etc.), biological features (large coral colonies, seagrass patches, conspicuous sponges, etc), and other seascape landmarks (marker buoy anchor point, flagged rebar, etc.) should be mapped along the transects. This will allow establishing the orientation of the site and the relative position of transects. The heading, length of the transects, and distances among transects should be marked in the diagram as well. An example of such a diagram is provided in Figure 3.

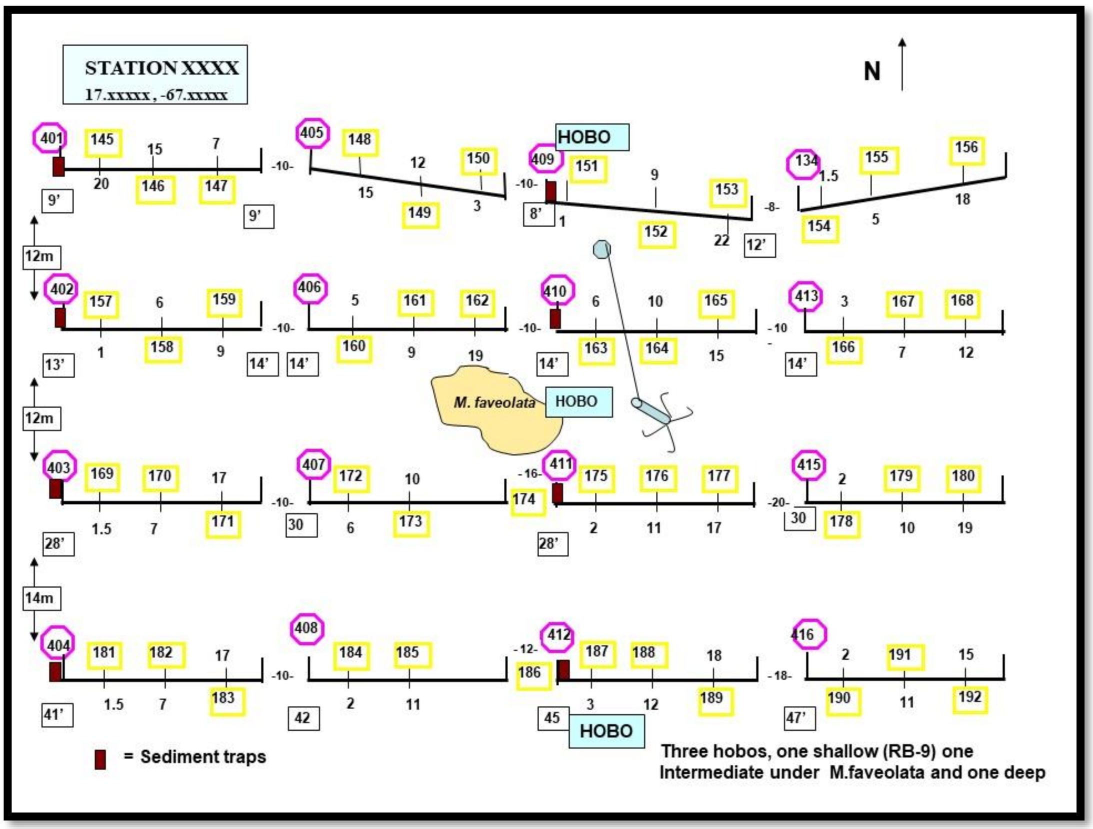

Figure 3. Example of permanent transects arrangement within a monitoring station. Different underwater landmarks are identified (larger corals, buoy anchor, sensors). Each transect is labeled with a number and subsamples are marked. Source: Project NOAA-CRES, La Parguera.

In deep stations (20-30m) it is recommended to install a **visible marker** at the central area of the station. This marker can be a rope with a sunken buoy, for example, that will guide divers when locating individual transects. Such a marker would be mapped in the station transect diagram. In shallow stations (<10m), such as Acropora biotopes, **drilling stainless steel eye bolts** into consolidated hard substrates can provide a more permanent transect marker than rebars, but it is time-consuming and costly to do. However, stainless steel eye bolts are less prone to detachment due to surge and wave detonations during winter swells or hurricanes.

Second Step: Reestablishing transects rebar markers

Reestablishing transects is needed if the reconnaissance diver fails to locate the transect after a significant effort has been done. The amount of time invested in locating transects in the field will vary across sites due to differences in reef complexity, depth, weather conditions, and other factors. The fieldwork team must always have **spare transect markers** in the boat to reestablish a monitoring station if damage or transect loss occurs during the surveys.

When possible, the transects should be **reinstalled in the same depth, habitat, and use the same GPS coordinates** as the previous transects to continue monitoring of the site without drastically changing the type of community surveyed. Therefore, it is key to have coordinates for each transect and a diagram that can be taken in the field.

If a disturbance has modified the benthic seascape in a way that there is not much live coral cover left at the previous GPS coordinates, the team can locate a **nearby reef area with a similar community** and habitat as the previous station and install transects there (Ex. Gallardo Reef after hurricane María).

If some of the old transects in a station are still standing, reestablishment of new transects should be done in the same relative position to transects that are still standing.

All transect rebar **markers and numeric tags should be cleaned** during each survey (biofouling removed) and replaced if their condition might lead to transect loss by the next monitoring event. This will avoid the loss of transects and reinstallation in a different section of the reef.

New coordinates per transect will be collected if the transect line is moved by **more than 3 meters** from the previous transect coordinates.

Third Step: Reporting and updating the PRCRMP database

After a station has been reestablished, it is important to **report any changes and update the station metadata** (station name, transect coordinates, etc.), if needed. In PRCRMP reports, any installation of new transects should be logged and the circumstances in which transects were not found should be described in the results and discussion section. This will provide more context to report readers and database users on possible changes observed during that monitoring event.

If **new coordinates** are taken for individual transects or the central coordinates of the station, they should be provided to the DNER as well to update the metadata available in the **PRCRMP Habitat Classification Database**.

If transect rebar markers are replaced in the same position as the previous marker, it should be reported as well, but no changes in the station name or transect coordinates are needed.

If transects rebar makers are replaced in a different position (shifting the previous position by more than 1 meter), it should be reported, and the **name of the station** should be updated (new coordinates needed only if shifted by more than 3m). The new station name should consist of two parts: (1) the previous station name and (2) the year in which transects were re-established in parenthesis (Ex. Palominos (1999) changes to Palominos (2016) if a change in transect marker position of more than 1m was made in 2016). This name change will allow identifying [in the database] that a station transect arrangement update has been done. The station remains in the same reef site, but a new baseline has been set with the reinstallation of transect markers by which the chain will be guided when doing benthic surveys.

In time-series analyses although the stations remain in the same reef site after reestablishment, each station (before and after reinstallation of transects) should be treated as **different benthic baselines,** and thus, transects after reinstallation are not part of the same time series.

If the PRCRMP fieldwork team understands that the change in transect location will not introduce a **confounding effect to time series analyses**, the rationale for this should be reported in PRCRMP annual reports along with the circumstances in which the transects were reinstalled (cause of transect loss, new transect coordinates if taken, etc.).

Table 1. List of reef sites where transect rebar markers have been reestablished at variable points in time (years in parenthesis in Station). Additional metadata of the station is provided. Source: PRCRMP Site Classification Database. Temporal analyses with these stations should consider transect reestablishment events.

Reef Site	Station	Location	Latitude	Longitude	Baseline Year	Most Recent Survey	Mean Depth_(m)	Habitat Type	Coral Biotope
Boya Vieja	Boya Vieja (2000)	La Parguera	17.89751	-66.9903	2000	2000	20	Spur & Groove	O. annularis complex
	Boya Vieja (2015)	La Parguera	17.88827	-66.9978	2015	2019	20	Spur & Groove	High-Moderate Hard Coral
Cayo Caribes	Cayo Caribes (2013)	Salinas	17.92292	-66.20331	2013	2013	10	Aggregate reef	High-Moderate Hard Coral
	Cayo Caribes (2016)	Salinas	17.915435	-66.214007	2016	2019	10	Aggregate reef	High-Moderate Hard Coral
Cayo Diablo	Cayo Diablo (1999)	Fajardo	18.36003	-65.53237	1999	1999	10	Aggregate reef	O. annularis complex
	Cayo Diablo (2016)	Fajardo	18.36033	-65.53089	2016	2018	5	Aggregate reef	High-Moderate Hard Coral
Cibuco	Cibuco (2011)	Vega Baja	18.48916	-66.3736	2011	2011	5	Aggregate reef	O. annularis complex
	Cibuco (2013)	Vega Baja	18.48955	-66.3736	2013	2018	10	Patch reef	O. annularis complex
Gallardo	Gallardo (2000)	Cabo Rojo	18.00498	-67.32975	2000	2000	10	Patch reef	High-Moderate Hard Coral
	Gallardo (2013)	Cabo Rojo	18.00138	-67.32993	2013	2017	5	Patch reef	A. palmata
	Gallardo (2019)	Cabo Rojo	18.0017	-67.3299	2019	2019	5	Patch reef	A. palmata
Maria Langa 10m	Maria Langa 10m (2001)	Guayanilla	17.96703	-66.75103	2001	2001	10	Aggregate reef	High-Moderate Hard Coral
	Maria Langa 10m (2016)	Guayanilla	17.96093	-66.75284	2016	2019	10	Aggregate reef	High-Moderate Hard Coral
Maria Langa 20m	Maria Langa 20m (2001)	Guayanilla	17.96234	-66.7492	2001	2001	15	Aggregate reef	High-Moderate Hard Coral
	Maria Langa 20m (2016)	Guayanilla	17.95955	-66.74697	2016	2019	15	Spur & Groove	Octocoral canopy
Palominitos	Palominitos (1999)	Fajardo	18.3357	-65.56573	1999	1999	10	Spur & Groove	O. annularis complex
	Palominitos (2016)	Fajardo	18.33537	-65.56555	2016	2018	10	Spur & Groove	O. annularis complex
Palominos	Palominos (1999)	Fajardo	18.35555	-65.57112	1999	1999	10	Aggregate reef	High-Moderate Hard Coral
	Palominos (2016)	Fajardo	18.35466	-65.56711	2016	2018	20	Aggregate reef	High-Moderate Hard Coral
Sardinera	Sardinera (2008)	Isla Mona	18.09474	-67.94926	2008	2010	30	Pavement	Low Coral
	Sardinera (2020)	Isla Mona	18.098323	-67.95077	2020	2020	30	Pavement	Low Coral

References:

Department of Natural and Environmental Resources (DNER). 2001, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2014, 2015, 2016, 2017, 2018. Puerto Rico Coral Reef Monitoring Program Reports (prepared by Garcia-Sais et al.). San Juan, Puerto Rico. Available at: http://drna.pr.gov/coralpr/

Rogers, C.S., T.H. Suchanek, and F.A. Pecora. 1982. Effects of hurricanes David and Frederic (1979) in shallow *Acropora palmata* communities: St. Croix, U.S. Virgin Islands. Bulletin of Marine Science 32: 532-548