Experimental design for assessing SCTLD impacts and treatment effects in Puerto Rico

Submitted to the DNER Coral Reef Conservation and Management Program (CRCMP)

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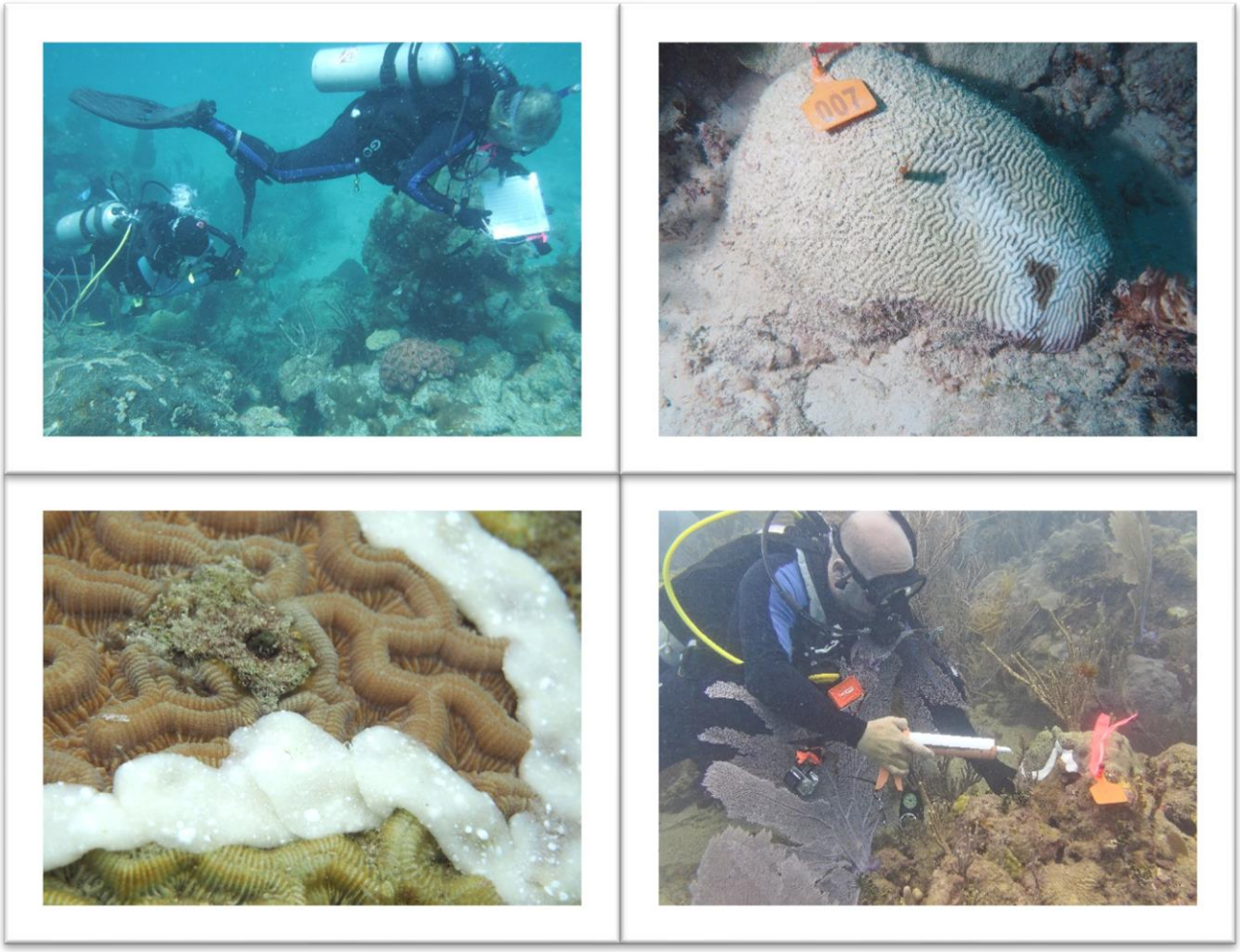

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Justification and rationale

Stony Coral Tissue Loss Disease (SCTLD) is a highly virulent disease affecting most Caribbean reef-building scleractinian species. An outbreak with signs similar to previous reports of SCTLD in other Caribbean jurisdictions (high prevalence, fast spread, and species affected) was reported for the first time in the Puerto Rican archipelago around reefs of the Luis Peña Canal reserve at Culebra Island. Since then, the Puerto Rico Department of Natural and Environmental Resources (PR_DNER) has created an SCTLD response working group composed of members of academia, NGOs, and government representatives to coordinate planning, field efforts, and reporting. In the field, opportunistic roving dive surveys have been performed to confirm the presence of preliminary SCTLD reports, and antibiotic treatments have been applied to dozens of colonies across sites where logistical and ecological criteria have rendered priority. In each site where roving dives and antibiotic treatment efforts are undertaken, data on disease prevalence, colony sizes, number of lesions, and percent mortality are noted. Data collected by various entities have been compiled in a database under the custody of the PR_DNER. The purpose of informing management decision making and updating stakeholders, such as NOAA and other potential sponsors of the SCTLD response in Puerto Rico, regarding the known status of the disease, response efforts implemented, and management needs. However, these efforts have not been coordinated under a common research question to be addressed or evaluated using the data collected. These questions must be formulated to develop an experimental design that can lead to statistical analyses in support of evidence-based management decision making.

Monitoring both SCTLD impacts on coral communities and treatment effectiveness are common research priorities between Florida and the U.S. Virgin Island. Mixed results of treatment effectiveness across locations, species treated, and employed techniques warrant the careful evaluation of treatment priorities in each jurisdiction to maximize the impact of limited resources. Likewise, spatiotemporal trends of coral cover before, during, and after SCTLD outbreaks can help to identify potential areas with resistance to this event and prioritize future restoration efforts.

In this report, a set of experimental designs based on common priority research questions are formulated to aid in the implementation of research priorities that inform management of the SCTLD event. The impacts of this event on stony coral populations and the long-term effectiveness of the antibiotic treatments are priority research topics that are the focus of these questions. Thus, the following questions are proposed as foundational for two basic experimental designs:

1. **What are the changes in coral reef benthic community structure and composition (abundances and diversity) occurring due to the SCTLD outbreaks?**
2. **How do disease progression and colony mortality change depending on antibiotic treatment, depth, and sites over one year of monitoring?**

Experimental Designs

Question 1: What are the changes in coral reef benthic community structure and composition (abundances and diversity) occurring due to the SCTLD outbreaks?

Factor 1 (fixed): SCTLD Status

To evaluate the impacts of SCTLD outbreaks on stony coral assemblages it is important to identify reef sites that have not been impacted by the disease at the start of monitoring. These can be chosen based on the prevalence of the disease which is expected to increase over time when it first appears on a site (characteristic of SCTLD outbreaks). A temporal factor would be essential for this design and can have three time periods: "***before"*** (when the disease shows less than 2% total prevalence), "***during***" (disease total prevalence above 10%), and "***after"*** (total prevalence falls back to less than 10%). The precise timing of the before and during level will depend on the reef site and will require frequent monitoring surveys where coral disease prevalence should be estimated via roving diver surveys.

Factor 2 (fixed): Locality

A set of 4 geographical areas (localities) should be monitored during each time period (i.e., before, during, after). This spatial factor serves to evaluate potential spatial patterns of variation in SCTLD impacts at kilometers scales. In Puerto Rico, since the disease has already been reported as causing outbreaks in many localities (Ex. Fajardo, Culebra, Vieques, Salinas, Ponce, San Juan, Vega Baja), careful selection of localities for this design should be employed. By April 2021, coral reef localities where SCTLD outbreaks have not been reported include areas in the western half of the Puerto Rican archipelago such as Guayanilla, Guanica, Lajas, Cabo Rojo, Mayaguez, Rincón, Aguadilla, Isabela, and Arecibo. It is recommended to select at least four localities covering a broad geographical area (50-80 kilometers).

Factor 3 (random): Reef Sites

Each locality should be replicated by at least three sites randomly selected to get a representative sample of the locality. However, each site should have a relatively high coral cover (high abundance of SCTLD susceptible species) and be accessible. To get random sites with these characteristics, a pool of potential sites should be identified for each locality from which three reef sites can be randomly selected. The use of already established permanent monitoring stations should be explored (Ex. UPRM Marine Sciences Department, DNER PRCRMP stations,

among others) as there will be historical data available for analysis. For a list of sites with known permanent stations and a high abundance of susceptible species data from existing monitoring programs such as NCRMP and PRCRMP as well as local knowledge from researchers should be relied upon.

Factor 4 (fixed): Transects

Sets of permanent transects should be established at each reef site so that replicate surveys can be conducted each visit. A set of five transects per reef site could provide a moderate replicate number to perform analyses of variance to test for spatio-temporal patterns. If resources allow, this number should be increased.

Methods and metrics

Methods selected should provide accurate estimations of key indicators of SCTLD impacts while being feasibly implemented in the field. Metrics to be monitored as indicators of SCTLD impacts and the data collection methods can be:

- Coral cover per species (coral cover %)
    - 10-meter line-point-intercept or chain transects
        - If aligning methodologies with PRCRMP choose chain transects.
        - Fieldwork is intensive but data is readily available (collected *in situ*).
    - 10-meter photo transect
        - Provides a permanent photographic record of the event.
        - Reduces time in the field but time-consuming in-office/lab due to image processing/data extraction.
- Coral density per species (colonies per m^2)
- Coral sizes per colony per species (height, maximum diameter, perpendicular diameter)
- Coral recent mortality per colony per species (colony recent tissue mortality %)
- Disease prevalence by species
    - 10 m x 1 m belt transect.
    - All colonies of susceptible species larger than 5cm (in maximum diameter or height) are counted, measured, and inspected for active disease and recent mortality.

Question 2: How do disease progression and colony mortality change depending on antibiotic treatment, depth, and sites over one year of monitoring?

Factor 1 (random): Reef Sites

Sites for monitoring of treatment effects should be selected randomly out of a pool of sites identified as priorities for SCTLD interventions based on a set of ecological and logistical criteria. Sites should be in a similar state with respect to the disease (ex. all be in the invasive stage of the disease when monitoring starts to reduce differences in treatment effects between sites potentially related to the progression of the disease. Sites selected should have similar environmental conditions such as topographic complexity, depth intervals, water turbidity regimes, wave exposure, and stony coral community structure and composition. Accessibility and proximity between sites are desired to maximize any available resources for data collection (ex. sites within the same area or locality where research groups will work). A set of 4 sites can be selected to evaluate spatial differences at less than 10-kilometer scales.

Factor 2 (fixed): Depth

The role of depth in the effect of treatments and disease progression should not be overlooked. At shallow depths (<6m), turbulence due to wind stress and groundswells have been observed to reduce the adhesion of the Base2B polymer to stony coral skeletal surfaces compared to deeper habitats. Furthermore, it is likely that under high turbulence conditions, the dissolution of Amoxicillin in the water column is greater and thus, might reduce the time of diseased coral tissue exposure. To evaluate this, it is recommended to factorize for depth, adding at least two depth strata (“shallow” from 3 to 8 meters and “deep” from 10 to 15 meters).

Factor 3 (fixed): Treatment

Treatments should consist of a set of tagged colonies where various treatment conditions will be applied. Three levels of treatments can provide information on the effectiveness of Amoxicillin + Base2B ointment treatments and the difference between the different number of treatment reapplications over time. One “control” group should not be treated for the duration of the project. One “treated” group should be treated only once, representing colonies that are commonly treated in efforts that do not ensure the relocation of colonies for monitoring and treatment reapplication. One “re-treated” group should be treated multiple times during one year. All colonies from all groups will be reassessed over time and only the “retreated” colonies will be treated multiple times if lesions remain active or if new lesions appear.

Factor 4 (fixed): Colony

Priority should be given to tag 3 colonies of meandroid tissue integration (*Meandrina* spp., *Colpophyllia natans,* and *Pseudodiploria* spp.) and 3 colonies of plocoid tissue integration (*Orbicella* spp. and *Montastraea cavernosa*) per treatment group per depth, if available at a site (total 24 colonies per site). It is important to consider that given the natural variability of scleractinian community structure

and composition across sites and depths it is very likely that tagged colonies will represent different species for each site and depth. A decision can be made to treat only one species to control for differences in treatment effects on colony mortality due to the species.

Factor 5 (fixed): Visit

The number of visits to each site will depend on available resources for interventions to assess the research question. However, it is recommended to distribute these visits across at least one year to evaluate the survival of treated colonies during an epizootic event. For this design, 9 visits over 12 months are recommended. During each visit, tagged corals should be inspected, and the selected metrics of treatment effectiveness/colony mortality should be collected. The frequency between visits can be variable based on the starting point of the monitoring effort, as has been recommended by other jurisdictions. Appendix I includes a diagram with the suggested frequency of visits.

Methods and metrics

At each site, a map or diagram with the position of each tagged colony relative to one another should be developed for ease of colony location. For this, it is recommended to use a GPS unit attached to a diver safety buoy, synchronized with the diver's clock watch, and with tracking mode enabled with the frequency of georeferencing set to 10-second intervals. When a colony is tagged the diver must record the tag number and the time (hour: minutes) so the coordinates can be extracted from the GPS track. Colonies should be within a polygon of no more than 2,000 m^2 per site to maximize air consumption and dive time. For each tagged colony, the following qualitative and quantitative data should be collected during each visit:

- Tag number
- Colony status (treated or non-treated)
- Size measurements (height, max. diameter, perp. diameter)
- Lesions/disease status: ("healthy", "diseased")
- Number of lesions treated
- Colony percent recent mortality (estimated over the entire colony surface area with a photographic record each visit)
- Distance advanced by the lesion (selecting between one and three lesions per colony to mark with a nail and measure lesions progression each visit)

Appendix I – Experimental design diagrams

Q1: What are the changes in coral reef benthic community structure and composition (abundances and diversity) occurring due to the SCTLD outbreaks?

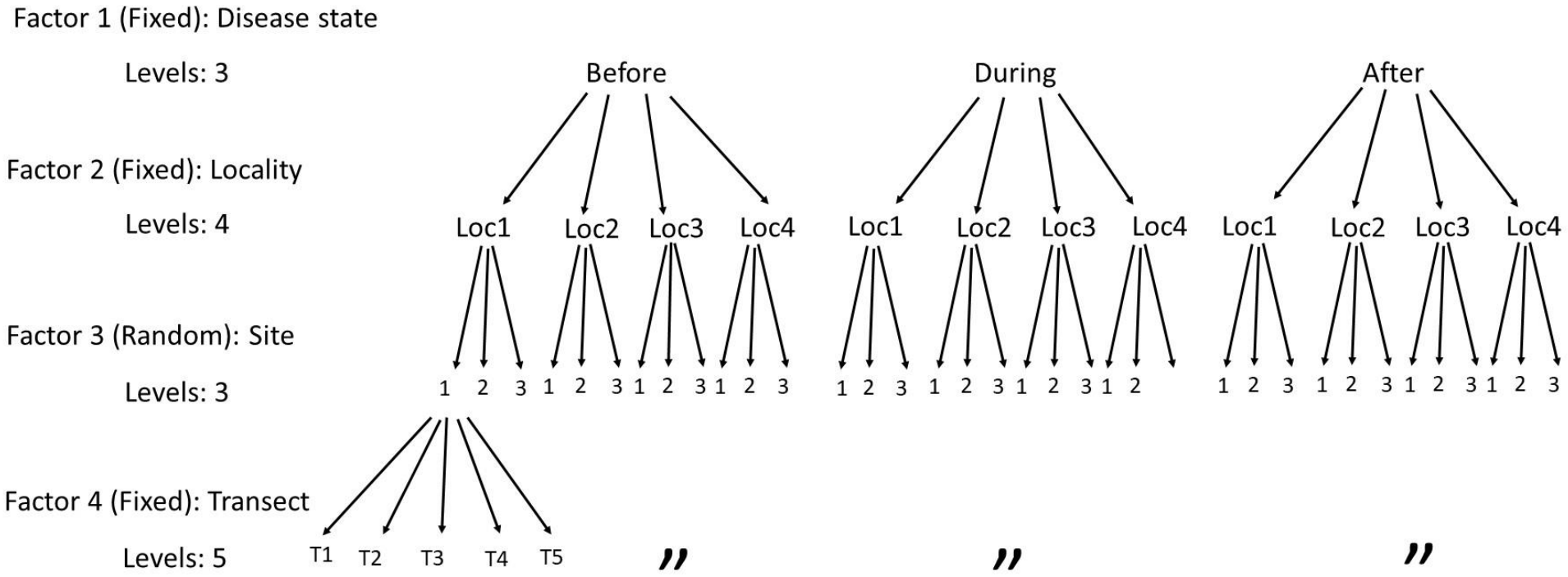

Figure 1. Experimental design diagram to assess the impacts of SCTLD in scleractinian assemblages structure and composition. Each factor is divided in levels with are replicated by levels of other factors. Localities examples can be municipalities such as Guayanilla, Guanica, Lajas, Cabo Rojo, Mayaguez, Rincón, Aguadilla, Isabela, and Arecibo, while sites can be selected with further evaluation by the Puerto Rico Coral Disease Response Group.

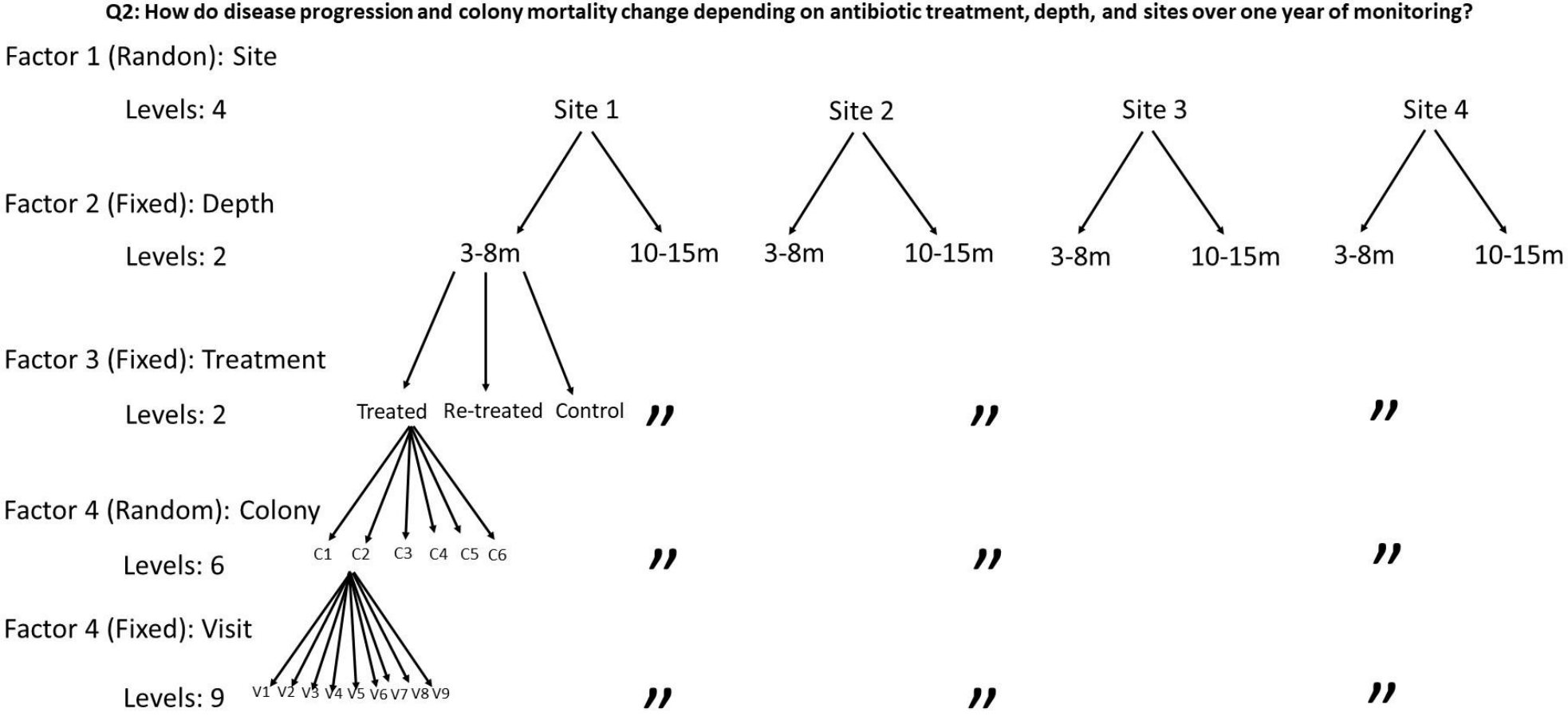

Figure 2. Experimental design diagram to assess disease progression and treatment effects. Each factor is divided in levels with are replicated by levels of other factors. Sites can be selected randomly with further evaluation by the Puerto Rico Coral Disease Response Group.

Suggested frequency of visits to evaluate colony mortality in treated and non-treated corals

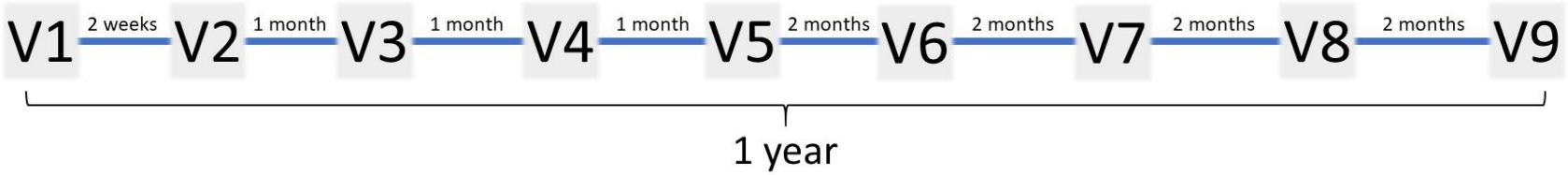

Figure 3. Number of visits across one year and the suggested time between visits. The number of visits and time between visits can be adjusted as needed given budget and other logistical constrains.