

Standard Operating Procedure (SOP) #17

Settlement Analysis

Version 2.03 (April 29, 2021)

Change History

New Version #	Revision Date	Author	Changes Made	Reasons for Change	Previous Version #
2.0	6/30/2007	Eric Brown	See Track Changes	Response to reviewer's	1.0
2.01	7/20/2007	Larry Basch	See Track Changes	Corrections, additions	2.0
2.02	8/22/2019	Kelly Kozar	Updated SOP #s	Added a geospatial SOP and had to renumber subsequent SOPs.	2.01
2.03	4/29/2021	Sheila McKenna	Updated info on images of juvenile coral and other sessile invertebrates photo guide.	KALA is the only park to have done coral settlement work with the tiles.	2.02

Only changes in this specific SOP will be logged here. Version numbers increase incrementally by hundredths (e.g., version 1.01, version 1.02) for minor changes. Major revisions should be designated with the next whole number (e.g., version 2.0, 3.0, 4.0). Record the previous version number, date of revision, author of the revision, changes made, and reason for the change along with the new version number.

Purpose

This SOP details the processing of CSAs using bleach and the methodology for locating, identifying, counting and documenting coral settlers. This SOP also includes procedures for cleaning the tiles in preparation for the next deployment. Data handling and quality assurance/quality control for all monitoring protocols implemented by the PACN monitoring program are detailed in the program's Data Management Plan¹. Microsoft Access® is the primary software environment for managing benthic data and the associated metadata. ArcGIS® Desktop software (as of publication, the version in use is ArcGIS Pro® 2.7.3) serves as a tool for validation of spatial data residing in Access®. The use of products identified in this protocol does not imply endorsement, effectiveness, or warranty by NPS.

¹ PACN Data Management Plan, <https://irma.nps.gov/DataStore/Reference/Profile/633300> (accessed 29 April 2021).

A master equipment list for the entire Benthic Marine Community Vital Sign Monitoring Protocol can be found in Appendix SOP 1.a. of [SOP #1 Before the Field Season](#). The master equipment list should be updated simultaneously if any SOP requiring an equipment list is revised.

Laboratory Analysis of Coral Settlement Plates

This SOP is to be followed once Coral Settlement Arrays (CSAs) have been collected from the field, properly stored, processed, and returned to the laboratory for analyses (see [SOP #9 Conducting Coral Settlement Sampling](#)). The time required to prepare the tiles for analysis takes approximately two days. The equipment needed for the preparation and analysis of the terracotta settlement plates can be found in [Appendix SOP 17.a](#). The coral settlement data forms (Appendix SOP 7.c., [SOP #7 Selecting and Marking Subtidal Sampling Transect Locations](#) or Appendix SOP 9.b., [SOP #9 Conducting Coral Settlement Sampling](#)) used during the collection of the CSAs should also be available for consultation.

Preparing Plates

The processing of the settlement plates is an important step and should be completed, with no delay, immediately as part of the daily post dive procedures. Do not allow the plates to sit longer than necessary, or overnight before processing. Handle the plates as little as possible and only by lifting up or lowering down while holding the edges. Excessive or improper handling can damage or destroy the coral juveniles. The equipment needed for the preparation of the plates can be found in [Appendix SOP 17.a](#).

1. Prepare a 10% bleach solution by mixing 1 part of standard household bleach with nine parts of fresh water. Pour enough solution into plastic trays so the tiles will be completely immersed. Flat-bottom shallow trays with raised ridges on the bottom (such as photographic development trays) allow tiles to rest with minimal contact to their surfaces and expose tile bottom surfaces to bleach solution.
2. Check each plate to make sure site code and other information is legible on plate edge. If necessary, re-write this information so that it is clear. Then, gently place the plates in trays filled with the bleach solution.
3. Leave plates in the bleach solution for at least 24 hours. Remove from the bleach and rinse off in gently flowing fresh water to remove algae and sediment.
4. Optional: Take macro photos of each plate after bleaching. See [SOP #15 Managing Photographic Images](#) for more information on photo storage.
5. Allow the plates to air dry. Ensure that site code and other information is legible. If necessary, retouch the site code with an ultra-fine Sharpie marker.

6. Wrap each tile in bubble wrap and store in box in a safe place until microscopic examination is possible.

Locating, Identifying, and Counting Coral Settlers

The following procedure outlines the routine used to find, identify, and count coral juveniles on the dried terra cotta plates. The amount of time needed to complete one plate varies, and could differ by season, soak duration, settlement intensity of sessile benthos, by transect site, park, and other variables. Pay particular attention to crevices/cracks and edges of tiles – Montiporid juveniles tend to occur in the crevices. The equipment needed for the microscopic analysis of the plates can be found in [Appendix SOP 17.a](#).

1. Set up a dissecting microscope mounted on a stable “arm” stand according to its instructions. Set up in a comfortable location ensuring proper ergonomics. Make sure lighting is adequate. Fiber optic lights (2) that can be focused and positioned at various angles to the plate are needed. Two extra bulbs need to be kept available for these lights and reordered when one burns out.
2. Always handling each plate carefully by the edges, unwrap from bubble wrap and place bubble wrap or other flat cushioned object (closed cell foam, a flat piece of neoprene, etc.) on microscope base. Always use a cushion of uniform thickness to avoid changes in magnification that would cause measurement error. Using the dissecting microscope at 6-10X magnification, systematically search the bottom then top plate surfaces, moving the plate edges with fingertips. “Raster scan” entire tile twice, once side-to-side and once top-to-bottom. Keep track of where you are on the tile using the thin permanent marker to make hash marks on the periphery.
3. For each coral juvenile encountered, identify it to the lowest possible taxonomic level (typically genus) and record it in the appropriate location on the datasheet ([Appendix SOP 17.b](#)). Make sure that the appropriate site code, CSA number, plate number, and surface (upper top, upper bottom, lower top, lower bottom, and side; see Figure SOP 9.1 in [SOP #9 Conducting Coral Settlement Sampling](#)) has been noted for each observed juvenile. Record the approximate dimensions of each settler (in millimeters) using a calibrated ocular micrometer for particular magnification settings. An ocular micrometer and a stage micrometer (for calibrating) need to be installed and calibrated for each magnification used (which should be recorded with each measurement). A small table including magnifications, ocular micrometer units, and corresponding absolute units (e.g., micrometers μm) can be written on a piece of paper and taped to a nearby work surface for easy reference. If the microscope (e.g., Leica® MZ 12.5) has this feature, use the click stop setting on the magnification knob to ensure that discrete magnification settings are used. Continuous focus (non-click stop magnification knobs) can lead to magnification drift and error. If the microscope has the latter type of magnification knob, be careful to look at magnification knob and re-set it to the closest magnification mark before making each measurement. Coral

juveniles of several taxa can be easily tallied using a multi-place lab bench counter available from scientific or laboratory equipment suppliers.

4. To keep from recounting juveniles, (and to relocate them more easily as needed) circle each juvenile that has been tallied with a blue sharpie fine point pen. If notes on particular juveniles are desired and juveniles need to be relocated for any reason, note tile and surface (e.g., UT) and use a coordinate system to quickly relocate particular juveniles that have been circled. For example, on UT, the left, short side of the tile corresponds to the Y axis, and the bottom, long side, the X axis. A metric ruler held on these sides can be used to quickly record X and Y distances in cm or mm from the origin at the bottom left of the tile.
5. Once all plate surfaces have been searched, re-wrap the plate in bubble wrap, and store as above until at least one year after all data have been verified, validated, and reported, at which time samples can be assessed, re-stored (only if needed), and disposed of.
6. Review your work by quickly scanning plates, taking care to note site locations, CSA numbers, species identifications, size measurements, and plate surfaces for each coral juvenile.

Entering Data

For detailed procedures of data entry into the Benthic Marine and Marine Fish Database, including quality control and verification, please see [SOP #14 Data Entry, Verification, and Certification](#).

Cleaning Plates

Once all plates have been searched, data has been entered and verified, plates should be cleaned and stored for re-deployment at a future date. The equipment needed for cleaning the plates can be found in [Appendix SOP 17.a](#).

1. Completely submerge plates in household vinegar for at least 24 hours or until all CaCO₃ material has dissolved from the plate surface. This can be confirmed through a thorough visual inspection of the plate surface using a microscope or a hand lens.
2. Use a toothbrush or other soft plastic bristle brush to clean difficult spots. Rinse in freshwater. Dry for 24 hours in a clean place.
3. Wrap each tile in bubble wrap and store until needed.

Photo-guide of Coral Juveniles and Other Sessile Invertebrates

Create a photographic reference guide of each park's coral juveniles and other sessile invertebrates. This guide should be assembled during the first year of monitoring and serve as a living document that is updated over time. The guide should include photographs of each coral juvenile taxon observed over the course of the year. In most cases, multiple individuals of each taxon should be

photographed to record the range of natural variation in morphology, size, and development stage for each taxon. The photos should be assembled into a printable guide or photo archived for future reference. Each photograph should be large enough to illustrate the juvenile and be accompanied by a caption detailing the taxon name, the time period the individual was collected, and an appropriate scale bar. Prior to including any photos in this guide or photo archive, confirm the taxonomic identification by using the published scientific literature or a (preferably local) taxonomic specialist. Because coral juveniles can be easily confused with other benthic sessile marine organisms, it is helpful to include examples of locally common bryozoans and foraminifera. This guide is invaluable for training new observers and confirming identifications. Please see [SOP #2 Training Observers](#) for an updated list of park taxonomic, including photographic guides. [Appendix SOP 17.c.](#) shows an example of coral juveniles and locally common other invertebrates from Hawaii. This guide can be virtual and kept online (for example in PowerPoint®) for access and revisions.

Data Verification

Prior to sending the working copy of the database to the PACN Data Manager it is important to check the plate surface locations, number of coral juveniles, juvenile measurements, and taxon identifications on at least 10% of the tile pairs. This effort equates to five tile pairs annually for each park. Typically, the marine ecologist for each park conducts the initial examination of the tile pairs. Therefore, the marine ecologist is responsible for selecting another natural resources staff member to complete the re-examination of the tile pairs.

1. After the marine ecologist has finished examining the 45 tile pairs for their park s/he randomly selects five tile pairs for re-examination by another natural resources staff member (usually a marine biotechnician).
2. Random selection can be done using the Excel® “RANDBETWEEN” function or simply picking numbers out of a hat. For example, in Excel® assign the 45 tile pairs to 45 cells and label them sequentially from one to 45 in another column. Then in a third column highlight five cells and enter the formula =Randbetween(1,5). Select the tile pairs whose number is generated in the five cells.
3. The person conducting the re-examination verifies that the correct number of juveniles have been recorded on the datasheet ([Appendix SOP 17.b.](#)) corresponding to the pre-marked tiles. In addition, taxon identification and sampling information written on tiles also needs to be validated.
4. If the measurement error is less than 10% for both the incorrect number of settlers AND the number of misidentified settlers, then proceed to Step 7.
5. If the measurement error is greater than 10%, either for the incorrect number of settlers OR the number of misidentified settlers, then the initial examiner must re-examine the remaining tile pairs.

6. New sets of five tile pairs will be subsequently selected and re-examined by the natural resources staff member until the measurement error is less than 10%.
7. All corrections should be completed on the working database copy for that park which will subsequently be sent to the PACN Data Manager by the park marine ecologist.

Appendix SOP 17.a. Equipment needed to conduct laboratory analysis for CSAs

Master equipment list is located in [SOP #1 Before the Field Season](#). If making any changes to this SOP, ensure that master equipment list is updated.

Table SOP 17.a.1. Equipment list for processing coral settlement arrays (CSAs).

Equipment (#/quantity)	Preparation & Maintenance
10% bleach solution (3-4 gal/tray)	Solution can be re-used
Trays for bleach solution (1 per 8)	One tray per 8 tile pairs is needed (e.g., Rubbermaid® 30 qt, 28.3 L – dimensions 24" x 16" x 6")
Bubble wrap (1 sheet per plate)	Used for plate storage

Table SOP 17.a.2. Equipment list for microscopic analysis of coral settlement arrays (CSAs).

Equipment (#/quantity)	Preparation & Maintenance
Dissecting microscope (6-50x)	Ensure has swing arm stand and ocular micrometer
Light source (1) and extra bulbs (2)	Ensure equipment is ready and available
Magnification table for measurements	Ensure equipment is ready and available
Lens paper and optical cleaning fluid	Ensure equipment is ready and available
Taxonomic reference sheets	Ensure equipment is ready and available
Bench counter	Ensure equipment is ready and available
Foam pad/bubble wrap (2-3)	Used for resting tile on
Blue or black fine tip permanent marker (2)	Ensure equipment is ready and available
Probe for cleaning debris on tile (2)	Ensure equipment is ready and available

Table SOP 17.a.3. Equipment list for cleaning coral settlement plates.

Equipment (#/quantity)	Preparation & Maintenance
Household vinegar (3-4 gal/tray)	Ensure equipment is ready and available
Trays for vinegar (1 per 8 tile pairs)	Ensure equipment is ready and available
Bubble wrap (1 sheet per plate)	Used for plate storage; ensure equipment is ready and available
Toothbrush (1)	Ensure equipment is ready and available
Microscope or hand lens (1)	Ensure equipment is ready and available

Appendix SOP 17.b. Datasheets used to record settlement data from terra cotta tiles

Codes for the plate surfaces are as follows: UT = Upper Top, UB = Upper Bottom, LT = Lower Top, LB = Lower Bottom, E – Edge. Enter Notes on reverse side if necessary.

Settlement Data Entry

Recorder:

Year:

[illegible]

Appendix SOP 17.c. Examples of photographs for inclusion in the settlement and other sessile invertebrates photo-guide or image archive.

Photos should include pictures of common local coral juveniles as well as photographs of benthic sessile non-coral organisms that commonly occur on settlement tiles. The identities of all photographs should be confirmed either by comparison with scientific literature or by a subject area specialist.

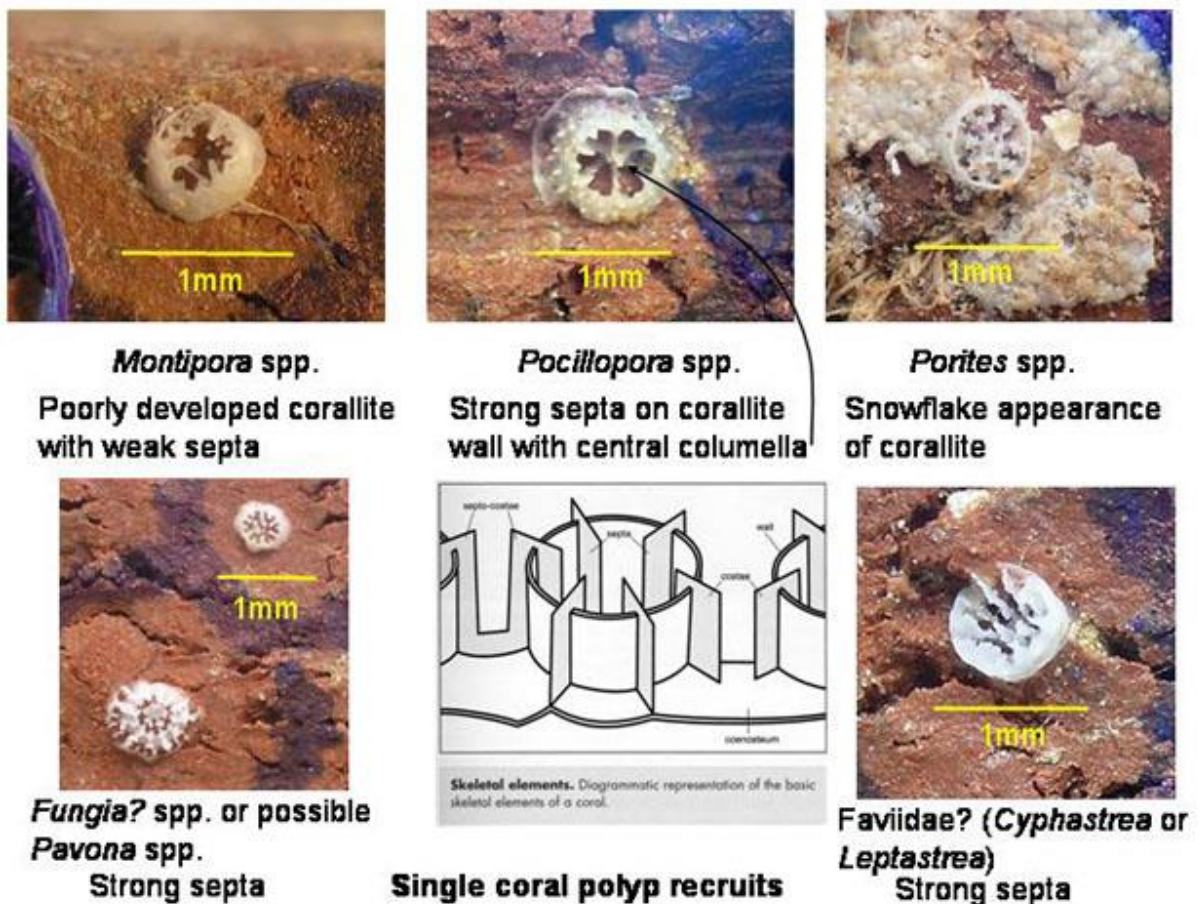


Figure SOP 17.c.1. Common coral juveniles observed on terra cotta plates in Hawaii. Photos by E. Brown, NPS.

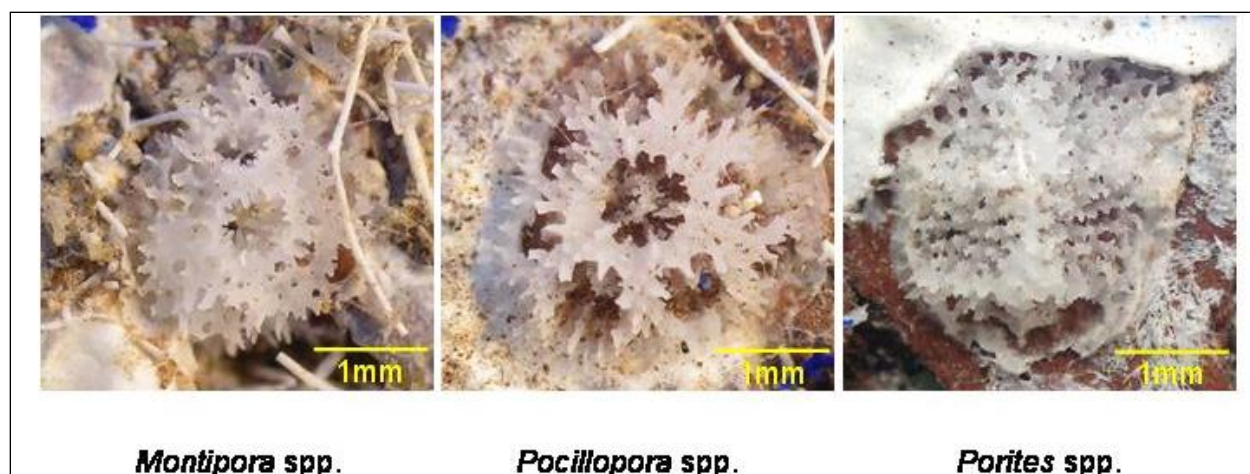


Figure SOP 17.c.2. Common juvenile coral colonies observed on terra cotta plates in Hawaii. Photos by E. Brown, NPS.

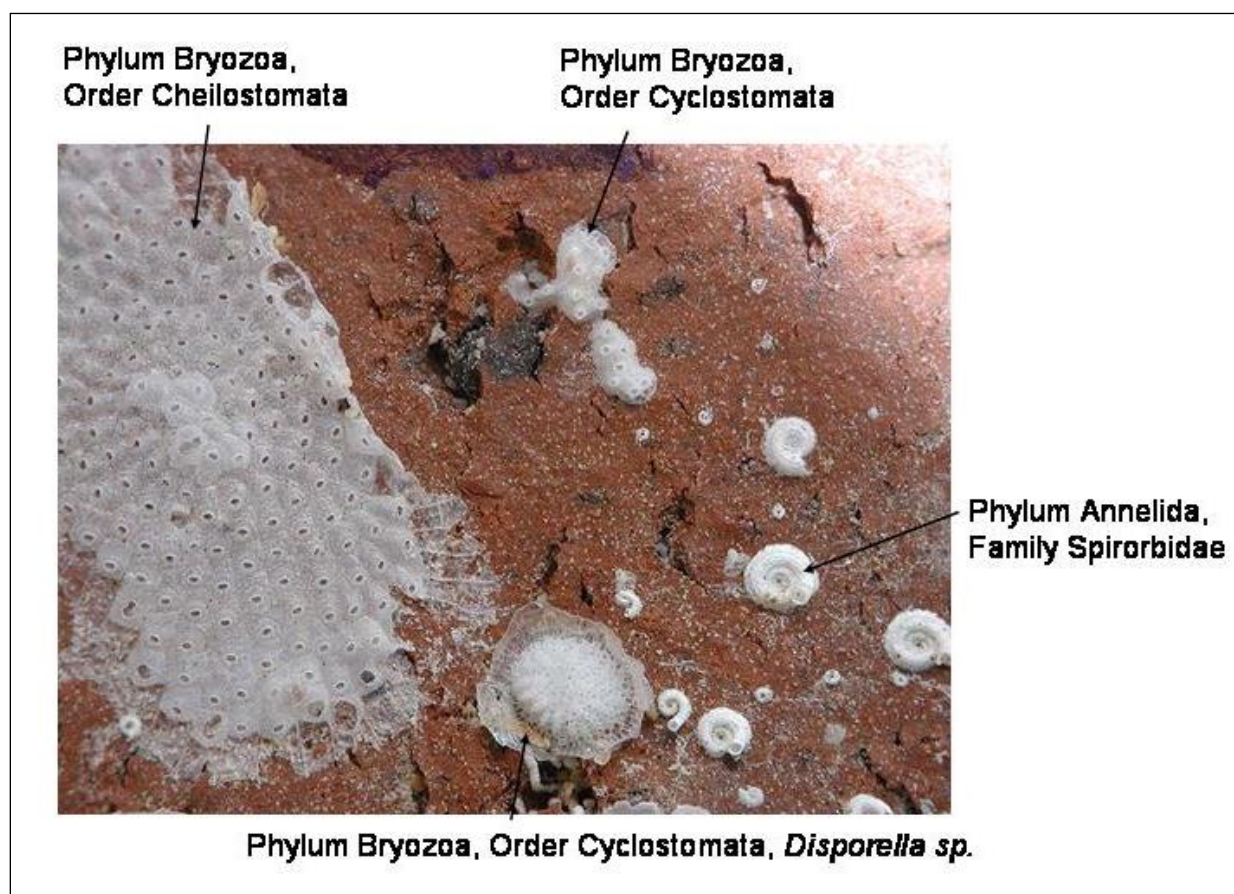


Figure SOP 17.c.3. Common benthic sessile non-coral organisms present on terra cotta plates after six months of deployment in Hawaii. Photo by E. Brown, NPS.