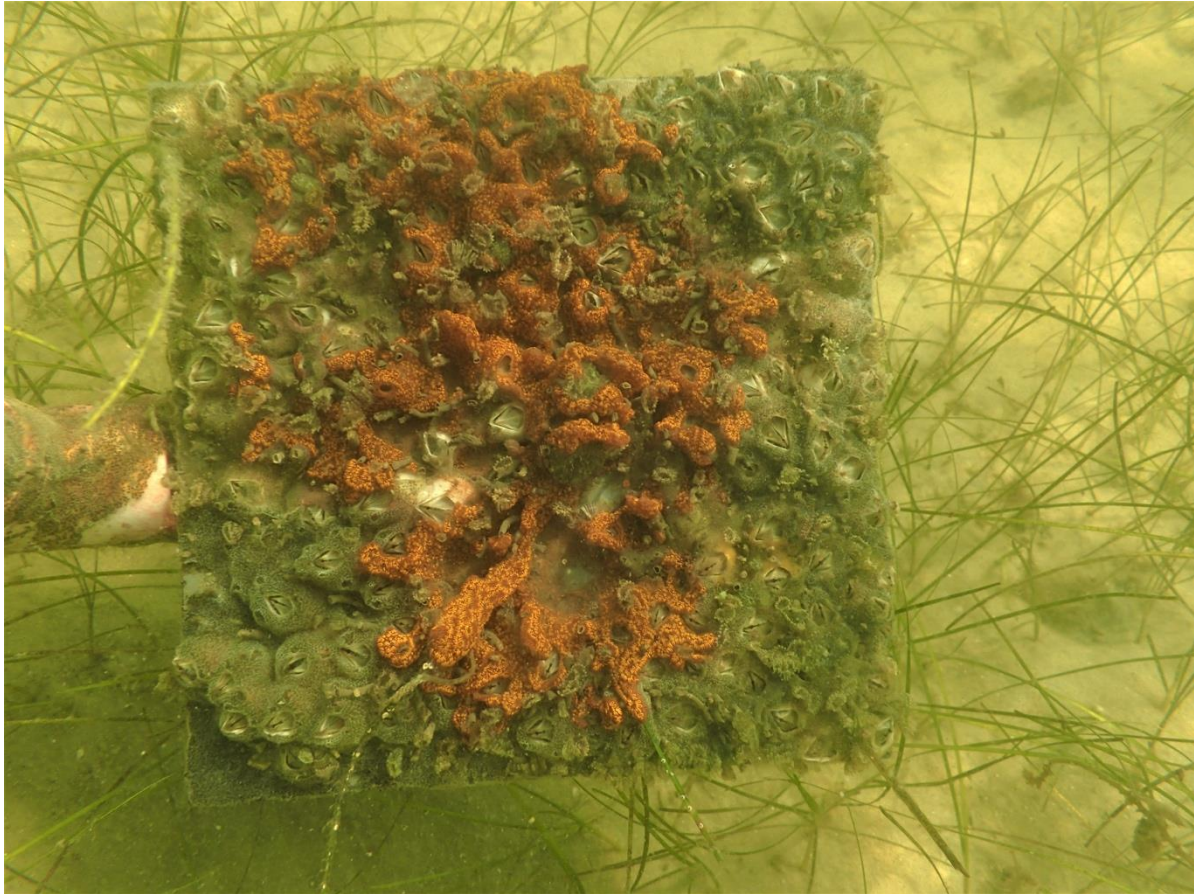


Protocol: Fouling Community Monitoring



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Smithsonian Institution

Introduction

Fouling communities are a diverse group of marine species that inhabit hard surfaces and are ubiquitous throughout coastal waters. These communities have long been used as a model system for a variety of ecological questions including community assembly, impacts of non-native species and invasion resistance, disturbance, and predator-prey interactions.

Fouling communities are commonly associated with artificial structures including docks and seawalls. By virtue of close proximity to areas of high human activities, these habitats form a hub for non-native species and in general, many of the species encountered are introduced. Given the abundance of artificial structures and hardened shorelines found throughout the world, these areas are particularly important to monitor for new introductions and range expansions.

The study of fouling communities can be extended to a variety of different habitats where hard substrate can be deployed in the form of settlement or fouling panels. The ease of standardization and deployment of fouling panels allow for unique spatio-temporal comparisons and can serve as a baseline for a variety of manipulative studies. Fouling species are generally fast-growing, short-lived and tightly tied to the environment. Continued deployments can provide useful insights into how communities change overtime with changing environmental forcing factors (e.g. temperature, rainfall, pollution, etc.).

In this document, we provide MarineGEO's standard design for fouling community monitoring. Protocols listed below are recommended for MarineGEO partners:

- Water quality
- Percent cover of fouling species during monthly intervals (30, 60, and 90 days)
- Detailed list of all sessile fauna
- Biomass of the entire community
- Identification and enumeration of associated mobile fauna (optional)

Methods

MarineGEO protocols offer a standardized set of measurements for characterizing the biodiversity of fouling communities within a locality, which provides useful and comparative data on community development over the course of a field season. Data are gathered from standardized settling plates, which allow fouling species to recruit naturally. It is recommended that within the partner site, panel deployments be prioritized for artificial substrate (e.g. local docks or marinas), and additional deployments are suggested for other habitats currently being monitored through other protocols (e.g. seagrass beds, mangroves, reefs, etc.).

Fouling community monitoring has 4 components:

1. Photographs taken of panels to assess community composition via percent cover.
2. A detailed list of sessile fauna and their origin (i.e. native or non-native) on panels after 90 days.
3. Biomass of the entire community.
4. Identification and enumeration of small mobile associated fauna (optional).

Brief Summary of Core Modules

1) Fouling panel deployment and monthly photography

Overview

Fouling panels are deployed individually ($n = 6$) per site. Each site is classified by its habitat type (e.g. dock, seagrass, mangrove, etc.) and there should be 3 sites per habitat ($n = 18$ panels per habitat). MarineGEO suggests that different habitats be used to capture as much biodiversity as possible (not all species are found at a single habitat) and as a means to compare community metrics (richness, diversity, composition, introduced species, and development) over space and time. However, if resources are limited, it is recommended that artificial habitats (e.g. docks) be first choice. The majority of data from this protocol comes in the form of photographs of the communities over time. It is vital that photographs be both clear and labeled properly to be useful for post-processing. In many cases, the identification of these species can be difficult and comparisons can be done between sites, habitats, and regions on functional or taxonomic groups, which can greatly reduce the workflow at each partner site. Please see the following sections for how to process photos using point counts to quantify community development and composition. All labeled photographs should be supplied to MarineGEO for storage and backup.

Measured Parameters

- Photographs

2) Fouling panel retrieval and post-processing

Overview

Within sub-tropical and temperate regions, 90 days is an adequate length of time to capture community development during the most productive time of the year and is reflective of a typical sampling season. In tropical locations, recruitment and growth are generally reduced, however to remain consistent, we request tropical sites to not exceed 90 days. To quantify community development, photographs are taken of communities at 30, 60, and 90 days as noted above. This section is for retrieval and post-processing at 90 days. After 90 days, panels are retrieved and brought back to the lab. In the lab, panels are first weighed to obtain a biomass of the community, useful for estimating standing stock. Panels are next rinsed in a 500 μm sieve and all mobile fauna are collected and preserved for later identification (optional). To reduce the workload during the busy summer months, panels can be individually bagged and labeled in the field and then frozen and processed at a later date.

A key question within this protocol focuses on the richness and diversity of communities between habitats and regions. To get an accurate count of the species present within these communities, panels must be examined under a dissecting microscope and destructively sampled (either freshly retrieved or frozen). Photographs are useful for obtaining percent cover but generally miss many cryptic species and any diversity indices obtained from photographs can be misleading.

Measured Parameters

- Species richness and diversity of the sessile community
- Community biomass

3) Community development and composition

Overview

Within the sub-tropical and temperate regions, fouling community development is a relatively fast-paced process. Bays and estuaries can enhance this process because of higher nutrient loads. The goal of this section is to provide a standardized process for quantifying colonization and growth of a community within a particular habitat. The species found within these diverse communities have rapid colonization rates, are fast-growing, and are useful for cross-regional comparisons. Several basic ecological processes shape these communities including both environmental factors as well as predation, and fouling communities can be easily manipulated to study a variety of mechanisms encompassing community assembly. To quantify community composition and development, photographs of panels are analyzed via point counts using a stratified random grid of 100 points per panel.

Measured Parameters

- Community development and composition using percent cover

4) Associated mobile fauna (Optional)

Overview

Although fouling communities have been used as a model system for decades, very little work has been done on the small, mobile fauna found associated within these communities. This group is similar to mesograzers found in seagrasses though unlike seagrass mesograzers, there is very little known about the role this group plays within these communities. When fouling panels are retrieved from the field at 90 days, they are individually bagged and at the lab, washed with fresh- or saltwater to remove all mobile fauna. This process is done over a 500 μ m sieve and all retained species are preserved in 70% ethanol and enumerated at a later date. The requirements for this are optional as this can be a time-consuming task and requires taxonomic knowledge of difficult fauna.

Measured Parameters

- Mobile fauna abundance and diversity

Summary Workflow

Preparation:

1. Identify and become familiar with the required modules listed above.
2. Download copies of protocols, field datasheets, and data entry templates.
3. Contact marinegeo-protocols@si.edu to schedule a brief conversation to discuss site selection and partner needs.
4. Acquire any necessary permits and/or permissions at your planned sites.
5. Review the necessary safety requirements from your institution. MarineGEO is not responsible for any loss or injury incurred during sampling.

Site Selection:

1. Identify 3 separate sites for each habitat that is planned to be used for monitoring. Sites should be typical of your region, reasonably accessible and safe, and persistent over time.
2. Contact marinegeo-protocols@si.edu to verify your sites with our team and to receive permanent site codes to be used when submitting data.

Fieldwork:

1. Acquire all necessary supplies needed to deploy 6 replicate fouling panels at each site (a list of materials needed can be found within the protocol documents).
2. Take initial detailed notes on the site that is being used including the type of habitat and any pertinent features, extent or size of habitat, depth of water and depth of fouling panels, what deployment material is being used, and other information that could be useful.
3. Measure environmental parameters (see Environmental Sampling Protocol) and note GPS location.
4. Deploy fouling panels with the experimental surface facing the seafloor. These will either be hung individually using rope or attached individually to PVC depending on the site. Each panel should be labeled so that each individual community can be followed through time. See protocol for details.
5. Photograph panels every 30 days for 3 months. If this is too time consuming, it is recommended that at least the 90-day photos be taken, as that would be the most useful time point for comparisons.
6. After 90 days, remove panels and bring them to the lab for post-processing. At the lab, panels are washed in either fresh- or seawater, and all small mobile fauna are sieved and preserved in 70% ethanol.
7. Take wet weight of panels for biomass.
8. Examine communities under a microscope and identify all species found. Vouchers and barcodes can be obtained at this time.
9. Identify and count associated mobile fauna (optional).
10. Assess percent cover for each community via point counts from photographs.

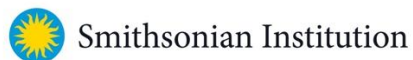
Data Submission

1. Scan the completed field data sheets and save both paper and electronic versions locally. We do not require you to submit the scanned forms.
2. Enter data into the provided data entry template. Each template is an Excel spreadsheet. Please provide as much protocol and sample metadata as possible, such as the protocol version and contact information. Use the “notes” columns to provide additional information or context if a relevant column doesn’t already exist, rather than renaming or creating columns.
3. Use our online submission portal to upload the Excel Spreadsheet:
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4. Contact us if you have any questions: marinegeo-protocols@si.edu

Fouling Community Survey



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Introduction

This protocol provides methods on deployment, retrieval, and standardized data collection for the development and composition of fouling communities. Development here is defined as colonization and growth of species over monthly intervals during the most productive season in a given region (June - August for northern temperate and tropical sites and December - February for southern temperate sites). Sites are selected based on habitat type and where other MarineGEO activities occur. It is recommended that 3 different sites be used for each habitat of interest. Habitats include but are not limited to docks or marinas (i.e. artificial habitat), seagrass beds, soft-sediment or non-vegetated, mangroves, oyster reefs, and coral reefs. It is recommended that at a minimum, docks or marinas be used as these are important for monitoring introduced species and have analogous hard surfaces similar to fouling panels. Docks are also one of the few habitats that can be found throughout the world and are therefore are useful for large scale comparisons.

Measured Parameters:

- Community development and composition
- Species richness and diversity of the sessile community
- Total community biomass
- Optional: Mobile fauna abundance and diversity

Requirements

Personnel: 1-2 people

Estimated Total Time Per Location (n = 3 sites per habitat):

Preparation: 1 person x < 1 day

Field work: 1-2 people x < 1 day per location

Post processing: 1-2 people x 5 days

Data processing: 1 person x 5 days

*Estimated times will vary by site and conditions

Replication: At least three (3) sites per habitat, the number of habitats is decided by the partner site.

Fieldwork:

- ☐ GPS
- ☐ Multiparameter sonde or similar to measure temperature and salinity
- ☐ Field sheets
- ☐ PVC fouling panels (n = 6), 13 x 13 cm, roughened with sandpaper on experimental side, thickness can vary from 0.5 – 1 cm
- ☐ Colored zip ties to identify individual panels at each site
- ☐ Camera
- ☐ Zip ties – large 8 -13"
- ☐ Rope
- ☐ Bricks
- ☐ PVC frames for benthic deployments if needed



Figure 1: Photo of a 13 x 13 cm fouling panel.

Post-Processing:

- ☐ Scissors and/or cable tie cutters
- ☐ 1-gallon sealable plastic bags or containers labeled by site and color
- ☐ Paper labels (waterproof) with site and color
- ☐ Data sheets
- ☐ Container with ice or buckets for transport
- ☐ Metal paint scraper
- ☐ Labelled vials
- ☐ Forceps
- ☐ Dissecting microscope

Methods

Fully review this and any additional protocols necessary for the sampling excursion. Address any questions or concerns to marinegeo-protocols@si.edu before beginning this protocol.

Preparation:

1. Review the MarineGEO Fouling Community Survey Design for selection of permanent sites.
2. Become familiar with GPS equipment and test the device to make sure it works.
3. Prior to deployment, weigh several clean panels to obtain an average weight of a clean panel. This weight will be used when panels are retrieved to obtain community biomass.

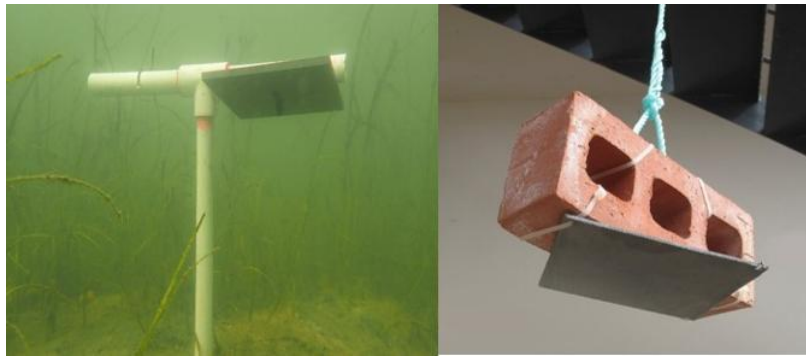


Figure 2: Photos of benthic deployment on a PVC (left) and a dock deployment using a brick (right).

Fieldwork:

Deployment in Artificial Habitats (e.g. docks and marina)

1. Attach each panel to a brick (or half brick) with a cable tie going through the panel connected tightly to the brick (Figure 2). The experimental surface of the panel should be horizontal, facing the seafloor. Attach a rope to the brick with enough line to tie down to the dock. Panels should hang at least 1 m below mean low water. It is recommended that panels be further from shore and closer to flow if possible. Panels should also be at least 0.5 m from the seafloor. *Panels can also be hung using a float or buoy if necessary.*
2. Each panel must be labeled. It is recommended that a colored cable tie be used or a label be attached for this rather than engraving the panel. Generally, a single colored cable tie will last for 90 days. Six colors would be required for 6 replicate panels per site. However, it is up to the practitioner on how panels are labeled.
3. Panels should be at least 1 m for each other.

Deployment in Benthic Habitats

1. Attach a panel to a T-shaped PCV frame (Figure 2). Frames can be assembled using $\frac{3}{4}$ " - 1" PVC pipe and glued with PVC glue to a matching tee socket. Holes large enough to fit cable ties need to be drilled into the PVC prior to deployment.
2. Each panel must be labeled. It is recommended that a colored cable tie be used for this rather than engraving the panel or marking the PVC frame, but this is up to the site.
3. Push the PVC frame into the sediment until it is secure. Panels should remain at least 0.5 m above the seafloor. Panels also need to be deep enough that they will NOT be exposed during a low tide. In some cases, the sediment is too coarse in the immediate area for deployment (e.g. reef habitat) and panels can be deployed adjacent to the habitat of interest. *As an example, for an oyster reef, the nearest location that is closest to the reef and has soft-enough sediment for deployment of frames and where panels remain subtidal during low tide. In shallow areas, floats or buoys could provide a better means of deployment.*
4. PVC frames should be deployed at least 1 m for each other. It is generally easier to find them if they are in a line parallel to the shore.



Figure 3: Field photos of each colored tag and associated panel.

Photography and Environmental Monitoring

1. Perform the following steps at 30, 60, and 90 days after the initial deployment.
2. Record environmental measurements. **See environmental measurement protocol for details.**
3. Remove the panel from the water. Shake any sediment off the panel. It is acceptable to photograph panels underwater as long as details are evident. Regardless of where photos are taken, **panels do not need to be removed from PVC frame or bricks.**
4. First photograph the colored cable tie and then photograph the panel making sure the panel fills the entire camera frame.
5. Take several photos of the panel to ensure at least one is in focus.
6. If warranted, take photos of individual species to assist in identifications.
7. Return each panel to the water. Panels do not need to be placed in previous exact location.
8. When back at the lab, pick the best photo of each panel and relabel with the site_age_cabletiecolor. Organize photos by year, site, and age.
9. See the **Fouling Community Photo Analysis protocol** for photo processing.

Retrieval After 90 Days

1. After taking 90-day photos in the field, detach panels from either the PVC frame or bricks.
2. Place the panel in labeled bags or containers with enough fresh seawater to keep moist. Each bag should be labeled on the outside and a paper label should be on the inside. The backs of the panels should be cleaned of fouling species with a paint scraper either in the lab or in the field as these are not quantified. In some cases, it may be necessary to do this in the field to reduce lab work. However, if your site will be collecting mobile fauna (see below), it is suggested to not scrape the back of each panel until back at the lab.

3. The easiest method of transport is to place all panels in a cooler with ice for travel back to the lab. In some cases, if travel time is short, panels in bags with fresh seawater can simply be placed in buckets and returned to the lab.
4. Depending on the number of panels and time available at the site, panels can either be processed live or frozen. If freezing, place the plastic bag with seawater directly in a freezer.

Post-Processing

1. Pour the contents of a single plastic bag and panel gently into a 500 μ m sieve and rinse with fresh- or saltwater. After a thorough rinsing, any material on the back side of the panel needs to be scrapped off and discarded. Do not scrape this into the sieve. Once the back material is scrapped off and discarded, re-rinse panel in the sieve. Let the panel hang vertically for 1 minute to allow water to drip off. Place the entire panel on a scale to obtain a wet weight (g). Basic food scales are inexpensive and can be used and are relatively accurate to 0.1 g. A piece of wax paper can be placed on top of the scale to prevent any excess water from dripping on the scale. Note that this weight contains the panel itself, which can be subtracted during data entry from weights obtained prior to deployment.
2. For optional small mobile fauna, wash the contents of the sieve into a vial with 70% ethanol. Place a label of the panel inside the vial as well as write the label on the outside of the vial (see Associated Mobile Fauna protocol for further details).
3. Once the panel is washed and weighed, place the panel in a dish with fresh seawater and examine it under a dissecting microscope. If panels were previously frozen, they can go into tap water. Identify all sessile species found and place any mobile fauna into associated vial. **Be careful with tube-dwelling fauna (corophiid amphipods, sabellid or serpulid worms, etc.) as these are considered to be part of the sessile community and get quantified as percent cover from the photographs.** If species are difficult to ID, at least give each a unique identifier. The overall goal is to acquire a species list and a count for total species richness and therefore, there is no need to count the number of individuals on the same panel.
4. At this point, the community can be destructively sampled to find hidden or cryptic species.
5. Take photos of unknown species. Photographs can be shared with the network for help in identifications. Also, a photo library is beneficial to have for incoming techs or students to assist in identifications. Within the first sampling year, the majority of fauna will be captured, and this makes the next season's sampling go faster.

Data Submission

1. Scan the completed field data sheets and save both paper and electronic versions locally. We do not require you to submit the scanned forms.
2. Enter data into the provided data entry template. Each template is an Excel spreadsheet. Please provide as much protocol and sample metadata as possible, such as the protocol version and contact information. Use the “notes” columns to provide additional information or context if a relevant column doesn’t already exist, rather than renaming or creating columns.
3. Use our online submission portal to upload the Excel Spreadsheet:
<https://marinegeo.github.io/data-submission>
4. Contact us if you have any questions: marinegeo-protocols@si.edu

Fouling Community Photo Analysis



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Introduction

This protocol provides standardized methods on how to best estimate percent cover via point counts from photographs taken of fouling communities.

Measured Parameters

- Percent cover of fouling panel communities

Requirements

Personnel: 1-2 people

Estimated Total Time Per Location ($n = 3$ sites per habitat):

 Data processing: 1 person x 5 days

Replication: At least three (3) sites per habitat, the number of habitats is decided by the partner site.

Recommended Software

Several programs are available to quantify percent cover and are listed below. The most useful program is CPCe, built for quantifying benthic cover though only available for Windows computers. Training sessions can be made through MarineGEO for help with this and other programs.

- [CPCe with excel extensions](#) (Windows, free)
- [Paparazzi](#) (Windows and Mac, free)
- [Image J](#) (Windows and Mac, free)
- Adobe Photoshop (Windows and Mac)
- [CoralNET](#) for automated image processing

Methods

Fully review this and any additional protocols necessary for the sampling excursion. Address any questions or concerns to marinegeo-protocols@si.edu before beginning this protocol.

Photo Processing:

1. Make sure photographs are labeled with names to indicate site_age_cabletiecolor.
 - a. Site: acronym for the site
 - b. Age: either 30, 60, or 90 days
 - c. Cabletiecolor: color of cable tie used to identify each panel
2. Photos should be cropped, and color corrected (if necessary) for light prior to analysis.
3. Place the photo in the program of choice.
4. Overlay a 100-point grid on the panel. There are several methods on the distribution or arrangement of points. It is recommended that a stratified random assortment (random but still covers all of panel) of points be placed on the panel (Figure 1).
5. Under each point, identify the species to the lowest possible taxonomic group. When filling our data sheets, we require that each species be also categorized into a taxonomic category using Table 1). If a point falls on sediment or open space, count that point as either sediment or open space. If a point falls upon a species growing on top of another (e.g. barnacles on top of oyster), count the species that is on the upper most layer. However, the user is encouraged to use their best judgement on what species should be counted that best reflects what is most dominant. Points can exceed 100.
6. All data should be entered into the supplied data templates to be uploaded to MarineGEO.



Figure 1: Estimating percent cover using a stratified random overlay.

Table 1: Taxonomic Categories

Code	Name	Details
OS	Open Space	available space for colonization
Sed	Sediment	sediment
Spg	Sponges	all families of sponges
C_H	Corals	hard and soft corals
Hyd	Hydroids	hydroids
An	Anemones	anemones
P_S	Soft tube-building polychaetes	mud- or pebble-built tubes: Sabellidae, Terebellidae, Spionidae, etc
P_H	Hard tube-building polychaetes	calcium-built tubes: Serpulidae
Oy	Oysters	oysters
Biv	Bivalves	mussels, clams, brachiopods
S_G	Sessile gastropods	Crepidula, Vermetidae, etc.
B_A	Arborescent bryozoans	upright bryozoans including soft ctenostomes
B_E	Encrusting bryozoans	encrusting hard bryozoans and soft ctenostomes
Bn	Barnacles	barnacles
Amp	Tube-building amphipods	Corophium, Eriochthonius, etc
Ech	Echinodermata	holothuroids, ophiuroids
A_S	Solitary ascidians	solitary and social forms
A_C	Colonial ascidians	colonial forms
F	Foraminifera	all forms of forams
Turf	Turf	small turf algae, various groups
Green	Green algae	all forms
Red	Red algae	filamentous, foliose non-CCA encrusting
CCA	Crustose coralline	All forms of coralline
Brown	Brown algae	All forms
Micro	Micro algae	sessile diatoms and dinoflagellates

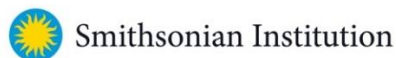
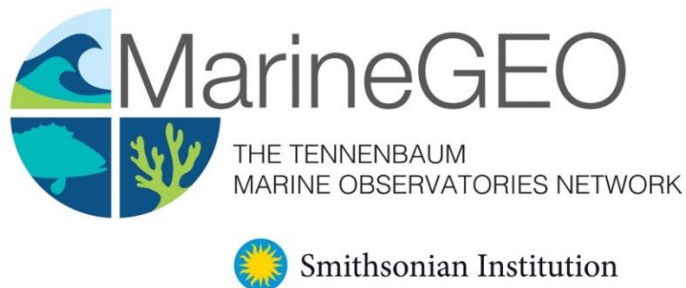
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3. Use our online submission portal to upload the Excel Spreadsheet:
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4. Contact us if you have any questions: marinegeo-protocols@si.edu

Associated Mobile Fauna (Optional)



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Introduction

Despite a lengthy history of research done on fouling communities, there exists very little data on the associated mobile fauna (non-tube building) found within these communities. Here we provide an optional auxiliary protocol to sampling this group of species in a standardized way.

Measured Parameters:

- Mobile fauna abundance and diversity

Requirements

Personnel: 1-2 people

Estimated Total Time Per Location ($n = 3$ sites per habitat):

Lab work: 1-2 people x 5 days

Data entry: 1 person x <1 days

*Estimated times will vary by site and conditions

Replication: At least three (3) sites per habitat, the number of habitats is decided by the partner site.

Materials:

- ☐ Forceps
- ☐ Dissecting microscope
- ☐ Data sheets

Methods

Fully review this and any additional protocols necessary for the sampling excursion. Address any questions or concerns to marinegeo-protocols@si.edu before beginning this protocol.

1. Please see previous protocols for methodology on how to sample mobile fauna from communities. The following steps occur after samples have been collected.
2. Pour contents of a single vial into a dish and sort all complete species or at least those with the anterior portion intact. Remove and discard all non-living material. Identify and count all mobile fauna. All species should be identified to the possible taxonomic level. However, if this is not possible, place species into lowest taxonomic categories (amphipods, polychaetes, crabs, shrimp, etc.). We recommend that each new species at least be given a unique identifier. This is important to estimate richness and diversity of the mobile community. Photos can also be taken of unknown species and shared with the network. *Be sure to ignore any tube-building fauna (e.g. corophiid amphipods, sabellid and or serpulid polychaetes).*

3. For counting purposes, only record species that are complete or at least have the anterior region present (“head-counts”). For mangled or incomplete species without the anterior region, do not count and discard. For gastropods, make sure the animal is present either by looking for the foot or crushing the shell.
4. Once enumerated, samples should be retained for potential further studies.

Data Submission

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