Protocol: Seagrass Biomass Cores

V 0.0.1

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1. Introduction

This protocol provides standardized data on seagrass biomass and infauna including *above- and belowground biomass*, and *infaunal community structure*. The information from these variables will provide insight into the belowground characteristics of the seagrass ecosystem, and how these might affect aboveground processes characterized through other surveys.

Three (3) core samples are taken along three (3) transects (total $N = 9$). Samples are post-processed to quantify above- and belowground seagrass biomass and to characterize infauna.

Additional copies of this protocol, field datasheets, data entry templates, instructional videos, literature, and more can be found on the Seagrass section of the MarineGEO protocol website: https://marinegeo.github.io/seagrass-habitat.

2. Measured Parameters

- Aboveground macrophyte biomass (mg)
- Belowground macrophyte biomass (mg)
- Infaunal abundance (individuals)

3. Requirements

Personnel: 2 persons

Time:
Preparation: 2 persons *x* 0.5 hr.
Field work: 2 persons *x* 0.5 days.
Post processing: 1 persons *x* 3 days.
Data processing: 1 persons *x* 5 hr.

Replication: 3 locations *x* 3 transects = 9 samples

Materials Checklist:

Fieldwork:
- ☐ 9 draw-string mesh bags (X mm mesh size) with internal labels
- ☐ Sediment corer (round; 10 cm diameter-*x*-20 cm length)
- ☐ 1 plastic cooler (with ice)

Post-processing:
- ☐ Pre-weighed foil tins (≥ 20)
- ☐ 1.0 mm mesh sieve
- ☐ Sorting tray
- ☐ 9+ scintillation vials (20-mL) with lids
- ☐ 70% ethanol (0.25-0.5 L)
- ☐ Petri dishes
- ☐ Forceps (fine-tip)
- ☐ Pen/pencil
- ☐ Ruler (mm)
- ☐ Drying oven
- ☐ *OPTIONAL:* Combustion furnace

4. Methods

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

4.1 Preparation

1. Place 9 internal labels written on waterproof paper with the sampling location, transect, and replicate number inside 9 mesh bags (X mm mesh size).
2. Fill a cooler with ice immediately before departing for the field.

4.2 Fieldwork

1. Sampling locations should align with one-fourth of the 36 quadrat locations as determined in the Seagrass Quadrats Protocol (link). The cores will be taken *every third* point along the transect (e.g., replicate 1, 4, 7, etc.). (Alternately, the 9 sampling locations can be determined haphazardly within the full area of the bed.)
2. At each sampling location, identify a replicate vegetated patch near the transect and select the corresponding labeled mesh bag.
3. Lower the sediment corer over the sediment surface, using your hands to guide the seagrass canopy through the opening to ensure that no seagrass blades are cut.
4. Push the corer ~10 cm into the sediment, then gently pry the corer (with sediment) up and away from the benthos. To prevent the sediment from falling out of the core, you can insert a plunger in the top to create suction, or work your hand under the corer and use it to support the sediment within the core.

5. Deposit the contents of the corer into the mesh bag, and close the opening using the drawstring. Gently agitate the sample to remove loss sediment and other fine particles, then place on ice in the cooler and transport back to the lab for processing.

4.3 Sample Processing

1. Samples are best processed within 24 hours upon returning from the field. Samples can be stored for longer in the freezer but risks damaging the organisms.
2. Use a scale to pre-weigh foil tins (either manufactured, or made by folding an aluminum foil square over on itself and sealing the sides). Record the weight of the tin directly on the foil using a pen.
3. Open a mesh bag and record the metadata from the internal label on the lab data sheet.
4. Gently rinse the contents of the bag into a shallow sorting tray with water.
5. Divide all macrophytes by species.
6. Further physically separate the seagrasses into above- and belowground components by gently pinching at the intersection of the shoots and rhizomes until they separate.
7. Place the shoots and rhizomes for each seagrass species, as well as any other macrophytes (like macroalgae), into separate pre-weighed tins and record the tin weight(s) on the lab data sheet. Be very careful that no animals are transferred with the macrophytes! This may require picking animals one-by-one out of more complex substrates.
8. Once no plant or algal material remains, pass the contents through the 1.0 mm mesh sieve. Gently rinse any loose material through the sieve, and then transfer the remaining contents to an internally and externally labeled 20-mL vial filled with 70% ethanol. Set the vial aside for processing at a later date.
9. Place the labeled foil tins containing the macrophytes in a drying oven at 60°C and dry to constant weight (usually 1-3 days, depending on the volume of material).
10. When the samples have dried thoroughly, remove the foil container from the oven, weigh to nearest mg, and record the dry mass (including foil) on the lab data sheet.
11. *OPTIONAL:* If a combustion furnace is available, combust the samples at 450°C for 4 hours. Allow the samples to cool in the drying oven, then weigh the combusted sample to the nearest mg and record the ash-free dry mass (including foil) on the lab data sheet.
12. At your leisure, transfer the contents of a 20-mL vial into a 10-cm Petri dish or similar vessel for sorting. If the sample is large, begin with a portion of the sample, and repeat until the sample is completed.
13. Prepare the sample for photographic measurement of infaunal body sizes: remove as much debris as possible and spread the animals out so they are separated on the bottom of the dish. If there are too many animals to separate clearly, return some to the vial.
14. Place a centimeter rule under or adjacent to the dish to provide a scale for measurement.
15. Take one or more digital photographs to capture all animals in the dish at sufficient resolution that their body sizes can later be measured from the image using the included scale. Photographs may be captured with a camera attached to the microscope or with a cell phone fitted with a macro lens.

16. After photographing the organisms, count all individuals of a given species. If you cannot reliably identify the taxon to species, identify it to the lowest taxon that you feel confident in, and give it a provisional name, (e.g., Nereid polychaete A).

    Note: Only species with heads should be counted (amphipods and worms); bivalves should be checked to make sure that they were alive when collected (break shell and look for tissue; check all mud tubes for inhabitants.

17. Record the taxon identification and abundance on the lab data sheet.
18. Record photographs of unidentified species at the highest magnification possible. Label these images with the sample information and provisional species name you assigned on the data sheet. These images can be used to later clarify the species' identity.
19. Return all specimens to the labeled 20-mL vial, fill with 70% ethanol, and seal for long-term storage.

4.4 Data Submission

1. Enter data into provided data entry templates (link).
2. Scan lab data sheets.
3. E-mail data entry file and scanned lab data sheets to: marinegeo-data@si.edu
4. Upload photos to XXX