Protocol: Seagrass Shoots

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MarineGEO
THE TENNENBAUM
MARINE OBSERVATORIES NETWORK

Smithsonian Institution

Introduction

This protocol provides standardized data on characteristics of the seagrass canopy and fouling/sessile (attached) organisms on the seagrass blades from shoot collections. Additional copies of this protocol, field datasheets, data entry templates, literature, and more can be found on the Seagrass section of the MarineGEO protocol website: https://marinegeo.github.io/modules/seagrass-shoots.

Measured Parameters

This assay quantifies physical characteristics of seagrass blades and the associated fouling community, measured as:

- Individual blade length (mm)
- Individual blade width (mm)
- Sheath length (mm)
- Total blade mass (mg)
- Grazing scars (number)
- Total fouling biomass (mg)

Requirements

Personnel: 2 people

Estimated Total Time Per Location ($n = 3$ transects):

Preparation: 1 person x 1 day
Field work: 2 people x 1 day
Post processing: 1 person x 3-5 days
Data processing: 1 person x 1 day

*Estimated times will vary by site and conditions

Replication: Six (6) shoot samples (1 shoot of each of the dominant species) taken along three (3) transects (total $n = 18$).

Materials:

Survey Design:

- ☐ 1 50-m metric transect tape
- ☐ Hand-held GPS unit
- ☐ 2 PVC marker poles (diameter and length as needed)

Fieldwork:

- ☐ 18 plastic bags with external and internal labels (example)
- ☐ 1 cooler with ice (optional)

Post-Processing:

- ☐ 72+ pre-weighed foil tins (example)

- ☐ Sorting tray
- ☐ Pencil/pen
- ☐ Permanent marker
- ☐ Microscope slide or other scraping instrument
- ☐ Ruler (mm)
- ☐ Drying oven

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.

Preparation:

1. Review the MarineGEO Seagrass Habitats Survey Design for site selection and setup. This protocol assumes $n = 6$ shoots taken every 8 m along a 50-m transect, replicated along 3 separate transects.
2. Label 18 disposable plastic bags with the sampling location, transect, and replicate number using a permanent marker.
3. Place 18 internal labels with the same metadata written on water-proof paper inside the corresponding plastic bag (Fig. 1).
4. Fill a container with ice immediately before departing for the field.

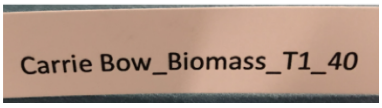

Figure 1: Example label with site (Carrie Bow), method (biomass), transect (1), and replicate (40 m).

Fieldwork:

1. At each predetermined point along the transect where the sample is to be collected, randomly select a patch ~1 m to any side of the transect. Be sure *NOT* to sample within the 0.5-0.5 m quadrat used for quantifying percent cover and shoot density, as this may affect cover and density surveys in subsequent years.
2. Use your fingers to gently break off a single seagrass shoot at the base of the shoot at the rhizome (Fig. 2), being careful not to disturb any material on the shoot. For some species this may require digging into the sediment to acquire the entire sheath. Place the shoot and any attached material into the corresponding labeled plastic bag.
3. If a quadrat contains more than 1 seagrass species, repeat this procedure for each seagrass species and store in the same labeled plastic bag.
4. Place the bag and contents on ice in the container.
5. Repeat steps 1-4 at the at the remaining 5 sampling locations along the transect.
6. Repeat steps 1-5 for the remaining two transects.
7. Transport container with samples back to the lab for immediate processing.

Post-Processing:

Samples are best processed immediately (within 24 hours) upon returning from the field. Samples can be stored for longer in the freezer but risks decay.

1. Print lab data sheets.
2. Weigh foil tins and record the weight of the tin directly on the foil using a pen. Tins can be either pre-made, or constructed by folding an aluminum foil square over on itself and sealing the sides.
3. Select a labeled bag and record the metadata on the lab data sheet.
4. Gently transfer the shoot from the bag into a shallow sorting tray without any water.
5. Separate seagrasses by species (if more than one). If any belowground material was accidentally sampled, separate by gently pinching at the meristem (the intersection of the shoots and rhizomes) and discard it.
6. For each seagrass species, select a pre-weighed tin and label with the sample metadata (replicate number, date, location) and species name (to lowest taxonomic group), and contents (fouling material).

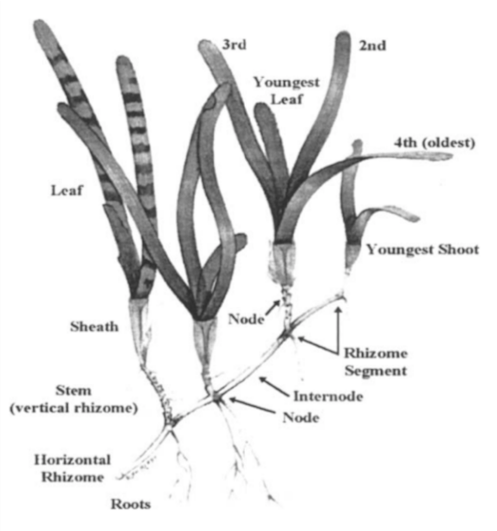

Figure 2: Morphology of seagrasses (*Cymodocea serulata* pictured). From: Short, F. T., & Coles, R. G. (Eds.). (2001). Global seagrass research methods (Vol. 33). Elsevier.

7. Lightly scrape the fouling material, including epiphytic algae and sessile invertebrates, from the surface of the blades into one of the pre-weighed tins. *Be careful that no mobile (non-sessile) animals are transferred with the scraped material. This may require picking animals one-by-one out of more complex samples.* For sites with highly abundant epifaunal communities, you can gently submerge the shoot in freshwater for 30-60 seconds to remove any mobile animals (being careful not to dislodge the attached material).
8. Next, for each shoot of each species, measure and record:
    a. The length, width, and rank of each leaf (Fig. 2); and
    b. the sheath length: from the top of the sheath surrounding the leaf bundle to the meristem (the visible constriction at the shoot base) (Fig. 2).
9. Examine each blade for any evidence of grazing scars and record the presence/absence on the lab sheet.
10. Transfer the scraped blades into a pre-weighed tin labeled with the sample metadata (replicate number, date, location) and species name (to lowest taxonomic group), and contents (blades). If the sample contains more than one species of seagrass, weigh each species in a separate tin.
11. Place all the tins (fouling material and blades) in a drying oven at 60°C. Dry samples until they register a constant weight (usually 1-3 days, depending on the volume of material).
12. Remove tins from the oven and weigh each to the nearest mg. Record this dry mass (including foil) on the lab data sheet. Note: you will have *at least* two weights per sample: fouling dry-mass, and blade dry-mass of 1 or more seagrass species.

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@si.edu