Protocol: Seagrass Shoot Collections

V 0.0.1

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1. Introduction

This protocol provides standardized data on seagrass canopy characteristics and sessile epibionts living on the seagrass blades used from **shoot collections**. The information from these variables helps characterize the quality and quantity of seagrass habitat through measurements of seagrass blades, and provide estimates of primary production. They also describe the amount of fouling material on the seagrass blade, which can inhibit photosynthesis.

Three (3) shoots of each of the dominant seagrass species are collected from twelve (12) locations along three (3) transects (total $N = 108$). Shoots are post-processed to measure leaf characteristics, fouling biomass, shoot dry mass.

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

- Blade length (mm)
- Blade width (mm)
- Sheath length (mm)
- Disease lesions (number and length in mm)
- Grazing scars (number)
- Fouling biomass (mg)

3. Requirements

Personnel: 2 persons

Time:

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

Replication: 3 shoots *x* ≥1 species *x* 12 locations *x* 3 transects = ≥108 samples

Materials Checklist:

Field:
- ☐ 36 plastic bags with external and internal labels
- ☐ 1 plastic cooler (with ice)

Post-processing
- ☐ Sorting tray
- ☐ Pre-weighed foil tins (≥ 72)
- ☐ Pencil/pen
- ☐ Microscope slide
- ☐ 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. Label 36 disposable plastic bags with the sampling location, transect, and replicate number.
2. Place 36 internal labels with the same metadata written on waterproof paper inside the corresponding plastic bag.
3. Fill a cooler with ice immediately before departing for the field.

4.2 Fieldwork

1. Sampling locations should align with the 36 quadrat locations (12 replicates *x* 3 transects) as determined in the Seagrass Quadrats Protocol (link). (Alternately, the 36 sampling locations can be determined haphazardly within the full area of the bed.)
2. At each location, use your fingers to gently break off 3 seagrass shoots of the dominant seagrass species, being careful not to disturb attached material. If the sampling location contains equal cover of >2 species, repeat this procedure for all dominant species.
3. Gently place the shoots and attached material into the corresponding labeled plastic bag.
4. Place the bag and contents on ice in the cooler.
5. Repeat steps 2-4 at the next location along the first transect until all 12 replicates are taken.
6. Repeat steps 2-5 for the remaining two transects. Transport cooler with samples back to the lab for immediate processing.

4.3 Sample Processing

1. Samples are best processed immediately upon returning from the field. However, samples can be stored for up to 24 hours in the refrigerator; any longer risks decay.
2. Print lab data sheets.
3. 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. At least 36 tins are required, although more may be necessary depending on how many species were sampled in the bed.
4. Select a labeled bag and record the metadata on the lab data sheet.
5. Gently transfer the shoot from the bag into a shallow sorting tray without any water.
6. Separate seagrass above-ground biomass from any below-ground biomass by cutting the shoot where color changes from green (above-ground) to white/clear (below-ground). Discard any below-ground material.
7. Use the microscope slide to lightly scrape attached material from the surface of the blades.
8. Label one of the pre-weighed tins in pen, record its weight on the datasheet, and transfer into it all fouling material that is removed.
9. From each shoot of each species, measure the length and width of the single *longest* leaf and record on the lab data sheet for that sample.
10. Record the sheath length from the meristem (visible as a constriction at base of shoot) to the top of the sheath surrounding the leaf bundle for each shoot (Figure 1).
11. Examine the blades for any evidence of disease lesions (Figure 2). Record the presence and measure the length in mm of the largest lesion.
12. Examine the blades for any evidence of grazing scars, and record the number on the lab data sheet.
13. Transfer the blades of each species into labeled pre-weighed tins and record the tin weight(s) on the lab data sheet for that sample.
14. Place all the labeled foil tins (blades and epibionts) in a drying oven at 60°C and dry to constant weight (usually 1-3 days, depending on the volume of material).

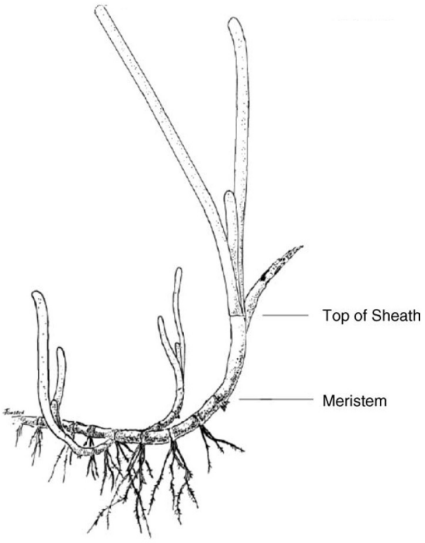

Figure 1. Morphology of an eelgrass (*Zostera marina*) shoot, showing leaves and sheath. Adapted from Gaeckle et al. (2006).

Figure 2. Example of diseased lesions on the blades of eelgrass (*Zostera marina*). From Ralph & Short (2002).

15. 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.
16. *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.

4.4 Data Submission

1. Enter data into provided data entry templates.
2. Scan lab data sheets.
3. E-mail data entry file and scanned lab data sheets to: marinegeo-data@si.edu