MarineGEO
THE TENNENBAUM MARINE OBSERVATORIES NETWORK

Protocol: Seagrass Shoot Collections

V 0.0.1

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1. Introduction

This protocol provides standardized data on characteristics of the seagrass canopy and sessile (attached) organisms on the seagrass blades from **shoot collections**. The density and lengths of seagrass blades provide information on the quality and quantity of seagrass habitat for animals, and measurement of sheath length provides a proxy estimate of leaf growth rate (primary production). The protocol also measures the amount of fouling material on the seagrass blades, which can inhibit photosynthesis and provide food for animals.

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 mass, 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 (per seagrass species) 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 (https://marinegeo.github.io/modules/seagrass-quadrats). (Alternatively, 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 (or uproot) 3 seagrass shoots of the dominant seagrass species at the sediment surface, being careful not to disturb attached material. If the sampling location contains equal cover of >2 seagrass species, repeat this procedure for each dominant seagrass 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 balance 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 (available from MarineGEO GitHub website).
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. If

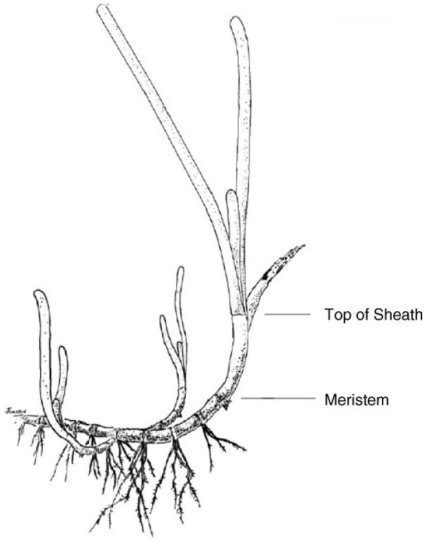

Figure 1. Morphology of an eelgrass (*Zostera marina*) shoot, showing leaves and sheath. Adapted from Gaeckle et al. (2006, Aquatic botany 843:226-232).

Figure 2. Example of diseased lesions on the blades of eelgrass (*Zostera marina*). From Ralph & Short (2002, Marine Ecology Progress Series 226: 265-271).

possible, record photos of representative grazing scars (e.g., with a phone camera)

13. Transfer the scraped blades into a labeled pre-weighed tin and record the tin weight(s) on the lab data sheet for that sample. If the sample contains more than one species of seagrass, weigh each in a separate tin.
14. Place all the labeled foil tins (blades and fouling material) in a drying oven at 60°C and dry to constant weight (usually 1-3 days, depending on the volume of material).
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 remaining ash mass (including foil) on the lab data sheet. Ash-free dry mass (an estimate of organic matter in the sample) can be calculated by subtracting the ash mass from the dry mass.

4.4 Data Submission

1. Enter data into provided data entry template (https://marinegeo.github.io/modules/seagrass-shoots).
2. Scan the completed lab data sheets and save both paper and electronic versions.
3. E-mail data entry file and scanned lab data sheets to: marinegeo-data@si.edu