MarineGEO
THE TENNENBAUM
MARINE OBSERVATORIES NETWORK

Seagrass Biomass

v 0.1.0

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Introduction

This protocol provides data on seagrass biomass and infaunal community structure. These data helps MarineGEO and our partners characterize the seagrass ecosystem; additionally, the data can be connected to other aboveground processes characterized by other surveys.

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.

Measured Parameters

This assay quantifies seagrass biomass and infaunual community structure, measured as:

- Above-ground macrophyte biomass (mg)
- Below-ground macrophyte biomass (mg)
- Infaunal abundance (individuals)

Requirements

Personnel: 2 persons

Time: Preparation: 2 people x 0.5 hours
Field work: 2 people x 0.5 days
Post processing: 1 person x 3 days
Data processing: 1 person x 5 hours

Replication: Six (6) core samples are taken along three (3) transects (total N = 18)

Materials Checklist:

Fieldwork:

- ☐ 18 draw-string mesh bags (~1 mm mesh size)
- ☐ Waterproof paper (for internal labels)
- ☐ Sediment corer (round; 10 cm diameter-x-20 cm length)
- ☐ 1 plastic cooler (with ice)

Post-processing:

- ☐ 20+ pre-weighed foil tins
- ☐ 1.0 mm mesh sieve
- ☐ Sorting tray
- ☐ 18+ scintillation vials (20-mL) with lids
- ☐ 70% ethanol (0.25-0.5 L)
- ☐ Petri dishes
- ☐ Forceps (fine-tip)
- ☐ Pen/pencil
- ☐ Ruler (mm)
- ☐ Drying oven
- ☐ *RECOMMENDED* – Combustion furnace

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. Identify sampling scheme. If following the MarineGEO survey design, review the materials here (6 replicates *x* 3 transects = 18 replicates total). Alternately, samples can be taken haphazardly within the bed (if done, record GPS coordinates of each sample)
2. Place 18 internal labels written on waterproof paper with the sampling location, transect, and replicate number inside each mesh bag
3. Fill a cooler with ice immediately before departing for the field

Fieldwork:

1. At each sampling location, identify a vegetated patch at the corresponding point the transect (if following the MarineGEO survey design) and select the corresponding labeled mesh bag.
2. Place the sediment corer over the sediment surface. Guide the seagrass below the corer through the corer opening to ensure that no seagrass blades are cut
3. Push the corer into the sediment ~10 cm
4. Gently pry the corer 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. Alternatively, work your hand under the corer and use it to support the sediment within the corer as you lift up
5. Deposit the contents of the corer into the mesh bag. Close the opening using the drawstring. Gently agitate the sample to remove sediment
6. Repeat steps 2-5 at the at the remaining 5 sampling locations along the transect

7. Repeat steps 2-6 for the remaining two transects
8. Place on ice in the cooler and transport back to the lab for processing

Post-processing:

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 and making them difficult to identify

Step 1: Macrophyte dry-mass

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. Gently rinse the mesh bag to remove any loose sediment. Open the bag and record the metadata from the internal label on the lab data sheet
4. Gently transfer the contents of the bag into a shallow sorting tray with water
5. Sort all seagrasses and macroalgae by species
6. Separate seagrasses into above- and belowground components by gently pinching at the meristem (the intersection of the shoots and rhizomes) until they separate (Fig 1)
7. For each seagrass species, select a pre-weighed tin and label with the sample metadata (replicate number, date, location), species name (to lowest taxonomic group), and contents (above- or belowground material). Place the macrophytes into the corresponding tins. For each non-seagrass macrophyte species (e.g., unrooted macroalga), place entire individuals into labeled tins
    - *Be careful that no animals are transferred with the macrophytes*. This may require picking animals one-by-one out of more complex substrates
8. For each taxon sorted above: record the sample data, species name, and the empty tin weight on the lab data sheet
9. Once no plant or algal material remains in the sorting tray, pass the contents through a 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. Multiple vials may be required for larger samples or organisms. Set the vial aside for infaunal processing at a later date
10. Repeat for each sample
11. Place tins containing macrophytes into a drying oven. Dry at 60°C to constant weight (usually 1-3 days, depending on the volume of material)

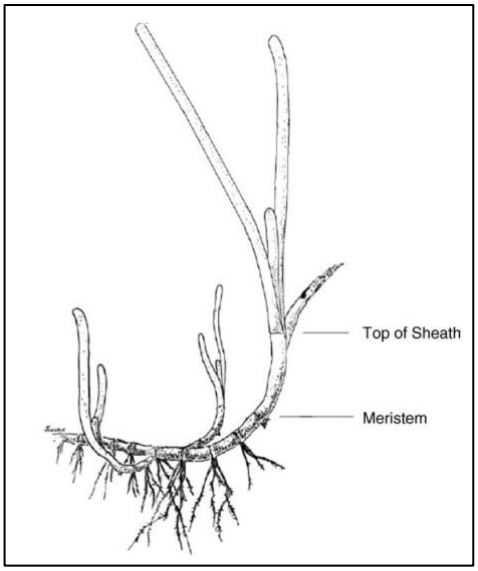

Figure 1. Morphology of an eelgrass (*Zostera marina*) shoot, showing leaves and sheath. *Adapted from: Gaeckle et al. (2006),* Aquatic Botany *843:226-232.*

12. Once dried, remove all tins from the oven and weigh to nearest mg. Record this weight (including tin weight) on lab data sheet
13. *RECOMMENDED* – 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

Step 2: Infaunal community

This step can be done at your leisure as organisms are now preserved in ethanol (Step 1, #8)

Note: to prevent overestimating abundances, only count individuals *with heads intact*. "Headless" organisms such as bivalves and tube dwellers should be checked to ensure that they were alive when collected by opening the covering and looking for intact tissue

1. Print lab data sheets
2. Transfer the contents of one of the 20-mL vials with infauna (Step 1, #8) into a 10-cm Petri dish or similar vessel for sorting
3. Record the sample information on the lab datasheet
4. Prepare the sample for photographic measurement of infaunas' body sizes by removing as much debris as possible from the Petri dish
5. Spread the animals out so they are separated from one another. If there are too many animals to separate clearly, return some to the vial
6. Place a centimeter ruler under or adjacent to the dish to provide scale.
7. 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. Photographs may be captured with a camera attached to the microscope or with a cell phone fitted with a macro lens
8. Identify each taxon in the sample to species and record the species name on the lab datasheet

   If you cannot reliably identify a taxon to species, identify it to the lowest taxonomic group that you feel confident. Then, give it a provisional name (e.g., Nereid polychaete A). Photograph unidentified species and label image file names with the sample information and the provisional species name you assigned on the data sheet. These images can be used to later clarify the species' identity. *Be sure to maintain the naming scheme for all future samples* (especially if samples are processed by different people)
9. Count and record the number of individuals for each species.
10. Return all specimens to the labeled 20-mL vial, fill with 70% ethanol, and seal for long-term storage

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Data Submission

1. Enter data into provided data entry templates

2. Scan the completed lab data sheets and save both paper and electronic versions
3. E-mail data entry file, any photos, and scanned lab data sheets to: marinegeo-data@si.edu