Protocol: Seagrass Epifauna (Mesh Bags)

V 0.0.1

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1. Introduction

This protocol provides standardized data on seagrass mobile epifauna including *community composition*, *diversity*, and estimates of *abundance*/*biomass* and/or *secondary production*. The information from these variables will help link plant characteristics to the animal community, and how these properties affect higher trophic levels.

Six (6) grab samples are taken using mesh bags along three (3) transects (total $N = 18$). Samples are post-processed to quantify seagrass biomass and estimate the biomass/secondary production of mobile epifauna.

In post-processing, we recommend using a series of stacked sieves of increasingly smaller mesh sizes. The method from Edgar (1990. JEMBE 137:195-214) provides an accurate and non-destructive alternative to obtaining estimates of both ash-free dry mass and secondary productivity from size-fractionated abundances. Alternately, you can identity, count, and measure (using photographs) each individual of each species. Both sets of instructions are provided in this guide.

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

- Macrophyte biomass (mg)
- Faunal abundance (individuals)
- Faunal biomass (mg or mg ash-free dry mass)

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* 30 days.
Data processing: 1 persons *x* 10 hr.

Replication: 6 locations *x* 3 transects = 18 samples

Materials Checklist:

Field:
☐ 18 draw-string mesh bags (500 µm mesh size, dimensions: x cm *x* x cm and *x* cm opening)
☐ Waterproof paper for internal labels
☐ 1 plastic cooler (with ice)

Post-processing:
☐ 20+ pre-weighed foil tins
☐ 500 µm mesh sieve
☐ Sorting tray
☐ 20+ scintillation vials (20-mL) with lids
☐ 70% ethanol (0.5-1 L)
☐ Petri dishes
☐ Forceps (fine-tip)
☐ Pen/pencil
☐ Drying oven
☐ *OPTIONAL*: sieve tower with appropriate mesh sizes (8.0, 5.6, 4.0, 2.8, 2.0, 1.4, 1.0, 0.71 and 0.5 mm)

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 18 internal labels written on waterproof paper with the sampling location, transect, and replicate number inside 18 mesh bags (500 µm mesh size).
2. Fill a cooler with ice immediately before departing for the field.

4.2 Fieldwork

1. Sampling locations should align with half of the 36 quadrat locations as determined in the Seagrass Quadrats Protocol (link). The faunal samples will be taken *every other* point along the transect (e.g., replicate 1, 3, 5, etc.). (Alternately, the 16 sampling locations can be determined haphazardly within the full area of the bed.)
2. At each sampling location, first select the corresponding labeled mesh bag.

3. Position the mesh bag over the canopy and gently lower it over the seagrass, being careful not to disturb or dislodge any organisms or macroalgae. It may be necessary to move the bag from side-to-side to aid the shoots in passing through the opening.
4. Once the opening of the bag is *just above* the surface of the sediment, close the bag by pulling the drawstring and either cut or tear the exposed shoots to release them into the bag.
5. Invert the bag and move it up through the empty water column to flush the contents fully into the bag, and then tie the mesh into a knot to prevent the drawstring from opening.
6. Place the bag and contents on ice in the cooler.
7. Repeat steps 2-6 at the next location along the first transect until all 6 replicates are taken.
8. Repeat steps 2-7 for the remaining two transects for a total of 18 samples.
9. Transport cooler with samples 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. Separate macrophytes (e.g., seagrass, macroalgae) by species and place into separate pre-weighed tins and record the tin weight(s) on the lab data sheet for that sample. Be very careful that no animals are transferred with the macrophytes! This may require picking animals one-by-one out of more complex substrates.
6. Once no plant or algal material remains, pass the contents through the 500 μm mesh sieve. Gently rinse any loose material through the sieve, and then transfer the remaining contents to a labeled 20-mL vial filled with 70% ethanol. Set the vial aside for processing at a later date.
7. 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).
8. 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.

<u>If you have a sieve tower, conduct *Sieve Processing*; if not, conduct *Photographic Sizing*.</u>
<u>Conduct only one of the two, not both.</u>

Sieve Processing

9. Stack the sieves from smallest on the bottom to largest on top (0.5, 0.71, 1.0, 1.4, 2.0, 2.8, 4.0, 5.6, 8.0 mm).
10. Select a sample vial and gently invert it over the top sieve, allowing the contents to pass onto the sieve tower.

11. Use a hose or squirt bottle to gently rinse out the vial with water and empty its contents into the sieve tower until it is clean.
12. With the same water source, gently rinse the animals down the sieve tower. Take care not to use too much pressure (it may damage the specimens).
13. Transfer the contents of each sieve into a separate labeled Petri dish filled with 70% ethanol. A squirt bottle of 70% ethanol can be used to gently rinse the animals into the dish. Forceps will be useful in extracting some of the smaller or more articulated organisms.
14. Identity and count the number of species in each sieve size class, and record this information (including sieve size) on the lab data sheet.

    Note: Only species with heads should be counted (amphipods and worms).

15. 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.
16. Return all specimens to the labeled 20-mL vial, fill with 70% ethanol, and seal for long-term storage.

Photographic Sizing

9. 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.
10. Prepare the sample for photographic measurement of epifaunal 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 position so that none are touching, return some to the vial to clear space.
11. Place a centimeter rule under or adjacent to the dish to provide a scale for measurement.
12. 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.
13. 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).

14. Record the taxon identification and abundance on the lab data sheet.
15. Place any unidentified species in a separate vessel and photograph 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.
16. Return all specimens to the labeled 20-mL vial and seal for long-term storage.

4.4 Data Submission

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