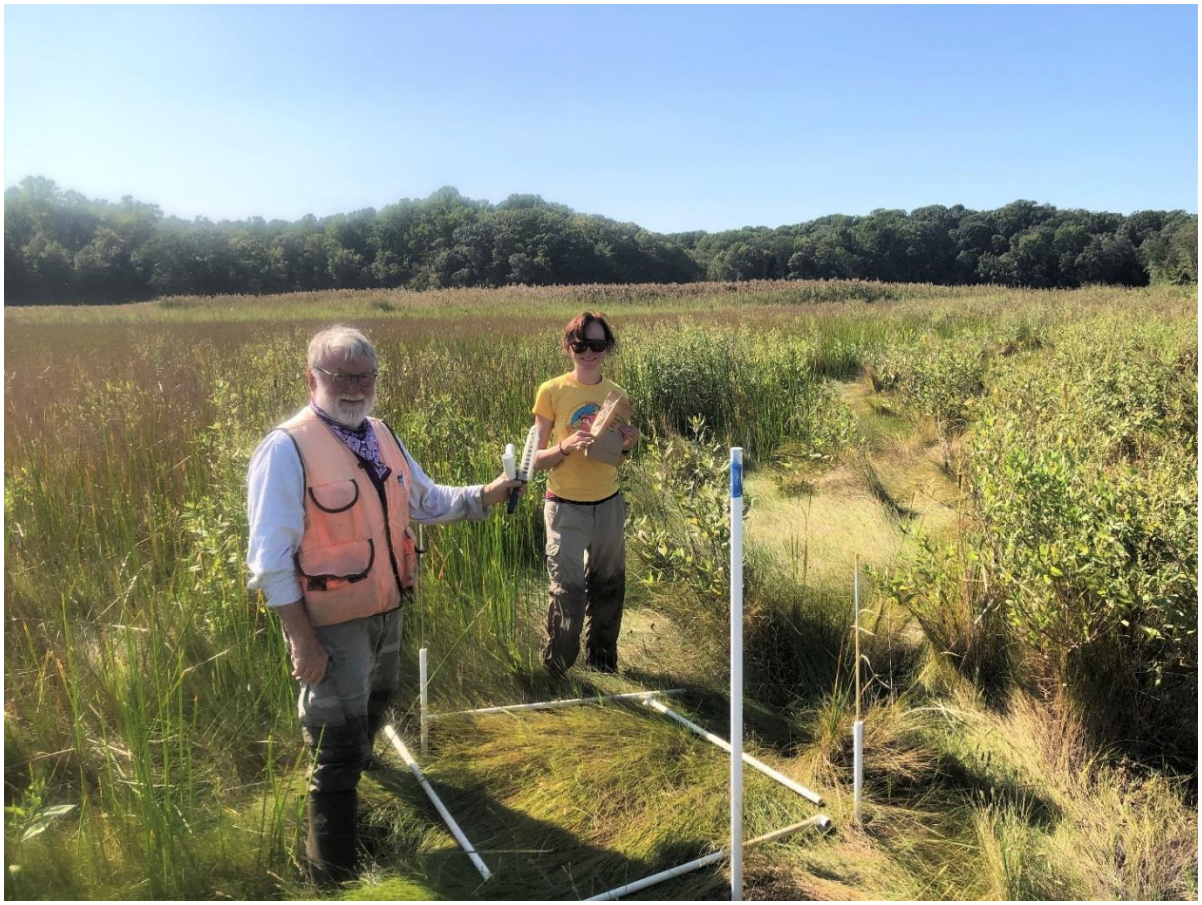
MarineGEO Salt Marsh Habitat Monitoring Protocol



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Background

Coastal salt marshes are biogenic wetland habitats defined by regular inundation with salt water from tides. Plant species diversity is typically low and comprised of salt and submersion-tolerant species. Salt marshes provide a variety of services including erosion protection, essential habitats for fish and invertebrates, nutrient filtering, and carbon sequestration. Principal threats include coastal development, accelerating sea level rise, and pollution from land-based sources. Salt marshes have a near-global distribution although they are most prevalent along protected shorelines in the mid to high latitudes. Given their widespread occurrence and position at the land-sea interface, salt marshes are ideal ecosystems to examine responses to global change.

This document provides an overview of MarineGEO's standardized methodology for estimating key ecological parameters in salt marshes including plant species composition, above-ground primary productivity, infaunal and epifaunal diversity and abundance, consumption rates, and sediment organic matter. Also provided are site selection and establishment procedures and an integrated workflow. Marsh vegetation surveys are conducted during the period of annual maximum standing biomass in the late summer or early fall. Prior to data collection, marsh transects must be selected and plots established for permanent sampling.

We thank Merryl Alber, Matt Ferner, and Christine Angelini for thoughtful feedback on the initial draft of these protocols.

Protocols

Core protocols below are **required** for MarineGEO partners:

- Sampling Event & Environmental Monitoring
- Marsh plant species cover and allometry (biotic and abiotic cover, species composition, stem widths, stem heights, live stem density)
- Saltmarsh fauna (infauna/epifauna species composition, infauna/epifauna abundance, crab burrow counts)
- Sediment organic matter (bulk density, organic carbon)
- Predation Assay (bait loss, "Squidpops")

Recommended protocols:

- Beach seine, Trawl, and/or Visual census (mobile fish and invertebrate abundance, length, composition)

Requirements

Personnel: 3 people

Estimated Total Time Per Marsh Site (*n* = 3 transects)

Preparation: 1-person x 1 day
Fieldwork: 3 people x 1 day
Sample Post-processing: Variable - see individual protocols
Data processing: 1-person x 1-2 days

Materials:

- ☐ 150 cm length of PVC (15/marsh)
- ☐ 80 cm length of PVC (30/marsh)
- ☐ Aluminum tags for quadrat identification (15/marsh)
- ☐ Handheld GPS unit
- ☐ 50-meter transect tape
- ☐ Small cable ties (15/marsh)
- ☐ All materials from core and recommended modules (see individual protocols)

Workflow

Preparation

1. Using a stamp tool or similar, inscribe unique identification codes onto the metal tags for every plot to be established (*n* = 45) (Ex: marsh A, transect 1, plot 1 = “A1-1”) (Fig. 2).

Site Selection and Establishment

1. Identify 3 salt marshes, each in separate bays or sub-estuaries, for permanent sampling. Each marsh should be typical of your region, reasonably accessible, at least 60 meters long (along shore) and 40 meters wide (perpendicular to shore)
   - Transects should run approximately parallel to the dominant environmental gradient (e.g., tidal elevation, salinity) (Fig. 1).
2. Within each marsh, identify 3 transect locations containing 40 linear meters of vegetated area perpendicular to shore and separated from one another by at least 20 meters.
   - If marshes in your area are narrower than 40 m, contact MarineGEO (marinegeo-protocols@si.edu) for help developing an alternative transect design.

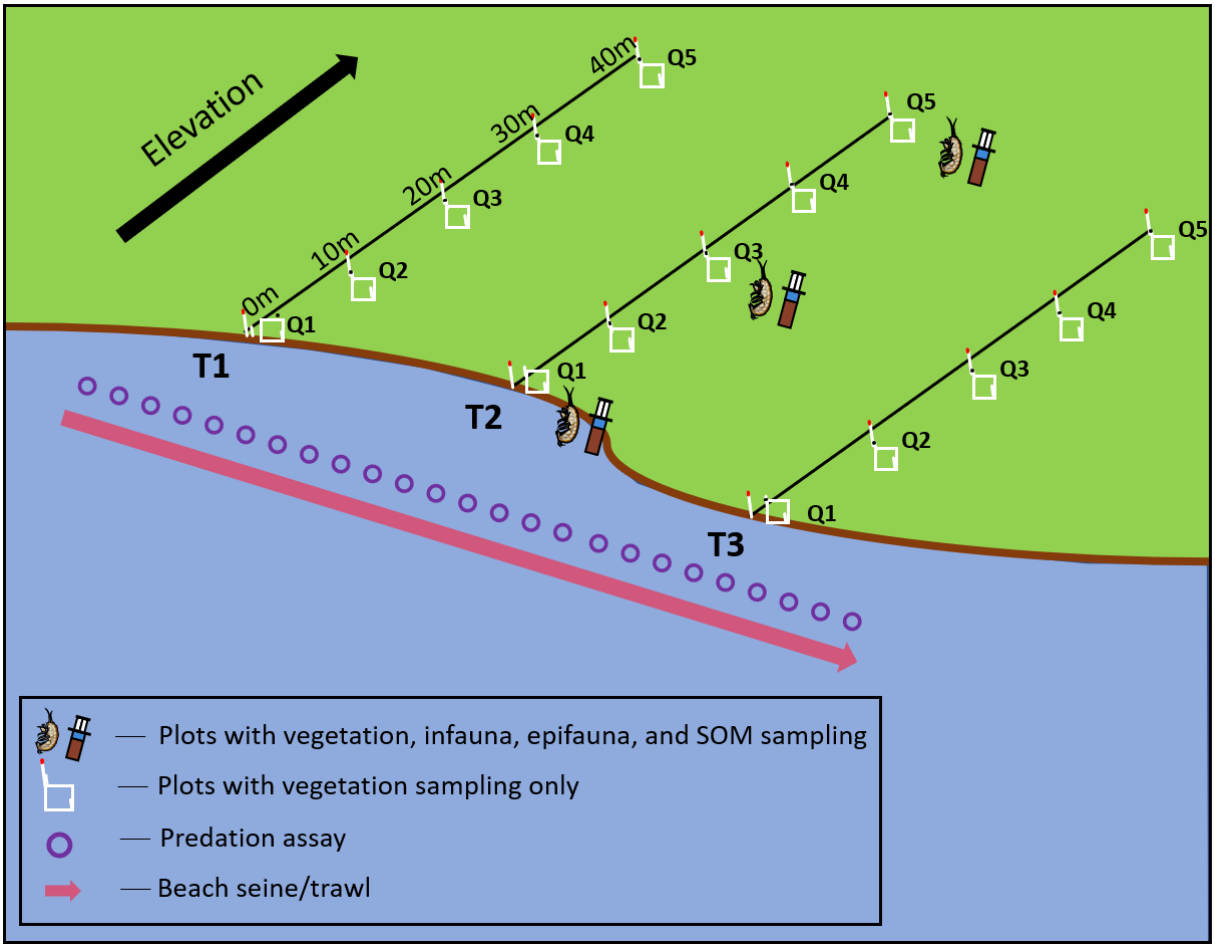

Figure 1. Sampling diagram of a single marsh site showing replicate transects and relative placement of surveys for core and recommended modules. Note that transects are separated by a minimum of 20 meters and quadrats are spaced at 10-meter intervals.

3. At each transect location, measure 40 meters perpendicular to shore heading into the marsh using a transect tape. Beginning at the shore (0 m), mark plot locations every 10 meters on the righthand side of the tape (facing away from shoreline) using 150 cm PVC sight poles to mark the near corner of each plot (Fig. 1; Fig. 2). Record plot coordinates with a handheld GPS unit.
    - If a 10-meter increment falls over a tidal creek or other non-living obstacle (e.g. boulder, un-vegetated mud flat, large piece of wrack), make note and reposition the plot as close as possible to the original location on the far side of the obstacle along the transect, heading away from shore.
4. Mark 2 diagonal corners quadrats of each 1m x 1m quadrat with 80 cm lengths of PVC driven into the marsh (Fig. 2).
5. Using a small cable tie, attach an aluminum tag engraved with respective transect and plot numbers to the PVC corner post adjacent to the sight pole in each plot (Fig. 2).

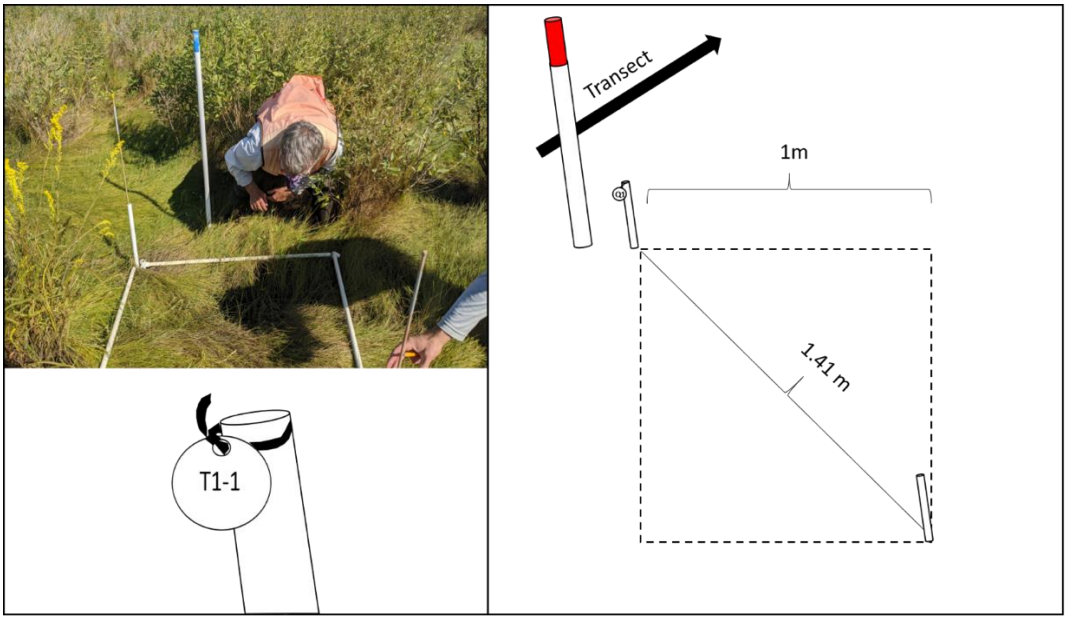

Figure 2. Diagram of a 1m^2 plot with painted PVC sight pole, diagonal corner posts, and engraved metal identification tag attached to a corner post.

Fieldwork Overview: Vegetation, invertebrates, sediment organic matter (SOM)

1. At each site, first record site metadata and measure environmental conditions using the Sampling Event and Environmental Monitoring Protocol.
2. At each plot, conduct vegetation sampling (*Marsh plant species cover and allometry protocol*).
3. For plots with invertebrate and SOM samples (1st, 3rd, 5th plot of the middle transect of each marsh site; Fig.1), conduct epifaunal surveys, take infaunal cores, and take SOM samples following the respective MarineGEO protocols.
4. Repeat for all transects and marsh sites.

Fieldwork Overview: Predation assay, beach seines/trawls

1. Once per field season (late summer/early fall) conduct a predation assay at each marsh site (*n* = 25 'squidpops').
   - Position squidpops roughly every 2m, running along shore, perpendicular to vegetation transects (Fig. 1).
2. *RECOMMENDED:* After retrieving squidpops, quantify fishes and large mobile invertebrates by conducting either a beach seine or trawl along shore in front of marsh vegetation transects (Fig. 1)

Sample post-processing:

1. Vegetation surveys (marsh plant species cover and allometry) require no post-processing.
2. Infaunal cores should be pre-processed within 24-h. Once preserved, infauna may be processed at leisure.
3. Sediment organic matter samples should be processed within 1-3 days.

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. 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-protocols@si.edu

Marsh Plant Species Composition and Allometry

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Introduction

Long-term monitoring of salt marsh plant species composition and above-ground biomass provides information on the condition of a salt marsh and the quality of ecosystem functioning. Over time, such data allows for analysis of community shifts in the context of changing environmental conditions. This protocol provides standardized methods for estimating plant cover, species composition, and annual production of above-ground biomass. Allometric equations used to estimate biomass will need to be adapted or developed for species present at each MarineGEO location conducting salt marsh monitoring. Contact marinegeo-protocols@si.edu to discuss adapting this protocol to your local marsh species.

Measured Parameters:

- Percent cover
- Stem density, height, basal diameter, stem diameter at 40 cm (stems/m^2, cm, mm, mm)

Requirements

Personnel: 3 people

Estimated Total Time Per Location (*n* = 3)

Preparation: 1-person x 1-2 hours
Fieldwork: 3 people x 1 day per marsh
Post processing: 1-person x 0.5 day
Data processing: 1-person x 0.5 day

Replication: 5 replicate quadrats per transect, 3 replicate transects per marsh, 3 marshes per region

Materials:

Fieldwork

- ☐ 1 m x 1 m collapsible PVC quadrat
- ☐ 10 cm x 10 cm fixed PVC quadrat
- ☐ 150 mm dial caliper (2)
- ☐ 3 m folding ruler (3)
- ☐ Species cover datasheets
- ☐ Species allometry datasheets
- ☐ Hand-held GPS pre-loaded with transect and plot coordinates
- ☐ Permanent markers

Methods

Fully review this and any additional protocols necessary for the sampling excursion. Address any questions or concerns to marinegeo-protocols@si.edu before beginning this protocol.

Preparation:

1. Review and complete salt marsh survey design protocol.
2. Review and print this protocol.
3. Print datasheets on waterproof paper.
4. Plan to sample at low tide when marsh is not inundated.
5. Surveys are to be conducted during the period of annual maximum standing biomass in the late summer or early fall. All vegetation surveys should be conducted within a period of 2-3 weeks.

Fieldwork Part 1: Species composition

1. Once the first plot in a transect is located, position the 4 PVC segments of the collapsible 1 m^2 quadrat within the diagonal corner posts of the plot, making sure to maintain 90° angles at each corner (Fig. 1).
2. Identify all plant taxa rooted within or overhanging the plot to the species level.
    - Visually estimate the two-dimensional cover of all species and assign into discrete cover-class bins (Table 1).
3. It may help to estimate cover by subsections of plot and combine for a final estimate
4. Estimate non-living cover of the following categories:
    - Surface litter (leaf litter)
    - Standing litter
    - Bare substrate
    - Open water
    - Wrack
5. If there has been a physical disturbance within the plot (e.g. animal digging, fallen tree, shoreline erosion) make a note on the datasheet.

Table 1. Modified Braun-Blanquet cover class bins and corresponding percentages.

Cover Class Bin	Cover (%)
0	0
+	<1
1	1-5
2	6-25
3	26 -50
4	51-75
5	76-100

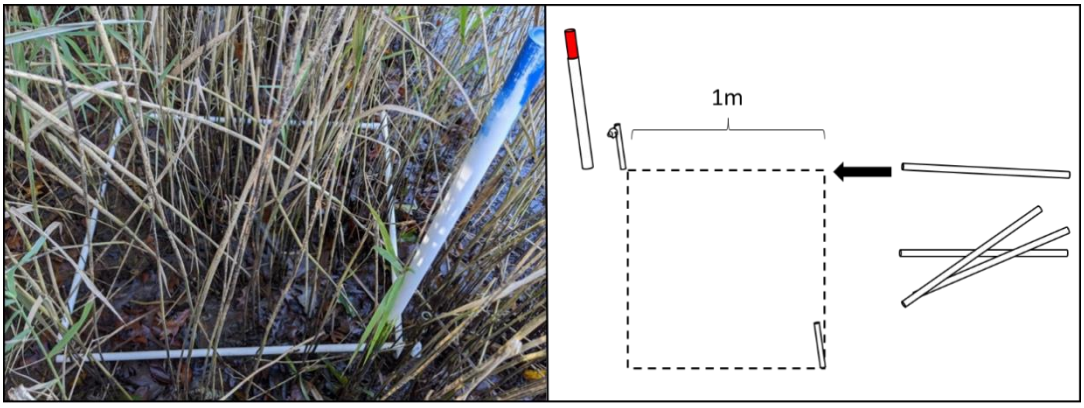

Figure 1. Deployment of collapsible 1m^2 PVC quadrat.

Fieldwork Part 2: Species density and allometry

1. After finishing the species cover protocol in each plot, leave the collapsible quadrat in position on ground and place an extended ruler across plot, roughly dividing it in half.
2. Two people count living stems by species for all plants rooted inside of the plot; each person restricting their counts to half of the plot as marked by the ruler.
3. After stems have been counted for all species *(*see Fieldwork Part 2a and 2b*), haphazardly choose 8 representative individuals of each species to measure.
4. For each plant selected, measure its maximum stem height (cm), stem diameter at ground level (mm), and stem diameter (mm) at 40cm above the ground. If stem does not extend to 40cm, take only the basal diameter but make note on the datasheet.
5. Repeat Fieldwork Parts 1 and 2 for each plot (*n* = 5), transect (*n* = 3), and marsh site (*n* = 3).

** Fieldwork Part 2a: Highly abundant species density and allometry*

1. In some cases, a species may be so abundant (> 500 stems/plot) that counting all stems is overly time consuming. In such situations it is acceptable to take a random subsample of stem densities, heights, and widths.
2. First, using the random number table provided (Table 2), close your eyes and point to a single digit on the table. The number you choose corresponds to a quadrant within the larger 1m^2 plot (Fig. 2).
3. Drop a pencil into the selected quadrant from shoulder height.
4. Note the position of pencil's tip and place the small, 10 cm x 10 cm PVC quadrat such that is it centered over that point.
5. Count all living stems of the species in question which are rooted within the small quadrat.
6. Haphazardly select 8 individual plants of that species rooted within the small quadrat and for each, measure maximum stem height (cm), stem diameter at ground level (mm), and stem diameter (mm) at 40cm above the ground. If a stem does not extend to 40cm, take only the basal diameter but make note on the datasheet.

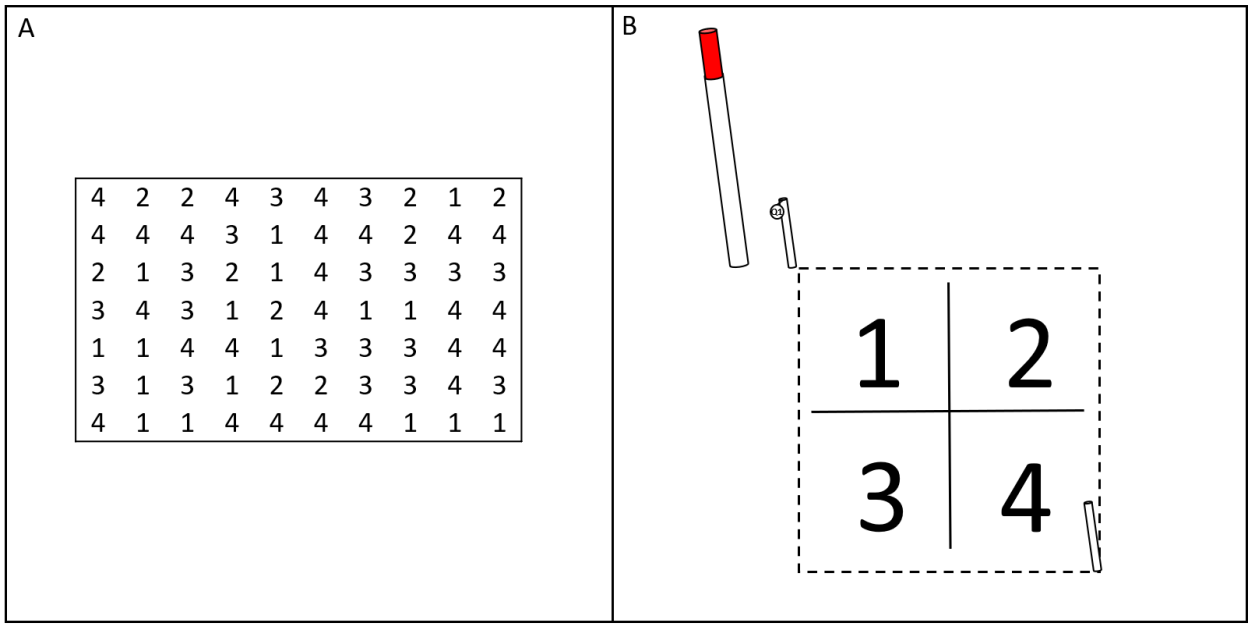

Figure 2. (A) Random number table (RNT) for selection of quadrants for highly abundant species subsampling. (B) Quadrant number assignments relative to position of PVC sight pole.

** Fieldwork Part 2b: Shrub allometry*

1. For shrub species (i.e. perennial, woody), identify the number of distinct bushes within the plot.
2. For each bush, record the total number of live and dead stems rooted within the plot.
3. Measure the diameter (mm) at 40 cm for 6 stems per bush, maintaining an equal proportion of live/dead measurements if possible (i.e. 3/3). If there aren't enough live or dead stems to do so, measure all stems of the limiting category and substitute the remainder with the other category.
    - If a bush does not have 6 total stems, measure the diameter of all stems present regardless if they are alive or dead.
4. Measure the maximum height (cm) of each bush.
5. Measure the maximum dimensions of the canopy of each bush in two dimensions (cm x cm) (Fig. 3).

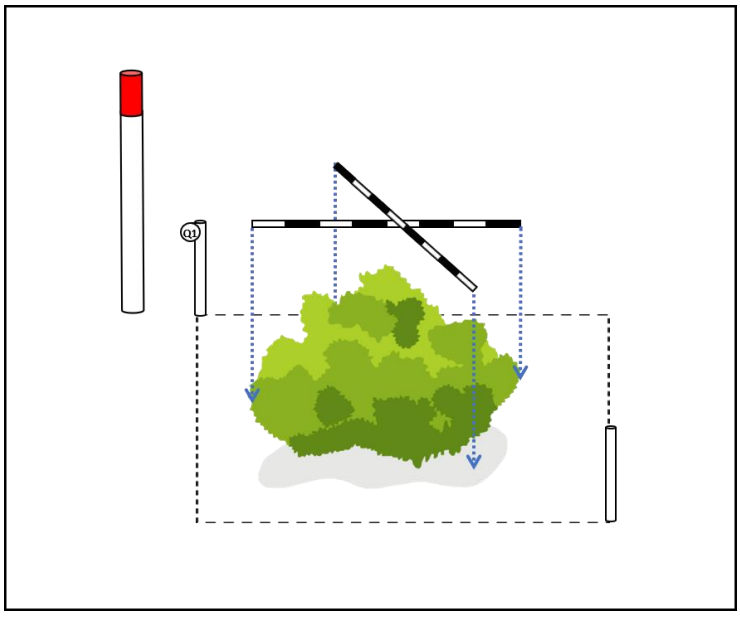

Figure 3. Example of the procedure for taking two-dimensional shrub canopy measurements from a single bush using folding rulers.

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. 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-protocols@si.edu

Salt Marsh Fauna

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Introduction

Tidal salt marshes support a variety of invertebrate species living within and among vegetation, root structures, and sediments. Marsh ecosystem structure and function depend, in part, on the abundance and diversity of these taxa and the services they provide. This protocol provides a standardized methodology for estimating species composition and abundance of salt marsh invertebrate infauna and epifauna. Prior to starting fieldwork, permanent marsh transects should be established using the Salt Marsh Survey Design Protocol. Infaunal samples are taken with a hand coring device designed to collect a standard volume of sediment for calculation of infaunal densities. Epifauna are surveyed visually within a 0.25m^2 area of select plots. Lab processing steps entail separating infauna from detritus, identifying species, and counting individuals.

Measured Parameters:

- Infaunal density (individuals / cm^3) and taxonomic composition
- Epifaunal density (individuals / 0.25m^2) and taxonomic composition
- Crab burrow counts (burrows / 0.25m^2)
- Bivalve/gastropod shell length (mm)
- Crab carapace width (mm)

Requirements

Personnel: 3 people

Estimated Total Time Per Location (*n* = 3)

Preparation: 1-person x 1-2 hours
Fieldwork: 3 people x 1 day per marsh
Post processing: 1-person x 0.5 day
Lab work: 1-person x 1 week
Data processing: 1-person x 0.5 day

Replication: 3 replicate plots per marsh, 3 marshes per region

Materials:

Fieldwork

- ☐ 6.3 cm (2.5 inch) diameter aluminum sediment corer
- ☐ 3.78 L (1 gallon) sealable bags (9)
- ☐ 3 m folding ruler (3)
- ☐ Epifaunal abundance datasheets
- ☐ 150 m dial caliper (2)
- ☐ Hand-held GPS pre-loaded with transect and plot coordinates
- ☐ Water-proof labels (9)
- ☐ Permanent marker
- ☐ Small cooler with ice

Lab Work

- ☐ 70% ETOH + 10% rose bengal solution
- ☐ 20 mL glass scintillation vials (9)
- ☐ 1.0 mm sieve
- ☐ Fine tip squeeze bottle
- ☐ Petri dishes
- ☐ Dissecting microscope
- ☐ Forceps

Methods

Fully review this and any additional protocols necessary for the sampling excursion. Address any questions or concerns to marinegeo-protocols@si.edu before beginning this protocol.

Preparation

1. Review and complete the Salt Marsh Survey Design Protocol for selection and establishment of permanent sampling sites.
2. Review this protocol and print datasheets on waterproof paper prior to starting fieldwork.
3. Print waterproof labels (n = 9) including sampling date, marsh site, transect number, and plot number for each infauna sample to be taken and label zip-locks bags with permanent marker.
4. Measure 10 cm from the opening of the hand corer and make a horizontal mark with permanent marker (Fig. 1).
5. Before heading into the field, fill a container with ice for sample storage.
6. Plan to sample at low tide when marsh is not inundated.

Fieldwork Part 1: Infauna

1. Along the middle transect of each marsh site, infauna cores are taken at the 1st, 3rd, and 5th plots (Fig. 1), (*n* = 9 cores).
2. Using the random number table provided (Table 1), close your eyes and choose a number from the table. This number corresponds to a quadrant within the larger 1m^2 plot. Take the sediment core diagonally adjacent to this quadrant (Fig. 2) in each plot.
3. Using the aluminum hand corer, take a 10-cm deep, 6.3-cm diameter sediment core approximately 50 cm away from the selected corner of the plot (Fig. 2). Press the hand corer vertically into the substrate to the 10 cm depth line, twisting if necessary to cut through roots.
    - If there is standing water at your chosen sample point, move left or right to the nearest patch of exposed substrate.
4. Place the sediment sample in a pre-labeled gallon-size (3.78L) zip-lock bag along with the associated water-proof label for that plot. Place each bag in the ice-filled cooler for transport back to the lab.
    - Infaunal samples should be processed within 24-h of collection.

Figure 1. Example of 6.3 cm diameter hand corer marked with 10 cm depth line.

A

4	2	2	4	3	4	3	2	1	2
4	4	4	3	1	4	4	2	4	4
2	1	3	2	1	4	3	3	3	3
3	4	3	1	2	4	1	1	4	4
1	1	4	4	1	3	3	3	4	4
3	1	3	1	2	2	3	3	4	3
4	1	1	4	4	4	4	1	1	1

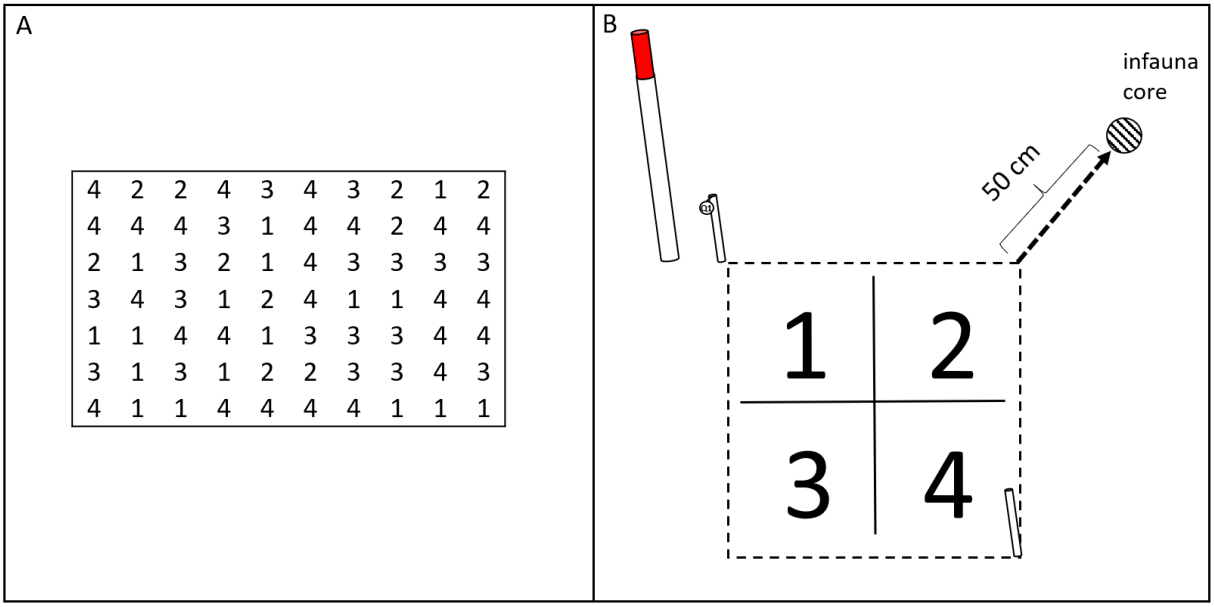

Figure 2. (A) Random number table (RNT) for selection of quadrant for infauna and epifauna sampling. (B) Example location of infauna core given random selection of “2” from RNT. Note that random numbers need only be selected once per transect.

Fieldwork Part 2: Epifauna

1. Epifaunal surveys are conducted along with infaunal sampling at the 1st, 3rd, and 5th quadrat of the middle transect of each marsh site (*n* = 9 surveys).
2. Epifauna are surveyed within the 0.25m^2 quadrant selected in Fieldwork Part 1 (Fig. 2)
3. To demarcate the 0.5m x 0.5m area of the larger plot to survey, subdivide the plot into quarters using 2 foldable rulers lain perpendicular to each other across the plot.
4. Identify and count all macroinvertebrate taxa present on the substrate surface and clinging to vegetation within the selected 0.25m^2 area.
    - For bivalves and shelled gastropods, measure total length (mm; Fig.3) of the first 25 individuals per species and count the rest.
    - For crabs, measure carapace width (mm; Fig. 3).
    - Count all other epifauna encountered
    - Release all animals after processing
    - If unidentifiable species are encountered, photograph each for potential identification later.
        i. Photograph individuals from multiple angles and with a ruler in frame, if possible.
5. If crab burrows are present, count and record the number of burrows within the 0.25m^2 area.
6. Repeat Fieldwork Parts 1 and 2 for the 1st, 3rd, and 5th plot of the middle transect of each marsh site.

Lab work: Part 1

1. Print all lab data sheets and scintillation vial labels containing the sampling date, marsh name, transect number, and plot number.
2. Pour the contents of each zip-lock bag into a 1mm sieve and gently rinse using a squeeze bottle with tap water.
3. Continue rinsing, taking care not to use too much pressure to avoid damaging fragile specimens, until all sediment is washed away and only animals and larger pieces of debris remain in the sieve.
4. Discard any large debris then gently transfer all animals into a 20 ml scintillation vial containing 70% ETOH / 10% Rose Bengal solution.
5. For each vial, attach an exterior label and include an interior waterproof label.
6. Repeat for all samples, making sure to thoroughly rinse sieve between uses.
7. Store vials until the end of the field season.

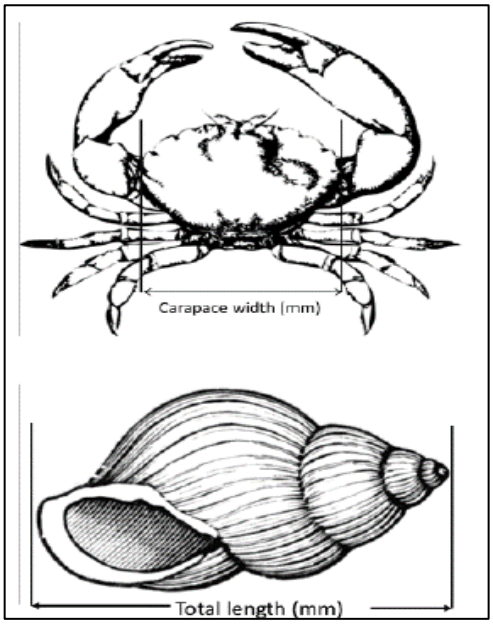

Figure 3. Length measurement diagram for crabs and shelled molluscs.

Lab work: Part 2

1. Transfer the contents of each vial into a 10-cm Petri dish for identification.
2. Using a dissecting microscope, identify each taxon in each sample to species and record the species name on the infaunal abundance 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).
    a. Count only macroinfauna and exclude meiofauna (e.g. nematodes and copepods).
    b. Only count animals that were alive at the time of collection: discard exoskeletons.
        - For gastropods, gently break open shells to verify that the individual was alive when collected. Only count shells that have tissue inside.
    c. Count only full specimens or those with anterior portions intact. Discard disembodied limbs, posterior ends of polychaetes, amphipods, etc.
3. Count and record the number of individuals of each species on the provided lab sheet.
4. Return all specimens to the labeled 20-mL vial, fill with 70% ethanol, and seal for long-term storage.

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. Use the "notes" columns to provide additional information or context if a relevant column doesn't already exist, rather than renaming or creating columns.
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