Byrnes Lab Salt Marsh Sampling Protocols

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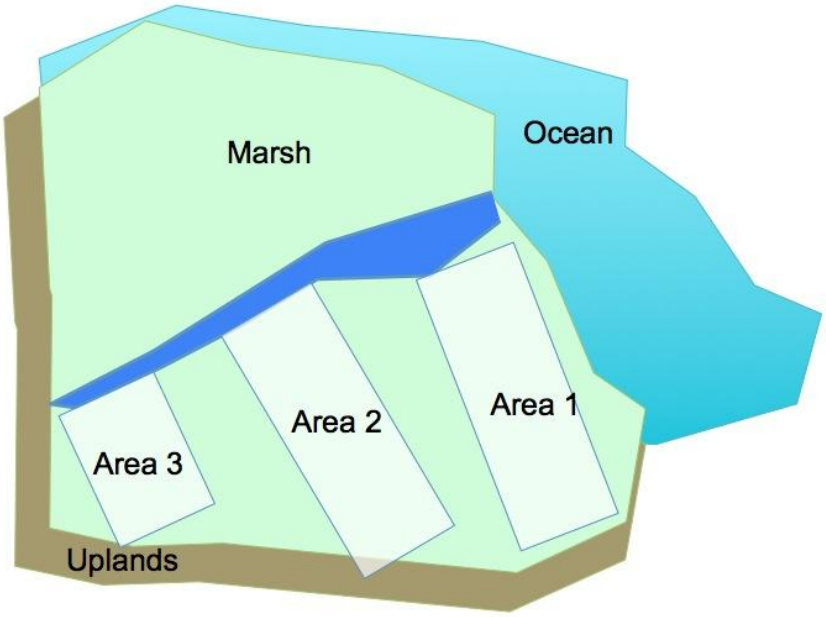
Site Selection Protocols

Area Selection

Every marsh has three areas sampled with four transects per area. All sampling uses transects within marsh areas as a reference point for all other sampling protocols. Begin by selecting areas within a marsh at the seaward edge, towards the back end of the marsh, and in between.



Transect Placement*

Within each area, randomly select four transects. Looking at the creekbank in an area, visually divide it into 100 pieces (think percent along a line). To select transects, choose four numbers between 0 and 100 using a random number generator. Place the start of transects at these points. If two transects are <10m from each other, re-choose the numbers. Transects should run from the creek bank perpendicular edge to the nearest upland area.

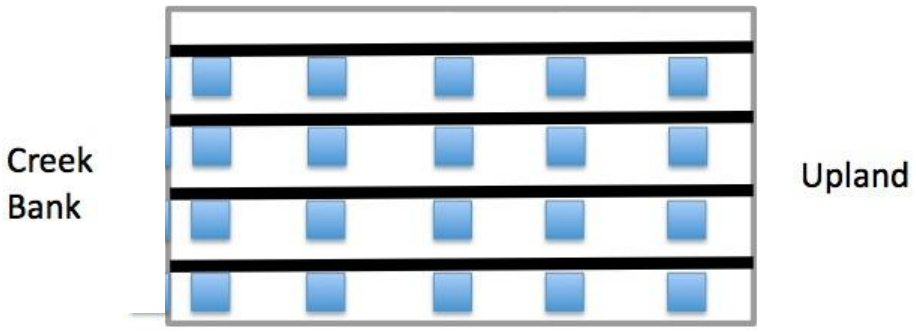
*For PIE sampling, we will be reducing the number of transects sampled to four total, in whatever the predetermined area will be.

Marsh Biota

Marsh Vegetation Sampling

Quadrat placement

Within each marsh area, we will sample five quadrats along a transect line. The first will be on the edge of the creek bank itself, allowing for sampling of algal cover on the bank. That is, the edge of the quadrat should begin at the edge of the grass. Other quadrats will be evenly spaced between the bank and the upland area. Quadrats should be placed 1m to the left of the transect line at the selected point. Additionally, use a flag to mark off a spot 1m to the right of the transect line. This is our "ecosystem function plot" where we will place the bite map, sedimentation paper, and decomposition plug.



Vegetation Sampling

Within each quadrat, quantify the percent cover of all marsh plants and algae species. To facilitate this, use a 4x4 strung quadrat where each cell is 6.25%. The most common plants will be pre-printed on the data sheet but use the species codes printed at the bottom of the datasheet for any other plant species that is found. These species should be recorded in the "SP1" or "SP2" box in the data sheet. Additionally, quantify the percent cover of detritus (i.e. what % of the plot is dead grass), wrack (dead, non-standing Spartina stems), and bare ground. Record these data in the appropriate boxes marked detritus, wrack and bare %

Spartina stem density and heights

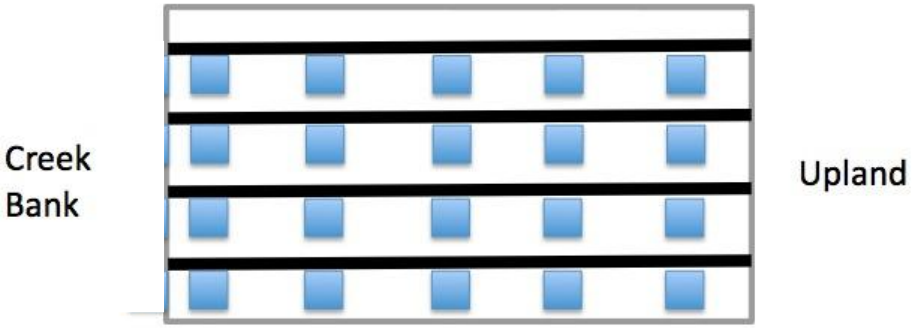
To provide further quantitative measurement of the cover of *Spartina alterniflora*, within plots dominated by these species, perform an additional count within a $0.125m^2$ quadrat measuring the number of stems. Repeat this in 2 more quadrat sections (3 total), and record in the "SPA Density" boxes. Additionally, record

the heights of 10 random *Spartina alterniflora* stems within the whole quadrat. To select these stems, chose the first 10 stems that are touching one of the strings in the quadrat, or that rest along the PVC. Record these 10 heights in the "SPA Height" boxes

Marsh Marine Invertebrate Sampling

Quadrat placement

Within each marsh area, we will sample five quadrats along a transect line. The first will be on the edge of the creek bank itself, allowing for sampling of algal cover on the bank. That is, the edge of the quadrat should begin at the edge of the grass. All other quadrats will be evenly spaced between the bank and the upland area. $1m^2$ Quadrats should be placed 1m to the left of the transect line at the selected point.



Quadrat Marine Macroinvertebrate Counts

In each $1m^2$ quadrat, count and identify to lowest taxonomic level all large conspicuous snails (*Litorina littorea)* on all grass blades or on the sediment (sometimes in algae). Visually search through the plot to enumerate all crabs or other large (>2cm) conspicuous macroinvertebrates. In addition to any actual invertebrates, also record the number of crab burrows in a $0.5m^2$ subset of the quadrat. Crab burrows of all sizes should be recorded, excluding burrows less than 0.5cm in diameter. Record these numbers in the "Snail" and "Crab Burrows" boxes on the data sheet

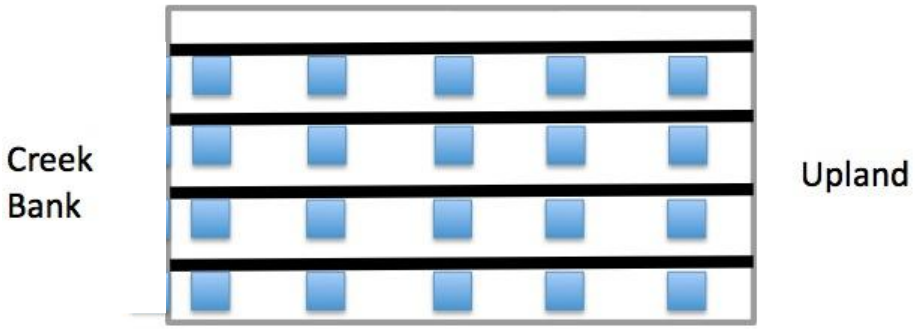
Microinvertebrate Counts

As numerous invertebrates (e.g., *Melampus bidentata)* are exceedingly small and difficult to census within grass or algal beds, these invertebrates should be censused using four $100cm^2$ quadrats, with one placed within each quadrant of the sampled $1m^2$. Each quadrat should be intensively combed through, and all small invertebrates identified to the lowest level possible. This should likely be only *Melampus* as amphipods and most other are quantified through suction sampling. Record this data in the "Melampus" box on the data sheet

Marsh Terrestrial Invertebrate Sampling

Quadrat placement

Within each marsh area, we will sample five quadrats along a transect line. The first will be on the edge of the creek bank itself, allowing for sampling of algal cover on the bank. That is, the edge of the quadrat should begin at the edge of the grass. All other quadrats will be evenly spaced between the bank and the upland area. $1m^2$ Quadrats should be placed 1m to the left of the transect line at the selected point.



Insect Pitfall Traps

Place one, non-holed pitfall trap at the rear of each Q5 (end) quadrat filled with a mixture of 70% ethanol, one tablespoon of sugar, and a dollop of dish soap (to kill, attract, and prevent escape, respectively). Let it stay out for six hours, or right before the tide comes in. Retrieve and either count invertebrates or decant those requiring additional identification into 50ml vial to preserve in ethanol. Traps should be left out (if tide permits) or replaced each day for 5 days. Organisms in traps will be quantified at the end of the sampling week, so cap and store the traps in the fridge/freezer until then.

Organisms will be identified to the lowest taxonomic level, recording the abundance of each species

Insect Sticky Traps

Elevate one sticky trap at Q5 at the beginning of the sampling week. These traps can hang from a strung line between two PVC poles and should be at least as tall as the grass (i.e. 25 cm+ above the sediment). If tides are very high, you may need to collect these traps before the high tide. Leave these traps out for 5 days. Place the traps, labeled with what transect they came from, back into the boxes and freeze, if possible.

Organisms will be identified to the lowest taxonomic level, recording the abundance of each species.

Quadrat Sweep Nets

In each $1m^2$ quadrat, before vegetation sampling, pass a 15" sweep net back and forth across the grasses at their tips five times. Empty the contents of the bag into a zip-lock bag. In order to ensure predatory insects/spiders don't eat the other organisms, place a few acetone or ethanol soaked cotton balls into the bag.

Suction Samples

Before vegetation sampling, each plot will be suction sampled with a Stihl 55 leaf blower with a suction sampling attachment. Within the suction end, secure a piece of coarse muslin underneath a cut-out flower pot using large binder clips. For the

sampler, mentally divide each plot into a 4x4 grid. Moving against the grain of the plants, have a partner make sure the plants have been pushed upright. Place the sampler over the patch of plants, then tip the sampler back, vaccuming up and down the plant leaves. Take one final sample at the base of the plants before moving on to the next patch in the grid.

When suction sampling is complete, while the suction is still going, tip the suction edge upwards. Undo the binder clips and carefully remove the flower pot while gathering the edges of the muslin together, ensuring that no insects escape or are lost into the suction sampler. Place the bag into the same marked zip-lock bag as the above sweep sample. Seal it, and transfer all bugs into marked 15ml vials back in the lab.

If a sample is lost, move the quadrat 1-2m within the same zone and sample again.

Protocol for counting arthropods and other species from the vacuum sample

1) Spread your sample evenly in a gridded dish.
2) Enumerate all arthropods > 1cm in length across the entire dish. Record them on your data spreadsheet with the >1cm field notes as "yes". For the 'square' entry, enter "sample".
3) Choose 15 random numbers between and 1 and 36. If you don't have a random number generator of choice, you can get them from the Random Sample Generator in the Vacuum Sample Master. Copy and paste them into a new tab in your working data sheet, just to make sure you keep track of them.
4) Count all of the invertebrates in the first square. Record the abundance of each invertebrate taxa for that square on your data sheet, making sure to note the square number
5) When finished return the sample to its original vial and put it in a "completed samples" box

Cross-Ecosystem Animal Surveys

Crab Traps

Using crab traps with at most 1cm^2 mesh size, throw a baited (squid) trap into the center of the creek from Q1. Anchor the trap line near Q1. Check and record the presence of any animal (i.e. green crab, fish) in each trap every day of the sampling week. Check each trap daily after the tide has fallen and rebait as necessary.

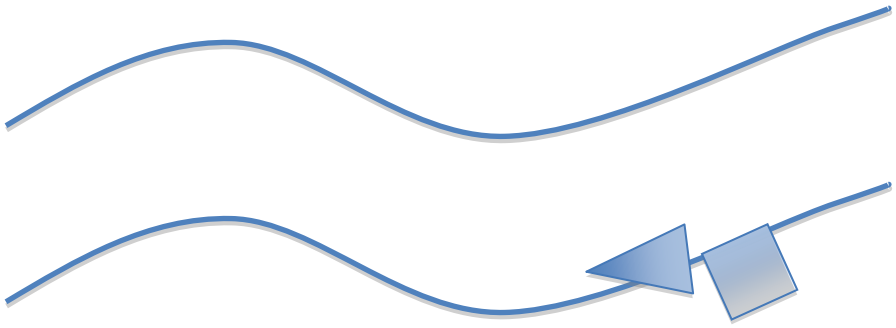
Minnow Traps

Following the same protocol as above, throw an unbaited minnow trap onto the creekbank near Q1 of each transect as the tide is coming up. Check each minnow trap after the tide has fallen. Repeat this procedure daily, for each day in the sampling week

GoPro Survey of Marine Animals

Note: this procedure will only work for tidal waters with at least 20 cm of visibility!

As the tide begins to come onto the marsh platform, place one GoPro on the sediment near the vegetated edge of Q1. GoPro's should be implemented when at least 2 cm of water has come on to the creek edge. Place the GoPro perpendicular to the creek bank, so that the camera will be able to see both the vegetation, and the bare sediment in front of the vegetation. Exact placement (i.e. angle, distance from plot) will vary from plot to plot but the goal of this placement is to see fish and shrimp as they swim into the plots with the rising tide. Record video from when the tidal water begins to cover the lens until the battery runs out. Be sure to clear any impediments (e.g. grass) out of the way of the camera lens



Video recording time will depend on the quality of camera and temperature of the water, but generally lasts between 1-2 hours

Video Data Collection

Load the videos onto a computer or hard drive and immediately name the file Site_Date_CameraLocation. When watching the videos, identify any fish, crabs, shrimp, and snails seen by species and mark at what time in the video you saw them. Also record what the activity of said organism is (i.e. feeding, swimming, crawling, hunting, hiding, burrowing, looking at camera). We have a detailed spreadsheet for this data collection.

Bird Surveys

We will conduct bird observations of each of the sampled marsh areas (Zone 1, 2, 3) within a marsh site. Surveys will be done at low and high tide for each zone. In each zone, three observation points will be selected to provide three complementary views of the zone. These observation points should make sure to cover the marsh platform, creekside marsh edge, and upland-side marsh edge.

Perform a 10 minute scan from each observation point (thus 3 total per marsh zone per tide) using binoculars. During the scan, note the following about any bird observed: species, activity (flying, standing in marsh, standing in creek, hunting, nesting), and it's location within an area (upland edge, midmarsh, creekbank, mudflat, in creek). Only count each individual once during a 10 minute scan. With each of the three observation points, there will be 30 total minutes of observation time within each zone. Repeat this activity in each zone for high and low tide.

Ecosystem Functions

FRACTURE/SLUMP

Within 10 m on either side of Q1, count the number of fractures and, if possible, measure the width and length of the fracture. Additionally, record the number of slumps within this zone. For slumps, mark if the slump is vegetated or unvegetated

Decomposition rate

Collect standing dead *Spartina alterniflora*, rinse all salt/mud, and dry at 60 C for 48 hours. Select 3-5 stems with a mass of 5.5 g (you can choose to control for number of stems if available), and tightly zip tie them to a PVC flag pole. Place this decomposition plug into the 'function' plot at Q1 for all transects. Mark this location well with flags so it will be easy to find! After 1 month, retrieve each plug, rinse, and dry at 60 C for 48 hours. Calculate the mass lost to estimate decomposition rates per month

Sedimentation rate

Place two filter papers (should be ~0.5g each but pre-weigh each paper to make sure) into a plastic 9cm diameter petri dish so that the papers are flush with the bottom of the petri dish. Using a bent metal plant pin (or other appropriate anchor), securely anchor the sediment trap onto the bare sediment in the function plot at Q1 for all transects. The anchor should firmly hold the trap onto the marsh while ensuring that the paper does not float away. Collect the sediment traps after one week (or longer, if possible) on the marsh. Remove the filter papers from the dish and allow to dry for 24 hours (drying oven, 60 C, 24 hours). Discard the bottom filter paper and weigh the paper with sediment. Calculate g of sediment added/week from this measurement

Herbivory & Predation: Bite Map

Adapted from Emmett Duffy's MBON initiative

Obtain dried kelp (kombu) and dried squid. Cut squid into $1cm^2$ chunks and cut kelp into 4 x 4 cm chunks. Using a wooden dowel (or really any long thin pole) as an anchor point, poke a small hole in the squid chunk and tie a 10 cm piece of fishing line through this hole. Tie (or tie and glue) this string of squid onto a .5m length wooden dowel (or some type of pole). Make the knot at about 15 cm up the dowel, because this piece of squid will need to touch the marsh surface. At 10 cm up the dowel, use wire to fasten the piece of kelp to the dowel. Both the kelp and squid should touch the marsh surface. All specs can change as long as the kelp and squid are both touching the mud.

Net Primary Production (*Spartina alterniflora*)

Biomass OR growth rate is used as a proxy for NPP here. If time/resources permit, revisit your marsh site after 3-4 months and either (i) harvest a 50 x 50 cm plot of grass from each (Q1-5) original plot or (ii) remeasure *Spartina* density and height as in "Vegetation Survey". Biomass or new morphometric data can be used to estimate plant growth throughout the season.

Soil Respiration (LICOR)

To estimate soil respiration, first (rubber) hammer a 10cm diameter, 10 cm height PVC segment (collar) into each Q1, 3, 5 in each transect. This collar should be placed in a spot of representative vegetation. Remove any vegetation inside of the collar and wait at least 12 hours. Cover the ends of stems with Vaseline. Place the LICOR chamber over the collar and begin sampling according to unit instructions.