Massachusetts *MarineFisheries* Standard Operating Procedure
Monitoring eelgrass *(Zostera marina)* at SeagrassNet site Salem Sound MS 45.1

Last updated 6/15/16 TE

POINT OF CONTACT
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I. OBJECTIVE

Quarterly monitoring of Salem Sound MS 45.1 (off of West Beach in Beverly) site as part of a global monitoring program.

II. GEAR LIST

A. Field Gear

- Divers
  - Personal Diving gear
  - Tanks/ Tank Racks
  - Safety gear, dive float/flag
- Electronics:
  - Drop camera, charged (July only)
  - Underwater digital camera, charged
  - GoPro, extra batteries
  - HOBO, launched (do not store in plastic)
- Blue Cooler:
  - Clip boards (metal and blue)
  - Transect tapes A, B, C
  - WD 40
  - Duct tape
  - Mesh bags nested 1-12 (all 3 sets)
  - Extra mesh bag for voucher
  - Metal labeler
  - Labeled zip loc bags for samples (3 voucher, 3 sediment, 36 biomass (12 for each A, B, and C))
  - Bags of ice for Samples & Extra Cooler
  - Refractometer
- Small Box In Cooler:
  - Sediment syringes and stoppers (A, B, and C)
  - Ziptie cutters or knife
  - Water vials (A, B, and C)
  - Extra plastic bags
  - Flagging tape
  - Electrical tape
  - Scissors
  - 4” and 8”cable ties
  - Pens, pencils and sharpies

(Field Gear continued)

- Tote:
  - 2 large mouth catch bags with pocket
  - 2 meter sticks
  - 2 brass clip (for meter sticks)
  - 3 screw anchors
  - Re-Bar for screw anchors
  - 2 x 0.25m^2 quadrats
  - 1 x 0.0625m^2 quadrat
  - 3 mushroom weights
  - 3 large surface buoys (A,B,C)
  - 2 subsurface toggle buoys (replacements)
  - Extra line for replacement if needed
- Extra in blue building:
  - Spool of potwort line
  - 6 large surface buoys on a rope
  - 2 loose buoys
  - Yellow string
  - Scissors

B. Laboratory

- Dissecting scalpel or razors
- Forceps
- Lab dry-paper mats
- Tin drying oven cups
- Drying oven
- Digital electronic scale
- Brown paper bags, labeled
- Sharpies
- Voucher sample press
- Voucher paper
- Wax paper
- Desiccator

Elastic bands

III. SCHEDULE

Monitoring events occur 4 times per year: January, April, July and October if weather permits

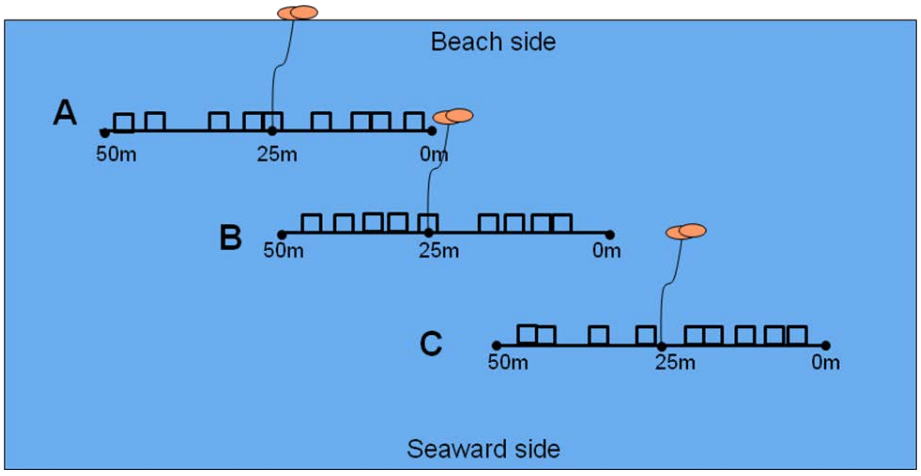
IV. FIELD METHODS:

A. Pre-dive To-do List

- WD-40 all metal equipment
- Make sure Hobo desiccant is charged (dark blue), hobos are grouped, batteries are charged and cases are replaced as needed
- Launch Hobos directly before heading into the field
- Hobos can malfunction while they are reading if they come into contact with plastic (due to static electricity), so keep wrapped in cloth
- Prep Gear, sign out vehicles and boat
- Doodle poll if more divers are needed
- Recharge desiccant in dessicator and prepare lid with petroleum jelly

B. Locating the Site

Drop sash weighted buoys at the mid-point (25m) of each transect, where a screw anchor is already installed.



C. Setting the Transect

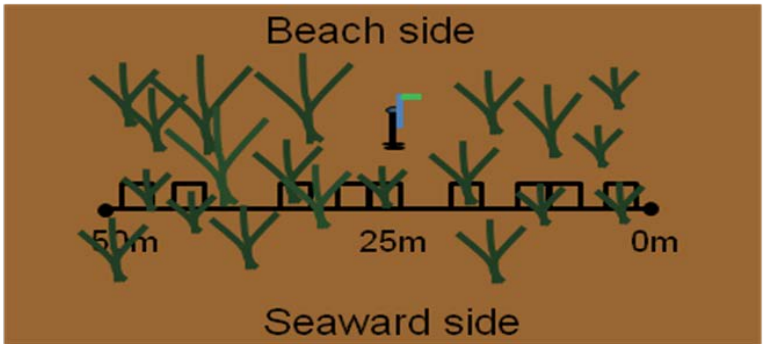
Divers descend along the buoy line and search for the mid-point screw anchor, If not found within a few minutes, diver clips a transect tape or search reel to the mushroom anchor and begins a circular search pattern until the 25m screw anchor is found. Diver 1 clips the transect tape to the center screw anchor and navigates to the 50m screw anchor with the handle of the tape, attaching the handle to the screw anchor when found. The tape is left with the reel open and facing upward to allow it to spool out additional tape. Diver 1 swims back to the center to meet with Diver 2, who during this time has found the HOBO screw anchor approximately 0.5m beachside of the mid-point and replaced the HOBO (shallow and deep stations only). The Hobo is placed above the canopy to prevent shading from plants, and in a horizontal, south-facing direction. It is attached with a PVC coupler to the screw anchor using zip ties, and is replaced 4 times per year. Diver 2 also collects the following in the area around the midpoint of the transect:

- One syringe sediment sample to 15cm. To do this, use the syringe to take a small core in the top layer of sediment. Position the syringe in the top 1mm of sediment and pull the plunger until sediment fills the syringe up to the black line or pull the plunger first and then push the core down into the sediment, in order to get 15 cm of core. Remove carefully and cover with the stopper. Make sure there is a good amount of sediment and not water in the syringe. Repeat if

necessary until desired sample is collected. Put the sediment filled syringe into the mesh catch bag or plastic bag.

- One water vial sample. Open the water vial in an area immediately above the eelgrass canopy and close it tightly. Put the water vial in catch bag.
- One voucher specimen. Collect one full plant with 2 inches of rhizome and put in unlabelled mesh bag. Be sure not to collect a plant from within a monitoring quadrat.

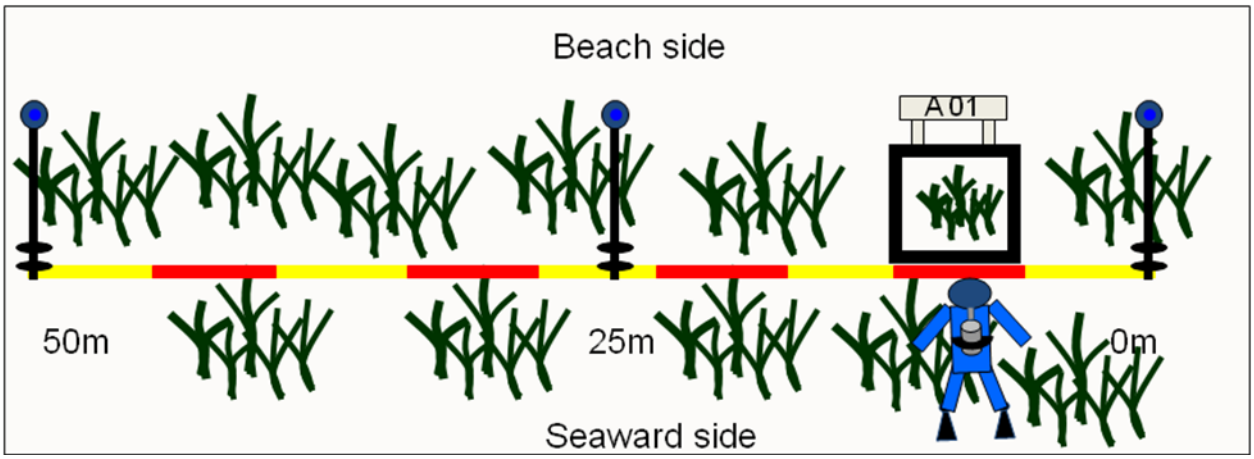
Both divers now swim together with the clip-end of the tape to the 0m screw anchor to finish laying the transect.



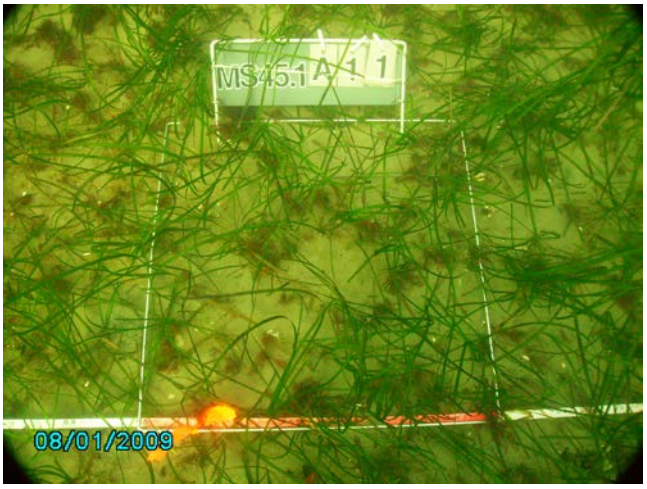
D. Monitoring – this method requires two to three divers in the water and one topside person.

- **Diver 1** – Takes Pictures at each Quadrat

Start at the 0m mark on the tape measure (staying on the seaward side of the tape to avoid stirring up sediment), and swim the transect line until you come across a highlighted portion of tape. Place quadrat on the beach-side of the highlighted tape, according to pre-determined location written on the datasheet. The metal labeler has the meter marking numbers on the back if you need to double check. Place the metal label at the upper outside extent of the quadrat and move the tabs to the correct transect, cross transect and quadrat number. Use a rubber band around the labeler if labels are moving around.



When the quadrat and labeler are in place, remove any unattached algae or large mobile animals (but make sure to note their presence). Make sure the quadrat is not smothering any plants, so pull the grass blades of the “innies” into the quadrat and the “outies” out. Take a picture of the whole quadrat with the label and the tape in view. The picture should be as straight as possible.



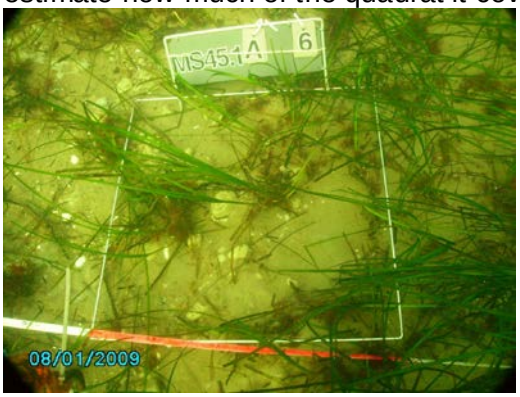
Diver 1 takes the metal labeler and the second quadrat and goes to next location so Diver 2 can work on the biological sampling at quadrat 1. At the next marked spot set up the labeler and quadrat and take a picture. When Diver 2 catches up take their quadrat and go to next location, leaving quadrat 2 set up. Divers should verify the meter marking on the data sheet and the back of the labeler.

▪ **Diver 2** – Collecting Biosamples and Data

Diver 2 collects biological data within each quadrat and records it using the data sheet. This data includes percent plant cover, density (shoot count), canopy height (cm), ID of algae or invasives, grazing evidence, and reproductive shoot count.

- Percent cover:

  Record the percent coverage of eelgrass when viewed aerially in the water. To quantify this, imagine that all the grass is pushed to one area of the quadrat and estimate how much of the quadrat it covers. See examples below.

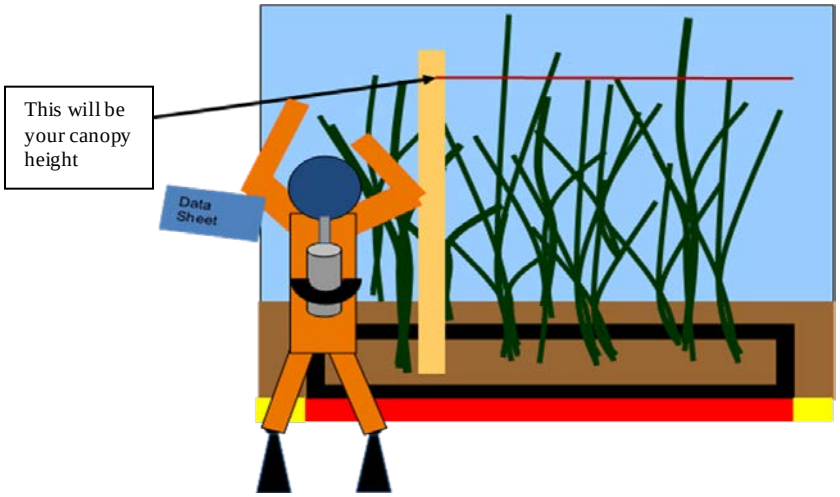


Percent cover approx 20%

Percent cover approx 85%

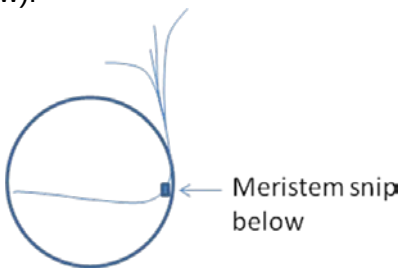
- Canopy height:

  Place the meter stick vertically within the quadrat and use your arms to fan the grass upward. Record the height (cm) that describes 80% of the tallest leaves (i.e. ignore the tallest 20%). In July, plants are likely to be >1m in height so you may need to slide the meter stick up.



- Density/Shoot count:
  Use the 0.0625m^2 quadrat by placing it in a representative area within the 0.25m^2 quadrat (i.e. if percent cover is 70% in the 0.25m^2, your 0.0625m^2 sample should also have 70% cover). Count the number of individual shoots rooted within the quadrat. A good method is to start from one corner and work your way around systematically, holding down the plants you have counted with your arm.
- Reproductive shoot count:
  Count the number of individual reproductive shoots within the quadrat, mostly just in July.
- Evindence of grazers: record anything notable including bite marks from amphipods or brant geese in the area, or chewed, clipped plants indicating green crabs.
- Other observations, algae and invasives

After taking biological data at a quadrat, Diver 2 (or 3*) takes a biomass core by swimming 0.5m landward, and selecting a few shoots that are representative of the ones inside the quadrat and in a representative area. First remove the plants by breaking below the meristem and be aware of the location of the extending rhizome. Put the core down on the exact place where the plant(s) were removed with the point where you snipped the plant at the edge of the core and the extending rhizome across the core (see figure below).



Push and twist the corer into the sediment to 10cm depth (to line on corer). If the plants are really large you can just sample one plant with at least 7cm of rhizome by hand (based on global SGN methods). Put contents of core in the mesh bag labeled with the quadrat number and make sure to use the zip closure on bags or plants can float away. Shake gently to remove fine sediments.

An alternative biomass sampling method that we have been using since 2015 is harvesting one shoot and 10 cm of rhizome.

* A third diver can help by doing all the cores, doing mimic invasive species monitoring, taking pictures and/or video and performing maintenance on any screw anchors, buoys, line or bricks.

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Diver 2 also records the sediment type along the transect for left, center and right (near each screw anchor) by picking up sediment and characterizing it as mud, fine sand, sand, course sand, shell, gravel, or rock.

- **Topside person:**
  - Continually check for bubbles and keep your eye on the diver's position
  - Record data for dive log
  - Transfer biomass samples from mesh to labeled plastic bags, and place in cooler full of ice. Try to shake away as much sediment as you can before transferring, but be careful of roots. With the one shoot harvested method it may not be necessary to transfer shoots to a plastic bag. However, shoots should immediately be placed on ice in the cooler and should be kept wet, cold and dark until they reach the lab. A plastic bag may help if there is a concern that the shoots may dry out.
  - Use the refractometer to test the salinity of the divers' water samples at each transect. Calibrate first with fresh water. If needed, salinity testing can be performed in the lab immediately upon return. Do not leave salinity sample in the fridge, it must be recorded immediately before any evaporation.
  - Prep mesh bags and gear for divers, help divers with their gear
  - Set up and break down the dive ladder and dive flag
  - Keep an eye out for other boats in the area
  - Throw current line to divers when they surface

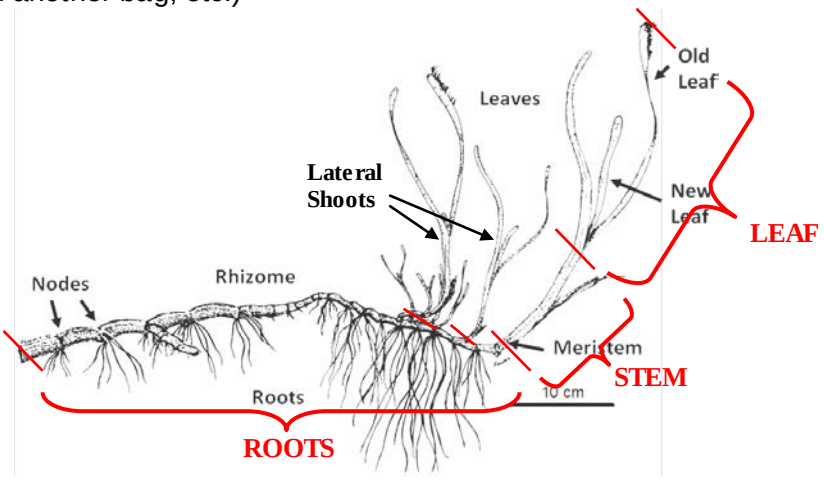
E. Extent of Bed (July only)
After all three transects are complete, use the drop camera to measure the distance to the edge of the bed. Measure distance from transect A to the shallow bed edge and from transect C to the deep bed edge. Mark edge on GPS. Note: if water is clear enough, edge of bed may be visible from the boat.

F. Mimic protocol
At each screw anchor (0, 25 and 50m), place the quadrat on the shore side of the transect with the screw achor in the bottom right-hand corner. Within the quadrat and anywhere on the screw anchor and floating buoy, record presence/absence of the invasive species listed on the MIMIC datasheet. Take photographs of each quadrat where species are present.

V. LABORATORY METHODS

- Rinse gear w/ fresh water and hang to dry
- Salinity Samples – Use the refractometer to determine salinity of the three water samples if it was not done in the field.
- Sediment Samples – Empty the entire contents of the syringe, including any water at the top of the core, into a metal tin inside a clip seal plastic bag. Open one end of the bag and allow the sample to air-dry. Put in labeled plastic bag to send to UNH or store at Kate's desk.
- Rinse biomass samples with fresh cool water in the plastic bag, drain bag of all water and put in fridge at 5 degrees C. if not processing immediately.
- Rinse voucher specimens and press the same day using the wooden press, a sheet of white press paper and a sheet of wax paper. Lay the plant out flat on the white paper and bend leaves as necessary to get the entire plant on the page. Label with date and site name.
- Download Hobo Data and update existing spreadsheets.
  - Plug Hobo into coupler USB device.
  - Export data as an excel file to the folder W:\Habitat Project\Habitat Research\Seagrass\SeagrassNet\hobos (create a new file for that month and year)
  - Make a copy of the raw spreadsheet and clip the data down to the 2 weeks following deployment. Also clip each daily data to a period between 10am and 2pm.
- Process biomass samples within 24 hours. For each bagged sample:

- Place shoot(s) in a shallow fresh water tub and count the number of meristems in the sample – this is your shoot number. Count all laterals as individual shoots if they are out of the terminal shoot's sheath bundle. Record number of shoots on the biomass data sheet.
- Remove one shoot at a time onto a dissecting surface.
- Carefully scrape epiphytes from the shoot using the razor blade.
- Cut the shoot at the meristem and at the sheath to get root/rhizome, stem and leaves (see figure below).
- Collect "live" pieces of plant from the tub – use forceps to pick out live roots (tend to be light in color and float) or living pieces of leaves.
- Place each part in the designated pre-labeled paper bag (all roots can go together in one bag, all stems in another bag, etc.)



- Place bags in a preheated drying oven at 50-60˚C (120˚F) for approximately 48 hours or until dried. Do not leave samples in the oven longer than is necessary to dry them.
- Transfer samples to the desiccator to cool to room temperature. Maintain desiccant according to package instructions, recharging in the drying oven as needed, and lubricate desiccators rim with petroleum jelly. After all samples are processed clean the desiccator and store with a paper towel in between the lid and the jar to prevent it from permanently sticking. Desiccant will need to be recharged before the next use. Weigh samples on a balance that has been recently calibrated (balance in growth lab) and record data to three decimal places (i.e. 0.123) both on the datasheet and on the individual paper bags. Enter data into our Access database and also on SeagrassNet site. See details in the Data Management section.

V. TROUBLESHOOTING NOTES:

VI. PHOTOS:

Photos are organized by sampling month and year and stored at W:\Habitat Project\Habitat Research\Seagrass\SeagrassNet\Photos. Several quadrats have been selected to be used in a photo-progression series which is a powerpoint file called "SGN Quadrat Progression" and saved in the folder linked above.

VII. DATA MANAGEMENT:

Field datasheets can be found here: W:\Habitat Project\Habitat Research\Seagrass\SeagrassNet\DATA

MarineFisheries previously maintained an excel database housing all SeagrassNet data and calculations. In 2013, and Access database was developed and is now the most current database. Step by step instructions can be found here: W:\Habitat Project\Habitat Research\Seagrass\SOPs\SGN Access Database instructions.doc

Data entry: *SeagrassNet* global database

1. Go to http://www.seagrassnet.org/global-monitoring and "download field data entry forms" and "download biomass data entry forms". Follow instructions provided on the site.
2. Use the PDF's linked to the website for entry so it's in the Internet Explorer browser (i.e. NOT a saved pdf copy on the desktop…lesson learned)
3. Enter field data FIRST before biomass data…submit them after both are filled out
4. Order is important: Do 'A' Field data THEN 'A' Biomass data; THEN 'B' field and 'B' biomass etc
5. GPS – fill in as degrees / minutes/ NSEW
6. Percent Cover – only row to fill in is the percent cover/density since we are only working with Zm
7. Total SG Biomass – the value (in yellow) is based on core volume, as follows:
    a. Core = 0.0035m^2
    b. Mass per core = Leaf mass + shoot mass + root mass
    c. Mass per m^2 = Mass per core/0.0035m^2
    d. This is mainly for reference…you don't need to actually do the math, but the excel spreadsheets for our personal use are now updated with the same row (also in yellow). I'd suggest using it to double-check your data entry.
    e. Note – when double-checking data on the SeagrassNet spreadsheet, the 'total' cell (originally yellow, equivalent to the yellow row in OUR data spreadsheet) for number 8 is wrong on SeagrassNet's end. I did inform them in January but as of May 29 it is still wrong.
8. Submit data using ID: wppseagnet, Password: r1Xsps
9. Print the sheets – Go to Upload → Retrieve data → User: Evans, Pwd: evans
10. File sheets in SGN folder in filing cabinet.

MIMIC data entry…..TBD, under development

VIII. STATISTICAL METHODS:

Refer to the Results section of the 6-year monitoring report. Analytical graphs are located in the Access database pivot chart files.

IX. REFERENCES:

http://www.seagrassnet.org/global-monitoring
W: Drive \ Habitat Project \ Technical Research \ Seagrass \ SeagrassNet \ Guide to SeagrassNet