Massachusetts Marine Fisheries Standard Operating Procedure
The Horizontal Rhizome Method: planting and monitoring eelgrass (Zostera marina)

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I. OBJECTIVE:
Transplanting eelgrass using the Horizontal Rhizome Method (Davis and Short 1997) and monitoring success at HUB3 and other restoration sites (i.e. Woodbury Point 2011 sites)

II. GEAR LIST:
(Per 2-person dive team)
- Dive gear
- Safety gear, dive float
- Field notebook
- Drop Buoys
- Mushroom Anchors
- 2 mesh bags
- Rubber bands
- 2 x screw anchors
- Rebar for screw anchors
- Wooden skewers (pre-soaked for at least 24 hours)
- 1m² quadrat
- 0.25m² quadrat
- 2 transect tapes
- Tote or cooler

III. METHODS:
A. Pre-dive
Soak wood skewers for at least 48 hours prior to dive. This allows them to become waterlogged, thereby reducing their buoyancy, and also allowing them to bend more easily.

B. Site Reconnaissance and Layout
After a suitable site has been identified through site selection models and test plots, the planting site can be laid out by divers. In the field, divers determine the best location and orientation for the plots based on site bathymetry, substrate composition and presence of eelgrass, algae or rocks; but each plot should be as close to aligned with the cardinal directions as possible to make navigation on subsequent dives easier. Divers will use transect tapes to measure and record all the boundaries of the planting area, and install screw anchors or oak stakes at each corner. Marker buoys are placed next to the screw anchors so that GPS coordinates can be recorded for each corner.

C. Harvest
Using a harvest site that has not recently been used, harvest plants (24 or 50 plants for each planted m² quadrat, plus 10% extra) using the methods outlined in SOP entitled "Massachusetts Marine Fisheries Standard Operating Procedure".

D. Planting
Divers arrive at the site and use a surface buoy to mark the location, following it down to the seafloor. Using a transect tape, delineate the planting site and find the first planting area. Laying the 1m² quadrat at the appropriate meter mark, Diver 1 places two shoots together with rhizomes facing in opposite directions (Figure 1) and pushes the plants 1-2cm into the sediment. Diver 2 “staples” them to the sediment with a bent bamboo skewer in the “Λ” position. The skewer must be pushed all the way down (without snapping the rhizomes) to securely anchor the plants (Figure 1 and Photo 1).

Distribute 12 or 25 pairs of plants evenly within each quadrat. A checkered planting design allows plants to expand into bare areas (Figure 2).

Figure 1. Horizontal Rhizome method uses a bent wooden skewer to anchor two eelgrass shoots to the sediment

Figure 2. Checkered planting design allows plants to expand into bare areas.
Be sure to draw a map of the planted areas and meter markings on your dive slate and transcribe into the field notebook at the surface.

E. Monitoring

After eelgrass is transplanted, monitoring occurs at intervals of one month, six months and one year for the first year, and once annually for at least five additional years.

Post-planting monitoring, 1 month interval

Percent survival will be quantified by collecting biological measurements from 10% of the planted squares; or 14 randomly selected squares per 144-square plot. A random number generator is used prior to each monitoring event to select sampling squares.

From the boat, a marker buoy is deployed at one of the four corners of the site. Once in the water, divers follow the buoy line to the bottom and find the screw anchor marking the corner of the site. Using compasses for navigation, the transect tape is laid out and divers locate the meter marking for the randomly-selected squares that will be monitored. For newly planted plots, divers should be able to see distinct squares and determine which one was selected for monitoring. If for some reason distinct squares are not obvious (poor visibility, plants are gone, or plots have coalesced (for later-stage monitoring)) line the quadrat up at the transect tape and flip it over until you have reached the chosen distance from the tape.

At each sampling quadrat, divers use their slates to record the following measurements:
• Percent cover:
  Place the 1m² quadrat on the sampling location and record the percent coverage of eelgrass
  when viewed aerially in the water. To quantify this, imagine that all the blades of grass have
  been pushed to the seafloor and estimate how much of the quadrat area they would cover.
• 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 the tallest 80% of the plants.
• Shoot count:
  Count the number of individual shoots within the 1m² quadrat.
• Any additional notes (grazers, epiphytes, sediment characteristics, etc)

Divers should take a few moments to note the general health of the plot as a whole.

Post-planting monitoring, 6 month and annual intervals
Divers follow the steps above to randomly select and sample 10% of the planted squares. Two
additional tasks are to record the surface sediment type (mud, fine sand, sand, course sand, shell-
hash, gravel, cobble), and take a picture of the quadrat.

If percent cover is greater than 50%, use the 0.25m² quadrat by placing it in a representative area
within the 1m² quadrat (i.e. if percent cover is 70% in the 1m², your 0.25m² sample should also have
70% cover). Make note of what quadrat you are using on your dive slate and in the notebook.

Note: Annual monitoring:
In the event that the site is not highly successful, using the above monitoring protocol can be overly
tedious and waste time if all the randomly selected quadrats are empty and navigating to each one is
too time consuming. The current protocol is for divers to lay transect tapes along the two long-ends
of the plot. Then, two to three divers line up near the 0m marking, spacing themselves out appropriately to
be able to still see each other and a two to five meters of ground cover. Divers use their compasses
and to navigate to the opposite transect, recording any grass or other features along the swath and
collect density measurements. After a swath, divers move down the transect and repeat the process
until the entire site is completed. At the surface, a detailed map is drawn, compiling the data collected
by all the divers.

Expansion monitoring
At one year monitoring, measure the distance from the checkered plot to the edge of the plot on all four
sides to look for expansion into the unplanted border of the checkered plot. After evidence of expansion
is noted, monitoring will follow the same protocol as above. Divers should make special note of
growth/expansion into unplanted areas.

Success Criteria
A reference bed must be monitored to assess the success of the planted site. Success will be
measured for individual parameters compared to reference levels measured at the natural sites or
“reference beds”. Refer to SOP entitled Monitoring eelgrass (Zostera marina) in a reference bed and
Short et al. (2000) for more information.

IV. TROUBLESHOOTING NOTES:
If sediment is too dense or rocky to push skewers all the way down, break skewers in half and try them at a
smaller size.

V. PHOTOS:
VI. DATA MANAGEMENT:
Monitoring data are entered and quality-control checked in the Access database titled *HUB3 Database* stored here [W:\Habitat Project\Habitat Research\Seagrass\HUB3 Eelgrass restoration\Monitoring data](W:\Habitat Project\Habitat Research\Seagrass\HUB3 Eelgrass restoration\Monitoring data).

VII. REFERENCES: