

STANDARD OPERATING PROCEDURE
FOR DEPLOYMENT AND COLLECTION OF
LIGHT AND TEMPERATURE CONDITIONS
IN EELGRASS BEDS USING HOBO PENDANTS

SOP 50.1
Revision 1.0
July, 2010
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POINT OF CONTACT:

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I. OBJECTIVE

To characterize water temperatures (year-round) and quantify canopy light relative to surface light (for a two week period) for each eelgrass collection site.

Overview: The HOBO Pendant light and temperature logger has a waterproof housing and can record both temperature and light intensity. Each collection site receives 3 HOBO Pendants to collect light and temperature data. All loggers are pre-launched (at UNH), then placed in the field. For light, one logger is deployed in an eelgrass meadow and one above the water surface nearby; they are set up to record light levels for 2 weeks, then they are retrieved and downloaded (at UNH) to establish light levels at each site. For temperature, one underwater HOBO Pendant is attached to the eye anchor at the sediment surface at the time of light logger deployment, and is left to continue recording water temperatures for one year before it is retrieved.

II. MATERIALS AND EQUIPMENT

For each site:

- 3 HOBO Pendant loggers
- 1 PVC support rods
- 1 Eye anchor
- 1 Pipe as a handle to install anchor
- 10 Zip ties

III. METHODS

A. Deployment and Retrieval of the HOBO Pendant (Light/Temperature):

1. HOBO Pendants (3) to log light and temperature should be deployed at the time of eelgrass sampling. Two Hobo Pendants are deployed in the water (one for light and one for temperature) attached to a eye anchor in the area sampled for eelgrass. The third pendant is deployed on land (in the air), attached to a post or other un-shaded, permanent structure.
2. Send two cable (zip) ties through the HOBO Pendant bail, in opposite directions. Slide both ends of both cable ties through the PVC support rods.
3. In the field, using the cable ties, attach the HOBO Pendant assigned for light measurements to the PVC light pole and attach the pole to the eye anchor, orienting the HOBO Pendant toward the Equator (i.e., facing south in the Northern Hemisphere) and with the Pendant at the top of the seagrass canopy. If the canopy is greater than 50 cm above the bottom, the eye anchor and post will not be tall enough to evaluate the light without obstruction from the plants, so install the Pendants in an unvegetated 'hole' within the meadow. Sunlight reaching the HOBO Pendant should not be obstructed by shadows; the horizontal surface with sensors and flashing light should be level and face the sky. Cut off the unused ends of the cable ties. In a similar fashion, deploy the second HOBO Pendant for temperature collection on the eye anchor itself, just above the sediment surface (1 cm). Record the time and GPS location of deployment for each logger on data sheet (attached).
4. The final Pendant for light collection is deployed on land (in the air) in a convenient but protected location without shadows throughout the day. Attach it to the PVC light pole, then use cable ties to attach the light pole to a solid structure (fence post, dock piling, or similar). Record the time and GPS location of the logger on the data sheet.

5. After the HOBO Pendants have been deployed for 2 weeks, collect the Pendant and the PVC light pole from the eye anchor as well as the Pendant attached on land. Rinse in fresh water, dry with paper towel, and mail back to UNH immediately, within 24 hours of retrieval, for data download.
6. The full-year temperature sensors should be collected after one year. Rinse in fresh water, dry with paper towel, and return to UNH.
7. Mail all sensors to Nikki Sarrette, Jackson Estuarine Laboratory, 85 Adams Point Road, Durham, NH 03824, 603-862-5125.

IV. TROUBLE SHOOTING / HINTS

1. Write the location of the deployed pendants from the GPS unit on data sheet or book immediately upon collection.

V. STATISTICAL ANALYSIS AND DATA USAGE

No data reduction or statistical analyses are required at this stage. Pendants with data will be sent to Nikki Sarrette.

VI. REFERENCES

Short, F.T. and R.G. Coles (eds.). 2001. Global Seagrass Research Methods. Elsevier Science B.V., Amsterdam. 473 pp.

Short, F.T., L.J. McKenzie, R.G. Coles, K.P. Vidler, and J.L. Gaeckle. 2006. SeagrassNet Manual for Scientific Monitoring of Seagrass Habitat – Worldwide Edition. University of New Hampshire Publication, Durham, NH, USA. 76 pp.

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[Data sheet for recording Pendant sensor information]

Deployment Team Members: _____

Site Name and Site number: _____

LAND LIGHT PENDANT

Description: _____

Pendant serial number:		
Date and Time Deployed	GPS (N)	GPS (W)
Date and Time Retrieved		

UNDERWATER LIGHT PENDANT

Pendant serial number:		
Date and Time Deployed	GPS (N)	GPS (W)
Date and Time Retrieved		

UNDERWATER TEMPERATURE PENDANT

Pendant serial number:		
Date and Time Deployed	GPS (N)	GPS (W)
Date and Time Retrieved		



STANDARD OPERATING PROCEDURE
FOR SAMPLE COLLECTION OF EELGRASS
FOR MORPHOLOGICAL, PHYSIOLOGICAL
AND GENETIC ANALYSES

SOP 50.2
Revision 0.0
May, 2010
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POINT OF CONTACT:

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I. OBJECTIVE

To assess the health and diversity of eelgrass meadows, a procedure is outlined to collect plants and limited physical data from a discrete meadow.

Overview: Air and water temperature data, water salinity and five sediment samples are collected from each sampling site along with 55+ plants. Preparation of the light and temperature pendants and the eelgrass plants for specific analyses are outlined in other SOPs.

II. MATERIALS AND EQUIPMENT

- Boat (appropriate for sampling needs and exposure) and safety equipment
- Navigation map
- Eelgrass meadow map and sampling map
- GPS unit
- Snorkel/SCUBA gear, dive flag, weight belt
- Sample Collection Data sheet, 2B pencils and clipboard using unique ID # System
- Pre-labeled collection bags for eelgrass (quart-size freezer bags)
- 2 gallons fresh water (for rinsing)
- Sediment piston core sampler (20 cc) and 5 sample bags
- Marker buoys and weights (2 each)
- Flotation basket and cooler
- Vials (60 ml) for salinity samples (5)
- Calibration solution for refractometer (to be used at lab)
- Optical refractometer (temperature corrected; to be used at lab)

III. METHODS

A. Field Collection

1. The eelgrass bed of the proposed sampling site is located and navigated to. Eelgrass

and other measures will be sampled at 50 stations within each collection site.

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2. The bed is visually inspected from the boat or in the water, depending on depth and size, to determine sampling boundaries. Select a 100-meter sampling path that traverses the middle of the depth range for the eelgrass meadow. Any deviations from expected meadow dimensions are recorded on the sampling map.
3. A sampling plan is chosen that will effectively sample eelgrass for genetic analyses, with stations about 2 meters apart (or more) and containing 50 sample stations (Olsen et al. 2004, Coyer et al. 2008). (If 'holes' in the bed are encountered, move on.) The sampling goal is to sample a portion of the bed about 100 m in length (Figure 1), unless the bed is small, and in that case the sampling path may curve around a portion of the bed. Safety, water depth and predicted tide currents and depths are considered in determining collection method (wading, snorkeling, SCUBA).
4. As soon as you arrive at the location, find the first station for sampling and drop a temporary marker buoy with weight to mark the site. At the weight, screw in an eye anchor to be used for attaching the HOBO Pendant temperature logger and the light logger on its PVC rod. Pendant loggers to measure light and temperature are deployed (see SOP 50.1) and the GPS position is written on the data sheet at the site before plant and sediment sampling. Be sure to note time of deployment on the data sheet.
5. A two-person team, one with pre-labeled collection bags and GPS, the other focused on sampling, get into the water with any other needed gear (e.g., dive flag, float to hold samples, flotation basket and cooler). GPS is marked at beginning and end points and waypoints are entered for each of the 50 collection stations. Be sure to record the GPS beginning and endpoint on the data sheet.

6. Sampling:

- 6.1 For the genetics only sample stations (50 stations per site), one terminal shoot is uprooted at the sediment surface with a small section of rhizome and placed in the sample bag. Refer to the sample form attached to the bags.

- 6.2 For the Wasting Index, NPI & N15 stations (every 4 stations: # 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, and 45), a longer section of rhizome is collected with the terminal shoot and one to two lateral shoots (all connected by rhizome). Three to four lateral shoots may be needed if the leaves are diminutive (less than 4 mm wide and 20 cm in height). All shoots for each station are placed in one bag.

- 6.3 At five stations (# 1, 13, 25, 37, 45), sediment and salinity samples are collected, as well as a second eelgrass shoot (with rhizome) for a herbarium voucher. A piston corer fashioned from a 20 ml plastic syringe is used to capture about 50 grams of sediment from the top 10 cm. Remove the piston and insert the tube into the sediment until the flange is reached. Insert the piston just to the top of the coring tube and withdraw the sample from the sediment. Above the water, extrude the sediment from the core into a labeled plastic bag. The sediment is kept cool until return to laboratory, where samples are air dried at room temperature (open bag and thumb tack it to a wall until all visible liquid has evaporated) and then shipped to UNH for analysis. Upon arrival it will be kept cool (4°C) until analyzed for organic matter using the Loss-on-Ignition method.

- 6.4 At the same five stations (# 1, 13, 25, 37, 45), a small pre-labeled 60 ml vial is used to collect water at mid depth for determination of salinity. The vials are returned to the lab and salinity measured with a temperature-corrected optical refractometer. The refractometer is calibrated with 15 ppt salinity immediately prior to measurements.
7. Once all plant samples are collected, the collection team boards the vessel, and retrieves the marker buoy with weight. Then, on the boat, plants are rinsed in fresh water and then placed in coolers to keep dark and cool until processing begins (SOP 50.3-50.5). To rinse, add ~50 ml of tap water to each plastic sample bag, slosh gently, and turn upside down holding the plant in the bag to drain. First and last station GPS positions are recorded on the data sheet. GPS coordinates for beginning and end points of the collection are also recorded in the GPS.
8. Once all samples are processed for a site (see SOP 50.3-50.5) - mail soil and plant samples to Nikki Sarrette, Jackson Estuarine Laboratory, 85 Adams Point Road, Durham, NH 03824 (603-862-5125). Record the Sent Date on the data sheet.

IV. TROUBLE SHOOTING / HINTS

1. The eelgrass meadow being sampled may not be continuous for a variety of reasons. If a station cannot be sampled, the team will move on to the next available sample station. No waypoint will be recorded for 'missing' stations.
2. If the team needs to leave the water for any reason, mark the endpoint with a small buoy (as well as the GPS) to aid in finding the location when sampling resumes.

V. STATISTICAL ANALYSIS AND DATA USAGE

No Data reduction procedures or statistical tests are planned for eelgrass sample and physical data collection.

VI. REFERENCES

- Coyer, J.A., K.A. Miller, J.M. Engle, J. Veldsink, A. Cabello-Pasini, W. T. Stam and J.L. Olsen. 2008. Eelgrass meadows in the California Channel Islands and adjacent coast reveal a mosaic of two species, evidence for introgression and variable clonality. *Annals of Botany* 101: 73–87.
- Olsen, J. L., W. T. Stam, J. A. Coyer, T. B. H. Reusch, M. Billingham *et al.* 2004. North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L. *Molecular Ecology* 13: 1923-1941.

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UNH - TNC Eelgrass Assessment: Field and COC Form				State:	
Date Collected:		GPS: Lat.	Long.	Site Name:	
Date Processed:		Start =		Site Number:	
		End =			
Station	Sampling	Processed	Station	Sampling	Processed
series start #					
#	1	2x (g + laterals) +			
	2	g	26	g	
	3	g	27	g	
	4	g	28	g	
	5	g + laterals	29	g + laterals	
	6	g	30	g	
	7	g	31	g	
	8	g	32	g	
	9	g + laterals	33	g + laterals	
	10	g	34	g	
	11	g	35	g	
	12	g	36	g	
	13	2x (g + laterals) +	37	2x (g + laterals) +	
	14	g	38	g	
	15	g	39	g	
	16	g	40	g	
	17	g + laterals	41	g + laterals	
	18	g	42	g	
	19	g	43	g	
	20	g	44	g	
	21	g + laterals	45	2x (g + laterals) +	
	22	g	46	g	
	23	g	47	g	
	24	g	48	g	
			49	g	
	25	2x (g + laterals) +	50	g	

g = sample for genetics
g + laterals = additional attached shoots if small shoots (all from the same rhizome)
2x (g + laterals) + = extra shoot(s) for herbarium vouchers + salinity and sediment

Send original with samples after making a PDF file to:
 Nikki Sarrette, JEL, 85 Adams Point Road, Durham, NH 03824

Email copies to: Fred.Short@unh.edu, Anita.Klein@unh.edu

STANDARD OPERATING PROCEDURE
FOR SAMPLE PREPARATION OF EELGRASS
FOR GENETIC ANALYSES

SOP 50.3
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May, 2010
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I. OBJECTIVE

To prepare eelgrass samples for genetic analysis.

Overview: Approximately 50 individual eelgrass shoots will have been collected from each site to determine the genetic diversity within, and at a larger scale, among sites in Southern New England. To prepare the tissue for genetic analysis, care must be taken to provide dry plant material.

II. MATERIALS AND EQUIPMENT

- Eelgrass samples with unique site/station labels from field collections
- Eelgrass Tissue Transfer Data Sheets
- Scissors
- Straight edge razor blades
- Forceps
- Kimwipes to remove epiphytes
- Soft, absorbent paper towels
- Microcentrifuge tubes, 2 ml, numbered, half-filled with fresh silica crystals
- Extra silica crystals to top off the tubes

III. METHODS

A. Field Collection has been covered previously in SOP 50.2.

B. Laboratory Processing

1. Enter the date, site and unique station numbers for each eelgrass sample on the Eelgrass Tissue Transfer Data Sheet.

2. For station numbers 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, and 45 (every fourth sample), the shoots must also be processed according to the Wasting Index/NPI & N¹⁵ processing instructions (SOP 50.4).
3. For all other stations, select the terminal vegetative shoot from the plastic bag, remove excess water with a paper towel, and place on bench top.
4. Using scissors, collect approximately a 10 – 20 cm long section of leaf from the youngest leaf. The material may come from within the sheath as well as from the recently emerged leaf.
5. Use a Kimwipe to wipe the plant tissue free of surface contamination and blotted dry. Cut the cleaned leaf section in 1 cm pieces with a razor blade. The aim is to collect 2 cm² of leaf tissue; this will amount to 4 pieces of leaf about 1 cm long if the leaf width is about 0.5 cm.
6. Open the labeled sample 2 ml tube containing dessicant and insert the sections of leaf tissue using the forceps. Cap the tube and shake. Be careful not to allow the leaf pieces to stick together – each piece should be surrounded by the silica gel crystals. Uncap the tube and add sufficient silica to nearly fill the tube. Then cap the tube a final time.
7. Once a site is completed, prepared plant samples for genetic analysis are sent to Nikki Sarrette, Jackson Estuarine Laboratory, 85 Adams Point Road, Durham, NH 03824, 603-862-5125.

IV. TROUBLE SHOOTING / HINTS

1. Silica crystals will absorb the water out of the section of eelgrass leaf very quickly. It is essential to blot the plant material as much as possible before putting it in the silica. The trick is to use enough silica crystals and to expose the maximum surface area of the leaf to the drying substance. "Top up" each vial completely with silica after putting in the leaf sample.
2. Plants should be processed as described above within 24 hours of collection. In the field, plants should be stored in the dark in coolers and then refrigerated immediately after return to the lab.
3. Have all bags and sample tubes marked ahead of time with the unique ID #s.
4. Always mail the sample(s) in their tubes with silica intact. If not, the samples will rehydrate and be ruined.
5. Do not air-dry samples for genetic analysis before putting in silica crystals.

V. STATISTICAL ANALYSIS AND DATA USAGE

No data reduction procedures or statistical tests are appropriate for this stage of analysis.

VI. REFERENCES

Burdick, D. M., and G. A. Kendrick. 2001 Standards for seagrass collection, identification and sample design. Pp. 79-100 *In*: Short, F.T. and R.G. Coles (eds.) *Global Methods in Seagrass Research*. Elsevier Science.

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FOR GENETIC ANALYSES

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Coyer, J.A., K.A. Miller, J.M. Engle, J. Veldsink, A. Cabello-Pasini, W. T. Stam and J.L. Olsen. 2008. Eelgrass meadows in the California Channel Islands and adjacent coast reveal a mosaic of two species, evidence for introgression and variable clonality. *Annals of Botany* 101: 73–87.

Olsen, J. L., W. T. Stam, J. A. Coyer, T. B. H. Reusch, M. Billingham *et al.* 2004. North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L. *Molecular Ecology* 13: 1923-1941.

STANDARD OPERATING PROCEDURE
FOR SAMPLE PREPARATION OF EELGRASS
FOR THE WASTING INDEX, NUTRIENT POLLUTION
INDICATOR (NPI) AND STABLE ISOTOPES

SOP 50.4
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May, 2010
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POINT OF CONTACT:

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I. OBJECTIVE

To measure amount of wasting disease on eelgrass plants, as well as processing eelgrass for nutrient pollution indicator (NPI) and stable isotope (N15) measurement.

Overview: Nutrient loads and wasting disease both can stress eelgrass. Wasting disease is responsible for destroying eelgrass populations in epidemic outbreaks (Short et al. 1987, Muelhstein et al. 1990), and can be an important stressor, limiting ecological success (Burdick et al. 1993). The procedure to assess the Wasting Index provides basic morphological data useful for characterizing the size and shape of individual plants in a specific eelgrass bed (Burdick et al. 1993). The eelgrass NPI is an indicator of nutrient enrichment to an estuary (Lee et al. 2004) and can be used to determine stress associated with excessive nutrient loading (Short et al. 1995) to an eelgrass meadow. Stable isotopes can reveal nutrient sources in an estuary (Tewfik et al. 2005). In combination, these three measures can provide important information regarding estuarine nutrient sources, nutrient and Wasting Disease stresses to eelgrass and eelgrass plant morphology.

II. MATERIALS AND EQUIPMENT

- Eelgrass plants in labeled bags
- Data Sheet: UNH – TNC Eelgrass Assessment Data Form
- Ruler to 1 meter (in mm)
- Calipers (to 0.1 mm)
- Straight edge razor blades
- Sample tubes (10 ml) filled with silica beads for N analyses

III. METHODS

A. Field Collection is covered in SOP 50.2.

B. Laboratory Processing

1. For station numbers 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, and 45 (every fourth sample) process as below for Wasting Index, NPI & Isotopes (N15) as well as for genetics. For all other samples, process only for genetics, according to the Genetics Protocol (SOP 50.3 Section III.B.).

2. Assessment of Wasting Index (WI)

2.1 Enter the date, site name and site number and the unique station number for the eelgrass sample on the data sheet. Select a terminal vegetative shoot and place on bench top.

2.2 Measure the height of the sheath length to the nearest mm: measure from the top of the youngest visible sheath (usually encloses the youngest 2 - 3 leaves) to the youngest root node in cm and record on the data sheet.

2.3 Visualize numbering the leaves for each shoot from youngest (1) to oldest.

2.4 In order of youngest to oldest leaf, measure and record the length of each leaf in cm to the nearest mm: measure from the leaf tip to the root node. If a tip is broken, record its length and indicate the broken tip with an asterisk.

2.5 Again starting with the youngest leaf, measure the Wasting Index for each leaf: using the Wasting Index Key (below) as a guide, enter the percentage of the wasting disease on the leaf under 'WI' on the data sheet. The percentage of disease on a leaf

is estimated by examining the portion of the leaf from the top of the sheath to the tip, then comparing the area of disease on the leaf to those shown on the Key (leaf areas 0, 1, 10, 20, 40 and 80% infection from disease are shown). Interpolate if the leaf appears to have a percentage of diseased area between those pictured on the Key.

3. Procedure for preparing eelgrass leaf tissue for the NPI and for Stable Isotope (N15) analysis (second half of the data form)

3.1 Use the Wasting Index shoot (above) or, if needed, remove an additional, lateral shoot from along the rhizome and cut the shoot(s) from the rhizome at the meristem above the rhizome node.

3.2 Remove the two oldest leaves from the shoot(s) with their sheaths and discard, keeping the youngest leaves for analysis.

3.3 Remove all surface water and material (slime, sediments) from the youngest leaves using a Kimwipe or paper towel.

3.4 Measure the shoot width at the top of the sheath to the nearest 0.1 mm with the calipers. Record the width under Shoot 1 on the data sheet.

3.5 To obtain the plant tissue samples for the NPI, cut the shoot exactly 20 cm above the top of the sheath, again at 10cm above the sheath and finally at the top of the sheath. Then, from the 0 – 10 cm part of these leaves (the part nearest the sheath), discard the youngest leaf (which will likely end at an intact leaf tip) and select the second-youngest leaf for genetic analysis (see SOP 50.3, Section III.B.).

Gather the third and fourth youngest leaf sections from the 0 – 10 cm part and the 3 to 4 youngest leaf sections from the 10 – 20 cm part for NPI and isotope analysis. Avoid using any floppy, immature leaf tissue, and avoid any grazed, damaged, or epiphytized leaf pieces. From these four to six leaf sections, choose a sufficient number of 10 cm sections to create leaf area to equal 20 cm² for analysis.

If there is insufficient material available from the original shoot (Shoot 1), select and section a second (and/or third) lateral shoot from the attached horizontal rhizome in order to obtain sufficient leaf area. Measure the shoot width at the top of the sheath to the nearest 0.1 mm with the calipers. Record the width under Shoot 2 or 3, as appropriate, on the data sheet.

Record on the data sheet the number of 10 cm leaf sections included in the NPI sample from each shoot. If need be, sections shorter than 10 cm can be used and recorded as partials with their lengths (i.e., 5.6 cm section).

3.6 When collecting leaf material, only mature plant material from within the sheath should be used. Do not include the leaf tip in the NPI/isotope samples. Do not use any plant material that is visibly epiphytized or diseased.

3.7 Cut the 10 cm sections in half and insert all the leaf sections into a 10 ml tube with silica beads. Top off the tube with silica beads after inserting the leaves. Tighten cap and check label, include unique ID #, and place it into a plastic bag.

4. Once a site is completed, prepared plant samples are sent to Nikki Sarrette, Jackson Estuarine Laboratory, 85 Adams Point Road, Durham, NH 03824, (603) 862-5125.

IV. TROUBLE SHOOTING / HINTS

1. The freshwater rinse performed in the field is critical for accurate assessment of wasting disease.
2. Plants should be processed within 24 hours of collection.

V. STATISTICAL ANALYSIS AND DATA USAGE

No data reduction procedures or statistical tests are appropriate for this stage of analysis.

VI. REFERENCES

- Burdick, D. M., F. T. Short, and J. Wolf. 1993. An index to assess and monitor the progression of wasting disease in eelgrass *Zostera marina*. Marine Ecology Progress Series 94:83-90.
- Burdick, D. M., and G. A. Kendrick. 2001 Standards for seagrass collection, identification and sample design. Pp. 79-100 *In*: Short, F.T. and R.G. Coles (eds.) Global Methods in Seagrass Research. Elsevier Science.
- Lee, K., F. T. Short and D. M. Burdick. 2004. Development of a nutrient pollution indicator using the seagrass, *Zostera marina*, along nutrient gradients in three New England estuaries. Aquatic Botany 78: 197-216.
- Muehlstein, L.K., D. Porter, and F.T. Short. 1991. *Labyrinthula zosterae* sp. Nov., the causative agent of wasting disease of eelgrass, *Zostera marina*. Mycologia, 83(2):180-191.
- Short, F.T. and Burdick D.M. 2003. Eelgrass as an indicator of nutrient over-enrichment in estuaries: a final report submitted to the NOAA/UNH Cooperative Institute for Coastal and Estuarine Environmental Technology. University of New Hampshire, Durham, NH 60 pp. +CD-ROM. [web NPI - Nutrient Pollution Indicator Manual: <http://marine.unh.edu/jel/faculty/fred2/fredshort.htm>]
- Short, F.T., L.K. Muehlstein and D. Porter. 1987. Eelgrass wasting disease: cause and recurrence of a marine epidemic. Biological Bulletin 173:557-562.

STANDARD OPERATING PROCEDURE SOP 50.4
FOR SAMPLE PREPARATION OF EELGRASS
FOR THE WASTING INDEX, NUTRIENT POLLUTION
INDICATOR (NPI) AND STABLE ISOTOPES

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Short, F. T., D. M. Burdick, and J. E. Kaldy, III. 1995. Mesocosm experiments quantify the effects of coastal eutrophication on eelgrass, *Zostera marina* L. *Limnology and Oceanography* 40:740-749.

Tewfik, A., J.B. Rasmussen, K.S. McCann. 2005. Anthropogenic enrichment alters a marine benthic food web. *Ecology* 86: 2726–2736.

STANDARD OPERATING PROCEDURE
FOR PREPARATION OF ARCHIVAL
EELGRASS HERBARIUM SPECIMENS

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I. OBJECTIVE

To prepare and press eelgrass as herbarium specimens that will be archived at UNH for later study and use.

Overview: The permanent record of eelgrass plants from specific sites and with known microsatellite distributions (genetic diversity) may be valuable for interpretation of results of the Eelgrass Genetic study and even more valuable for unknown studies conducted in the future (Burdick and Kendrick 2001).

II. MATERIALS AND EQUIPMENT

- Plant press
- Blotting paper / newspaper
- Herbarium labels
- Herbarium paper
- Floor fan

III. METHODS

A. Field Collection for herbarium samples is covered in SOP 50.2. For station numbers 1, 13, 25, 37, and 45, a shoot is collected for a herbarium archive.

B. Laboratory Processing (abstracted from Burdick and Kendrick 2001)

1. Five terminal shoots are collected from each sample site for archiving as a herbarium specimen. Eelgrass samples are pressed fresh, with a freshwater rinse, but without chemical treatment.

2. Place each plant separately on herbarium paper and arrange to clearly show the different tissues (rhizome, roots, shoot, reproductive portions, etc.). Fill out a herbarium label using collection data, including unique ID # and enclose with plant (see sample label below).

3. Separate each sample with blotter plus ventilation boards (corrugated cardboard) and place in a press. Dry in a well-ventilated room. A floor fan is ideal for improving ventilation.

4. Change the blotters daily until dry (2 to 3 days).

5. Once a site is completed, mail the press with the herbarium sheets to Nikki Sarrette, Jackson Estuarine Laboratory, 85 Adams Point Road, Durham, NH 03824, 603-862-5125.

V. STATISTICAL ANALYSIS AND DATA USAGE

No data reduction procedures or statistical tests are appropriate for this stage of analysis.

VI. REFERENCES

Burdick, D. M., and G. A. Kendrick. 2001 Standards for seagrass collection, identification and sample design. Pp. 79-100 *In*: Short, F.T. and R.G. Coles (eds.) *Global Methods in Seagrass Research*. Elsevier Science.

Dawes, C.J. 1981. *Marine Botany*. John Wiley, New York. 628 pp.

Herbarium Label:

Species Name: *Zostera marina* L. and Eelgrass

Collection Site: Use Site Name, Site Number for SNE Project
Lat/Long from GPS of the Site:

Collection Date: [dd MMM yyyy] 23 Jun 2010

Collector with Contact Information:

Unique ID# for Station:

STANDARD OPERATING PROCEDURE
FOR SEDIMENT ANALYSIS TO DETERMINE
ORGANIC CONTENT AND GRAIN SIZE

SOP 50.6
Revision 0.1
February, 2011
Page 1 of 4

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I. OBJECTIVE

To determine sediment organic content determined by loss on ignition and characterize distribution of sediment size classes (gravel, sand and silt/clay fractions). See Erftemeijer and Koch 2001.

II. MATERIALS AND EQUIPMENT

- Drying oven
- Muffle furnace
- Aluminum weighing dishes
- Temperature-corrected optical refractometer
- Dessicator
- 1L graduate cylinder
- Mortar and pestle
- 63 μ and 2 mm sieves with catch pan
- Deionized water
- Squeeze bottle with deionized water
- Small beakers (50 mL)
- Glass stirring rod, spatula, brush
- Plastic funnel

III. METHODS

1. Label the underside of the aluminum pan by etching the unique serial number for the sample you are processing. Any ink or pencil labels will be lost upon combustion.
2. Place sediment sample into pre-weighed aluminum pan; use glass stirring rod to disperse the sample evenly inside the pan. Add 10 ml of deionized water while picking out any large shell material using forceps and rinse; stir with glass rod; be careful not to lose any of the sediment. Set the sample aside to settle until water is clear, then measure the salinity using a few drops of the water and decant and discard the rest of the clear water fraction. Weigh sediment sample to the nearest 0.0001 g to determine the wet weight.
3. Dry in oven at 60° C for 24 hours or until completely dry. Place sample in dessicator until cooled to room temperature. Reweigh the sample to obtain the dry weight.
4. Heat sample in muffle furnace at 450° C for 4 hours to determine organic matter content. Place sample in dessicator until cooled to room temperature. Reweigh sample once it is sufficiently cooled to obtain the combusted weight.
5. Then make a calculation to remove the weight due to salinity from the dry weight and from the combusted weight: calculate the water weight in the sample (wet weight – dry weight). Multiply this weight by the salinity expressed in ppt; then multiply the water weight by 0.001 to determine the weight of salt. Calculate the corrected weight of the samples by subtracting the salt weight from the dry weight and the salt weight from the combusted weight, yielding the salt-free weights “dry wt” and “combusted wt”

6. Loss on Ignition is calculated as % weight loss after combustion using (Erfteimeijer and Koch 2001):

$$\text{LOI} = 100 * ((\text{dry wt} - \text{combusted wt}) / \text{dry wt})$$

7. Now that the organic content has been burned out of the sample, the sample must be gently ground by use of mortar and pestle to break up aggregates caused by combustion. Place sample into mortar and gently grind apart the aggregates using only the weight of the pestle.
8. After the sample has been gently disaggregated, dry sieve the sample through a stack of two sieves consisting of a 2mm and a 63 μ stainless steel sieve plus a catch pan. Be careful not to touch the sieve with your hand or anything that will damage the mesh. Put the disaggregated sample into the 2mm sieve on top, cover and shake with a circular motion and tap until the sand fraction has passed through the 2 mm sieve, leaving the shell and gravel material on top of the 2mm sieve. The sand fraction is left on the 63 μ sieve, and the silt-clay fraction (finest) is in the catch pan. Transfer the three grain size fractions into separate pre-weighed pans. Re-dry for an hour, then weigh when cool. The weight minus the weight of the pre-weighed pan yields the weight for each fraction.
9. The weights of the three grain size fractions: 1) gravel/shell, 2) sand, and 3) silt/clay are summed to provide the total weight and the percent of each fraction by weight is calculated.

IV. TROUBLE SHOOTING / HINTS

1. The salts do not affect estimates of grain size distribution.
2. Drying of sediment samples can be checked by placing them back in the drying oven and reweighing the following day.

3. Dry samples must be cooled in a dessicator before weighing. Dried and combusted samples will gain moisture from the atmosphere rapidly. Also, warm samples will give erroneous readings by creating convection currents around the pan of the balance.

V. STATISTICAL ANALYSIS AND DATA USAGE

1. Input pan weight, wet weight, salinity, dry weight, combusted weight and dried sediment fractions to spreadsheet. Calculate % LOI and size fractions using appropriate formulae (above).
2. Calculate mean and standard error of five sediment samples for each eelgrass collection site.

VI. REFERENCES

Erftemeijer, P.L.A., E.W. Koch. 2001. Sediment geology methods for seagrass habitat, p. 345-368, In Short, F.T. and R.G. Coles (eds.) Global Seagrass Research Methods. Elsevier Science B.V., Amsterdam. 473 pp.

UNH -TNC Eelgrass Assessment: Data Form														Date:		Site Name:		GPS:		Lat.		
														Site Number:		Long.						
Wasting Index														NPI & N15								
youngest														oldest		Shoot 1		Shoot 2		Shoot 3		Leaf Mass
no	Station	Sheath length cm	#1 Leaf length (cm)	WI %	#2 Leaf length (cm)	WI %	#3 Leaf length (cm)	WI %	#4 Leaf length (cm)	WI %	#5 Leaf length (cm)	WI %	#6 Leaf length (cm)	WI %	no. of 10 cm sections	Leaf width mm	no. of 10 cm sections	Leaf width mm	no. of 10 cm sections	Leaf width mm	Weight all sections combined mg	
1	1																					
2	5																					
3	9																					
4	13																					
5	17																					
6	21																					
7	25																					
8	29																					
9	33																					
10	37																					
11	41																					
12	45																					

* broken leaf tip

box indicates collect extra eelgrass shoot for herbarium and samples for sediment and salinity