Deepwater Horizon/Mississippi Canyon 252 Spill

Sampling and Monitoring Plan for the Assessment of MC252 Oil Impacts to Coastal Wetland Vegetation in the Gulf of Mexico

Approval of this work plan is for the purposes of obtaining data for the Natural Resource Damage Assessment (NRDA). Each party reserves its right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

Each laboratory shall simultaneously deliver raw data, including all necessary metadata, generated as part of this work plan as a Laboratory Analytical Data Package (LADP) to the trustee Data Management Team (DMT), the Louisiana Oil Spill Coordinator's Office (LOSCO) on behalf of the State of Louisiana, and to BP (or Cardno ENTRIX on behalf of BP). The electronic data deliverable (EDD) spreadsheet with pre-validated analytical results, which is a component of the complete LADP, will also be delivered to the secure FTP drop box maintained by the trustees' Data Management Team (DMT). Any preliminary data distributed to the DMT shall also be distributed to LOSCO and to BP (or Cardno ENTRIX on behalf of BP). Thereafter, the DMT will validate and perform quality assurance/quality control (QA/QC) procedures on the LADP consistent with the authorized Analytical Quality Assurance Plan, after which time the validated/QA/QC'd data shall be made available simultaneously to all trustees and BP (or Cardno ENTRIX on behalf of BP). Any questions raised on the validated/QA/QC results shall be handled per the procedures in the Analytical Quality Assurance Plan and the issue and results shall be distributed to all parties. In the interest of maintaining one consistent data set for use by all parties, only the validated/QA/QC'd data set released by the DMT shall be considered the consensus data set. In order to ensure reliability of the consensus data and full review by the parties, no party shall publish consensus data until 7 days after such data has been made available to the parties. Also, the LADP shall not be released by the DMT, LOSCO, BP or Cardno ENTRIX prior to validation/QA/QC absent a showing of critical operational need. Should any party show a critical operational need for data prior to validation/QA/QC, any released data will be clearly marked "preliminary/unvalidated" and will be made available equally to all trustees and to BP (or Cardno ENTRIX on behalf of BP).This plan will be implemented consistent with existing trustee regulations and policies. All applicable state and federal permits must be obtained prior to conducting work.

Approval of this work plan is for the purposes of obtaining data for the Natural Resource Damage Assessment (NRDA). Each party reserves its right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

APPROVED:

9/02/2011

Louisiana Trustee Representative: **Date**

Laura W. Tobe 8.23.2011

BP Representative: **Date**

8/10/2011

NOAA Trustee Representative **Date**

(on behalf of all other trustees)

Sampling and Monitoring Plan for the Assessment of MC252 Oil Impacts to Coastal Wetland Vegetation in the Gulf of Mexico

August 4, 2011

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This document presents a sampling and monitoring plan for use in assessing the impacts of MC252 oil on coastal wetland vegetation as well as soil characteristics and elevation along the Gulf of Mexico coast, particularly in Louisiana. The plan may be implemented before oil from the MC252 incident(s) reaches an area (to the extent feasible), and also thereafter. The plan specifically addresses the following topics:

I. **Introduction and objectives.** This section describes the overall purpose and objectives for a coastal wetland vegetation assessment.

II. **Health and safety.** This section summarizes pertinent health and safety protocols applicable to this effort. It includes a number of procedures by reference, all of which should be carefully reviewed and adhered to by all team members.

III. **Site location selection.** This section describes the proposed approach to identifying sites for evaluation.

IV. **Survey timing and frequency.** This section describes the proposed approach with respect to timing issues: when surveys can be executed, and at what frequency subsequent monitoring may occur.

V. **General survey procedures.** This section notes, by reference, case-wide protocols for use as part of this sampling plan.

VI. **Establishing transects and study plots.** This section describes how to establish transects and plots at the selected sites, and within representative types of Gulf Coast coastal wetland vegetation.

VII. **Characterization of herbaceous coastal wetland vegetation health.** This section describes specific activities and procedures pertaining to site characterization and sample collection in this habitat.

VIII. **Characterization of Louisiana (stunted) black mangrove health.** This section describes specific activities and procedures pertaining to site characterization and sample collection in this habitat.

IX. **Soils - general characterization.** This section describes specific activities and procedures that will generate information on soil physical and chemical characteristics including contaminants.

X. **Soil sampling for contaminant characterization.** This section references the case-wide protocol for soil collection.

XI. **Post-survey management of samples and data.** This section includes the procedures to be used in managing data and samples after collection.

XII. **Quality Assurance Project Plan.**

XIII. **Coastal wetland vegetation elevation survey.** This section describes specific activities and procedures pertaining to collection of shoreline and coastal wetland vegetation elevation data.

XIV. **References**

Appendix A – Acknowledgements

Appendix B – Fall 2010 Datasheets

Appendix C – Fall 2010 Quick Reference Guides

Appendix D – Spring 2011 Datasheets

Appendix E – Spring 2011 Quick Reference Guides

Appendix F - Budget

I. INTRODUCTION AND OBJECTIVES

This initial phase of sampling covers the first three monitoring events; Fall 2010, Spring 2011, and Fall 2011. Data collected during these events will be used to refine the plan for future years. This plan may be extended to 2012 and beyond by executing yearly addenda. This sampling and monitoring plan provides a detailed practical methodology for collecting ephemeral data for use in assessing the potential effects of MC252 oil on coastal wetland vegetation along the Gulf of Mexico (GOM) coast as part of the NRDA for the MC252/Deepwater Horizon oil spill. It also aims to provide information and data that can assist in identifying, designing and implementing further procedures as may be needed to complete the NRDA process for assessing the impact of MC252 oil on coastal wetland vegetation. The data collected under this plan may be used to establish unoiled and post-impact conditions and to monitor recovery. Results from implementation of this plan may be combined with results from related efforts (e.g., analysis of aerial imagery/remote sensing data) to produce an overall understanding of the potential effects of the Deepwater Horizon spill on coastal wetland vegetation habitat. The Trustees reserve the right to perform additional analyses on samples collected under this plan, or any addendum to this plan, independently or as part of a trustee/BP cooperative sampling effort.

The plan's objectives are:

A. To collect and evaluate ephemeral and other data that will assist in the evaluation and assessment of the potential effects of MC252 oil on herbaceous coastal wetland vegetation health and in design and implementation of additional assessment activities appropriate to the purpose;

B. To collect and evaluate ephemeral and other data that will assist in the design and implementation of other assessment activities related to Louisiana (stunted) black mangrove health; and

C. To provide data that will assist in the design and implementation of other activities as may be needed to characterize and assess the physical and chemical characteristics of soils and sediments[1], including contaminants in so far as they relate to MC252 impacts.

This sampling and monitoring plan is specific to Louisiana coastal wetland habitats, but is broadly applicable to coastal wetland vegetation in general. Sampling efforts outside of Louisiana will be addressed through addenda. Regional variation in dominant coastal vegetation types in the Gulf of Mexico may require slight modifications of the sampling design and protocol to accurately assess coastal wetland vegetation on a broader spatial scale.

The procedures described in this document may be used in the context of both unoiled and post-oiling data collections. The specific protocols are focused on biological characterization unique to coastal wetland vegetation and refer to other protocols for general case-wide procedures (e.g., GPS use, photography guidance, etc.) as well as to case-wide protocols associated with chemical characterization of soils and sediments.

Since coastal wetland habitats provide a variety of ecological services, there are many potential metrics that can be used to assess impacts to these habitats due to oiling. The current plan focuses on metrics relating to the health of macrophytes (Table 1). Additional metrics (e.g., for faunal analysis) may be considered in the future in a separate plan or addendum.

[1] Note that the terms soil and sediment are used nearly interchangeably throughout the document; their use is not intended to create a distinction between different matrices.

Table 1. Proposed measures of ecological function and services for coastal wetland vegetation habitats.

Ecological Service	Measure	Proposed Sampling Frequency
Primary production	**Herbaceous coastal wetland vegetation metrics**	
	Light-adapted fluorescence (unitless)	A
	Chlorophyll content (SPAD)	
	Oiling impact extent	
	Vegetation condition index	A
	Perpendicular penetration of oil into marsh (m)	
	Sediment surface oiling (%)	
	Oiling extent index	
	Total aboveground biomass (g/m^2; live and dead by species)	B
	Stem height (cm per stem and cumulative cm/m^2)	
	Stem density (# per m^2)	
	Belowground biomass (g/m^2)	A
	Louisiana mangrove metrics	
	Adult Trees ($\geq$ 50 cm)	
	Mangrove maximum height (in 4-m^2 plot; cm)	A
	Number of trees (all tagged in 1-m^2 subplot; # per $m^{2)}$	
	Oiling impact extent (in 4-m^2 plot)	
	Vegetation condition index	
	Perpendicular penetration of oil into marsh (m)	
	Sediment surface oiling (%)	
	Oiling extent index	
	Belowground biomass (g/m^2)	
	Area of tree canopies based on perpendicular diameter measurements (in 1-m^2 subplot; m^2)	
	Light-adapted fluorescence (in 4-m^2 plot; unitless)	
	Chlorophyll content (SPAD) (in 4-m^2 plot)	
	Height of oiling on pneumatophores (in 1-m^2 subplot; cm)	
	Height of each tree (in 1-m^2 subplot; cm)	
	Survival (in 1-m^2 subplot; % of tagged trees)	
	Number of branches per tree, leaf bearing and bare (in 1-m^2 subplot; #)	
	Pneumatophore density (in 1-m^2 subplot; # per m^2)	
	Pneumatophore height (in 1-m^2 subplot; cm)	
	Main stem diameter of each tree (in 1-m^2 subplot; cm)	
	Height of oiling on adult stems (in 1-m^2 subplot; cm)	
	Propagule production index (in 1-m^2 plot)	B
	Seedlings (< 50 cm, all tagged in 1-m^2 subplot)	
	Number of seedlings (in 1-m^2 subplot; # per m^2)	A
	Height of each seedling (in 1-m^2 subplot; cm)	
	Number of main stem nodes (in 1-m^2 subplot; #)	

Ecological Service	Measure	Proposed Sampling Frequency
	Internodal distance along main stem (in 1-m^2 subplot; cm)	
	Survival (in 1-m^2 subplot; % of tagged seedlings)	
	Oiling impact extent (in 4-m^2 plot)	
	Vegetation condition index	
	Perpendicular penetration of oil into marsh (m)	
	Sediment surface oiling (%)	
	Oiling extent index	
	Height of oiling on seedlings (in 4-m^2 plot; cm)	
	Canopy extent (in 1-m^2 subplot; total number of leaves, # per seedling)	
Provision of	**Herbaceous coastal wetland vegetation metrics**	
marsh habitat	Visually-estimated live and dead cover of plant species (%)	A
	Average canopy height of dominant species (cm)	
	Oiling impact extent	
	Vegetation condition index	
	Perpendicular penetration of oil into marsh (m)	
	Sediment surface oiling (%)	
	Oiling extent index	
	Oiling height on stems (cm)	
	Louisiana mangrove metrics	
	Adult Trees ($\geq$ 50 cm, all tagged in 1-m^2 subplot)	
	Oiling impact extent (in 4-m^2 plot)	A
	Vegetation condition index	
	Perpendicular penetration of oil into marsh (m)	
	Sediment surface oiling (%)	
	Oiling extent index	
	Visually-estimated live and dead cover of mangrove and other species (in 4-m^2 plot; %)	
	Pneumatophore density (in 1-m^2 subplot; # per m^2)	
	Oiled pneumatophores (in 1-m^2 subplot; # per m^2)	
	Pneumatophore height (in 1-m^2 subplot; cm)	
	Pneumatophore oiling height (in 1-m^2 subplot; cm)	
	Seedlings (< 50 cm, all tagged in 1-m^2 subplot)	
	Oiling impact extent (in 4-m^2 plot)	A
	Vegetation condition index	
	Perpendicular penetration of oil into marsh (m)	
	Sediment surface oiling (%)	
	Oiling extent index	
Marsh	Plot elevation (measured by RTK and tied to NAVD88/ GULFNet)	B
sustainability	Shoreline change (cm/year)	
	Soil bulk density (g/cm^3)	A
Carbon sequestration	Soil organic matter (%)	A
Nutrient cycling	Extractable KCl, nitrate-nitrite, ammonia (ppm)	A
Biogeochemical	Extractable salinity (ppt), pH	A
processes	Extractable sulfate (ppm)	

Ecological Service	Measure	Proposed Sampling Frequency
	Soil Eh (mV)	
	Extractable elements (ppm)	
	Sand, silt, clay composition	
	TPH and PAH concentrations in soils (ppm)	

Proposed Sampling Frequency

A = at 0, 0.5, 1, 2, 3, 5 and 10 years

B = at 0, 1, 2, 3, 5 and 10 years

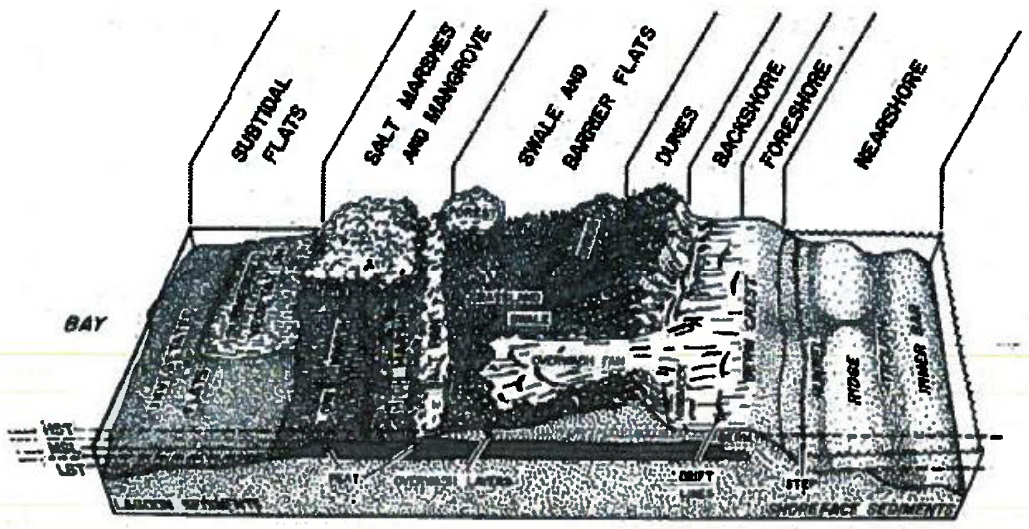
Notes:

Some measures of ecosystem processes contribute to more than one ecosystem service and therefore appear more than once in the table above.

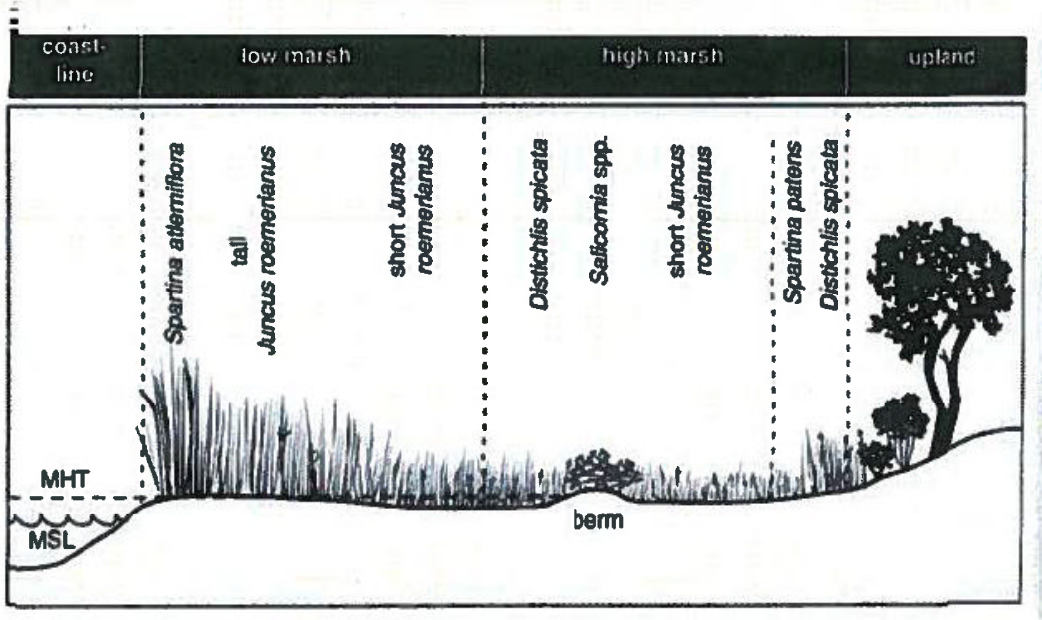
Measures of fisheries and food web support ecosystem services, although important, are not directly addressed in this sampling plan.

Coastal wetland vegetation can vary in plant composition across regions, and within different hydrogeomorphic settings within a region. Figure 1 presents some typical coastal wetland macrophyte distributions along idealized transects from water's edge towards interior. In Louisiana, *Spartina alterniflora* (smooth cordgrass) is generally the dominant lower inter-tidal macrophyte, although *Juncus roemerianus* (black needle rush) is present in many of Louisiana's coastal wetland habitats. *Avicennia germinans* (black mangrove) is present in barrier island (back barrier) coastal wetland habitats and also in the more southern coastal wetland habitats, both as continuous stands of stunted black mangrove and in association with smooth cordgrass. Across the Northern Gulf of Mexico into Florida there are often some shifts in herbaceous coastal wetland macrophyte dominance from *Spartina alterniflora* (smooth cordgrass) to *Juncus roemerianus*.

A



B



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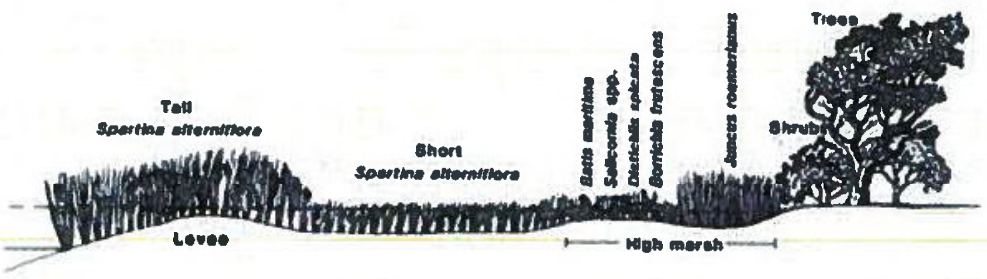

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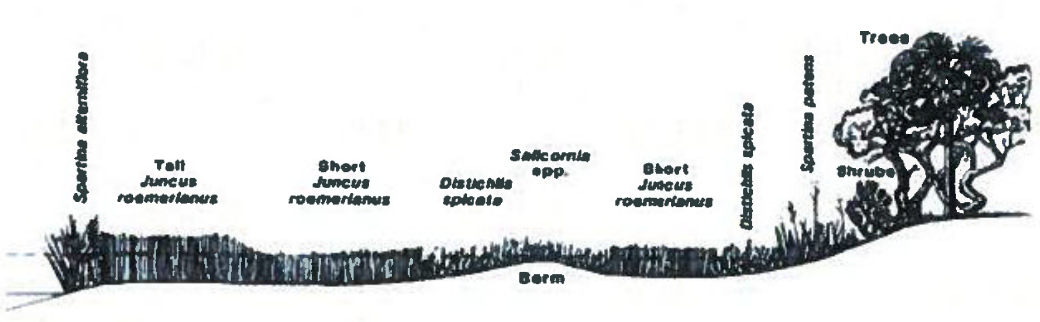

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II. HEALTH AND SAFETY

1. The team leader and field crew parties should have completed all applicable health and safety training as directed by NOAA or state agency oil spill policy.
2. All field team members must complete the NOAA safety training and documentation requirements set forth in "Safety Requirements for All Personnel Working on NOAA-led NRDA teams for MS Canyon 252 Incident" (02 - Safety Documentation Requirements.doc in the Resource Catalog on the case's website, noaanrda.org).
3. All field team members should read all of the documents in the Safety directory of the Resource Catalog of noaanrda.org. Exception: if site collection activities do not include use of a boat or helicopter, then familiarity with the safety documents for these vehicles is not required.
4. Each field team must submit a plan, not later than the night prior to going into the field. This plan must specify:
    - The team leader;
    - Names of all team members;
    - The sampling location(s)-- please use the grid coordinates provided in the site Safety Maps (available in the Safety section of the Resource Catalog of noaanrda.org);
    - What kind of sampling they are doing;
    - Expected arrival time at sampling area (daily);
    - Expected departure from sampling area (daily);
    - Team deployment date;
    - Team return date.

    This information may be reported in one of two ways:

    - Fill out the Excel spreadsheet "04 - Team Member Information Form – Sampling and Safety.xls (available in the Safety section of the Resource Catalog of noaanrda.org) and send it to dwhnrda@gmail.com. Please use one tab for each team.
    - If you cannot submit this spreadsheet electronically, you can call in and report the information using this number: (985) 746-4916.
5. Field teams must adhere to all procedures set forth in the MC252 Site Safety Plan ("NRDA MC 252 Site Safety Plan_6.22.10.pdf" and 12/8/2010 revision, available in the Resource Catalog of noaanrda.org).
6. If participating in a cruise: Each cruise may have additional required health and safety procedures, which must be observed.
7. The safety procedures outlined here are subject to change at the direction of the Deepwater Horizon NRDA Field Operations team. Such changes will be applicable to field teams under this plan without the need for an amendment to this plan.

III. SITE SELECTION

The site selection process involves the following steps. First, based on available data, the targeted coastline will be divided into a few broad "sampling regions." For example, Louisiana coastal wetland vegetation may be divided into a number of regions based on combinations of location and dominant vegetation (e.g., mainland-herbaceous salt marshes) to reduce environmental variation not associated with this spill. Additional combinations may be added depending on impacted areas, if needed. For this spill mainland herbaceous salt marshes, barrier island salt marshes, mangroves habitat and *Phragmites* habitat were sampled.

Second, a pool of possible sampling sites will be identified based on the review of earlier preassessment data, SCAT reports, aerial photography and other available data in each sampling region. In this process, beach locations devoid of coastal wetland vegetation, sites impacted by human activities, locations along man-made canals, as well as sites entailing lengthy permitting process will be excluded. To the extent possible, the identified locations should be dominated by the targeted vegetation type.

Third, based on the review of SCAT data and quantitative information provided by earlier preassessment observations, each possible sampling site will receive an oiling extent designation. Oiling extent is defined as the ratio of the observed oil band height on the vegetation relative to the average vegetation height (expressed as a percentage). Considered oiling extent categories include:

1. Unoiled: no oiling from this spill reported from SCAT or other pre-assessment activity and no visible signs of oil on the vegetation, on or in the soil, or on the nearby water.

2. Trace to 10%: Vegetation, standing or prone, with 1% to less than 10% of the visible plant surface coated with oil, with or without oil detectable on or in the soil. This category also includes locations with no visible oil on the vegetation, but observed oil on or in the soil. Given observations of this condition in the field, we believe it is reasonable to include this condition within the trace to 10% category.

3. 10% to less than 50%: Vegetation, standing or prone, with 10% to less than 50% of the visible plant surface coated with oil, with or without oil detectable on or in the soil.

4. 50% to less than 90%: Vegetation, standing or prone, or stubble with 50% to less than 90% of the visible plant surface coated with oil, with or without oil detectable on or in the soil.

5. 90% to 100%: Vegetation, standing, prone, or stubble, with greater than 90% of the visible plant surface coated with oil, with or without oil detectable on or in the soil.

The above cited oiling categories may be adjusted according to the extent of observed oiling within specific sampling regions.

Fourth, to the extent possible, from the pool of sampling sites within each sampling region, a number of sites will be randomly selected from each oiling extent category.[2] Selected locations of sites will be distributed to maximize the sampling coverage of the region. Additional specific site selection considerations will include the availability of sampling sites with existing pre-incident data, such as the CRMS sites along the Louisiana coast, the ability to coordinate locations with planned sampling efforts in adjacent habitats (e.g., SAV beds, oyster reef, and/or general nearshore subtidal areas), and the ability to obtain landowner permission for access. Given the fact that in some locations such as on National Wildlife Refuges coastal wetland vegetation sampling entails permitting requirements, every attempt must be made to fix the location of selected sites for all subsequent monitoring efforts.

In addition to the selected sites, alternative locations will be determined from the pool of sampling sites within each sampling region to allow for substitution of sites if necessary. For instance, field teams may find that designated unoiled sites are oiled. In that event, the field team will instead proceed to a predesignated alternative site.

The Field Chief will assign a unique site code to each site. After consideration of other sources of shoreline oiling data (such as SCAT records and photos) and initial data collection and analysis (including chemical analytical data), the original classification and/or the category of each selected site will be reviewed, and if necessary, will be corrected consistent with field observations. Upon confirmation of site classifications and categories, the statistical design will be reassessed and determinations will be made as to whether changes (e.g., evaluating additional sites) are required to provide greater statistical rigor, if deemed necessary or appropriate.

IV. SURVEY TIMING AND FREQUENCY

This data collected under this plan may be used to establish unoiled conditions, post-impact conditions and to monitor recovery.

Under this plan, sampling will be conducted at 0 and 1 years subsequent to oiling (Table 1). For certain variables, an additional sampling effort will be conducted at year 0.5. Results from past sampling efforts will be used to help determine the need for any future sampling and monitoring in order to assess the impacts of oil on coastal wetland flora. Additional monitoring efforts will be proposed as addenda to this sampling plan.

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[2] For example, for mainland-herbaceous salt marshes, 14 transects within each of the five (5) oiling categories will be established as follows: (a) Seven (7) *Spartina alterniflora*-dominated transects where *Spartina alterniflora* contributes the greatest species cover to the total vegetation cover in each plot (i.e., *Spartina alterniflora* is the dominant species in each transect plot); and (b) Seven (7) *Spartina alterniflora*-present transects where *Spartina alterniflora* is present in the immediate transect area, but is not required to be dominant or present in a transect plot.

V. GENERAL SURVEY PROCEDURES

Field staff will receive training prior to each field effort.

Several case-wide protocols and associated forms have been developed for common activities, including:

- Chain of Custody procedures and documentation;
- Sample collection forms;
- GPS setup and use;
- Field photography guidance;
- Photologger forms; and
- Data management.

All field team members should familiarize themselves with these documents, available in the Data Management section of the Resource Catalog of noaanrda.org[3], prior to commencing fieldwork. As needed, team members should participate in NRDA data management training to learn about these protocols, and to have any questions answered.

Copies of all appropriate forms and datasheets, including those mentioned above as well as those specific to the current sampling plan, should be assembled as part of the equipment needed for the day's activities. Sample and data transfer/upload procedures should be followed after completion of the day's field work activities.

Aside from case-wide photography guidance, specific guidance has been developed for this plan, organized by activities completed by forward and plot teams. Forward teams identify sites and set stakes for the plot teams. Plot teams collect measurements outlined in the plan protocols.

Recommended Photography Procedure

"Forward Team": For each site, the first photo should be a photo of a whiteboard with the site ID written. Then, take four site photographs following placement of the shoreline and interior stakes. The first photograph should be taken from the interior stake facing directly toward the shoreline stake. The second and third photographs should be taken at the shoreline stake facing parallel to the shoreline in both directions. The fourth photograph should be taken from just offshore (1-2 m) with both the shoreline and interior stake in the photo frame.

"Plot Team": Take two photographs of each cover plot and each productivity plot. A whiteboard with the site name and plot number should be placed in the lower right-hand corner (as you are

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[3] http://www.noaanrda.org/twg-resource.nsf/dx/Data_Management_NRDA_Photos_and_GPS_Guidance and http://www.noaanrda.org/twg-resource.nsf/dx/Data_Management_Field_Sample_Forms

facing the plot from the transect) of the plot and should be visible in each picture. The first photograph (90° photo) should be taken with your back to the shoreline from directly above the plot ensuring that the whole plot and the whiteboard are visible. The second photograph (45° photo) should be taken facing the plot from the transect line, aligning yourself with the center of the plot. For a site with 3 paired plots a total of 12 plot photographs will be taken (2 photographs x 3 cover plots plus 2 photographs x 3 productivity plots). For a site with 2 paired plots a total of 8 plot photographs will be taken (2 photographs x 2 cover plots plus 2 photographs x 2 productivity plots). For a site with 1 paired plots a total of 4 plot photographs will be taken (2 photographs x 1 cover plot plus 2 photographs x 1 productivity plot).

Take one photograph of each belowground biomass core. Orient the belowground biomass core horizontally (relative to the photographer) and place a measuring device below and parallel to the core. Place a whiteboard or label with the site number and plot number above the core. Take the photograph from above each core ensuring that the core, measuring device, and label are all visible in the photograph. For *Spartina alterniflora* and mangrove-dominated communities, there will be one core photograph taken at each productivity plot since one core will be taken at each plot[4]. For a site with 3 paired plots a total of 3 core photographs will be taken (1 photograph x 1 belowground biomass core x 3 productivity plots). For a site with 2 paired plots a total of 2 core photographs will be taken (1 photograph x 1 belowground biomass core x 2 productivity plots). For a site with 1 paired plot a total of 1 core photographs will be taken (1 photograph x 1 belowground biomass core x 1 productivity plot).

VI. ESTABLISHING TRANSECTS AND STUDY PLOTS

General Procedures

At each site, a transect shall be established that is approximately perpendicular to the shoreline and proceeds inland into the coastal wetland vegetation. Because the transect must end in the interior of the coastal wetland vegetation, and not near another shoreline, the transect must be relocated if it does not terminate within the coastal wetland vegetation interior. The ends of the transect shall be staked using a PVC pipe and the coordinates recorded on the "Site Visit/Set Up Datasheet". Transects should be placed so as to avoid open water (i.e., pond) areas. In particular, the edge of plots should be located a minimum of 5 meters from the edge of the open water area (with the exception of the shoreline edge of shoreline plots, which is located 1 m from the shoreline). Further, to the extent possible, transects should be placed to minimize the occurrence of oil boom, wrack, and debris in the intended plot locations.

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[4] For *Phragmites* communities, two cores will be taken per plot; each core will be photographed separately and then placed into the same bag (see section Additional Protocols for *Phragmites* Sampling).

The appearance of all sites and plots will be thoroughly documented using digital cameras. GPS readings will be recorded for the lower left corner of each plot (facing inland, the left-hand seaward corner) and should be recorded on the appropriate plot datasheet.

In general, detailed photographic and GPS logs will be maintained as described in the latest MC252 NRDA guidance (available on www.noaanrda.org).

Due to the inherent variation in all coastal wetland vegetation metrics among the environmental settings, it is important to select appropriate unoiled areas. As discussed here, "reference" areas are unoiled coastal wetland vegetation that are similar (to the extent possible) in hydrogeomorphic setting, vegetation type, past environmental history, etc., and for which data can be obtained for key metrics in the same general area where oiling impacts are assessed. These unoiled sites will be studied using the same methods as at oiled sites, so that temporal comparisons can be made between oiled and unoiled measures. Note that certain unoiled herbaceous coastal wetland vegetation plots will be paired with an additional set of unoiled plots in which the vegetation is temporarily "laid over" by hand using PVC poles. This extra set of unoiled plots will only have the suite of vegetative cover measurements performed. This additional evaluation of vegetative cover in unoiled plots, where vegetation is standing and then laid over, will serve to enable interpretation of oiled plot vegetative cover measurements where vegetation is laid over and cannot be stood up.

Herbaceous Coastal Wetland Vegetation Areas

General Procedures

At specified points along the transect, two permanent plots will be established as follows: a 1 meter x 1 meter "cover plot" and 1 meter x 2 meter "productivity plot". Analysis of these plots will follow the herbaceous coastal wetland vegetation protocols described in Section VII. Each productivity plot will be established 2 meters to the right (facing inland) of the transect, while its paired permanent vegetative cover plot shall be placed 2 meters to the left of the transect. (These distances may exceed 2 m to attain better similarity with respect to species cover and composition between the plots.)

The length of the transect and the placement of plots along the transect length is a function of whether the site is an oiled site or an unoiled site, as described below. Figure 2 depicts the layout of transects and study plots in herbaceous coastal wetland vegetation areas.

Oiled Sites

The forward team shall proceed to the GPS coordinates of the selected site. The team shall visually examine the site, including up to approximately 50 meters to each side of the coordinates. A specific location for the transect shall be selected to be most representative of the

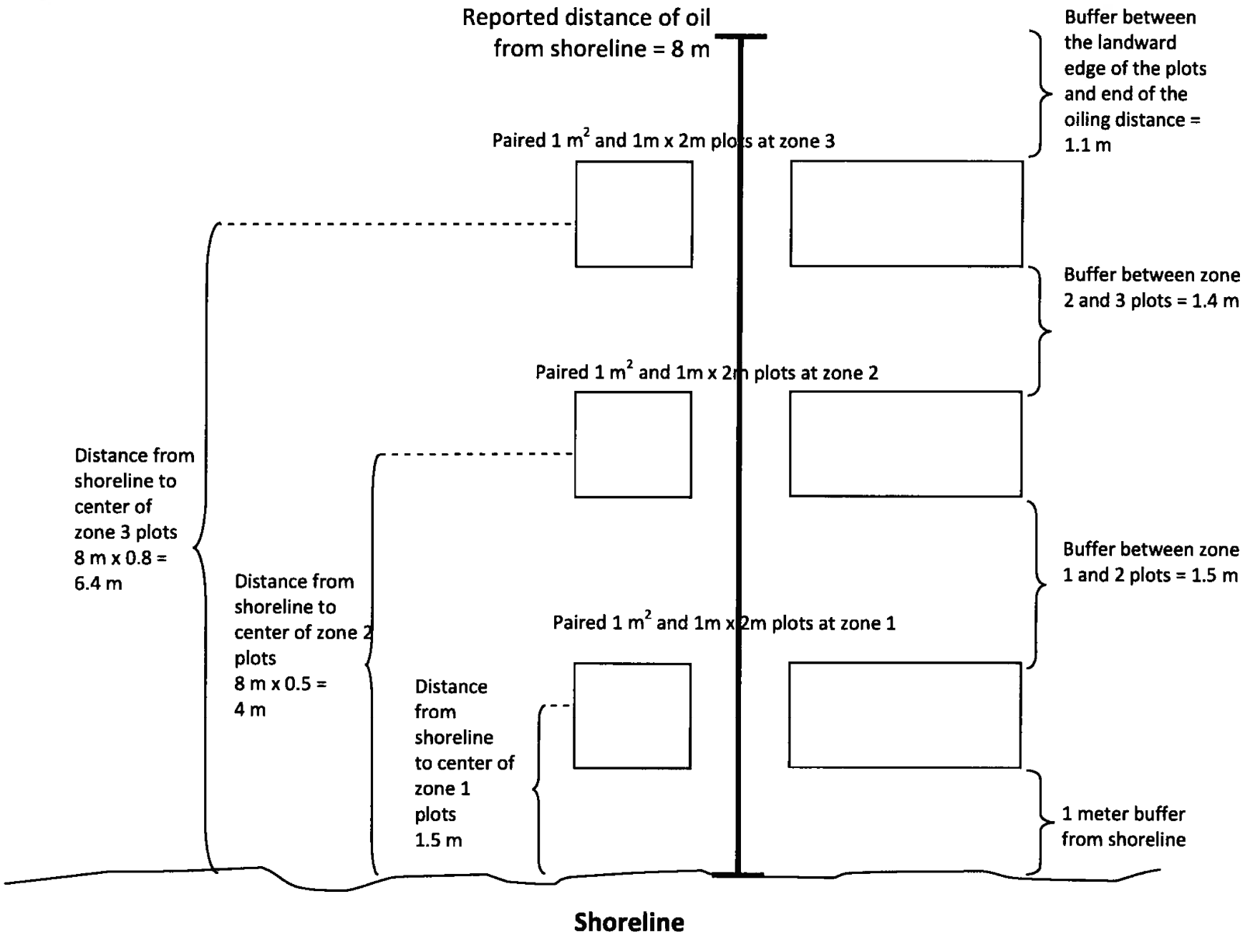

Figure 2. Example of a hypothetical transect for an herbaceous marsh area with oiling distance into the marsh 8 meters from the shoreline. Note the placement of plot centers at 1.5 m, 4 m, and 6.4 m and the maintenance of a minimum landward buffer of 0.5 meters to the end of the transect. The distance from the transect line to edge of each plot is 2.0 m. (Drawing is not to scale.)

area's natural (i.e., non-oil related) features (e.g., area with special microgeography such as small pools/ponds, and areas that are clearly disparate in terms of vegetation composition or density shall be avoided). Additionally, the transect location should be representative of that specific oiled shoreline in both the extent of penetration into the coastal wetland vegetation and the extent of vegetation oiling.

At each site one transect should be established. The length of the transect at an oiled site (i.e., all selected sites except unoiled sites) should be the greater of: (a) the length of oil penetration into the coastal wetland vegetation as reported during earlier preassessment observations or (b) the length of observed oil penetration into the coastal wetland vegetation at the time of the present survey up to a maximum length of 30 m. If 10% or greater oiling of visible plant surface is observed at 30 m, this will be recorded on the datasheet to indicate that further assessment may be required.

Upon arriving at any plot designated as "herbaceous only", the plot will be initially inspected for the presence of mangroves. If any adult mangroves ($\geq$ 50 cm in height) or more than 3 mangrove seedlings < 50 cm in height are found, then the entire plot will be shifted to the left or right (facing inland), depending on which side of the transect the plot is located, until the requirements of a "herbaceous only" plot are met.

Note that when establishing plots, the first plot pair (i.e., cover plot and productivity plot) on a transect will always start 1 meter in from the shoreline; this plot pair will be referred to as the "shoreline edge" or "zone 1" plot pair. The second and third plot pair for that site, if relevant, will be referred to as the "zone 2" and "zone 3" plot pairs, respectively. There will be a minimum buffer of 0.5 meters from the landward edge of the last plot pair of the transect and the end of the oiling distance from the shoreline. Please see Table 2 for a summary of criteria for plot placement and Figure 2 for an example transect layout schematic. Table 3 provides example plot locations based on oiling distances from the shoreline.

For transects less than 2.5m: Insufficient space is available to establish plots.

For transects equal to or greater than 2.5 meters but less than 5m: One set of plot pairs will be established, representing the shoreline zone. The shoreline zone plots shall be established such that the seaward edge of the associated permanent plots is 1 meter in from the shoreline (i.e., plot centers will be 1.5 meters in from the shoreline).

For transects equal to or greater than 5 meters but less than 7 meters: Two sets of plot pairs will be established (zone 1 and zone 2). The zone 1 (shoreline edge) plots shall be established as described above. The zone 2 plots will be established such that the plot centers are 80% of the oiling distance (i.e., transect length) from the shoreline. Note that the distance from the center of the zone 2 plots to the end of the oiling distance from the shoreline must be at least 1 meter.

Particularly when plots are relatively closely spaced, as is the case for transects of this length, field personnel should exercise extra care to avoid inadvertently trampling the edge of one plot while inspecting another.

For transects equal to or greater than 7m: Three sets of plot pairs will be established (zones 1, 2, and 3). The zone 1 plots shall be established as described above. The zone 2 plots will be established with the plot centers at 50% of the oiling distance from the shoreline and the zone 3 plots will be established with the plot centers at 80% of the oiling distance from the shoreline. Note that the distance from the center of the zone 3 plots to the end of the oiling distance from the shoreline must be at least 1 meter.

Table 2. Matrix of the number of herbaceous plot pairs to establish for a given range of oiling distances from the shoreline.

Oiling distance from shoreline	< 2.5 m	2.5 m to < 5 m	5 m to < 7 m	≥ 7 m
Number of plot pairs to establish	0	1	2	3

Table 3. Matrix of example herbaceous plot pair center distances from the shoreline based on oiling distances from the shoreline with buffer sizes.

Oiling distance from shoreline	**2 m**	**2.5 m**	**4.5 m**	**5 m**	**6.5 m**	**7 m**	**10 m**	**15 m**	**20 m**	**30 m**
Buffer: shoreline to zone 1 plots	NA	1.0 m	1.0 m	1.0 m	1.0 m	1.0 m	1.0 m	1.0 m	1.0 m	1.0 m
Center of zone 1 plots	NA	1.5 m	1.5 m	1.5 m	1.5 m	1.5 m	1.5 m	1.5 m	1.5 m	1.5 m
Buffer: zone 1 plots to zone 2 plots	NA	NA	NA	1.5 m	2.7 m	1.0 m	2.5 m	5.0 m	7.5 m	12.5 m
Center of zone 2 plots	NA	NA	NA	4.0 m	5.2 m	3.5 m	5.0 m	7.5 m	10.0 m	15.0 m
Buffer: zone 2 plots to zone 3 plots	NA	NA	NA	NA	NA	1.1 m	2.0 m	3.5 m	5.0 m	8.0 m
Center of zone 3 plots	NA	NA	NA	NA	NA	5.6 m	8.0 m	12.0 m	16.0 m	24.0 m
Buffer: most landward plots to transect end	NA	0.5 m	2.5 m	0.5	0.8 m	0.9 m	1.5 m	2.5 m	3.5 m	5.5 m

Unoiled Sites

Transect lengths and the placement of plots along these transects in unoiled sites are intended to match as closely as reasonably achievable, the positioning of plots along transects in oiled areas. Towards this goal, the length of transect at these sites should be 20 meters: 20 meters is the 95 percentile value of reported coastal wetland vegetation oil penetration lengths as observed during

earlier preassessment investigations. (Of note, the length of transects in no-oiling/none sites may be revised based on initial penetration data collected during study implementation.)

Unoiled site transects will include three plots along a transect:

- The zone 1 (edge) plots shall be established as for oiled coastal wetland vegetation areas.
- The zone 2 coastal wetland vegetation plots shall be established with plot centers at 50% of the total transect length (e.g., at 10 meters, for a 20 meter transect).
- The zone 3 coastal wetland vegetation plots shall be established with plot centers at 80% of the total transect length (e.g., at 16 meters, for a 20 meter transect).

Additional Protocols for Establishing *Phragmites* Plots

Due to the unique challenges associated with sampling in *Phragmites australis* communities (e.g., water depth, unconsolidated sediment), the following additional protocols are provided for establishing transects and study plots in *Phragmites* habitat.

1. Shoreline edge determination: Approach the *Phragmites* from the water (whether by boat or foot) with the shoreline roughly perpendicular to your approach. As you slowly approach, visually look towards the horizon at both your right and left sides (180 degrees) while observing the space between stems and clumps as the vegetation becomes denser. That point at which your view to the horizon becomes partially obscured by a fairly uniform field of stems to both sides (rather than clumps that remain visually distinguishable) should be used to determine the position of the shoreline transect pole(s).

2. Datasheets will include entries for whether the transect was established from the boat or by foot and whether it was sampled from the boat or by foot.

3. Whenever safe to do so, three *Phragmites* plot pairs should be established according to the SOP as a 20 m transect with plot *centers* as follows: zone 1 plot pair centers 1.5 m from shoreline; zone 2 plot pair centers 10 m from shore; zone 3 plot pair centers at 16 m from shoreline (i.e., 80% of 20 m transect length).
    - It may not be possible to establish all three plot pairs when:
        - Unsafe to do so
        - The coastal wetland vegetation location does not have sufficient width between shorelines (i.e., a coastal wetland vegetation peninsula; see additional info below) because an 'interior plot pair' cannot be established beyond the midpoint between shorelines.
    - Specifically, a coastal wetland vegetation peninsula width must be at least 3 m to establish a zone 1 plot pair. If not, reject that location.
    - If peninsula width is <32 m; only 2 plot pairs can be established: zone 1 plot pair *centers* at 1.5 m from vegetation edge, zone 2 plot pair centers at a target distance

of 50% of the peninsula width (e.g., if peninsula is 24 m wide, zone 2 plot pair centers should be attempted to be establish at 12 m from shoreline).
- If peninsulas are > 32 m, all 3 plot pairs should be attempted to be established according to SOP as a 20 m transect (e.g., the zone 3 plot centers would be at 80% of 20 m, which is 16 m).

4. Conditions may limit the length of a transect (e.g., excessive water depth, inability of an airboat to penetrate further). The following guidelines should then be followed
   - A minimum of two plot pairs should always be attempted to be established when safe to do so.
   - The zone 2 plot pair centers should be established at 10 m from the shoreline, but if not possible, a distance of > 5m can be used and be recorded on the datasheet diagram.
   - If a zone 2 plot pair is successfully established at 10 m, the zone 2 plot pair centers should be established at a distance of 16 m from the shoreline. If that is not possible, the zone 2 plot pair centers can be established at a distance >13 m.

5. Review recent aerial photography prior to visiting potential transect establishment sites to estimate width of coastal wetland vegetation between water bodies (i.e., *Phragmites* peninsula widths). Pre-estimates of peninsula width will assist with determination of whether 1, 2 or 3 plot pairs can be established.

Black Mangrove Coastal Wetland Vegetation Areas

General Procedures

At specified intervals along the transect, single 1 meter x 4 meter permanent plots will be established. Plot placement along a transect for Louisiana stunted black mangrove areas will follow the same rules as for herbaceous coastal wetland vegetation with the exception that a single 1 meter by 4 meter permanent plot will be established rather than paired plots. Please see Figure 3 for an example layout. Assessment of these plots will follow the Louisiana black mangrove protocols described in Section VIII.

Oiled Sites

Transects in oiled coastal wetland vegetation will extend from the shoreline (i.e., the most seaward edge of the coastal wetland vegetation) into the coastal wetland vegetation for a total transect length of 20 m.

Unoiled Sites

The same procedure as for herbaceous coastal wetland vegetation should be followed in establishing a 20 m transect and situating 1 x 4 meter plots along its length. Plots will be located to the right (facing inland) of the transect line.

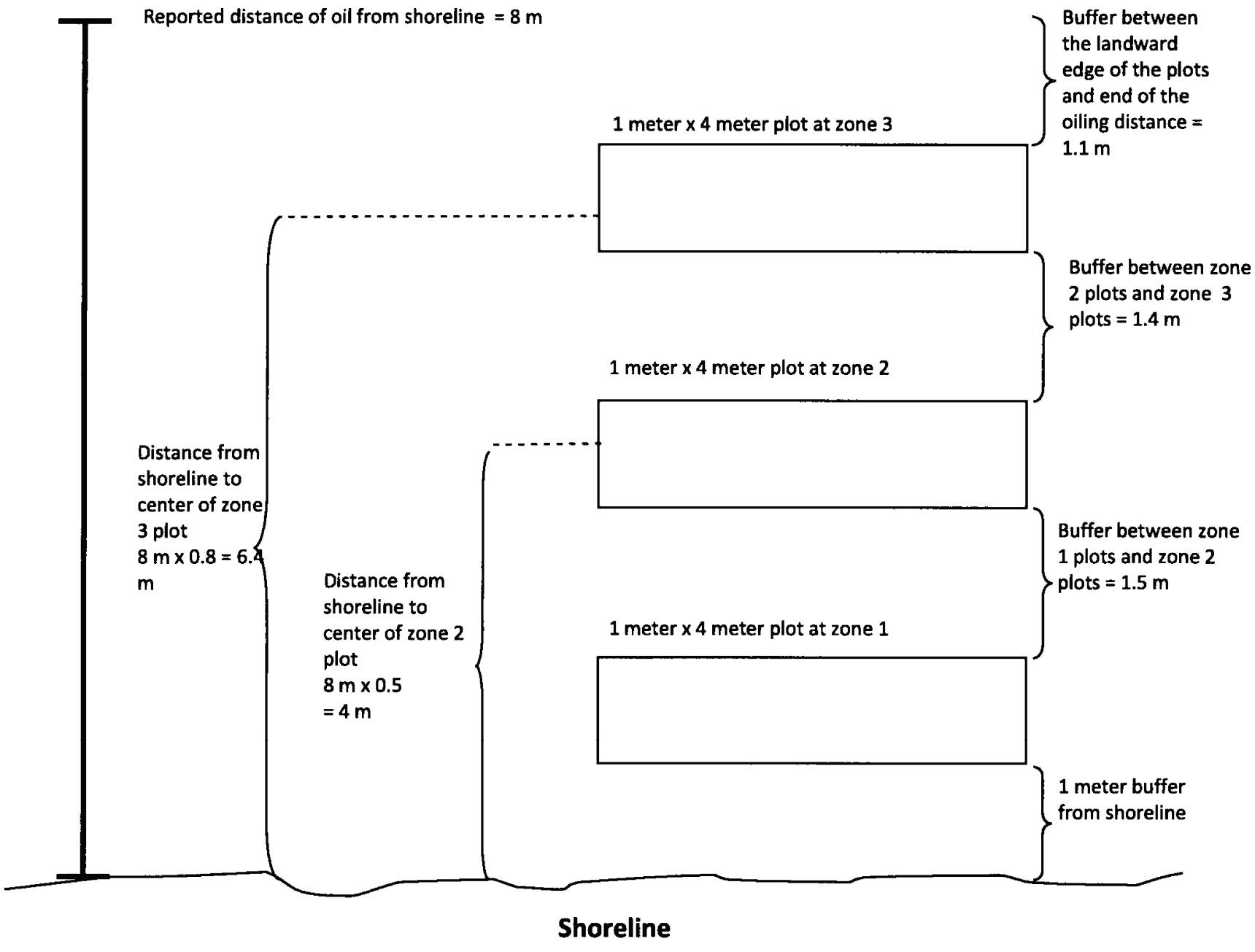

Figure 3. Example of a hypothetical transect for a Louisiana stunted black mangrove area with oiling distance into the marsh 8 meters from the shoreline. Note the placement of plot centers at 1.5 m, 4 m, and 6.4 m and the maintenance of a minimum landward buffer of 0.5 meters to the end of the transect. (Drawing is not to scale.)

Site Set-up Verification

For sampling events following the initial establishment of plots (i.e., Year 0.5 and beyond for most sites), revisited plots will need to be checked for the presence of plot markers and signs of erosion. The following guidance should be used.

Contingency Plan for Missing Plot Markers

1. Upon reaching a transect, the "Site Set-up Verification Datasheet" should be completed.
    a. Note: "Site Visit/Set Up Datasheet" for each site is provided in a booklet to each team.

2. If either the shoreline PVC stake or inland PVC stake are missing, but not both, then the missing PVC stake should be replaced. Consult the "Site Visit/Set Up Datasheet" to determine the appropriate bearing and distance from the remaining PVC stake to the location where the replacement stake is to be placed. Note that bearings were recorded from the Shoreline PVC stake to the Inland PVC stake. Therefore if the Shoreline stake is missing then teams will need to add 180° if the bearing is between 0° and 180° and subtract 180° if the bearing is between 180° and 360°.

3. For any plot, if 1 or 2 PVC poles that mark the plot are missing, then replacement PVC poles should be employed to re-mark plots fully.

4. For any plot, if 3 PVC poles that mark the plot are missing, then replacement PVC poles should be carefully employed to re-mark plots fully according to the following constraints. Plots must be carefully re-established in such a fashion that they do not include former areas of destructive sampling, such as soil core collection or walk paths outside of the plot. Consult the "Site Visit/Set Up Datasheet" to verify distances as appropriate.

5. If all the PVC poles marking a plot are missing, then review the situations below.
    a. If PVC poles are missing due to erosion and this erosion is consistent across the adjacent shoreline (i.e., all portions of the adjacent shoreline have experienced land loss) then this plot cannot be re-established.
    b. If PVC poles are missing due to erosion, but this erosion is NOT consistent across the adjacent shoreline (i.e., if it can be reliably determined by the field teams that portions of the adjacent shoreline have not eroded and there is coastal wetland vegetation at the same distance from the shoreline as the previously established plot) then this plot should be established at the same distance from the shoreline as the previously established plot and at sufficient distance from the original plots

to be sufficiently buffered from previous foot traffic as determined by the field teams.

c. If PVC poles are missing due to factors other than erosion (e.g., vandalism), there is clear evidence of the location of the plot (e.g., markings from PVC poles or previously collected cores), and the area does not appear trampled, the plot should be re-established in the same location, and the "Site Set-up Verification Datasheet" should be completed.
d. If PVC poles are missing due to factors other than erosion (e.g., vandalism) and the plot appears trampled, then the plot should be re-established at an appropriate distance from the shoreline and at sufficient distance from the original plot to be sufficiently buffered from previous foot traffic.

6. In the case of re-establishing plots ensure that the bottom portion of the "Site Set-up Verification Datasheet" is completed.

7. Any re-established plot must be located in a representative area of the same coastal wetland vegetation zone in which the previous plot was located.

Contingency Plan for Signs of Erosion/Submerged Plots

If a plot is submerged or partially submerged, it should be sampled to the extent feasible and should not be re-established at a different location. Any signs of erosion should be noted on the datasheets and in the field notebook. If the water depth exceeds 15 cm at a *Spartina* or mangrove plot, the plot should not be sampled. Rather, the water depth should be recorded on the datasheet, and the plot should be revisited when the water level is lower, if possible. This water depth restriction does not apply to *Phragmites* plots.

Equipment Checklist for Herbaceous Coastal Wetland Vegetation and Louisiana (Stunted) Black Mangrove Plots (vegetation and soil measurements)

1. Sufficient 10-ft PVC poles (3/4" in diameter, schedule 40) for marking the corners of all vegetative cover and productivity plots, as well as stunted mangrove plots and subplots, to be established
2. PVC poles (2 per site, 1 1/4", 10-ft length)
3. Wooden stakes (for Refuge property)
4. Pole driver
5. 1-m^2 quadrat, with increment markings (for establishing vegetative cover, productivity plots, and subsampling of mangrove plot)
6. 0.25-m^2 quadrat (for sequential harvesting of herbaceous productivity plots)
7. 4-m^2 quadrat (1 m x 4 m quadrat for black mangrove plots in stunted black mangrove areas in Louisiana)
8. GPS (Garmin 76/60 or equivalent)
9. Digital camera
10. Extra Batteries (AA & AAA lithium and any other size required by field equipment)
11. Compass

12. Spot tracker
13. 700 MHz radio w/ charger
14. VHF radio w/ charger
15. Trimble GeoXH
16. Clean large-size freezer bags for collection of belowground biomass sample
17. Clean white plastic garbage sacks for collection of unknown plant specimens for identification
18. Compactor bag equivalents for harvest collection
19. Sample containers (pre-labeled and pre-weighed, clean plastic bags and pre-cleaned glass jars to hold extruded soil from cores and soil contaminant sample, respectively; note that if bags are not pre-weighed, empty bags should be sent to lab for "tare" weight)
20. Labels
21. Waterproof note cards for labeling and placing inside plant collection bags
22. Aluminum tree tags with aluminum attachment wire
23. Flagging
24. Belowground biomass core sampler (15.5-cm diameter marked at 30 cm)
    a. Wooden cap/plug
    b. Clamps x 4
    c. Wooden plunger
    d. 5 gal. bucket
    e. 1 gal. bucket
25. Sharpened aluminum corer (7.2-cm diameter) for collection of soil cores for physical characterization and chemical characterization
    a. Plug
    b. Plunger
    c. 2 x 4 w/ hole
    d. Metal file for maintaining sharp outer edge of aluminum corers
26. Russian peat corer (5-cm diameter) for sampling in *Phragmites*
27. Trowel shovel (2)
28. Harvesting shears (2)
29. One- and two-meter measuring sticks
30. Tape measure (50 m & 8 m)
31. Level rods (telescoping, minimum 5 m) with increment markings (for adult mangrove height and canopy measurements)
32. White boards
33. Dry erase markers
34. Counters
35. FluorPen FP 100 handheld fluorometer or equivalent with extra batteries
36. Minolta SPAD 502 plus chlorophyll meter or equivalent with extra batteries
37. Digital calipers with extra batteries
38. Soil combination redox measurement electrodes (minimum of 12 per team)
39. Waterproof pH/mV meters (2) with extra batteries
40. Wooden dowel (10 cm length) for redox probe placement
41. Apparatus for maintaining redox probes upright
42. DI water
43. Calibration solution
44. Tape (duct, clean, electrical)

45. Cooler with pre-chilled cold packs and/or ice
46. Heavy duty paper towels
47. Chem wipes
48. Lens cleaning solutions
49. Trash bags or drum liners
50. Sample decontamination supplies (spray bottles, rags, Alconox cleaner, DI water - laboratory grade, acetone, and hexane)
51. Safety forms (HASP, TSA, JSA)
52. Waterproof datasheets, chain of custody form
53. Clipboard
54. Field notebook with waterproof paper
55. Permits
56. Quick Reference Guides
57. Laminated oiling impact to vegetation index score sheet
58. Laminated list of plant species that may be encountered in that habitat type
59. Plant identification guides
60. Laminated sheets with species-specific fluorometer settings
61. Site map with GPS coordinates
62. Pencils, write in the rain pens
63. Black permanent marker for marking sampling bags
64. Personal floatation device (PFD)
65. Pelican box
66. Nitrile gloves
67. Tyvek suits
68. Boot covers
69. Knee boots
70. Hip boots
71. Chest waders
72. Ear protection
73. Backpack
74. Sun screen
75. Bug spray
76. Rain gear
77. Drinking water/snacks
78. Sunglasses
79. Hat
80. First-aid kit
81. Pocket knife/multi-tool

VII. CHARACTERIZATION OF HERBACEOUS COASTAL WETLAND VEGETATION HEALTH

Considerations

1. Note that soil cores will be collected immediately outside of plots (see diagram on the "Herbaceous Marsh Sample Collection Datasheet") to reduce sampling impacts to the plots.
2. After sites have been selected, but prior to sampling, lists of plant species likely to occur will be developed to facilitate species identification.
3. See Hester and Mendelssohn (2000) as well as Peterson et al. (2008) for further discussion of the below techniques.
4. For sampling in *Phragmites* coastal wetland vegetation, the standard herbaceous protocol has been modified according to the instructions at the end of this section.

Methods

Different measurements are to be made on the productivity plots versus the cover plots. In particular, the productivity plots are to be sampled for vegetation (aboveground clip plots, belowground biomass cores, and also soil samples). The permanent cover plots are to be used for all other measurements described below, in addition to the collection of some soil cores immediately outside of these plots, as described below. Note that for tall plant species, gentle bending of stems may be required for the collection of metrics such as the top 1/3rd of the stem for chlorophyll content and fluorescence measurements, as well as height. For productivity plots, stem density measurements will need to be recorded in the field for tall plant species (e.g., *Phragmites*) since biomass collected from these clip plots will need to be broken into several pieces to fit inside collection bags.

To the extent feasible, it is recommended that sampling occur in the late summer (September/October) timeframe. For endpoints that are to be sampled twice a year, late spring (typically, April/May) and late summer are suggested for optimally describing plant responses, although sampling events do not need to be exactly 6 months apart.

Cover Plot Methods

Cover plots will be established using PVC poles at each corner and always to the left of the transect stakes to aid in differentiating these plots from the destructively sampled vegetation productivity plots, which will be designated by PVC poles at each corner and always to the right of the transect stakes. The lower left PVC pole of each plot type may be taller than those in the other three corners to a) provide guidance to avoid trampling as the plot is approached and b) assist with reestablishing the plot area if some poles are lost over time.

Note: Fluorescence and chlorophyll measurements should be performed on the same leaves from the same plants. The person taking the measurements should determine fluorescence and then determine chlorophyll content on each leaf and record the results before proceeding to the next leaf. Note that leaves should be cleaned of any foreign material (oil residue, mud, etc.) with kimwipes prior to measurements. The instrument lens should be cleaned with soft lens grade cloth and cleaning solution if contamination of the measuring lens is suspected. Calibration should be performed subsequent to measurements on oiled leaves if noticeable change in instrument performance is detected. No measurements will be performed on dead or highly oiled leaves that cannot be cleaned.

1. Light-adapted fluorescence (Note: this procedure is not possible to implement on *Juncus roemerianus* without resulting in damage to the round stem).
    a. Enter information into the "Light-Adapted Fluorescence and Chlorophyll Content Datasheet".
    b. Fluorescence measurements will be accomplished using a PSI systems FluorPen FP 100 or equivalent.
    c. In each plot, three stems of the dominant plant species (e.g., *Spartina*) that appear to be representative of those in the plot will be selected, and each stem will be visually divided into three vertical segments (top third, middle third, and bottom third) from which representative live leaves will be selected for fluorescence measurements. A minimum of 2 leaves in each segment of each of the three stems will be measured per permanent cover plot.
    d. Leaves selected will be fully expanded and measured in the center of the leaf, but avoiding any large veins or midribs.
    e. Fluorescence measurements will be performed on the top surface of leaves.
    f. Instrument settings for actinic and measuring light levels are species-specific and will be provided on laminated sheets.

2. Chlorophyll content (Note: this procedure is not possible to implement on *Juncus roemerianus*.)
    a. Enter information into the "Light-Adapted Fluorescence and Chlorophyll Content Datasheet".
    b. Chlorophyll content will be determined using a Minolta SPAD 502+ or equivalent.
    c. The chlorophyll meter should be recalibrated each time it is switched off.

d. Chlorophyll measurements shall be made on the same plants and leaves for which fluorescence is measured as described above in the "Note" section.
e. Leaves selected will be fully expanded and measurements will be taken in the center of the leaf.
f. Chlorophyll content will be determined on the top surface of leaves.

3. Vegetation condition index (considering all plant leaf area within the plot)
   a. Enter information into the "Herbaceous Marsh Cover Plot Datasheet."
   b. 0 = vegetation having a natural appearance, stem and leaf chlorosis not exceeding a slight mottling or occasional yellowing as observed in unoiled plots.
   c. 0.5 = vegetation having an intense speckled chlorosis.
   d. 1.0 = vegetation green but with considerable chlorosis (<50% chlorosis).
   e. 2.0 = vegetation having >50% yellowing (chlorosis) of leaves and stems.
   f. 3.0 = vegetation dead; no green aboveground tissue visible.
   g. Note: estimates will be independently generated by one BP representative, if available, and at least one trustee representative, discussed, and a consensus value determined. Vegetation condition index should be determined on live tissue (green or yellow tissue) only, unless all vegetation is dead (vegetation condition index = 3.0).

4. Oiling extent
   a. Enter information into the "Herbaceous Marsh Cover Plot Datasheet."

   b. Sediment surface oiling coverage
      i. Visual estimation of sediment oiling coverage will proceed in 1% increments up to 5% and 5% increments thereafter.
      ii. Oiling coverage will focus only on the amount of surface area covered by oil and will not include other characteristics (e.g., oiling color, depth, etc.).
      iii. Note: estimates will be independently generated by one BP representative, if available, and at least one trustee representative, discussed, and a consensus value determined.
   c. Vegetation oiling extent index:
      i. 0 = no oil evident anywhere in the plot.
      ii. 0.5 = oil intermittently present on plant stems.
      iii. 1.0 = oil present on 5% - 25% of plant stems.
      iv. 2.0 = oil present >25% - 50% of plant stems.
      v. 3.0 = oil present on > 50% of plant stems.
      vi. Note: estimates will be independently generated by one BP representative, if available, and at least one trustee representative, discussed, and a consensus value determined. Vegetation oiling extent index should be determined by the presence/absence of oil observed on a percentage of stems within a plot, not by oiling degree.

d. Oiling height on vegetation (cm). The highest point on the stem where oiling is visible, measured from the sediment surface, shall be recorded.

5. Visual estimation of the species composition (including live and dead coverage of vegetation present)
    a. Enter information into the "Herbaceous Marsh Cover Plot Datasheet."
    b. This is to include at a minimum:
        i. Total (live plus dead) percent vegetative cover.
        ii. Dead percent vegetative cover (i.e., total dead percent vegetative cover in plot).
        iii. Live percent vegetative cover by species.
        iv. Dead percent vegetative cover by species.
        v. Wrack percent cover*.
        vi. Oil Boom percent cover*.
        vii. Debris percent cover (i.e., debris that is not wrack or oil boom)*.
        *Note: transect placement and subsequent plot placement within a transect should attempt to minimize the occurrence of these items in a plot to the extent possible.
        viii. Vegetation stature (standing – ST, or laid over – LO).
    c. Practical aspects of implementation:
        i. When possible, vegetation should be "stood up" by the field team to allow for a more accurate estimate of coverage. In areas where it is not possible to stand vegetation up (e.g., heavy oiling) then cover will be estimated with vegetation laid over, a note will be made to this effect in the datasheet, and a correction factor generated from additional laid over unoiled plots will be employed during interpretation.
        ii. Visual estimation will proceed in 1% increments up to 5% and 5% increments thereafter.
        iii. Greater than 100% cover may occur because of canopy overlap of species.
        iv. Note: estimates will be independently generated by one BP representative, if available, and at least one trustee representative, discussed, and a consensus value determined.
        v. Estimates of "dead vegetative cover" whether at the whole plot or species level, should include any dead leaves that are attached to stems in the plot (whether the stem is totally dead or not), as well as any portions of dead stem tissues or stubble as long as they are rooted in the plot.

6. Dominant species canopy height
    a. Enter information into the "Herbaceous Marsh Cover Plot Datasheet."
    b. Visual estimation of the average height of the canopy of the dominant species.

c. Note that this value is intended to estimate the average canopy height rather than the maximum canopy height and should focus on the dominant species only.

Productivity Plot Methods

Enter information into the "Herbaceous Marsh Sample Collection Datasheet."

1. Total aboveground biomass, stem height, and oiling height
   a. As noted previously, a 1 meter x 2 meter productivity plot for the determination of aboveground biomass and stem height will be established 2 meters to right (facing inland) of the permanent vegetative cover plot described above. This distance may be > 2 m to attain uniformity with species cover and composition of the permanent plot.
   b. Productivity plots will be established using PVC poles at each corner and always to the right of the transect stakes.
   c. The apparent height of oiling will be determined on the taller (mature) stems in the permanent cover plot as well as the taller stems in the 0.25-m^2 productivity subsample prior to clipping vegetation in the 0.25-m^2 subsample of the productivity plot. Taller stems will be used to ensure accurate determination of oiling height (i.e., to ensure that oiling height is not greater than stem height).
   d. A 0.25-m^2 subsample of the productivity plot will be collected sequentially in the order described on the "Herbaceous Marsh Sample Collection Datasheet." Subsequent samplings may occur either seasonally or annually as determined by the shoreline team and statisticians.
   e. The location of the 0.25-m^2 subsample will be denoted on the field datasheets to prevent re-sampling of a previously harvested area.
   f. The number of live stems in the 0.25-m^2 subsample from the productivity plot will be determined. This measurement will be performed in the lab for *Spartina alterniflora*-dominated communities. (Note: for *Phragmites* communities, this measurement will be performed in the field, as described in the section Additional Protocols for *Phragmites* Sampling, below.)
   g. A cumulative stem height metric (sum of live harvested stems) will be generated using an average of several representative longest and shortest stems or the length of each stem from live vegetation in the productivity plot subsample (process based on lab-specific SOP).
   h. Clipping is best performed with large garden shears (loppers)

i. Clipping will be performed with one person holding the vegetation to be harvested erect and a second person carefully clipping the vegetation directly at the base, being mindful of the other person's hand locations at all times.
j. All attached vegetation, both live and dead, will be harvested.
k. Harvested biomass will be placed into high strength plastic bags (trash compactor equivalent), which will be appropriately labeled on a bottom corner section and tied and/or taped shut to ensure that the contents do not get wet during cooling and transport.
l. Bags of harvested material will be kept cold (~4° C) and wet weight determined within 14 days of collection.
m. In the laboratory, plant material will be rinsed of any foreign material (e.g., soil, oil, etc.) in accordance with laboratory protocols, sorted into live and dead partitions by species, the number of live stems determined, and the length of each stem in the live partitions determined and recorded into appropriate datasheets. Plant material will be dried to a constant weight in a temperature-controlled oven consistent with laboratory standard operating procedures (SOPs), which may dry at 65°C or 103 - 105° C (EPA Method 160.3). Note that if a stem has any green coloration (i.e., observable green external tissue) on it, it will be considered live.
n. Dried biomass will be weighed using a balance with readability of 0.01g or better.
o. Plant weights for each partition will be recorded into appropriate datasheets.

2. Belowground biomass
   a. A core for belowground biomass will be collected in the center of the 0.25-m^2 productivity sub plot after aboveground tissue is clipped and bagged (see diagram on the "Herbaceous Marsh Sample Collection Datasheet"). Note that belowground biomass sampling in *Phragmites* habitats proceeds in a different fashion; please refer to the "Additional Protocols for *Phragmites* Sampling" below for details.
   b. One core per plot will be collected for belowground biomass characterization.
   c. Belowground biomass will be collected using cores applicable to the area being sampled; generally, a 15.5 cm diameter core to a depth of 30 cm and extruded into clean plastic bags in the field.
   d. Belowground biomass samples will be kept cold (~4° C) and wet weight determined within 14 days of collection. At the laboratory,

they will be separated into live and dead components, rinsed of non-root material, and root-material will be dried.

e. Clean root material for belowground biomass determination will be dried to a constant temperature in a temperature-controlled oven consistent with laboratory standard operating procedures (SOPs), which may dry at 65°C or 103 - 105° C (EPA Method 160.3). Dried weight will be recorded using a balance with 0.01 readability or better. (Please refer to Darby and Turner (2008) for further discussion of this technique.)

Additional Protocols for *Phragmites* Sampling

The following protocols provide additional guidance specific to sampling in *Phragmites* coastal wetland vegetation. Where different from the above protocols, the protocols below take precedent for *Phragmites* sampling.

1. **If water depth precludes safely getting out of boat and walking on the marsh surface:**
    a. An airboat[5] will be utilized to move into and sample the marsh (a bay boat will be present to assist with pulling the airboat back out along the transect when sampling is complete)
    b. Two shoreline transect poles will be utilized to mark both sides of the boat path (rather than a single transect pole marking the center of a foot path).
    c. Plot pairs will be sampled from both sides of the airboat (cover plot from port side and productivity plot from starboard side). The plots should be established to allow some space between the plot side and the side of the boat to the extent safely possible to create a buffer zone.
    d. Plot PVC marker pole locations will be marked on datasheet plot diagrams (e.g., when plots established by boat, the 2 corners of the plot closest to the boat will have to be marked by PVC poles: top right and bottom right corners of cover plots and top left and bottom left corners of productivity plots).
    e. Soil core locations for the cover plot will have to be re-located to the boat side of the plot and marked on the plot diagram.
2. Whenever water depth permits safe access from boat onto marsh surface, sampling should be done from the marsh surface.
3. All sampling metrics on the datasheets should be attempted to be collected whenever it is safe to do so.
    a. All visual metrics/indices should be conducted at all sites with the exception of metrics that require seeing the sediment surface when it is underwater.
    b. Productivity clip plots should follow the instructions provided in this plan and must utilize a 0.25m^2 PVC quadrat (0.5 m x 0.5 m). Water depth greater than 1.5

[5] The use of airboats and terms of use are subject to individual landowner access agreements.

– 2 feet may preclude the team member from clipping and collecting an aboveground biomass sample.

c. Because *Phragmites* has long stems that may break into two or more pieces when bagged, *prior to bagging the clipped stems, the number of live stems (green tissue present on at least part of the stem) and number of dead stems (no green tissue visible on stem) will be counted and recorded on the datasheets.*
d. Cover plots must utilize a 1m x 1m PVC quadrat.
e. A Russian peat corer will be used for the collection of all soil samples, even when the soil is under several feet of water according to guidelines below.

4. The core chamber is 50 cm in length. As such, the core chamber should be inserted into the soil to a depth *not* exceeding 40 – 45 cm.
   a. Upon extraction, the soil core should be inspected to ensure that it is an intact core with soil surface present.
   b. Associated with each plot (i.e., each cover and each productivity plot), one core will be collected for a) soil physical characterization and b) soil chemical characterization. Each of these cores should be trimmed to a *total length of 10 cm from the surface*, sealed and stored according to this plan.
   c. Soil cores for hydrocarbon analyses will have the surface 5 cm of the core collected and placed into appropriate sample containers as specified in the SOP.
   d. Cores for belowground productivity will utilize the upper 30 cm of the core collected from the specified productivity plot area; duplicate cores will be taken and each core placed in labeled Ziploc freezer bag, sealed and stored as specified in the SOP.

VIII. CHARACTERIZATION OF LOUISIANA (STUNTED) BLACK MANGROVE HEALTH

General considerations

1. Black mangroves occurring along the Louisiana coast require additional measurements to fully garner information as to the health of the coastal wetland vegetation.

2. Live mangrove trees in 1-m^2 subplots of the 1 m x 4 m mangrove plots will be tagged with unique ID numbers by placing permanently embossed aluminum tree tags on the main stem of the mangrove trunk using aluminum wire and be further identified using flagging tape. Mangrove trees will be categorized as seedlings if they are $\leq$ 0.5 m or adult trees if they are $\geq$ 0.5 m. **Note that some measurements are performed on the entire 4-m^2 plot, whereas others are performed on the 1-m^2 subplot,** which will be selected as the left 1-m^2 corner of the 4-m^2 mangrove plot as one faces inland and then marked with an additional PVC pole (the location of which is also recorded on the datasheet, assuming the researcher is facing inland).

3. Potential logistical constraints as well as seasonality considerations may limit the ability of the field teams to collect, for all sites, the full set of information and samples described in the protocols below.

Methods

1. *Visual estimation of mangrove and other species composition (including live and dead coverage of vegetation present, whether standing or not; 4-m^2 plot)
    a. Visual estimation of mangrove and other species composition (including live and dead coverage of vegetation present) will be performed using the method described above in the herbaceous coastal wetland vegetation metric section.

2. *Mangrove maximum height (4-m^2 plot)
    a. The maximum height of the tallest black mangrove will be determined.

3. *Vegetation condition index (4-m^2 plot) (for adult mangroves and seedlings separately, considering all plant leaf area within the plot)
    a. 0 = vegetation having a natural appearance, stem and leaf chlorosis not exceeding a slight mottling or occasional yellowing as observed in unoiled plots
    b. 0.5 = vegetation having an intense speckled chlorosis
    c. 1.0 = vegetation green but with considerable chlorosis (<50% chlorosis).
    d. 2.0 = vegetation having >50% chlorosis of leaves and stems.
    e. 3.0 = vegetation dead; no green aboveground tissue visible
    f. Note: estimates will be independently generated by one BP representative, if available, and at least one trustee representative, discussed, and a consensus value determined. Vegetation condition index should be determined on live tissue (green or yellow tissue) only, unless all vegetation is dead (vegetation condition index = 3.0).

4. *Visually-estimated oiling (4-m^2 plot)
    a. Perpendicular penetration of oil into marsh (from marsh shoreline).
        i. The furthest extent of visible oiling along the transect will be determined utilizing a meter tape.
    b. Sediment surface oiling coverage.
        i. Visual estimation of sediment oiling coverage will proceed in 1% increments up to 5% and 5% increments thereafter.
        ii. Oiling coverage will focus only on the amount of surface area covered by oil and will not include other characteristics (e.g., oiling color, depth, etc.).
        iii. Note: estimates will be independently generated by one BP representative, if available, and at least one trustee representative, discussed, and a consensus value determined.
    c. Vegetation oiling extent index for adult mangroves and seedlings separately:
        i. 0 = no oil evident anywhere in the plot.

ii. 0.5 = oil intermittently present on plant stems.
iii. 1.0 = oil present on 5% - 25% of plant stems.
iv. 2.0 = oil present >25% - 50% of plant stems.
v. 3.0 = oil present on > 50% of plant stems.
vi. Note: estimates will be independently generated by one BP representative, if available, and at least one trustee representative, discussed, and a consensus value determined. Vegetation oiling extent index should be determined by the presence/absence of oil observed on a percentage of stems within a plot, not by oiling degree.

5. Belowground biomass (collected immediately outside 4-m^2 plot)
    a. A core for belowground biomass will be collected immediately outside of the 4-m^2 mangrove plot (see diagram on the relevant mangrove datasheet).
    b. One core per plot will be collected for belowground biomass characterization.
    c. Belowground biomass will be collected using a corer applicable to the area being sampled; generally, a 15.5 cm diameter core to a depth of 30 cm, and be extruded into clean plastic bags in the field.
    d. Belowground biomass samples will be maintained under chilled (i.e., ice or refrigerated) conditions until processed at the laboratory. At the laboratory, they will be separated into live and dead components, rinsed of non-root material and dried.
    e. Clean root material for belowground biomass determination will be dried to a constant temperature in a temperature-controlled oven consistent with laboratory standard operating procedures (SOPs), which may dry at 65°C or 103 - 105° C (EPA Method 160.3). Dried weight will be recorded using a balance with 0.01 readability or better.
    (Please refer to Darby and Turner (2008) for further discussion of this technique.)

6. *Light-adapted fluorescence (4-m^2 plot)
    a. Enter information into the "Light-Adapted Fluorescence and Chlorophyll Content Datasheet".
    b. Light-adapted fluorescence will be performed on adult mangroves following the protocols described in the herbaceous coastal wetland vegetation protocols.

7. *Chlorophyll content (4-m^2 plot)
    a. Enter information into the "Light-Adapted Fluorescence and Chlorophyll Content Datasheet".
    b. Chlorophyll content will be determined for adult mangroves following the protocols described in the herbaceous coastal wetland vegetation protocol.

8. *Propagule production index (1-m^2 plot)
    a. Alteration of reproductive output could be a sensitive indicator of mangrove population health.

b. The number of propagules in the 1-m^2 plot will be visually estimated as being in one of the following categories: <100, 100 to <500, 500-1,000, and >1,000.

9. *Number of mangrove trees (1-m^2 subplot)
   a. The number of all mangrove trees (including seedlings) occurring in mangrove plot will be determined.

10. Height of each mangrove tree (1-m^2 subplot)
   a. The height of each mangrove tree (both adult trees and seedlings) including branch length in the 1-m^2 subplot will be determined using appropriate devices (e.g., 1-meter stick, 2-meter stick, telescoping level rod with length markings).

11. *Mangrove survival (1-m^2 subplot)
   a. The number of live mangroves (% of live trees that were initially tagged for both adult trees and seedlings) in the 1-m^2 subplot of each mangrove plot will be determined at each sampling period subsequent to the initial tagging.

12. *Mangrove tree canopy area estimate (based on 2 diameter measurements;1-m^2 subplot)
   a. The area of the canopy of all mangrove trees that are 0.5 meters or taller ($\geq$ 50 cm) in the 1-m^2 subplot of each mangrove plot will be estimated by inserting two meter sticks (or level rods if canopy diameter is greater than 1 meter) at right angles to each other through the canopy such that one meter stick traverses the longest canopy width and meter sticks intercept immediately adjacent to the main stem of the mangrove.
   b. Perpendicular mangrove measurements will be recorded in the field and mangrove canopy area estimates will be calculated based on the area formula for an ellipse in the lab.
   c. Although mangroves often have relatively homogeneous canopies, for reproducibility one of the meter sticks will always be inserted through the widest apparent section of the canopy.
   d. Note: estimates will be independently generated by one BP representative, if available, and at least one trustee representative, discussed, and a consensus value determined. The canopy of any tree rooted within the plot may extend outside the plot.

13. Mangrove tree branch number (leaf bearing and bare; 1-m^2 subplot)
   a. The number of live (leaf bearing) and dead (bare) branches for each adult mangrove tree in the 1-m^2 subplot of each mangrove plot will be determined. (Only primary branches off the main stem should be counted.)

14. Number of main stem nodes (1-m^2 subplot)
    a. The number of nodes along the main stem of each mangrove seedling in the 1-m^2 subplot of each mangrove plot will be quantified.

15. Internodal distance along main stem (1-m^2 subplot)
    a. The internodal distance along the main stem of each mangrove seedling in the 1-m^2 subplot of each mangrove plot will be determined.

16. *Mangrove pneumatophore density (1-m^2 subplot)
    a. The number of all mangrove pneumatophores in the 1-m^2 subplot will be determined.

17. *Oiled pneumatophore number (1-m^2 subplot)
    a. The number of all mangrove pneumatophores that are oiled in the 1-m^2 subplot will be determined.

18. *Average pneumatophore height (1-m^2 subplot)
    a. The average height of mangrove pneumatophores in the 1-m^2 subplot of each mangrove plot will be visually estimated.

19. Mangrove main stem(s) diameter (1-m^2 subplot)
    a. The diameter of the main stem(s) of all mangrove trees in the 1-m^2 subplot of each mangrove plot that are between 0.3 m – 0.5 m (30 cm – 50 cm) in height will be determined with calipers at a height of 5.0 cm above the soil surface. The diameter of the main stem(s) of all mangrove trees $\geq$ 0.5 m ($\geq$ 50 cm) in the 1-m^2 subplot of each mangrove plot will be measured at 10 cm above the soil surface.

20. *Height of oiling on mangroves (1-m^2 subplot)
    a. The apparent height of oiling on both mangroves, including mangrove seedlings, and pneumatophores will be determined in the 1-m^2 subplot of each mangrove plot.

21. Seedling mangrove canopy extent (1-m^2 subplot)
    a. Seedling mangrove canopy extent will be determined based on the total number of live (any green coloration) leaves on each seedling in the 1-m^2 subplot of each mangrove plot.

IX. SOILS - GENERAL CHARACTERIZATION

The general characterization of soils (excluding contaminant analysis) includes several components:

- Measurement of soil redox potential in the field;
- The collection of soil samples for laboratory analysis of bulk density and soil organic matter as well as sand-silt-clay composition (i.e., physical characterization cores);
- The collection of soil samples for laboratory analysis of pH, salinity, nutrients and elements (i.e., chemical characterization cores).
- Real time kinematic (RTK) elevation measurements at individual plots.

The following paragraphs set forth the procedures related to the above.

Determination of Soil Redox Potential (field procedure conducted in the herbaceous coastal wetland vegetation cover plot and in the black mangrove permanent plot)

Note: Inspection and calibration will be performed prior to each sampling run.

Inspection: Probes will be inspected for any wear that will impair measurements (e.g., loose components, etc.)

Cleaning: Probes will be cleaned prior to field deployment using detergent and a stiff grade brush

Calibration: Calibration will proceed using the pre-fabricated ORP (Oxidation-Reduction Potential) standard manufactured by Thermo Orion. Follow manufacturer's instructions for ORP standard. If the probe fails to read correctly, clean vigorously then recheck. If the probe still fails to read correctly, remove from circulation as a functional field equipment item and contact manufacturer.

<u>Field use of probes</u>

Note: Be sure all probes have been inspected, cleaned and calibrated before using. Manufactured combination redox electrodes will be used to facilitate ease of measurements. To reduce inherent variation, three probes will be used to determine surface readings and three probes will be used to determine deep readings. The three readings each for surface and deep will be averaged to generate a better estimate for surface and deep soil redox potentials. Soil redox potential will be determined immediately outside permanent cover plots (in herbaceous coastal wetland vegetation areas) or mangrove plots as appropriate (see field sheets for a diagram of locations relative to plots).

Prior to installation of electrodes, make a hole of appropriate depth with an appropriately sized wooden dowel. The dowel's diameter should be slightly smaller than the probe to allow for a tight fit without needing to use force when inserting the probe.

Insert redox electrode into coastal wetland vegetation soil to the appropriate depth, 1 cm (for surface readings) or 10 cm (for deep readings). Be sure that redox electrode remains erect for surface readings this may require a brace for the redox electrode.

For field measurements electrodes will be allowed to equilibrate for 20 minutes prior to readings being taken.

Collection of soil cores for physical characterization (soil bulk density, organic matter content, and sand-silt-clay composition)

Potential logistical constraints as well as seasonality considerations may limit the ability of the field teams to collect the full set of information and samples described in the protocols below, for all sites. In that event, the samples for bulk density shall be accorded a lower priority.

General Considerations

1. Cores for soil physical characterization will be identified on sample bags as "SCP" for soil core - physical (see plot datasheets for locations of samples relative to the plots).
   - **For herbaceous plots**, one soil core will be collected immediately outside the permanent cover plot and a second soil core will be collected immediately outside of the productivity plot[6]. Soil core collection areas will be noted on the datasheet in relation to plot diagram(s).
   - **For mangrove plots**, one soil core will be collected immediately outside the mangrove permanent plot. Soil core collection areas will be noted on the datasheet in relation to plot diagram.
2. Proper attention to chain of custody procedures and forms is crucial; consult the latest MS252 NRDA guidance (available on www.noaanrda.org) for review of procedures.
3. Soil cores are taken to a depth of 10 cm.
4. Sampling container: Heavy duty plastic 1 quart freezer bags; should be pre-weighed and pre-labeled with bag weights recorded directly on the bag. If not pre-weighed, empty bag weights should be given to laboratory as "tare" weights for calculations.
5. Soil cores: Core devices will be applicable to the area being sampled. For instance, "Russian Peat Corer" cores may be used in *Phragmites* areas, whereas wider-diameter aluminum cores may be used in *Spartina* and other areas. Core types used will be recorded for each sample collected. Cores will either be capped and placed into clean Ziploc (or equivalent) bags, extruded into clean Ziploc (or equivalent) bags, or extruded into pre-cleaned glass sample jars.
6. Aluminum soil corers will be sharpened to the outside as this prevents compaction and binding of soil core. NOTE: Sharpen only the soil end of the corer.

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[6] For *Phragmites* communities, two cores will be collected immediately outside of each plot using the Russian peat corer and placed in separate bags.

7. Subsequent to sample collection, soil coring devices for samples other than hydrocarbon analysis will be wiped cleaned with rags and rinsed with site water to prevent contamination of other sites. If oil is visible on coring devices then detergent will be employed for cleaning followed by rinsing with site water.
8. See Soil Testing and Plant Analysis Council (2000) for further discussion of the below techniques.

<u>Specific techniques</u>

1. Clear area to be cored of live vegetation and other debris that will interfere with characterization (e.g., logs, sticks and other materials than cannot truly be considered part of a soil horizon).
2. Place sharpened corer perpendicular to area to be cored and insert with a twisting motion up to the appropriate depth mark (10 cm).
3. Work gloved hand down beside corer while it is still inserted into the ground and position hand on bottom of corer. Carefully remove core from soil and place bottom of corer into labeled sampling container prior to releasing hand from bottom of corer.
4. Extrude sample fully from corer into sampling container using corer extruder, examining core as extruded to be sure that core is not anomalous (e.g., obviously short, long, etc).
5. If the core appears anomalous, then it will be discarded and a new core taken as described above.
6. Once samples are collected, sample containers will be placed in coolers with pre-chilled gel packs and maintained at 4°C.
7. DO NOT store soil samples in cooler with ice/water unless you have a mechanism to prevent water from leaking into the sample container through the seal and affecting characteristics. This can be accomplished by double-bagging samples or placing them in pre-cleaned glass jars that are then tightly sealed.
8. Holding Time: soil samples for physical characterization (dry weight for soil bulk density and soil organic matter) should be placed into an oven for drying within 7 days of collection.
9. Soil Dry Weight: Samples will be dried to a constant weight in a temperature-controlled oven consistent with laboratory standard operating procedures (SOPs), which may dry at 65°C or 103 - 105° C (EPA Method 160.3). Depending on laboratory SOPs, either the entire sample or a well-mixed sub-sample of the soil may be dried to determine dry-weight (also equivalent to Total Solids reported as Percent Solids). Dried weight will be recorded using a balance with 0.01 g readability or better.

It is expected that the laboratory(ies) responsible for the analyses of the collected samples will have their own established protocols (SOPs) for the measurement of soil bulk density, organic matter, and sand-silt-clay composition, including QA/QC procedures. For general guidance purposes, however, the following is provided.

Determine **Soil Bulk Density** (laboratory procedure) by the following formula:

Soil Bulk Density = dry weight / volume of core

Volume of core is that of a cylinder for those particular measurements, i.e.,

Volume of core = $(\Pi)(r^2)(h)$

where r = radius of corer inside diameter and h = height of core. Units for bulk density are g/cm^3.

Determination of **Soil Organic Matter** content (laboratory procedure).

Equipment needed:

1. Muffle furnace
2. Pre-combusted, labeled (pencil marks on crucible bottom are effective) porcelain crucible or other combustion vessel
3. Analytical balance (minimum of 0.01 g sensitivity)
4. Oven gloves and tongs

Procedure:

1. Dry all soil samples (65°-105°C range; see procedure under #9 above)
2. Homogenize each soil sample: e.g., manual stirring, Wiley Mill, mortar and pestle, etc.
3. Weigh crucible empty (tare weight) and record weight.
4. Weigh ~5.0 g of dried, homogenized soil sample and transfer into a crucible.
5. Weigh crucible with sample and record total weight.
6. Place crucible into muffle furnace that has reached 500°C and allow to combust for 5 hours.
7. Remove crucibles and allow to cool to room temperature.
8. Weigh ashed sample and determine % organic matter as

((crucible + sample post-combustion weight- empty crucible weight)/
(crucible + sample pre-combustion weight- empty crucible weight)) *100

= Soil Organic Matter (%)

Collection of soil samples for chemical characterization (pH, salinity, nutrients, and element analysis)

Two soil samples for chemical characterization of herbaceous coastal wetland vegetation plots will be collected, one sample will be collected outside the cover plot and the second will be collected outside the productivity plot[7] (see plot datasheets for a diagram of locations). One soil sample for chemical characterization of mangrove plots will be collected outside of the mangrove plot. Samples for chemical characterization will be designated "SCC" on the sample bags or containers to distinguish them from the soil samples collected for physical analyses (designated "SCP").

1. Follow steps under "General Considerations" and "Specific Techniques" steps 1 through 7, above, using same procedure to collect cores as used for physical analyses.
2. All soil samples for chemical characterization should be maintained at 4° C during holding and transport.
3. Holding Times: Soil samples for chemical analysis should be kept cold (4 °C or on ice) and extracted within 7 days of collection. If extraction of soil samples within 7 days of collection is not possible, soil samples should be frozen at minus 5 °C for up to 2 months. After extraction, soil leachates generated for nutrients (KCl leachate) should be maintained at 4 °C and nutrients (ammonia and nitrate-nitrite) analyzed within 28 days. Soil leachates (DI leachate) generated for pH & salinity should be analyzed as soon as possible after leachate preparation (preferably the same day); DI leachate for phosphorus and sulfate should be kept cold (4 °C) and analyzed within 28 days of leachate preparation. Soil leachates generated for elements (DI leachate) must be preserved to pH <2 and ICP elements analyzed within 6 months of leachate preparation.

It is expected that the laboratory(ies) responsible for the analyses of the collected samples will have their own established protocols (SOPs) for the preservation and measurement of salinity, pH, nutrient, and elemental analyses in these samples, including QA/QC procedures. For general guidance purposes, however, the following is provided for these determinations

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[7] For *Phragmites* communities, two cores will be collected immediately outside of each plot using a Russian peat corer and placed in the same bag.

DI Leachate: Extraction of soil samples for pH, salinity, and element analysis & analysis methods

1. Weigh approximately 40.0 g of homogenized soil sample into an appropriate pre-cleaned bottle If insufficient sample is available (< 40g), record weight used. Lab will try to maintain soil:leachate ratio of 1:2. Narrate in report.
2. Add 80.0 ml of de-ionized water (or amount to maintain soil:leachate ratio of 1:2).
3. Place samples in a laboratory shaker for 20 minutes.
4. Place bottles on laboratory counter and allow suspension to settle for approximately 10 minutes, pour off approximately 15 ml of supernatant (leachate) for pH, Salinity, Total Phosphorus, and Sulfate measurements using laboratory SOPs and the following EPA methods or equivalent:

pH: EPA 150.1
Salinity: Salinometer manufacturer's instructions plus laboratory SOP
Total Phosphorus: EPA Method 365.3
Sulfate: EPA Method 300.0

Alternately, if the laboratory has the capability, Total Phosphorus (P) and Sulfur (S), rather than sulfate, can be measured using ICP-AES, using leachate prepared as described below.

5. Filter remaining leachate through 0.45 micron filters. Transfer the filtered DI leachate into appropriate pre-cleaned containers and acidify with nitric acid to pH < 2 for element analysis by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES).
6. ICP-AES analysis by EPA Method 200.7 or EPA SW846 Method 6010 for the following elements:

Aluminum
Boron
Barium
Calcium
Cadmium
Chromium
Copper
Iron
Lead
Nickel
Potassium
Magnesium
Manganese
Molybdenum
Sodium
Zinc

(Phosphorus and Sulfur can be analyzed by ICP-AES rather than methods above, if lab has instrument capability for these elements)

KCl Leachate: Extraction of soil samples for nutrient analysis & analysis methods

1. Weigh approximately 40.0 g of homogenized soil sample into an appropriate pre-cleaned bottle. If insufficient sample is available (< 40g), record weight used. Lab will try to maintain soil:leachate ratio of 1:2. Narrate in report.
2. Add 80.0 ml of 2 N KCl (or amount to maintain soil:leachate ratio of 1:2).
3. Place samples in a laboratory shaker for 20 minutes.
4. Place bottles on laboratory counter and allow suspension to settle for approximately 10 minutes; then filter supernatant (leachate) through 0.45 micron filters.
5. Transfer filtered leachate into appropriate pre-cleaned containers for nutrient analysis.
6. KCl leachate analysis for nutrients using the following EPA methods or equivalent:

Ammonia: EPA 350.1
Nitrate-Nitrite: EPA 353.2

X. SOIL SAMPLING FOR CONTAMINANT CHARACTERIZATION

Four samples per plot[8] will be collected at each site (see field datasheets for guidance on placement of these samples) for contaminant characterization. Specifically, the top 2 cm of soil will be carefully collected by hand using clean nitrile gloves, and in general accordance with the case-wide intertidal sediment collection protocol set forth in "Sediment and Water Collection and Analyses for NRDA Purposes in Louisiana", available in the Resource Catalog of www.noaanrda.org.

These samples will not be composited in the field; whether some may be composited in the future will be determined at a later date.

XI. POST-SURVEY MANAGEMENT OF SAMPLES AND DATA

Soil samples for contaminant characterization need to be kept cold (4 °C) during transportation and frozen as soon as possible. Soil samples for physical characterization should be kept cold (4 °C) during holding and placed within a drying oven in accordance with the holding times defined above under "Collection of Soil Samples for Physical Characterization". Soil samples for chemical characterization should be kept cold (4 °C) during holding and be processed in accordance with holding times set forth in the Analytical Quality Assurance Plan (AQAP, July

[8] Sampling in herbaceous marshes will occur in association with the productivity plot rather than the cover plot.

2010) and defined above under "Collection of Soil Samples for Chemical Characterization" (Section XI. Soils – General Characterization.) Above and belowground biomass samples must be kept cold (4 °C) during transportation and holding, then sorted and placed in the drying oven in accordance with the analytical laboratory SOPs. For shipping instructions, refer to the NRDA Sample Shipping Instructions document (available on www.noaanrda.org).

NRDA Sample Collection forms need to be completed before leaving the site. Sample data and data from the field datasheets will need to be entered into the appropriate electronic spreadsheet as soon as possible after the fieldwork (preferably on the same day). Chain of custody forms must be completed rigorously. Photographs and GPS tracks shall be managed in accordance with case-wide protocols. Appendix B contains further information on these topics.

XII. QUALITY ASSURANCE PROJECT PLAN

Data Quality Objectives

The sampling program described in this document addresses the collection of the data and information relevant to characterize herbaceous coastal wetland vegetation physically, chemically, and biologically: in (a) post-impact and (b) unoiled areas. Specific sampling objectives include:

i. Characterizing the severity and extent of MC252 petroleum contamination in coastal wetland vegetation; and
ii. Characterizing the effects of petroleum contamination on coastal wetland vegetation.

Specific data types relevant to achieving these objectives and measurement performance criteria for Data Quality Indicators are described in this section and in the MC252 Data Validation Plan (July 2010).

Data Quality Indicators

Data developed in this study must meet acceptable standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity described in this section. Each of these data quality indicators, some of which are not readily quantifiable, is discussed below with specific reference to the current study.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristics. Precision for laboratory contaminant analyses of hydrocarbons and PAHs is addressed in Section 5.1 of the MC 252 AQAP (January 2011). Precision for this study will be assessed quantitatively using the measurement objectives for duplicate analyses defined in the EPA method references and laboratory SOPs. Precision may also be assessed by evaluating the range or standard deviation of duplicate measures of the same parameter such as soil redox, light-adapted fluorescence, and chlorophyll content.

Accuracy is defined as the agreement of a measure with its true value. Accuracy for laboratory contaminant analyses of hydrocarbons and PAHs is addressed in Section 5.2 of the MC 252 AQAP (January 2011). Accuracy for soil core analyses (physical and chemical) is improved by following the EPA method references and laboratory SOPs in terms of calibration requirements. Accuracy is estimated from laboratory control sample measurements, and method blank measurements. Accuracy in vegetation measurements and species identification (e.g., in clip plot samples) is controlled through use of laboratory SOPs.

Completeness is defined as the percentage of the planned samples actually collected and processed (analyzed) to give valid results. The objective for overall completeness for this program is set at 90%, though specific measurements may have different completeness objectives (see contaminant analysis, below) and some of the collected samples may intentionally remain unanalyzed. Completeness can be evaluated for all components of this study. In particular, for all sites visited, it can be determined whether all specified measurements were recorded, and whether samples were acquired from all sites for which sampling was planned through review of datasheets and chain of custody forms. Completeness can also be evaluated with respect to the proposed sampling strategy—e.g., whether the targeted number of sites per region are visited. Of note, logistical factors, including but not limited to the ability to obtain landowner permissions, may result in numbers of sites visited that differ from the presented target numbers. Completeness in the context of the laboratory contaminant analyses of hydrocarbons and PAHs is a measure of the planned data versus the amount of valid or usable data generated, defined as 95% completeness, in Section 5.4 of the MC 252 AQAP (January 2011).

In this plan, representativeness refers to the degree to which the data accurately reflect the broader populations investigated by the sampling effort. The investigated populations include specific wetland communities impacted by various levels of oiling and un-oiled communities. The stratified and random selection of sites for evaluation, among all possible sites, and the positioning of specific sampling locations within sites, has been designed using statistical considerations intended to enhance the representativeness of the selected sites.

Representativeness will be improved by proper handling and storage of samples and analysis within accepted holding times to maintain sample integrity from the field to the laboratory so that the material analyzed reflects the material collected.

Additionally, to reduce inherent variation, replicate field measurements will be performed and averaged to represent a plot location value for soil redox, light-adapted fluorescence, and chlorophyll content.

Comparability expresses the confidence with which one data set can be compared to another. Comparability for this project will not be quantified, but will be addressed through the use of consistent field and laboratory methods. Comparability of laboratory contaminant analyses of

hydrocarbons and PAHs is addressed in Section 5.3 of the MC 252 AQAP (January 2011). Comparability can also be assessed qualitatively by evaluating data compared to appropriate results from prior studies at similar locations, where applicable.

Sensitivity is the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest. The detection limits and reporting limits (RL) for chemistry contaminant parameters are addressed in the MC 252 AQAP (January 2011) and for other physical and chemical parameters in the laboratory SOPs and summarized in Table 4. These, in conjunction with the measured biological parameters, will provide sufficient sensitivity for the purpose of providing insight into the potential for the measured contaminants to impact the coastal wetland vegetation community.

Data verification and data validation of field observations, field measurements, and laboratory measurements will be performed to assess the achievement of the measurement performance objectives for Data Quality Indicators as defined above and in relation to data quality indicators for similar studies. Verification and data validation processes are described in the MC252 DV Plan (July 2010) and addenda.

XIII. COASTAL WETLAND VEGETATION ELEVATION SURVEY[9]

Coastal wetland vegetation elevation is one of several factors that governs the composition and health of coastal wetland vegetation communities in coastal Louisiana. Therefore, it is important to measure coastal wetland vegetation elevation at sites where composition, cover and productivity will be sampled so that substrate stability and elevation dynamics can be quantified and assessed over time. This section provides a description of the horizontal and vertical coordinate data that will be collected at and in the vicinity of the vegetation stations (plots) to ascertain information on substrate and coastal wetland vegetation elevation as well as evaluate topographic and bathymetric changes in the vicinity of the coastal wetland vegetation edge.

As described in previous sections, cover and productivity measurements will be taken along a vegetation transect established at 151 sampling sites across the coast. These data will be used to determine the composition and health of the coastal wetland vegetation communities and soil characteristics at the sites. Elevation transects will be established adjacent to each vegetation transect and have a length of 45 meters. Three elevation transects will be located 10, 15, and 25 meters on the left and right side of the center vegetative transect for a total of 6 elevation transects and one vegetation transect per site. All transects will be established to begin in the water body approximately 15 meters from the vegetative edge and extend landward 30 meters

[9] By signing this work plan and agreeing to fund the work outlined, BP is not endorsing the sample design as it relates to the elevation transect data as proposed under Section XIII. Coastal Wetland Vegetation Elevation Survey.

into the coastal wetland vegetation interior (Figure 4). Transects will extend further landward than shoreward to quantify potential coastal wetland vegetation edge retreat and elevation changes over time. To ensure sufficient coverage of data points along the elevation transect, horizontal and vertical coordinates will be collected at significant breaks in slope or every 3 meters, whichever is a shorter distance. Significant breaks in slope are defined as a change in elevation of 8 cm or more over the distance of 3 meters or less. By collecting coordinate data at this frequency, a more precise depiction of the substrate profile and coastal wetland vegetation edge can be delineated; thus decreasing compounding errors associated with comparing data sets over time. Also, an elevation point will be collected in each of the cover and productivity stations that have been established along the vegetative transect.

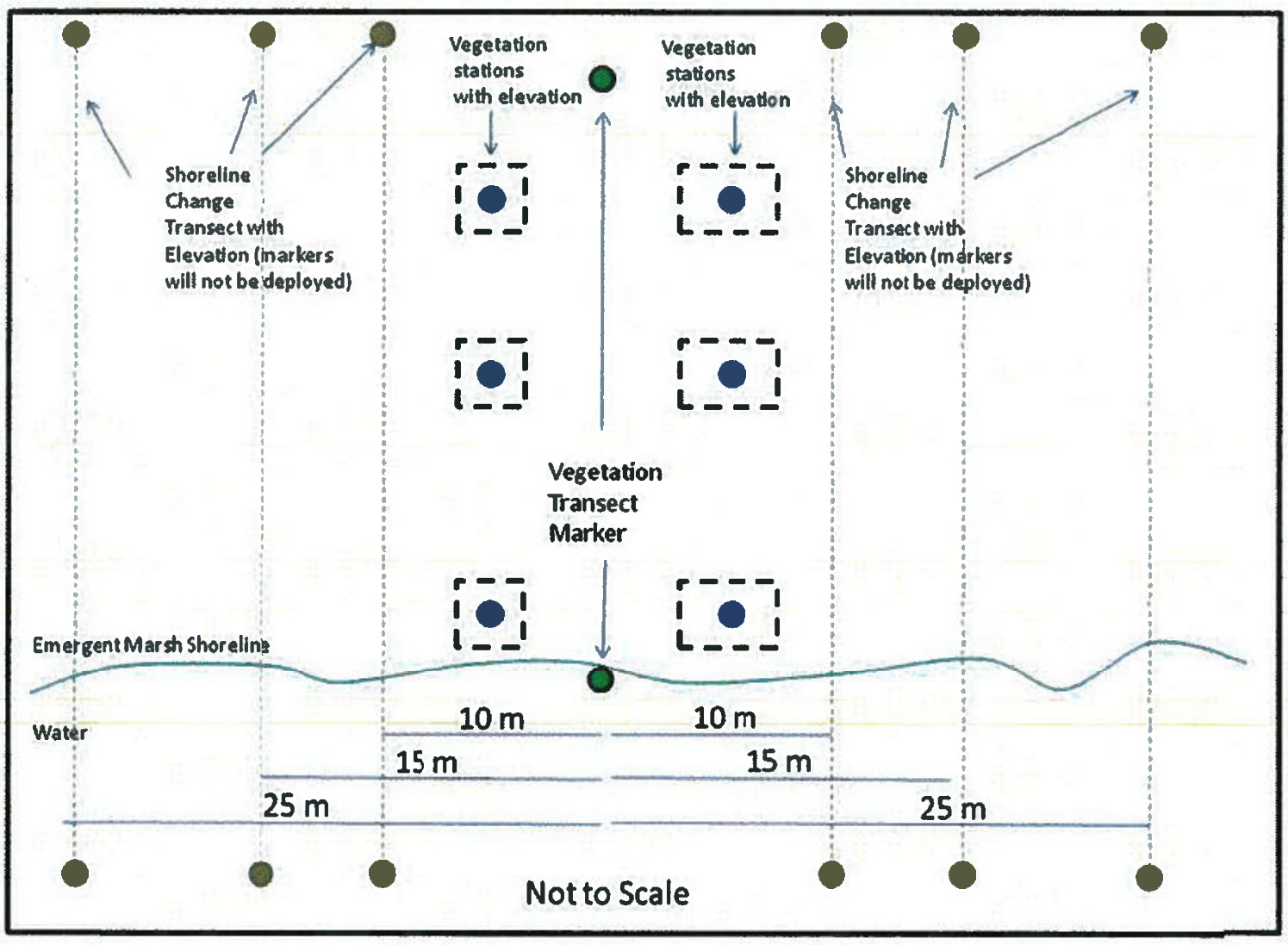

Figure 4. Depiction of a typical assessment site showing the location of the survey points in the cover (left of vegetation transect) and productivity (right of vegetation transect) stations and the six transect locations.

Coordinate data will be acquired by a professional land surveying company using Real Time Kinematic (RTK) methods. When possible, primary or secondary benchmarks established by the Office of Coastal Protection and Restoration (former Louisiana Department of Natural Resources, Coastal Engineering Division) through the Louisiana Coastal Zone Primary GPS Network will be utilized; however, temporary benchmarks may be established following the methods explained in the manual "A Contractor's Guide to Minimum Standards for Contractors Performing GPS Surveys & Establishing GPS Derived Orthometric Heights within the Louisiana Coastal Zone" when necessary. Using this method produces surveying results that have an expected accuracy of 1-2 cm horizontally and 2-5 cm vertically. However, final accuracies are not verifiable until the field data is processed. These values will be published in the final survey report as a deliverable product of the survey. The final report and deliverables will be stamped by a professional land survey with a Louisiana license.

To ensure the most accurate horizontal and vertical positions are acquired, the RTK survey must be conducted within a maximum of 6.4 km of a secondary benchmark. Temporary monuments will be established when sampling locations are outside the 6.4 km limit of any previously established monuments.

Data collected from the base unit at the temporary monument will be processed for horizontal and vertical positions and elevation.

RTK surveys will occur at the frequency described in Table 1, Proposed measures of ecological function and services for Gulf Coast coastal wetland vegetation habitats; plot elevation and shoreline change will be measured at the same time of the year as the data being collected at the cover and productivity stations. Survey data will be collected and processed using the same methodology as the initial data collection effort to ensure consistency for data comparison. Also, information contained within the survey report will be utilized to interpret the survey data.

Initial survey data set will yield the following:

a. Elevations at the cover and productivity plots will be compared to elevations obtained on the coastal wetland vegetation platform along the 6 elevation transects
b. Horizontal coordinate and elevation data will ensure accurate delineation of the substrate profile and morphology at each transect.

Multiple survey data sets will yield the following:

a. Document elevation change(s) at the cover and productivity stations, if any.
b. Document elevation change(s) along the elevation transects, if any.
c. Compare elevations at the cover and productivity plots to the transect elevation data. This will show if the elevations near the plots are responding as areas in the adjacent coastal wetland vegetation.
d. Quantify profile and coastal wetland vegetation edge changes at the sites. Shoreline change will be determined using both horizontal and vertical coordinate data.