Mississippi Canyon 252 Oil Spill
Submerged Aquatic Vegetation Tier 2 Pre-Assessment
Post Spill Exposure Characterization Plan

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For the
MC 252 NRDA Submerged Aquatic Vegetation Technical Working Group

Mississippi Canyon 252 Trustees

Final Version

November 8, 2011

First Drafted

May 25, 2010

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Mississippi Canyon 252 Incident
Submerged Aquatic Vegetation Tier 2 Pre-Assessment
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Approval of this Tier 2 Post-Spill Exposure Characterization Plan is for the purposes of obtaining data for the Natural Resource Damage Assessment. Each party reserves it's right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

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.

Unless otherwise agreed upon by the Trustees and BP, all samples will be sent to Alpha Analytical Lab.

The Trustees have developed preliminary conclusions about the result of data collected to date. These preliminary conclusions have informed the Trustees' decision to pursue the studies outlined in the work plan and addenda. By signing this work plan and agreeing to fund the work outlined, BP is not endorsing the preliminary conclusions articulated in the work plan or addenda, including addenda developed subsequent to the signature date.

APPROVED:

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Department of Commerce Trustee Representative: Date

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Summary

This document presents a plan to collect data concerning the post-spill condition of Submerged Aquatic Vegetation (SAV) resources in the north-central Gulf of Mexico (GOM), extending from the coastal areas and islands of Louisiana, Mississippi, and Alabama, through the panhandle of Florida to evaluate whether SAV habitats and communities were exposed to MC252 oil or related products. This plan builds on the "Mississippi Canyon 252 Oil Spill Submerged Aquatic Vegetation Tier 1 Pre-Assessment Plan Pre-Impact Baseline Characterization" (herein **Tier 1**, or Tier 1 plan) with some additional parameters added to evaluate potential exposure.

This plan is intended for use, to the extent feasible, to document conditions after oil from the Deepwater Horizon/Mississippi Canyon 252 Oil Spill (MC 252 Oil Spill) may have reached some SAV habitats. The activities outlined in this plan "Mississippi Canyon 252 Oil Spill Submerged Aquatic Vegetation Tier 2 Pre-Assessment Post Spill Exposure Characterization Plan" (herein **Tier 2**, or Tier 2 plan) are part of the pre-assessment phase of the Natural Resource Damage Assessment (NRDA) process for the MC 252 Oil Spill. The data collection described in this plan targets ephemeral **exposure** data—data that is anticipated to change or be lost within a relatively short period time (even while the spill was ongoing) (15 C.F.R. §990.43). The plan specifically addresses the following components:

1. **Introduction.** This section describes the overall purpose and objectives of an SAV assessment. It also identifies the ecosystem services and SAV metrics that will be considered through the NRDA data collection effort.

2. **Investigative and Sampling Approach.** This section describes the specific tasks to be conducted to obtain data on the SAV metrics. It also provides information on how sampling locations will be determined, an overview of guidance for sample processing, health and safety requirements, and documentation requirements.

3. **Quality Assurance Project Plan.** This section provides an overview of the field and laboratory procedures that will be followed to maintain sample integrity.

4. **Budget.** This section provides an estimate of resources required to accomplish the objectives of this work plan.

5. **Literature Cited.** This section provides the references cited in the text.

   Appendices:

   Appendix 1 SAV list: List of SAV species found in the northern GOM
   Appendix 2 SOPs: Detailed standard operating procedures (SOPs) for each of the tasks and SAV metrics
   Appendix 3 Sampling Maps
   Appendix 4 NRDA Baseline Assessment for SAV: Florida
   Appendix 5 Data Sheets

1. Introduction

1.1 Purpose/Objectives

SAV are rooted vascular plants that, except for some flowering structures, live and grow below the water surface. The term "SAV" includes seagrasses growing in the open GOM and saline estuaries as well as brackish and freshwater plant species. There are over 26 species of SAV within the GOM (Appendix 1). In Florida alone, approximately 2.5 million acres of seagrass have been mapped in estuarine and nearshore waters, but, when deep water seagrass beds growing in water too deep to easily map are included, the total area of seagrasses within Florida waters and adjacent federal waters is over 3 million acres (Handley et al. 2007). Important ecological services provided by SAV include, among others, food and habitat for many aquatic animals, maintenance and improvement of water quality, sediment stabilization and shoreline erosion protection. As a result, SAV plays an important role in the marine and estuarine ecology of the GOM.

As a result of the MC 252 Oil Spill, marine and estuarine ecosystems from Louisiana to Florida may have been exposed to and injured from oil discharged from the wellhead as well as from chemical dispersants. Accordingly, baseline and/or post-spill samples, as well as reference samples, within SAV beds *have been collected* under the **Tier 1 plan** and *will continue to be collected* under the **Tier 2 plan**, to document exposure to and presence of petroleum hydrocarbons and dispersants for the purposes of NRDA. Types of MC 252-specific contaminants that could occur during the life the MC 252 Oil Spill include (1) the crude oil product itself, (2) weathered oil – i.e., oil that has been exposed to the environment and is in a "degraded" state, (3) dispersed oil – oil that has been treated with dispersants and now is in an altered state, and (4) the dispersants themselves. Potential impacts of oil and dispersants on SAV include complete mortality (Jackson et al. 1989; Sandulli et al. 1998, Thorhaug and Marcus 1987; Scarlett et al. 2005, for example) and sublethal stress and chronic impairment of seagrass and SAV metabolism and function (Hatcher and Larkum 1982; Ralph and Burchett 1998; Peirano et al. 2005).

Overall Framework

The SAV Technical Work Group (TWG) has established a tiered approach that mirrors phases of the NRDA framework established by the Oil Pollution Act of 1990 (OPA), which includes the following phased elements:

a. Pre-assessment
b. Restoration Planning
    a. Injury Assessment
    b. Restoration Selection
c. Restoration Implementation

The SAV workgroup has divided the NRDA into five tiers. Each tier identifies related tasks to collect information to assist in the NRDA process. The tiers include:

Tier l Characterization of baseline and reference conditions
Tier 2 Preassessment/characterization of initial post- spill conditions
Tier 3 Injury assessment
Tier 4 Scaling and Restoration planning and selection
Tier 5 Restoration Implementation

Each Tier may have one or more associated plans. This **Tier 2 plan** addresses pre-assessment activities and uses many of the SOPs from **Tier 1**.

Underlying Question:

Have SAV beds and their associated biological communities been exposed to the MC 252 Oil Spill?

Goal:

Determine the spatial extent of exposure to MC 252 oil and related products on SAV resources during the time that oil is present on the surface of the water and in the vicinity of SAV beds. Monitoring stations for assessment of exposure will be representative of the major SAV habitat types (see Appendix 1 for species list) and degrees of oiling on adjacent shorelines based on SCAT observations. It will be necessary during the pre-assessment phase to identify locations of the major habitat types. Under this plan, the data collected will assist in determining which locations warrant further study for injury assessment and potential restoration.

Protocols and operational criteria contained within this plan are based on NOAA protocols developed for all NRDA activities associated with the MC 252 Oil Spill (see SOPs in Appendix 2). Protocols will be followed consistently for all samples collected whether before, during, or after impact from the offshore oil source. Many of the SOPs also duplicate those referenced in the Tier 1. There are also some additions, including the SAV Rapid Assessment (SOP 6) to collect information on the condition of seagrasses and the Polyethelyne Memebrane Device (PEMD) (SOP 7) to assist in documenting exposure.

An SAV Technical Working Group (TWG) of experts from government and academia, along with natural resource trustee agency representatives, has been assembled to draft this work plan and more broadly to implement exposure and post- exposure assessments of SAV beds throughout the northern GOM from Louisiana to the panhandle of Florida for the NRDA process established by OPA. Additionally, BP has participated in plan review and field implementation.

The geographical scope of the area of concern for SAV in this event consists of over 3500 linear kilometers of shoreline from Louisiana to Florida and offshore areas, requiring a broad-scale assessment. Because environmental conditions vary among regions and water bodies within the geographic scope of the MC 252 Oil Spill, sampling methods for SAV also will necessarily vary

accordingly. Some sites may require additional sampling stations based on the configuration and species composition of the bed. Also, some sites may require field staff to enter the water or strictly collect from vessels. The ultimate goal however, is to maintain a consistent set of methods to facilitate subsequent analyses.

This Tier 2 plan for exposure and post-spill assessments and has six objectives:

1) To continue collecting relevant data from existing SAV mapping and monitoring programs;

2) To review existing information and identify spatial, temporal and/or attribute data gaps relative to the suite of SAV metrics identified herein;

3) To conduct targeted analytical sampling to evaluate potential exposure of SAV beds and their associated faunal communities to MC 252 oil and related products;

4) To conduct targeted observational sampling to evaluate potential exposure of SAV beds and their associated faunal communities to MC 252 oil and related products;

5) To determine the extent of any oiling to the beds; and

6) To compile existing aerial imagery and/or acquire new imagery in support of mapping the areal extent of SAV resources at risk and or exposed in the northern Gulf of Mexico.

1.2 Geographic Scope

The geographic scope of the SAV NRDA for the MC 252 Oil Spill includes the nearshore and estuarine environments containing SAV habitats along the northern GOM from eastern Louisiana to the Florida Panhandle (see Tier 1 plan). For this Tier 2 assessment, areas potentially exposed to MC 252 oil or related products were identified based on data compiled by Shoreline Cleanup Assessment Technique (SCAT) data, NOAA surface oil trajectories, and NRDA shoreline assessments. The site selection process is discussed in Section 2.1.

1.3 SAV Metrics

SAV provides numerous ecological services. The SAV TWG has selected a suite of metrics that can be used to characterize key SAV physical, biological, and chemical attributes necessary to provide these ecological services, with the specific goal of supporting the NRDA. The metrics were chosen based on their widespread use for characterizing the ecological condition of SAV resources in peer-reviewed studies as well as their ability to serve as useful indicators of potential exposure to MC 252 oil and dispersant and/or adverse spill-related changes that could serve as a basis for quantifying injury (Table 1).

Table 1. Selected SAV Metrics

Metric	Task‡ (Sampling Program) and SOP	States Collecting Data under Existing Programs
Extent and coverage as determined from aerial observations	Task 1; SOP 1	LA, AL, MS, FL*
Areal coverage – visual estimates	Task 1; SOP 1, SOP 2, SOP 6	LA, AL, MS, FL*
SAV biomass and shoot density	Task 2; SOP 2; SOP4; SOP 6	LA, AL, MS, FL*
SAV species composition	Task 2; SOP 2; SOP 4; SOP 6	LA, AL, MS, FL
Sediment infauna abundance and diversity	Task 4; SOP 2; SOP 4	LA, AL, MS, FL*
SAV associated fauna (fish and mobile macroinvertebrate) diversity and relative abundance	Task 5; SOP 2; SOP 5	LA, AL, MS, FL*
Exposure metrics		
Sediment chemistry	Task 3; SOP 2; SOP 3A	LA, AL, MS, FL
Vegetation tissue chemistry	Task 3; SOP 2; SOP 3B	LA, AL, MS, FL
Invertebrate tissue chemistry	Task 3; SOP 2; SOP 3C	LA, AL, MS
Presence/absence of oiling	Task 6: SOP 7	LA, AL, MS, FL
Habitat characterization metrics		
Optical conditions	Task 2; SOP 2	LA, AL, MS, FL*
Conductivity/salinity	Task 3; SOP 2	LA, AL, MS, FL*
Depth	Task 2; SOP 2	LA, AL, MS, FL*
Temperature	Task 2; SOP 2	LA, AL, MS, FL*
Dissolved oxygen	Task 2; SOP 2	LA, AL, MS, FL*

* At some locations in Florida, variations of the SOPs were used in order to maintain consistency with existing datasets (see Appendix 3).
‡ See Section 2.2 for a description of the tasks.

1.4 General Approach

Federal, state and local resource management agencies and non-governmental organizations conduct SAV mapping and monitoring on a routine (regular or semi-regular) basis within areas of the GOM potentially impacted by the MC 252 Oil Spill. Examples of such organizations are provided in Table 2. This table will be augmented as additional relevant datasets are identified. These programs provide recent and historic data collected over varying spatial and temporal scales and frequencies that may be useful for documenting baseline conditions of at-risk and oiled SAV resources, as well as potential reference areas. Sampling designs, field methods, and assessment metrics differ across programs due to varying conditions among regions and waterbodies and differences in organizational objectives. However, most existing monitoring programs share common elements and possess significant overlap with the NRDA metrics identified in Table 1.

Table 2: Entities Conducting SAV monitoring in the Northern GOM & South Florida

Dauphin Island Sea Lab (DISL)
Florida Fish & Wildlife Research Institute (FWRI)
Grand Bay National Estuarine Research Reserve
Gulf Coast Research Laboratory
Mobile Bay National Estuary Program
U.S. Geological Survey
Florida Department of Environmental Protection
Florida International University (Florida Keys National Marine Sanctuary)
Apalachicola National Estuarine Research Reserve
South Florida Water Management District
Southwest Florida Water Management District

To the maximum extent possible, data from these existing SAV monitoring programs, along with the data collected during the Tier 1 sampling, will be utilized to characterize baseline conditions of SAV resources potentially affected by the MC 252 Oil Spill. This approach is intended to leverage existing information and focus new data collection efforts toward filling relevant data gaps.

This Tier 2 plan provides SOPs for the collection, processing, and management of field data needed to fulfill Tier 2 objectives (Appendix 2). The SOPs contained in Appendix 2 may be applied at Tier 2 sampling sites that are part of an existing monitoring program but at which data have not previously been collected for a metric identified in Table 1. Additionally, the SOPs in Appendix 2 may be applied at Tier 2 sampling sites that are not part of an existing monitoring program. The goal is to collect samples that are consistent and compatible with any existing local data sets in order to leverage the existing data with respect to data analysis.

2. Investigative and Sampling Approach

Sampling sites will be accessed by water using a vessel of appropriate size and configuration for the waterbody as well as for anticipated weather conditions and sea state. At this time it is anticipated that the majority of in-water sampling will be performed at depths accessible by snorkeling. Collection of certain data types, such as physiochemical water quality parameters and trawl surveys, will be conducted from onboard the sampling vessel.

In addition, rapid assessment of the status of seagrass beds in areas where adjacent shorelines were documented by SCAT teams to have experienced different degrees of oiling will also occur. The assessment will be implemented in a phased series of sampling events and evaluation steps that will examine the health and growth condition of seagrasses at five sites (see section 2.1) potentially exposed to different degrees of oiling (based upon SCAT information) at different spatial scales.

The initial assessment of these sites is designed to address the pathway of potential exposure where surface oil sheens passed across the deeper seagrass beds into shallow water reaching the upper limits of seagrass distribution in the shallow subtidal. During this process oil, weathered oil, derivatives of oil and dispersants may have been mixed into the water column and settled into the seagrass bed and on the seagrass plants and associated sediments. The SAV TWG will proceed on the assumption that, of seagrass beds in areas documented through trajectory and/or SCAT information to have experienced oiling, the seagrass beds in very shallow water (< 1.0m – intertidal) had the highest probability of exposure to oil.

The initial sampling event will consist of a rapid visual assessment of the health and condition of the seagrass beds along shore normal transects and at discrete sample points identified by association with surface oil trajectories, SCAT data and NRDA shoreline data. Condition of the seagrass beds includes an assessment of seagrass species composition, presence/absence of healthy growing apical meristem, new shoot growth, and new rhizome and root growth.

All sampling teams will use GPS and digital photography to document site conditions:

- Sampling teams will use a GPS device to document geographic positions (Waypoints) of all sample locations. The datum on GPS units should be set to WGS84. Meta data will be maintained according to protocols. (See **Basic_GPS_Skills_Final_0223_2010.doc**, available in the Documentation section of [redacted])

- Digital photographs will be taken to document general site location/conditions. Photos will be linked to latitude and longitude coordinates using GPS photolinking software (see **NRDA Field Photography Guidance**, available in the Documentation section of [redacted].

2.1 Sampling Locations and Design

In Tier 1, 19 sites were sampled for baseline pre-oiling data. As information on oiled shorelines was acquired, SAV beds that were not receiving oil could be systematically excluded from future pre-assessment and injury assessment unless new observations/documentation of oil exposure were identified. This process occurred under this Tier 2 plan and occurred in phases.

The first phase used NOAA surface oil trajectories to reduce the area of interest from the original 19 sites to 14 sites. SCAT data was then used to reduce the number of potentially exposed sites to 9 at which adjacent shoreline oiling (marshes and beaches) was observed, but at which it was unknown whether SAV beds were being exposed. To evaluate whether SAV beds were being exposed, the 9 sites (Table 3) were more closely examined for the presence of oil and level of exposure using submerged oil sentinels (see the draft Submerged Oil Sentinel Plan, Fish/Nearshore TWG). Based on the results of these observations, five of the 9 sites were identified as sites that needed further documentation on exposure (Table 3).

Five of the sites showed evidence of potential exposure and were further evaluated under Phase 2 using Polyethylene Membrane Devices (PEMDs) and the collection of sediment samples for chemistry analysis as part of the rapid assessment protocol.

Table 3: Phase 1 and Phase 2 Sites under Tier 2

Tier 2 Sites	Phase 1	Phase 2
Chandeleur Islands , LA	X	X
Horn Island, MS	X	X
Petit Bois Island; MS	X	X
Perdido Pass (Robinson Is), AL	X	X
Pensacola Bay (Big Lagoon), FL	X	X
Cat Island, MS	X	
Ship Island, MS	X	
Mobile Bay, AL	X	
Grand Bay , AL	X	

Phase 1: Identified as potentially exposed using oil trajectories and SCAT data. Further evaluated using submerged oil sentinels
Phase 2: Evidence of exposure from snare sentinels in Phase 1. Further evaluated for exposure with PEMDs and sediment chemistry samples.

For purposes of characterizing the conditions of SAV post- spill under Phase 2 of Tier 2, sampling will follow the Tier 1 sampling design. However, as new oiling information continues to be reported, additional sampling stations at the five sites may be added to cover the range of the oiling footprint. Reference areas (un-oiled areas) will also be considered for sampling. Such areas include locations where available oiling information indicates consistent absence of any form of post-spill SAV and/or shoreline oiling. Targeted stations within oiled or reference areas should be situated no more than 400 to 500 meters apart for establishing sediment, invertebrate, and vegetation data. If warranted by new oiling information, additional sites and or stations within sites will be added. For example, if the exposed SAV beds in a site proved to be more patchy than originally expected, then additional stations between existing stations will be added to enhance the coverage of the collected data. Each station will yield one set of sediment, invertebrate, and vegetation samples from the intertidal zone in regions where SAV occurs in the shallow subtidal zone.

2.2 Sampling tasks

The Tier 2 plan replicates many of the tasks and SOPs of the Tier 1 plan. The Tier 2 plan is intended to continue the collection of data under preassessment as described under NRDA. The exception is that post-spill data is now the focus of the plan rather than the documentation of baseline conditions. The SAV TWG identified six discrete tasks to accomplish the six objectives (Section 1.1) of this Tier 2 plan. The six tasks are described below; any differences between the Tier 1 and Tier 2 plans are identified by *italics.*

Task 1: SAV aerial and areal coverage (SOP 1)

- Continue to compile presently-available areal extent data for the sites identified in Section 2.1 using the following sources:
    - Literature
    - Historic aerial imagery

- Acquire new SAV coverage data through:
    - Imagery acquisition
        - *Collect low altitude aerial imagery for seagrass mapping for oil spill damage assessment and to aid strategic planning for the Deep Horizon NRDA. Please refer to the work plan entitled "**Low Altitude Aerial Photography of the Seagrass Beds of Southeastern Louisiana and Coastal Mississippi".***

        - *Under the Aerial Imagery TWG, a plan was developed to acquire high altitude imagery data for both SAV and marsh vegetation. Please refer to the **Technical specifications and scope of work services for aerial image acquisition and image processing in support of the MC 252 NRDA process – Fall 2010 – Spring 2012 plan** for details on imagery acquisition*

Task 2: SAV biological characterization (SOP 2 and 6)

- *Continue to* compile available SAV monitoring data on density, biomass, productivity, and species composition at selected sites (see Section 2.1).

- Acquire *post*- Spill data on SAV coverage, density, biomass, and species composition, including composite vegetative tissue samples representing the species of SAV present at selected sites (see Section 2.1). (For more information, see Appendix 2, SOP 2.)

- *Acquire rapid visual assessment data (observational) on the health and condition of the SAV beds at discrete sample points stratified perpendicular or aligned along shore in association with surface oil trajectories, SCAT data, and NRDA shoreline data at selected sites. (For more information, see Section 2.1 and Appendix 2, SOP 6.)*

Task 3: Chemistry (SOPs 3A–3C)

- *Collect* sediment, SAV, detritus, and invertebrate samples for chemical (PAH and dispersants) analyses in the areas that warrant further evaluation based on documentation of shoreline oiling, NOAA trajectories submerged oil sentinels and PEMDs described in Section 2.1.

    - *Sediment*, SAV, detritus, and invertebrate samples will be analyzed for hydrocarbon contaminants, including total petroleum hydrocarbons (TPH),

polycyclic aromatic hydrocarbons (PAHs), and other constituents as appropriate. (For more information, see Appendix 2, SOPs 2 and 3A–3C.) Sediment composition will also be assessed through analysis of grain size and total organic carbon.

- *Collect* sediment, SAV, and invertebrate samples for chemical (PAH and dispersants) analyses in reference areas that have been documented as un- exposed to MC 252 oil and related products. (For more information, see Appendix 2, SOPs 2 and 3A and3B.)

- Record and photograph all species collected within a sample. This will enable chemistry results to be evaluated based on species present in each sample.

- Coordinate with other NRDA TWGs and state-led NRDA efforts to collect samples in all necessary locations.

- *Continue to perform* SAV invertebrate tissue sampling in conjunction with otter trawls. (For more information, see Appendix 2, SOP 3C.)

Task 4: Invertebrate (benthic and epibenthic) densities and species composition (SOPs 2 and 4)

- *Continue to compile* available data on the density and species composition of SAV-associated benthic and epibenthic invertebrates (e.g., from published literature, monitoring data) in the vicinity of identified SAV beds for those parts of the GOM region that may potentially be affected by the MC 252 Oil Spill.

- *Continue to acquire*, where not available, data on the density and species composition of SAV-associated benthic and epibenthic invertebrates (using site-appropriate methods). Coordinate with other NRDA TWGs and state-led NRDA efforts to collect samples in all necessary locations. (For more information, see Appendix 2, SOP 4.)

Task 5: SAV-associated mobile fauna (fish and mobile macroinvertebrates) (SOPs 2 and 5)

- *Continue to* compile available data on the density and species composition of SAV-associated fish and mobile macroinvertebrates (e.g., from the published literature, monitoring data) in the vicinity of identified SAV beds for those parts of the GOM region that may potentially be affected by the MC 252 Oil Spill.

- *Continue to acquire*, where not available, data on the density and species composition of SAV-associated fish and mobile macroinvertebrates using site-appropriate methods, including trawling. Coordinate with other NRDA TWGs and state-led NRDA efforts to collect samples in all necessary locations. (For more information, see Appendix 2, SOP 5.)

Task 6: Additional exposure documentation methods and compilation of exposure data. (SOP 7)

The purpose of this task is to implement two additional methods for evaluating potential exposure 1) submerged oil sentinels (or pom poms) and 2) Poly Ethylene Membrane Devices (PEMDs). The pom pom devices are described in detail in the draft plan, "Nearshore Ephemeral Data Collections: Submerged Oil Characterization Across Multiple Habitats Deepwater Horizon Oil Spill" (version October 23, 2010). In addition, an SAV oil mapping product may be developed based on analysis of these techniques as well as the use of other data sources and products (ERMA, submerged oil sentinels, PEMDs, SPEMDs, snorkel SCAT, surface oiling maps, etc.).

- Deploy submerged oil sentinels (pom poms) at the nine sites that have been identified as having the potential of exposure based on oil trajectory and SCAT data**.**

- Deploy subsurface PEMDs at four locations that showed evidence of oil exposure based on the submerged oil sentinel monitoring. A total of 144 PEMDs are to be deployed on 72 moorings at the same sampling stations identified in the Tier 1 plan. After deployment, transport samples to NOAA Auke Bay laboratory for GCMS analysis. (For more information, see Appendix 2, SOP 7.)

- Produce SAV potential oiling maps based on SCAT, shoreline NRDA data and oil mapping products, submerged oil sentinels, PEMDs, SPEMDs, submerged oil snorkel surveys (response), etc.

2.3 Sample processing requirements

After completing all field sampling activities for a given day, the field team must take the collected samples, datasheets and electronic information (including photographs and GPS track log) to an appropriate sample processing center.

At this center, the following activities will take place:

- Appropriately package and prepare all samples for shipment to the receiving laboratory(ies).
- Complete **Chain-of-custody** forms.
- Enter all data from all field forms into the appropriate Excel file format (Forms or Flat version) either by the field sampler or a data management team member. Once the file is completed, submit it to the data management team for incorporation into the database.
- Archive all photographs in accordance with the instructions in the **NOAA Field Photography Guidance** in the Documentation section of [redacted].
- Synchronize the photos with the GPS track in accordance with the instructions in the **GPS Photolink Guidance** in the Documentation section of [redacted]

- Import the photos into the ORR PhotoLogger database. (This will allow the photos to be uploaded to ERMA.) See the **PhotoLogger** instructions in the Documentation section of [redacted] for more information.
- Scan all field data sheets and store originals in a secure location.

2.4 Health and Safety

All personnel will participate in training modules required by Incident Command (IC) and BP Exploration and Production Inc. (BP). Float plans will be filed with the IC for each day's activities on the water. All necessary personal protective equipment (PPE) will be used.

- **The team leader and field crew parties must complete all applicable health and safety training as directed by NOAA or state agency oil spill policy.**

- **All field team members must complete the NOAA safety training and documentation requirements** as set forth in "Safety Requirements for All Personnel Working on NOAA-led NRDA teams for MS Canyon 252 Incident" (NOAA Safety Documentation Requirements_0327_Without Safety Plan.pdf available on the Field Ops wiki page under the "New Hire Pre-Gulf Documents and Guidance" link).

- **All field team members must read all of the health and safety documents on the Field Ops wiki page and in the Safety Protocols directory in the Documentation section of** [redacted]. Exception: if field activities do not include use of a helicopter, then familiarity with the safety documents for these vehicles is not required.

- **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);
  - What kind of sampling they are doing;
  - Expected arrival time at sampling area (daily);
  - Expected departure from sampling area (daily);
  - Team deployment date; and
  - Team return date.

  This information may be reported in one of two ways:
  1. Fill out the Excel spreadsheet "04 - Team Member Information Form - Sampling and Safety.xls" (available in the Resource Catalog of [redacted] and send it to [redacted]. Please use one tab for each team.
  2. If you cannot submit this spreadsheet electronically, you can call in and report the information using this number: [redacted]

- **Field teams must adhere to all procedures set forth in the MC 252 Site Safety Plan ("NRDA Field Ops Safety Plan 1-28-11.pdf").** This document can be found on the

Field Ops wiki page under the "General Safety and Guidance Documents" link on [redacted]

2.5 Documentation Requirements

All team members should familiarize themselves with case-wide protocols for data collection and documentation and should adhere to these. Currently available case-wide documentation procedures include (but are not necessarily limited to):

- GPS setup, use, and archiving;
- Camera setup and use;
- Electronic data downloads;
- Sample collection documentation;
- Photo documentation (including in-field photo logging, post-field photo archiving; synchronization with GPS tracks, and importing into the ORR PhotoLogger database),
- Sample packaging and shipping; and
- Chain-of-custody documentation, including for electronic records (e.g., photographs, databases).

These protocols are available in the Documentation section of [redacted]

Additional case-wide data documentation requirements are expected, including requirements for transferring hard copy field data into electronic form.

In addition, all SOP-specific documentation requirements should be reviewed and followed.

3. Quality Assurance Project Plan

3.1 Data Quality Indicators

Data developed in this study must meet acceptable standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity. Each of these data quality indicators, several of which have quantitative measures and several only qualitative measures, is discussed next 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 this study is assessed through the use of field duplicates for those data types that are amenable to duplicate measurements (e.g., field duplicates are to be taken at a 5% rate for samples collected for chemical analysis) and by taking records of multiple in-situ measurements where this is possible. Precision in the context of laboratory analysis is described in Section 5.1 of the MC 252 AQAP (July 2010).

Accuracy, or bias, is defined as the agreement of a measure with its true value. Accuracy in the context of laboratory chemical analyses is addressed in Section 5.2 of the MC 252 AQAP (July 2010) using, for example, laboratory control samples, standard reference materials, matrix spike samples, and matrix spike duplicate samples. Accuracy in species identification and in abundance measurements (e.g., in core samples) will be estimated by subjecting a proportion of samples (5%) to re-analysis by a second reviewer. Accuracy of *in-situ* field measurements may

be estimated by repeated measurements (at the same time) at a proportion of stations by a second field surveyor.

Completeness is defined as the percentage of the planned samples actually collected and processed (analyzed) to provide valid results. 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. Completeness can also be evaluated with respect to the proposed sampling strategy — e.g., establishment of selected SAV sampling sites to represent SAV habitat areas of the northern GOM SAV beds (final number of sites to be determined). Completeness in the context of the analytical chemistry measurements is a measure of the planned data vs. the amount of valid or usable data generated, as described in Section 5.4 of the MC 252 AQAP (July 2010).

Representativeness refers to the degree to which the data accurately reflect the broader community represented by the sampling effort. The careful 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 allow results to be representative. Representativeness also will be ensured by proper handling and storage of samples and analysis within accepted holding times so that the material analyzed reflects the material collected as accurately as possible. Additionally, a quantitative measure of representativeness is the relative percent difference of field duplicate results.

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, particularly with respect to geographical areas to maintain continuity and consistency with historical data sets. Additional discussion of comparability is presented in Section 5.3 of the MC 252 AQAP (July 2010).

Sensitivity, the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest, is largely not applicable to the biological parameters. The detection limits for chemistry parameters are addressed in Section 6.0 of the MC 252 AQAP (July 2010). 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 SAV community.

Field quality control samples will be collected as described in Table 4 to support the above data quality indicators.

Table 4. Summary of Field Quality Control Samples to Support Data Quality Indicators

Sample Media/Type	Field Duplicate frequency of collection	Additional Sample Collection for Matrix Spike / Matrix Spike Duplicate or Lab Matrix Duplicate
Sediment: Grain Size	1 per 20 field samples	1 Lab Matrix Duplicate per station or per 20 field samples, whichever is more frequent
Sediment: TOC	1 per 20 field samples	1 MS/MSD per station or per 20 field samples, whichever is more frequent
Sediment: Contaminants	1 per 20 field samples	1 MS/MSD per station or per 20 field samples, whichever is more frequent
Vegetation: Contaminants	1 per 20 field samples	1 MS/MSD per station or per 20 field samples, whichever is more frequent
Tissue: Contaminants	N/A	1 MS/MSD per station or per 20 field samples, whichever is more frequent

3.2 Project Management

Project organization, roles, and responsibilities help ensure that individuals are aware of specific areas of responsibility as well as internal lines of communication and authority. Overall authority for project management will rest with the Trustee Council. Currently, Trustee representatives have divided their staff into a number of TWGs, which are overseeing the development of specific plans for the evaluation and generation of information of relevance for the ongoing NRDA. The current leaders of the SAV TWG are Natalie Manning of NOAA and Eva DiDonato of the National Park Service. Representatives from the States of Louisiana, Mississippi, Alabama, and Florida are participating in the TWG. Furthermore, the Trustees are currently engaged in a cooperative effort with BP, whose representatives are also participating in the TWG. TWG participants have contributed to the development of this report.

Under the auspices of the SAV TWG, field teams will be organized to implement this plan. Field team members have partially overlapping and partially distinct areas of responsibility. All field team members are responsible for ensuring that they are adequately trained with respect to health and safety requirements, requirements relating to the implementation of study-specific data generation activities, and adherence to case-wide protocols on topics including (but not necessarily limited to) chain-of-custody documentation, sample collection documentation, use of

camera and GPS equipment, sample handling, packaging, and shipping requirements (see Section 2.5).

Designated field team leaders have additional responsibilities, including overall responsibility for the activities of the field teams while they are deployed. Field team leaders have responsibility for communication with designated contacts on the status and safety of their teams. They are also responsible for ensuring the accuracy of information and the integrity of samples collected during field activities, and to make sure samples are appropriately handled and delivered, under chain-of-custody, to designated locations where they will be temporarily stored prior to shipment to an appropriate laboratory. Field team leaders are also responsible for ensuring complete collection of all information, data, and samples, as specified in the SOPs. They have responsibility for ensuring that electronic data (e.g., from cameras and GPS units) are appropriately archived and uploaded into Trustee databases, and that hard copy data are transcribed into case-wide databases.

3.3 Data Generation and Acquisition

The SOPs included in this document, and included by reference, as well as Sections 3.0 and 7.0 of the MC 252 AQAP (July 2010), provide full details about how data will be generated, including sampling methods, sample handling, chain-of-custody requirements, and data reporting. All data generated will be compiled in a GIS-compatible electronic database such as the Environmental Response Management Application (ERMA) which will be accessible to all parties.

3.4 Assessment and Oversight

All field data collected pursuant to this work plan, including that of the RP, will be recorded in forms kept in loose leaf notebooks and will be signed and dated. The Field Team Leader will supervise day-to-day field investigations, including sample collection, field observations, and field measurements and generally is responsible for all field quality assurance procedures. The Field Team Leader will review all forms for accuracy prior to their submittal at the end of the field day. The field forms, including those of the RP, will be scanned and archived, and data from the forms will be entered into the case-wide database [redacted] and distributed, as needed, to the Trustees and RP representatives participating in the field effort.

If technically and logistically feasible, during the course of the field work, an external audit will be conducted by a Trustee-designated member of the QA team to evaluate adherence to relevant protocols and ensure that procedures are in place for proper sample handling, processing, and documentation of results. Laboratory audits are also anticipated. The RP reserves the right to conduct independent audits, or monitor the Trustee audits of the laboratories.

If, during the course of any field or laboratory audits, the QA auditor identifies deficiencies and other non-conforming conditions, the QA auditor or designee shall document these issues and shall formulate recommendations for corrective actions, which shall be communicated to the

responsible team members (including the analytical laboratory and field personnel, as applicable), designated TWG representatives, RP representative(s), and/or Trustees representatives. Implementation of corrective actions will be the responsibility of the NOAA QA Manager. Corrective action recommendations and documentation that the corrective actions have been implemented will be provided to the RP within two weeks of notification to the Trustees.

3.5 Data Validation, Usability, and Sharing

The chemistry data will be subjected to formal data validation prior to use, in accordance with the requirements in Section 7.0 of the MC 252 AQAP (July 2010). For non-chemistry measurement data, the Trustees and RP will jointly develop performance criteria and will mutually evaluate the data based on these criteria. Any data that do not meet the performance criteria for measurement data will be flagged appropriately to indicate potential uncertainty in the data. Descriptions of potential bias and reason for the uncertainty will be documented.

The data generated in this study will be compiled in a GIS-compatible electronic database. The accuracy of data transcriptions will be evaluated by conducting 100% transcription verification. This evaluation level will be increased if any errors are encountered during the initial evaluation of the data.

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 Quality Assurance Project 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 Quality Assurance Project 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 assure 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).

4. Budget

COST ELEMENTS SAV Tier 2				
Labor: All labor under NOAA contract, NOAA, NPS, USFWS staff or State Reps is recoverable under NRDA but not calculated here.				
Laboratory Analysis (Chemistry cost not included)				
Item	**Unit**	**Rate**	**Number of samples***	**Total**
Sediment forensic chemistry	per sample	1,000	70	70,000
Vegetation forensic chemistry	per sample	800	100	80,000
Invertebrate forensic chemistry	per sample	800	52	41,600
Sediment epifauna/infaunal taxonomic ID	per sample	$300	100	30,000
PEMDS Budget: (first 100/100 samplers)				
Preparation of pucks and bare passive samplers				15,000
Spectrofluorometric analyses of all samples			200	5,000
GCMS of 40 samples			40	20,000
Shipping				2,000
Laboratory Analysis Total				**263,600**
Other Direct Costs				
Item	**Unit**	**Rate**	**Number of days**	**Total**
Boat rental, including gas	per team-day	$2,000	30	60,000
Other transport	per team-day	$200	10	2,000
Other equipment rental	per team-day	$100	10	1,000
SUBTOTAL				**63,000**
Additional Costs				
Item		Rate		
Project management		0%		
Contingency		0%		
TOTAL				**$326,600**
Cost Not Explicitly Included:				
Costs for sampling plan development				
Reanalysis costs if QA/QC goals are not met.				
Costs for audits or other QA/QC measures.				
Report development/data analysis.				
Travel costs, including per diem, from outside of the immediate area				
Costs for sample management team and data management team support			,	

*Not all samples may be processed. The number of samples reflects the high end of samples that could be processed and analyzed.

The Parties acknowledge that this budget is an estimate, and that actual costs may prove to be higher. BP's commitment to fund the costs of this work includes any additional reasonable costs within the scope of this work plan that may arise. The Trustees will make a good faith effort to notify BP in advance of any such increased costs.

5. Literature Cited

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Appendix 1.
SAV list

Table 1. List of Species found in Alabama, Louisiana, Mississippi, and Florida

Seagrass:
Halodule wrightii Asch. shoal grass
Halophila decipiens Paddle Grass
Halophila engelmannii Star grass
Halophila johnsonii – Johnson's Seagrass, if oil is found between south Miami and Sebastian FL
Thalassia testudinum Banks & Sol. ex J. König turtle grass
Syringodium filiforme Manatee grass
Ruppiaceae
Ruppia maritima L. widgeon grass

Other:
Cabombaceae
Cabomba caroliniana A. Gray Carolina fanwort
Ceratophyllaceae
Ceratophyllum demersum L. coon's tail
Cymodoceaceae
Cyperaceae
Eleocharis elongata Chapm slim spikerush
Haloragaceae
Myriophyllum heterophyllum Michx. twoleaf watermilfoil
Myriophyllum spicatum L. Eurasian watermilfoil †
Hydrocharitaceae
Hydrilla verticillata (L.f.) Royle hydrilla †
Najas guadelupensis (Spreng.) Magnus southern naiad
Najas minor All. brittle waternymph †
Vallisneria neotropicalis Marie-Victorin. wild celery
Lentibulariaceae
Utricularia foliosa L. leafy bladderwort
Utricularia gibba L. humped bladderwort
Poaceae
Luziola fluitans (Michx.) Terrell & H. Rob southern watergrass
Pontederiaceae
Heteranthera dubia (Jacq.) MacMill. water stargrass
Potamogetonaceae
Potamogeton crispus L. curly pondweed †
Potamogeton illinoensis Morong Illinois pondweed
Potamogeton pusillus L. small pondweed
Stuckenia pectinata (L.) Böerner sago pondweed
Zannichellia palustris L. horned pondweed

Appendix 2.

SOPs

1. SOP for SAV Aerial and Areal Coverage
2. SOP for Site Characterization of SAV beds
3. SOPs for Sediment, Water, Vegetation and Invertebrate Chemistry
    - 3A. SOP for SAV Sediment Chemistry
    - 3B SOP for SAV Vegetation Chemistry
    - 3C. SOP for SAV Invertebrate Chemistry
4. SOP for SAV and Associated Epifauna and Infauna cores
5. SOP for SAV Associated Fauna (fish and mobile macroinvertebrates) trawls
6. SOP for the rapid assessment of the status of seagrass beds potentially experiencing exposure to different degrees of oiling
7. SOP for Polyethylene Membrane Devices (PEMDs)

All materials associated with the collection or analysis of samples under these protocols or pursuant to any approved work plan, except those consumed as a consequence of the applicable sampling or analytical process, must be retained unless and until approval is given for their disposal in accordance with the retention requirements set forth in paragraph 14 of Pretrial Order # 1 (issued August 10, 2010) and any other applicable Court Orders governing tangible items that are or may be issued in MDL No. 2179 IN RE: Oil Spill by the Oil Rig "DEEPWATER HORIZON" (E.D. LA 2010). Such approval to dispose must be given in writing and by a person authorized to direct such action on behalf of the state or federal agency whose employees or contractors are in possession or control of such materials.

1. SOP for SAV Aerial and Areal Coverage

A. Intertidal and Shallow Subtidal Aerial SAV Surveys

Available and acquired aerial imagery will first be evaluated in the following manner: 1) determination of baseline year for imagery; 2) determination that the spatial coverage and quality of the imagery are adequate for baseline SAV cover assessment. This will be accomplished by georectification and overlays with existing map products, and visual inspection for glare, water column turbidity and other visual impairments; 3) determine whether the resolution of the imagery will support finer-scale damage assessment than was planned for its original mapping use. If imagery does not meet at least the first two criteria, then the SAV TWG will request additional imagery acquisition. For areas likely to be oiled, imagery should meet criteria #3 as well.

Locations for imagery to be acquired:

Initially, the SAV TWG has identified the following locations that are a priority for imagery acquisition:

1. Chandeleurs to Pensacola
2. Tortugas, Marquesas, and Lower Keys;
3. Upper Keys and Florida Bay;
4. Big Bend ; and
5. Ten Thousand Islands.

The most recent imagery for these areas was acquired in 2006. An Aerial Imagery TWG was assembled.to assist the SAV and shoreline TWGs in high resolution imagery collection. For details on imagery acquisition, please see the plan titled **Technical specifications and scope of work services for aerial image acquisition and image processing in support of the MC 252 NRDA process – Fall 2010 – Spring 2012 plan.**

B. Baseline assessment using on site assessment techniques (quadrats):

Sampling methods vary by agency, scientific group and location throughout the GOM region, but all groups determine percent bottom cover by species. Some sampling programs use a variant of the Braun/Blanquet quadrat assessment method. This method produces a quantitative assessment of species composition, shoot density, and overall bed density and is often used along a transect running through a seagrass bed from closest shore limit to the deep edge, where evaluation is done at specific intervals (5, 10 meters, for example). Spatially-distributed random sampling designs determine percent bottom cover by evaluating several quadrats (3-8) at randomly chosen, spatially distributed sampling points within an estuary or coastal waters. Within a specific estuary or subregion, before and after event data sets can be statistically analysed if the same quadrat method is used throughout the study. Where data has not been collected that can serve as baseline assessment, the SAV TWG will select appropriate methods for those particular estuaries or regions that provide consistent data going forward (See the Site

Characterization Form in Appendix 4 and the SOP 2 in Appendix 2). Use of the same sampling methodology is recommended, however, there may be minor variations in these methods from one area to another within the GOM.

C. Coarse scale deepwater seagrass surveys[1]

Extensive areas of deepwater seagrass habitats can be visualized and recorded for analysis using a towed camera and GPS. A large (2 x 3m) benthic sled would be deployed from a suitable vessel (must have towing frame capable of swinging on-and over-board a 200lb object of these dimensions) equipped with a video camera. Cameras will be mounted obliquely on the sled creating a visual track of the seafloor 1m wide with ~0.5cm resolution. Differential GPS (DGPS) information will be collected in tandem with video, allowing for geo-rectification of all collected video. Calculations may be necessary to determine the precise location of the sled from the DGPS unit, all information needed to make these calculations, including water depth, the angle and amount of tow cable deployed, will be recorded.

The dominant habitat within the frame of view will be recorded using a present or absent score. Adjacent but non-overlapping frames will be analyzed, ensuring each area is classified only once. Dominant coverage will be determined following Fonseca et al. (2008) and previously developed visual assessment techniques[2]. These habitats are:

1. hard coral,
2. macroalgae (non-drift – typically calcareous green),
3. mixed reef (hard and soft corals),
4. sand,
5. seagrass (*Halophila decipiens* or other deep water seagrasses as a vector of 0's and 1's indicates absence of *H. decipiens*)
6. soft coral and
7. bioturbation (mounded sand or excavation pit).

2. **SOP for Site Characterization (Appendix 4; Data sheet #1))**

This SOP describes the standard parameters to be collected at a site.

Equipment

- Sampling Points (if pre-determined)
- GPS with extra batteries
- Digital camera with extra batteries
- Secchi disk
- PAR sensor with datalogger (LICOR spherical sensors, one for air one for water, and LICOR 1400 datalogger, or equivalent setup)
- Refractometer
- Thermometer
- Dissolved oxygen meter
- Meter stick or weighted transect tape (or boat equipped with depth sounder)
- 0.25 m^2 quadrat - Quadrat can be constructed from 4 pieces of regular plastic plumbing pipe (not too flexible) with right-angle elbow joints. Ensure that internal dimension of each side is 50 centimeters.
- Waterproof pens
- Waterproof forms (SAV Site Characterization Form, Chain-of-Custody, NRDA Sample Collection Forms for both tissues and sediments, PhotoLogger Form)
- *For equipment specific to collecting samples, see the relevant SOPs.*

Set up and use the GPS unit in accordance with case-wide protocols (see Basic_GPS_Skills_Final_0223_2010.doc, available on the case FTP site).

Samplers should complete all portions of the **SAV Site Characterization Form** (Appendix 5). The following descriptions correspond to the sections of the SAV Site Characterization Form:

1. *Site Description*

The site name (general geographic location or established sampling area) along with the latitude and longitude coordinates obtained via a GPS should be noted. Coordinates should be recorded in decimal degrees with WGS84 as the datum. The time of day and date should be noted next.

Next, the habitat setting of the SAV bed should be indicated. The habitat setting is a reference to the tidal regime the bed normally experiences (intertidal or subtidal). If the bed is located subtidally, indicate the depth at the time of sampling, in meters. The overall visual condition of the bed should also be described--for example, whether the bed appears to be impacted by oiling, disease, or scarring and to what extent.

2. *Physical/Chemical Parameters*

Because SAV distribution and abundance are influenced by a range of physical and chemical parameters, several variables should be measured, as indicated in the **SAV Site Characterization Form # 1 (Appendix 5)**, including salinity and water temperature. If beds are subtidal, bottom water samples are the most appropriate to measure. If the beds are intertidal, the nearest source of tidal water should be used if the beds are not flooded at the time sampling is performed.

The standard protocol for rapidly assessing optical conditions in the water column that affect SAV is the Secchi disk and measurements of light attenuation using quantum sensors. These measures are of paramount importance and should be taken as described below.

Secchi depth: Secchi depth is measured using a Secchi disk, a round black and white weighted disc (20 cm) that is lowered through the water until the distinction between white and black quadrants is no longer visible to the human eye. The disk is attached to a non-stretching rope, marked at appropriate intervals (5 and/or 10 cm apart). The observer lowers the disk over the side of the boat facing the sun and not in the shadow of the vessel, until the disk disappears, then raises it until it reappears and records this depth. At the time of the measurement record the time of day, cloud cover, and wave height. Do not wear sunglasses when taking the measurement.

Light Attenuation (irradiance): For teams possessing the appropriate equipment, light attenuation in the water will be calculated using either a 2pi or 4 pi quantum sensors attached to a data recorder. The sensor is lowered in the water column to obtain a profile of light readings. A sub-surface reading denoted I_o is taken just below the water surface and then at least three additional readings with depth down to the bottom. Readings are taken closer together near the surface, as this is where light attenuates the fastest. For each depth, record the irradiance value displayed on the data logger. At the time of the measurement record the time of day, cloud cover, and wave height. Perform three profiles per station. For calculating light attenuation (k_d) in each profile take the natural log of the irradiance values and regress light on depth. The attenuation coefficient is the absolute value of the lope of the line. Note: Under oiled conditions the sensor should be wrapped in plastic wrap.

Oiling (if applicable). Several descriptors are given for the sampler to denote the relative amount of oil present within the area sampled. The list should be thought of as a range of oiled conditions from none to the most saturated.

3. *Seagrass percent cover*

If water clarity allows for a visual survey of SAV abundance to occur, haphazardly toss the $0.25m^2$ quadrat within the SAV habitat a minimum of three times but preferably ten times. Estimate seagrass vegetative cover visually (first total cover, and then, if multiple species are present, estimate seagrass cover for each species; the cover estimates for the individual species must equal the total cover) on a percent cover scale (0-100%).

Look for the presence of any flowering shoots and record their presence/absence.

4. *Sample Collection and Disposition*

For detailed sample collection protocols, see the relevant SOP included in this work plan.

If samples are collected for a site, the individual who collected the sample should be specified on the field data form. If more than one person, list the field party leader and the person who entered the data (if different).

Sample IDs should be clearly listed under each category. If no samples of a given type are taken, write "none". Sample IDs should be assigned in accordance with the instructions in the **NOAA Field Sampling Workbooks** (available on the case's FTP site[3]).

Samples must also be recorded in the appropriate case-wide NRDA Sample Collection Form (also available on the case's FTP site).

Field duplicates should be clearly marked and Field duplicates are separate samples, so should be assigned a new sample number distinct from the original duplicated sample. On the sample form, use the Sample QA/QC Type column to indicate that the sample is a duplicate. The associated parent sample number can be identified in the Sample Notes column (the entire Sample ID should not be required in most situations since the location ID, matrix, and data should be the same). If a particular type of sample is not collected at a site, enter "none" for that sample type.

5. Trawl Data

For detailed trawl data collection protocol, see the relevant SOP indicated in this work plan. See instructions about Sample IDs in (4) above. If no trawl is conducted, enter "none" in this area.

6. Photographs

Set up the camera in accordance with NRDA Field Photography Guidance (NRDA_Field_Photographpy_Guidance.doc, available on the case FTP site). **Always begin by taking a photo of the operating GPS screen showing the date and time to synchronize the photos with the GPS track.**

Take photographs of the site and sample collection itself if possible; make sure each photograph or series can be later associated with the corresponding sampling locations (e.g. through use of GPS Photolink software or by keeping a detailed photo log). Do not delete or alter any photographs, the numbering sequence of photos uploaded from your camera must not have any gaps (see separate NRDA Field Photography Guidance).

Enter all photographs into the **NOAA NRDA Trustees Sampler Photo Logger Form**. Follow all required Chain of Custody procedures, as indicated in the data management Chain of Custody training session. Original photo files must either be left on flash cards and placed in locked in storage or uploaded to a hard drive and not opened. A copy can be made of the original, and that file may then be opened.

3. SOPs for Sediment, Vegetation and Invertebrate Chemistry

The following protocols will be followed to ensure sediment, vegetation, and invertebrate data collection is done consistently with other media sampling efforts as well as other sediment data collections that may occur opportunistically with other NRDA TWG activities. At this time, locations include inshore and offshore SAV coastal areas across the northern Gulf of Mexico from Louisiana to the Florida panhandle.

Equipment

- (2) 20' boats
- (6) trained personnel (staff recommended)
- (8) 12 hr days for sampling per boat
- (8) 8 hr days for sample prep, handling, and shipping
- (4) 150qt ice chests
- (8) 80 qt ice chests
- (6) boxes Nitrile gloves, Nomex coveralls
- (2) Eckman dredges mounted on poles
- Sample containers as described in the protocols below, i.e.:
    - 500 mL (16 oz) or 250 ml (8 oz) glass jars certified-clean to be organic-free (solvent rinsed), with Teflon-lined lids(for sediment chemistry samples)
    - 4 oz glass jars or sealable plastic bags (for grain size analysis samples)
    - Pre-cleaned aluminum foils (to make packets for various biota samples) and plastic bags
    - Sample bags (Ziploc quart or gallon size depending on coring device size)
- Laboratory grade detergent, nylon brushes, paper towel
- Sorbent pads
- Plastic sheeting
- Pre-cleaned metal spoons or spatulas
- Food/water for remote deployment of personnel
- 3 GPS units with extra batteries
- 3 digital cameras with extra batteries
- Sampling device (dredge, grab, or core)
- Disposable aluminum pan, on aluminum foil, or on other disposable, non-contaminating material (for mixing samples prior to distribution into jars, if necessary)
- Clear tape
- Chain-of-custody forms
- Sample collection forms
- Waterproof forms: Chain-of-Custody, NRDA Sample Collection Forms, PhotoLogger Forms
- Waterproof pens
- Waterproof labels

3 A. SOP for SAV Sediment Chemistry

Purpose/Objectives

- To determine the concentration and source of any oil compounds in the sediments collected.
- To measure sediment characteristics for interpreting chemical and biological results.
- To maintain the integrity the sample(s) during sampling, transport, and storage.

Methods

Target sample volume for TPH/THC and PAH analysis: two 250 ml (8 oz) glass jars filled ¾ full or one 500 ml (16 oz) jar filled ¾ full.

Target sample volume for grain size analysis: 100 g in sealed plastic bag or 4 oz jar

Samplers should use disposable surgical gloves and pre-cleaned metal spoons or spatulas.

- Sediment samples \ should be placed in glass containers, certified-clean to be organic-free (solvent rinsed), with Teflon- or aluminum foil-lined lids.
- Decontaminate all sampling gear before using and between sampling stations by washing with laboratory-grade detergent and clean water.
- For subtidal samples when SCUBA is not feasible, lower and retrieve the sampling device at a controlled speed of ~1 foot per second. Sampling devices may include dredges, grabs and cores.
- The device should contact the bottom gently; only its weight or piston mechanism should be used to penetrate the sediment. It is important to minimize disturbance to the surface floc which may contain oil contaminants.
- Inspect the sample to make sure that it meets the following criteria:
    - The sampler is not overfilled; the sediment surface is not pressed against the sampler top.
    - Overlying water is present, indicating minimal leakage.
    - Sediment surface is undisturbed, indicating lack of channeling or sample washout.
    - The desired penetration depth is achieved (e.g., 4-5 cm for a 2 cm sample).
- Siphon off the overlying water near one side of the sampler.
- Using a pre-cleaned flat spoon or spatula, accurately collect the top 2 cm, avoiding sediments in contact with the sides of the sampler. Use a new spoon or spatula for each station. Collect other intervals, per the sampling plan.
- If placing sediment in more than one jar, or if compositing samples from more than one location, the sample must be mixed before placing in the jar(s). This should be performed in a disposable aluminum pan, on aluminum foil, or on other disposable, non-contaminating material.

Labeling / Documentation / Other Considerations

- Prepare sample labels following sample ID protocol provided in the instructions from the trustee data management team.
- Affix sample ID labels to each container and cover with clear tape wrapped around the entire container circumference.
- Apply tape around lid to secure.
- Note collection of sample both in the **SAV Site Characterization Form (Appendix 4)** and in the **NRDA Sample Collection Form for Soils and Sediments**.
- Field duplicates should be clearly marked and Field duplicates are separate samples, so should be assigned a new sample number distinct from the original duplicated sample. On the sample form, use the Sample QA/QC Type column to indicate that the sample is a duplicate. The associated parent sample number can be identified in the Sample Notes column (the entire Sample ID should not be required in most situations since the location ID, matrix, and data should be the same).
- Preserve all original field notebooks, forms, and notes, which should be signed and dated. If crossing out or correcting any entries, date and initial when making the changes. Documentation is critical; original records will be gathered and kept on file by the trustees.
- Ship known oil-contaminated samples separate from non-contaminated or low contaminated samples to reduce risk of cross-contamination.
- See related NRDA protocol documents for specific sample shipping and notification/ sampling documentation instructions.

Preservation/Holding Times

- Immediately place all samples in cooler and keep at 4°C. Freeze as soon as possible.

- Please see the Analytical Quality Assurance Plan for the MS Canyon 252 (Deepwater Horizon) Natural Resource Damage Assessment (AQAP) for further details on storage and holding times.

3 B. SOP for SAV Vegetation Chemistry

Purpose/Sampling Objectives

- To determine the concentration and source of oil compounds (fingerprinting) in/on SAV samples collected.
- To document the presence or absence of oil.
- To maintain the integrity the sample(s) during sampling, transport, and storage.

Treatment of samples will be given the same consideration as those collected for sediment. Vegetation will be collected from each sampling station. Samples (blades) are to be collected by hand or clipped. This will also prevent any bottom sediments from being disturbed. Vegetation samples for hydrocarbon analysis should be collected in 8-ounce (250 mL) wide-mouth glass jars (certified clean to be organic free). The minimum target sample volume for vegetation is 30 grams (wet weight) although 50 grams is desirable. If the jars are filled approximately 3/4 full the minimum volumes are assuredly achieved. Composite a sufficient number of plants to fill the sample jars approximately 3/4 full. Visibly oiled vegetation requires less volume than unoiled (background)vegetation. Excess sediment adhered to vegetation will be physically removed or avoided. Immediately place all samples in a cooler and keep at between 2-6 degrees C.

Sampling using glass jars is preferred, however, if necessary, pre-cleaned aluminum foil and plastic Ziploc bags can be used instead of glass jars. Solvent-rinsed aluminum foil is available from [redacted]. (Use of aluminum foil that has not been solvent [Dichloromethane. PR (pesticide research) or HPLC grade] rinsed is undesirable as it contains contaminants that interfere with low level hydrocarbon analysis.)

Each vegetation sample should be photographed and the genus and species should be recorded. If oiling was observed, a marker (pointer of some sort) should be used in the photograph to indicate the observed oil.

3 C. SOP for SAV Invertebrate Chemistry

Purpose/Sampling Objectives

- To determine the concentration and source of oil compounds (fingerprinting) in/on biota collected within, or within close proximity (~ 1 m) of the SAV beds.
- To document the presence or absence of oil.
- To maintain the integrity the sample(s) during sampling, transport, and storage.

Treatment of samples will be given the same consideration as those collected for sediment. Invertebrates will be collected from the blades of SAV or within the beds and will be collected of the same sampling stations for the collection of SAV. Invertebrate samples for hydrocarbon analysis should be collected in 8-ounce (250 mL) wide-mouth glass jars (certified clean to be organic free). The minimum target sample volume for invertebrates is 30 grams (wet weight) although 50 grams is desirable. If the jars are filled approximately 3/4 full the minimum volumes will be achieved. Composite a sufficient number of individuals to fill the sample jars approximately 3/4 full. Excess sediment adhered to invertebrates should be physically removed or avoided to the degree practical. Immediately place all samples in a cooler and keep at between 2-6 degrees C.

Sampling using glass jars is preferred, however, if necessary, pre-cleaned aluminum foil and plastic Ziploc bags can be used instead of glass jars. Solvent-rinsed aluminum foil will be available from [redacted]. (Use of aluminum foil that has not been solvent [Dichloromethane. PR (pesticide research) or HPLC grade] rinsed is undesirable as it contains contaminants that interfere with low level hydrocarbon analysis.)

Each invertebrate sample should be photographed and the genus and species should be recorded. If oiling was observed, a marker (pointer of some sort) should be used in the photograph to indicate the observed oil.

Please note: If collecting small invertebrates, you will need a significant amount of bodies (especially amphipods (e.g. caprellids) and isopods to obtain the number of grams needed).

4. SOP for Submerged Aquatic Vegetation (data sheet #2) and Associated Epifauna and Infauna

Note: The following protocol can be used for both epi- and infaunal macroinvertebrates sampling events (i.e. one sample will yield data for both objectives).

Purpose/Objective

- To document the presence/absence and species composition of SAV and associated epi- and infaunal macroinvertebrates for baseline and post- Spill comparison
- To provide qualitative and quantitative estimates of SAV abundance
- To provide quantitative estimates of SAV- associated epi- and infaunal macroinvertebrates
- To provide preliminary information that is easily compared with data gathered by prior studies, and to help evaluate the need for more comprehensive studies

Sampling parameters

- SAV presence/absence & species composition
- SAV biomass and shoot density
- SAV-associated epi- and infaunal macroinvertebrate presence/absence & species composition
- SAV-associated epi- and infaunal macroinvertebrate relative abundance distributions and densities

Equipment

- Hand coring device (cylindrical, 7.6cm (3") or 15.2cm (6") inner diameter) with hole near the top for attaching a rubber stopper
- Sieve with 0.5mm mesh
- Large tub or bucket (optional)
- 10% formalin solution

Sample labeling and chain of custody forms

Follow NRDA case-wide protocols

Methods (assuming sites are accessed by vessel)

- After arriving at site, look for
    - Presence/absence of SAV bed. If no SAV patch occurs at pre-determined location but can be found within the near vicinity, move to the new patch.
- If SAV is patchy and not present as a continuous bed, take sample in the center of the patch; avoid taking samples on patch edges.

- To take a core, place cylindrical coring device on the sediment surface, making sure to the best of your ability that the leaves of the shoots within the coring area are inside the coring device and those shoots outside the coring area are outside the coring device.
- Push the coring device into the sediment to a depth of approximately 15cm. It is helpful to pre-mark or etch the outside of the corer to the appropriate depth to ensure that core does not exceed 15 cm in depth.
- Put the rubber stopper into the hole and gently rock the corer back and forth to break it loose. Pull the core up and make sure that you place your hand underneath the opening once the corer is above the sediment surface.
- Samples are preferably sieved in the field, using a 0.5 mm mesh sieve. A large bucket / tub of water may be useful, particularly in rough seas. Take care to avoid techniques that might force soft bodied animals through the sieve or splash them out of sieve.
- With the core opening over the sieve, remove the rubber stopper and allow the cored sediment to fall into the sieve. When sieving, it is best to force water up through the bottom of the sieve, by bobbing the sieve up and down in a large bucket or tub of water, thus preventing forcing animals through the sieve.
- After sieving, place remaining sediment, seagrass and animals in a sample bag or jar, using a minimum amount of water.
- Place a waterproof label with the station location, sample number, and date inside the sample bag or jar.
- Place sample bag or jar in cooler with ice.

Laboratory Protocols :

Supplies:

2 glass bowls	0.5mm sieve	Rose Bengal
Paper drying bags	Razor blades	Sharpie
Glass plate	Analytical balance (readability of 0.0001g)	
Magnifying lamp	Drying oven at 60^{o}C	Ruler
Aluminum weighing tins	Forceps	plastic sorting tray
Glass petri dish	Dissecting microscope	
Datasheet	Pencils	
25mL scintillation vial*	label tape*	

*Not needed for every sample.

Definitions *(adapted from Kuo, J. and den Hartog, C. 2006. Seagrass Morphology, Anatomy, and Ultrastructure. In: Larkum, AWD., Orth, RJ, and Duarte, CM. Seagrasses: Biology, Ecology and Conservation, pp 51-87. Springer, Netherlands.)*

Leaves: intact green portions of the plant distal from the basal leaf sheath ends.
Shoots: The shoot contains several foliage units at differing developmental stages. The stem (also known as the vertical rhizome), if present, is part of the rhizome structure. These structures connect the leaves to the belowground plant structures
Roots and rhizomes: Belowground plant structures
Single leaf: a solitary blade not attached to a stem or rhizome

GENERAL CORE PROTOCOL:
Samples are kept in the locked COC freezer.
Remove only samples to be processed that day and document them on the COC sheet.
Samples are to be thawed in their collection bag.
Once thawed, samples are to be rinsed onto a 0.5 mm sieve.
The SAV should be put into a glass bowl containing tap water while remaining sediment/detritus from the 0.5 mm sieve is placed into another glass bowl.
The information from the sample tag should be copied to the datasheet and the original tag left with the sediment/detritus portion of the sample until it is processed. The sediment/detritus portion of the sample will be sorted for infauna and SAV seeds, fruit, fruiting bodies, spathes, bracks, flowers, and other fruiting parts, and processing should occur immediately following the SAV processing.

SAV:
Four (4) paper bags are required per SAV species in each core. Bags are for: leaves, shoots, roots & rhizomes, and single leaves. Label each bag using a sharpie with the site information, collection date and SAV tissues contained in the bag (e.g., Hal – leaves; Hal – single leaves). For shoots and roots & rhizomes, rinse any sediment from these tissues. Blot all tissues dry BEFORE putting them in the bag- they dry better if they don't go in the bag soaking wet.

Place each group of bags upright and open into the drying oven at 60^{o}C. Dry samples for at least three (3) days. When retrieving the paper bags from the oven, take care not to let any of the contents fall out. Weigh each tissue sample on the analytical balance (readability of balance is 0.0001g) by removing the tissue from the paper bag and placing in a tared plastic weight boat. Be sure that the bag is empty. Record the weight onto the datasheet and place the dried tissues back into the paper bag. Fold the top of the bag over three times and place samples aside for dry storage.

For the first sample done in each location (e.g., of locations: Chandeleur Island, Horn Island, Big Lagoon) some initial additional work is needed. Epiphytes are scraped from the leaves of intact shoots on a glass plate only using a non-rusted razor blade and put into a LABELED, PRE-WEIGHED 25ml scintillation vial. Scintillation vials should be weighed on the analytical balance (readability of balance is 0.0001g). Use label tape to mark the vials and weigh the vials with the tape already in place and the caps off. Using the razor blade to scoop them up, place the epiphytes into the labeled, preweighed scintillation vial. Use DI water to rinse remaining epiphytes from the blade if needed, adding only a minimal amount of water to the vial. Vials are then placed into the 60^{o}C drying oven with the cap cracked open to allow the sample to dry (if the cap is on too tight the sample will not dry). Keep the vials upright so as not to spill the contents. Once the epiphyte sample is dry, record final weight of vial on datasheet.

All other samples in that location set will not be scraped, unless a new species is encountered. If a new species is found, only epiphytes from that species need to be scraped and saved (e.g., the first sample from Chandeleurs has Thalassia only- scrape leaves from shoots in the sample, saving the epiphytes; second sample from Chandeleurs has Thalassia only- do not scrape anything- just count, measure and separate tissues; third sample from the Chandeleurs has Thalassia and Halodule- only scrape the Halodule).

In any sample, only scrape leaves that are from shoots- DO NOT scrape single leaves.

Single leaves are blotted dry and placed into their respective bag. (**Be sure the single leaves are not detritus- they should still have a green color to them).

If the sample was taken post- Spill, record the presence/absence of oil during the sample processing (see datasheet).

From each core record:
-Shoot Density:
- # of shoots
- # of shoots without leaves, if present in sample (i.e., stem/vertical rhizome and/or basal leaf sheath with no leaves attached)
- # of leaves per shoot from 10 random shoots

-Canopy Height: Measure in millimeters the longest leaf from the cut point (see below for species specific instructions) from 10 random shoots. The longest leaf may or may not be the oldest.

Species specific directions:

Thalassia

Cut the leaves at the white/green point of the oldest leaf, discarding the sheath

Syringodium

Cut the leaves at the white/green point of the leaf. It can also be cut at the place where two leaves fork – this may not be the green/white area.

Halodule

Cut the leaves at the white/green point of the leaf. These leaves are more delicate than those of *Thalassia* so care is required when scraping them.

Halophila

These leaves are very delicate and will tear easily.

Ruppia .

These blades are delicate and fork frequently.

SEEDS, OTHER REPRODUCTIVE STRUCTURES, AND INFAUNA:

Take the sediment/detritus retained on the 0.5 mm sieve, place in the glass bowl and stain with Rose Bengal for approximately 30 minutes (staining should be done while the SAV portion of the core is being processed).

Place small portions of the sample into a sorting tray, pick out all organisms and reproductive structures including seeds under a magnifying light and put into a glass petri dish.
Under the dissecting microscope, separate, identify to the lowest practical unit and count all organisms present (e.g. as polychaetes, bivalves, etc).
Place each group into a labeled, pre-weighed aluminum tin (see datasheet).
Place tins in a 60^{o}C drying oven for 36-48 hours and reweigh the tin, recording weights onto the datasheet.
Fold tin into a closed "taco" and put into a muffle furnace for 4 hours at 500^{o}C for calculation of ash free dry weight (AFDW).
For any reproductive structures including seeds found in the sample, identify, count and place in a labeled 7mL scintillation vial for storage.

5. SOP for Submerged Aquatic Vegetation Associated Fauna (fish and mobile macroinvertebrates) (Data sheet # 3)

Purpose/Objective

- To document the presence/absence and species composition of SAV-associated faunal community for baseline and post- Spill comparison
- To provide quantitative estimates of SAV- associated fauna.
- To provide preliminary information that is easily compared with prior studies, and to help evaluate the need for more comprehensive studies

Sampling parameters

- SAV-associated fauna presence/absence & composition
- SAV-associated fauna abundance or density

Equipment

- Watch
- 16' Otter Trawl (3/4" mesh wings, and ¼" liner)
- Work gloves
- Sieve with 1 mm mesh (optional)
- Large tub or bucket (2-3 are sufficient)
- Sample bags (gallon size)

Sample labeling and chain of custody forms

Follow NRDA case-wide protocols

Methods (assuming sites are accessed by vessel)

- After arriving at site, look for presence/absence of SAV bed. If no SAV patch occurs at pre-determined location but can be found within the near vicinity, move to the new patch.
- Collect physical data (water depth (cm or m), salinity (ppt), temperature (^{o}C), and time).
- With the vessel moving in a tight circle around the starting seagrass patch or bed, toss out the net portion of the otter trawl. Once the net is fully deployed, straighten out the boat and place the doors of the trawl in the water. Be sure that the doors and the lines attaching the trawl to the boat do not cross.
- Once the doors are deployed, record starting coordinates (lat/long in decimal degrees, WGS84), for the sampling site. Ensure that GPS is giving accuracy < 5meters. Record the start time for the trawl.
- Begin trawling. Trawl times are 2 minutes and if the SAV is not a continuous bed, but instead patchy, steer the vessel so that you cover as many patches as possible within the 2 minutes. Vessel speed should be relatively slow (approximately 2-3 knots) and

- consistent. Record RPM of vessel. **Note: our trawling speed is usually around 1600 RPM to 2500 RPM.
- Once 2 minutes has passed, stop the vessel and begin pulling in the trawl. Once the trawl doors are at the boat, record the ending coordinates (lat/long in decimal degrees, WGS84), for the sampling site. Ensure that GPS is giving accuracy < 5meters.
- With the trawl doors and lead and float lines of the trawl in the vessel, begin to "shake" down any animals in the trawl net as you pull it into the boat. Release the trawl over a large tub or bucket.
- Sort the "catch", identify and enumerate to species level (common name).
- Place any fauna to be retained (e.g. commercially important fish and invertebrates) into foil and label.
- Place a waterproof label with the station location, sample number, and date inside the sample bag or jar.
- Place sample bag or jar in cooler with ice.

6. SOP for the rapid assessment of the status of seagrass beds that warrant further evaluation .

Summary

This document describes the phase 1 of an SOP for Task 2 of the SAV Tier 2 Work plan. This plan primarily addresses an assessment of the status of the marine seagrasses in the northern Gulf of Mexico potentially impacted by the Spill. The plan will be implemented in a phased series of sampling events and evaluation steps that will examine the health and growth condition of seagrasses at five sites (see locations below) potentially exposed to different levels of oiling at different spatial scales.

The initial assessment of these sites is designed to address the pathway of potential exposure where surface oil sheens passed across the deeper seagrass beds into shallow water reaching the upper limits of seagrass distribution in the shallow subtidal. During this process oil, weathered oil, derivatives of oil and dispersants may have been mixed into the water column and settled into the seagrass bed and on the seagrass plants and associated sediments. The SAV TWG will proceed on the assumption that, of seagrass beds in areas that warrant further evaluation , the seagrass beds in very shallow water (< 1.0m – intertidal) have had the highest probability of exposure to oil. The scale of exposure may have included the seagrass canopy in the water column and the sediments beneath and around the canopy. Additionally, weathered oil and beached oil retained in these nearshore locations may still be impacting the shallow seagrass beds. This SOP will also complement work occurring with the SOP for the collection of analytical samples.

The initial sampling event will consist of a rapid visual assessment of the health and condition of the seagrass beds along shore normal transects and at discrete sample points identified by association with surface oil trajectories, SCAT data and NRDA shoreline data. Condition of the seagrass beds includes an assessment of seagrass species composition, presence/absence of healthy growing apical meristem, new shoot growth, and new rhizome and root growth. As per the consensus of the SAV TWG, five sites with known presence of seagrass, Tier 1 data, and evidence of surface and shoreline oiling have been selected as candidates for rapid assessment (See appendix 1). The selection of these sites is based on criteria identified in subsection 2.1 of the SAV Tier 2 work plan including historical information about seagrass distribution, known surface oil trajectory data, shoreline scat data, NRDA shoreline data, direct observations of oiling using snare sentinels, and Tier 1 data. The four locations are:

1. Chandeleur Islands, La. (surface sheen and light, moderate, heavy oiling, no oiling)
2. Horn Island Miss. (surface sheen, light to moderate oiling, no oiling)
3. Petit Bois Island, Miss. (surface sheen, light to moderate oiling, no oiling)
4. Robinson Island inside Perdido Pass, Al. (surface sheen, light to moderate oiling, no oiling)
5. Big Lagoon, Fl. (light to moderate oiling, surface sheen)

Based on the observations and results of this first phase of rapid assessments the SAV TWG will design and implement a second phase of assessment to assess injury to the seagrass beds at specific sites. This second phase will include amplification of Tier 1 core data metrics and additional replicate sampling of metrics for seagrass abundance, growth and mortality and will evaluate the health and condition of the seagrass against baseline conditions for Tier 1 data and reference seagrass beds of similar species composition and distribution in the Gulf of Mexico not exposed to oiling.

Investigative and Sampling Approach (Phase 1)

Selection of sample sites

Sample locations for conducting rapid assessments were determined by spatially associating data from the record of surface oil trajectories, shoreline oiling (SCAT, NRDA and direct observations), location of Tier 1 sampling stations, and available data on seagrass distribution (see sample sites section below). Data were compiled and projected in ARC GIS layers to assign sample points. The Chandeleur Islands were stratified into three general categories of associated oiling condition (heavy, light to moderate, and no oiling) based on the SCAT classification of the adjacent shoreline. Each strata was further divided into ten sampling transects oriented approximately perpendicular to shore along the north-south axis of the island chain and identified by two waypoints at each end of the transect. One waypoint was positioned at the offshore end and the second waypoint at the inshore end. Horn and Petit Bois Islands were assigned 29 sample points nearly uniformly distributed along the northern shoreline and overlapping with all of the Tier 1 sampling stations. The Robinson Island site has 10 stations divided among three geographically associated islands just inside Perdido Pass and overlapping with Tier 1 stations. Big Lagoon has 17 stations nearly uniformly distributed along both the northern and southern shorelines in the eastern end of the lagoon.

Rapid assessment methods

At each sampling station we will record the general conditions of the site including habitat setting, water depth, seagrass species present, bed size and form, and the overall condition of the bed (see field data sheet section below). The physical characteristics of the sampling site will be described with regard to the substrate type and the spatial association of oiled conditions and seagrass bed distribution. We will characterize the water and the sediments in and around the sample site to determine if there is visible presence of oil and if there is oil contacting the seagrass plants and the sediments they are growing in. We will also note whether oil or products of weathered oil are present in the vicinity of the sample site.

The general condition of the seagrass plants at the sample site will be characterized by visually determining if there is oil on seagrass plants and sediments beneath the seagrass plants (see field data sheet below). We will use a checklist of plant health descriptors to document the visible condition of the seagrass leaves and shoots with respect to potential Spill-related oiling according to the condition of the leaves. Subsamples of belowground tissue (rhizomes and roots) will be examined to assess the presence of new shoots, new rhizome and new root growth on

new rhizomes as well as the visible health and condition of rhizome apical meristems. Where possible, estimates of seagrass cover and shoot density will be obtained using a PVC quadrat (either 0.25 m^2 and/or 0.04 m^2) placed on the sediment surface.

Depending on water visibility we will photo document the in situ condition of the seagrass beds and the sediments at each sample site. Where feasible, plant and sediment conditions will be photo documented separately using subsamples obtained at the sample site.

Subsamples of sediment, biological tissue and water (analytical samples) will be taken by Newfields staff. Sample selection will be determined based on the site inspection and consultation between Newfields and NOAA field staff.

Sampling schedule

The sample schedule, for 2010, is designed to prioritize areas in the vicinity of heaviest documented shoreline oiling. The initial sampling event will occur between August 23 and September 2 in two trips utilizing a live aboard vessel provided by NRDA. The first trip will sample Horn and Petit Bois Islands on August 24, 25 and 26. Pending completion of sampling at this site, sample intake can begin as early as August 26 and be completed on August 27. The second trip will sample the Chandeleur Islands between August 29 and Sept. 1 with sample intake on the evening of Sept 1 and the morning of Sept. 2.

The second sampling event will take place in September at Robinson Island, Al and Big Lagoon, FL. Alternatively, if weather or any other unexpected interruptions prevent us from sampling at Horn, Petit Bois and Chandeleur Islands during the first sampling event in August we will coordinate with the SAV TWG and NERDA staff to rearrange the vessel schedule to sample at Robinson Island and Big Lagoon between August 24 and Sept. 1.

Costs

Salary, travel and equipment costs for staff from the Center for Coastal Fisheries and Habitat Research, NCCOS, NOS, NOAA for implementing this plan are covered by a BOP from NOAAs Office of Response and Restoration to the Center for Coastal Fisheries and Habitat Research to conduct sampling and assessments for the SAV Tier 1 and Tier 2 Workgroup Plan under NRDA. Additional costs to be incurred by NRDA would include: 1) vessel support to access the sample sites, 2) the costs associated with data intake, 3) sample statistical design analytical support provided by Newfields, and 4) salary costs incurred by TWG lead coordination.

7. SOP for PEMDs

Introduction

Passive accumulation devices (PADs) are typically hydrophilic membranes with or without hydrophilic reservoirs and they are designed to sample non-polar hydrophobic hydrocarbons, including polynuclear aromatic hydrocarbons (PAH) and persistent organic pollutants (POPs) from air, water, and sediment. A commercially available PAD commonly available in the USA is the semi-permeable membrane device (SPMD); its central reservoir is triolein (e.g., Huckins et al. 1990). Hydrocarbons in SPMDs diffuse through pores in the membranes and are trapped in the central triolein matrix, mimicking uptake by living organisms; additional hydrocarbons are retained by the membrane. Advantages of passive sampling are that they can sample large volumes of water, amplify trace hydrocarbon quantities (part-per-billion or part-per-trillion) to detectable levels, and average the signal over time. In addition, they are cheaper and easier to analyze than biological tissue and can be deployed over a greater range of environmental conditions.

At low ambient hydrocarbon concentrations, low-density polyethylene membrane devices (PEMDs) deployed without inclusion of the central hydrocarbon reservoir are simpler and less expensive sampling devices than SPMDs, yet provide the same benefits (Carls et al. 2004). Loss of accumulated PAH is slow, thus PEMDs reliably capture sporadic or fluctuating events. Composition of PAH accumulated by PEMDs can be used to identify hydrocarbon sources in situations not complicated by multiple sources. At the Auke Bay Laboratory, we also refer to PEMDs as LDPEs (low density polyethylene devices) or PMDs (polyethylene membrane devices). A universal moniker has not yet been established in the literature.

Laboratory preparation

Low-density polyethylene tubing (98 µm × 4.9 cm × 50 cm) is sonicated twice in pentane to remove hydrocarbons, placed in aluminum samplers (11.5 diameter × 6.6 cm with perforated endplates, 3 mm holes spaced 4.8 mm apart, precleaned in dichloromethane), wrapped with two layers of aluminum foil, heat-sealed in two plastic bags, and frozen until shipment (Fig. 1; Carls et al. 2004).

Fig. 1. Example PEMD wrapped in foil and placed in ziplock bags. This example was wrapped with a single layer of foil which has torn, illustrating the need to be careful and double wrap each canister.

Caution

These devices are incredibly sensitive!!! Be careful!!!

PEMDs sample both air and water and tiny unwanted quantities can swamp the target signal! For example, a venting gas tank in a skiff is very bad. (Solution – do not fill the tanks full!) The person who deploys or retrieves the PEMDs should NOT be the same one running the engine, handling the trailer, or fiddling with gas!!! Petroleum products have a way of migrating from hands/clothing to the PEMDs. Open the devices only when ready to deploy without delay; wrap and bag them without delay upon retrieval. Practice your moves and carefully arrange your tools ahead of time to minimize time and the possibility of contamination. Wear disposable gloves and change them between every set or retrieval. If available, an assistant (also with clean gloves) can open or close pails/bags. Do not allow devices to come in contact with clothing, the boat etc; it should go straight from bag to water and vice versa. Design your fastening system to work with minimal effort – rusty shackles that require wrenches add time and increase the odds of unwanted contamination. Any necessary tools should be cleaned ahead of time; this may include the boat. Oil or gas weeping from a boat will ruin samples!

Deployment & Retrieval

The general strategy is to determine where the devices are to be located, place suitable anchors for them, then deploy. All hardware must be very clean; hydrocarbons that leach from anchor cable, for example, will become the sample, compromising the study. "Very clean" typically means solvent-washed in the laboratory and transported appropriately (e.g., in ziplock bags) so that hydrocarbons are not accumulated along the way. Anchors, other hardware, floats, and rope can be reused repeatedly without further cleaning assuming that it is not contaminated during exchanges. New rope (nylon, polypropylene) does not cause contamination. Do not use old rope that has an unknown history or rope that has been exposed to bilge water, etc. Rope should be placed in suitable bags (e.g., garbage or ziplock bags) when transported to avoid contamination! The same is true for transportation of every other item and tool.

Clean bags are acceptable storage for PEMDs, tools, & gear. Start with new clean ziplock and garbage bags and keep them clean in other bags & in pails.

Five gallon pails are an excellent for transportation and collection. Buy new ones and keep the insides clean! Stacking pails with hydrocarbons stuck to the outside will contaminate pail insides! (Buckets can be stacked if lined with clean garbage bags.) Screw-top lids are very useful in this context and a variety of colors is recommended so buckets are easily distinguished (see Table 1 for example bucket contents). Two or three half-gallon plastic drinking containers placed inside can provide structure for pails used for tools and small gear (Fig. 2). Place bagged PEMDs inside garbage bags inside pails. When open, place lids

Fig. 2. Example equipment bucket with internal structure.

upside down (inside showing) on the ground or other surfaces to avoid transferring potential external contamination into buckets. Keep buckets closed as much as possible.

Deployment. Do not open a PEMD until you are ready to place it. Put on clean disposable gloves. Tear through the two heat-sealed ziplock bags and remove the aluminum foil. A second person to manage the waste can be helpful, particularly under windy conditions. Fasten the PEMD onto its anchoring device and back/drift away. (Do not contaminate newly installed devices by standing upstream of them, running boat exhaust by them etc.)

Retrieval. Retrieval is essentially the reverse of deployment and should occur first when swapping. 'Collection kits' are needed; these are simply pre-cut aluminum foil sheets placed in ziplock bags. Arrange collection gear to optimize efficiency and minimize time; put on clean disposable gloves. Retrieve the PEMD; this may require tools, such as wire cutters, wrenches, etc. Be sure to swirl sediment out of the canister with the water it has been in. Fold the shackle to the canister and place the PEMD at the center of an aluminum sheet; fold to cover completely. Repeat with a second sheet; starting at the opposite side. (A helper make this process easier, particularly in wind. Avoid contact with clothing!) Then place the PEMD in a ziplock bag; close (with as little air as practical). Place this in a second ziplock bag. Add a label and seal. Put this package in a garbage bag inside a bucket. Depending on time and sampling circumstances, you might wish to bundle groups of PEMDs in separate garbage bags, tied shut at intervals to minimize any possibility of contamination. Freeze as soon as possible.

Blanks. Site blanks are a necessary quality assurance technique. Open one container per site / trip, depending upon agreed-upon design. Expose to air about 1 minute, then re-bag, label, and freeze as above.

Labels & record keeping. Make labels out of paper; "Rite in the rain" all weather paper is nice. Pre-printed labels with a minimum of necessary identification information are nice; keep them clean in a small ziplock bag. Each label should have a sample number; use a pencil to write the information. Place labels outside of the inner PEMD bag and inside the second bag. A complete record of collection, including location and time should be kept in a separate notebook. In addition, complete chain of custody forms; these are required by the Auke Bay Laboratory for record keeping and processing (Table 2).

Deployment

Place anchors on polypropylene rope in desired depth of water. Consider using chain on Danforth anchors, then rope (Fig. 3). Alternatively, fasten the rope to the anchor and place a lead cannonball weight about 10' up the rope: this should force the anchor to dig. When tying rope to anchors, be sure to smooth sharp edges on the anchor if present – or use a shackle. Make eye splices with protective sleeves if possible, or reweave rope ends back into the rope several times after knotting (e.g., bowlin or rewoven figure eight). Use 3/8" line or larger. Keep float sizes small to reduce lift on the anchors – but large enough to float the hardware and big enough that you can find them. Bullet or seine floats are ok. Assuming the anchors (& other bottom hardware) are reasonably clean (consider a detergent wash) and that there are several meters between the

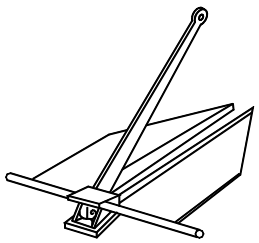


Fig. 3. Danforth anchor.

anchor and buoy, solvent cleaning isn't needed. Allow enough line for tidal fluctuation, extra slack, etc. Consider weighting partway down so it sinks to prevent navigation hazard.

Gear has a better chance of surviving if placed outside the surf zone and on a broad flat shelf. Lines will eventually part if there is too much wave energy, so inspect them periodically for wear. Anchors have a habit of moving around even if they initially appear to be well set; if they slide too far down a slope the buoys will be pulled under and the gear will be lost.

Deploy/retrieve sequence: set anchor, mark position with GPS. Shut engine off!!! Swing on the anchor until fumes clear from the air. No smoking; it will contaminate the sampler. Only then, deploy (or retrieve) the PEMD.

Fig. 4. Anchor line looped around PEMD shackle

Subsurface deployment. PEMDs deployed below the surface are designed to assess average hydrocarbon concentrations in various water layers. We typically deploy 1 m below the surface to characterize this layer. The most efficient method is to pass a loop of anchor rope through the shackle on the PEMD and around the PEMD, then cinch it tight (Fig. 4). In our experience, the PEMD will stay put (not sink) on 3/8" polypropylene rope and 3/8" may be the largest rope diameter possible for this method. To preclude sinking, consider hanging the PEMD from a loop attached directly to the buoy; the disadvantage is that this can snarl. Another way to stop potential sinking is to place a knot in the anchor rope below the device loop. For line larger than 3/8" a second, larger shackle will be necessary (precleaned, of course). Obviously, a knot (e.g., butterfly) can be placed in the anchor line at the appropriate depth (e.g., 1 m below the buoy) and the PEMD can be shackled to it. Shackles can add time at retrieval and are difficult when rusty. One solution is to place a rope loop between both shackles, again passed around the PEMD. (An effective knot for making loops is the double fisherman's bend; Fig. 5). Shackles could be potentially linked together with zip ties, but this has not yet been tried with this type of deployment and is not recommended.

Surface deployment. PEMDs deployed at the surface are designed to sample the surface microlayer. Well known is the propensity for hydrophobic compounds to accumulate in this layer, increasing the probability of detecting very low

hydrocarbon levels. A technique we previously used was to pass a threaded shaft through the center of the PEMD canister; floats were placed at each end of the shaft and the PEMD shackle was tied to the anchor rope. A major disadvantage with this system was that field assembly was necessary and slow. A suggested modification is to revise the sequence; place the two floats near the center of the shaft with a gap for a shackle; this will connect to the anchor rope (Fig 6). Place a dummy (or replicate) PEMD at one end of the shaft; place another PEMD at the other end. These will be held in place with nylon-bushed nuts to prevent unthreading. Two nuts at each end are advisable and an ancillary rope or cable from the PEMD shackle to the anchor line is possible.

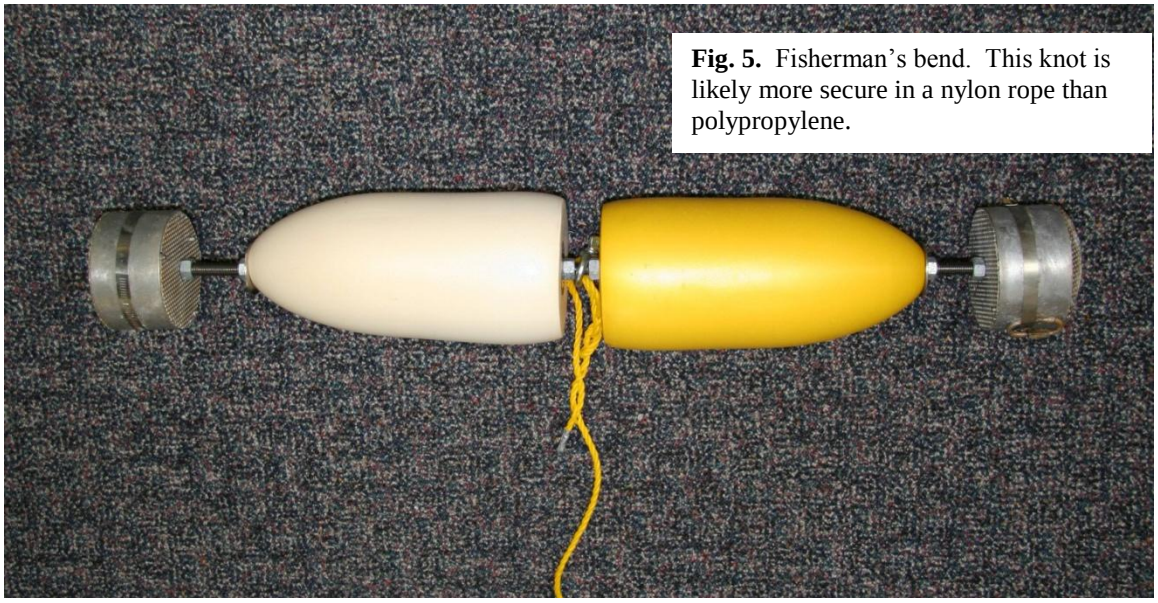
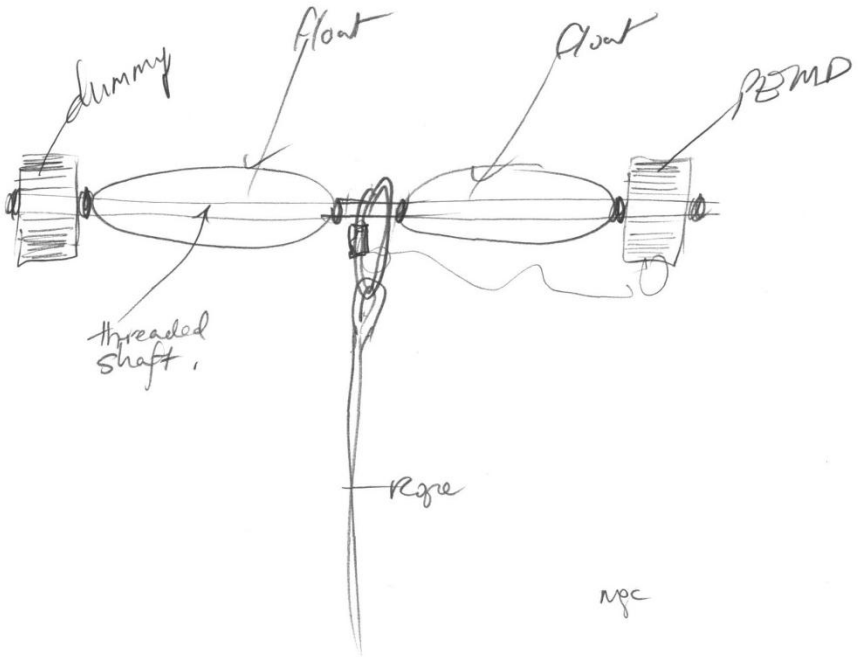


Fig. 5. Fisherman's bend. This knot is likely more secure in a nylon rope than polypropylene.

Fig. 6. Configuration for surface sampling. PEMDs are fastened on the ends of the shaft with nylon nuts.



Shipping

PEMDs in buckets and bagged as outlined above can be shipped via Alaska Air Freight. Indicate that the buckets are to be kept frozen. General delivery is ok under these conditions. If freezers aren't working or are not available, then ship priority (or Gold Streak).

Laboratory Analysis

PEMDs are extracted with organic solvent after wiping to remove gross surface contamination. Membranes are placed in centrifuge tubes and spiked with six deuterated PAH standards (Carls et al. 2004). Spike solvent (hexane) is allowed to evaporate, then the tubes are placed in a sonic bath, and extracted in 80:20 ml pentane/dichloromethane for 130 min. The sonicator is on for the first 20 min of each 50 min period. The PEMDs are rinsed with pentane as they were removed without delay after the final sonication. Extracts are concentrated to 20 to 30 ml, dried with 2 to 4 g of sodium sulfate, concentrated to 1 to 2 ml in hexane, and passed through 1.5 g silica gel columns. All extracts were spiked with an internal standard (hexamethyl benzene) and frozen pending analysis.

Extracts are analyzed by gas chromatography equipped with a mass selective detector and PAH concentrations are determined by the internal standard method (Short et al. 1996). Experimentally determined method detection limits (MDL) are generally 0.18 to 3.94 ng/g in PEMDs. The accuracy of the hydrocarbon analyses is typically about ∀ 15% based on comparison with National Institute of Standards and Technology values, and precision expressed as coefficient of variation is usually less than about 20%, depending on the PAH. Samples with questionable internal standard recoveries (< 25% or > 150%) are typically excluded from analyses unless the results can be independently corroborated by other data.

References

Carls, M.G., L.G. Holland, J.W. Short, R. A. Heintz, and S. D. Rice. 2004. Monitoring polynuclear aromatic hydrocarbons in aqueous environments with passive low-density polyethylene membrane devices. Environ Toxicol Chem 23:1416-1424.

Huckins JN, Tubergen MW, Manuweera GK. 1990. Semipermeable membrane devices containing model lipid: a new approach to monitoring the bioavailability of lipophilic contaminants and estimating their bioconcentration potential. *Chemosphere* 20:533-552.

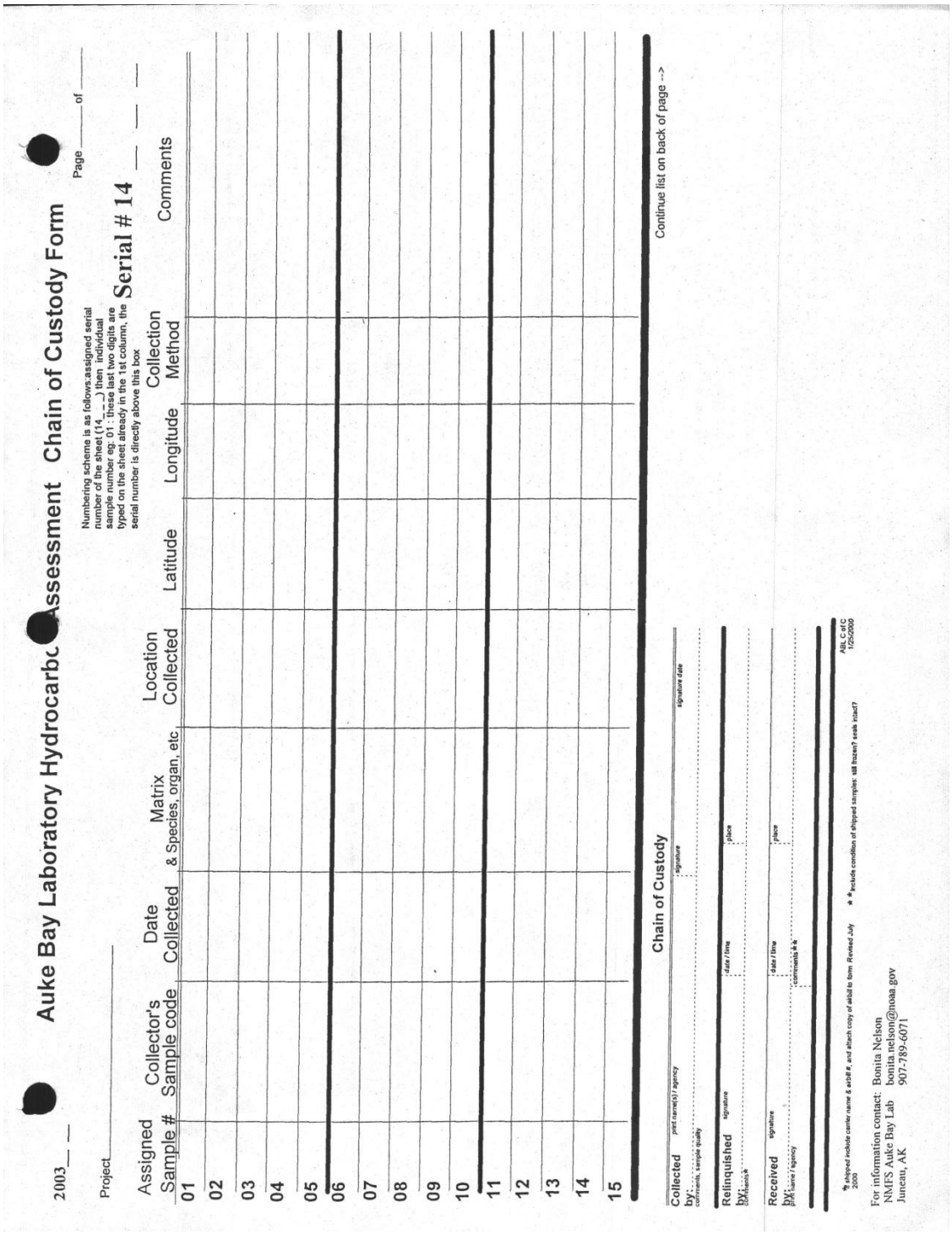
Short JW, Jackson TJ, Larsen ML, Wade TL. 1996. Analytical methods used for the analysis of hydrocarbons in crude oil, tissues, sediments, and seawater collected for the natural resources damage assessment of the *Exxon Valdez* oil spill. Am Fish Soc Symp 18:140-148.

Table 1. Example equipment and supply lists, arranged by bucket. Buckets with colored screw-top lids are one convenient way to transport gear and retrieve PEMDs. A four-bucket system is used in this example, each dedicated to a specific purpose. On a first trip, an installation bucket with appropriate gear might be substituted for the pickup kit.

Yellow	Red
Pickup kit	**Equipment kit**
field notebook	YSI salinity, temperature meter
labels	Sonar (handheld)
Aluminum foil	GPS
Disposable gloves	VHF radio
ziplock bags	pens, pencils, markers
garbage bags, large, black	knife
garbage bags, small, white	wrench(es)
	nut driver
	cutters
Blue	wire cutters
PEMD deployment kit	screw drivers
New PEMDs	spare batteries
	marlinspike or fid or plastic stake

White
PEMD retrieval kit
garbage bags
retrieved PEMDs

Table 2. Example chain of custody form required by the Auke Bay Laboratory as a data record and for analytical processing.



Auke Bay Laboratory Hydrocarbon Assessment Chain of Custody Form

2003__ __ Page ____ of ____

Project _____

Numbering scheme is as follows:assigned serial number of the sheet (14___) then individual sample number eg: 01. These last two digits are typed on the sheet already in the 1st column, the serial number is directly above this box

Serial # 14 ___ ___ ___

Assigned Sample #	Collector's Sample code	Date Collected	Matrix & Species, organ, etc	Location Collected	Latitude	Longitude	Collection Method	Comments
01								
02								
03								
04								
05								
06								
07								
08								
09								
10								
11								
12								
13								
14								
15								

Continue list on back of page -->

Chain of Custody

Collected by: print name(s) / agency / signature / signature date
(affiliation, sample quality)

Relinquished by: signature / date / time / place
(affiliation)

Received by: signature / date / time / place
print name / agency / comments #'s

*If shipped include carrier name & airbill #, and attach copy of airbill to form. Revised July 2000

** Include condition of shipped samples: still frozen? seals intact?

ABL C-41C 1/25/2000

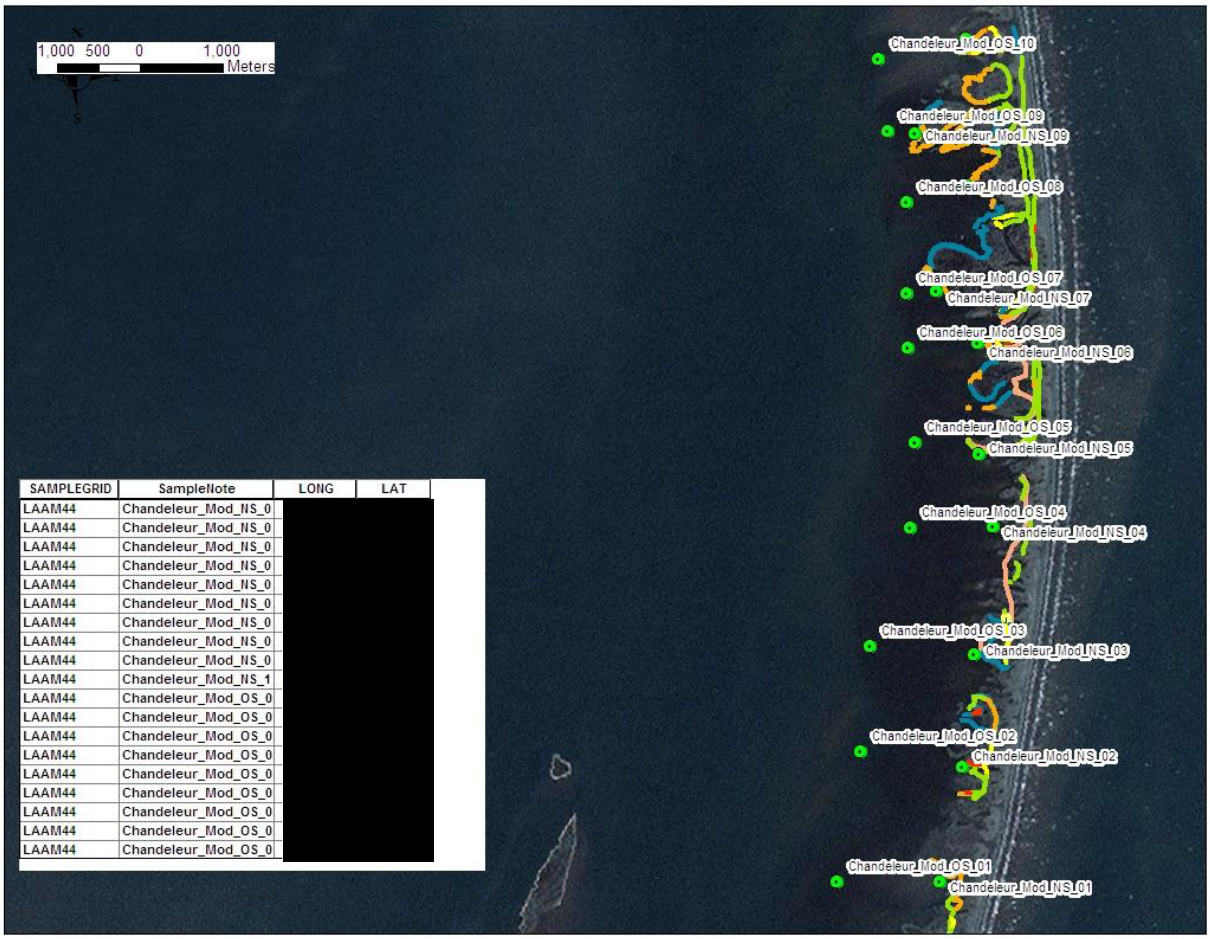
For information contact: Bonita Nelson
NMFS Auke Bay Lab bonita.nelson@noaa.gov
Juneau, AK 907-789-6071

Appendix 3.

Sampling Sites and Locations for Tier 2

SAMPLEGRID	SAMPLENOTE	LONG	LAT
LAAN44	Chandeleur_Heavy_NS_01	[redacted]	
LAAN44	Chandeleur_Heavy_NS_02		
LAAN44	Chandeleur_Heavy_NS_03		
LAAN44	Chandeleur_Heavy_NS_04		
LAAN44	Chandeleur_Heavy_NS_05		
LAAN44	Chandeleur_Heavy_NS_06		
LAAN44	Chandeleur_Heavy_NS_07		
LAAN44	Chandeleur_Heavy_NS_08		
LAAN44	Chandeleur_Heavy_NS_09		
LAAN44	Chandeleur_Heavy_NS_10		
LAAN44	Chandeleur_Heavy_OS_01		
LAAN44	Chandeleur_Heavy_OS_02		
LAAN44	Chandeleur_Heavy_OS_03		
LAAN44	Chandeleur_Heavy_OS_04		
LAAN44	Chandeleur_Heavy_OS_05		
LAAN44	Chandeleur_Heavy_OS_06		
LAAN44	Chandeleur_Heavy_OS_07		
LAAN44	Chandeleur_Heavy_OS_08		
LAAN44	Chandeleur_Heavy_OS_09		
LAAN44	Chandeleur_Heavy_OS_10		

Figure1. Tier 2 sampling sites for south section Chandeleur Islands, Louisiana. Colored lines represent level of oiling on adjacent shorelines, Red = Heavy, Yellow = Light, Blue = no oiling.



SAMPLEGRID	SampleNote	LONG	LAT
LAAM44	Chandeleur_Mod_NS_0	[redacted]	
LAAM44	Chandeleur_Mod_NS_0		
LAAM44	Chandeleur_Mod_NS_0		
LAAM44	Chandeleur_Mod_NS_0		
LAAM44	Chandeleur_Mod_NS_0		
LAAM44	Chandeleur_Mod_NS_0		
LAAM44	Chandeleur_Mod_NS_0		
LAAM44	Chandeleur_Mod_NS_0		
LAAM44	Chandeleur_Mod_NS_0		
LAAM44	Chandeleur_Mod_NS_1		
LAAM44	Chandeleur_Mod_OS_0		
LAAM44	Chandeleur_Mod_OS_0		
LAAM44	Chandeleur_Mod_OS_0		
LAAM44	Chandeleur_Mod_OS_0		
LAAM44	Chandeleur_Mod_OS_0		
LAAM44	Chandeleur_Mod_OS_0		
LAAM44	Chandeleur_Mod_OS_0		
LAAM44	Chandeleur_Mod_OS_0		
LAAM44	Chandeleur_Mod_OS_0		

Figure 2. Tier 2 sampling sites for central section Chandeleur Islands, Louisiana. Colored lines represent level of oiling on adjacent shorelines, Red = Heavy, Yellow = Light, Blue = no oiling.

SAMPLEGRID	SampleNote	LONG	LAT
LAAM44	Chandeleur_NOO_NS_01	[redacted]	
LAAM44	Chandeleur_NOO_NS_02		
LAAM44	Chandeleur_NOO_NS_03		
LAAM44	Chandeleur_NOO_NS_04		
LAAM44	Chandeleur_NOO_NS_05		
LAAM44	Chandeleur_NOO_NS_06		
LAAM44	Chandeleur_NOO_NS_07		
LAAM44	Chandeleur_NOO_NS_08		
LAAL44	Chandeleur_NOO_NS_09		
LAAL44	Chandeleur_NOO_NS_10		
LAAM44	Chandeleur_NOO_OS_01		
LAAM44	Chandeleur_NOO_OS_02		
LAAM44	Chandeleur_NOO_OS_03		
LAAM44	Chandeleur_NOO_OS_04		
LAAM44	Chandeleur_NOO_OS_05		
LAAM44	Chandeleur_NOO_OS_06		
LAAM44	Chandeleur_NOO_OS_07		
LAAM44	Chandeleur_NOO_OS_08		
LAAL44	Chandeleur_NOO_OS_09		
LAAL44	Chandeleur_NOO_OS_10		

Figure 3. Tier 2 sampling sites for northern section Chandeleur Islands, Louisiana. Colored lines represent level of oiling on adjacent shorelines, Red = Heavy, Yellow = Light, Blue = no oiling.

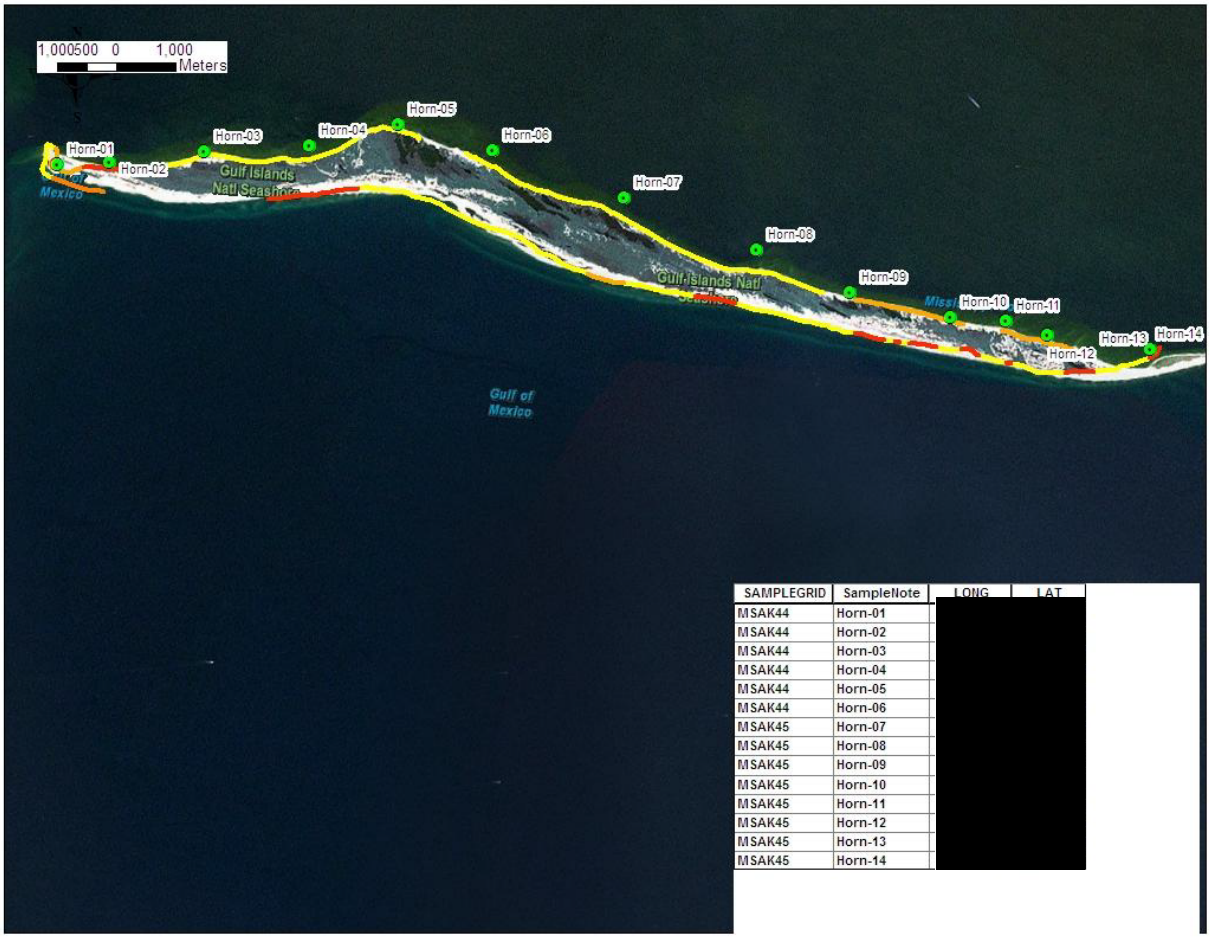
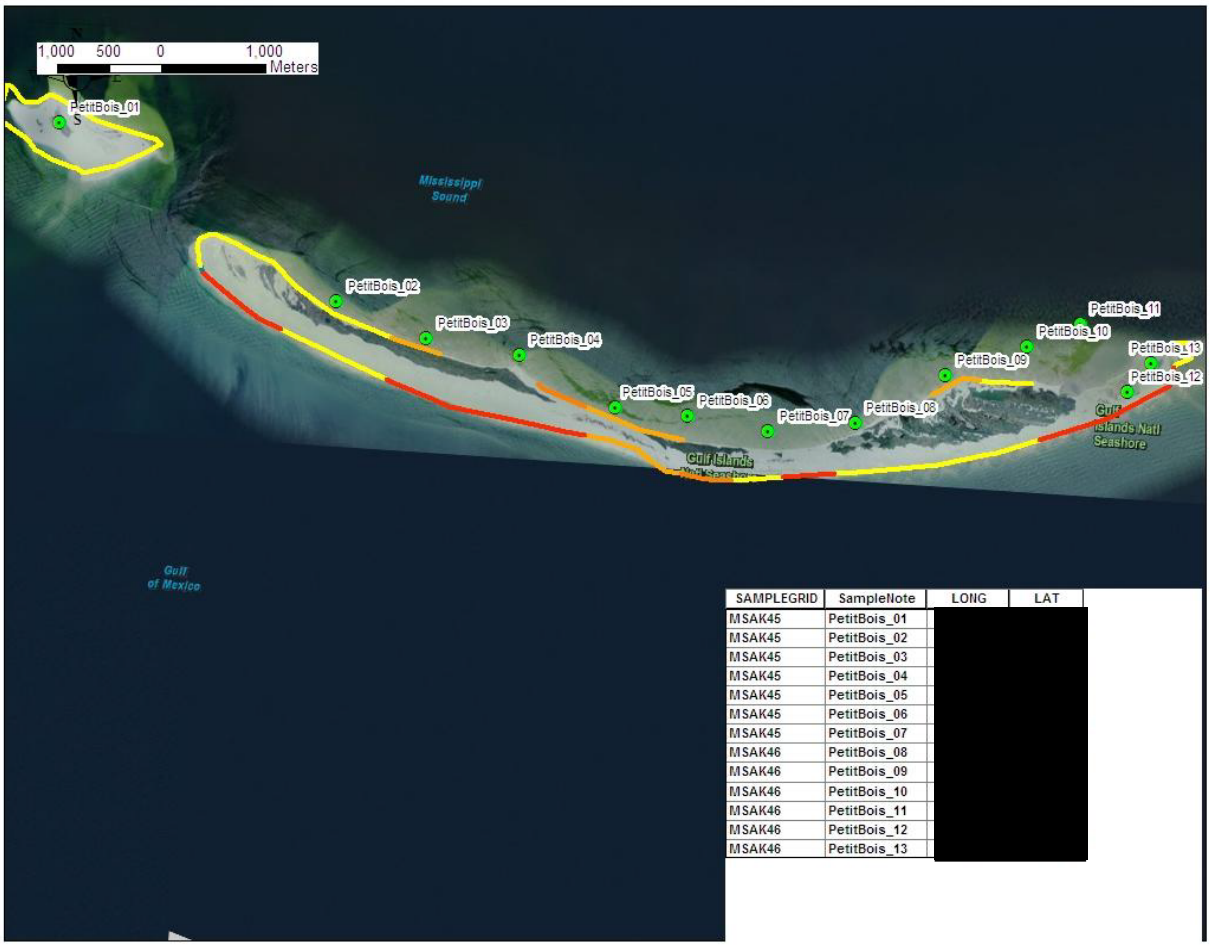


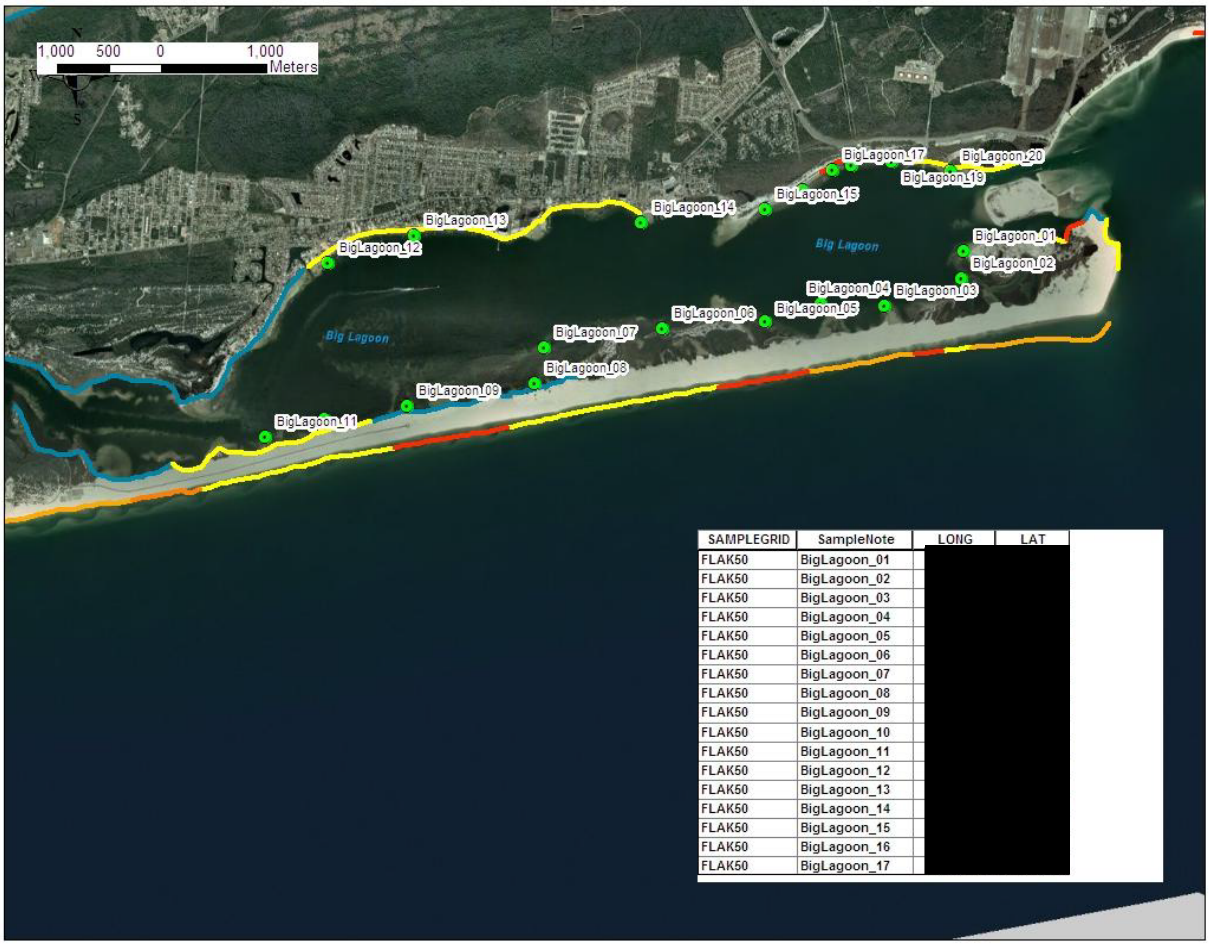
Figure 4. Tier 2 sampling sites for Horn Is, Mississippi. Colored lines represent level of oiling on adjacent shorelines, Red = Heavy, Yellow = Light, Blue = no oiling.



SAMPLEGRID	SampleNote	LONG	LAT
MSAK45	PetitBois_01	[redacted]	
MSAK45	PetitBois_02		
MSAK45	PetitBois_03		
MSAK45	PetitBois_04		
MSAK45	PetitBois_05		
MSAK45	PetitBois_06		
MSAK45	PetitBois_07		
MSAK46	PetitBois_08		
MSAK46	PetitBois_09		
MSAK46	PetitBois_10		
MSAK46	PetitBois_11		
MSAK46	PetitBois_12		
MSAK46	PetitBois_13		

Figure 5. Tier 2 sampling sites for Petite Bois Is, Mississippi. Colored lines represent level of oiling on adjacent shorelines, Red = Heavy, Yellow = Light, Blue = no oiling.

Figure 6. Tier 2 sampling locations for Robinson Island, Alabama. Colored lines represent level of oiling on adjacent shorelines, Yellow = Light, Blue = no oiling.



SAMPLEGRID	SampleNote	LONG	LAT
FLAK50	BigLagoon_01		
FLAK50	BigLagoon_02		
FLAK50	BigLagoon_03		
FLAK50	BigLagoon_04		
FLAK50	BigLagoon_05		
FLAK50	BigLagoon_06		
FLAK50	BigLagoon_07		
FLAK50	BigLagoon_08		
FLAK50	BigLagoon_09		
FLAK50	BigLagoon_10		
FLAK50	BigLagoon_11		
FLAK50	BigLagoon_12		
FLAK50	BigLagoon_13		
FLAK50	BigLagoon_14		
FLAK50	BigLagoon_15		
FLAK50	BigLagoon_16		
FLAK50	BigLagoon_17		

Figure 7. Tier 2 sampling sites for Big Lagoon, Pensacola Bay, Florida. Colored lines represent level of oiling on adjacent shorelines, Red = Heavy, Orange= Moderate, Yellow = Light, Blue = no oiling.

Appendix 4.

NRDA Baseline Assessment for SAV: Florida

Table 1: Seagrass Imagery and Mapping Status for Florida

Bay System	Most Recent Imagery	Agency	Most Recent Maps
Perdido Bay	2010	NASA, NOAA	2003
Big Lagoon	2010	NASA, NOAA	2003
Pensacola Bay System	2010	FWC FWRI SIMM	2003
Santa Rosa Sound	2010	FWC FWRI SIMM	2003
Choctawhatchee Bay	2010	FWC FWRI SIMM	2007
St. Andrews Bay	2010	GCCC	2003
St. Joseph Bay	2010	FDEP CAMA	2006
Franklin County	2010	FWC FWRI SIMM	1992
Big Bend Region	2006	FWC FWRI SIMM	2006
Cedar Keys and Waccasassa	2001	SRWMD	2001
Springs Coast	2007	SWFWMD	2007
Tampa Bay	2010	SWFWMD	2008
Sarasota Bay	2010	SWFWMD	2008
Lemon Bay	2010	SWFWMD	2008
Charlotte Harbor North	2010	SWFWMD	2006
Pine Island Sound	2008	SFWMD	2006
Matlacha Pass	2008	SFWMD	2006
Caloosahatchee Estuary	2008	SFWMD	2006
Estero Bay	2008	SFWMD	2006
Rookery Bay	2009	FDEP CAMA	Unknown
Ten Thousand Islands	2009	None	Unknown
Florida Bay	2004	FWC FWRI SIMM	2004
Gulf Upper Keys	2006	NOAA NCCOS	1992
Gulf Lower Keys, Marquesas	2006	NOAA NCCOS	1992
Tortugas	2006	NOAA NCCOS	1992
Atlantic Lower Keys	2006	NOAA NCCOS	1992
Atlantic Upper Keys	2006	NOAA NCCOS	1992
Biscayne Bay	2005	FWC FWRI SIMM	1992
Palm Beach County	2009	Palm Beach Co	2007
Southern Indian River Lagoon	2009	SFWMD	1999
Northern Indian River Lagoon	2009	SJRWMD	2007

Table 2: Seagrass Monitoring Programs in Florida

Estuary	Lead Agency	Most Recent Sampling	Sampling Frequency
Perdido Bay	DISL	May 2010	Event driven
Big Lagoon	DISL	May 2010	Event driven
Pensacola Bay	DISL	May 2010	Event driven
Santa Rosa Sound	DISL?	May 2010?	Event driven
Choctawhatchee Bay	FWRI	August 2009	Annual
St. Joe Bay	FWRI	August 2009	Annual
St. Joe Bay	DEP/CAMA	June 2009	Annual
St. Andrew Bay	FWRI	August 2009	Annual
St. Andrew Bay	GCCC	June 2009	Annual
Apalachicola Bay	ANERR	Unknown	Uncertain
St. Georges Sound	FWRI	June 2009	Annual
Franklin County	FWRI	June 2009	Annual
Ochlockonee Bay	None	None	None
St Marks	FWRI	June 2009	Annual
St Marks	DEP/CAMA	Summer 2009	Annual
Big Bend	FWRI	June 2009	Annual
Steinhatchee	DEP/CAMA	Summer 2009	Annual
Cedar Key	DEP/CAMA	Summer 2009	Annual
Waccasassa Bay	None	None	none
St. Martins Marsh	DEP/CAMA	Summer 2009	Annual
Homosassa	FWRI	August 2008	Sporadic
Springs Coast	DEP/CAMA	Summer 2009	Annual
Western Pinellas	Pinellas County	Fall 2009	Annual
Tampa Bay	City of Tampa	Fall 2009	Annual
Sarasota Bay	Sarasota County	February 2010	2X a year
Sarasota Bay	DEP/CAMA	Fall 2009	Annual
Lemon Bay	Sarasota County	February 2010	
Charlotte Harbor	DEP/CAMA	Fall 2009	Annual
Estero Bay	DEP/CAMA	February 2010	Twice yearly
Ten Thousand Islands	USGS/NOAA	May 2010	none
Florida Bay	FWRI	May 2010	Twice yearly
FKNMS	FIU	March 2010	quarterly
Biscayne Bay	FWRI	May 2010	Twice yearly
Biscayne Bay	Miami-Dade DERM	June 2009	Annual
Palm Beach	Palm Beach County	Summer 2009	Annual
South Indian River	SFWMD	February 2010	Bimonthly
North Indian River	SJRWMD	February 2010	Twice yearly

Table 3: Seagrass Metrics Used by Monitoring Programs in Florida

Estuary	Lead Agency	Visual Density	Spp Comp	Shoot Counts	Biomass	Sediment Contam	Benthic Inverts
Perdido Bay	DISL	No	Yes	Yes	Yes	??	Yes
Big Lagoon	DISL	No	Yes	Yes	Yes	??	Yes
Pensacola Bay	DISL	No	Yes	Yes	Yes	??	Yes
Santa Rosa Sound	DISL?	No	Yes	Yes	Yes	??	Yes
Choctowhatchee Bay	FWRI	No	Yes	No	No	No	No
St. Joe Bay	FWRI	No	Yes	Yes	No	No	No
St. Joe Bay	DEP/CAMA	Yes	Yes	Yes	Yes	No	No
St. Andrew Bay	FWRI	No	Yes	No	No	No	No
St. Andrew Bay	GCCC	No	Yes	Yes	No	No	No
Apalachicola Bay	ANERR						
St. Georges Sound	FWRI	No	Yes	No	No	No	No
Franklin County	FWRI	No	Yes	No	No	No	No
Ochlockonee Bay	None						
St Marks	FWRI	No	Yes	No	No	No	No
St Marks	DEP/CAMA	Yes	Yes	??	Yes	No	No
Big Bend	FWRI	No	Yes	No	No	No	No
Steinhatchee	DEP/CAMA	Yes	Yes	??	Yes	No	No
Cedar Key	DEP/CAMA	Yes	Yes	??	Yes	No	No
Waccasassa Bay	None						
St. Martins Marsh	DEP/CAMA	Yes	Yes	??	Yes	No	No
Homosassa	FWRI	Yes	Yes	No	No	No	No
Springs Coast	DEP/CAMA	Yes	Yes	??	Yes	No	No
Western Pinellas	Pinellas County	No	Yes	Yes	No	No	No
Tampa Bay	City of Tampa	Yes	Yes	??	No	No	No
Sarasota Bay	Sarasota County	No	Yes	Yes	No	No	No
Sarasota Bay	DEP/CAMA	No	Yes	No	No	No	No
Lemon Bay	Sarasota County	No	Yes	Yes	No	No	No
Charlotte Harbor	DEP/CAMA	Yes	Yes	Yes	No	No	No
Estero Bay	DEP/CAMA	Yes	Yes	Yes	No	No	No
Ten Thousand Islands	USGS/NOAA	Yes	Yes	??	??	No	??
Florida Bay	FWRI	Yes	Yes	Yes	Yes	No	No
FKNMS	FIU	Yes	Yes	No	Some	No	No
Biscayne Bay	FWRI	Yes	Yes	Yes	Yes	No	No
Biscayne Bay	Miami-Dade	Yes	Yes	Yes	Yes	No	No
Palm Beach	Palm Beach Co	Yes	Yes	Yes	No	No	No
South Indian River	SFWMD	Yes	Yes	Yes	No	No	No

Appendix 5.

Data Sheets

1. SAV Site Characterization and Trawl Form

(Adapted from Dauphin Islands Sea lab long-term monitoring program)

2. SAV Lab processing form

(Adapted from Dauphin Islands Sea lab long-term monitoring program)

3. SAV Rapid Assessment Form (created by NOAA Beaufort lab for NRDA)

SAV Site Characterization and Trawl Form [*Page 1 of 3*]

Survey Team ID: _____

Field Crew Leader: _____ _____

Data Entry: _____ _____
(Name) (Agency)

1. Site Descriptors

Site Name/ID:_____ Lat:_____ Lon:_____
Time:_____ Date:_____
Habitat Setting (check one) ____ Intertidal _____ Subtidal (Depth (m) _____)
Bed size: _____ Width (m) _____ Length (m)
Location of samples with respect to bed:_____
Overall bed condition:_____
_____

2. Physical/Chemical Parameters

Bottom Salinity (ppt): _____ Air Temperature (C): _____
Bottom Temperature (C)_____ Bottom Dissolved Oxygen (mg/L):_____
Weather/Cloud Cover: _____ Wave height (m): _____
PAR (uEm^{-2}s^{-1}) :_____ Secchi depth (cm):_____
Irradiance: _____ (value 1) _____ (value 2) _____ (value 3)
Depth: _____ (value 1) _____ (value 2) _____ (value 3)

Oiled Condition (check one): _____ None _____ Sheen _____ Light
_____ Moderate _____ Heavy

3. Seagrass percent cover: *Fill in table below, or check if visibility is too poor to estimate:* _____

Species	Quadrat 1	Quadrat 2	Quadrat 3
Overall			

Flowering shoots: _____yes _____no

SAV Site Characterization and Trawl Form [*Page 2 of 3]*

Site Name/ID:_____ Lat:_____ Lon:_____

Date:_____ Survey Team ID: _____

4. Point Sample Collection and Disposition

The following subsamples were collected [list all sample IDs for each, indicating any that are field duplicates, as well as geographic coordinates in decimal degrees]

Sediment samples for contaminant analysis:

Sample ID	Latitude	Longitude

Sediment samples for grain size analysis:

Water samples for contaminant analysis:

Vegetation samples for contaminant analysis:

Invertebrate samples for contaminant analysis:

Vegetation/faunal core samples for species and abundance metrics: **Core Diameter (cm)**: _____

Other (Please Describe):_____

SAV Site Characterization and Trawl Form [*Page 3 of 3]*

Site Name:_____ Lat:_____ Lon:_____

Date:_____ Survey Team ID: _____

5. Trawl Sample Collection and Disposition [*Enter "none" if no trawl conducted*]

Collected by: _____ _____

Field Crew Leader: _____ _____

Data Entry:_ _____ _____

(Name) (Agency)

Trawl Details

Trawl No.	Starting Lat	Starting Lon	Ending Lat	Ending Lon	RPM	Sample ID
1						
2						

Trawl 1 Sample Data and Disposition			Trawl 2 Sample Data and Disposition		
Species	Number	Sample Retained? (y/n)	Species	Number	Sample Retained? (y/n)

Other Site Notes: _____

SAV Lab Processing Form [*Page 1 of 1]*

Location:	**Site:**
Date sample taken:	**Core size:**
Date sample processed:	**Processed By:**

For post-oiling samples: Any oil residue visible during sample processing: (circle one) YES NO

Species 1:	**# shoots**	**# shoots without leaves:**	**# of single leaves:**	**# of leaves per shoot (n=10):**	
	Wt of leaves (g)	**Wt of shoots (g)**	**Wt of single leaves (g)**	**Wt of roots & rhizomes (g)**	
Length of longest leaves, mm (n = 10):					

Species 2:	**# shoots**	**# shoots without leaves:**	**# of single leaves:**	**# of leaves per shoot (n=10):**	
	Wt of leaves (g)	**Wt of shoots (g)**	**Wt of single leaves (g)**	**Wt of roots & rhizomes (g)**	
Length of longest leaves, mm (n = 10):					

Species 3:	**# shoots**	**# shoots without leaves:**	**# of single leaves:**	**# of leaves per shoot (n=10):**	
	Wt of leaves (g)	**Wt of shoots (g)**	**Wt of single leaves (g)**	**Wt of roots & rhizomes (g)**	
Length of longest leaves, mm (n = 10):					

Epiphyte Vial	**Pre-weight**	**Post-weight**
Species #: ____		
Species #: ____		
Species #: ____		

SEEDS	**# of seeds**
Species #: ____	
Species #: ____	
Species #: ____	

Infauna	**Counts**	**Pan #**	**Dry wt (g)**	**Muffled wt. (g)**	**Calculated AFDW**

SAV Tier 2 - Rapid Assessment Form

Survey Team ID: 42

Field Crew Leader: Jud Kenworthy NOAA

Data Entry: _____ _____
(Name) (Agency)

1. Site Descriptors

Sample ID/Note:_____ Lat:_____ Lon:_____
Time:_____ Date:_____
Water depth (check one) ☐ Intertidal ☐ Subtidal Depth (m) _____
Seagrass species present (Circle each) ☐ none ☐ Thalassia testudinum ☐ Halodule wrightii
☐ Syringodium filiforme ☐ Halophila engelmanii ☐ Ruppia maritima
Bed size: _____ Width (m) _____ Length (m)
Bed form: _____ Patchy _____ Continuous
Location of samples with respect to bed:_____
Comments :_____
_____

2. Physical/Chemical Parameters

Substrate: _____
Oiled visible on sediments (check one): None Sheen Light Moderate Heavy
Oil band dimensions: length _____m width _____m
Surface oil distribution: ○ none ○ <1% ○ 1-10% ○ 11-50% ○ 51-90% ○ 91-100%
Surface oil type: ☐ none ☐ Fresh ☐ Mousse
☐ Tar ☐ Balls/Patties ☐ Residue ☐ Asphalt/Pavement
Sediment subsurface oil condition (check one)

- ○ **Not applicable**
- ○ **Sheen appears only after sediment is disturbed (i.e. handful of sed. is removed)**
- ○ **Sheen on sediment surface prior to disturbance**
- ○ **Oil buried in sediment (describe type)_____**

3. Biological Parameters

Oil visible on seagrass plants (check one): ○ yes ○ no ○ NA
Form of oil if it appears on plants: ☐ none ☐ Sheen ☐ Globules or drops ☐ Tar ☐ Mousse
Seagrass shoot condition: (check one): If vegetation is dead, roots will still be examined.

- ☐ **vegetation appears natural, shoot appearance only slight mottling with minimal dead tissue/desiccation restricted to ends of older leaves.**
- ☐ **vegetation appears natural but intense leaf chlorosis.**
- ☐ **vegetation green but with considerable yellowing (≤ 50% chlorosis).**
- ☐ **vegetation having > 50% yellowing or blanching of leaves.**
- ☐ **vegetation dead.**

new shoots ○ yes ○ no new roots ○ yes ○ no
new rhizome growth ○ yes ○ no new leaf growth ○ yes ○ no
Seagrass root/rhizome condition:

4. General observations and comments