MC252 Deepwater Horizon Oil Spill
Deepwater Benthic Communities and Water Column Data Collection

July-September 2011 *HOS Sweetwater*
ROV Sediment and Bottom-Water Sampling Cruise Plan

July 15, 2011

Cruise Dates: July 14 – August 7, August 22 – September 1, and September 10 – 25, 2011

HOS Sweetwater 4
Leg 1 July 14– August 7 (25 days), Dave Valentine, Chief Scientist

HOS Sweetwater 6
Leg 1 August 22 – September 1 (11 days), Jim Payne, Chief Scientist
Leg 2 September 10-25 (16 days), Jim Payne, Chief Scientist

Prepared by: Deborah French-McCay (ASA), Christopher Lewis (IEC), David Valentine (UCSB), James Payne (PECI), on behalf of Trustees

Reviewed by: Dan Hahn, Rob Ricker, John Quinlan (NOAA), Laura Riege, Jody Kubitz (Cardno ENTRIX), on behalf of BP

Overview

This Cruise Plan, developed as a collaborative effort by representatives of both the Deep Benthic Communities and Water Column Technical Working Groups, will collect photographic/video imagery, sediment and near-bottom water column data in the vicinity of the MC252 well site and areas to the southwest. The choice of sampling stations includes those locations where: 1) there was potential contact of suspended and dissolved MC252 hydrocarbons with the continental slope and numerous bathymetric features (salt domes) in the plume depth horizon, and 2) there was potential sediment deposition of MC252 hydrocarbons down-current from the well head. These processes are referred to as the bathtub ring (BR) and fallout plume (FP) for differentiation and purposes of station identification. Surrounding Slope (SLP) locations and slope areas where red crabs were historically sampled will also be sampled. The station locations identified in this plan (Figure 1 and 2, also see Attachment 1) will be sampled with a Remotely Operated Vehicle (ROV): 1) Sediment push cores will be taken from the ROV and processed for chemical analyses; 2) near-bottom water samples will be taken above these cores, filtered, and both filters and filtrates chemically analyzed; 3) samples of flocculent material will be collected with the ROV using a slurp system and chemically analyzed; 4) CTD, dissolved oxygen (DO) and fluorescence will be measured using sensors mounted on the ROV; 5) red crabs and other megafauna found along transects opportunistically collected for exposure assessment (hydrocarbons, dispersants, and metals, see Attachment 18); 6) occurrence and locations of hardgrounds and/or sessile megafauna will be documented; and 7) bacterial samples will be

collected from the sediment and near-bottom water column and archived in a secure facility under trustee control for future possible analysis. If fluorescence or DO profiles indicate relative maxima (fluorescence) or minima (DO) in waters below 200m, samples will be taken in bounding these peaks. As warranted, near bottom water samples and sediment cores may also be collected in other locations where indications of potential sedimented oil or potential flocculent oil layers (floc) are noted during bottom surveys with the ROV.

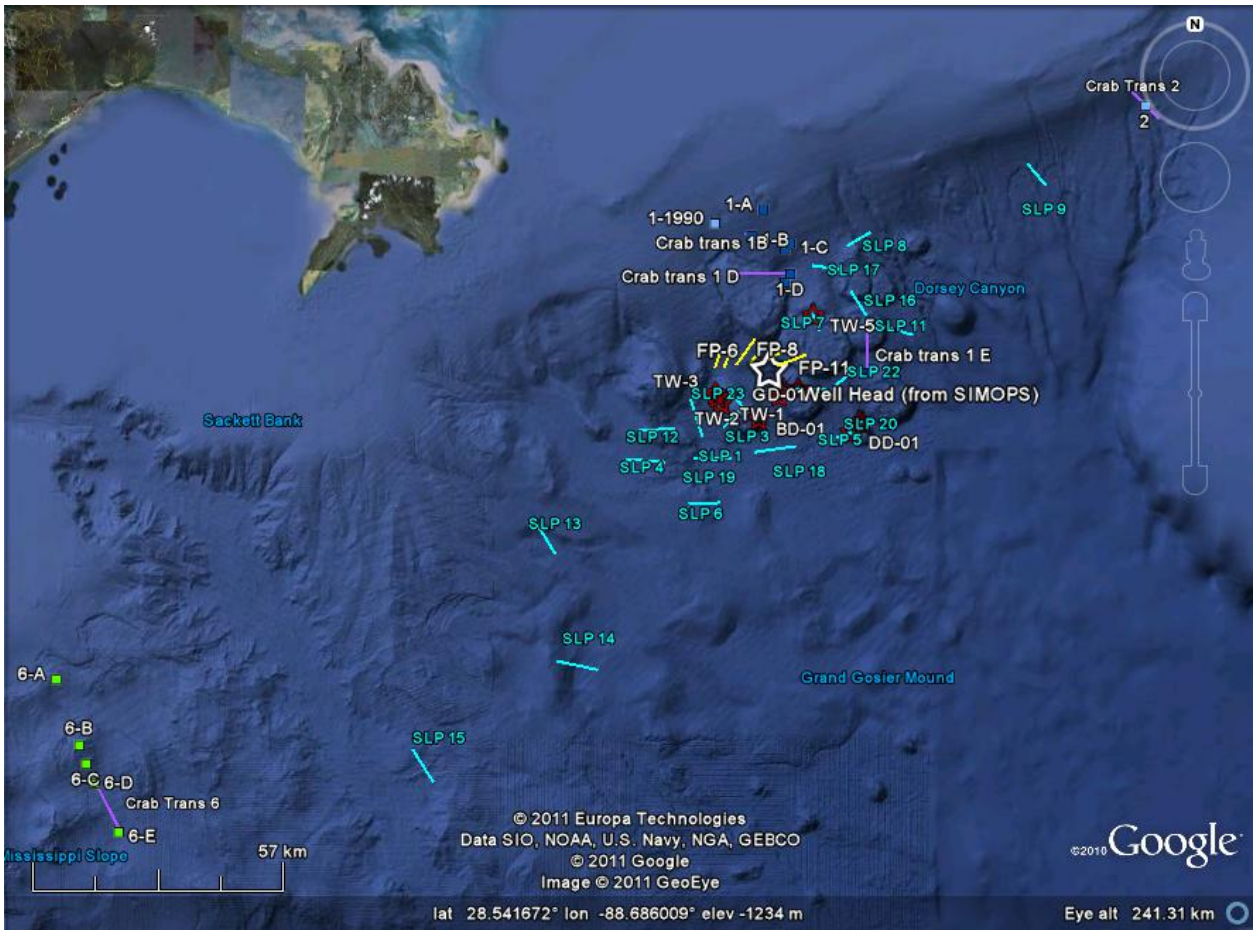

Figure 1. Overview of all proposed *HOS Sweet Water* Cruise 4 and 6 transects: fallout plume FP-6 to FP-11; crab transects 1B, 1D, 1E, 2 and 6 (purple) and slope transects (blue, SLP). The white star is the location of the wellhead. The brown star icons represent potential seep recon sites.

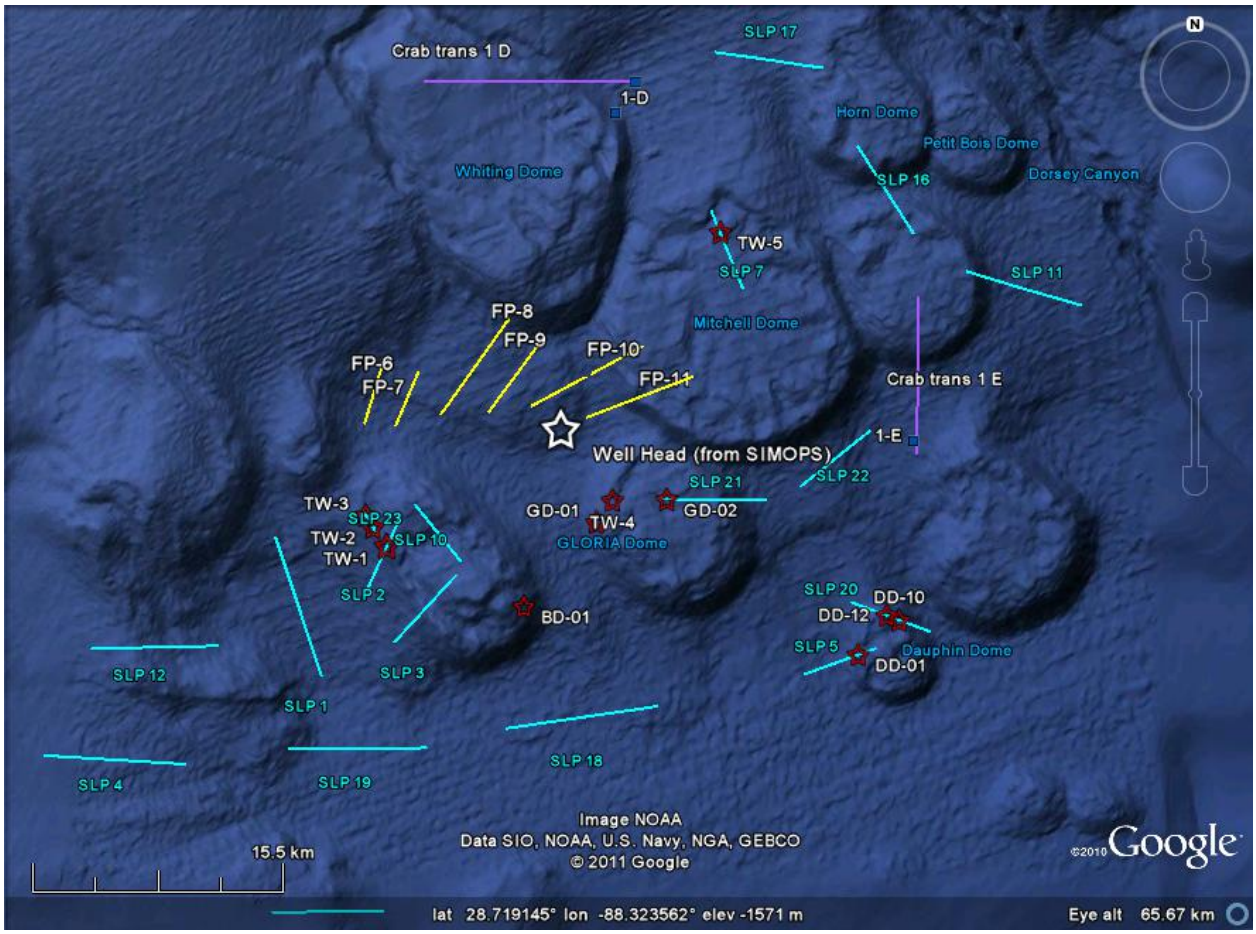

Figure 2. Proposed *HOS Sweet Water* Cruise 4 and 6 near-field transects: fallout plume FP-6 to FP-11; crab transects 1B, 1D, and 1E (purple) and near-field slope transects (blue, SLP). The white star is the location of the wellhead. The brown star icons represent potential seep recon sites on/near Dauphin Dome (DD-01, DD-10, DD-12), Biloxi Dome (BD-01, TW-1, TW-2 and TW-3), and Gloria Dome (GD, TW-4).

In addition, several transects are planned in cruise 4 (i.e. Leg 1) to allow early reconnaissance of selected seep locations (Figure 2) to inform approaches to seep sampling and flux measurements during this and later cruises. To avoid confusion with the previous station locations occupied during legs 2 and 3 of the *HOS Sweetwater* 2 cruise, the proposed transects for this cruise plan (see Figures 3 to 8 in Attachment 1) are identified as:

1. Fallout Plume transects: FP-6 through FP-11. These are the yellow fallout plume transects to the north of the wellhead in the relatively flat plain surrounded by Whiting, Mitchell and Gloria Domes (Figure 2).
2. Crab Transects: Crab Trans 1B, 1D, 1E, 2, and 6 (Figure 1; Figures 4, 5, and 6 in Attachment 1). Crab Trans 1B and 1D are transects to the north of the wellhead on and north of Whiting Dome, respectively; whereas Crab Trans 1E is to the east of Mitchell Dome. Crab Trans 2 is at the head of DeSoto Canyon approximately 100 km to the northeast of the wellhead and Crab Trans 6 is near Mississippi Canyon approximately

180 km to the southwest of the well head. Sampling for red crabs has historically been conducted at each of these stations by Harriet Perry (USM, pers comm., see references for bibliography of published information). These stations are being occupied to provide sediment and floc concentration data in support of a separate cruise to these areas planned for later in the summer.

3. Surrounding Slope Stations (colored blue and designated as SLP-1, SLP-2, etc, Figures 1 and 2; Figure 6 in Attachment 1), to examine potential "bathtub ring" and "fallout plume" accumulation further afield (refer to the *ROV Sediment and Bottom-Water Sampling Cruise Plan/ March-April 2011 HOS Sweetwater* Study Plan for a definition of these terms). Some of these stations also pass near to locations of suspected seeps (Figure 2). Locations along these transects where a seep is visibly observed using the ROV cameras will provide accurate locations for a potential follow up dives and sampling.
4. Short seep recognizance transects near seeps identified from acoustic data collected on the *Nick Skansi* November and December 2010 cruises or by Thomas Weber (UNH, pers. com, Jun 2011) based on acoustic data collected in summer of 2010. (Figure 2, TW indicates identified by Thomas Weber; other seeps were identified on the *Nick Skansi* cruises).

Applicable protocols are provided as appendices to this plan (e.g. ROV operations, sediment and water sampling; sampling handling, naming conventions, COC templates, QA/QC procedures, and NOAA QAP; Attachments 2-10, 17, 18). Health and safety and related procedures are described in Attachments 11-16.

Attachments 19 and 19A through 19E provide standard operating procedures (SOPs) for the protection and conservation of marine mammals and any species listed under the Endangered Species Act as appropriate for the vessel and sampling equipment operations to be conducted on this cruise.

Cruise Objectives and Approach

The cruise is designed to address the following objectives:

1. Examine potential locations where hydrocarbons and dispersants related to the MC252 oil spill and particulate matter may have settled on the sea floor by sampling surface sediments and flocculent material in the "bathtub ring", the "fallout plume", and surrounding slope areas defined above and in Attachment 1.
2. Evaluate potential concentrations of MC252-related hydrocarbons and dispersants in bottom waters that may be related to any sediment contamination by sampling water overlying locations of sediment samples in the "bathtub ring", the "fallout plume", and surrounding slope areas.
3. Opportunistically collect red crabs and other megafauna found along transects for exposure assessment (hydrocarbons, dispersants, and metals, see Attachment 18).
4. Document occurrence and location of hardgrounds and/or sessile megafauna potentially exposed to MC252 oil at and enroute to survey locations that were selected to address the primary objectives listed in #1 and 2 above.

The overall focus of this collaborative study is to evaluate the possible presence, concentrations and sources of oil in surficial sediments and overlying water at specified locations and water depths. We will sample surface sediments to examine potential locations where MC252 oil, particulate matter and water column biota may have settled on the sea floor. We will use the ROV video and sampling to examine the sea floor for potential evidence of water column exposure to MC252 oil and any effects of exposure such as settled water column biota or visual evidence of impact to corals. Core sampling of surface sediments by ROV will be collected for chemical analysis. Sampling locations will be in areas within 180 nm of the well and primarily to the south and southwest, in areas where sensors on previous water column studies have suggested subsurface oil was/is present and at locations previously selected by the Deepwater Benthic Communities TWG as being of interest because of the presence of anomalies indicating hard bottom.

Sediment samples will be analyzed for PAH and dispersant concentrations as described below and in Attachment 4. In addition, we will collect water samples at locations and depths where sensors indicate potential presence of hydrocarbons, focusing on bottom waters in the vicinity of coring stations. In addition to collecting samples for measuring hydrocarbons, biomarker analysis and dispersant indicators, we will also take samples for bacterial analysis. Samples for bacterial analysis will be collected from water and surface sediment and preserved in such a way that DNA can be later extracted and the microbial community analyzed using any of a number of existing techniques. Water samples will be analyzed for PAH and dispersant concentrations as described below and in Attachment 4.

Specific components of sample and data collection proposed in this cruise plan include:

1. Measurement of Conductivity, Temperature, Depth (CTD), particle density by transmissometer, and dissolved oxygen (DO) *in situ* to measure the physical characteristics and vertical density structure of the water column (e.g. thermoclines and pycnoclines).
2. Measurement of UV fluorescence *in situ* and real-time using sensors packaged with the CTD instrument. Two fluorometers will be employed simultaneously: WetLabs CDOM and Chelsea AquaTracka. These instruments utilize different excitation and emission wavelengths, with the AquaTracka designed to be sensitive to fluorescence from hydrocarbons (Attachment 5). The CDOM is designed to measure fluorescence from dissolved organic matter.
3. Collection of water-column and near-bottom water samples with 10 L Go Flo Bottles mounted on the Remotely Operated Vehicle (ROV) at locations and depths where sensors indicate potential presence of hydrocarbons for measurement of the following chemicals in accordance with the attached protocols and NOAA Analytical Quality Assurance Plan (QAP); Attachments 7-9:
    - Extended PAH (parent plus alkylated PAHs) as described in QAP Table 1.1a;
    - Saturated Hydrocarbons and Total Hydrocarbons as described in QAP Table 1.1b
    - VOCs as described in QAP Table 1.1c, unless a joint BP/Trustee agreement to discontinue VOC analyses project-wide is implemented.
    - Total suspended solids (TSS), Carbon-Hydrogen-Nitrogen (CHN) analysis

- Dispersant concentrations by LC/MS/MS (DOSS), and semi-quantitative analysis of monitoring ion profiles by GC/MS-SIM for dispersant indicator compounds (DPnB m/z 59 and 103)

4. Water and sediment samples will be preserved for potential microbial community analysis.
5. Real-time ROV video and digital still camera imaging for general seafloor observations (Attachment 2).
6. Surface sediment sampling from the sea floor using push-coring devices deployed on the ROV (Attachment 4) and direct floc sampling using a slurp-gun type sampling system controlled by the ROV manipulators. Microbial samples will be preserved for potential future analysis.
7. Red crabs and other megafauna may be opportunistically collected for exposure assessment (hydrocarbons, dispersants, and metals, see Attachment 18).
8. An ROV-deployable chamber for seep flux measurements will be tested by deploying the inverted-funnel device over visibly observable oil/gas seepage for a timed period, and then volumetrically measuring the collected oil and gas. This will require moving the ROV up to a depth where any gas hydrates formed in the collection device would dissociate to free gas in order to make the measurements. (See Attachment 20 for details). Data collected as part of this "methods shakedown" will be considered provisional, i.e., the parties reserve the right to reject the data as invalid based on the provisional nature of the methodology.

Sampling Plan

As illustrated in Figures 1-2, and detailed in Attachment 1, data and samples will be collected near and in several areas within 180 km of the MC252 well site. Sediment cores will be taken at stations listed in Attachment 1 and also may be taken from the ROV on the *HOS Sweetwater* at locations identified visually (using the ROV video feed) as of interest based upon one or more of the following criteria: Proximity to known or observed seeps (as suggested by observed oil or gas plumes, extensive *Beggiatoa* mats, or chemosynthetic mussel communities), in areas where oil may be present based upon information from previous cruises or other sources, in areas where affected biota may be present, or in areas historically sampled for red crabs.

We plan to collect sediment, particulate matter and water samples from the ROV deployed off the *HOS Sweetwater* at approximately 35 transects, targeting sampling at 1 transect per 24-hour cycle (See Attachment 1). The primary sampling plan consists of transects of coring sites progressing up-slope through the plume-depth horizon along several salt dome and slope features surrounding the Macondo well head, and transects of coring stations progressing to the southwest and east of the well head. The up-slope transects will be centered at approximately 1050 - 1150m, and will be bracketed by depths as great as 1500m and as shallow as 900m, depending on local topography (Figures 1 and 2). We plan to occupy 35 transects along the salt-dome slopes and in the vicinity of the Macondo well head, with each transect containing up to 5 stations. We plan to conduct each transect as a single deployment and dive of the ROV.

For each transect we plan to lower the ROV at the deepest station. If warranted (by AquaTracka and/or DO profiles) water-column samples may be collected around sensor indicators at two depths during the descent, sampling above, in and below the sensor feature (as in previous cooperative Water Column cruises). Upon reaching the sea floor the ROV will be released from the TMS, and it will be used to search out undisturbed sediment in the immediate vicinity to collect the first samples. The precise location of sampling is determined in practice from the video footage by the chief scientist in consultation with the ROV operator, as modulated by visibility and current flow. As the ROV moves into position a sample of the bottom water will be collected with a GoFlo bottle mounted to the ROV, taking care not to sample flocculent material possibly kicked up by the ROV. If observed, surface floc will be collected with the slurp gun, and then the ROV will slowly move into position and hold position over the sediment to collect 3 push cores for chemical analyses. Once these sampling activities are performed, the HOS Sweetwater (while still on dynamic positioning (DP)) will be directed to move in an up-slope direction (specific to each station) along the transect line at 0.25 to 1.0 knots. During this time, the ROV will typically be flown along the bottom at 1-2 m, transmitting real-time video back to the ship for recording and observation by the Scientific Party (Chief Scientist and CSA/Entrix representatives). Real time positioning and depth of the ROV will be tracked and recorded. The plan will be to collect sediment and additional water samples at four depth intervals from the deeper starting position to the eventual end-point of each transect near the top of each feature. With the real-time video feed, it will also be possible to sample anomalous features of any deposits that may appear to be oil along each transect. Starting and ending positions for each transect are presented in Tables 1-3 of Attachment 1. All near-bottom water and sediment sampling stations will be documented by HD video and high resolution still photography.

This sampling approach and the order of sampling activities (near-bottom water, settled floc, and finally triplicate sediments) will be exactly the same at each station, so even though there will be two Chief Scientists on sequential legs of the cruise, comparable samples will be collected throughout the month-long effort. The general transect locations have been pre-selected (Attachment 1, Tables 1-3), so the only activity left to the discretion of the Chief Scientists will be specific station selection.

Criteria for station location along the transect will include: 1) soft sediment for coring; 2) observations of surface floc; 3) high visibility; 4) observation of other features or anomalies (e.g., piles of drilling mud, accumulations of mucus-like oil agglomerates, etc.); 5) the presence of hard-bottom and coral assemblages (in particular for slurp-gun floc collection if the bottom is too hard for coring); and 6) avoidance of other obstructions (pipelines or platform debris). Both Drs. Valentine and Payne have completed these types of operations previously, and following each day's dives, satellite telephone communications will be established between them and other members of the Water Column and Deepwater Benthic Communities TWGs to discuss preliminary findings and provide guidance to ensure continuity.

Methods and Instrumentation

To meet the objectives of this proposed cruise, the vessel will be outfitted with the following instrumentation to allow the acquisition of oceanographic data, collection of water samples using the TMS/ROV bundled with instruments. Adaptive sampling locations will be selected on the basis of the preliminary data collected real-time and *in situ* by these instruments with a consideration of the extent of the area to be sampled, rate of sampling, and available crew hours. The chief scientists for the two legs will develop a consensus approach to adaptive sampling based on ROV performance and experiences during the first leg. It is anticipated that, under good conditions, one transect of four to five stations will be sampled per day on the *HOS Sweetwater,* enabling all 34 transects plus the 6 seep recognizance stations performed during cruise 4 and 6 to be sampled during the 52 operational cruise days (including 6 days of transit to/from port), while still allowing for two days of operational delays. Should this not be possible, then fewer stations will be sampled, as determined by the Chief Scientist. At a minimum, whole water samples as close as possible to the sea floor and sediments will be collected at every designated sampling location.

Ship-Board Instrumentation and Water Sampling Equipment

Equipment aboard the *HOS Sweetwater* (Table 1) is listed below. Use of these instruments will enable collection of oceanographic data to characterize water column properties, including *in situ* measurements of the following chemical and physical parameters:

- Conductivity (and so salinity and density), temperature, and depth (CTD)
- Dissolved oxygen (DO)
- Fluorescence (AquaTracka, CDOM, ECO-FL)
- Turbidity (transmissometer)
- pH sensor

Table 1. Equipment aboard the M/V *HOS Sweetwater*.

Canyon Triton XL Remotely Operated Vehicle equipped with six 10 L water sampling Go Flo Bottles	(Attachment 3)	2,500 m approximate depth range
Sediment core sampling gear for ROV	(Attachment 4)	
Multi-chamber slurp system	(Attachment 17)	
Seabird 19V2 CTD with DO sensor	Seabird	Water depths to 5,000 m
Chelsea AquaTracka Fluorometer	(Attachment 5)	Water depths to 5,000 m
Chlorophyll (ECO-FL) + Turbidity Sensor	Wetlabs	
CDOM Fluorometer	Wetlabs	
Seabird pH sensor	Seabird	
Portable Large Volume Water Sampling System (PLVWSS)	(Payne et al., 1999, Attachment 7)	

On-board equipment and monitors on the vessel will convert and display real-time data, gather and record all raw data, and provide the survey team with information relative to the presence of chemical and physical features in the water column such as dissolved oxygen minima and fluorescence maxima. Instruments are factory-calibrated. They will be used to guide the locations at which targeted water column samples will be collected according to sampling protocols (described below).

Water Sampling

Attachment 1 contains a list of locations identified for sampling during the cruise. Whole water samples will be collected using 10L Go Flo Bottles mounted on the ROV at all stations along each transect. The focus for water sampling will be to take one water sample near the sea floor as close as possible to each of the sediment sampling sites (and as close as operationally safe: typically within 1-1.5 m). The water sample will be collected from as close to the bottom as possible, prior to coring, and in a way that avoids resuspension of sediments into the water. The Go-FLO will be mounted on the ROV and actuated as we approach the coring site.

In addition, the real-time, *in situ* fluorescence and CTD/DO observations will be used as screening tools to identify any other depths at which water-column samples will be collected according to the criteria described below. The science leads (Chief Scientists and their designated watch leads) will review the data, select the depths for sampling, and Go-Flo bottles will be tripped at the appropriate locations by the ROV controller.

If either a distinct depth-zone fluorescence peak is observed and/or a distinct decrease in dissolved oxygen (DO) is observed (relative to background), then water samples can be collected as follows:

- above or below the indicator depth zone (fluorescence peak and/or DO minimum)
- at the maximum deflection or mid-point of the indicator depth zone (peak or minimum)

Samples collected above and/or below the indicator shall be collected immediately adjacent to the observed indicator (fluorescence peak or DO minimum) but at a vertical location that is clearly outside the feature (i.e. on the background or ambient trend line of the parameter outside the influence of the perturbation). The actual depths of sample collection are at the discretion of the trustee scientific leads on each vessel based on the real-time assessment of the CTD/DO/CDOM/AquaTracka profiles and in accordance with the above criteria. However, as the focus is on sediment and near-bottom water sampling, priority will be given to those activities over mid-water-column sampling.

If the AquaTracka signal increases near the sea floor, or if fluorescence is observed on the sea floor using a black light and video camera on the ROV, additional water samples (as feasible) will be taken near the seafloor (as close as operationally safe: typically within 1-1.5m). If no indications of fluorescence are observed, one water sample will be taken near the sea floor.

Standard operating procedures for water sampling and handling will be followed, and care will be taken to preserve sample integrity for hydrocarbon analyses (see Attachments 4, 6-10

regarding sampling, handling, and decontamination procedures). Near-bottom field duplicates will be collected for 10% of samples or when no water-column samples were collected during the descent such that additional, unused Go Flo bottles are available. Equipment blanks will be collected once per day (or after sampling in a particularly heavy AquaTracka lens) from different pieces of equipment. Trip blanks and temperature blanks will be included in samples for at-sea transfer and shipping.

For each sample, sufficient volumes will be collected to satisfy all analytical procedures in accordance with the NOAA MC252 Analytical QAP V2.2 (Attachment 8): Table 1.1a (extended PAH); Table 1.1b (alkane/isoprenoid and TEH); Table 1.1c (volatile aromatic hydrocarbons); Table 1.1e and f (quantitative and qualitative petroleum biomarkers); and dispersant concentrations (by LC/MS/MS). Biomarker analyses will be conducted only if there are detectable hydrocarbons.

Water sampling volumes, jar requirements, and handling procedures for each of the primary analytes are summarized in Table 2. Whole water samples for PAH and TPH analyses will be placed in 1-L I-Chem Certified Clean amber glass jars. Whole water samples for total suspended solids (TSS) and organic carbon, hydrogen, and nitrogen (CHN) analyses will be placed in 1-L non-acidified amber glass jars, clearly labeled for this dual intent. The CHN analysis will be conducted after the non-destructive TSS analysis using an elemental analyzer (micro-Dumas method). Water samples for volatile aromatic hydrocarbons (Standard List of VOCs given in AQAP v2.2 Table 1.1c) will be collected in duplicate (in 40 mL pre-acidified VOA vials with septa).

Water Filtration

The Portable Large Volume Water Sampling System (PLVWSS, Payne et al., 1999; Attachment 7) will be employed on both legs of the cruise in order to provide measurements of particulate and dissolved hydrocarbon concentrations. In order to maximize the number of stations that can be sampled during this cruise, the focus will be on filtering samples from locations at which the presence of subsurface oil is suggested by indicators (e.g. distinct fluorescence maxima and/or dissolved oxygen minima) and all samples taken just above the sea floor.

Water Sample Containers

To supply ships with the appropriate sample containers, approximately 1-2 water samples are planned for each sampling location, plus ample additional containers to accommodate discretionary sampling and equipment blanks. The requirements for glassware are broken out in detail in Table 4 of Attachment 1. Whole water sample collection, sample bottle labeling, equipment decontamination, and chain of custody procedures will be conducted in accordance with the protocols provided as appendices.

Sediment Sampling

The ROV will collect a minimum of three cores at each station for chemical analysis, to allow for an assessment of variability in chemical distributions and/or archiving of samples. Cores will

be collected from sediment undisturbed by the bow wave of the ROV, as determined by the chief scientist through viewing of the ROV's video feed. The precise placement of the cores will be determined by the chief scientist with logistical input from the ROV operator. Several approaches can and will be used to minimize bow wave disturbance, including: 1) adjusting the buoyancy of the ROV to be slightly positive so that only top-side vertical thrusters are required to hold position above the bottom; 2) approaching a site slowly from the down current direction; 3) stopping the ROV short of the site and inching forward at a slow pace; and 4) turning the ROV slowly into a site. Such efforts will be aided by the closed loop DVL navigation available for the ROV. Both Drs. Valentine and Payne have considerable experience in completing such coring operations with an ROV, and arrangements have been made to facilitate direct (elbow-to-elbow) communication with the ROV pilots. All three cores will be collected in close proximity, using the closed loop Doppler capabilities of the ROV, and this will further minimize sediment disruption. Ideally all three cores will be inserted, prior to removal of the first, to avoid cavitation of the hole and flocculent release.

Cores will be taken for chemical analysis only. Protocols for taking these samples and analytes/analytical methods are provided in Attachment 4. Attachment 1 contains a list of locations already identified for sampling, based on existing information. Upon returning to the *HOS Sweetwater*, the cores will be sectioned into five layers as follows: 1) overlying water; 2) 0-1 cm; 3) 1-3 cm; 4) 3-5 cm; and 5) 5-7 cm. We anticipate analyzing the upper 4 layers from one of the replicate cores, and holding all the other core sections in frozen archive for additional analyses if warranted.

Benthic Flocculent and Particulate Sampling

The ROV will collect samples of flocculent and particulate matter suspended at the sea floor, when such material is observed at each station. Sampling will be performed with a slurp-type system in which the suspended matter is drawn by vacuum into a sealed chamber outfitted with glass-fiber filters. These samples will be processed in the same way as the flocculent from sediment cores, as described in Attachment 17.

Sediment Bacterial Sampling

Samples of the bacterial community will be collected from the sediment at one station on every third transect for later extraction and analysis. Approximately 5g of sediment from select depth intervals in chemistry-designated cores will be collected into sterile containers and stored at -80^{o}C for the duration of the cruise. Samples preserved in this way can be used for extraction and purification of bacterial DNA, and can be used for identification of the microbial community.

- Identification of bacteria through sequencing of the 16SrRNA gene or other approaches,
- Quantification of bacterial abundance or metabolic potential through quantitative PCR,
- Identification of metabolic potential through metagenomic sequencing.

Sample Analysis

This Plan includes provision for the collection of various samples including: sediment, water, floc, red crab and other biota tissues, natural hydrocarbon seep gases and microbial samples.

Any agreement between the Trustees and BP regarding analysis of the collected samples will be determined in subsequently developed, reviewed and approved cooperative work plan(s). The Trustees shall provide a draft sample analysis plan or plans for this cruise's samples to BP by August 1, 2011, with the exception of the microbial sample analysis plan that will be provided by September 1, 2011 (because the Principal Investigator is the Chief Scientist on HOS Sweetwater 4 and will be at sea until August 7). BP and the Trustees agree to work together in good faith to cooperatively develop and finalize this plan or plans by September 1, 2011, or October 1, 2011 for the microbial analyses, and agree that the intent of the plan(s) is to include 1) analyses for Polycyclic Aromatic Hydrocarbons (PAHs), saturated hydrocarbons and total hydrocarbons, TSS, CHN, metals, fingerprinting for MC252 oil, dispersants, whole crab/biota or tissue samples and microbial analyses where technically practicable, 2) a prioritization scheme for sample analysis, 3) identification of data deliverables, and 4) a reasonable schedule of delivery for the data deliverables. With the exception of sample preparation and analysis required to ensure holding times are met, samples collected under this workplan where the laboratory and methods are not already cooperatively agreed upon will be archived pending signature of the Sample Analysis Plan(s) unless otherwise agreed.

However, if BP and the Trustees are unable to reach consensus on any individual element(s) of the Sample Analysis Plan(s) by September 1, 2011, with the exception of the microbial sample analysis plan where October 1, 2011 will apply, the Trustees reserve the right to proceed independently on those elements on which no agreement was reached. Regardless, the Trustees agree to provide BP with all laboratory results for PAH and fingerprinting for MC252 oil for samples collected under this Plan.

Sample Retention

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.

Data Management and Trustee Oversight

All data and imagery (including navigation, video files, instrument data, field logs, photographs and photo logs, and documentation), and other electronic data will be saved to an on-board computer, and all data shall be migrated to dedicated external hard drives. The data will be controlled and managed by the NOAA NRDA data manager under project protocols, including Chain-of-Custody tracking of the external hard-drives. Data is generally organized by sampling station and all electronic data files will be filed into this structure by NOAA NRDA data manager with the assistance of the operator/data logger. The hard drive will be duplicated in full immediately following the cruise, and duplicates of the hard drive will be provided to (1) the Louisiana Oil Spill Coordinator's Office (LOSCO) on behalf of the State of Louisiana, and to (2)

Cardno ENTRIX on behalf of BP. The original hard drive shall be kept in a secure facility in trustee custody.

Under the direction of the Chief Scientist, a NOAA Data Manager on board the vessel will summarize sampling activities and scientific observations throughout the day and email NOAA NRDA [redacted]) by midnight, with the following materials:

- daily report (according to the example provided as Attachment 16 to this plan);
- PDFs of each CTD cast conducted that day (according to the example provided as an attachment to this plan).

By the end of the cruise, all documentation, including COCs, field notes, sampling logs, sampling forms, photos, photo logs, ship logs, and GPS tracking shall be transferred to the NRDA Sample Intake Team to upload to the NOAA_NRDA ftp following NRDA data management protocols. An identical copy of all documentation will be provided to LOSCO and BP/Cardno ENTRIX at the end of the cruise.

All photographic documentation will be logged as per NRDA protocol and two copies submitted to the NRDA sample intake team after the cruise and copies provided to BP/Cardno ENTRIX. Results of the unvalidated sediment and water analytical chemistry data will be delivered to BP/Cardno ENTRIX and LOSCO as generated and pursuant to the data sharing agreement (see "Distribution of Laboratory Results" paragraph below).

Logistics

Cruise Schedule

The proposed schedule for personnel and crew of the *HOS Sweetwater* is July 14 – September 25, 2011, divided into three legs: July 14– August 7 (a minor crew change will occur about July 28), August 22 – September 1 and September 10-25. The 52 days of ship time includes six days of transit time, and 46 full days of sampling activities; 24-hour operations are planned. Below is an outline of the anticipated order of sampling, which will be subject to logistical and weather constraints.

HOS Sweetwater 4 (July 2011)

1) Perform the 6 transects that go through seep areas: SLP23, SLP2, SLP21, SLP20, SLP5, and SLP7. It is expected that this will require 6 days sampling.
2) Select up to 6 seep locations and sample them with short radial transects (see Attachment 1) and perform flux measurements as time and logistics allow. This requires a change on the ROV from the slurp gun to the flux chamber. If there are problems with the flux chamber or remodeling/reconfiguring is required, postpone flux measurements at seeps until the August leg of the HOS Sweetwater 6 cruise after remodeling is accomplished. It is expected that the seeps transects and flux measurements will involve 6 days of sampling.
3) Continue to end of HOS Sweetwater 4 sampling the crab locations (Crab Trans 1B, 1D, 1E, 2, and 6), with other SLP transects in-route, as logistics dictate feasible. This allows

time to for crab station samples to be worked up before red crab cruise work to be performed later. [There will be at most 12 days left at sea after the seep work. The order and transects sampled will be subject to logistics and required trips to shore.]

HOS Sweetwater 6

1) August 2011: Continue seep flux measurements at 6 selected locations if this work could not be accomplished in the HOS Sweetwater 4 (July) cruise.
2) Perform all FP transects, remaining SLP, and any remaining Crab transects.
3) September 2011: Repeat flux measurements at up to 6 seep locations sampled in HOS Sweetwater 4 (or in August).

At-Sea Transfer of Samples

Multiple at-sea transfers of supplies and samples will be necessary to maintain the integrity of the samples and to meet laboratory hold times. Assuming water samples have a maximum hold time of seven (7) days from the time of collection, at-sea transfers will be scheduled to occur after two days of sampling. A Chain of Custody (COC) will be maintained by ensuring that both a NOAA representative and a Cardno ENTRIX representative are present on all transfers. Protocols for COC procedures and at-sea transfers are attached as appendices to this cruise plan (NOAA Attachments 9, 10, 14; Cardno ENTRIX Appendix "Transfer of Material at Sea"). At-sea transfers will be performed by the M/V *Emily Bordelon* (140') or similar vessel operated by Bordelon Marine, Houma, LA. Supplies for NOAA personnel will be delivered to the Houma boat yard for at-sea transfer from the support vessel to the receiving vessel. Samples under NOAA Chain of Custody will be unloaded at the Houma yard and taken to a secure sample processing facility under trustee control in Baton Rouge. Cardno ENTRIX resupply needs and sample intake will occur out of the Houma yard, supported by CSA according to standard cruise operations.

Sampling Equipment and Containers

Equipment (ROV mounted):
Sampling deployment gear to sample at full depths (to 2500 m) (CSA)
Seabird CTD with dissolved oxygen sensor and CDOM fluorometer to full depths (CSA)
Chelsea Labs AquaTracka *in situ* fluorometer (CSA)
ECO-FL *in situ* fluorometer (CSA)
Go-Flo bottle samplers (CSA)

Sample Containers
Estimated jars and containers, including field blanks and field duplicates – see Table 4, Attachment 1
Coolers

Personnel

The allocation of personnel aboard the *HOS Sweetwater* is as follows:

8 NOAA contractors:
Dr. David Valentine (UCSB) or Dr. James Payne, Chief Scientist
1 Co-Lead scientist
2 Red Crab Specialists
6 Water/Sediment Samplers
3 Data Managers
7 ROV Technicians

1 Operation Supervisor (CSA)
2 Survey/Navigation technician (CSA)
1 field technician (CSA)
2 Cardno ENTRIX Personnel

Vessels

All instrumentation and sampling operations will be conducted aboard the *HOS Sweetwater*. At-sea transfers of supplies and samples will be performed by the *Emily Bordelon* or similar supply vessel operated by Bordelon Marine, Houma, LA.

Safety Plans

BP's full operations and safety plans are attached as appendices. A HASP binder is provided to each vessel. In addition, the NOAA incident site safety plan (which all NOAA employees and contractors must sign prior to the cruise) is attached (Attachment 11). Vessels will report in daily using the attached situation report (Attachment 16).

Distribution of Laboratory Results

Water samples (whole water samples, filtered water, and associated filters) for VOC, Total Hydrocarbons, and PAH analysis will be sent to Alpha Analytical Laboratories in Mansfield, MA (Table 2) under NOAA Chain of Custody (COC). Likewise, sediment for Total Hydrocarbons and PAH analysis will also be sent to Alpha Analytical under NOAA COC. Water samples for dispersant analyses (in 15 mL centrifuge tubes) and water in 1 L containers for TSS and CHN analyses will be sent to Columbia Analytical Services under NOAA COC.

The following provision will apply to 1) any laboratory analysis of samples generated pursuant to cooperative agreement; and 2) in the absence of a cooperative agreement for sample analysis, laboratory results for PAH and fingerprinting MC 252 oil to be provided to BP pursuant to the Sample Analysis section of this Plan.

Each laboratory shall simultaneously deliver raw data, including all necessary metadata, generated as part of this work plan as a Laboratory Analytical Data Package (LADP) to the trustee Data Management Team (DMT), the Louisiana Oil Spill Coordinator's Office (LOSCO) on behalf of the State of Louisiana and to BP (or Cardno ENTRIX on behalf of BP). The electronic data deliverable (EDD) spreadsheet with pre-validated analytical results, which is a component of the complete LADP, will also be delivered to the secure FTP drop box maintained

by the trustees' Data Management Team (DMT). Any preliminary data distributed to the DMT shall also be distributed to LOSCO and to BP (or Cardno ENTRIX on behalf of BP). Thereafter, the DMT will validate and perform quality assurance/quality control (QA/QC) procedures on the LADP consistent with the authorized Analytical Quality Assurance Plan, after which time the validated/QA/QC'd data shall be made available simultaneously to all trustees and BP (or Cardno ENTRIX on behalf of BP). Any questions raised on the validated/QA/QC results shall be handled per the procedures in the Analytical Quality Assurance Plan and the issue and results shall be distributed to all parties. In the interest of maintaining one consistent data set for use by all parties, only the validated/QA/QC'd data set released by the DMT shall be considered the consensus data set. In order to 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. 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).

Table 2. Summary of water sample volumes, containers, and handling procedures required for primary analytes. Details are provided in the Water Sampling Protocol (Attachments 6-7). All analytes will be under NOAA COC and sample handling procedures.

Analyte	Sample Volume	Sample Container	Sample Handling	Holding Time	Lab
Water: PAH (extended) TEH, Dispersant indicators (DPnB)	1 L	Amber Glass, Chem Certified Clean	4° C (refrigerate)	7 days	Alpha (Mansfield, MA)
Water: PAH (extended) TEH, Dispersant indicators (DPnB)	4 L	Amber Glass, Chem Certified Clean	4° C (refrigerate)	7 days	Alpha (Mansfield, MA)
Dispersant (DOSS)	4 x 15 mL	Centrifuge tubes	0° C (freeze)	N/A	CAS (Kelso, WA
TSS/CHN	1 L	Amber Glass, Chem Certified Clean	4° C (refrigerate	7 days	CAS (Kelso, WA
Water: VOA	80 mL	2 x 40 mL pre-acidified (HCl) vials w/ septa	4° C (refrigerate)	14 days	Alpha (Mansfield, MA)
Filtration samples (Payne filtering products)	150 mL	Glass fiber filters associated with each 3.5 L (4 L amber glass jug) are frozen in 150 mL jars immediately after collection.	0° C (freeze)	N/A	Alpha (Mansfield, MA)
Sediment samples: PAH (extended) TEH, Dispersant indicators (DPnB), TOC	(various)	Subsamples from sediment cores	0° C (freeze)	N/A	Alpha (Mansfield, MA)
Microbial samples	(various)	Subsamples from water and sediment cores	-80° C (freeze)	N/A	UCSB

Budgeting

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 approved work plan that may arise. The trustees will make a good faith effort to notify BP in advance of any such increased costs.

The field survey costs, miscellaneous costs, and travel costs indicated in Budget Chart # 1 below shall be reimbursed by BP upon receipt of written invoices submitted by the Trustees. The Vessel Costs indicated in Budget Chart # 2 shall be paid directly by BP.

Budget Chart #1 Survey Costs (NOAA).

Field Survey Costs	Hrs/Days/Trips	Day/Hr Rate	Total
NOAA Labor (days):			
David Valentine (UCSB)	[redacted]	[redacted]	$75,000
James Payne (PECI)	[redacted]	[redacted]	$75,000
Co-lead scientists	[redacted]	[redacted]	$130,000
Scientist/biologist	[redacted]	[redacted]	$104,000
[redacted] Water/Sediment Samplers	[redacted]	[redacted]	$208,000
[redacted] Data Managers	[redacted]	[redacted]	$156,000
Misc Costs Sample Handling and Supplies	1	$25,000	$25,000
Travel	1	$20,000	$20,000
TOTAL			**$793,000**

Days/Trips based on [redacted] in field, plus preparation time for Chief Scientists
Labor is estimated cost and hours

Budget Chart #2 Vessel Costs (CSA).

Vessel Cost Table	Total
Mobilization Costs	$270,000
Vessel Costs	$5,718,839
Fleet Mgmt / Shore Support	$400,000
Total Estimated Vessel Cost	$6,388,839

Total Cost: $7,181,839

BP agrees to pay the costs of any laboratory results for PAH and fingerprinting for MC 252 oil that are provided to BP outside of any executed cooperative analytical addendum.

References

Payne, J.R., T.J. Reilly, and D.P. French, "Fabrication of a Portable Large-volume Water Sampling System to Support Oil Spill NRDA Efforts," in *Proceedings of the 1999 Oil Spill Conference*, American Petroleum Institute, Washington, D.C., pp. 1179-1184, 1999.

Perry, Harriet (University of Southern Mississippi, pers comm.). Fisheries Center, Gulf Coast Research Laboratory, Ocean Springs, MS. See the following publications for further information:

Lockhart, F.D., W.J. Lindberg, N.J. Blake, R. B. Erdman, H.M. Perry, and R.S. Waller. 1990. Distributional differences and population similarities for two deep-sea crabs (family Geryonidae) in the northeastern Gulf of Mexico. Can. J. Fish. Aquat. Sci. 47(11): 2112-2122.

Waller, R., H. Perry, C. Trigg, J. McBee, R. Erdman, and N. Blake. 1995. Estimates of harvest potential of the deep sea red crab, *Chaceon quinquedens*, in the north central Gulf of Mexico. Gulf Res. Repts. 9(2): 75-84.

Weber, Thomas, 2011. Personal communication, University of New Hampshire, Jun 2011.

List of Appendices (NOAA)

Attachment 1.Targeted Sampling Locations and Prioritization for the *HOS Sweetwater* Cruises 4 and 6 Sediment/Near-Bottom Water Sampling
Attachment 2. ROV Operations
Attachment 3. Canyon ROV Triton XL info
Attachment 4. ROV_Sediment_ Collection
Attachment 5. Chelsea AquaTracka Fluorometer
Attachment 6. Water Sample Handling Procedures 2011_03_16
Attachment 7. PLVWSS sampling protocols in support of NRDA Cruises_050510
Attachment 8. MC252 Analytical QAP V2.2
Attachment 9. Quality Assurance Guidelines for NRDA Water Column Chemistry
Attachment 10. NRDA_Field_Sampler_Data_Management_Protocol_7_5_2010
Attachment 11. NOAA-NRDA_MC_252_Site_Safety_Plan_5.13.10
Attachment 12. CSA-Sweetwater HSE Plan Rev 005_Final
Attachment 13. MC252 HSSE Incident Reporting Final 02 May 10 rev 1
Attachment 14. Transfer of Personnel and Material at Sea 070510
Attachment 15. NRDA Offshore Vessel Reporting 071311
Attachment 16. DWH Vessel Daily SitRep
Attachment 17. Slurp-gun operations and sample collection
Attachment 18. ROV_Biota_Collection
Attachment 19. Protected Species Interaction Prevention Procedures for No-impact Gear Types
Attachment 19A. NMFS Protocol for Dead Entangled Small Cetaceans
Attachment 19B. Sea Turtle Retrieval Resuscitation Protocols
Attachment 19C. Turtle Stranding Report Forms_STSSN
Attachment 19D. Vessel Strike Avoidance Guidance
Attachment 19E. 201106016_Final acoustic measures_NRDA BMPs
Attachment 20. HOS Sweetwater 4/6 Seep Flux Quantification Testing

List of Appendices (CardnoENTRIX)

MC252 NRDA Water Column Cruise Appendices
COCs
CardnoENTRIX MC252 Chain of Custody Instructions
CardnoENTRIX MC252 Chain of Custody Template and Example CAS 103110
CardnoENTRIX MC252 Photograph and GPS COC
CardnoENTRIX Electronic Data CoC
Data Sheets
MC252 ROV datasheet
Sampling and Data Management
CardnoENTRIX Marine Assessment Shipboard Data Management Procedures 103110
CardnoENTRIX MC252 NRDA Checklist for Electronic Data Transfer at Cruise Completion
CardnoENTRIX MC252 NRDA Water Column Cruise Daily Cruise Report (DCR) Template
Safety
BP IH Short Form
BP IIR Short Form
CardnoENTRIX CSA Next of Kin List
CardnoENTRIX MC252 NRDA HSE Directions from dock to hospital
CardnoENTRIX MC252 NRDA Water Column Transfer of Material at Sea
CardnoENTRIX MC252 Tailgate Safety QA Meeting Form
HSE Plan CardnoENTRIX NRDA Water Column Cruise 103010
Houma Incident Command PFD Requirements Jul 2010
ICS 213 Deepwater Horizon Heat Stress Management Plan 052810
Material Safety Data Sheet: Hexane_MSDS_03-16-2010
Material Safety Data Sheet: Hydrochloric Acid_MSDS_Feb 2010
Material Safety Data Sheet: Liquinox_MSDS_english_ansi
Material Safety Data Sheet: Methanol_MSDS_US_06-25-2010
MC252 Incident Reporting Standing Order
MC252 Lightning and Tornado Plan Jun 2010
MC252_Incident_SIMOPS_Plan_May10_2010_Rev2
NOLA UAC Heat Stress Plan Aug 2010
"Used Material" Label
"Hazardous Material" Label
SIC Protocols
SIC NRDA SOPs PowerPoint Slides (11)
SIC NRDA SOPs Word Documents (11)
Contact With Questions
Jodi Harney, [redacted]

MC252 Deepwater Horizon Oil Spill
Deepwater Benthic Communities and Water Column Data Collection

July-September 2011 *HOS Sweetwater*
ROV Sediment and Bottom-Water Sampling Cruise Plan

July 15, 2011

HOS Sweetwater 4
Leg 1 July 14– August 7 (25 days), Dave Valentine, Chief Scientist

HOS Sweetwater 6
Leg 1 August 22 – September 1 (11 days), Jim Payne, Chief Scientist
Leg 2 September 10-25 (16 days), Jim Payne, Chief Scientist

Approvals

Approval of this work plan is for the purposes of obtaining data for the Natural Resource Damage Assessment. Each of the parties reserve its right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan. Signature by Trustee representatives is not intended to and should not be taken to approve or endorse Cardno ENTRIX appendices.

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.

The trustees have developed a preliminary conceptual model of the DWH release, potential pathways and routes of exposure, and potential receptors. This preliminary model has informed the trustees' decision to pursue the studies outlined in the work plan. By signing this work plan and agreeing to fund the work outlined, BP is not endorsing the model articulated in the work plan.

BP Approval:

Printed Name	Signature	Date
Lawrence Malnar	LDH	11/10/2011

Federal Trustee Approval:

Printed Name	Signature	Date
ROBERT W. RICKER	Robert W. Ricker	8/22/2011

Louisiana Trustee Approval:

Printed Name	Signature	Date
DEBUSSCHERE	FOR ROLAND GUIDRY	11/29/11