Study Plan for NRDA-Phase II Project

Deepwater Sediment Sampling to Assess Post-Spill Benthic Impacts from the Deepwater Horizon Oil Spill

Deepwater Benthic Communities Technical Working Group

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1.0 Background and Objectives:

The Deepwater Horizon (DWH) incident in the northern Gulf of Mexico occurred on April 20, 2010 at a water depth of 1525 meters in Mississippi Canyon Block 252. While some of the oil from DWH incident would be expected to float (average density of 0.849, API 35.2), portions potentially could have moved into offshore and deepwater (> 200 meters) sediments via multiple hypothesized pathways — e.g., sinking of oil and/or dispersed oil droplets adsorbed onto suspended particles, or incorporated into copepod fecal pellets, in either surface or sub-surface layers; onshore-offshore transport of oil-laden particles; sinking of heavier oil by-products resulting from the burning of oil; or settling of oil-mud complexes resulting from the injection of drilling mud during top-kill operations (Figure 1). In addition, drill cuttings, drill fluids, and other containment fluids may have been released and deposited to the bottom during the blowout[1]. Preliminary observations and measurements from prior cruises have noted the presence of oil or oil-like material in bottom sediments at several deepwater locations near the DWH well-head and within the potential paths of oil movement.

An Implementation Plan for enhanced subsurface monitoring was developed by the Unified Area Command (UAC, Final 13 November 2010) as a basis for assessing the presence of actionable oil posing a threat to public health or the environment and to serve as a framework for transitioning from response to injury assessment and recovery phases. The Implementation Plan emphasized the need for sampling in offshore and deepwater sediments where oil may have migrated and where gaps in sampling efforts exist. Pursuant to this plan, a series of initial Response cruises in offshore and deeper waters were conducted through October 2010. Two such field missions — the Gyre (September 16 through October 19, 2010) and Ocean Veritas (September 24 through October 30, 2010) — are of particular interest as the majority of the sampling locations were at depths greater than 200 meters. Both cruises collected sediment samples for analysis of oil, benthic communities, and toxicity (Microtox) at near-field sites around the well-head (within a radial/bulls-eye array) and additional far-field sites under known surface water slick areas, beneath subsurface dispersed oil, and at historic sampling sites with pre-spill benthic reference data (Figure 2, Appendix A). The pre-spill sampling sites are from a MMS (BOEMRE) sponsored study (Deep Gulf of Mexico Benthos Program, DGoMB)

[1] BP's approval of this work plan shall not be construed as an admission of the accuracy of the conceptual model of fate/transport of hydrocarbons/oil presented in this background description. This reservation applies to all references to trustee models or data interpretations contained herein.

conducted in 2000-2002 (Rowe and Kennicutt 2008, 2009). On both the Gyre and Ocean Veritas cruises, a multi-corer system (OSIL Bowers and Connelly Multiple Corer) was used to collect the sediment samples. This unique system is designed to collect undisturbed samples of seabed sediment and overlying supernatant water and minimizes the risk of a bow-wave effect that might otherwise displace the upper surface layer of sediments or flocculant and any associated oil and thus compromise the results.

The proposed study is intended to build upon results of the Implementation Plan efforts by making use of samples already collected and conducting follow-up sampling in deepwater areas to help identify any potential oil-related impacts on sediments and resident benthic fauna. The sampling design will focus on sites where oil was observed, that are in paths of oil exposure predicted by the Trustee conceptual model, and that serve as anticipated reference sites (including the historic DGoMB sites and other areas where MC252 oil has not been found) and is based on the Implementation Plan results as presented in the recent OSAT (2010) Report.

The overall goal of this study is to help identify any potential impacts of the DWH oil spill on deepwater sediments and resident benthic fauna in support of the NRDA injury-assessment process. There are two fundamental questions to be answered in addressing this goal. First, are sediments in areas with a greater likelihood of exposure, such as near the well-head, under the former surface sea slick, or under the dispersed sub-surface hydrocarbons, impacted by hydrocarbons traceable to the oil spill? If the answer is yes, the second question is, do living benthic resources show evidence of a difference in community indices or other injuries that can be related to exposure to the hydrocarbons? To help address such questions, the study will focus on three primary objectives discussed in further detail below.

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 and will 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, paragraph 6 of Pretrial Order #30, the entirety of Pretrial order 16 which details the retention of metadata, 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.

This plan will be implemented consistent with existing Trustee regulations and policies. All applicable state and Federal permits must and will be obtained prior to conducting work.

2.0 Methods and Approach:

2.1. Objective 1: Examine the spatial pattern and magnitude of hydrocarbon exposure in deepwater sediments to help inform follow-up sediment sampling designs and facilitate biological interpretations.

The OSAT (2010) report presents results of hydrocarbon analyses performed through Response efforts on 127 sediment samples from deepwater (> 200 m) locations. The samples are among those collected in fall 2010 on the Gyre and Ocean Veritas cruises (Figure 2, Appendix A; 169 sample sites total including sites < 200m). As part of these analyses, concentrations of polynuclear aromatic hydrocarbons (PAHs) were measured in each sample and compared to corresponding EPA aquatic life benchmarks for PAHs in sediments. Samples that exceeded the benchmarks were examined in further detail using oil fingerprinting methods to assess the likelihood that exceedances were due to MC252 oil. Results of these analyses are discussed in the OSAT (2010) report. In addition, the data (i.e., total PAH concentrations) can be accessed through the Environmental Response Management Application® (ERMA) database [redacted] a web-based GIS tool maintained by NOAA/ORR, University of New Hampshire, and EPA.

PAH sediment exceedances were found at a total of seven stations all located within 3 km of the well-head (OSAT 2010). All seven samples had hydrocarbons consistent with MC252 oil. Concentrations of total PAHs in these samples ranged from 9,900 – 28,000 ng/g (OSAT 2010). Two additional nearfield sites within this 3-km zone were not listed as PAH hits, but had concentrations in excess of other published Sediment Quality Guidelines (SQGs) for total PAHs: either the Effects Range Low (ERL) value of 4,022 ng/g (Long et al. 1995) or Threshold Effect Level (TEL) value of 1684 ng/g (MacDonald et al. 1996). For comparison, total PAHs in sediments from pre-spill reference sites (Rowe and Kennicutt 2009) ranged from 0 – 1033 ng/g (mean of 140 ng/g). An additional pre-spill study in 2000 – 2003 at 45 locations within Lease Blocks VK916, GB516, GB602, and MC 292 (Continental Shelf Associates 2006) found total PAHs in farfield sediments at concentrations ranging from 93.8 – 748 (mean of 237 ng/g) across a range of reference stations; and two samples within 300 m of the GB516 drill site had concentrations of 3,470 and 23,840 ng/g. Another conclusion of the OSAT (2010) report was that sediment PAH concentrations were above average reference levels at some locations within 9 km of the well-head.

Results of the above hydrocarbon analyses have been used in our planning thus far to help identify a subset of the fall 2010 Response samples to work up initially for benthic community analysis and to develop the sampling design for follow-up field sampling (Objectives 2 and 3 of the present study); however, the Trustees do not believe that the data presented in the OSAT (2010) report are necessarily informative or conclusive with regard to natural resource injuries. Because, according to the OSAT (2010) report, the highest concentrations of oil consistent with MC252 oil were found within 3 km of the well-head, one of the emphases is on samples within this nearfield zone. These and other priority sites (total of 65) were selected based on the following overall criteria:

- Nearfield sites within ~ 3 km of well-head (17 sites) — Area where OSAT data have shown sediments containing oil consistent with MC252 in excess of EPA aquatic life benchmarks (OSAT 2010).
- Mid-field sites within 25 km of well-head (23 sites) — Area within suggested path of oil movement in various directions, based on subsurface trajectory modelling results

performed by NOAA contractors[2]; also includes the portion within 9 km of the well-head where sediment PAH concentrations were above average reference levels (OSAT 2010). Preliminary verbal reporting of laboratory data from the HOS Davis NRDA Cooperative cruise in December 2010 also suggests the presence of MC252 oil in sediments at two sites within this region.

- Farfield sites > 25 km of well-head within suggested paths of oil movement based on subsurface trajectory modelling results performed by NOAA contractors (15 sites) — 14 sites located to the southwest and one site (DO43S) about 50 km to the northeast.
- Farfield sites > 25 km NW of well-head (2 sites) — Path of potential transport of oil to seafloor due to possible interaction of surface oil with the sediment-laden Mississippi River plume; also, qualitative indications of oil were found in some sediment samples from this area (OSAT 2010).
- Pre-spill DGoMB reference sites (Rowe and Kennicutt 2009) (8 sites) — Areas where baseline sample results exist for pre-spill/post-spill comparisons.

Of the above 65 selected sites, seven were listed in the OSAT (2010) report as containing PAHs consistent with MC252 at levels above EPA aquatic life benchmarks (see Appendix A herein) and two more (ALTNF015, LBNL1) had relatively high levels of total PAHs in excess of other published SQGs; 31 are likely to be non- to lesser-contaminated sites at comparable depths (1300 - 1700 m, with most between 1400 – 1600 m) as the more heavily oiled sites (1493 - 1607 m); eight are historic/pre-spill DGOMB sites; and the remaining sites (17) provide additional spatial coverage over a wider range of depths in comparison to sites where oil has been observed at elevated levels.

It is noted that initial Response efforts only included oil fingerprinting on sediment samples that contained PAHs in excess of EPA aquatic life benchmarks. As benthic samples are processed in the present study, it may become necessary to have additional laboratory analyses performed on some samples to support the interpretation of biological results. In addition, while the ERMA website provides access to data on total PAH concentrations, data on individual PAH concentrations (which should be available) and laboratory QA/QC packages will be needed to facilitate biological and chemical interpretations.

Data from the above Response efforts and other potentially relevant information on hydrocarbon concentrations within the region, as they become available, may be used to assess potential impacts of the oil spill on benthic communities. Maps depicting the spatial pattern and magnitude of oil exposure in sediments also will be prepared to help depict potential linkages between presence of MC252 oil and results of the benthic analysis. Additional hydrocarbon data resulting from the analysis of sediment samples collected in spring 2011, as part of the present study, will provide a basis for assessing how the pattern and levels of hydrocarbons may have persisted or changed relative to prior sampling efforts.

2.2. Objective 2: Assess potential impacts of the spill on benthic infauna from archived samples collected in Fall 2010 (Gyre and Ocean Veritas cruises).

[2] BP's approval of this work plan shall not be construed as an admission of the accuracy of the NOAA models or data interpretations relied on in selecting sites for surveys. This reservation applies to all references to trustee models or data interpretations contained herein.

This work will involve the processing of the archived fall 2010 benthic infaunal samples (both macrofauna and meiofauna), focusing on the subset of priority samples discussed above. There is a total of 65 of these priority samples: 17 from nearfield sites within 3 km of the well-head, 23 additional mid-field sites within 25 km of the well-head, 15 farfield sites > 25 km of the well-head (14 southwest of the well-head and one site ~50 km to the northeast), two farfield sites > 25 km NW of the well-head, and eight pre-spill DGoMB reference sites from the Rowe and Kennicutt (2009) study (Figures 3a, 3b, Appendix A). If it is determined that additional data from any of the remaining 104, fall 2010, sampling sites are needed as the project evolves, then these samples may be processed as well (through an addendum to this work plan).

Macrofaunal samples from the fall 2010 cruises were collected and processed in the following manner: (1) three sediment cores (0.01 m^2 each) collected from a single multi-core drop at each station; (2) each core extruded into two vertical sections (0-5 and 5-10 cm deep); and (3) samples preserved in the field in 4% buffered formalin with Rose Bengal, sieved in the laboratory on a 0.3-mm mesh screen, and transferred to 70% ethanol. Individual cores from the same multi-corer drop, though processed separately, will be pooled mathematically to serve as an individual sample (0.03 m^2) for data-analysis purposes. Meiofaunal samples from the fall 2010 cruises were collected and processed in the following manner: (1) 1 sediment core (0.01 m^2) collected from a single multi-core drop at each station; (2) each core extruded into two vertical sections (0-1 cm and 1-3 cm deep); and (3) samples relaxed in the field in 7% $MgCl_2$ and preserved in 4% buffered formalin with Rose Bengal, sieved in the laboratory on a 0.042-mm mesh screen, and transferred to 70% ethanol.

Macrofauna from each of the above samples will be identified to lowest possible taxonomic level and enumerated. It is anticipated that for most taxa this will be at the family level. Identifying species to the family level is justified for three reasons: (1) it increases the speed at which the analyses can be performed; (2) very few of the deep-sea species are known to species level — for example, only 40% (207 of 517) of polychaete species and 25% (31 of 124) of the amphipod species found in the DGOMB study could be identified to the species level; and (3) benthic data at the family level have been shown to detect the same basic patterns as those developed to the species level and often with less noise due to eliminating the influence of individual rarer species (Heip et al. 1988, Warwick 1988, Warwick et al. 1988, Montagna and Harper 1996). More obvious dominant taxa may, however, be identified to genus or species as appropriate. Also, all specimens will be archived so that identifications to lower taxonomic levels can be made at a later date if that is deemed desirable. To help inform such a decision, a subset of the fall 2010 samples — 10% (7) of the above 65 priority sites selected over a broad range of habitats — will be processed to a finer taxonomic resolution, i.e. species level wherever possible. The results will be used to test whether the ability to detect among-station differences is improved significantly with the finer-resolution taxonomic data. A synthesis of the literature by Peterson et al. (1996) of benthic responses to marine pollution suggests that macroinfaunal and meiofaunal communities exhibit repeatable patterns of response to sedimentary contamination generally detectable at high taxonomic levels, even the phylum level.

Meiofauna will be counted and identified to the lowest possible taxonomic level (family level for Harpacticoida and higher taxa levels for other meiofaunal groups that lack reliable species-level

systematics for the Gulf of Mexico). Meiofaunal biomass also will be measured using a semi-automated microphotographic technique (Baguley et al. 2004, 2008) used successfully by members of the project team in prior Gulf of Mexico deep-benthic studies.

Biological response variables will include common benthic attributes such as numbers of taxa, diversity, total density, species abundances, community structure, Swartz's Dominance Index, biomass (for meiofauna only), and percentages of sensitive vs. tolerant species (e.g., based on family guild structure and known responses) that have been proven in previous studies to serve as sensitive indicators of pollution-induced disturbances (Engle et al. 1994, Van Dolah et al. 1999, Simboura and Zenetos 2002, Peterson et al. 1996). For example, bioindicators of contaminant exposure around platforms in the Gulf of Mexico include the relative percentages of sensitive species such as echinoderms and crustaceans (especially amphipods and harpacticoids) versus other more tolerant species such as polychaetes, oligochaetes, and nematodes (especially non-selective deposit feeders) that are often enhanced by presence of contaminants (Peterson et al. 1996). In addition, the biota will be examined for evidence of visible abnormalities, such as oil-coated appendages, high incidence of empty shells or other animal parts, lesions, relatively high incidence of parasites, and any other abnormal appearances that may be apparent. As a specific example, harpacticoids will be inspected to determine if the external egg sacs are oiled or exhibit signs of deterioration. Potential effects of pressure changes in bringing these invertebrate animals to the surface should be minimal (e.g., due to lack of swim bladders present in fishes) and thus should not interfere with the ability to detect signs of such oil-related abnormalities if present.

Taking samples along potential exposure gradients in different directions from the incident site has been shown in the peer-reviewed literature to be a scientifically effective method for detecting long-term environmental impacts (Kennicutt et al. 1996a). This kind of sampling design is often referred to as a "bulls-eye" design because transects extend in radial patterns from the source of the contamination and the statistical analysis of such designs is well known (Kennicutt et al. 1996a). The present sampling design includes stations in a bulls-eye configuration with an increasing concentration of stations moving closer to the well-head (Fig. 3b). Additional stations > 25 km to the SW of the well-head provides another means to look for gradients of response with increasing distance from the well-head (Fig. 3a).

Among-station spatial comparisons will also be facilitated by applying appropriate multivariate data-analysis methods — e.g., combinations of numerical classification (cluster analysis), multidimensional scaling, principal components analysis, and discriminant analysis to define groups of stations with similar faunal composition and then to identify which of the measured abiotic environmental variables (including oil concentrations) are the most correlated with any observed among-group differences (e.g., see Green and Vascotto 1978, Green and Montagna 1996, Hyland et al. 1991) (also see Objective 3 below).

The inclusion of samples coinciding with historical/pre-spill DGOMB sampling sites (Rowe and Kennicutt 2008, 2009) also provides a basis to test for temporal differences in benthic fauna before versus after the spill at specific locations around the study area. This information will help provide some reference to natural temporal variability in these fauna that may be useful in evaluating the significance of potential biological impacts associated with the oil spill. For

example, such information could be valuable in addressing the relevant question: if there are observed variations in benthic fauna between oiled versus non-oiled sites, based on spatial comparisons of samples collected after the spill, then how different are these variations from the normal ranges of temporal variability within the region? Note that because different sampling gear and methods were used to collect benthic samples at DGOMB stations during the original, 2000-2002, pre-spill study (Rowe and Kennecutt 2008,2009) vs. post-spill (fall 2010) Response cruises, the sample sizes (surface area of the sampling units) vary between collections (e.g., macrofauna: 0.17 m^2 for 2000-2002 study, 0.03 m^2 for fall 2010 effort) and adjustment factors may need to be applied.

2.3. Objective 3: Conduct a follow-up cruise in spring 2011 to assess potential oil-related impacts on deepwater sediments and benthic infauna approximately one year after the DWH oil spill.

Overall Scope. A follow-up cruise will be conducted in spring 2011 (May 23 – June 16 proposed), as part of a proposed time series, to assess potential oil-related impacts on deepwater sediments and associated benthic fauna at a subset of the stations sampled previously in fall 2010 **(**including oiled and comparable reference stations). A request for a ship and additional equipment, including a multi-corer (12 core system) and conductivity-temperature-depth (CTD) instrument, has been presented to the Vessel Coordination Committee in support of this mission. The M/V Sarah Bordelon has been proposed by the Vessel Coordination Committee. The cruise will consist of two legs of approximately 12 days each with a 1-day port call in-between. Some contingency time (2.5 days total) is included to cover potential weather/equipment downtime and/or opportunistic sampling in support of other collaborative project needs. In addition, a preliminary "shake down" cruise is anticipated, to be conducted from May 17th through the 19th, to ensure that all equipment will be functioning properly prior to the cruise. No samples will be collected for analysis as part of the "shake down" cruise.

A total of 38 core sampling sites have been selected for this task (Figure 4a, 4b). They include:

- 17 nearfield Response sites within ~ 3 km of the well-head in a bulls-eye pattern — Inclusive of all seven sites listed in OSAT 2010 report with PAHs in excess of EPA aquatic life benchmarks and three additional sites with PAHs in excess of other published, ERL or TEL, Sediment Quality Guidelines. Remaining seven stations are at comparable depths (1439-1585 m) as the above oiled sites (1493-1607 m).
- Seven Response sites within 3 to 25 km of the well-head — These stations (D050S, LBNL14, D019S, D024S, 2.21, FF010, LBNL7), which are located in various directions from the well-head and have depths ranging from 1356-1697 m, were selected to expand spatial coverage within the 25-km radius of the well-head while staying at depths as comparable as possible to the above oiled sites. Also, one of the below-listed historic/pre-spill sampling sites, MC292 (CSA 2006), provides additional spatial coverage within the 25-km radius, though at a shallower depth (1025 m) than other targeted sites within this zone.
- Four Response sites beyond 25 km of the well-head— These consist of three sites (LBNL9, LBNL10, D062S) to the southwest and one site (D043S) to the northeast of the

well-head, which are at depths (1303-1516 m) as comparable as possible to the above oiled sites.

- Seven historic/pre-spill reference sites — Five DGoMB (Rowe and Kennicutt 2009) sites (S36, D002S, HiPro, FFMT4, FFMT3) and two CSA (2006) sites (MC292, VK916). One of these sites (MC292) also provides additional spatial coverage within 25 km to the northwest of the well-head, two (HiPro and FFMT4) provide additional spatial coverage beyond 25 km to the southwest of the well-head, and three (VK916, S36, D002S) provide additional spatial coverage to the northeast and east of the well-head.
- Three Response sites within 3 to 9 km of well-head — These stations (LBNL17, LBNL3, and LBNL4; Figs 4a, 4b) are at similar depths (1422-1595 m) as the well-head and provide additional mid-field spatial coverage to the southwest.

The present scope of work and budget covers the collection and processing of samples from each of the above 38 fixed/repeated sampling sites for the spring 2011 cruise. After this mission is completed and preliminary results become available, we will evaluate whether there is a need to continue the time series beyond the spring 2011 sampling period or collect additional samples to better understand the geographic distribution of oiling and any potential associated adverse effects.

At each station, a multi-corer (12 core system) will be used to collect sediment samples for analysis of macrofauna, meiofauna, hydrocarbons, metals, pore-water chemistry (Eh, sulfides, ammonia), and other basic sediment properties (total carbon [TC], total organic carbon [TOC], total inorganic carbon [TIC], total nitrogen [TN], grain size). Additional samples for microbial analysis and sediment toxicity testing will be collected opportunistically from the multi-corer to be analyzed outside the scope and budget of this project. A CTD with dissolved oxygen (DO) and Aquatracka fluorometer sensors also will be deployed to obtain water-column profiles of salinity, temperature, DO, pH, fluorescence, and depth.

Required NRDA QA/QC procedures for field sampling, including logbook documentation of sampling activities and sample-custody tracking, will be followed throughout the cruise mission. Procedures to protect personnel safety during field operations will follow guidelines provided in the NOAA Deepwater Horizon NRDA Field Safety Plan, latest 1/28/2011 version (NOAA 2011). Decontamination procedures during field operations will follow steps summarized in Appendix C, which are based on relevant methods from a combination of the following guidance documents: (1) NOAA/Office of Marine and Aviation Operations's Procedure 1110-01/Version 1, "NOAA Ship Operations Near Deepwater Horizon Effluents" (NOAA/OMAO 2010); (2) NOAA National Status & Trends field operations manual (Lauenstein and Young 1986); (3) U.S. EPA EMAP/National Coastal Assessment Quality Assurance Project Plan (U.S. EPA 2001); and (4) U.S.EPA Region II CERCLA Quality Assurance Manual, Revision 1 (U.S.EPA/Region II 1989).

Multi-corer Deployments. A multi-corer will sample up to 12 individual cores (diameter of 110 mm) in each deployment with the ability to obtain relatively undisturbed sediment samples and overlying supernatant water. Sediments extruded from the cores will be processed for infaunal (macrofauna and meiofauna) community analyses, measurement of sediment properties and pore-water chemistry, and analysis of hydrocarbon and metal concentrations (Table 1). The larger 12-core multi-corer system is proposed for this study, because of its ability to collect

additional spare cores to provide backup material or to support other potential analyses outside the present scope of work and budget (e.g., sediment bioassays and microbial analyses). Core locations in the corer system will be sequentially numbered and cores will be assigned to study elements using a random number generator. At each station, three replicate sets of samples (from three separate multi-corer drops) will be collected for each of the sample types.

Sampling and Analysis of Sediment Properties. One of the cores from each of the multi-core drops will be used for the analysis of sediment properties: total carbon (TC), total organic carbon (TOC), total inorganic carbon (TIC), total nitrogen (TN) and grain size. Sediment from each core will be extruded and divided into four vertical sections (0 – 1 cm, 1 – 3 cm, 3-5 cm, and 5-10 cm deep). Samples for the analysis of elemental carbon and nitrogen (TC, TOC, TIC, TN) will be placed in clean 4-oz glass jars with Teflon lid liners and stored at -20 °C. Samples for grain size will be placed in whirl-pack bags and stored at 4 °C. TC, TOC, TIC, and TN content of dried samples will be determined using a CHN (Carbon/Hydrogen/Nitrogen) analyzer. For grain-size analyses, standard sieve and hydrometer methods will be used (as described in ASTM D-422) to determine the relative percentages of various particle size fractions based on sediment classification procedures of Folk (1974). For consistency with other pre-spill benthic studies in the region (Rowe and Kennicutt 2008, 2009; CSA 2006), results for each sample will be reported as percent gravel, sand, silt, and clay. Thus, the sieving procedure will not include separating the sand fraction into finer subdivisions (i.e., coarse sand, medium sand, fine sand, very fine sand). This latter option should not be necessary since sediments throughout the sampling area are predominantly silt and clay (based on the above pre-spill studies).

Sampling and Analysis of Macrofauna. Three of the cores from each multi-corer drop will be used for the analysis of macro-infauna. The surface area of each individual core is ~0.01 m^2. In order to be consistent with protocols followed on the fall 2010 sampling cruises, the individual cores from the same multi-corer drop, although processed separately, will be pooled mathematically to serve as an individual sample (0.03 m^2) for data-analysis purposes. Three replicate samples, derived from three separate multi-corer drops, will be collected at each station.

Sediment from each core will be extruded into four vertical sections (0 – 3 cm, 3 – 5 cm, and 5 – 10, and 10-15 cm), preserved at sea in 4% buffered formalin with Rose Bengal, and sieved later on-shore. The upper three sections will be processed as part of the this project and the lowest section will be archived for possible subsequent analysis (outside the scope of this plan). On return to the laboratory, the formalin-Rose Bengal solution will be changed to a 70% EtOH solution. Samples will be sieved using a 0.3-mm mesh and a gentle wash.

Macrofauna in these various samples will be identified to lowest possible taxonomic level and enumerated. As for the analysis of fall 2010 benthic samples (previous section), it is anticipated that for most taxa this will be at the family level. As before, some of the more obvious dominant taxa may be identified to genus or species as appropriate. Also, all specimens will be archived so that identifications to lower taxonomic levels can be made at a later date if that is deemed desirable. Numbers of individuals will be recorded to lowest taxon (mostly family level) in each vertical section, from each individual core (within same drop), from each of the three replicate samples (separate drops) at a station.

The following benthic response variables may be calculated for each replicate sample:

- Infaunal abundance (total number of individuals per station)
- Infaunal density (total number of individuals per square meter)
- Densities of individual key taxa (e.g., numerical dominants)
- Species richness (e.g., number of taxa, Margalef's D)
- Taxa diversity (Shannon's Index H´)
- Evenness (Pielou's Index J´)
- Swartz's Dominance Index

In addition, as mentioned in the previous section, the biota also will be examined for evidence of visible abnormalities. Such effects could include oil-coated appendages, high incidence of empty shells or other animal parts, lesions, high incidence of parasites, and any other abnormal appearances that may be apparent.

A suite of standard univariate and multivariate data-analysis methods can be used to characterize benthic communities and examine patterns in relation to oil and other measured environmental variables. Multivariate analysis may include ordination of station species abundance data by multi-dimensional scaling using the Bray-Curtis similarity measure and hierarchical clustering of similarity values using the group-average sorting strategy. A test of the significance of dissimilarities determined by the ordination may be conducted using a non-parametric permutation procedure on the ordination similarity matrix (Clarke and Gorley 2006). Results are used to depict spatial and/or temporal groupings of samples. Principal components analysis (PCA) can then be applied in conjunction with these analyses to help determine whether the separation of groups can be explained by various measured abiotic environmental variables including levels of oil (*sensu* Green and Vascotto 1978, Green and Montagna 1996, Hyland et al. 1991). The analysis derives a reduced set of variables that best describe the variance, based on data represented by the different abiotic environmental variables. Variable loads, which are the correlations between the original variables and the PCA scores on each variable, provide a measure of the relative contribution of each variable to group separation. Sample scores provide a new derived multivariate variable containing information about the load of each sample. Each of the above biotic univariate metrics (density, diversity, number of sensitive taxa, etc.) can be regressed against the PCA scores for each sample (Green and Montagna 1996, Long et al. 2003) and analyzed using an appropriate test of significance to determine statistically significant differences among various station groups or group/time interactions (Kennicutt et al. 1996a, Montagna and Harper 1996). Also, because replicate samples (three) will be collected at each station during the spring 2011 sampling effort, potential impacts can be examined from spatial comparisons of benthic response variables between oiled versus reference sites of comparable depths using ANOVA (e.g., nested ANOVA to test for differences in benthic variables between oiled stations and reference stations, with three replicate measurements per each station; Sokal and Rohlf 1969).

Comparisons of post-spill benthic data with pre-spill data from DGOMB sites may focus on response variables and analysis approaches known to be relatively independent of sample-size differences (e.g., density/m^2, Hurlbert's $E(S_n)$ and related rarefaction methods). A similar approach may need to be used for comparisons with pre-spill data from the two CSA (2006) sites (MC292, VK916), which also were based on a different sample size (0.1 m^2).

Sampling and Analysis of Meiofauna. One of the sediment cores from each multi-corer drop will be used for meiofaunal analysis. Sediment from each 0.01-m^2 core will be extruded and divided into five vertical sections (0 – 1 cm, 1 – 3 cm, 3-5 cm, 5-10 cm, and 10-15 cm deep). Each vertical section will be sub-sampled with a 5.5 cm diameter corer (to be consistent with the fall 2010 sampling methods). Once extruded and subdivided, the sediment will be relaxed in 7% $MgCl_2$, preserved in 4% buffered formalin with Rose Bengal, and sieved later on-shore with a 0.042-mm sieve. After sieving, the samples are transferred to 70% ethanol. The upper four sections will be processed as part of this plan and the remaining section will be archived for possible subsequent analysis (outside the scope of this plan). Meiofaunal specimens will be counted and identified to the lowest possible taxonomic level (family level for Harpacticoida and higher levels for other meiofaunal groups that lack reliable species-level systematics for the Gulf of Mexico). The methods are consistent with those used in previous Gulf of Mexico deepwater studies by members of the project team (e.g., Baguley et al. 2008). Biomass will be measured using a semi-automated photographic technique (Baguley et al. 2004). In addition, the meiofauna will be examined for evidence of visible abnormalities that could be related to adverse effects of the DWH incident (see previous section). Data analysis methods to assess meiofaunal patterns will be the same as those described above for macrofauna.

Sampling and Analysis of Chemical Contaminants. One of the cores from each of the replicate multi-corer drops will be used for the analysis of hydrocarbons. Sediment from each core will be extruded and divided into four vertical sections (0 – 1 cm, 1 – 3 cm, and 3-5, and 5-10 cm deep). Once extruded, the sediment will be placed in clean 4-oz glass jars with Teflon lid liners, stored onboard the ship at -20°C, and transported frozen to the NRDA/Trustees analytical chemistry contractor (Alpha Analytical Laboratory) for extraction and analysis of hydrocarbons using approved NRDA QAP chemistry analysis and laboratory methods. The following measurements should be included: total petroleum hydrocarbons (TPH), also referred to as total extractable hydrocarbons (TEH), representing the total aromatic and aliphatic hydrocarbon content of the sample; PAHs including individual parent and alkyl homologues; saturated hydrocarbons including alkanes and isoprenoids; and oil finger-printing diagnostics. Analyses should follow methods provided in the NOAA MC 252 Analytical Quality Assurance Plan/Version 2.2 (NOAA 2011). For consistency, analyses also should include analytes measured in the fall 2010 Response samples (e.g., the 41 individual PAH analytes listed in Table A-3 of OSAT 2010 Report).

In addition, one of the cores from each replicate multi-corer drop will be used for the analysis of metals (e.g., silver, barium, cadmium, mercury, lead, nickel, vanadium, and zinc) that are common constituents of drill cuttings and drill muds (Kennicutt et al. 1996b) and Corexit 9500/9527 dispersant indicators (e.g., dioctylsulfosuccinate sodium salt, Dipropylene Glycol n-Butyl Ether). As with the analysis of hydrocarbons, the analyses will be performed by the NRDA/Trustees analytical chemistry contractor using approved NRDA QAP methods. Sediment from these cores also will be divided into four vertical sections (0 – 1 cm, 1 – 3 cm, 3-5, and 5-10 cm deep), split evenly for the metals and dispersant (Corexit) analyses, and placed in clean 4-oz glass jars with Teflon lid liners. Subsamples for metals analysis will stored onboard the ship at -20 °C, and transported frozen to shore-based Alpha Analytical Laboratory for extraction and analysis. Subsamples for Corexit analysis will stored onboard the ship at -20 °C, and transported frozen to Columbia Analytical Services for extraction and analysis.

Sediment Pore-Water Chemistry. Two of the cores from each of the multi-corer drops will be used for the analysis of pore-water chemistry: sulfide, ammonia, pH, salinity, temperature, and oxidation-reduction potential (Eh). A total of 50 mL of pore water is required to complete all analyses. Pore water will be collected in one of two ways: (1) *in situ* using a suction filtration method in order to minimize disturbance to the sediment and exposure to oxygen (Winger and Lasier 1991, Cooksey and Hyland 2007); or (2) *ex situ* centrifugation of sediment, as a fall back, if suction filtration methods do not provide a large enough volume of pore water for testing all parameters. While a large variety of methods are available for pore-water extraction, both *in-situ* suction filtration and *ex-situ* centrifugation have been widely used with only minor differences in chemistry found between these methods (Winger et al. 1998). However, the first method is the preferred one and preliminary extractions with sediment from the region yielded successful results.

For *in-situ* suction filtration, two fused-glass air stones will be placed at a depth of 3 cm in each core and assumed to sample approximately the upper surrounding 0-5 cm. A vacuum will be applied with either a 50 mL syringe or vacuum pump to facilitate suctioning. For *ex-situ* centrifugation, the upper 0-5 cm will be extruded from the core and placed in clean centrifuge tubes and centrifuged at high speed (10,000 g) for a maximum of 30 minutes in a refrigerated bench-top centrifuge. Any open handling of pore water for analysis, or sediment for pore water extraction, will occur under a nitrogen environment. Regardless of the extraction method, we will attempt to collect the required volume of pore water by combining the upper 0-5 cm of sediment from both cores into a single composite for each replicate multi-corer drop at a station. If a sufficient volume of pore water cannot be obtained from the two composited cores within a single multi-core drop, then samples will be composited from all three replicate drops at a station.

Sulfide and ammonia will be measured on a HACH DR/890 colorimeter while in the field. Measurement of total ammonia concentrations will follow the salicylate method in HACH (2009). Un-ionized ammonia nitrogen (UAN), the form considered the most toxic to shallow aquatic fauna (US EPA, 1989), will be calculated based on the total ammonia concentration and the corresponding salinity, pH, and temperature of the sample (Bower and Bidwell, 1978). Measurement of sulfide will follow the methylene blue method in HACH (2009) adapted from APHA Standard Method 4500-S^{2-} (APHA, 1989). Un-ionized H_2S, the form considered the most toxic to shallow aquatic fauna (US EPA, 1986) will be calculated based on the sulfide (S^{2-}) concentration, pH of the sample, and pK′ provided in Standard Method 4500-S^2- (APHA, 1989). Temperature, pH, and oxidation-reduction potential (ORP) will be measured while in the field using a combination pH and ORP pen meter (Hanna Instruments model 98121). ORP is measured in mV and requires the addition of an offset voltage of 210 mV to convert the reading to Eh (Hanna Tech Service, personal communication). Salinity of the pore-water sample will be determined with a refractometer.

The data resulting from pore-water chemistry analysis may be able to be used to aid in the interpretation of toxicity results and biological patterns of infauna and to validate Sediment Profile Imaging (SPI) data collected at coinciding sites through a separate collaborative project effort.

Spare Cores: Sediment Toxicity Testing and Microbial Analysis.
Three of the 12 cores on the multi-core system will be available as spares to provide back-up material to replace any of the above types of cores that may have failed. To the extent that extra cores may be available, spare cores may be available for use in toxicity testing or microbial analysis. At this time, a specific sediment bioassay for toxicity testing has not been identified and thus is not included as a budgeted activity within the scope of the present plan. The top 3 cm of up to three spare cores from each replicate multi-core drop will be reserved for toxicity bioassay tests. Each core will be subdivided into two aliquots. One aliquot will be preserved at 4°C and will enter into BP chain of custody, and the other alliquot will be frozen on board the vessel at -80 °C, and transferred to Alpha Analytical Laboratory upon completion of the cruise, where they will be archived at -80 °C under Trustee chain of custody. Analysis of these cores is not addressed in this plan.

Up to one spare core from a subset of the sampling locations will be reserved for microbial analysis. Microbial cores will be retained intact (un-sectioned). Overlying water on the sediment core will be removed via siphoning (not poured) with only a couple of cm of water left in place. Cores will be capped and frozen upright at -80 °C. Once frozen, the cores may be stored horizontally. Cores will be shipped packed in dry ice to Alpha Analytical Laboratory for archive, until such time as a cooperatively-approved laboratory can be identified.

CTD Profiles. A CTD with DO and Aquatracka fluorometer sensors will be used to acquire continuous profiles of conductivity, temperature, pH, dissolved oxygen, depth, and fluorescence at each station as it is lowered and raised through the water column. The resulting data can be used to aid in the interpretation of biological patterns. The CTD/Aquatracka unit will be deployed either independently of the multi-corer system, within its own frame, or simultaneously by mounting the unit to the multi-corer frame. The latter approach would save time on station by eliminating a separate instrument deployment (typically minimum of 2 hrs roundtrip) and is identified by the multi-corer manufacturer as a viable option.

3.0 Milestones and Deliverables:

- Cruise Plan for spring 2011 cruise — Prior to cruise departure.
- Cruise Report — Within 2-4 weeks of cruise completion.
- Report on results of initial benthic sample analysis (from 2010 Gyre and Ocean Veritas cruises) — Approximately 6 months after approval to begin these analyses.
- Report on benthic results from spring 2011 field effort — Approximately 6 months after cruise completion.
- Periodic letter progress reports — To provide updates on progress and reports of any preliminary salient findings.
- Overall final report — Approximately 1 year after completion of spring 2011 cruise (pending availability of chemistry data).

4.0 Key Personnel:

- Project management —Jeff Hyland (NOAA/NCCOS), Paul Montagna (Texas A&M University-Corpus Christi; TAMU-CC), and Cynthia Cooksey (NOAA/NCCOS) as co-Project Leads.
- Field work —Paul Montagna (TAMU-CC), Rick Kalke (TAMU-CC), and Cynthia Cooksey (NOAA/NCCOS) will serve as Chief Scientist or 2nd Watch Leader on alternating legs of the spring 2011 cruise (May 23 – June 16, 2011, divided into two legs of ~12 days each with a 1-day port call in-between). The proposed vessel, M/V Sarah Bordelon, accommodates 30 people total, six of whom are required for basic ship operations (captain, engineer, cook, etc.). The following list is a breakdown of the remaining 24 crew slots (by institution) needed to support the science mission for each of the two cruise legs (each leg will consist of two 12-hr shifts):
    - CSA — 4 per watch (8 total) for various ship scientific support functions (1 winch operator, 1 Nav Tech, 2 deck hands one of whom also assists with Nav Tech functions);
    - TAMU-CC — 4 per watch (8 total): Chief Scientist/Watch Leader, processing of multi-core samples, misc. sampling activities;
    - NOAA-NCCOS — 2 per watch (4 total): Chief Scientist/Watch Leader, pore-water chemistry, misc. sampling activities;
    - Cardno ENTRIX — 1 per watch (2 total): RP representation, processing of multi-core samples, misc. sampling activities;
    - NOAA-Trustees Field Ops — 1 per watch (2 total): Data management and record keeping; misc. sampling activities.
    - Total — 12 per watch (24 total).
- Macrofauna analysis — Paul Montagna (TAMU-CC)
- Meiofauna analysis — Paul Montagna (TAMU-CC), Jeff Baguley (U. Nevada-Reno), Woncheol Lee (Hangyang U., South Korea)
- Sediment grain and elemental C/N analyses – Paul Montagna (TAMU-CC)
- Sediment chemistry analysis — NRDA Trustees Contractor to run analyses; Mike Fulton (NOAA/NCCOS) to work with chemistry Contractor and other project staff on interpretation and reporting of chemistry data.
- Data analysis and reporting — All of the above personnel; Others (e.g., Len Balthis and Ian Hartwell from NOAA/NCCOS)

5.0 Safety Plans:

A HASP binder containing all health and safety protocols is provided to each vessel. All well-established health and safety protocols will be followed and will be provided to the vessel in a dedicated binder. The M/V Sarah Bordelon is the vessel currently assigned for the upcoming spring 2011 cruise. The ship's operational safety procedures will be followed at all times. Also, all activities will follow protocols of NOAA's Deepwater Horizon NRDA Field Safety Plan, latest version 1/28/2011 (NOAA 2011), which will be available on the vessel. MSDS hazardous materials sheets will be posted as well. Principal investigators may merge these safety plans with other applicable university or participating organization practices.

6.0 Data Sharing:

6.1 Digital and Shipboard Data

All data and imagery (including navigation, instrument data, field logs, photographs and documentation), acoustic, and other electronic data will be saved to an on-board computer, and all data shall be migrated to a dedicated external hard drive. The data will be controlled and managed under project protocols, including Chain-of-Custody tracking of the external hard-drive. Upon return to port, the Data Manager shall produce identical copies of the raw and processed electronic media generated during the cruise and deliver one of those copies each to NOAA (or its contractor), Louisiana Oil Spill Coordinator's Office (LOSCO) on behalf of the State of Louisiana, and to Cardno ENTRIX.

6.2 Laboratory Data

Sediment samples for macrofauna, grain size, and elemental TOC/TC/TIC/TN analyses will be performed at TAMU-CC under the supervision of Dr. Paul Montagna. Sediment samples for meiofauna analysis will be processed under the supervision of Dr. Paul Montagna (TAMU-CC) and Dr. Jeff Baguley (U. of Nevada). The meiofauna samples will be sent to University of Nevada and extracted, sorted, enumerated to major taxonomic levels, and measured for biomass in Dr. Baguley's lab. Harpacticoid copepods from these meiofaunal samples will then be sent to Dr. Woncheol Lee of Hangyang University to be identified to family level. Drs. Baguley and Lee are former students and postdocs of Dr. Montagna and performed all of the prior DGOMB analyses. Sediment samples for the analysis of hydrocarbons and other chemical contaminants will be sent to the appropriate NRDA/Trustees contractor (Alpha Analytical Laboratories for hydrocarbons and metals, Columbia Analytical Services for dispersants). Excess sediment cores will be sent to Alpha Analytical Laboratories for archive.

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).

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. 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.

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References to the studies cited in this work plan are for background and context only. Approval of this work plan does not constitute endorsement of, or agreement with, the methods, analysis, or conclusions of any study cited herein.

8.0 Costs:

The Parties acknowledge that this budget is an estimate, and that actual costs may prove to be higher due to a number of potential factors. As soon as factors are identified that may increase the estimated cost, BP will be notified and a change order provided describing the nature and cause for the increase cost in addition to a revised budget for BP's consideration and review. The project costs indicated in Budget Chart 1 below are to be submitted by Trustees for reimbursement by BP. The Vessel Costs indicated in Budget Chart 2 shall be paid directly by BP.

Budget Chart 1. Non-vessel costs to be submitted by Trustees for reimbursement by BP.

Description	**Cost**	**Subtotal**
A. TAMU-CC		
1. Analysis of archived fall 2010 samples		
• Macrofauna (3 cores/drop x 1 drop/station at each of 65 priority stations)	$330,000	
• Meiofauna (1 core/drop x 1 drop/station at each of 65 priority stations)	$130,000	
• Macrofauna species-level taxonomy (3 cores from each of 7 stations)	$50,000	
• Subtotal		$510,000
2. Sampling & analysis of 2011 samples		
• Macrofauna processing (3 cores/drop x 3 drops/station x 38 stations)	$780,000	
• Meiofauna processing (1 core/drop x 3 drops/station x 38 stations)	$460,000	
• Sediment grain-size processing (1 core/drop x 4 sections/core x 3 drops/station x 38 stations)	$150,000	
• Sediment C/N processing (1 core/drop x 4 sections/core x 3 drops/station x 38 stations)	$76,000	
• Equipment (4 microscopes)	$105,000	
• Cruise labor, benefits, indirect & travel	$150,000	
• Subtotal		$1,721,000
3. Data reporting & analysis		
• Macrofauna (labor, benefits, indirect, travel)	$105,000	
• Meiofauna (labor, benefits, indirect, travel)	$100,000	
• Subtotal		$205,000
4. Total cost for TAMU-CC		$2,436,000
B. NOAA/NOS (NCCOS)		
• FY11: Participation in May-Jun 2011 cruise, program management, data analysis & reporting (Apr-Sep 2012)	$203,383	
• FY12: Program management, data analysis & reporting (Oct 2011 – Sep 2012)	$208,296	
• Subtotal		$411,679

D. Total cost*		**$2,847,679**

Budget Chart #2. Vessel Costs to be paid directly by BP.

Estimated Vessel Costs	**Total Estimated Price**
Fleet Mgmt / Shore Support	$157,500
Mobilization/Rigging	$210,000
Operational Ship Days	$1,085,456
Standby Days	$733,623
Fuel & Lube	$529,200
Satellite Communications	$6,300
Total Estimated Vessel Cost	**$2,722,709**

Operational days are based on a two-day mobilization, 29 survey days (including 25 "at sea" days and up to four days of "shake down" sea time) and a demobilization of one day. Costs are based on the 2011 Schedule. Stand-by dates are estimated based on a stand-by period from April 24 through May 16th.

Total Estimated Cost*: $5,570,388
*Excluding costs of chemical contaminant analyses by NOAA Trustees analytical chemistry laboratories (Alpha Analytical Laboratories and Columbia Analytical Services) to be covered separately outside project budget.

Table 1. Collection, allocation and sectioning of sediment cores per multi-corer drop. Any available spare cores may be used to provide extra backup material (ie., to replace failed cores) or to support other potential analyses outside the present scope of work and budget (e.g., sediment toxicity testing). (A) Core allocation, (B) Vertical core sections to be collected for each allocation.

A)

Core	Allocation
1	Macrofauna
2	Macrofauna
3	Macrofauna
4	Meiofauna
5	TC/TOC/TIC/TN, Grain Size
6	Hydrocarbons

Core	Allocation
7	Metals, Dispersants
8	Porewater Chemistry
9	Porewater Chemistry
10	Spare (Toxicity,Microbial)
11	Spare (Toxicity)
12	Spare (Toxicity)

B)

Allocation	Vertical Core Section (cm)				
	0 - 1	1 - 3	3 - 5	5 - 10	10 -15
Macrofauna	X		X	X	X
Meiofauna	X	X	X	X	X
Microbial, if available	Entire core, intact and capped				
Sediment Properties	X	X	X	X	
HC	X	X	X	X	
Trace Metals, dispersant	X	X	X	X	
Porewater Chemistry	X				
Toxicity, if available	X				

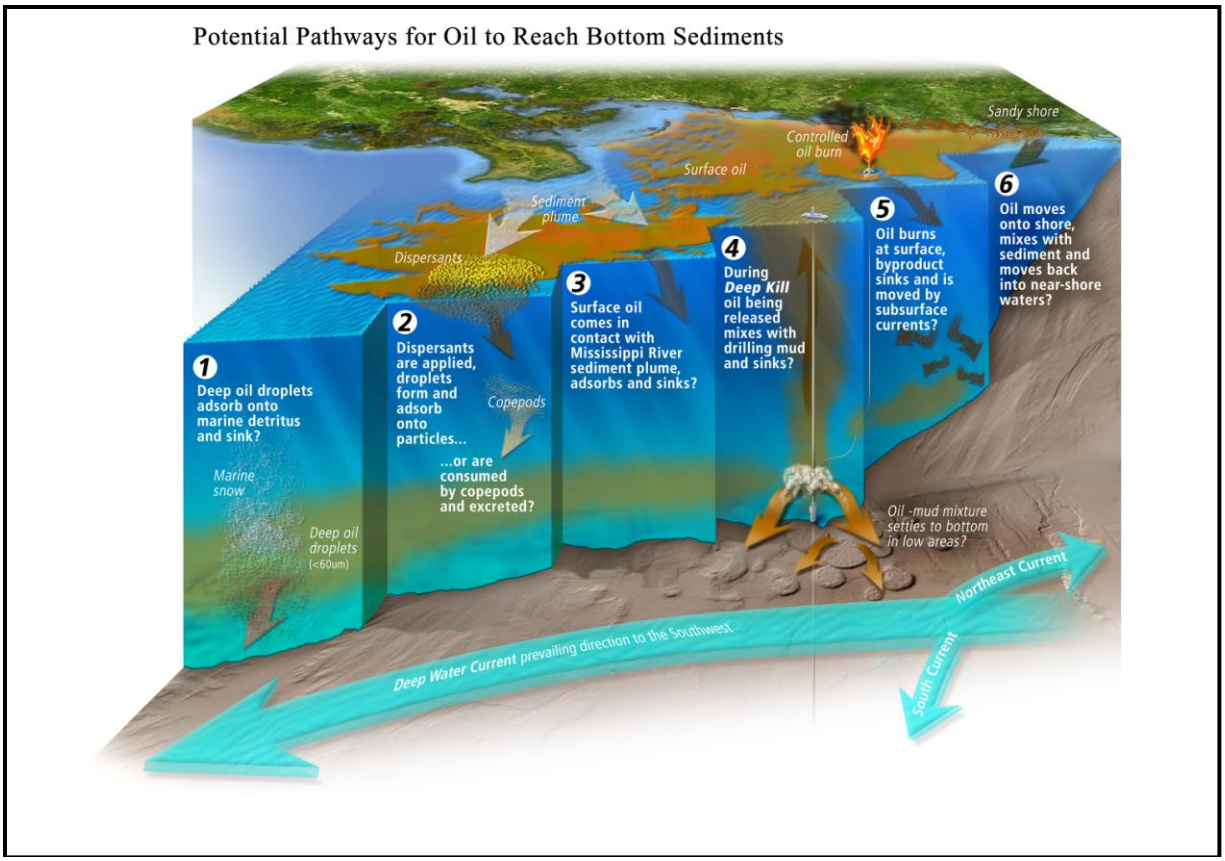

Figure 1. Potential pathways for oil to reach offshore and deepwater sediments (from UAC Implementation Plan for Subsurface Monitoring, 13 November 2010).

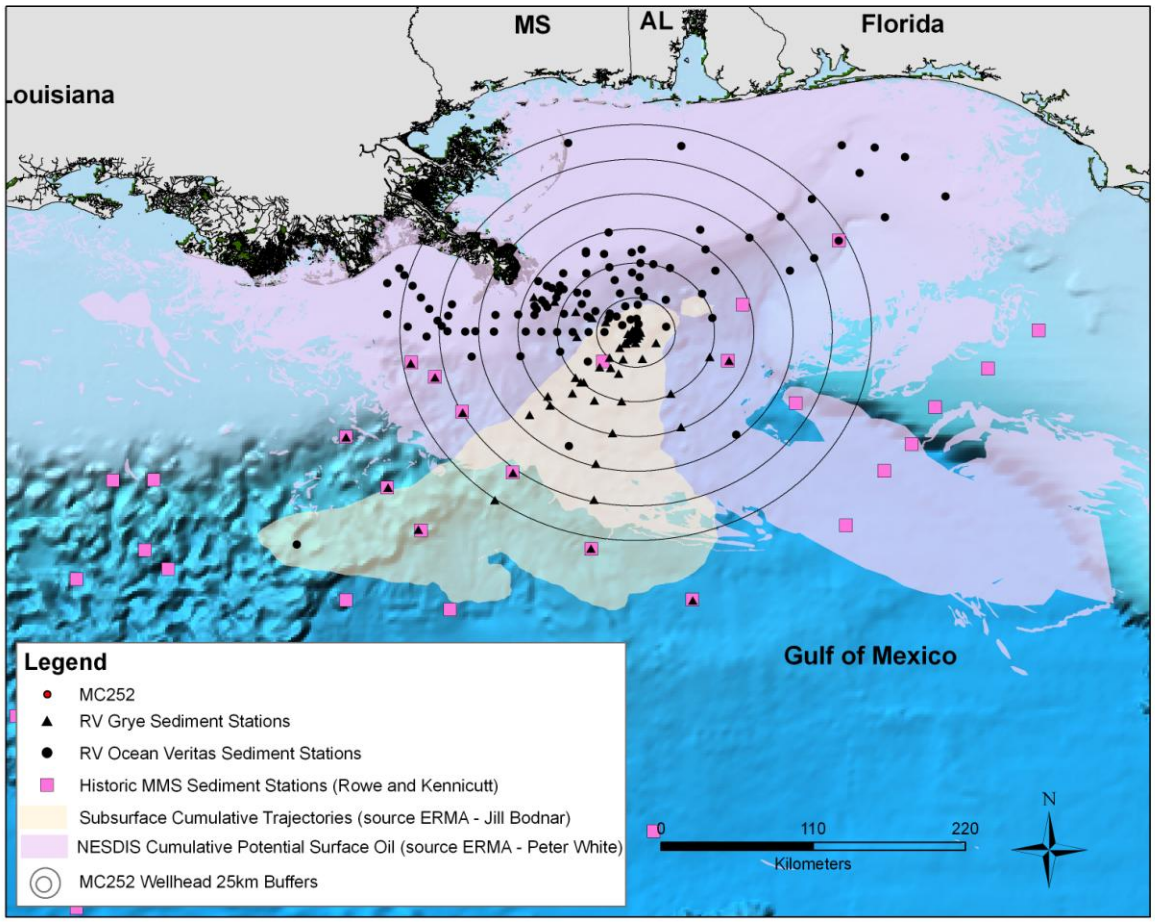

Figure 2. Overview of offshore and deepwater stations sampled on recent (2010) Gyre and Ocean Veritas cruises and prior 2000-2002 MMS (now BOEMR) sponsored cruises (Rowe and Kennicutt 2009, DGoMB sites). Rings centered around the well-head are 25 km apart. Sediment samples were collected with multi-corers at all sites.

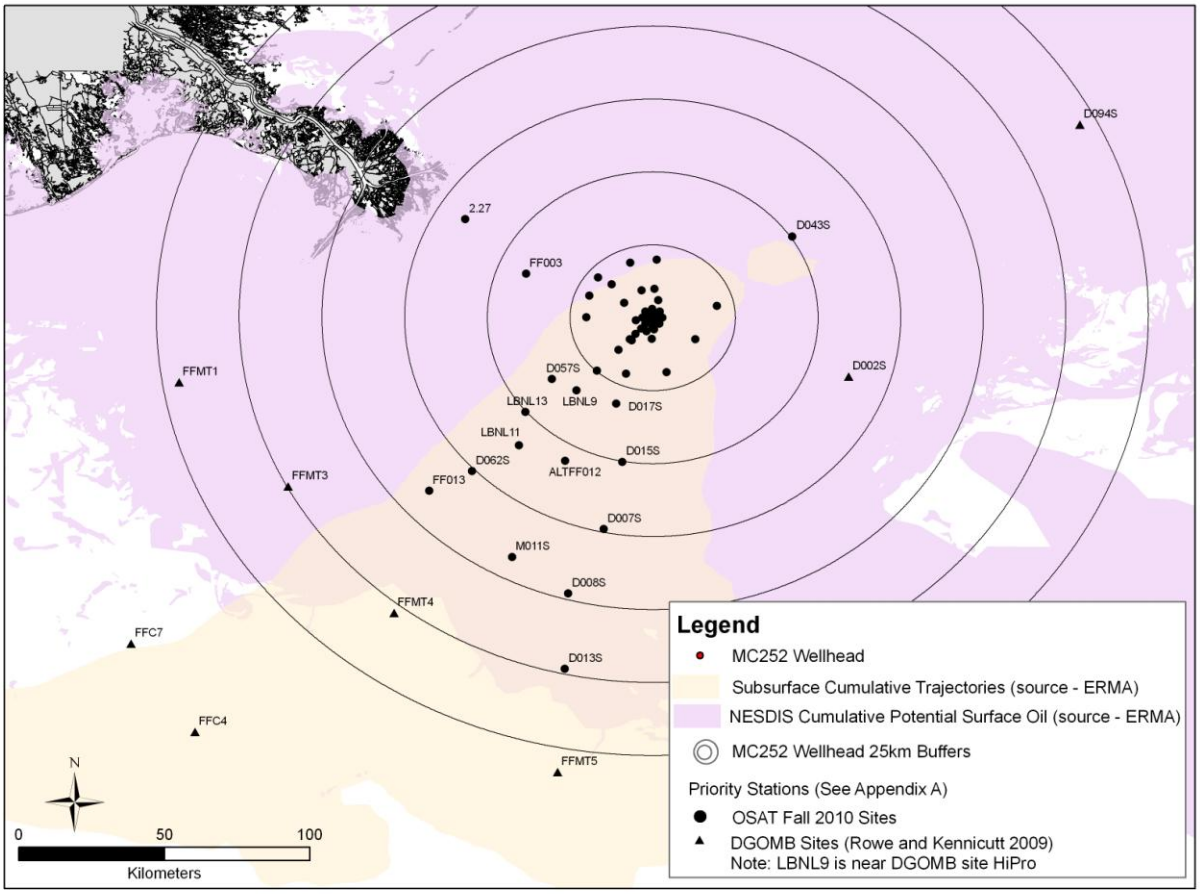

Figure 3a. Priority stations from fall 2010 Response cruises (Gyre and Ocean Veritas) that are selected for the initial suite of macrofaunal and meiofaunal analyses. Rings centered around the well-head are 25 km apart.

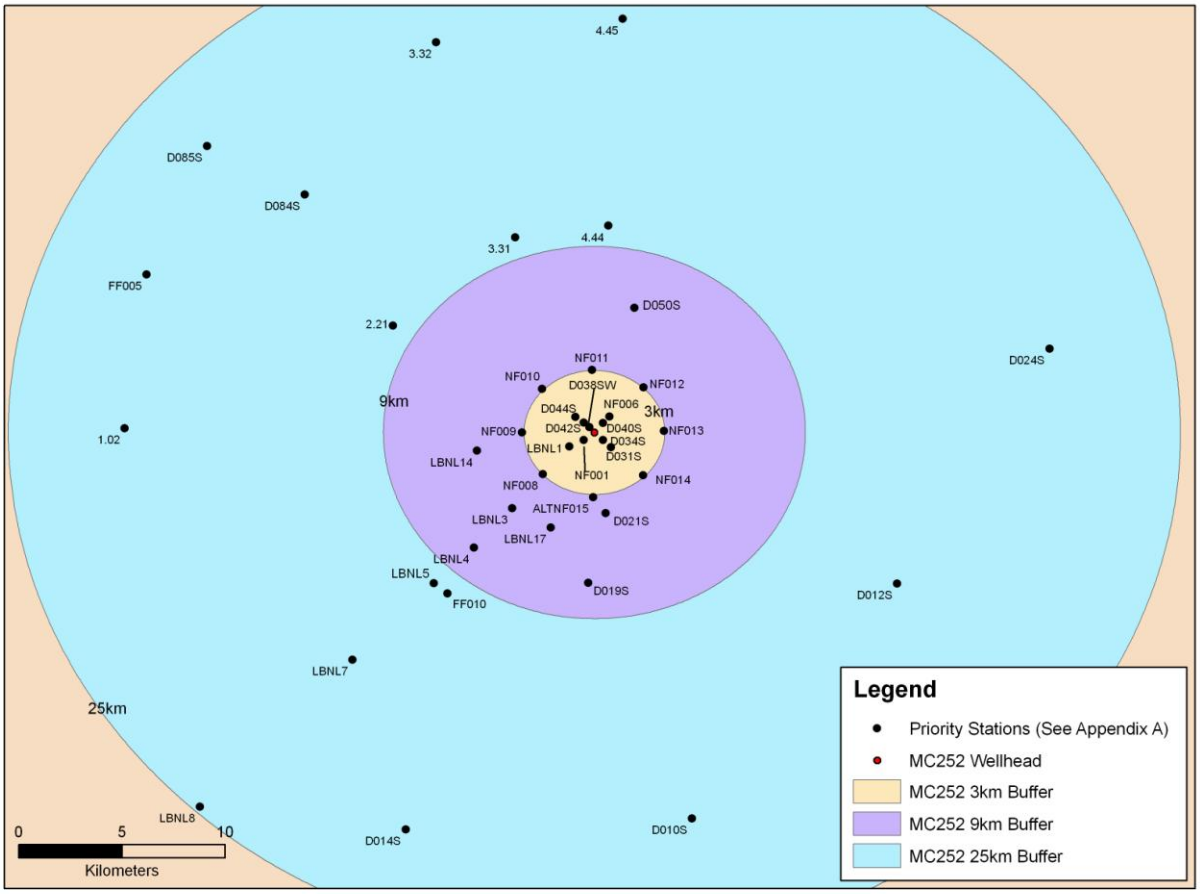

Figure 3b. Zoomed-in view of priority fall 2010 stations within 25 km of well-head that are selected for the initial suite of macrofaunal and meiofaunal analyses.

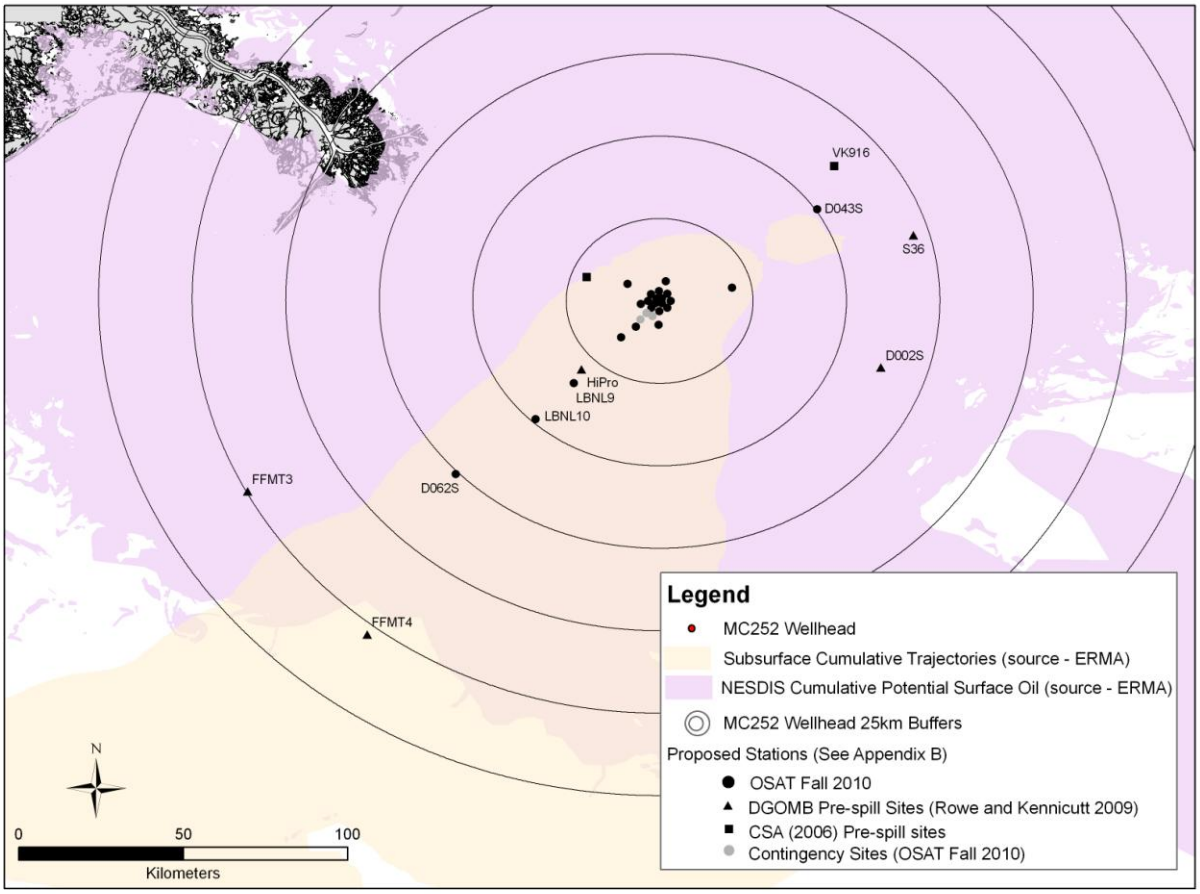

Figure 4a. Fixed/repeated sampling sites for spring 2011 cruise as part of a time series. Rings centered around the well-head are 25 km apart.

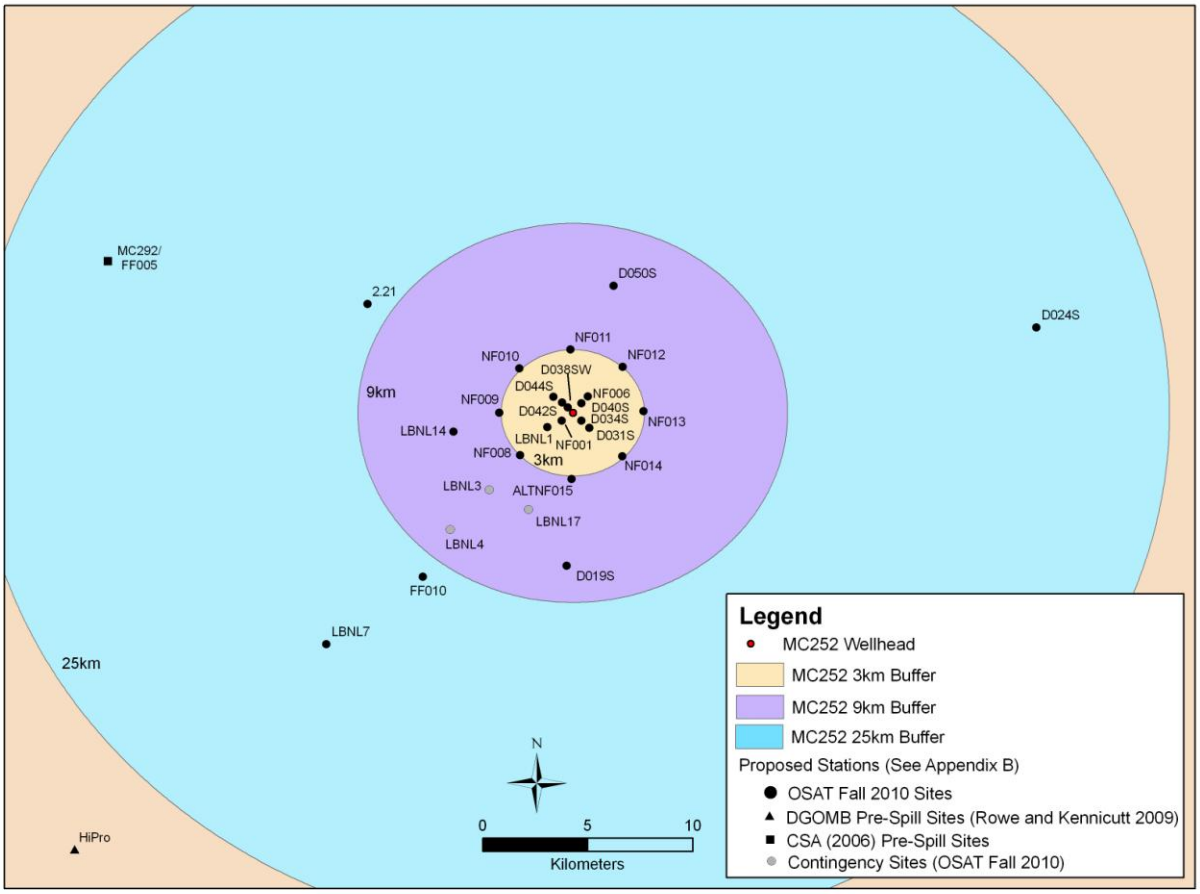

Figure 4b. Zoomed-in view of fixed/repeated sampling sites for the spring 2011 cruise within 25 km around the well-head.

Appendix A

Table of existing benthic macrofauna and meiofauna samples from fall 2010 Response cruises, along with corresponding station coordinates and depths. Rationale for priority analysis of first 65 samples listed is also presented. Abbreviations: GY = Gyre cruise (total of 65 stations from September-October 2010), OV = Ocean Veritas cruises (total of 104 stations from September-October 2010).

Ship	Station	Latitude	Longitude	Depth	Priority Rationale
GY	ALTNF001	[redacted]	[redacted]	1543	Oil contaminated from OSAT report
GY	D031S	[redacted]	[redacted]	1508	Oil contaminated from OSAT report
GY	D038SW	[redacted]	[redacted]	1509	Oil contaminated from OSAT report
GY	D040S	[redacted]	[redacted]	1517	Oil contaminated from OSAT report
GY	D042S	[redacted]	[redacted]	1502	Oil contaminated from OSAT report
GY	D044S	[redacted]	[redacted]	1493	Oil contaminated from OSAT report
GY	NF006MOD	[redacted]	[redacted]	1517	Oil contaminated from OSAT report
GY	ALTNF015	[redacted]	[redacted]	1607	< 3 km of well-head
GY	D034S	[redacted]	[redacted]	1544	< 3 km of well-head
GY	LBNL1	[redacted]	[redacted]	1578	< 3 km of well-head
GY	NF008	[redacted]	[redacted]	1585	< 3 km of well-head
GY	NF013	[redacted]	[redacted]	1567	< 3 km of well-head
GY	NF014	[redacted]	[redacted]	1579	< 3 km of well-head
OV	1.02	[redacted]	[redacted]	1129	< 25 km of well-head
OV	2.21	[redacted]	[redacted]	1367	< 25 km of well-head
OV	3.31	[redacted]	[redacted]	976	< 25 km of well-head
OV	3.32	[redacted]	[redacted]	854	< 25 km of well-head
OV	4.44	[redacted]	[redacted]	755	< 25 km of well-head
OV	4.45	[redacted]	[redacted]	755	< 25 km of well-head
GY	D010S	[redacted]	[redacted]	1884	< 25 km of well-head
GY	D012S	[redacted]	[redacted]	1819	< 25 km of well-head
GY	D014S	[redacted]	[redacted]	1760	< 25 km of well-head
GY	D019S	[redacted]	[redacted]	1656	< 25 km of well-head
GY	D021S	[redacted]	[redacted]	1618	< 25 km of well-head
OV	D024S	[redacted]	[redacted]	1697	< 25 km of well-head
OV	D050S	[redacted]	[redacted]	1432	< 25 km of well-head
OV	D084S	[redacted]	[redacted]	931	< 25 km of well-head
OV	D085S	[redacted]	[redacted]	842	< 25 km of well-head
GY	FF005	[redacted]	[redacted]	1003	< 25 km of well-head
GY	FF010	[redacted]	[redacted]	1356	< 25 km of well-head
GY	LBNL14	[redacted]	[redacted]	1535	< 25 km of well-head
GY	LBNL17	[redacted]	[redacted]	1595	< 25 km of well-head
GY	LBNL3	[redacted]	[redacted]	1585	< 25 km of well-head
GY	LBNL4	[redacted]	[redacted]	1422	< 25 km of well-head
GY	LBNL5	[redacted]	[redacted]	1350	< 25 km of well-head
GY	LBNL7	[redacted]	[redacted]	1577	< 25 km of well-head
GY	LBNL8	[redacted]	[redacted]	1578	< 25 km of well-head
GY	NF009	[redacted]	[redacted]	1489	< 25 km of well-head
GY	NF010	[redacted]	[redacted]	1439	< 25 km of well-head
GY	NF011	[redacted]	[redacted]	1449	< 25 km of well-head

Ship	Station	Latitude	Longitude	Depth	Priority Rationale
GY	NF012	[redacted]	[redacted]	1520	< 25 km of well-head
GY	D002S	[redacted]	[redacted]	2389	Historical DGOMB site S37
OV	D094S	[redacted]	[redacted]	668	Historical DGOMB site S35
GY	FFC4	[redacted]	[redacted]	1456	Historical DGOMB site C4
GY	FFC7	[redacted]	[redacted]	1015	Historical DGOMB site C7
GY	FFMT1	[redacted]	[redacted]	211	Historical DGOMB site MT1
GY	FFMT3	[redacted]	[redacted]	1002	Historical DGOMB site MT3
GY	FFMT4	[redacted]	[redacted]	1405	Historical DGOMB site MT4
GY	FFMT5	[redacted]	[redacted]	2259	Historical DGOMB site MT5
GY	D017S	[redacted]	[redacted]	1712	< 50 km of well-head
OV	D057S	[redacted]	[redacted]	1364	< 50 km of well-head
GY	LBNL9	[redacted]	[redacted]	1516	< 50 km of well-head
GY	ALTFF012	[redacted]	[redacted]	1738	< 75 km of well-head
GY	D007S	[redacted]	[redacted]	2052	< 75 km of well-head
GY	D015S	[redacted]	[redacted]	1576	< 75 km of well-head
OV	D043S	[redacted]	[redacted]	1492	< 75 km of well-head
GY	LBNL11	[redacted]	[redacted]	1438	< 75 km of well-head
GY	LBNL13	[redacted]	[redacted]	1286	< 75 km of well-head
GY	D008S	[redacted]	[redacted]	1606	< 100 km of well-head
GY	D062S	[redacted]	[redacted]	1303	< 100 km of well-head
GY	FF013	[redacted]	[redacted]	1213	< 100 km of well-head
OV	M011S	[redacted]	[redacted]	211	< 100 km of well-head
GY	D013S	[redacted]	[redacted]	1766	< 125 km of well-head
OV	2.27	[redacted]	[redacted]	76	NW of well-head in Miss. River Plume
GY	FF003	[redacted]	[redacted]	493	NW of well-head in Miss. River Plume
OV	1.01	[redacted]	[redacted]	735	
OV	1.03	[redacted]	[redacted]	1025	
OV	1.04	[redacted]	[redacted]	999	
OV	1.05	[redacted]	[redacted]	868	
OV	1.06	[redacted]	[redacted]	565	
OV	1.07	[redacted]	[redacted]	429	
OV	1.08	[redacted]	[redacted]	141	
OV	1.09	[redacted]	[redacted]	141	
OV	1.1	[redacted]	[redacted]	101	
OV	1.11	[redacted]	[redacted]	109	
OV	1.13	[redacted]	[redacted]	91	
OV	1.14	[redacted]	[redacted]	84	
OV	1.15	[redacted]	[redacted]	76	
OV	1.16	[redacted]	[redacted]	60	
OV	1.17	[redacted]	[redacted]	45	
OV	1.18	[redacted]	[redacted]	32	
OV	1.19	[redacted]	[redacted]	20	
OV	1.2	[redacted]	[redacted]	32	
OV	2.23	[redacted]	[redacted]	630	
OV	2.24	[redacted]	[redacted]	408	
OV	2.25	[redacted]	[redacted]	76	
OV	2.26	[redacted]	[redacted]	76	
OV	2.28	[redacted]	[redacted]	76	
OV	2.29	[redacted]	[redacted]	53	

Ship	Station	Latitude	Longitude	Depth	Priority Rationale
OV	2.3	[redacted]	[redacted]	32	
OV	3.33	[redacted]	[redacted]	710	
OV	3.34	[redacted]	[redacted]	371	
OV	3.35	[redacted]	[redacted]	178	
OV	3.36	[redacted]	[redacted]	72	
OV	4.46	[redacted]	[redacted]	755	
OV	4.47	[redacted]	[redacted]	437	
OV	4.48	[redacted]	[redacted]	221	
OV	4.49	[redacted]	[redacted]	82	
GY	ALTFF002	[redacted]	[redacted]	230	
GY	D003S	[redacted]	[redacted]	2286	
GY	D004S	[redacted]	[redacted]	2309	
GY	D006S	[redacted]	[redacted]	2127	
OV	D009S	[redacted]	[redacted]	1921	
OV	D046S	[redacted]	[redacted]	1458	
OV	D067S	[redacted]	[redacted]	1162	
GY	D068S	[redacted]	[redacted]	1172	
OV	D069S	[redacted]	[redacted]	1114	
OV	D070S	[redacted]	[redacted]	1074	
OV	D071S	[redacted]	[redacted]	1089	
OV	D072S	[redacted]	[redacted]	1085	
OV	D077S	[redacted]	[redacted]	1005	
OV	D089S	[redacted]	[redacted]	793	
OV	D090S	[redacted]	[redacted]	770	
OV	D096S	[redacted]	[redacted]	615	
OV	D100S	[redacted]	[redacted]	464	
OV	D101SW	[redacted]	[redacted]	460	
OV	D107SW	[redacted]	[redacted]	326	
OV	D108S	[redacted]	[redacted]	303	
OV	D300S	[redacted]	[redacted]	227	
OV	D301S	[redacted]	[redacted]	253	
OV	D302S	[redacted]	[redacted]	196	
GY	FF001	[redacted]	[redacted]	79	
GY	FF004	[redacted]	[redacted]	838	
OV	M001SW	[redacted]	[redacted]	316	
OV	M002SW	[redacted]	[redacted]	270	
OV	M004S	[redacted]	[redacted]	260	
OV	M005S	[redacted]	[redacted]	259	
OV	M008S	[redacted]	[redacted]	220	
OV	M009SW	[redacted]	[redacted]	210	
OV	M012S	[redacted]	[redacted]	260	
OV	M013S	[redacted]	[redacted]	187	
OV	M014S	[redacted]	[redacted]	175	
OV	M015S	[redacted]	[redacted]	168	
OV	M016SW	[redacted]	[redacted]	170	
OV	M019S	[redacted]	[redacted]	162	
OV	M020S	[redacted]	[redacted]	142	
OV	M022SW	[redacted]	[redacted]	126	
OV	M023S	[redacted]	[redacted]	124	

Ship	Station	Latitude	Longitude	Depth	Priority Rationale
OV	M025SW	[redacted]	[redacted]	119	
OV	M026SW	[redacted]	[redacted]	112	
OV	M031S	[redacted]	[redacted]	86	
OV	M034S	[redacted]	[redacted]	75	
OV	M037S	[redacted]	[redacted]	70	
OV	M039S	[redacted]	[redacted]	58	
OV	M201S	[redacted]	[redacted]	141	
OV	M202S	[redacted]	[redacted]	116	
OV	M205S	[redacted]	[redacted]	151	
OV	M206S	[redacted]	[redacted]	186	
OV	M207S	[redacted]	[redacted]	121	
OV	M208S	[redacted]	[redacted]	120	
OV	S016SW	[redacted]	[redacted]	58	
OV	S01S	[redacted]	[redacted]	106	
OV	S022S	[redacted]	[redacted]	57	
OV	S02SW	[redacted]	[redacted]	98	
OV	S03S	[redacted]	[redacted]	92	
OV	S04S	[redacted]	[redacted]	84	
OV	S05S	[redacted]	[redacted]	55	
OV	S12S	[redacted]	[redacted]	65	
OV	0	[redacted]	[redacted]	1367	
OV	2.22	[redacted]	[redacted]	956	
GY	D053S	[redacted]	[redacted]	1409	
OV	D064S	[redacted]	[redacted]	1200	
GY	LBNL12	[redacted]	[redacted]	1194	
GY	D055S	[redacted]	[redacted]	1376	
GY	FF011	[redacted]	[redacted]	1639	
GY	LBNL10	[redacted]	[redacted]	1402	
GY	FFMT6	[redacted]	[redacted]	2767	Historical DGOMB site MT6
GY	FFMT2	[redacted]	[redacted]	684	Historical DGOMB site MT2
GY	FFC1	[redacted]	[redacted]	325	Historical DGOMB site C1

Appendix B

Table of 38 sampling stations from the fall 2010 Response cruises to serve as proposed fixed sites for repeated sampling in spring 2011.

Station	Latitude	Longitude	Depth	Rationale
ALTNF001	[redacted]	[redacted]	1543	Oil contaminated from OSAT report
D031S	[redacted]	[redacted]	1508	Oil contaminated from OSAT report
D038SW	[redacted]	[redacted]	1509	Oil contaminated from OSAT report
D040S	[redacted]	[redacted]	1517	Oil contaminated from OSAT report
D042S	[redacted]	[redacted]	1502	Oil contaminated from OSAT report
D044S	[redacted]	[redacted]	1493	Oil contaminated from OSAT report
NF006MOD	[redacted]	[redacted]	1517	Oil contaminated from OSAT report
ALTNF015	[redacted]	[redacted]	1607	Within ~3 km of well-head
D034S	[redacted]	[redacted]	1544	Within ~3 km of well-head
LBNL1	[redacted]	[redacted]	1578	Within ~3 km of well-head
NF008	[redacted]	[redacted]	1585	Within ~3 km of well-head
NF013	[redacted]	[redacted]	1567	Within ~3 km of well-head
NF014	[redacted]	[redacted]	1579	Within ~3 km of well-head
NF009	[redacted]	[redacted]	1489	Within ~3 km of well-head
NF010	[redacted]	[redacted]	1439	Within ~3 km of well-head
NF011	[redacted]	[redacted]	1449	Within ~3 km of well-head
NF012	[redacted]	[redacted]	1520	Within ~3 km of well-head
FF010	[redacted]	[redacted]	1356	< 25 km of well-head
2.21	[redacted]	[redacted]	1367	< 25 km of well-head
D019S	[redacted]	[redacted]	1656	< 25 km of well-head
D024S	[redacted]	[redacted]	1697	< 25 km of well-head
LBNL14	[redacted]	[redacted]	1535	< 25 km of well-head
LBNL7	[redacted]	[redacted]	1577	< 25 km of well-head
D050S	[redacted]	[redacted]	1432	< 25 km of well-head
LBNL9	[redacted]	[redacted]	1516	< 50 km of well-head
LBNL10	[redacted]	[redacted]	1402	< 50 km of well-head
D043S	[redacted]	[redacted]	1492	< 75 km of well-head
D062S	[redacted]	[redacted]	1303	< 100 km of well-head
FFMT3	[redacted]	[redacted]	1002	Historical DGOMB site MT3
FFMT4	[redacted]	[redacted]	1405	Historical DGOMB site MT4
D002S	[redacted]	[redacted]	2389	Historical DGOMB site S37
HIPRO	[redacted]	[redacted]	1574	Historical DGOMB site HiPro
S36	[redacted]	[redacted]	1826	Historical DGOMB site S36
FF005	[redacted]	[redacted]	1003	CSA (2006) Site MC292, <25 km of well-head
VK916	[redacted]	[redacted]	1125	CSA (2006) Site VK916
LBNL17	[redacted]	[redacted]	1595	3-9 km SW of well-head
LBNL3	[redacted]	[redacted]	1585	3-9 km SW of well-head
LBNL4	[redacted]	[redacted]	1422	3-9 km SW of well-head

Appendix C
Cleaning and Decontamination Procedures for Field Sampling

Field sampling equipment coming into contact with targeted environmental samples will be kept as clean as possible to minimize the risk of cross-contamination that could jeopardize data integrity and lead to erroneous scientific conclusions. Cleaning and decontamination procedures are based on relevant methods from a combination of the following guidance documents: (1) NOAA/Office of Marine and Aviation Operations's Procedure 1110-01/Version 1, "NOAA Ship Operations Near Deepwater Horizon Effluents" (NOAA/OMAO 2010); (2) NOAA National Status & Trends field operations manual (Lauenstein and Young 1986); (3) U.S. EPA EMAP/National Coastal Assessment Quality Assurance Project Plan (U.S. EPA 2001); and (4) U.S.EPA Region II CERCLA Quality Assurance Manual, Revision 1 (U.S.EPA/Region II 1989).

Under no circumstances will sampling occur through a surface oil slick. If a surface slick is present at a station, measures will be taken to avoid deploying any sampling equipment in its path (e.g., relocating the ship until the slick has cleared). The multi-corer system for sediment sampling will be thoroughly cleaned between stations taking into consideration any specific manufacturer's instructions. The U.S.EPA EMAP/NCA protocol (U.S.EPA 2001) recommends that field equipment be cleaned using an Alconox scrub followed by a thorough rinsing with ambient seawater. In this study, clean tap water will be used instead of ambient seawater to rinse the equipment following the detergent scrub. In addition, critical parts that come into direct contact with the sample (e.g., core tubes) will be cleaned prior to initial use and between deployments using the following procedure, which is consistent with the NOAA NS&T (Lauenstein and Yound 1986) and EPA/Region II CERCLA (U.S.EPA/Region II 1989) protocols:

- Wash with low phosphate detergent (e.g., Alconox)
- Rinse with tap water
- Rinse with solvent (e.g., methanol followed by hexane) for parts used for hydrocarbon analyses, or with dilute (5-10%) nitric acid (HNO_3) or hydrochloric acid (HCl) for parts used for metals analyses. All solvents should be pesticide grade or better.
- Rinse with de-ionized (or distilled) water followed by air drying

The CTD unit should be washed down between deployments with fresh tap water. If the unit has encountered any oil, it should be scrubbed with detergent to remove the oil and then washed down with fresh tap water and rinsed with hexane.

Other delicate instruments and probes, including instruments that are used in the dry labs, should be cleaned in a manner appropriate for that equipment and that follows manufacturers' instructions.

Sampling utensils that come into direct contact with the sample should be made of non-contaminating materials (e.g., high-quality stainless steel or Teflon for hydrocarbon samples, or plastic for metals samples) and should be thoroughly cleaned between sampling events (e.g., Alconox scrub followed by tap water and solvent/acid rinses as described above) or replaced with new pre-cleaned disposable ones. Similarly, sample containers should be cleaned (or

purchased as certified pre-clean) before coming into contact with the sample and made of non-contaminating materials appropriate for the type of analysis (e.g., glass jars with Teflon lids for hydrocarbon samples, glass or plastic jars with Teflon lids for metals samples, whirl-pack bags for grain size samples). Once cleaned, utensils and other sampling devices should be covered in aluminum foil (shiny side out), or protected by other acceptable means, to prevent contamination between uses.

Any necessary decontamination of the vessel or its components (e.g., anchor, lines) will follow protocols of the vessel owner/operator as well as the spirit of the guidelines given in the above NOAA/OMAO (2010) manual for operations near the oil spill.

Procedures to protect personal safety during decontamination operations will follow the guidelines provided in the NOAA Deepwater Horizon NRDA Field Safety Plan, version 1/28/2011 (NOAA 2011). Solvents brought onboard for decontamination purposes will be stored in approved HAZMAT lockers and corresponding MSDS sheets will be readily available. Solvent wastes will be returned to shore-based facilities for appropriate disposal.

Field personnel will be responsible for documenting all decontamination activities occurring in the field. Data will be recorded in a field logbook and will include at a minimum the responsible person's name, date and time of activity, description of items decontaminated and the procedure used.

Mississippi Canyon 252 Oil Spill

NRDA Sampling Plan

Deepwater Sediment Sampling to Assess Potential Post-Spill Benthic Impacts from the Deepwater Horizon Oil Spill

Deepwater Benthic Communities (Deepwater Coral) Technical Working Group

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

APPROVED:

Robert W. Ricker — May 20, 2011

Department of Commerce Trustee Representative: — Date

May 24, 2011

DOI Representative: — Date

FOR KOLKMO GUIDRY

JUNE 6, 2011

Louisiana Trustee Representative: — Date