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## **Deepwater Horizon/Mississippi Canyon 252 Spill**

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As agreed upon by the Trustees and BP, all samples collected for contaminant analysis during the sampling plan described below will be sent to Alpha Analytical Laboratory, unless they are designated to be archived. Samples for other analyses, if not archived, will be sent to the laboratories indicated in the plan below.

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 ENTRIX 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 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-ed data shall be made available simultaneously to all trustees and BP (or 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-ed 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 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/un-validated" and will be made available equally to all trustees and to BP (or 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.

Mississippi Canyon 252 Spill Spring 2011 Oyster Recruitment Sampling Plan-Amendment 2  
September 23, 2011

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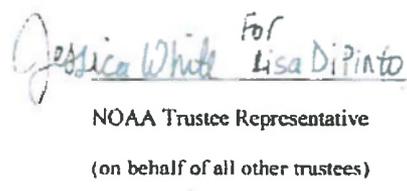
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Approval of this work plan is for the purposes of obtaining data for the Natural Resource Damage Assessment (NRDA). Parties each reserve its right to produce its own independent interpretation and analysis of any data collected pursuant to this work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

**APPROVED:**

 <sup>FOR ROGER MOQUIRY</sup> \_\_\_\_\_  
Date: 10/28/2011

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BP Representative: \_\_\_\_\_  
Date: 10/10/2011

 <sup>For</sup> \_\_\_\_\_  
NOAA Trustee Representative: Lisa DiPinto  
(on behalf of all other trustees)  
Date: 9/26/2011

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**Mississippi Canyon 252 Spill**  
**Spring 2011 Oyster Recruitment Sampling Plan**  
**-Amendment 2**

September 23, 2011

**Introduction and Summary**

This document (Amendment 2) further amends the Spring 2011 Oyster Recruitment Sampling Plan (Spring Plan). The original Spring Plan included three rounds of recruitment sampling starting in April 2011. Amendment 1 updated the Spring Plan to include additional rounds of recruitment sampling across all Spring Plan sites to gather additional data through the summer months. Amendment 2 expands the Spring Plan to include continued recruitment sampling into the fall of 2011. Under previous oyster sampling efforts (Phase I Amendment 2, Transition Plan, Spring Plan, and Spring Plan Amendment 1), teams have collected data on gonadal index and larval recruitment in the summer through late fall of 2010 and in the spring through summer of 2011.<sup>1</sup> Preliminary data collected in 2010 and preliminary results from the Spring Plan in 2011 show low or zero oyster settlement across much of the study area.

Because the initial preliminary data from the Spring Plan continue to show low or zero oyster settlement rates, the oyster working group has determined to continue injury assessment sampling. The purpose of this sampling is to estimate the degree and spatial and temporal extent of potential injury to oyster reproduction resulting from: 1) potential exposure of oysters to contaminants released into the environment as a result of the Deepwater Horizon Oil Spill; and/or 2) potential exposure of oysters to low salinities resulting from actions undertaken by the state of Louisiana in response to the spill. In addition, these samples may help us further characterize potential impacts to oyster settlement resulting from the opening of the Morganza and Bonnet Carre spillways by the Army Corps of Engineers in May 2011. Additional sampling under the Spring Plan will further characterize the spatial extent and temporal persistence of these low settlement rates, and when complete will provide a data set of oyster spat settlement (in spat per m<sup>2</sup> per day) in the Gulf in 2011 that encompasses both periods (spring and fall) of historically enhanced oyster reproductive activity.

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<sup>1</sup> These efforts also measured other metrics, including abundance, disease prevalence, tissue chemistry, and sediment chemistry.

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## Injury Metrics

The quantitative injury metrics covered by the fall 2011 Amendment 2 sampling are larval recruitment/settlement and disease and gonadal condition index. (See Table 1).

Under the Spring Plan and Amendment 1, recruitment sampling was paired with dredge samples that provided information on abundance, and also collected oysters for both disease/gonad analysis as well as contaminant analysis. The sampling to be conducted under Amendment 2 does not include a full dredge survey for assessing catch per unit effort (CPUE), because the Amendment 2 sampling will overlap with square meter/quadrat abundance sampling that the oyster working group expects to conduct this fall under a separate plan. Instead of a full dredge survey, sampling under Amendment 2 will consist of settlement plate sampling and oyster sampling via dredge for disease and gonad analysis. Dredges pulled under Amendment 2 will not be enumerated for abundance. Any dredges pulled will be taken solely for the collection of the first fifteen market-sized oysters obtained from the dredge for disease and gonad analysis. Thus, the minimum number of dredges at a site may be fewer than three if sufficient resource is obtained for the disease and gonad sample. An SOP for disease/gonad oyster sampling using dredge or tongs for the Amendment 2 sampling is presented at the end of this amendment. As in the Spring Plan and Amendment 1, gonad and disease sampling will be spread across the sampling rounds so that gonadal index data for a subset of sites are collected during each round.

This amendment seeks to provide continuous settlement monitoring through September at as many of the Spring Plan sites as feasible; it also seeks to continue sampling all Spring Plan sites in a timeframe similar to that of the 2010 Transition Plan. The amendment achieves the first goal by redeploying settlement plates at 73 Spring Plan sites that are currently being visited for Round 6 plate retrievals. It achieves the second goal by adding two additional full rounds of settlement plate sampling that begin around October 1 in order to generate data that could be compared against Transition Plan settlement results from the Fall of 2010. Structuring the amendment in this way provides three more rounds of settlement data at more than half the Spring Plan sites, while only requiring that three additional site visits be added to the Spring Plan.

The Spring plan and all its amendments, when complete, will result in 8 to 9 sampling rounds of settlement plates, three rounds of gonad and disease analysis, and two dredge surveys of oysters per site, during 2011, instead of the three sampling rounds and one dredge survey per site described in the original Spring Plan.

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Estimated samples from this activity:

The text in this section and Tables 1 and 2 of this Amendment updates the corresponding information in the Spring Plan as follows:

- Up to 272 dredge surveys of oysters (two sets of three replicates per site);
- Up to 272 composite oyster tissue samples (two per site, up to 6 market-sized oysters analyzed (or equivalent) per sample);
- Up to 408 oyster gonad/disease/condition samples (three per site, up to 15 market-sized oysters analyzed per sample); and
- Up to 1,161 sets of settlement plates (eight sampling events, with 136 sets collected each round, and an additional set of 73 plates collected to bridge the gap between Amendment 1 and Amendment 2 sampling at a subset of sites).

Cost Estimate

The text in the Cost Estimate section and Table 4 of the Spring Plan are updated as follows:

Table 4 provides the cost estimate for the Spring Recruitment Sampling Plan. The total cost associated with this level of field effort is \$2,323,990, including the Spring Plan and both Amendments. Analytical costs for samples collected as part of this plan add up to another \$510,000, including up to \$408,000 for tissue contaminant analysis, and up to \$102,000 for gonad/disease samples, bringing the total cost of the study to \$2,833,990.

The incremental costs of this Amendment are \$723,590, assuming all samples are analyzed.<sup>2</sup>

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. BP's commitment to fund the costs of this work includes any additional reasonable costs within the scope of this work plan that may arise because of any contingencies. The trustees will make a good faith effort to notify BP in advance of any such contingencies.

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<sup>2</sup>This cost assumes \$11,910 for the partial ninth round of 73 settlement plates, based on the additional cost of materials and analysis of the plates. Because deployment and retrieval of these plates would not require additional site visits, the incremental field effort associated with these samples is assumed to be minimal.

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**Table 1. Proposed metrics for the Spring Recruitment Sampling Plan**

<b>Metric</b>	<b>Proposed Frequency of Sampling</b>
<i>Effect Metrics</i>	
Disease	Three samples per site (first sample collected during one of three site visits in Spring 2011, second sample collected during one of three site visits in Summer 2011, and third sample collected in Fall 2011)
Gonadal condition	Three samples per site (first sample collected during one of three site visits in Spring 2011, second sample collected during one of three site visits in Summer 2011, and third sample collected in Fall 2011)
Larval settlement	Eight rounds of plate deployment/retrieval (spaced approximately three weeks apart), and an additional partial round of plates (for three weeks at up to 73 sites)
<i>Exposure metrics</i>	
Tissue concentrations	Two rounds of samples collected during six site visits (three in Spring and three in Summer 2011; not collected in fall sampling)
Oiling observations (qualitative)	Collected on each site visit

**Table 2: Estimated Sampling Activity for Spring Recruitment Sampling Plan**

Metric	Method	N = Sample Sizes (Potential Maximum # of sites)				Estimated subsamples per site	Estimated subsamples per event	Freq. of sampling	Estimated Total # of subsamples
		<i>LA</i>	<i>MS</i>	<i>AL</i>	<i>FL</i>				
Oyster Settlement	Settlement plate	103	15	7	11	2 samples (3 plates each) <sup>1</sup>	272	8-9 <sup>2</sup>	2,322 (1,161 analyzed)
Oyster Gonadal, Condition and Disease	Oysters	103	15	7	11	10-15 oysters	1,360 - 2,040 oysters (136 samples)	3	4,080 – 6,120 oysters
Tissue contaminant analysis	Oysters	103	15	7	11	6 oysters (1 composite)	816 oysters (136 composites)	2	1,632 oysters (272 composites)

<sup>1</sup> Two samples are deployed at each site in the event that one sample is lost during the deployment period. If both samples are retrieved, one sample from the pair will be selected randomly and analyzed, so that only one sample will be enumerated per site. Both the analyzed sample and the unanalyzed sample will be archived.

<sup>2</sup> A partial ninth round will be completed when logistics allow, which will generate an additional estimated 146 samples, 73 of which will be analyzed.

**Table 4. Costs for Spring Recruitment Sampling Plan (Assumes 136 Sites)**

Item	Unit cost	Units	Units	Costs (per event)	# (of events)	Total cost
<b>FIELD SAMPLING/PROCESSING</b>						
<b>Settlement Plate and Dredge Field Sampling</b>						
				\$207,400	8	\$1,659,200
Personnel	█	Person days	█	\$81,600		\$652,800
Boat charges	\$1,600	Days	68	\$108,800		\$870,400
Supplies	\$250	Days	68	\$17,000		\$136,000
<b>Settlement Plate Pickup, Rnds 6 and 8</b>						
				\$128,400	2	\$256,800
Personnel	█	Person days	█	\$54,400		\$108,800
Boat charges	\$1,600	Days	40	\$64,000		\$128,000
Supplies	\$250	Days	40	\$10,000		\$20,000
<b>Settlement Plate Processing</b>						
				\$22,840	8.5	\$194,630
Personnel	█	Person days	█	\$20,800		\$177,215
Supplies	\$5	Samples	136	\$680		\$5,805
Shipping and archive charges	\$5	Samples	272	\$1,360		\$11,610
<b>Dredge Processing</b>						
				\$85,680	2	\$171,360
Personnel	█	Person days	█	\$81,600		\$163,200
Supplies	\$5	Samples	408	\$2,040		\$4,080
Shipping and archive charges	\$5	Samples	408	\$2,040		\$4,080
<b>Cooler Rental (Maintained at Dauphin Island Sea Lab)</b>						
	\$2,000	Month	7	\$14,000	3	\$42,000
<b>Field Sampling/Processing Total</b>						<b>\$2,323,990</b>
<b>LABORATORY ANALYSIS</b>						
Sediment Contaminants	\$1,500	Sample	N/A	N/A	N/A	N/A
Oyster Contaminant	\$1,500	sample	136	\$204,000	2	\$408,000
Disease and Gonad	\$250	sample	136	\$34,000	3	\$102,000
<b>Laboratory Total</b>						<b>\$510,000</b>
<b>TOTAL</b>						<b>\$2,833,990</b>

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**Amended SOP for Tissue Collection for Gonadal Condition and Oyster Disease Analyses  
[APPLIES ONLY TO SAMPLES COLLECTED UNDER SPRING PLAN AMENDMENT 2]**

1. Sampling Objectives

- a. Sampling objectives include collection of oysters to determine the reproductive condition of oysters at each sampling site.
- b. Collection of oysters for oyster disease analysis.
- c. To maintain the integrity the sample(s) during sampling, transport, and storage.

2. Sample Size and pre-sampling activity

- a. At least 15 market-sized oysters for gonadal condition analysis. These same oysters will be used for oyster disease analysis.
- b. Clean dredges, knives, etc. between samples. If no oil is visible wash in ambient water. If the equipment was obviously contaminated, scrub with Alconox solution. Collect rinsate for proper disposal.

3. Take relevant photos at all sites, including a picture of the dredge once it is full including overall contents and visual appearance of size/condition of oysters/shells in dredge.

4. Adult Oyster Sampling Locations

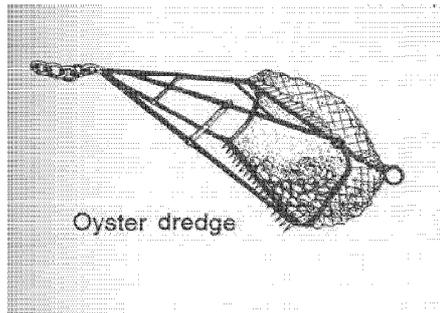
- a. Up to eight randomly generated contact points will be used to determine dredge sampling locations. These contact points will be generated as a random sample of points from transect segments identified during mapping of the cells under the Oyster Phase I and Oyster Transition sampling plans to contain Class III bottom surface.
- b. Field teams should begin dredging at the first contact point on the list and proceed to collect a sufficient number of dredges to obtain 15 market-sized oysters. Field teams may continue to dredge at the remaining contact points until 15 market-sized oysters have been collected or until two hours have elapsed, whichever comes first.

5. Sample Collection Methods

- a. Dredge harvesting using a 24 inch wide oyster dredge may be used to collect resource:
  - i. Deploy dredge from the beam or stern of the vessel.
  - ii. Record exact start and stop positions using a GPS. Start location is the point at which the dredge enters the water. Stop is the point at which the vessel stops moving in a forward direction (i.e., the stop point will be marked before the dredge is brought onboard).

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- iii. Drag dredge across the surface of the substrate for 3 minutes at 2 knots in a circular pattern.
  - iv. Additional dredge pulls beyond the initial pull may be performed if needed to obtain the target number of oysters. Additional dredges may be performed until the required number of live market size oysters (15) is collected (or until 2 hours have passed).
- b. Sample collection using tongs:
- i. In areas where dredging is not possible because of logistical or permit difficulties, oyster tongs may be used to collect oysters.
  - ii. Oyster tongs are generally 2-3 m long and constructed of two rakes welded or bolted together at the center point of the handles. The teeth on the rakes are generally 25 cm long and the head of the rake 1 m in length. The rakes are juxtaposed to form a small basket when closed (local variations on oyster tongs are common and measurements need not be exact).
  - iii. Once at a site, the tongs can be deployed over the side of the boat. Once placed on the bottom the tongs are opened and closed repeatedly to dislodge oyster from a small area.
  - iv. After 6-10 opening and closing events, the tongs are used to collect the dislodged oysters into one grab. The tongs are held closed and the operator withdraws the handles from the water and places the contents on the deck.
  - v. The entire procedure is repeated until the targeted number of oysters is collected.
  - vi. Four deployments should be made at each site; if no live oysters are collected, the boat should be repositioned 2 m away and the procedure repeated.
6. Sample preparation
- a. Retain the first fifteen (15) live, market-size (> 3 inches [75 mm] shell height) oysters encountered as samples for disease/gonad analysis. The remainder of each dredge's materials do not need to be retained.
  - b. Preparation of oysters for disease gonad analysis (15 oysters): Wearing clean latex or nitrile gloves Wrap each oyster individually in aluminum foil then place all wrapped oysters in a 2-gallon Ziploc bag. Close bag.
  - c. Samples should be tagged with an external (flagging tape with permanent marker) and internal flagging tape tag that prominently denotes sample code.
  - d. The sample code should be constructed of the location ID, date, matrix, unique sampler ID, Grid Cell ID and sample number along with information regarding sample type (for details, see the Oyster Sample ID Naming Convention, Appendix B).
  - e. Hold animals on ice until delivered to intake team.



- f. Wear nitrile or other non-contaminating gloves and change gloves after each sample to avoid cross-contamination. Record observations of any external evidence of contamination.
- g. Shellfish should not be opened in the field to minimize the risk of contamination.
- h. Avoid sources of contamination such as exhaust fumes and engine cooling systems on vessels. Work upwind of any exhausts. Segregate dirty/clean areas. Lay out clean substrates to work on and replace frequently. Take precautions so as not to introduce cross-contamination from oil on boots and shovels.
- i. Use packing material around sample containers to prevent breakage during handling and shipping.
- j. When accounting for other species present in the dredge on the field form it is important to document the presence of oyster drills in the sample; blue crabs and mud crabs will not be recorded.

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