

**Assessment of the Impacts of the Deep Water Horizon Oil Spill on  
Blue Crab Early Life History Stages: Addendum**

**PHASE II: SAMPLE SORTING AND IDENTIFICATION**

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Mississippi Canyon 252 (MC252)

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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 the right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

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

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

**APPROVED:**

*Tief Havel* 01/25/2012  
Mississippi Trustee Representative: Date

*Joyce Malley* 01/27/2012  
BP Representative: Date

*[Signature]* 2/15/2012  
FOR ROLAND GUYON  
Louisiana Trustee Representative: Date

**Assessment of the Impacts of the Deep Water Horizon Oil Spill on Blue Crab Early Life History Stages: Addendum**

**PHASE II: SAMPLE SORTING AND IDENTIFICATION**

This document presents Phase II of a monitoring and assessment plan for crab megalopae and ovigerous female crabs within the Mississippi waters in the north-central Gulf of Mexico (“Plan”). The Plan is intended to be implemented where MC 252 oil or dispersants (“MC 252 Oil” or “oil”) have been documented, or where historic megalopae collection areas may have been impacted. The data collection described in this Plan is intended to support the Deepwater Horizon natural resource damage assessment. The Plan specifically addresses the following topics:

- I. Purpose.** This section describes the overall purpose of and need for blue crab megalopal sample sorting, identification, and photo-documentation.
- II. Approach.** Defines the objectives of the Plan and provides an overview of the approach.
- III. Description of Metrics.** Provides a brief synopsis of the parameters to be measured and sampling units for the various collection metrics.
- IV. Sample and Data Handling**
- V. Integration with Other Approved or Pending Work Plans.**
- VI. Study Description**
- VII. Literature Cited**
- VIII. Detailed Standard Operating Procedures.** This section sets forth the standard operating procedures for laboratory activities.
- IX. Budget**

## **I. Purpose**

The overall study examines the impact of oil on early life history stages of blue crabs through monitoring and/or analysis of blue crab megalopae and ovigerous females from the natural environment, and through culture of blue crabs using ovigerous females taken from oil-impacted barrier island beaches and a control beach. The study is divided into three phases. Phase I has been implemented: it involved the collection of megalopae and ovigerous female tissues and the storage of both for subsequent examination and analysis. Phase II (this proposed Plan) involves the laboratory sorting, identification, and photo-documentation of megalopae collected in settlement samples in 2010 and 2011. Phase III involves chemical analysis of the samples to determine the composition of oily droplets which were found under the carapaces of blue crab megalopae from impacted areas, and may provide for the culture of blue crabs in the laboratory. This plan addresses activities to be conducted in Phase II of the study.

## **II. Approach**

This Phase II Plan involves the laboratory sorting, identification, and photo-documentation of megalopae collected in settlement samples in 2010 and 2011.

## **III. Description of Metrics**

Metrics include:

- a. Taxonomic identification of crab megalopae.
- b. Numbers and percentage of crab megalopae of each species exhibiting oily droplets.
- c. Number and volume of oily droplets in selected megalopae.

## **IV. Sample and Data Handling**

MC 252 NRDA chain-of-custody procedures will be observed at all times for all NRDA samples. All samples will be transferred with appropriate chain-of-custody forms. All field and laboratory data will be collected, managed and stored in accordance with US EPA Good Laboratory Practice regulations (GLPs) to the extent practicable. In accordance with GLPs, all field and laboratory work, and the calibration and use of field and laboratory equipment (e.g. scales, hand held GPS devices, etc.) shall be conducted using written Standard operating Procedures (SOPs). The appropriate training on particular equipment or in the conduct of specific field studies for all personnel involved with the project shall be documented, and those records kept on file by the implementing entity for the duration of this project. All data (including electronically archived data), and original data sheets or electronic files, will be

promptly transferred to MDEQ, with copies to NOAA, BP or its representative, and the Louisiana Oil Spill Coordinator's Office (LOSCO).

## **V. Integration with Approved or Pending Work Plans**

Results from this study Plan will be integrated with toxicity results obtained by the toxicity subgroup to determine whether blue crabs were injured due to exposure of crabs to weathered MC 252 Oil and dispersants (DOSS and related compounds). The Plan will also attempt to measure spawning success of crabs exposed to MC252 oil and components.

In addition, this Plan will supplement the "Assessment Plan for Marsh Edges and Sandy Shorelines," the "Work Plan for MC252 Oil Impacts to Marsh Fiddler Crabs and Periwinkles along the Gulf of Mexico," and potentially other work plans. The information collected by this sampling Plan is unique, however, and does not duplicate materials collected in either of these ongoing and planned activities.

## **VI. Study Description**

### **PHASE I (Implemented)**

Blue crab megalopae were sampled daily using standard settlement collectors made from air conditioner filters and PVC pipe (Perry et al., 1995; Metcalf et al., 1995). The collectors were deployed at two sites along the Mississippi coast (East Belle Fontaine area, Jackson County, and the I-110 overpass (Loop) in Harrison County). Deployment occurred from 1 September to October 31, 2011. Four hundred and eighteen samples were preserved by freezing and are archived at the Gulf Coast Research Laboratory (GCRL). Ovigerous females were collected from the Mississippi barrier islands of Cat, Ship and Horn, with sample locations chosen based on SCAT data from previously oiled sites.

### **PHASE II**

Megalopae processed under Phase II include those collected under Phase I of this plan in 2011, and from other sample collections performed in 2010. The samples from 2010 consist of approximately 800 samples currently archived in a locked freezer at the GCRL. These 2010 samples were collected under a National Science Foundation Rapid Response grant to investigate megalopal abundance following the MC 252 oil spill. Megalopae from these two sets of collections will be identified and sorted to the lowest possible taxonomic level, and will be

examined for the presence of oily droplets using microscopy. Selected megalopae with oily droplets will be photographed, the number of droplets determined and a volume of oil calculated.

In the laboratory, samples will be thawed and sorted to species (Bench Sheet 1). Each site has four replicate collectors which were sampled daily. Each replicate will be separated into oiled and un-oiled megalopae by species. For megalopae with droplets, the following aliquot protocol will be followed:

1. Get total number and pool megalopae of like species;
2. If total number is 10 or less, work up (as described below) all of them;
3. If numbers are 11–100, select 10 random megalopae to work up;
4. If numbers are 101–200 work up random 10%;
5. 201-500 work up random 5%;
6. 501+ work up random 2%.

Aliquoted megalopae will be photographed and the droplets counted, measured, and volume determined (Bench Sheet 2). This will be accomplished using a Nikon microscope equipped with [NIS Elements Imaging Software](#) tailored for facilitating image capture, object measurement and counting, databasing, and report generation. During the above procedures, [megalopae will be handled with small, artists brushes to minimize dislodgment of the droplets.](#) Following photo-documentation, all megalopae will be placed in trace clean vials with Teflon-coated lids and maintained in a locked freezer. Each week, megalopae from two randomly selected sample-days from each site will be chosen for analytical testing for PAHs and dispersant or dispersant constituents. These samples will be maintained in a separate locked freezer. All data will be stored in a locked cabinet.

## VII. Literature Cited

- Metcalf, K.S., J. van Montfrans, R.N. Lipcius, and R.J. Orth. 1995. Settlement indices for blue crab megalopae in the York River, Virginia: temporal relationships and statistical efficiency. *Bulletin of Marine Science* 57(3): 781-792.
- Perry, H.M., C.K. Eleuterius, C.B. Trigg, J.R. Warren. 1995. Settlement patterns of, *Callinectes sapidus*, megalopae in Mississippi Sound: 1991, 1992. *Bulletin of Marine Science* 57(3): 821-833.

## VIII. Standard Operating Procedures

*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.*

### **General Guidance for Megalopal Sorting and Identification**

- *Megalopal sorting*
  - Personnel will wear nitrile gloves during all laboratory activities and gloves will be changed between samples.
  - All glassware and sorting instruments will be decontaminated using an Alconox wash followed by a rinse with isopropyl alcohol and distilled, de-ionized water (DI water). Following the decontamination procedure described above, the instruments will be placed in a covered, clean glass container filled with distilled, de-ionized water and soaked overnight. The container will be decontaminated daily and the water changed. Enough sorting glassware and instruments will be cleaned to accommodate a day of sorting activities. All surfaces in contact with glassware (eg. microscope stage) will be wiped daily using isopropyl alcohol and DI water.
- *Labeling and Laboratory Data Sheets*
  - All data will be recorded on Bench Sheet 1 and 2. Bench Sheet 1 will be utilized for lab sorting and identification of species. Enumeration of megalopae with and without droplets will also be recorded. Bench Sheet 2 will be used with each sample aliquot to count, measure, and determine the volume of droplets in each megalopae.
  - As samples are processed in the laboratory, personnel will use the "Sample Split Composite Crosswalk" form, as requested and obtained by the NOAA NRDA data management team. This will maintain consistency when generating new sample IDs from sample aliquots.

### **IX. BUDGET**

As detailed in the budget spreadsheet attached hereto, the total field costs for this Plan is \$491,880. The Parties acknowledge that this budget is an estimate, and that actual costs may prove to be higher. BP's commitment to fund the costs of this work includes any additional

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

Harriet Perry & Darcie Graham  
 Blue Crab Recruitment 2010 & 2011 GCRL lab processing  
 MS DEQ - NRDA  
 10/5/2011

		Agency	
<b>SALARIES</b>	<b>Duties</b>		Months
H. Perry	PI		█
D. Graham	PI		█
T. VanDevender	Lab/Meg 2010-2011		█
R. Trigg	Lab/Meg 2010-2011		█
G. Mavar	Lab Technician		█
L. Collins	Lab/Meg 2010-2011		█
QA/QC Officer - TBD			█
Grad Student	Lab/Meg 2010-2011		█
		Sub	
			41,625
			10,192
			29,874
			23,587
			13,865
			22,932
			26,004
			19,200
			187,279
<b>FRINGE</b>			
H. Perry			█
D. Graham			█
T. VanDevender			█
R. Trigg			█
G. Mavar			█
L. Collins			█
QA/QC Officer			█
Grad			█
		Sub	
			3,746
			3,325
			2,689
			9,534
			5,220
			9,388
			9,965
			954
			44,821
<b>TOTAL PERSONNEL</b>			<b>232,100</b>
<b>TRAVEL</b>			2,000
Misc travel - supplies, etc.			2,000
<b>COMMODITIES/SUPPLIES</b>			38,000
Freezers			3,000
Trace Clean Jars			8,000
Trace Clean Vials			8,000
Storage Container for Vials			3,000
DI Water			6,000
Lab-Chemicals/sample work-up			6,000
Miscellaneous/office			4,000
<b>COMMUNICATIONS</b>			500
<b>EQUIPMENT (\$5,000)</b>			0
<b>PARTICIPANT COSTS</b>			0
<b>PROFESSIONAL FEES</b>			0
<b>SUBCONTRACTS</b>			0
<b>CONTRACTUAL SERVICES</b>			51,750
Nikon Image Analysis Microscope Rental - \$2875/month			51,750
<b>TUITION</b>			16,707
<b>Out of State Grad</b>			16,707
<b>TOTAL DIRECT COSTS</b>			341,057
<b>F&amp;A (Indirect) █████ MTDC</b>			150,823
<b>BOAT RENTALS</b>			0
<b>TOTAL PROJECTS COSTS</b>			491,880

**Bench Sheet 1.**

Team Leader Code: \_\_\_\_\_ Survey Team ID: \_\_\_\_\_

2011 Megalopal Settlement: Sort and Identification

Field Date: \_\_\_\_\_

Lab workup date: \_\_\_\_\_ Lab Technician: \_\_\_\_\_

Sample IDs:	# w/droplets					Total #	# in aliquot	# w/o droplets					Total #
	A	B	C	D	A			B	C	D			
<b>Callinectes sapidus:</b>													
Megalopae													
First Crab Stage													
<10 mm (CW)													
<20 mm (CW)													
Other:													
Other:													
<b>Callinectes similis:</b>													
Megalopae													
First Crab Stage													
<10 mm (CW)													
<20 mm (CW)													
Other:													
Other:													
<b>Other Species:</b>													

Notes: \_\_\_\_\_

