Assessment Plan for *Sargassum* Communities and Associated Fauna in the Northern Gulf of Mexico

Prepared By

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For the

Mississippi Canyon 252 Trustees

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Approval of this work plan is for the purposes of obtaining data for the Natural Resource Damage Assessment (NRDA). Each party reserves its rights to produce its own independent interpretations and analyses of any data collected pursuant to this work plan.

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

APPROVED:

NOAA Trustee Representative: _____ Date: 5/7/11

Louisiana Trustee Representative: _____ Date: 5/18/11

BP Representative: _____ Date: May 9, 2011

Summary

This document presents an injury assessment plan (Plan) for *Sargassum* communities associated with the north-central Gulf of Mexico. The data collection described in this Plan targets ephemeral data and can be reasonably expected to support a Natural Resource Damage Assessment under the Oil Pollution Act. The Plan specifically addresses the following topics:

I. **Approach and rationale.** This section describes the overall purpose of and need for a *Sargassum* injury assessment plan. Field work for this study is proposed to be initiated in May 2011 and continue through September 2011.

II. **Data needs and sources.** This section provides an overview of the types of data that will be needed to complete the assessment of *Sargassum* communities and fauna associated with these *Sargassum* communities.

III. **Health and safety.** This section summarizes health and safety protocols that are relevant to this effort. It includes a number of procedures by reference, all of which should be carefully reviewed and adhered to by all team members.

IV. **Site selection strategy.** This section describes a proposed approach to identifying sites for evaluation.

V. **Site procedures.** This section provides guidance on what to do with the samples and data gathered. This section makes frequent reference to protocols within this document and also to NRDA-wide procedures that are incorporated by reference. Field team members should make sure they understand and adhere to all procedures, whether included here directly or by reference.

VI. **Detailed Standard Operating Protocols (SOPs).** This section sets forth the standard operating procedures (SOPs) proposed for use during study execution.

I. Approach and rationale

Floating *Sargassum* is a key oceanic habitat that may have been impacted by the release of MC 252 oil and dispersants (hereafter referred to as "MC 252 oil"). *Sargassum* patches in the Gulf of Mexico are formed by the convergence and aggregation of two species of brown algae: *Sargassum natans* and/or *S. fluitans*. The Gulf of Mexico region may be the second most productive *Sargassum* system in the world (Gower and King 2008). The pelagic brown algae is an oasis of structure in the open ocean and supports an assemblage of organisms, including marine fish and invertebrates.

Because *Sargassum* patches (which may be mats, clumps, or convergence lines) are found in the neuston (floating surface layer of organisms), these habitats and associated fauna were, and may still be, potentially at risk of exposure to surface oil, sheens, and chemical dispersants introduced as a result of the MC 252 discharge. Although shipboard observation of *Sargassum* patches can be traced back centuries, little is known about the life history and ecology of floating *Sargassum* patches. Until recently, it was assumed that *Sargassum* originated in the Central North Atlantic Ocean and was advected to the Northern Gulf of Mexico (nGOM) by surface winds and ocean currents (SAFMC 2002). Recently, scientists examining satellite imagery have questioned this theory and proposed that the opposite pattern occurs, i.e. *Sargassum* originates in the nGOM and is advected to the North Atlantic (Gower and King 2008). Given that floating *Sargassum* plants may live for two years or more, and the nGOM may be a nursery for the algae, the effects of the MC 252 oil may last longer than the surface slick.

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Sargassum natans is far more abundant than *S. fluitans*, comprising up to 90% of the total global drift macroalgae. Limited quantities of several other benthic species, including *S. filipendula, S. hystrix, S. polycertium, S. platycarpum,* and *S. pteropleuron*, detached from coastal areas during storms, are also frequently encountered adrift. However, the drifting fragments of these benthic species soon perish (SAFMC 2002). The pelagic species are golden to brownish in color and typically 20 to 80 cm in diameter. *Sargassum* has a complex morphology in which the thallus branches into stripe-bearing leaf-like appendages (fronds) and gas-bladders resembling berries (pneumatocysts) (see Figure 1). Gametes are produced on special branches called "receptacles." These, in turn, have pits termed "conceptacles" in which the ova or sperm are produced. Propagation is achieved predominantly via vegetative fragmentation. As *Sargassum* ages, the plant darkens and the fronds become heavily encrusted with a variety of life forms. As the fronds grow heavier from encrusting animals, density exceeds the buoyancy provided by the *Sargassum's* gas bladders, and the alga begin to sink.

Floating *Sargassum* patches are an important habitat for a variety of invertebrate and vertebrate species. The algae provide substrate for attachment of a variety of encrusting invertebrates (e.g., bryozoans, barnacles, hydroids, etc.). In addition to the encrusting

community the plant supports, the pelagic brown algae complex of *Sargassum natans* and *S. fluitans* supports large and diverse assemblages of marine fish in early life history stages. Fish larvae and juveniles may utilize these pelagic habitats as protection from predators, but *Sargassum* may also provide enhanced feeding opportunities and serve to concentrate larvae and juveniles with flotsam-seeking behaviors (Rooker et al. 2006). *Sargassum* patches, therefore, serve as pelagic "nursery habitats" for many fishery species, including common dolphinfish, triggerfishes, tripletail, billfishes, tunas and amberjacks, as well as forage fish species, such as butterfishes and flyingfishes (Comyns et al. 2002; Hoffmayer et al. 2005). For these reasons, *Sargassum* has been designated an Essential Fish Habitat by both the South Atlantic and Gulf of Mexico Fishery Management Councils and by National Marine Fisheries Service (SAFMC 2002). As *Sargassum* patches are concentrated in the nGOM, these neustonic habitats and associated fauna may have been exposed to surface oil, sheens, and chemical dispersants introduced as a result of the MC 252 discharge. The overall evaluation of *Sargassum* will include juvenile sea turtles; however, this Plan is designed to address other components of the *Sargassum* community including invertebrates, fish of several life stages and *Sargassum* itself.

The main objectives of this study plan are to:

(1) Determine the 2011 areal extent and distribution of *Sargassum* in the north-central Gulf of Mexico via aerial surveys, and its spatial relationship to previously observed surface oil and dispersants in 2010 associated with the MC 252 discharge; and

(2) Document density, abundance and diversity of invertebrates and fishes associated with pelagic *Sargassum*, including assessment of any remaining degrees of MC 252 oil.

Timeline

The trustees and BP Exploration & Production, Inc. ("BP") intend to conduct, and BP agrees to fund, 8 sampling events beginning in May 2011. It is anticipated that monthly surveys will be conducted in May and June, followed by bi-weekly surveys from July through September.

Study Area

Principal study components of this proposal focus on areas potentially impacted by MC 252 oil that are immediately west and south of the Mississippi River Delta, in Louisiana and Mississippi. Even if a *Sargassum* patch may no longer be showing visual signs of MC 252 oil exposure, *Sargassum* patches and associated fauna encountered in these areas may have been exposed to and potentially injured by MC 252 oil.

This plan is designed to collect data to evaluate potential injury based on a surface floating community's potential risk of prior or ongoing exposure to MC 252 oil. The study design is intended to be sufficiently robust to support possible future empirical or modeling-based injury assessment approaches.

II. Data Needs and Sources

A. Aerial estimates of *Sargassum* distribution (Objective 1).

Aerial surveys are commonly used to assess the abundance of surface-occurring marine fauna (Craig and Reynolds 2004). *Sargassum* aggregations are easily seen from the air during calm weather. Aerial surveys will be used to determine the location of *Sargassum* patches in standardized survey grids. A systematic sample of equally spaced parallel transect lines (or strip transects) will be adopted and flown to determine the location of *Sargassum* patches (see section VI for details). Figure 2 is included as an illustration of the type of transects being proposed for the current study based on previous survey methods. These previous surveys were flown weekly in spring 2010 (April – June) in the northeastern GOM from the Chandeleur Islands to the Alabama/Florida state line with the support of a National Science Foundation (NSF) sponsored grant (S. Powers, U. South Alabama, unpublished data).

The aerial survey under this work plan is designed to include eight equally spaced transects that cover a total of 2000 square nautical miles (NM^2) per grid. A total of two grids have been identified to represent the highest probability of encountering Sargassum that may still be oiled, (Figure 3). These two survey grids are located in the nGOM extending between Louisiana and Alabama, specifically offshore of Venice, LA and Mobile, AL. It is assumed that *Sargassum* patches found between Venice, LA and Mobile, AL are more likely to have been exposed to MC 252 oil than *Sargassum* patches that may be found further east in the nGOM, which is why they are being targeted for this 2011 field work. However, the degree of any remaining MC 252 oil exposure to *Sargassum* patches in these areas will be determined during each sampling event based on visual evidence at sea and contaminant analyses of collected material.

Sargassum Locations

To direct ship-based sampling efforts, aerial grid surveys will be flown the day prior to the day of ship-based sampling. To the extent possible, aerial observers will photograph all *Sargassum* patches in the grid (e.g., see Figure 4), and distance off transect to each target will be estimated. Off transect distances and aerial line transect methodology will be used to estimate total number (or density) of potential targets in the study grid. Off transect distances in combination with the altitude of the aircraft and angle at which the patch was photographed will be used to estimate the size of each *Sargassum* patch that is a potential target for boat-based sampling. Using estimated density and average target size, the total extent (hectares) of *Sargassum* in the grid will be estimated.

Data necessary to estimate sightability (probability of detection) of *Sargassum* patches from the air will be collected on all flights. These data will consist of sightings made by two pairs of independent observers and data recorders, and the off-transect distance associated with each sighted target. Each observer/data recorder pair will focus on their side of the aircraft, with the data recorder serving as an additional observer to assess the probability of detecting *Sargassum* patches. The recorder and the observer on the same side of the aircraft will not communicate a target sighting until both have had ample opportunity to detect it. The number of targets observed by the data recorder and also observed by the regular observer will form the

basis of an estimate of sightability. Estimates of abundance and density of *Sargassum* will then be corrected for reduced distance-sightability and inherent observer sightability using standard distance sampling analyses.

Figure 2. Aerial surveys flown on June 10, 2010. Similar systematic surveys will provide information on *Sargassum* distribution (source S.P. Powers unpublished data).

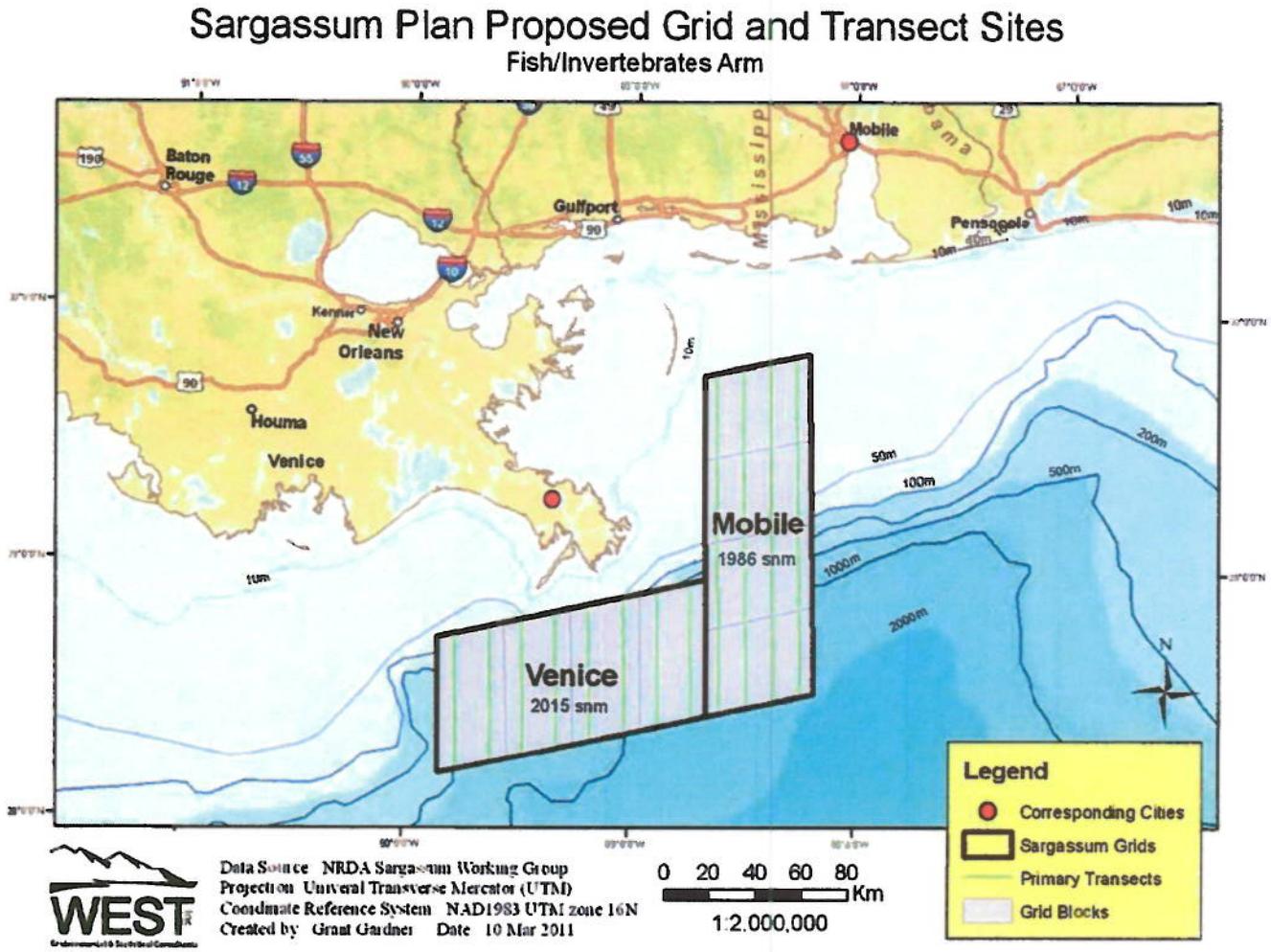

Figure 3: Two Sampling Grids: Venice, LA and Mobile, AL

Figure 4. Ship-based sampling of *Sargassum* mats identified by aerial surveys.

B. Quantification of associated fauna (Objective 2)

The sampling program will sample three types or classes of Sargassum patches: mats, clumps, and convergent lines. Following the aerial surveys, a number of potential sampling targets will be identified and classified as mats, clumps, or convergent lines. Fish and invertebrate surveys will be conducted in two survey grids: Venice, LA and Mobile, AL. See section V for site selection details. A BP/CardnoENTRIX representative will be invited to participate in all boat-based field surveys, if possible. If agreed-upon notification and communication procedures are followed regarding the schedule for sampling, yet circumstances prevent BP or its designated representative from participating in a field effort, the field effort may be carried out without BP or its designated representative's participation.

Within the testing sites, the following will be sampled:

(1) *Fishes and invertebrates*

(a) *Larval fish*

Early life history stages of many Gulf of Mexico marine fish utilize pelagic *Sargassum* as habitat (Comyns et al. 2002; Hoffmayer et al. 2005). Previous research suggests that *Sargassum* quantity is positively correlated with larval and juvenile fish abundance and richness (Casazza and Ross 2008). The faunal assemblage structure differs,

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however, with both the age and the geographic distribution of *Sargassum* patches (Stoner and Greening 1984). Larval fish associated with *Sargassum* will be sampled via ship-based 333 μm mesh bongo plankton nets with 60 cm net opening (towed under the canopy) (see Figure 5).

(b) *Juvenile fish and encrusting and small invertebrates*

Juvenile fish representing numerous species are associated with pelagic *Sargassum* in the Gulf of Mexico. Pelagic *Sargassum* also represents one of the few natural substrates available for sessile invertebrates (e.g., hydroids, polychaetes, bryozoans, etc) in open waters of the Gulf. In addition, smaller invertebrates with limited motility (copepods, gastropods, crustaceans) can utilize the refuge and food provided by the *Sargassum* fronds. This community of organisms represents potential prey items for higher trophic levels such as fish and sea turtles. A neuston net will be towed directly through each *Sargassum* patch at a random location for a distance of up to 2 m to collect samples of *Sargassum*, juvenile fish and invertebrates (encrusting and small invertebrates). The surface area (m^2) of *Sargassum* collected will be determined via direct visual estimation of the distance (length, m) of the neuston net tow X the known width (m) of the net frame. Juvenile fishes and invertebrates will be identified and enumerated in the lab and standardized to the biomass of *Sargassum* collected (see Section VI – SOPs). Samples of the *Sargassum* plant will also be collected from each patch for contaminant analysis as described more fully below.

(c) *Transient and resident large fishes*

1. ROV surveys: To collect census data on juvenile and adult fish associated with pelagic *Sargassum*, ROV video surveys will be conducted at *Sargassum* convergent lines, mats and clumps of sufficient size (at the discretion of the researchers in the field) for ROV sampling. Surveys will be made just beneath the *Sargassum* canopy, between a depth of 0.5 and 3 m, along three transects of at least 25 m in length. Video images will be recorded and archived for fish identification and counts (fish per m^3). Relatively small (suitcase-sized) ROVs can be used, provided the ROV has recordable video and a laser scaling system, which is necessary to determine fish size.

2. Pelagic longline surveys: To estimate the abundance (expressed as catch per unit effort, CPUE) of larger, highly mobile predatory pelagic fishes associated with *Sargassum,* and to collect specimens of large pelagic fishes for contaminant content analyses, a pelagic (near surface) longline will be deployed at *Sargassum* patches. A longline will be fished for one hour. The longline gear will consist of up to 4.8 km of 4.0 mm monofilament mainline sampled with up to 150 3.7 m long gangions, and 75 4.0 m droplines. Gangions consist of a longline snap and a #16 circle hook, baited with Atlantic mackerel (*Scomber scombrus*). Abundance will be expressed as fish per 100 hook hours. The length of the mainline, type and number of gangions and bait type is consistent with routine NMFS survey methodology designed to prevent incidental take of sea turtles. Use of consistent gear will allow for direct comparisons and additional utility (Driggers et al. 2008).

The Lead Investigators will obtain an HMS Exempted Fishing Permit for conducting the longline sets. As part of the permitting process, appropriate marine mammal and sea turtle impact mitigation measures, such as type of hook and duration of soaks will be specified. In addition, this study plan has been reviewed by NOAA protected resources staff to further ensure that appropriate mitigation measures are being taken. The Lead Investigators will be notified of any additional guidance on mitigation measures that may arise from this review prior to the first sampling cruise. Furthermore, at least one scientist on each cruise will have certification in the safe handling of protected resources.

C. Physical Parameters and Contaminant Analyses (Objectives 1 & 2)

(1) Water quality parameters

Abiotic variables will be collected in each sampling area, including vertical profiles of depth, surface and bottom temperature readings, salinity and dissolved oxygen to a maximum depth of 25m. These measurements will be done with standard oceanographic instrumentation (e.g., Seabird SBE911 plus, or an SBE 25 CTD). Standard observations of sea state, light, wind and turbidity will also be documented.

(2) Contaminant analyses

A suite of contaminant analyses will be performed on animals and plants collected during the study. Specifically, 75 g of tissue will be collected from two replicate *Sargassum* plants at each patch sampled and preserved at -20°C for subsequent contaminant analyses. Prior to freezing, the *Sargassum* samples will be photographed and their physical condition will be noted. In the laboratory, *Sargassum* tissue will be analyzed for hydrocarbons (including PAHs), weathered oil components, and other compounds that may help identify the source of any petroleum products. Forensic fingerprint analysis will be conducted on any oiled *Sargassum* collected, unless such analysis is not technically feasible. Samples of fish tissues and invertebrates will be collected from the most common fish and invertebrate taxa observed during neuston and longline surveys (as estimated by the PIs during at-sea operations). Up to 2 samples of the 5 most common species observed on each of these surveys will be collected from each of the patches sampled. Although not proposed as part of the current scope of work in this work plan, sampling and potential analyses of bile samples may be addressed separately by the Trustees.

III. Health and Safety

- The team leader and field crew shall have completed all applicable health and safety training as directed by NOAA or state agency oil spill policy.

- All field team members must complete the NOAA safety training and documentation requirements as set forth in "Safety Requirements for All Personnel Working on NOAA led NRDA teams for MS Canyon 252 Incident" (NOAA Safety Documentation Requirements.doc).

- Exception: if a specific team's site collection activities do not include use of either a boat or an aircraft, then familiarity with the safety documents for these vehicles is not required.

- At least **one week prior** to the start of field sampling, the field team leader will inform the NOAA NRDA Field Ops Team [redacted] of the intended operations. This notification will outline specific information regarding the operation, including at a minimum:
    - Point of Contact, including cell phone number and email,
    - Number of field teams and number of samplers per field team, including contact information for Federal Representatives,
    - Start date,
    - Number of anticipated field days,
    - Whether assistance in securing vessels will be needed,
    - General description of planned activities,
    - General operational area(s), and
    - Whether sample/data intake will be needed.

- Before conducting fieldwork, the field team leader will complete the following:

    - **Team Tracking Field Plan** - Updated daily to provide Field Ops with information about the operations (staff, operational area, launch, contact information)
    - **Site Safety Plan** - The field team will be expected to review and comply with the current Site Safety Plan accessed on noaanrda.org in the Documentation: Safety Protocols section. The field team leader will determine what required safety equipment the team needs
    - Field teams must adhere to all procedures set forth in the "Deepwater Horizon NRDA Site Safety Plan Version 12/08/2010," ("NRDA_OPS_Safety_Plan_08DEC2010.docx").[i]

IV. Site Selection Strategy

Sites selected for sampling should represent areas where we suspect MC 252 oil may still be found. For planning purposes, it is assumed that *Sargassum* patches found off Venice, LA and Mobile, AL have had a higher likelihood of encountering MC 252 oil than those patches found farther east in the nGOM. MC 252 oil exposure of each patch encountered will be determined for each sampling event based on both visual evidence at sea and contaminant analyses of collected material.

[i] This file is available on http://noaanrda.org

The day prior to planned boat sampling trips, aerial surveys of the subject grids will be conducted. Aerial grid surveys will be conducted for both areas: Venice, LA and Mobile, AL. During aerial surveys, fixed transect lines will be flown over the grid and sufficient data (e.g., off transect distances, size class, type, etc.) will be collected to allow estimation of *Sargassum* patch density. All sighted patches will be classified as either mats, clumps (isolated, small aggregations of algae floating at the surface not aggregated into distinct lines or mats), or convergence lines. Following aerial surveys, the observed *Sargassum* patches will be plotted on a set of four survey blocks superimposed on the grid (see Figure 3). For each of the survey blocks within the grid, the number of different types of *Sargassum* patches found (i.e., mats, clumps, and convergence lines) will be computed. For example, if two types of *Sargassum* patches were spotted in a particular survey block, the count for that block would be 2. Possible counts are 3, 2, 1, and 0. The 4 survey blocks within the grid will be sorted by number of *Sargassum* types present. To maximize efficiency of sampling in the field, boats will target those blocks with the most sighted types and suitable *Sargassum* patches. If two or more blocks tie for the highest numbers, one block from those with the same number will be chosen at random.

This information will be given to the planning staff so the ship-based surveys the following day can be directed to the selected sampling grid and the chosen block. Ship-based surveys will begin at the center point of the pre-selected block. The aircraft will join the effort and will rendezvous with the ship at the designated center point. The aircraft will then proceed to work in coordination with the ship and direct it to *Sargassum* patches for sampling. Working in coordination generally means the aircraft will direct the ship to the *Sargassum* patch that it deems nearest the current position of the ship and that is of sufficient size to allow sampling. If all three types of patches are not available to sample within a grid, the field team may choose to sample more than one patch of the same type of *Sargassum*.

V. Site Procedures

This Plan has seven sampling elements: (1) aerial survey assessment of distribution of *Sargassum* in the northcentral Gulf of Mexico; (2) collection of *Sargassum* plants to quantify the encrusting community and for contaminant analyses, (3) ship-based bongo and neuston net tows to quantify larval fish and juvenile fish and invertebrate densities; (4) ROV transects for transient and resident fish density and size frequency; (5) pelagic longline surveys for relative abundance and contaminant analyses of fish; (6) collection of fish and invertebrate samples for contaminant analysis; and (7) basic hydrographic measurements. The metrics associated with sampling elements 1-6 are detailed below in Table 1. Table 1 also summarizes the number of samples anticipated to be collected during the eight agreed upon sampling events in 2011.

Table 1: Summary of metrics and number of samples anticipated to be collected in 2011.

Study Component	Metric	Method	Replicates	# of patches*	# of samples
Areal estimates	Coverage	Aerial surveys	Multiple transects in each of the two grids	Dependent on field conditions	N/A
Sargassum, juvenile fish, small invertebrates, and encrusting community	Biomass & Density	Ship based neuston net	2 replicates	48	96
Larval fish	Density	Ship-based bongo nets	2 replicates	48	96
Transient and resident Fish	CPUE and frequency by size	Ship based Remotely Operated Vehicle (ROV)	3, > 25 m transects	48	144
Highly mobile, large fish	CPUE and frequency by size	Ship based pelagic longline	1 longline set	48	48
Contaminant Analyses	Concentration	Collection of plants and animals	75 g of *Sargassum* plants, including encrusted communities. Up to 2 samples of 5 fish and invertebrate species collected by neuston net Up to 2 samples of 5 species of fish collected by longline (and 30 g of muscle and liver tissue -- 30 g each)	48	48 patches * 2 replicates = 96 *Sargassum* samples. Fish and Invert. 48 patches * 10 species (5 for longline and 5 for neuston net) * 2 replicates = 960 samples.
***Fish and invertebrate sampling will be conducted at 2 grids, with 3 *Sargassum* classes targeted in each. The full study will include 8 sampling events at each of 2 grids for fish/invertebrates, a total of 48 *Sargassum* patches will be sampled (8 x 3 x 2).**					

VI. SOPs†

†See Attachment 1 for a detailed description of the sampling procedures. Data forms will be compiled for each element by the PIs of the fish arm
SOPs for the laboratory procedures for neuston and bongo net samples and interpretation of ROV tapes will be provided in an addendum. Costs associated with these activities have not been included in the budget for this plan.

A. Aerial Surveys for Sargassum abundance

Typically, an aircraft will survey one of the grids shown in Figure 3 the day before each boat-based survey within a grid is initiated. Each grid will consist of eight linear transects spaced at equal intervals; this pattern will cover approximately 2000 NM^2. Observers in the survey aircraft will photograph *Sargassum* accumulations, record information sufficient to estimate initial sighting distance off the transect being flown (e.g., inclinometer reading, altitude, and GPS waypoint), and assign each patch to one of three qualitative classes—scattered clumps, mats, or convergence lines.

B. Sargassum and Fish Sampling

Following the aerial surveys, ships will proceed to the center point of the block within the grid selected based on the results of the aerial survey observations (a process described above in Section IV -- Site Selection Strategy). The day after the aerial survey, the aircraft and ship will attempt to rendezvous at the designated center point and proceed to work in coordination to identify and sample three *Sargassum* patches, ideally one of each type –mats, clumps, and convergence lines. At each of the three *Sargassum* patches, ship-based observers will record dimensions or perimeter coordinates of the patch(es). Scattered clumps will be measured in diameter by recording GPS tracks along two bearings at right angles to each other. Mats will be measured using the method described above or using a digital inclinometer and known observer height. Length and width of convergence lines will be obtained using a GPS-measured track.

1. *Larval fishes:* To quantify *Sargassum* habitat use by larval fishes, bongo nets (333 µm mesh) will be towed simultaneously just under the area covered by the *Sargassum* patch, parallel to the vessel to avoid bow wakes. Bongo nets towed through the *Sargassum* surface will become quickly clogged, hence towing under the *Sargassum* patch is recommended to achieve a sample size of at least 25 m^3 (~100 m tow). Two bongo nets will be towed simultaneously, i.e., the bongo system has two separate nets that will provide the replicates. Samples will be preserved in 95% ethanol for storage (held between 20 – 30°C) until laboratory processing and analysis can proceed. Identification of larvae will follow NMFS SEAMAP guidelines and taxonomic resolution will be consistent with current SEAMAP ichthyoplankton collections. A total of up to 96 larval and juvenile fish samples are anticipated (48 patches x 2 duplicates).

2. *Juvenile fishes, encrusting and small invertebrates, and Sargassum:* A neuston net (1m x 2m, 3.2 mm mesh) will be towed directly through *Sargassum* patches to collect *Sargassum*, juvenile fishes and invertebrates. Two replicate samples will be collected at each patch. Samples of the *Sargassum* plant (75 g) will be collected from each neuston net tow and stored in aluminum foil on ice for no more than 48 hours after sample collection, at which point they must be frozen at -20°C for analysis at a later date.

Juvenile fishes and small invertebrates will be rinsed from the remaining *Sargassum*, collected and preserved in 95% ethanol for storage (held between 20 – 30°C) until laboratory processing can proceed. The weight of this *Sargassum*, its condition (extent to which it is weathered), and potential MC 252 oiling evidence will be recorded.

The samples of juvenile fishes and invertebrates, including encrusting and surface dwelling invertebrates, will be identified to the lowest possible taxonomic level. Per SEAMAP protocol, up to 20 fish of each species per sample will be measured. Resulting metrics will be expressed per unit weight of *Sargassum*. The total biomass per unit area can then be expressed per m^2. For definition of these standard practices, see American Fisheries Society Standard Methods. The biomass estimates can then be multiplied by aerial estimates of patch size to determine total biological resources. A total of up to 96 samples are anticipated (48 patches x 2 replicate samples).

3. *Transient and resident large fishes:*

a. ROV video surveys will be used to collect census data on large juvenile and adult fishes associated with *Sargassum*. The surveys will be collected just beneath the *Sargassum* canopy, between a depth of 0.5 and 3 m. Three ROV transects, of at least 25 m in length, will be surveyed at *Sargassum* patches. All recorded video images will be archived for fish identification and counts (fish per m^2 or m^3).

b. A pelagic (near surface) longline will be used to quantify the relative abundance (expressed as catch per unit effort, CPUE) of highly mobile, large predatory fishes and collect specimens for contaminant content analysis. Longline sets will be made at convergence lines with *Sargassum* present and at mats and clumps (based on the size of the mats and clump aggregations, as determined by the PIs on site), and only in areas with water depths greater than 100 m. Longlines will be fished for one hour. The longline gear will consist of up to 4.8 km of 4.0 mm monofilament mainline sampled with up to 150 3.7 m long gangions, and 75 4.0 m droplines. Gangions consist of a longline snap and a #16 circle hook, baited with Atlantic mackerel (*Scomber scombrus*).

All specimens will be identified to species and measured, following SEAMAP protocols. Abundance will be expressed as fish per 100 hook hours. The presence of Sargassum will be documented along the length of the longline based on hook number. A subsample will be kept for contaminant analysis (up to 2 individuals of the 5 most common species per longline set (10 fish total). A total of up to 480 sets of fish tissue samples (these will consist of whole bodies for fish up to two feet long and sub-samples of muscle (30 g) and liver (30 g) for fish larger than two feet) will be collected for contaminant analysis (48 patches x 10 fish x 1 longline set), which will be addressed by a separate plan.

4. *Additional specimen collection.* Smaller fish and crabs may be collected in the neuston net tows for processing and contaminant analysis in accordance with the NRDA fish tissue protocols. The five most common species identified in the neuston net tows will be targeted and two samples from each patch will be collected, if possible. If individual fish and crabs are smaller than 40g, multiple specimens will be collected to ensure sufficient sample for analysis (i.e., a composite sample of multiple whole specimens of the same species). A total of up to 480 tissue samples are anticipated (48 patches x 5 species x 2 samples).

The above metrics can be collected by one field crew on a vessel equipped with a hydraulic longline reel and ROV. For planning purposes, up to three days are anticipated to complete sampling activities at up to three *Sargassum* patches within a grid. One additional day (per grid) will be required to accommodate aerial surveys of the grids.

Data Handling and Sharing

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 written 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 shall be kept on file for the duration of this project.

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

This plan will be implemented consistent with existing trustee regulations and policies. All applicable state and federal permits will be obtained prior to conducting work. All analytical and non-analytical data will be provided to BP/CardnoENTRIX and all trustees within a reasonable timeframe. All samples collected pursuant to this plan will be submitted to an NRDA-approved laboratory.

Sample Retention

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

Lead Investigators and Description of Duties for *Sargassum* Fish and Invertebrate Component:

Name	Role
Dr. Sean Powers University of South Alabama & Center for Ecosystem Based Fisheries Management, Dauphin Island Sea Lab	Lead Investigator for *Sargassum* Fish Component
Dr. Frank Hernandez University of South Alabama & Center for Ecosystem Based Fisheries Management, Dauphin Island Sea Lab	Lead Investigator for *Sargassum* Fish Component – organizing May and June cruises
Mr. Read Hendon University of Southern Mississippi, Gulf Coast Research Lab	Co- Lead Investigator for *Sargassum* Fish Component – July through September cruises
Mr. Jim Franks University of Southern Mississippi, Gulf Coast Research Lab	Co-Lead Investigator for *Sargassum* Fish Component – July through September cruises
Dr. Bruce Comyns Independent Consultant	Co-Lead Investigator for Sargassum Fish Component - identification and enumeration of juvenile fish and invertebrates

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Estimated Budget for Sargassum Fish and Invertebrate Plan 2011 Sampling Cruise Activities

Labor Costs	Cost
GCRL Labor (Jim Franks, Read Hendon, Vessel Field Crew for July-September)	
Consultant Labor (DISL - Frank Hernandez, Vessel Field Crew for May and June)	
Aerial Observers	
Subtotal	**$ 497,800**
Gulf Coast Research Lab (GRCL) Direct Costs	
Travel	
Truck mileage of 1000 miles @ $0.50	$500
Materials and Supplies	
Sea-Gear Corp. bongo net frame @ $425 ea. x 2 (1 backup)	$850
Sea-Gear Corp. bongo nets @ $226 ea. x 4 (1 backup pair)	$904
Sea-Gear Corp. bongo net cod end buckets @ $189 ea. x 4	$756
Sea-Gear Corp. flowmeters @ $329 ea. x 4 (1 backup pair)	$1,316
Sea-Gear Corp. neuston net (1m x3m), 1/8 in. mesh net, @ $525 ea. x 2 (1 backup)	$1,050
Ethanol, sample jars, vials, dip nets, fish tissue processing materials	$8,500
Longline supplies (line, gangions, hooks, crimps, cutters, bait)	$7,500
Total Materials and Supplies	**$20,876**
Equipment Rental	

Longline winch (Lingren-Pittman Super Mini Spool) - 42 days @ $125/day	$5,250
Total Equipment Rental	**$5,250**
Indirect Cos [redacted]	
USM institutional indirect charge on direct costs - travel, materials	[redacted]
Total GCRL Direct and Indirect Costs	**$39,007**
ODCs for Cruises staffed by Consultants from DISL	
Materials and Supplies	
Sea-Gear bongo net frame @ $425 each x 2 (incl. 1 backup)	$850
Sea-Gear bongo nets @ $276 each x 4 (incl. 1 backup pair)	$1,104
Sea-Gear bongo net cod end buckets @ $189 each x 4 (incl. 1 backup pair)	$756
Sea-Gear flowmeters @ $329 each x 4 (incl. 1 backup pair)	$1,316
Sea-Gear neuston net (1 m x 2 m), 1/8 in. mesh net @ $525 each x 2 (incl. 1 backup)	$1,050
Neuston frame	$1,000
Larval fish preservation - ethanol, drum pump, carbuoys	$3,000
Shipboard ichthyoplankton processing - sieves, buckets, funnels, colanders, jars, etc.	$500
Longline sampling - bait, hooks, measuring boards, scales, storage bags, foil, etc.	$2,500
Contaminant analysis sampling - dipnets, gloves, scalpels, storage bags, foil, etc.	$500
Other - foul weather and safety gear, miscellaneous tools and	$3,000

supplies, etc.	
Data recording - internal and external labels, data sheets	$500
Total Materials and Supplies	**$16,076**
Equipment Rental	
Longline winch (Lingren-Pittman Super Mini Spool) - 16 days @ $125/day	$2,000
Total Equipment Rental	**$2,000**
Training Costs	
Training costs - HUET - 8 observers x $200 tuition plus $200 travel	**$3,200**
Total ODCs for DISL Consultant Staffed Cruises	**$21,276**
Total Project Cost for 2011	**$558,083**

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

Durable Equipment - All durable equipment (such as cameras, GPS, etc.) purchased by BP for this study will be returned to BP or their designated representatives at the conclusion of its use for this study.

Costs associated with the laboratory procedures for neuston and bongo net samples and review of the ROV tapes have not been included in the budget for this plan. The SOPs for these activities and estimated costs will be provided in an addendum.

ATTACHMENT 1
NRDA SARGASSUM FISH/INVERTEBRATE ARM FIELD SAMPLING ELEMENTS SOP

SAMPLING SEQUENCE

Field sampling elements conducted at pelagic *Sargassum* will occur in the following sequence upon arrival at selected *Sargassum* habitat types (mats, clumps, convergent lines). Descriptions of each sampling element are provided in the document entitled "Assessment Plan for *Sargassum* Communities and Associated Fauna in the Northern Gulf of Mexico": Section II. Data Needs and Sources, Sub-section B. Quantification of Associated Fauna (Objective 2); and Section VI SOPs:

A. Water quality parameters

Hydrographic measurements/abiotic parameters will be recorded prior to the biological sampling activities

B. Transient and large resident fishes

1. ROV video survey:
    a. An ROV video survey will be conducted along three transects to 1) collect census data on large juvenile and adult fishes, and 2) determine density and size frequency of transient and resident fish
    b. Recorded video images will be archived for laboratory determination of fish identifications and counts

2. Pelagic longline survey:
    a. A pelagic longline survey will be conducted to quantify relative abundance of highly mobile, large predatory fishes and collect samples for contaminant content analysis
    b. The longline will be deployed, fished (soaked) for 1 hour, then retrieved for the removal of pelagic fishes
    c. Fishes will be identified and measured
    d. Samples (tissue, etc.) will be collected from two individuals of each of the five most common species for purposes of contaminant content analysis; samples will be handled, placed in appropriate containers, and stored (frozen; -4°C) per approved NRDA fish tissue sampling, handling and storage protocols.

Longline Pelagic Surveys Fish Sampling/Subsampling SOP

1. The 5 most abundant fish species caught on the longline will be chosen for sampling: this abundance determination will be made by scientists on the boat, based on the specific haul.
2. Two of each of these 5 species will be sampled: if there are more than two, the first two of this species that come off the longline will be those that are sampled.
3. Place fish into a covered storage container until each can be subsampled or frozen (samples will spend as little time as possible sitting out in the open air on the deck of the ship)
4. Fish <2 feet in length should be wrapped in precleaned aluminum foil, placed in plastic freezer bag, and placed in freezer at -4°C for temporary storage
5. For fish >2 feet in length, subsamples must be taken
6. Clean table with Alconox solution and seawater
7. Put on a clean pair of nitrile gloves
8. Obtain a new disposable scalpel blade
9. Once fish is on the table, carefully clean the external area to be subsampled with diH_2O.
10. Subsampling: sampler should change scalpel blades between subsamples of each fish (e.g. if you are sampling muscle and liver, each of those samples should be taken using a new blade)
    a. Take 30 g sample of muscle tissue from behind the head
    b. Take 30 g of liver tissue from distal lobe
11. Wrap each sample independently in pre-cleaned aluminum foil immediately after sample is collected
12. Place aluminum foil-wrapped tissue in plastic freezer bag, let as much air as possible out of bag, seal bag
13. Place in freezer at -4°C for temporary storage

It is anticipated that during the one-hour longline set sampling (soak) period, one or both of the following sampling elements will be conducted. Otherwise, these elements (or remaining portions thereof yet to be completed) will be conducted following the longline sampling activities:

C. Larval fishes

1. Bongo plankton nets will be towed just under the *Sargassum* canopy to quantify use of *Sargassum* habitat by larval fishes

2. Contents of the nets will be removed (washed), placed in properly labeled containers, preserved in 95% ethanol, and stored for laboratory assessments

Bongo Plankton Net SOP

1. Bring nets onto deck
2. Wash the contents from each bongo net into separate buckets using seawater from hose.
3. Bring buckets into wet lab
4. Process samples individually; pour contents of bucket into a flexible, mesh sieve (same mesh as the plankton net) that is placed into a plastic funnel in order to drain water from the sample.
5. Place sample contents into sample jar with 95% EtOH for storage.

D. Juvenile fishes, encrusting and small invertebrates, and *Sargassum*

1. A neuston net will be towed directly through *Sargassum* habitat to collect *Sargassum*, juvenile fishes and invertebrates; two replicate neuston net samples will be collected
2. Samples of the *Sargassum* plant will be collected from each neuston net tow (two replicate plants per tow; 75g each sample), then placed in appropriate containers, and stored (frozen; -4°C) per approved NRDA sampling and storage protocols; *Sargassum* samples will be photographed and condition noted
3. Juvenile fishes and small invertebrates will be rinsed from the remaining *Sargassum* in the sample; specimens of whole fish (or tissues) and small invertebrates will be collected from two individuals of each of the five most common species for purposes of contaminant content analysis, and those samples will be placed in appropriate containers and stored (frozen; -4°C) per approved NRDA fish tissue sampling, handling and storage protocols
4. The remaining fish and invertebrates will be placed in properly labeled containers, preserved in 95% ethanol, and stored for laboratory assessments.

Neuston Net SOP

1. Bring net onto deck.
2. Put on a clean pair of nitrile gloves
3. Collect two samples (75g each) of the *Sargassum* plant; photograph and note condition of the samples; wrap the samples in pre-cleaned aluminum foil and store at -4°C.
4. Remove contents (remaining *Sargassum* and associated organisms) from net; place contents on the 'wash-down' table and rinse fish and invertebrates (using seawater from the hose) from the *Sargassum* into a flexible, mesh sieve that is placed into a large plastic bucket.
5. The 5 most abundant fish and invertebrate species caught in the net will be chosen for contaminant content analysis sampling: this abundance determination will be made by scientists on the boat, based on the specific haul; two of each of these 5 species will be sampled: if there are more than two, the first two of this species encountered in the sample will be those that are sampled.
6. Place these samples in a clean (Alconox/seawater washed) plastic bucket; bring plastic bucket into wet lab.
7. Wrap each sample independently in pre-cleaned aluminum foil.
8. Place aluminum foil-wrapped fish in plastic freezer bag, let as much air as possible out of bag and seal bag; place samples in freezer at -4°C for temporary storage
9. The remaining fish and invertebrates will be removed from the sieve and placed in properly labeled sample jar(s), preserved in 95% ethanol, and stored for laboratory assessments.
10. Record the total weight of *Sargassum* collected in the sample.
11. Clean table and sieve with Alconox solution and seawater in preparation for the next sample.