

### Deepwater Horizon/Mississippi Canyon 252 Spill

Approval of this work plan is for the purposes of obtaining ephemeral data to be used in evaluating the likelihood of injuries to oysters due to exposure to oil from the Deepwater Horizon/Deepwater Horizon Oil Spill (MC 252 Spill). Parties each reserve its right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

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. Samples for other analyses will be sent to the laboratories indicated in the plan below.

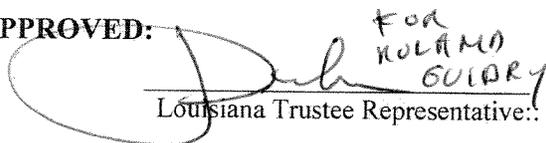
Each laboratory shall simultaneously deliver raw data, including all necessary metadata, generated as part of this work plan as a Laboratory Analytical Data Package (LADP) to the trustee Data Management Team (DMT), the Louisiana Oil Spill Coordinator's Office (LOSCO) on behalf of the State of Louisiana and to 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 ENTRIX. 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 to all trustees and ENTRIX. 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. 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/unvalidated" and will be made available equally to all trustees and ENTRIX.

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.

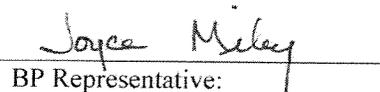
### DRAFT Oyster Sampling Plan – Phase I

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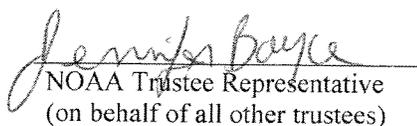
APPROVED:

  
Louisiana Trustee Representative:

2/22/11  
Date

  
BP Representative:

July 31, 2010  
Date

  
NOAA Trustee Representative  
(on behalf of all other trustees)

July 31, 2010  
Date

**Mississippi Canyon 252 Spill**  
**Oyster Sampling Plan**  
**Phase I – High Priority Sites**

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DRAFT Version 1.4  
July 30, 2010

Introduction

A Technical Working Group (“oyster working group”) of experts and trustee agency representatives has been assembled to develop work plans appropriate to carry out both baseline (pre-injury) and post-impact assessments of oysters throughout the northern Gulf of Mexico. This document presents the initial phase of a pre-assessment sampling plan (Phase I) for oysters in the north-central Gulf of Mexico.

This Phase I Plan is part of the pre-assessment phase of a Natural Resource Damage Assessment (NRDA) process under the Oil Pollution Act of 1990 (OPA). It represents the short-term, initial phase of the larger draft pre-assessment oyster sampling plan (“the Draft Pre-Assessment Plan”) developed by the oyster working group. It will use methods consistent with those described in the Draft Pre-Assessment Plan (version dated July 30, 2010) attached to this document.

Like the Draft Pre-Assessment Plan, the Phase I Plan provides for the collection of ephemeral data on the condition of oysters in the Gulf of Mexico - both baseline (pre-oiling) and post-oiling. These data will be collected for use in evaluating whether oysters may be or are being injured by oil or response actions associated with the Deepwater Horizon/Mississippi Canyon 252 incident (MC 252 Spill) and to assist in implementing future procedures that may be chosen to assess any such injuries to oysters, as appropriate. The collection of data outlined in this Plan is a pre-assessment phase activity with the Natural Resource Damage Assessment (NRDA) process for the MC 252 Spill that is underway pursuant to the Oil Pollution Act of 1990 (OPA). 15 C.F.R. §990.43.

The Plan specifically addresses the following topics:

- I. Approach and rationale.** This section describes the overall purpose and need for the initial targeted Phase I plan.
- II. Data Needs.** This section identifies the data needs for this initial targeted Phase I plan.

- III. Health and safety.** This section summarizes pertinent health and safety protocols applicable 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.** This section describes the proposed approach to identifying a limited number of priority sites for evaluation.
- V. Estimated Study Cost.** This section provides an estimate of the cost of implementing the Phase I sampling plan.

Additional details concerning the methods and approaches to be applied in the Phase I plan may be found in the attached Draft Pre-Assessment Plan.

### **I. Approach and rationale**

The Eastern Oyster, *Crassostrea virginica*, is an integral component of coastal ecosystems and local economies along the Gulf of Mexico. Biogenic reefs formed by the aggregation of this species provide numerous ecological benefits to estuarine systems and a valuable fishery resource. Oyster reefs also provide high quality habitat for numerous species of fishes and invertebrates many of which are themselves of commercial and recreational significance (*e.g.*, spotted sea trout, red drum, sheepshead, blue crabs). Oysters also serve as filters for estuarine water and influence energy flow and nutrient fluxes in estuarine environments. While any direct and long-term economic loss of the oyster fishery as a result of the MC 252 Spill is of paramount concern, equally important is the potential loss of ecological services provided by oyster reefs, whether harvested or not.

This Phase I sampling plan is an approximately two-month study intended to assess the ability of the Draft Pre-Assessment Plan study design to document exposure and/or injury to oyster resources, if any, in areas that have been oiled as the result of the MC 252 Spill. These data will be useful to the study team in identifying ways to streamline and focus the Draft Pre-Assessment Plan, if appropriate, without compromising the Draft Pre-Assessment Plan's ability to detect and characterize potential injuries to oyster resources resulting from the MC 252 Spill. The study team will use the Phase I results to evaluate the existing study design in terms of the suite of metrics used to document potential exposure and injury, sampling frequencies and duration, and the geographic scope of the study. The samples will be taken in accordance with the protocols and standard operating procedures (SOPs) presented at the end of the attached Draft Pre-Assessment Plan.

The oyster working group recognizes that in addition to the potential toxic (lethal) effects of oil, sub-lethal mechanisms of injury may also exist -- for instance, oil-stressed oysters may be more susceptible to disease. In addition, efforts to mitigate oil spill impacts may cause additional injury – for example, freshwater diversions intended to reduce oceanic input of water may decrease salinity and cause oyster mortality.

Overall, several key metrics were identified - abundance estimates by life stages, biological condition metrics, and environmental/chemical measurements - as important to characterize oysters and oyster reef health, and to explore potential links with sources of stress related to the MC 252 Spill. Metrics selected for measurement in Phase I (Table 1) are identical to those proposed in the attached Draft Pre-Assessment Plan. There is however, an increased frequency of sampling for some metrics compared to the Draft Pre-Assessment Plan, due to the short duration of Phase I (approximately two months).

With the exception of tissue contaminant analysis and larval counts, analysis of all metrics will be performed at the University of New Orleans. Samples for tissue contaminant analyses will be sent to Alpha Analytical Laboratory in Westborough, Massachusetts; samples for larval counts will be sent to the Fish & Wildlife Research Institute of the Florida Fish and Wildlife Conservation Commission in St. Petersburg, FL.

Oyster reefs are challenging habitats in which to collect quantitative samples because they occur at a wide range of depths (intertidal to deeper subtidal bars), form hummocks or clumps of live and dead animals and exist most often in highly turbid estuarine waters. Because of their exposure at low tide events, intertidal oyster reefs are comparatively easier to quantitatively sample. This is commonly accomplished by using a quadrat made of PVC that covers an area of ¼ meter square (0.5 m x 0.5 m). Shallow subtidal (< 0.5 m depth) oyster reefs may also be sampled by hand via quadrats. Quadrats in deeper areas are best sampled using SCUBA or surface assisted divers. When possible quadrat sampling is the preferred method because it achieves a highly quantitative sample (i.e. the area and effort of the sample are well defined). When in-water collection of samples is not feasible, small dredges are the best alternatives. These latter devices may only achieve a measurement of relative abundance (i.e. CPUE – catch per unit effort), and it is important to ensure that effort and methods are as consistent as practical from site to site. This initial targeted plan will include 12 subtidal and 12 intertidal sample sites.

## **II. Data needs**

Both data needs and potential sources of information relevant to documenting the impact of the spill and associated events on oysters were identified to assess further sampling needs. The oyster working group also developed standard operating procedures (SOPs), including data forms, to augment historic and baseline data. These additional data will provide important site-specific information useful in exploring linkages between changes in oyster communities before and after the MC 252 Spill. See the attached Draft Pre-Assessment Plan for details on existing baseline datasets (Section II), detailed site procedures (Section V), SOPs (Section VI), and data forms (Appendices A-D). The following data needs were identified for the initial targeted sampling effort. In addition, all sites and samples should be documented with photographs, in accordance with the procedures from the SOPs in the Draft Pre-Assessment Plan.

**A. Oyster density, size frequency and biomass** are fundamental measures of oyster community health. Biomass can be derived from density (#/unit area), if average wet weight of individuals is determined. If biomass data exist for size classes, potential injury can be estimated by size class. If size class data are not available, average biomass representative of the entire size distribution can be used.

**B. Larval supply and oyster settlement indices.** During the 2-3 week pelagic duration of oyster larvae may contact oil, potentially resulting in lethal and sublethal effects. The general life cycle of oysters is shown in Figure 1. Quantitative estimates of larval supply (# oyster veliger per liter) are rare in the literature because of the difficulty in identifying oyster larvae (i.e., all bivalve larvae look remarkably similar) and the large time commitment such a project entails. Oyster settlement metrics (e.g., settled spat on shells) are more frequently used; however, these are rarely a component of state monitoring programs due to the expense of collecting these regularly. Settlement metrics have the advantage of reflecting the result of all recruitment processes (larval supply, larval settlement, post-settlement mortality) but have more limited temporal resolution than the component metrics. Measurement of both larval supply and settlement metrics could aid in evaluating potential impacts of the oil spill and related events (Figure 2).

Larval supply and settlement metrics are both relevant measures of oyster reef health. In addition, they can be useful inputs in modeling impacts to oysters. The typical input for the model is average abundance (number/unit area) by depth region (on sediments or planktonic) by month. Some baseline (pre-impact) average abundances of oyster larvae by region (i.e., Mobile Bay versus Mississippi Sound versus offshore Louisiana) and depth zone are available; however, additional information—pre-oiling, if possible, as well as post-oiling—are valuable and can be used to determine potential population bottlenecks (e.g., larvae are unavailable, larvae are not competent to settle, early survivorship is low). Phase I sampling of larval abundance and settlement will provide information on the timing and magnitude of larval supply in the water column and settlement on the substrate.

**C. Contaminant data in oyster tissue and sediments** Currently, it appears that oil from the Deepwater Horizon will make landfall over a large geographic area and at highly variable concentrations. These factors necessitate that appropriate metrics for gauging oyster exposure to oil be collected synoptically with oyster measurements. Because depuration rate of hydrocarbon contaminants in oysters may be rapid (~30 days), it is useful to collect oyster tissue on a frequent basis (~2 weeks) (Sericano et al., 1996, Hwang et al., 2004).

Additionally, other NRDA sampling programs will occur concurrently with the oyster sampling program at the oyster sampling locations, specifically the Submerged Oil Characterization plan and the Louisiana DEQ Nearshore Sediment and Water Sampling Plan (LA DEQ Plan). Results from the first program will assist in the identification of oiled and baseline areas prior to sampling and sediment samples from the second will provide an additional exposure metric at oyster sampling sites. Prior to oyster sampling, targeted submerged oil assessment work will be undertaken at the oyster sampling locations (as described in the Submerged Oil Characterization work plan developed for the NRDA). Sorbent snare sentinel

deployments will provide a rapid assessment of the presence or absence of oil, and in areas where oiling is detected, sampling will characterize the extent of oiling and document exposure of the water column and benthos to hydrocarbons. Sediment samples will be collected for contaminant analysis both during sentinel deployment and during monthly oyster sampling events, according to the protocols set forth in the LA DEQ Plan (incorporated here by reference).

### III. Health and Safety

- **The team leader and field crew parties should 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).
- **All field team members should read all of the documents in the Safety directory on the case’s ftp site [REDACTED]**  
Exception: if site collection activities do not include use of a boat or helicopter, then familiarity with the safety documents for these vehicles is not required.
- **Each field team must submit a plan, not later than the night prior to going into the field.** This plan must specify:
  - The team leader;
  - Names of all team members;
  - The sampling location(s)-- please use the grid coordinates as shown in Tables 4 through 6;
  - What kind of sampling they are doing;
  - Expected arrival time at sampling area (daily);
  - Expected departure from sampling area (daily);
  - Team deployment date;
  - Team return date.

This information may be reported in one of two ways:

1. Fill out the Excel spreadsheet “Team Member Information Form – Excel.xls”<sup>1</sup> and send it to [REDACTED] Please use one tab for each team.
2. If you cannot submit this spreadsheet electronically, you can call in and report the information using this number: [REDACTED]

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<sup>1</sup> This file is available on the case’s ftp site:  
[REDACTED]

- **Field teams must adhere to all procedures set forth in the MC252 Site Safety Plan** (“NRDA MC 252 Site Safety Plan\_6.22.10.pdf”).<sup>2</sup>
- **If participating in a cruise:** Each cruise may have additional required health and safety procedures, that must be observed.
- **Diving:** SCUBA or surface-assisted diving, where used for sampling, will be conducted in accordance with existing Trustee dive safety programs.

#### IV. Site Selection

For the purposes of the Phase I study, sampling will be conducted in sites that have been characterized by oiling from the MC 252 Spill and reference sites that are not expected to have been oiled. The proposed Phase I sample sites are randomly chosen 4 ha (200 m x 200 m) grid cells in two Louisiana areas selected for their demonstrated degrees of oiling to date. Table 2 summarizes the various tiers and strata of sampling proposed for the Draft Pre-Assessment Plan (additional details on tier and stratum definition can be found in the attached Draft Pre-Assessment Plan). All Phase I sampling will be conducted within Tier 2 areas.

#### Proposed Sampling Locations

Figure 3 illustrates Strata A and B for Louisiana within which the proposed Phase I oiled and reference sample sites were selected. Barataria Bay and Lake Calcasieu were selected as the oiled and reference areas respectively based on consultation with the oyster working group and consideration of oiling observations from early to mid July. Due to time constraints, the oyster working group decided to select sample sites within Stratum A (mapped oyster reefs) where possible; however, due to the lack of mapped oyster reefs in oiled areas of Barataria Bay, sample sites for Barataria Bay were drawn within Stratum B (potential oyster habitat). Potential sample sites were randomly selected within these selected areas with the caveat that no two sites could be within 1,000 meters of another selected sample site. Barataria Bay contains numerous commercial leases, and though precise locations of oyster reefs are not known or mapped, the fact that commercial leases in the area exist suggests the presence of oyster reefs available for sampling. Because of the uncertainty associated with sampling Stratum B sites, however, we have also selected alternative sampling areas (described below) in the event that sampling of the Stratum B sites in the initial Barataria Bay sampling area shows no evidence of active oyster habitat (i.e., no evidence of live nor recently dead oysters).

Cells that cannot be accessed, e.g., private areas with no permission granted, will be labeled “missing data”, and replaced by the next alternate cells on the appropriate GRTS list.

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<sup>2</sup> This file is available on the case’s ftp site:



The recommended number of sites assumes that the cell has substrate suitable for oyster settlement within it. Cells that contain no oyster habitat based on the site survey will be logged as zeros for estimation of the total area of oyster habitat and new cell locations will be selected.

Table 3 summarizes the minimum number of sample sites and subsamples that will be collected for each metric as part of the Phase I study. The subsampling structure for each sampling event and metric is the same as for the Draft Pre-Assessment Plan. For Phase I, sample teams will collect one set of samples at a shallow subtidal oyster reef and one set, if possible, at the closest intertidal (<1m deep) oyster reef to the sample site, where a sample set is defined as in Table 3. Sampling will begin with the first sample site on the list. If no oyster reefs are located in a sample site, that sample site will be dropped from the list and the next sample site on the list sampled in its place. Sampling will continue until six sample sites have been sampled.

Figure 4 and Table 4 represent an initial proposed set of 24 oiled sample sites in Barataria Bay in Louisiana, chosen within Tier II, Stratum B. Due to uncertainty in the precise location of oil, the specific oiled oyster sample sites will be selected by the Lead Biologist on the sampling team, based on proximity to pre-selected sample sites and GIS maps of areas known to have incurred heavy oiling by MC 252 based on recent empirical data, and the sample team's observations concerning the presence of surface and/or submerged oil at the time of sampling.

The 12 proposed reference (un-oiled) sample sites in Calcasieu Lake (Figure 5 and Table 5) were chosen within Tier II, Stratum A. We therefore expect less uncertainty regarding the precise location of viable oyster reefs in Calcasieu Lake; however, to the extent that oil is observed at a particular sample site in the reference area, the Lead Biologist may choose to drop that site and proceed to the next sample site on the list where oiling is not observed.

The subjective selection of specific sampling locations is intended to increase the likelihood that the resulting metrics will reflect highly oiled and reference areas. Thus, the results of those Phase I measurements will be useful in assessing the potential value of those metrics relative to oiling, but the results will not be representative of the conditions across those sampling areas, or oiling polygons.

GIS coordinates for the sample sites (4 ha, 200 m x200 m grid cells) will be provided to help guide the field crew to the four corners of the corresponding 4 ha study sites.

### **Alternative Sampling Locations for Oiled Sites**

In the event that exploration and sampling of the proposed stratum B sites in Barataria Bay identifies no evidence of active oyster habitat (i.e., no live or recently dead oysters), we will investigate a set of alternative sites for sampling oysters in oiled areas (Figure 6 and Table 6). The alternative sites were selected based on consultation with the oyster working group and consideration of oiling observations from early to mid July. To maximize the likelihood that active oyster habitat will be found at the alternative site, we will conduct reconnaissance of these sites before selecting a sample location. The reconnaissance will include side-scan sonar mapping of the site to identify possible oyster reef (following the SOP in the Draft Pre-

assessment Plan), coupled with the collection of small dredge samples to assess whether live oysters or recently dead oysters are present. (Oysters collected during reconnaissance activities will not be retained.) In addition, we will deploy sorbent snare sentinels as described above to characterize the extent of oiling in these areas. Subsequent oiled sampling areas will be chosen based on indications of active oyster resource and presence of oiling based on team observations and/or sentinel results.

## **VI. Estimated Study Cost**

Our total estimated budget for implementing the Phase I plan described in this document, including physical measurements but not chemistry, is \$512,674. This estimate includes the costs of collecting, processing, and shipping the samples from Table 3. Because of the uncertainty associated with sampling the Stratum B sites, we have estimated these costs assuming the team will need to conduct reconnaissance of the alternative sites and identify and collect samples for at least one alternative site in addition to initial efforts at the sampling location in Figure 4. For additional detail concerning the cost estimate, please consult the attached Excel file, “Costmatrix\_Phase\_I\_Plan\_07.30.10.xlsx”.

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.

**Table 1. Proposed Phase I metrics.**

<b>Metric</b>	<b>Proposed Frequency of Sampling</b>
<i>Effect Metrics</i>	
Oyster abundance, by size class and by alive/dead status	Monthly
Abundance of associated species, by oyster size class	Monthly
Oyster biomass, by size class and by alive/dead status	Monthly
Biomass of associated species, by oyster size class	Monthly
Disease	Monthly
Gonadal condition	Every two weeks
Larval abundance (#/L)	Every two weeks
Larval settlement	Every two weeks
<i>Exposure metrics</i>	
Tissue concentrations	Every two weeks
Sediment concentrations	Monthly
Oiling observations (qualitative)	Every two weeks

**Table 2. Summary of sample site selection approach and rationale.**

<b>Tier</b>	<b>Stratum</b>	<b>Site selection</b>	<b>Rationale</b>	<b>Subsamples per grid cell<sup>1</sup></b>
1	None	<i>Fixed.</i> Sites are based on long-term monitoring locations of state agencies.	Pattern of recruitment and mortality (natural or fishing induced) can be inferred and potentially separated from oil spill mortality.	2 per fixed site - if historic methods differ from this protocol, 8 per grid cell
2	A	<i>Random in known oyster habitat-</i> boundaries designated by the state trustees for known oyster habitat (harvested and closed areas).	Much of oyster habitat is in mapped areas. Although oyster habitat within these large geographic areas is probably patchy.	up to 8 per grid cell
	B	Random in areas where oyster habitat may potentially occur but digitized GIS maps are not available. This stratum will include lease areas and shoreline areas in LA.	A portion of oyster habitat in each State occurs in scattered areas that have not been mapped.	up to 8 per grid cell
	C	Random in shoreline areas in MS, AL, and FL.	Oysters occur along many shoreline marshes, rip-rap, pilings and other hardened structures in the intertidal-shallow subtidal. These areas are likely to experience oiling.	up to 8 per grid cell
3	None	NOAA Musselwatch	Fixed station. Continuation and augmentation (in FL) of musselwatch sampling.	Follow Musselwatch protocols

<sup>1</sup> Assumes a site will have substrate suitable for oyster recruitment and growth.

**Table 3. Summary of oyster sampling procedures, minimum number of sites and replicates in Phase I sampling.**

Metric	Method	Minimum # of sites				Repl. per site	Est. Samples per event	Freq. of sampling	Total # of samples (estimate)	Ref. for SOP & Form in Attached Draft Pre-Assessment Plan
		<i>Subtidal (Tier II, Stratum A or B)</i>		<i>Intertidal Shoreline (Tier II, Stratum A or B)</i>						
		<i>Non-oiled (Baseline)</i>	<i>Oiled</i>	<i>Non-oiled (Baseline)</i>	<i>Oiled</i>					
Site Mapping	Side-scan sonar	0	24*	0	24*	1	1	1	N/A	Sec. VI. A. Form A
Adult and Juvenile Density	Quadrat or dredge	6	6	6	6	N = 8 quadrats	192	Monthly (3 total)	576	Sec. VI. B. Form B
Oyster Larvae	Water sample	6	6	6	6	N = 5	120	Biweekly (6 total)	720	Sec. VI. C. Form C
Oyster Settlement	Settlement plate	6	6	6	6	N = 3 plates.	72	Biweekly (6 total)	432	Sec. VI. D. Form D
Oyster Gonadal condition	Oysters	6	6	6	6	N = 10 oysters	240 oysters	Biweekly (6 total)	1,440 oysters	Sec. VI. E.
Tissue contaminant analysis	Oysters	6	6	6	6	N = 4 composite samples per grid cell	96	Biweekly (6 total)	576	Sec. VI. F
Sediment Contaminant analysis	Sediment	6	6	6	6	N = 4	96	Monthly (3 total)	288	Sec. VI. G
Oyster Disease	Oysters	6	6	6	6	N = 10 oysters	240 oysters	Monthly (3 total)	720 oysters	Sec. VI. H

\*Completed only in the event that no evidence of active oyster reef is found at the proposed oiled sample location.

**Table 4. Initial Proposed phase I oiled sample sites – Barataria Bay, LA (Figure 4).**  
Longitude and latitude coordinates are centers of 4 ha (200 m x 200 m) grid cells in datum WGS84.

Sample Site	Longitude	Latitude	Stratum
1			B
2			B
3			B
4			B
5			B
6			B
7			B
8			B
9			B
10			B
11			B
12			B
13			B
14			B
15			B
16			B
17			B
18			B
19			B
20			B
21			B
22			B
23			B
24			B

**Table 5. Initial Proposed phase I unoiled sampling locations – Lake Calcasieu, LA (Figure 5).** Longitude and latitude coordinates are centers of 4 ha (200 m x 200 m) grid cells in datum WGS84.

Sample Site	Longitude	Latitude	Stratum
1			A
2			A
3			A
4			A
5			A
6			A
7			A
8			A
9			A
10			A
11			A
12			A

**Table 6. Alternative Phase I Oiled Sampling Locations – Barataria Bay, LA (Figure 6).**  
Longitude and latitude coordinates are centers of 4 ha (200 m x 200 m) grid cells in datum WGS84.

Sample Area	Sample Site	Longitude	Latitude	Stratum
Hackberry Bay	1			A
	2			A
	3			A
	4			A
	5			A
	6			A
	7			A
	8			A
	9			A
	10			A
	11			A
	12			A
Government Reef	1			B
	2			B
	3			B
	4			B
	5			B
	6			B
	7			B
	8			B
	9			B
	10			B
	11			B
	12			B

**Table 6. Alternative Phase I Oiled Sampling Locations – Barataria Bay, LA (Continued).**

<b>Sample Area</b>	<b>Sample Site</b>	<b>Longitude</b>	<b>Latitude</b>	<b>Stratum</b>
Champagne Bay	1			B
	2			B
	3			B
	4			B
	5			B
	6			B
	7			B
	8			B
	9			B
	10			B
	11			B
	12			B
Bay Ronquille	1			B
	2			B
	3			B
	4			B
	5			B
	6			B
	7			B
	8			B
	9			B
	10			B
	11			B
	12			B

Figure 1. Life cycle of the eastern oyster, *Crassostrea virginica*.

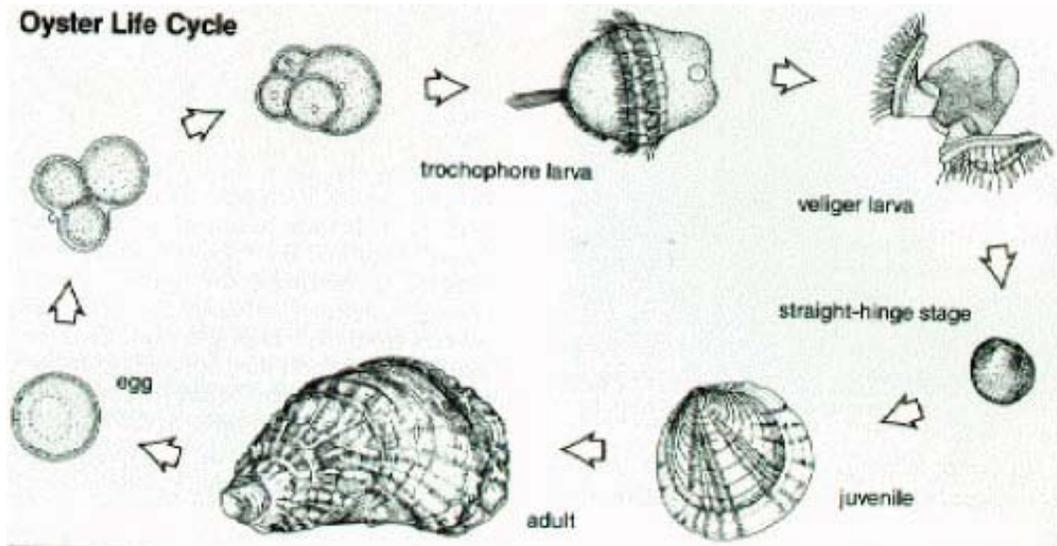
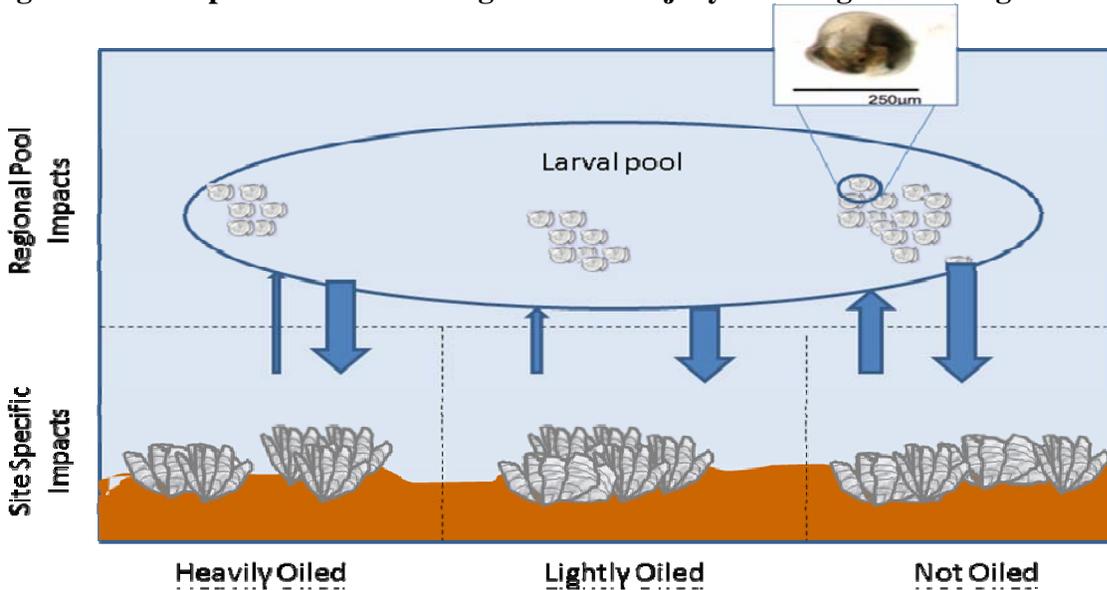


Figure 2. Some potential site and regional level injury resulting from oiling.



Direct mortality may result for sessile life stages (juvenile and adults). Reduction in reproductive output is also possible due to loss of adults or physiological impairment of adults, which decrease larval supply to the regional pool of larvae. This decrease in larval supply may have negative effects that extend beyond oiled sites.

Figure 3. Proposed Louisiana oiled and reference (un-oiled) sampling locations for Phase I.

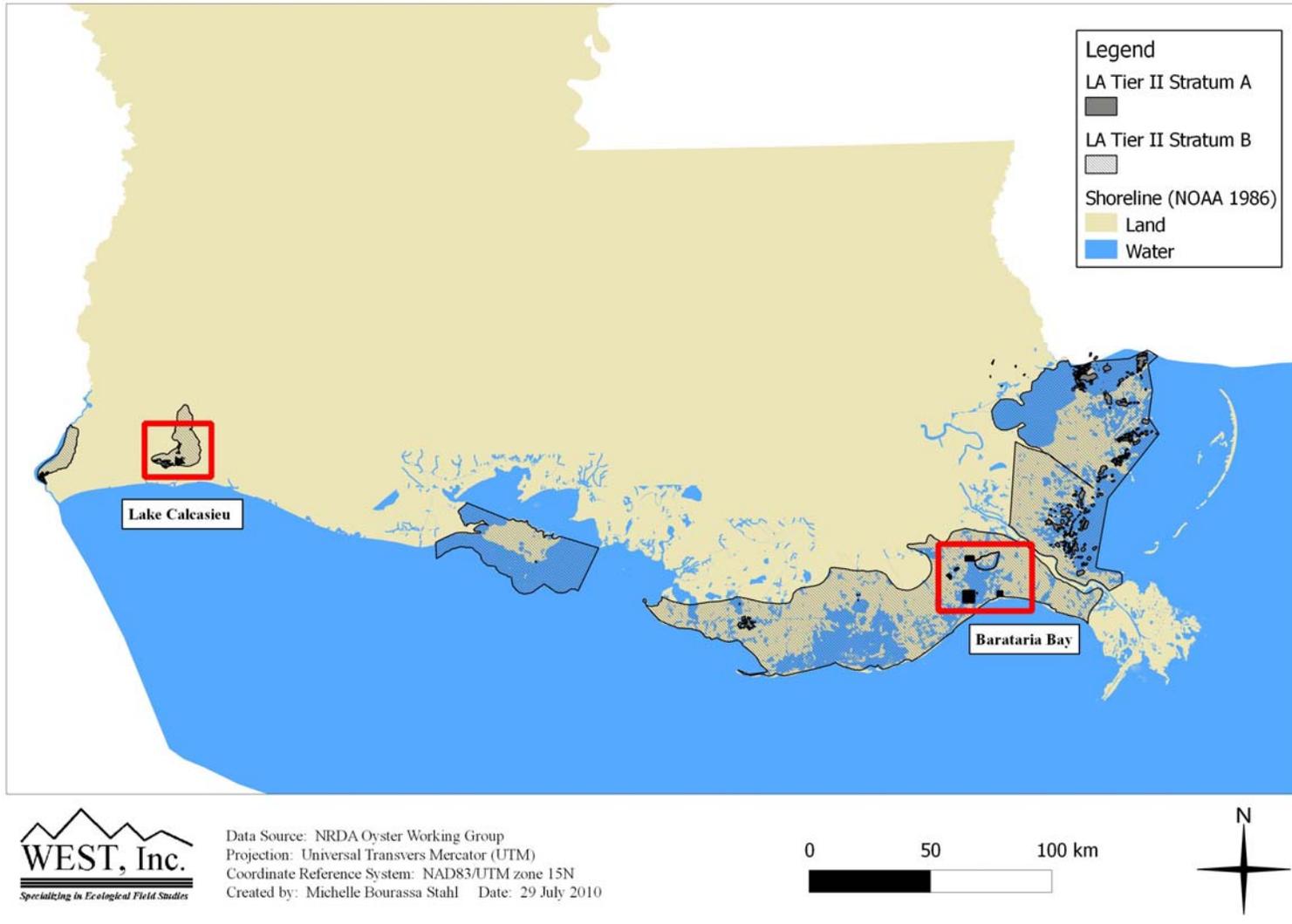


Figure 4. Proposed phase I oiled sample sites – Barataria Bay, LA (Table 4).

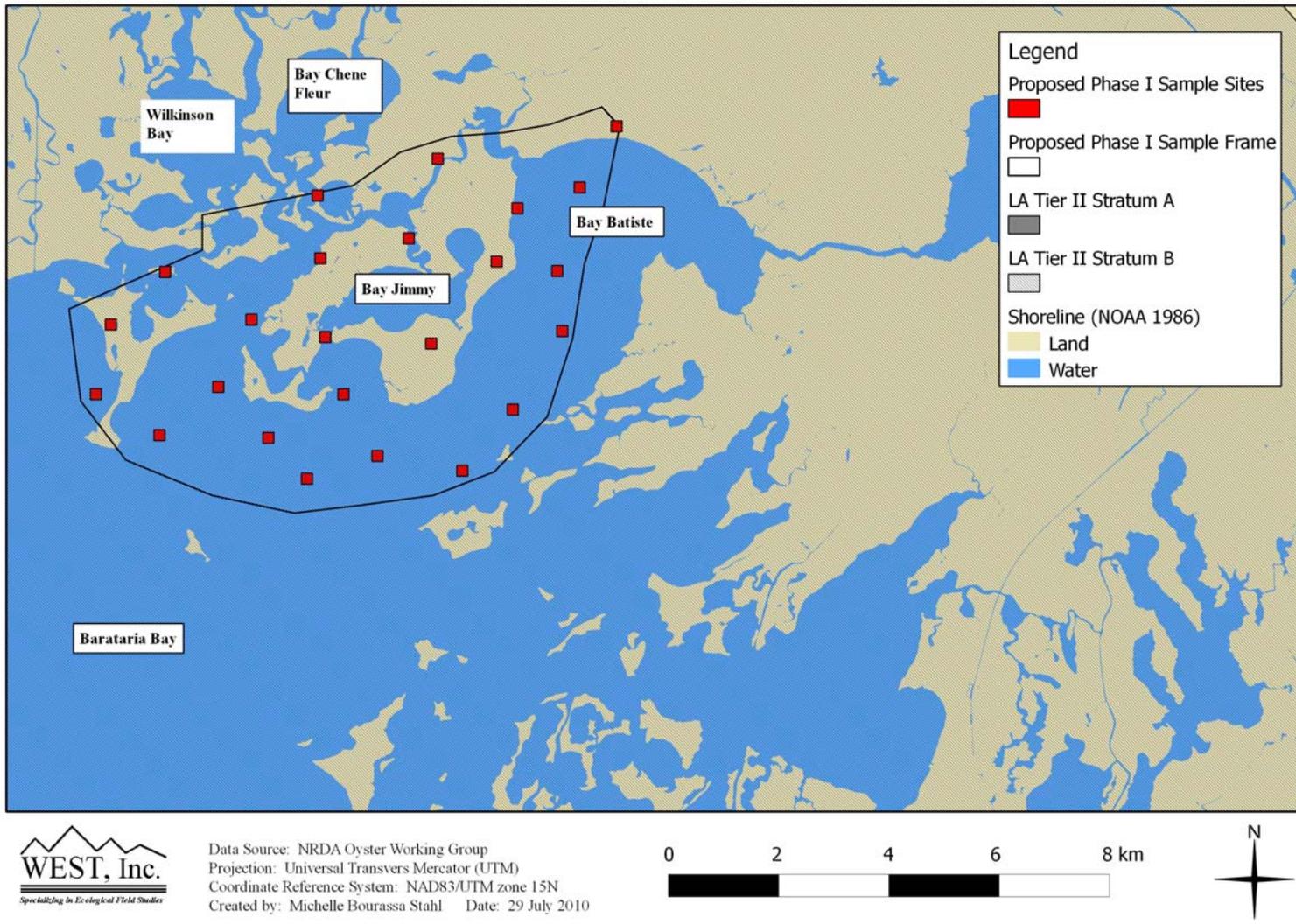


Figure 5. Proposed phase I reference (non-oiled) sampling locations – Lake Calcasieu, LA (Table 5).

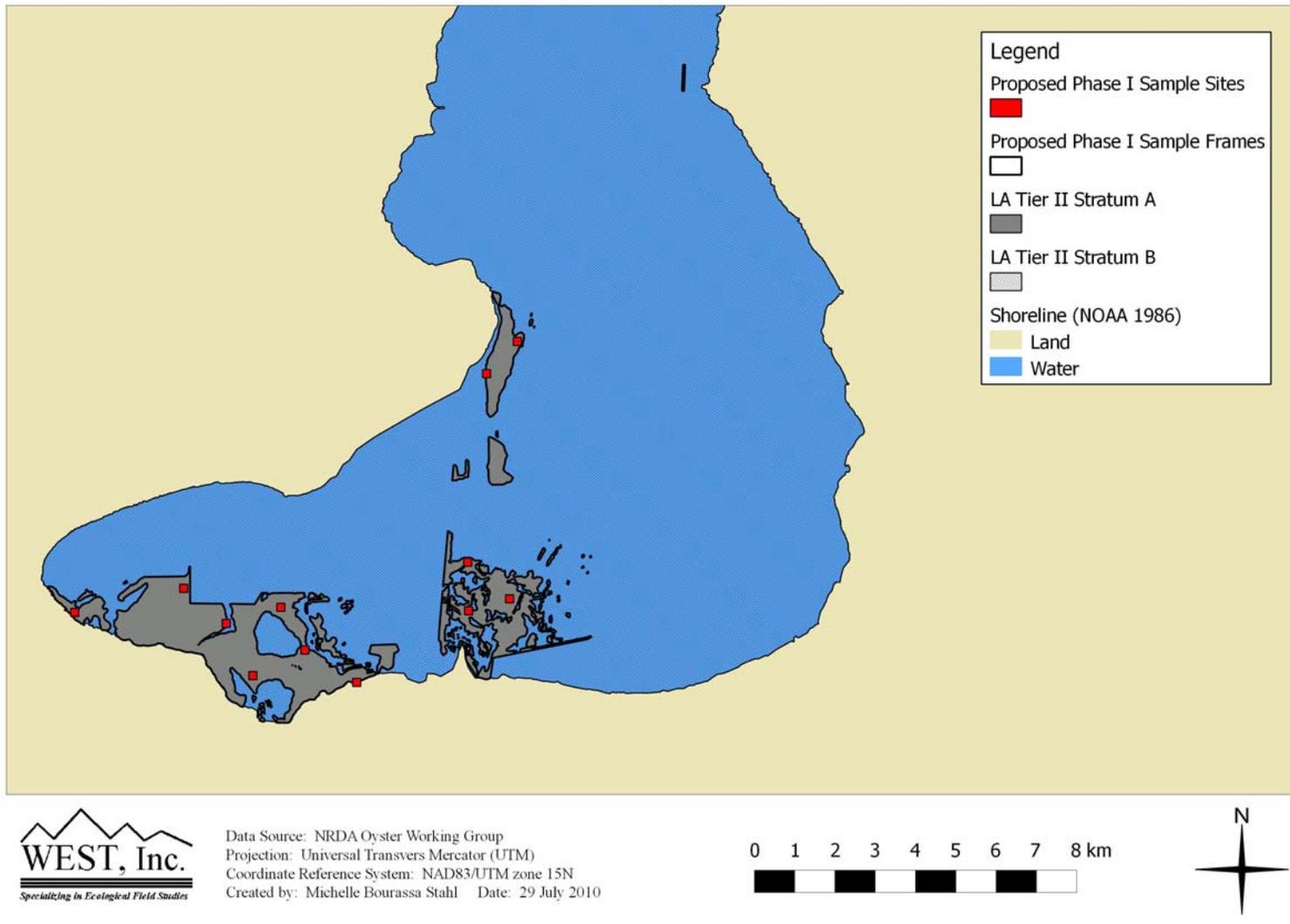
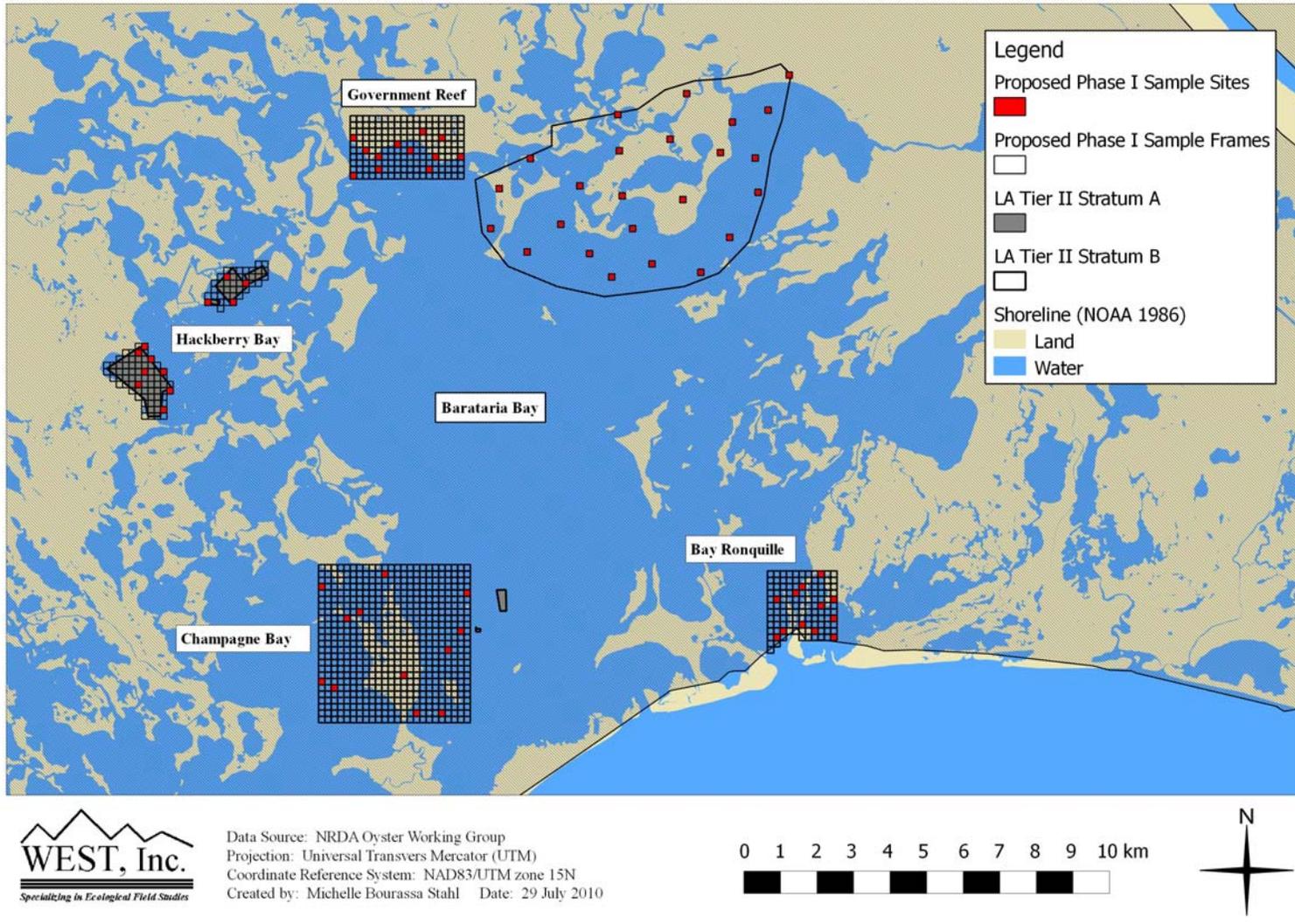


Figure 6. Alternative proposed phase I oiled sampling locations – Barataria Bay, LA. (Table 6)



Site Type	Phase	Field/Processing Cost
Baseline	1	\$240,450
Oiled	1	\$272,224
<b>Total</b>		<b>\$512,674</b>

Cost Type	Cost
Dive Labor	\$99,960
Non-Diver Labor	\$264,510
Boat Charges	\$122,364
Shipping and Supplies	\$25,840
<b>Total</b>	<b>\$512,674</b>

\*Assumes all samples will be analyzed

**Summary of key rates per day**

Personnel \$800 Blended rate of personnel from Federal, State and contractors  
 Dive Team and Equipment \$4,000 All inclusive day rate (equipment, personnel, post processing)  
 Field Boat \$1,800 From Vessel of Opportunity Program (up to 30 ft) OR from Dive company (equivalent estimates)

**Staffing Summary**

Team	Task	People in Team	# of cells (sites per day) per team	Total Cells	# of subsamples processed per day	Total Subsamples generated	# of days per sampling event or sample process	# of teams per sampling event	Frequency /yr
Side-scan sonar	Site Mapping/Reconnaissance	5	Unknown	Unknown			5.00	1.00	1.00
Quadrat (With Divers)	Quadrat sampling	5	3.00	24.00			4.00	2.00	3.00
Biweekly sampling	Recruitment/Contaminant	3	8.00	24.00			1.50	2.00	6.00
Lab A	Quadrat Sample processing	7			30.00	192.00	3.20	2.00	3.00
Lab B	Recruitment/Contaminant collections	5			90.00	192.00	2.13	1.00	6.00

Team	Task	People in Team	# of cell/sites per team	# of samples processed per day	Total Lab samples generated	# of days per sampling event or process	# of teams per sampling event	Frequency /yr
Non-Dive Quadrat	Quadrat sampling	2	3.00	12.00		4.00	1.00	3.00
Dive Quadrat	Quadrat sampling	3	3.00	12.00		4.00	1.00	3.00
Two-week	Recruitment/Contaminant	3	8.00	12.00		1.50	1.00	6.00
Lab A	Quadrat Sample processing	7		30.00	96.00	3.20	1.00	3.00
Lab B	Recruitment/Contaminant collections	5		90.00	96.00	1.07	1.00	6.00

Item	Unit cost	Units	Total per event	# of events	Total cost (Event Total * frequency)	Detail
<b>Quadrat Sampling</b>			<b>\$36,800</b>		<b>3.00</b>	<b>\$110,400 Subtotal</b>
Divers		Days	\$16,000			\$48,000
Non-Diving Personnel		Person days	\$6,400			\$19,200
Boat charges		Days	\$14,400			\$43,200
<b>Recruitment/contaminant team</b>			<b>\$6,675</b>		<b>6.00</b>	<b>\$40,050 Subtotal</b>
Personnel		Person days	\$3,600			\$21,600
Boat charges		Days	\$2,700			\$16,200
Supplies		Person days	\$375			\$2,250
<b>Quadrat Processing</b>			<b>\$18,880</b>		<b>3.00</b>	<b>\$56,640 Subtotal</b>
Personnel		Person days	\$17,920			\$53,760
Supplies		Person days	\$96			\$1,440
Shipping and archive charges		Person days	\$480			\$1,440
<b>Processing Recruitment/Contaminant</b>			<b>\$5,227</b>		<b>6.00</b>	<b>\$31,360 Subtotal</b>
Personnel		Person days	\$4,267			\$25,600
Supplies		Person days	\$480			\$2,880
Shipping and archive charges		Person days	\$480			\$2,880
<b>Cooler Rental</b>			<b>\$2,000</b>		<b>1.00</b>	<b>\$2,000 Subtotal</b>
<b>Field/Lab Total</b>						<b>\$240,450.00</b>

Dive Labor \$48,000  
 Non-Dive Labor \$120,160  
 Boat Charges \$59,400  
 Shipping and Supplies \$240,450

Team	Task	People in Team	# of cellisites per team	# of samples processed per day	Total Lab samples generated	# of days per sampling event or sample process	# of teams per sampling event	Frequency /yr
Side-scan sonar	Site Mapping/Reconnaissance	5	Unknown			5.00	1.00	1.00
Non-Dive Quadrat	Quadrat sampling	2	3.00	12.00		4.00	1.00	3.00
Dive Quadrat	Recruitment/Contaminant	3	3.00	12.00		4.00	1.00	3.00
Two-week		3	8.00	12.00		1.50	1.00	6.00
Lab A	Quadrat Sample processing	7		30.00	96.00	3.20	1.00	3.00
Lab B	Recruitment/Contaminant collections	5		90.00	96.00	1.07	1.00	6.00

Item	Unit cost	Units	Units (teams * days or samples)	Total per event	# of events	Total cost (Event Total * frequency)	Detail
<b>Side-Scan Sonar</b>		Days		\$19,800	1.00	\$19,800	Subtotal
<b>Quadrat Sampling</b>		Days		\$39,836	3.00	\$119,508	Subtotal
Divers		Person days		\$17,370		\$51,960	
Non-Diving Personnel		Person days		\$6,978		\$20,784	
Boat charges		Days		\$15,588		\$46,754	
<b>Recruitment/contaminant team</b>		Person days		\$3,600	6.00	\$40,050	Subtotal
Personnel		Person days		\$1,600		\$11,600	
Boat charges		Days		\$2,700		\$16,200	
Supplies		Days		\$375		\$2,250	
<b>Quadrat Processing</b>		Person days		\$19,835	3.00	\$59,506	Subtotal
Personnel		Person days		\$18,855		\$56,566	
Supplies		Person days		\$480		\$1,470	
Shipping and archive charges		Person days		\$90		\$2,880	
<b>Processing Recruitment/Contaminant</b>		Person days		\$5,227	6.00	\$31,360	Subtotal
Personnel		Person days		\$4,757		\$28,560	
Supplies		Person days		\$470		\$2,880	
Shipping and archive charges		Person days		\$480		\$2,880	
<b>Cooler Rental</b>		Person days		\$2,000	1.00	\$2,000	Subtotal
<b>Field/Lab Total</b>						\$272,223.60	

Dive Labor \$51,960  
 Non-Diver Labor \$144,350  
 Boat Charges \$62,964  
 Shipping and Supplies \$12,950  
 \$272,224

**Attachment**

### **Mississippi Canyon 252 Oil Spill**

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Approval of this work plan is for the purposes of obtaining oyster preassessment data as part of the Natural Resource Damage Assessment for the Deepwater Horizon Oil Spill. Each party reserves its right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

Unless otherwise agreed upon by the Trustees and BP, all samples will be sent to TDI Brooks Lab.

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 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 ENTRIX. 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 to all trustees and ENTRIX. 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. 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/unvalidated" and will be made available equally to all trustees and ENTRIX.

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.

### **DRAFT Oyster Sampling Plan**

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**APPROVED:**

\_\_\_\_\_  
Louisiana Trustee Representative::

\_\_\_\_\_  
Date

\_\_\_\_\_  
BP Representative:

\_\_\_\_\_  
Date

\_\_\_\_\_  
NOAA Trustee Representative  
(on behalf of all other trustees)

\_\_\_\_\_  
Date

## Mississippi Canyon 252 Oil Spill Oyster Sampling Plan

**Sean P. Powers, Jill Rowe, Alexandra van Geel, Patrick Banks, Lyman McDonald, &  
Kristopher Benson and Henry A. Roman**

DRAFT Version 2.1  
July 30, 2010

### Introduction

This Pre-Assessment Oyster Sampling Plan ("the Plan") provides for the collection of ephemeral data on the condition of oysters in the Gulf of Mexico (GOM) - both baseline (pre-oiling) and post-oiling - for use in evaluating whether oysters may be or are being injured by oil or response actions associated with the Deepwater Horizon/Mississippi Canyon 252 Oil Spill (MC 252 Spill) and to assist in implementing future procedures as may be chosen to assess any such injuries to oysters. This plan has been developed in consultation with a Technical Working Group ("oyster working group") of experts and trustee agency representatives. The collection of data outlined in this Plan is a pre-assessment phase activity of the Natural Resource Damage Assessment (NRDA) for the MC 252 Spill pursuant to the Oil Pollution Act of 1990 (OPA). 15 C.F.R. §990.43.

The Plan specifically addresses the following topics:

- I. Approach and rationale.** This section describes the overall purpose and need for an oyster sampling plan.
- II. Data needs and sources.** This section provides an overview of available datasets on oyster reef locations, extent, and condition, as well as identification of data needs.
- III. Health and safety.** This section summarizes pertinent health and safety protocols applicable 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.** This section describes a proposed tiered approach to identifying sites for evaluation.
- V. Site procedures.** This section: (a) references how field teams should set up their equipment prior to starting field work; (b) provides an overview of the protocols teams should follow once the team has reached a specific site to study, and (c) provides general guidance on what to do with the samples and data gathered during the course of the day. This section makes frequent reference to protocols within this document and also NRDA-

wide procedures that are incorporated by reference. Field team members should make sure to 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 site evaluation. It includes specific datasheets/forms to be completed by researchers and references appropriate case-wide protocols and forms.

### **I. Approach and rationale**

The Eastern Oyster, *Crassostrea virginica*, is an integral component of coastal ecosystems and local economies along the Gulf of Mexico. Biogenic reefs formed by the aggregation of this species provide numerous ecological benefits to estuarine systems and a valuable fishery resource. Oyster reefs also provide high-quality habitat for numerous species of fishes and invertebrates many of which are themselves of commercial and recreational significance (*e.g.*, spotted sea trout, red drum, sheepshead, blue crabs). Oysters also serve as filters for estuarine water and influence energy flow and nutrient fluxes in estuarine environments. Direct and long-term economic loss of the oyster fishery as a result of the Deepwater Horizon Oil Spill is of paramount concern, and equally important is the potential loss of ecological services provided by oyster reefs, whether harvested or not.

The Plan is intended to provide data for use in evaluating whether oysters in each State have been or may be injured due to MC 252 oiling and to provide a basis for implementing potential future assessment procedures related to any such injuries to oysters. The Plan presents a design-based approach intended to allow for inferences concerning the effects of MC 252 oil, if any, on demographic parameters and other metrics of oyster populations off the coasts of Louisiana, Mississippi, Alabama, and Florida. To this end, the oyster working group divided the areas into discrete sampling units, cells in a uniform grid, and generated generalized random tessellation stratified (GRTS) spatially balanced probabilistic samples of units (McDonald 2004, Stevens and Olsen 1999, 2004). The samples of units selected will be surveyed by the protocols and standard operating procedures (SOPs) presented in the Plan. It is anticipated that the data generated by this Plan will provide useful input to short-term statistical inferences and modeling and potentially to other more complex modeling efforts in future. The oyster working group adopted an approach that documents the full life cycle of oysters, and that is intended for application within areas that represent a continuum of potential impacts from unaffected to heavy. The oyster working group recognizes that in addition to the potential toxic (lethal) effects of oil, sublethal mechanisms of injury may also exist-- for instance, oil-stressed oysters may be more susceptible to disease. In addition, efforts to mitigate oil spill impacts may cause additional injury --for example, freshwater diversions intended to reduce oceanic input of water may decrease salinity and cause oyster mortality.

The group identified several key metrics - abundance estimates by life stages, biological condition metrics, and environmental/chemical measurements - as important to characterize

oysters and oyster reef health, and to explore potential linkages with sources of stress related to the MC252 spill. In particular, the group plans on measuring the metrics shown in Table 1.

**Table 1. Overview of Metrics to be Measured**

<b>Metric</b>	<b>Proposed Frequency of Sampling</b>
<i>Effect Metrics</i>	
Oyster abundance, by size class and by alive/dead status	Pre-oiling (if possible), Quarterly samples
Abundance of associated species, by oyster size class	Pre-oiling (if possible), Quarterly samples
Oyster biomass, by size class and by alive/dead status	Pre-oiling (if possible), Quarterly samples
Biomass of associated species, by oyster size class	Pre-oiling (if possible), Quarterly samples
Disease	Pre-oiling (if possible), Quarterly samples
Gonadal condition	Pre-oiling (if possible), every two weeks
Larval abundance (#/L)	Pre-oiling (if possible), every two weeks
Larval settlement	Pre-oiling (if possible), every two weeks
<i>Exposure metrics</i>	
Tissue concentrations	Pre-oiling (if possible), every two weeks
Sediment concentrations	Pre-oiling (if possible), Quarterly samples
Oiling observations (qualitative)	Pre-oiling (if possible), Quarterly samples

Oyster reefs are challenging habitats in which to collect quantitative samples because they occur at a wide range of depths (intertidal to deeper subtidal bars), form hummocks or clumps of live and dead animals, and exist most often in highly turbid estuarine waters. Because of their exposure at low tide events, intertidal oyster reefs are comparatively easier to quantitatively sample. This is most commonly accomplished by utilizing a quadrat made of PVC that covers an area of ¼ meter square (0.5 m x 0.5 m). Shallow subtidal (< 0.5 m depth) oyster reefs may also be sampled by hand via quadrats. Deeper areas are best sampled using SCUBA divers to harvest the contents within a quadrat. When possible, quadrat sampling is the preferred method because it achieves a highly quantitative sample (i.e. the area and effort of the sample are well defined). When collection of samples by SCUBA or surface assisted divers is not feasible, small dredges are the best alternatives. These latter devices may only achieve a measurement of relative abundance (i.e. CPUE – catch per unit effort), and it is important to ensure that effort and methods are consistent from site to site.

## II. Data needs and sources

The oyster working group identified both data needs and potential sources of information relevant to documenting the impact of the spill and associated events on oysters. The oyster working group also developed standard operating procedures (SOPs), including data forms, to augment historic and baseline data. These additional data will provide important site-specific information useful in exploring linkages between changes in oyster communities and the MC252 spill.

**A. Oyster density, size frequency and biomass** are fundamental measures of oyster community health. Biomass can be derived from density (#/unit area), if average wet weight of individuals is determined. If biomass data exists for size classes, injury can be estimated by size class. If size class data are not available, average biomass representative of the entire size distribution can be used.

*Existing Baseline Data for Average Oyster Reef Biomass.* Average oyster reef biomass data are available from the past few years for some locations (e.g., Louisiana public oyster areas and locations in Mobile Bay, Table 1). We propose to rely on post-Katrina data, which is likely to be more indicative of current pre-impact conditions of oyster reef biomass.

**Table 1.** Available datasets by state, and the contact person from whom to request the data.

State	Data Set Description	Locations	Years	Contact Person
Louisiana	Square-meter sampling provides oysters/m <sup>2</sup> ; raw data broken into size classes (5mm work groups)	LA public oyster seed grounds	Each July, most recent July 2009	Patrick Banks (LDWF)
	Dredge sampling; data broken into size classes	LA public oyster seed grounds	1992 to present	Patrick Banks (LDWF)
Mississippi	Quarterly dredge samples and yearly square meter samples	Mississippi commercial oyster reefs. Pass Christian and possibly the National Estuarine Research Reserve in Grand Bay, MS.	IJ samples. February 2010 square meter samples. July 2009 2010 samples in progress.	Bradley Randall (MDMR)
Alabama	Periodic oyster dredge surveys	Cedar Point Reef Complex	Non-continuous data beginning in 1970s	Kevin Anson, AMRD
	Quadrat surveys	Harvested and Non-Harvested Reefs 24 reefs within Mississippi Sound, Mobile Bay and Bon Secor Bay	Annual since 1971 Semi-annual since 2003	AMRD Sean Powers (DISL)
	Larval sampling	18 stations within Mississippi Sound, Mobile Bay and Bon Secor Bay	Monthly in 2006 only	Sean Powers (DISL) /Monty Graham
	Spat settlement on concrete tiles	18 stations within Mississippi Sound, Mobile Bay and Bon Secor Bay	Monthly 1968-1970; 2006-2008.	Sean Powers (DISL)
Florida	FL FWC conducted) monthly condition estimates and semi-annual abundance estimates	Tampa Bay Sebastian River Mosquito Lagoon Biscayne Bay	2005-2007	Steve Geiger
	FL FWC conducts monthly condition estimates and semi-annual abundance estimates	Multiple East Coast estuaries	2005-ongoing	Steve Geiger
	FL Gulf Coast University conducts monthly condition estimates and semi-annual abundance estimates	Caloosahatchee River Lostman's River	2002-ongoing 2009 - ongoing	Aswani Volety (via Steve Geiger)



**B. Larval supply and oyster settlement indices.** The 2-3 week pelagic duration of oyster larvae may result in significant lethal and sublethal effects from contact with oil (Figure 1). Quantitative estimates of larval supply (# oyster veliger per liter) are rare in the literature because of the difficulty in identifying oyster larvae (i.e., all bivalve larvae look remarkably similar) and the large time commitment such a project entails. Oyster settlement metrics (e.g., settled spat on shells) are more frequently used; however, these are rarely a component of state monitoring programs because of the expense. Settlement metrics have the advantage of reflecting the result of all recruitment processes (larval supply, larval settlement, post-settlement mortality) but have more limited temporal resolution than the component metrics. The oyster working group's view is that the measurement of both larval supply and settlement measurements could aid in evaluating potential impacts of the oil spill and related events (Figure 2).

Larval supply and settlement metrics are both relevant measures of oyster reef health. In addition, they can be useful inputs in modeling impacts to oysters. The typical input for a model is average abundance (number/unit area) by depth region (on sediments or planktonic) by month. Some baseline (pre-impact) average abundances (number/unit area) of oyster larvae by region (i.e., Mobile Bay vs Mississippi Sound vs. offshore Louisiana) and depth zone are available; however, additional information—pre-oiling, if possible, as well as post-oiling—are valuable and can be used to determine potential population bottlenecks influenced by the oil spill (e.g., larvae are unavailable, larvae are not competent to settle, early survivorship is low).

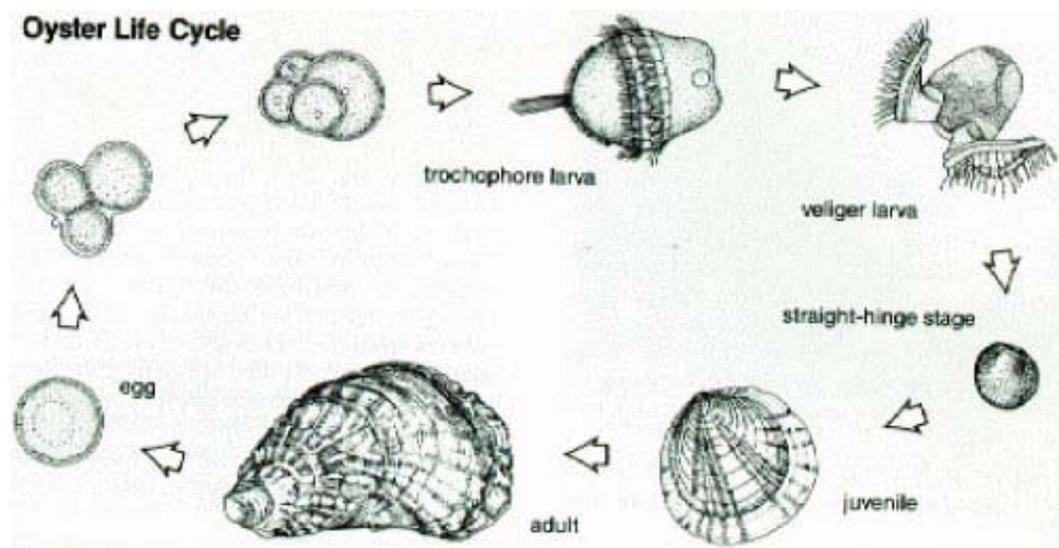


Figure 1. Life cycle of the eastern oyster, *Crassostrea virginica*.

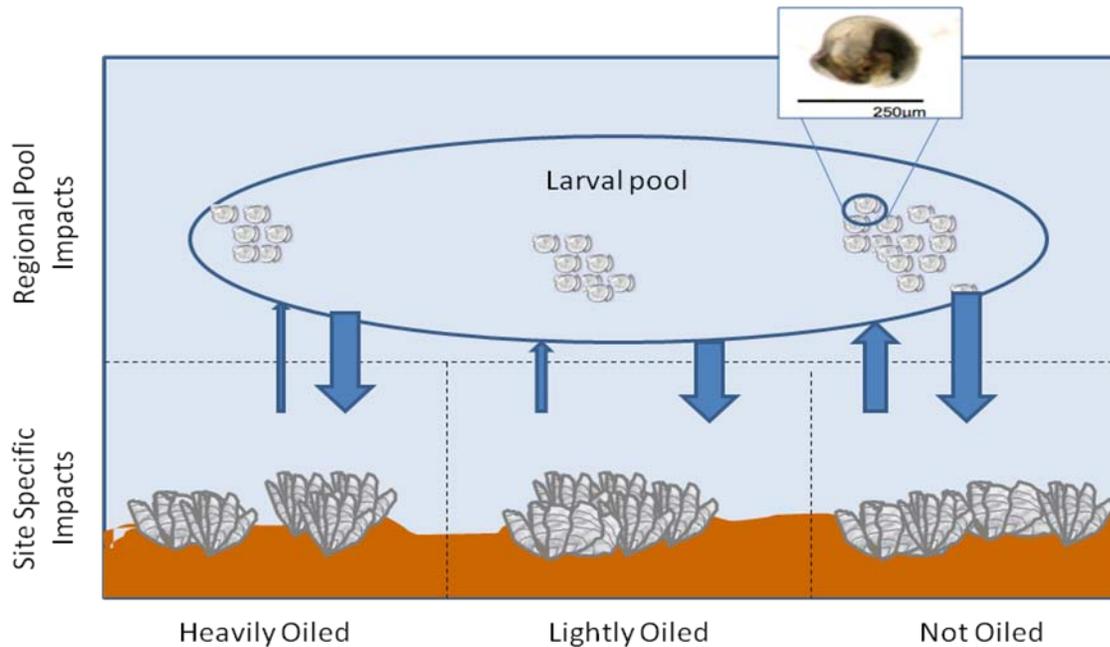


Figure 2. Some potential site and regional level injury resulting from oiling. Direct mortality may result for sessile life stages (juvenile and adults). Reduction in reproductive output is also possible due to loss of adults or physiological impairment of adults, which decrease larval supply to the regional pool of larvae. This decrease in larval supply may have negative effects that extend beyond oiled sites.

**Table 2.** Measurements of larval supply and larval settlement.

State	Data Set Description	Locations	Years	Contact Person
Alabama	Larval sampling	18 stations within Mississippi Sound, Mobile Bay and Bon Secor Bay	Monthly in 2006 only	Sean Powers (DISL) /Monty Graham
Alabama	Spat settlement on concrete tiles	18 stations within Mississippi Sound, Mobile Bay and Bon Secor Bay	Monthly 1968-1970; 2006-2008.	AMRD; Sean Powers (DISL)
Florida	Larval sampling FWC	Pensacola Bay – 9 stations, up to three depth strata	Monthly - May 07- July 08	Steve Geiger
Florida	Larval sampling FWC	Tampa Bay	2-3 times / wk, March 09 - July 09	Steve Geiger
Louisiana	Spat data captured in normal fisheries independent sampling (dredge and square-meter)	LA public oyster seed grounds	1992 to present	Patrick Banks (LDWF)

**C. Areal estimates of oyster reefs.** Mapped oyster reef habitat (location, extent, area (i.e. m<sup>2</sup>, km<sup>2</sup>) is useful in directing sampling activities and in scaling up site-specific measurements collected during the damage assessment.

*Mapped Oyster Reef Habitat GIS Layers.* Mapped oyster habitat GIS layers are available for most, if not all states. However, many states do not have complete GIS coverage of oyster resources within their respective waters. The table below summarizes which GIS layers are available by state, and the contact person for obtaining those layers. Data sets that could be adapted for GIS layers (i.e. georeferenced data) are also identified.

**Table 3.** Areal estimates of oyster reefs.

State	Locations	Years	Contact Person
Louisiana	Some public oyster areas	Variable	Patrick Banks (LDWF)
Mississippi	Pass Christian Commercial reefs Possibly NERRS	1998? 2009???	Bradley Randall (MDMR)
Alabama	Most public reefs and riparian lease grounds	1971, 1995, 2001	AMRD
	Side-scan imagery of major reef complexes (georeferenced)	2009-2011	Sean Powers (DISL)
Florida	Note from Steve Geiger : (I have contacted Steve Vanderkooy at Gulf states to see how complete their GIS layer is – it is currently being compiled as part of the revision of the oyster FMP.		

**D. Baseline contaminant data in oyster tissue and sediments**

Currently, it appears that oil from the Deepwater Horizon will make landfall over a large geographic area and at highly variable concentrations. These factors necessitate that appropriate metrics for gauging oyster exposure to oil be collected synoptically with oyster measurements. Because oysters may have some previous exposure to background levels of contaminants, establishing baseline contaminant exposure, through the use of pre-oiling data or reference area data, is necessary. Potential sources of pre-oiling data may already exist. Because depuration rate of hydrocarbon contaminants in oysters may be rapid (~30 days), it is critical to collect oyster tissue on a frequent basis (~2 weeks).

Many state and federal agencies have been collecting tissue, water and sediment samples since the Deepwater Horizon spill began. The majority of these tissue samples have been collected for seafood safety and sanitation purposes. In addition, NOAA’s Mussel Watch program (<http://ccma.nos.noaa.gov/about/coast/nsandt/musselwatch.html>) may be a source for baseline contaminants in oyster tissue and sediments (Figure 3). The State of Florida has requested an increase in the number of Mussel Watch sampling stations and that request is included in this plan. The Mussel Watch program also collects information on histology, disease and condition of oysters as well as benthic infaunal data. Additionally, other NRDA sampling programs will occur concurrently with the oyster sampling program. Information from these programs, such as the sediment/water sampling program, may be available for inclusion in the oyster impact analysis model. The availability of such data may reduce the need for similar information to be collected as part of this oyster sampling plan



**Figure 3.** Musselwatch collection sites in the northcentral Gulf of Mexico. (Source [http://www8.nos.noaa.gov/cit/nsandt/download/mw\\_monitoring.aspx](http://www8.nos.noaa.gov/cit/nsandt/download/mw_monitoring.aspx))

### III. Health and Safety

- **The team leader and field crew parties should 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 Oil Spill” (NOAA Safety Documentation Requirements.doc).

- **All field team members should read all of the documents in the Safety directory on the case’s ftp site** [REDACTED]  
Exception: if site collection activities do not include use of a boat or helicopter, then familiarity with the safety documents for these vehicles is not required.
- **Each field team must submit a plan, not later than the night prior to going into the field.** This plan must specify:
  - The team leader;
  - Names of all team members;
  - The sampling location(s)-- please use the grid coordinates as shown in Maps 1 to 3 below;
  - What kind of sampling they are doing;
  - Expected arrival time at sampling area (daily);
  - Expected departure from sampling area (daily);
  - Team deployment date;
  - Team return date.

This information may be reported in one of two ways:

1. Fill out the Excel spreadsheet “Team Member Information Form – Excel.xls”<sup>1</sup> and send it to [REDACTED]. Please use one tab for each team.
2. If you cannot submit this spreadsheet electronically, you can call in and report the information using this number: [REDACTED]

- **Field teams must adhere to all procedures set forth in the MC252 Site Safety Plan (“NRDA MC 252 Site Safety Plan\_5.13.10.pdf”).**<sup>2</sup>
- **If participating in a cruise:** Each cruise may have additional required health and safety procedures, that must be observed.
- **Diving:** SCUBA or surface-assisted diving, where used for sampling, will be conducted in accordance with existing Trustee dive safety programs.

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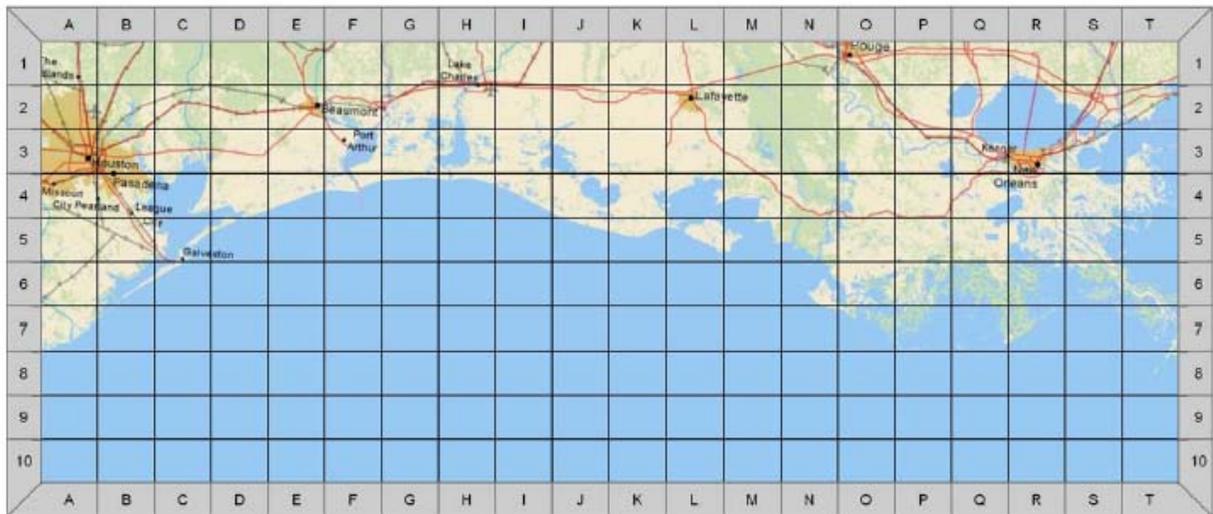
<sup>1</sup> This file is available on the case’s ftp site:  
[REDACTED]

<sup>2</sup> This file is available on the case’s ftp site:  
[REDACTED]

Please use these maps to fill out the sampling locations in your team member information forms. **IMPORTANT: When submitting grid coordinates, please include the map number as well (Example: “Map 1 – A3, A4; Map 2 – T3, T4”).**



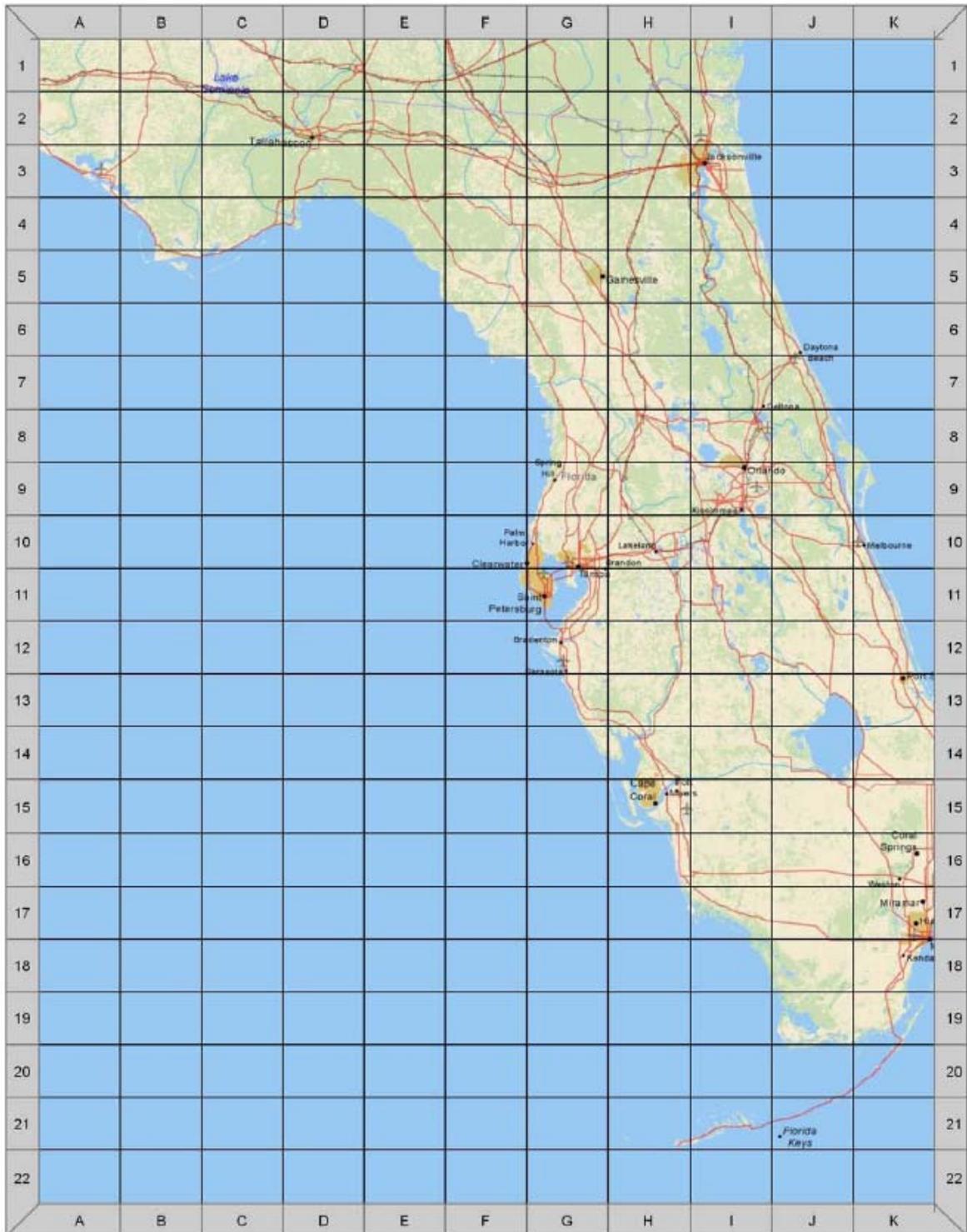
Map 1



Map 2



Map 3



#### IV. Site Selection

Currently, it appears that oil from the Deepwater Horizon will make landfall over a large geographic area and at highly variable concentrations. These factors argue for a large-scale sampling program with probabilistic selection of sites. Equally important is a sampling effort that preserves the long-term record of many state resource agencies. These latter data are considered fixed stations because the same site or area has been revisited over multiple years. Such data sets remove spatial confounding factors and allow for quantification of natural temporal variability. Because both factors are important in designing a sample plan (i.e., the ability to extrapolate to larger spatial scales [random sampling] and the need to explain normal temporal variation), the oyster working group has adopted a three-tiered selection procedure for sampling sites (Table 4).

##### A. Tier 1

Tier 1 (fixed sites) is designed to utilize long-term monitoring sites of state agencies (LDFW, MS DMR, AL MRD). (A separate tier, Tier 3, is included for Mussel Watch stations and additional stations included in the Florida requested for augmentation of Mussel Watch.) It is likely that these data are already being collected by the appropriate agencies, but need to be reviewed for consistency with NRDA sampling protocols. If samples are not available or do not comply with NRDA sampling protocols, then ideally the sites should be re-sampled as soon as possible. Data will be collected on historic sites (Table 1) that were sampled in previous years by state and federal agencies using the same standard operating procedures (SOPs) that were used for past data collections with emphasis placed on data collected in the last five years. The same metrics will be measured using the same field methods. In addition, if the historic methods differ significantly from the protocols described below, then the historic sites may be sampled by these new protocols, i.e., at least two quadrat subsamples (see below) would be collected on two transects across the oyster habitat in close proximity to the historic site.

**Table 4.** Summary of sample site selection approach and rationale.

Tier	Stratum	Site selection	Rationale	Minimum # of sites (Grid Cells) <sup>1</sup>	Subsamples per grid cell
1	None	<i>Fixed.</i> Sites are based on long-term monitoring locations of state agencies.	Pattern of recruitment and mortality (natural or fishing induced) can be inferred and potentially separated from oil spill mortality.	Known monitoring sites with emphasis on data from last 5 years.	2 per fixed site. If historic methods differ from this protocol, 8 per grid cell.
2	A	<i>Random in known oyster habitat-</i>	Much of oyster habitat is in mapped	60 per state <sup>2</sup>	up to 8 per grid cell

		boundaries designated by the state trustees for known oyster habitat (harvested and closed areas).	areas. Although oyster habitat within these large geographic areas is probably patchy.		
	B	Random in areas where oyster habitat may potentially occur but digitized GIS maps are not available. This stratum will include lease areas in LA.	A portion of oyster habitat in each State occurs in scattered areas that have not been mapped.	60 in LA 40 in MS 40 in AL 60 in FL <sup>2</sup>	up to 8 per grid cell
	C	Random in shoreline areas.	Oysters occur along many shoreline marshes, rip-rap, pilings and other hardened structures in the intertidal-shallow subtidal. These areas are likely to experience oiling.	0 in LA 40 in MS 40 in AL 60 in FL <sup>2</sup>	up to 8 per grid cell
3	None	NOAA Musselwatch	Fixed station. Continuation and augmentation (in FL) of musselwatch sampling.		Follow Musselwatch protocols

<sup>1</sup> Assumes a site will have substrate suitable for oyster recruitment and growth.

<sup>2</sup> This effort may be increased in all states, if sampling sites do not represent oiled areas sufficiently.

- **Data needs Tier 1:** GPS location, sampling history, and sampling protocol of long-term monitoring sites are needed. A more detailed synopsis of the sampling procedures and efforts will need to be submitted; however, the immediate need is for GPS location and the last 5 years of sampling history of long-term sites.

**B. Tier 2**

Tier 2 sampling consists of spatially balanced sampling from areas with known or potential oyster habitat, including areas experiencing oiling, for use in evaluating the nature and extent of impacts to oysters due to MC 252 oiling.

Tier II will be stratified into three strata: A (known oyster habitat); B (potential oyster habitat); and C (shoreline habitat). A grid of 4 ha (200 m x 200 m) grid cells will overlay the

strata and each grid cell assigned stratum membership based on the rules presented in Table X. Grid cells with the same stratum membership will make up the sample frame for the given stratum. Within each stratum, grid cells will be randomly selected from the stratum sample frame using the GRTS spatially balanced probabilistic sampling procedure (McDonald 2004, Stevens and Olsen 1999, 2004). Selected grid cells (sites) will be sub-sampled using the protocols and SOPs described below.

#### *Tier 2 Stratum A.*

Stratum A encompasses *known oyster habitat* with boundaries designated by the state trustees for oyster habitat and digitized as polygons in GIS data layers. A GRTS spatially balanced sample of sites (McDonald 2004, Stevens and Olsen 1999, 2004) will be selected with approximately 60 cells in Stratum A of each state (mapped and digitized oyster habitat: Louisiana, Mississippi, Alabama, and Florida). (Note that Stratum A is inclusive of harvested and closed areas and therefore many of the Tier 1 sites will occur within the known areas; however, the key difference for Tier 2 Stratum A sites is that a probabilistic procedure will be utilized for selection of sampling sites.

After cells are selected in Stratum A (mapped habitat) of a given state, they will be overlain on GIS maps (polygons) of areas known to have incurred heavy oiling by MC252, if available. If there are too few randomly selected cells in the heavily oiled categories for potential use in establishing a sufficient dose-response curve for the effect of MC252 oiling, then up to 10 additional grid cells may be selected. If GIS maps of areas known to have incurred heavy MC252 oiling are not available prior to the first round of sampling, then it may be necessary to supplement the sample of sites following initial sampling with both heavily MC 252 oiled sites as well as up to 10 sites receiving no or very light MC 252 oil. Supplemental grid cells in heavily oiled areas and in areas receiving no or very light MC 252 oil will be selected by taking the first grid cells on the GRTS list that are in the desired areas that on the GRTS list. The number of grid cells needed will be based on the distribution of exposure levels encountered on the grid cells in the initial sample of approximately 60 and on professional judgment to meet the objective of ensuring that some grid cells have both no (or very low) exposure and high exposure levels to better fit a dose-response curve. These supplemental cells will be used in fitting the dose response curve, and will only represent themselves in determining the extent of injury in Stratum A.

#### *Tier 2 Stratum B.*

Stratum B is inclusive of areas of potential oyster habitat, but for which little information is available on existence and location of colonies, i.e., no digitized, georeferenced polygons available. In many regions salinity regime may be the only factor that can be used to establish mapped boundaries. Figure 6 contains an hypothetical section of shoreline illustrating that sections of Stratum B are not necessarily adjacent. Stratum B will include lease areas that are not mapped. The “shoreline” boundary for Stratum B in Louisiana will be extended to include areas of the complex delta area that are determined by Louisiana Agency Biologists to

potentially contain oyster habitat, depending on salinity and other environmental variables. Similarly, part of the complex shoreline intertidal and shallow subtidal habitat in Mississippi, Alabama, and Florida may be included in Stratum B. Otherwise, cells with primarily subtidal areas of potential oyster habitat are placed in Stratum B. A GRTS spatially balanced sample of sites will be selected with approximately 60 cells in stratum B of Louisiana and Florida and approximately 40 cells in Stratum B of Mississippi and Alabama.

After cells are selected in Stratum B of a given State, they will be overlain on GIS maps (polygons) of areas known to have incurred heavy oiling by MC252, if available. If there are too few randomly selected cells in the heavily oiled categories for potential use in establishing a dose-response curve for the effect of MC252 oiling, then up to 10 additional grid cells may be selected. If GIS maps of areas known to have incurred heavy oiling by MC252 are not available in Louisiana for the first round of sampling, then it may be necessary to supplement the sample of sites later with both heavily MC 252 oiled sites as well as up to 10 sites receiving no or very light MC 252 oil. Supplemental grid cells in heavily oiled areas and in areas receiving no or very light MC 252 oil will be selected by taking the first grid cells on the GRTS list that are in the desired areas. The number of grid cells needed will be based on the distribution of exposure levels encountered on the grid cells in the initial sample of approximately 60 and on professional judgment to meet the objective of ensuring that some grid cells have both no (or very low) exposure and high exposure levels to better fit a dose-response curve. These supplemental cells will be used in fitting the dose response curve, and will only represent themselves in determining the extent of injury in Stratum B.

#### *Tier 2 Stratum C.*

Stratum C consists of the 4 ha grid cells that intersect the digitized “shoreline” intertidal and shallow subtidal habitat as defined and provided in GIS data layers by the States of Mississippi, Alabama, and Florida. Because of the complexity of the coastline and the vast expanse of shallow (<1m) habitat in Louisiana, Stratum C would be poorly defined in this state. Hence, these shallow areas for Louisiana are better represented in Stratum B. Figure 6 contains a hypothetical section of shoreline illustrating Stratum C (between the black lines and the dashed blue lines). A GRTS spatially balanced sample of sites will be selected with approximately 60 cells in Stratum C of Florida and approximately 40 cells in Stratum C of Mississippi and Alabama.

After cells are selected in Stratum C (shoreline habitat) of a given state, they will be overlain on GIS maps (shape file) of shoreline known to have incurred heavy oiling by MC252, if available. If there are too few randomly selected cells in the heavily oiled categories for potential use in establishing a dose-response curve for the effect of MC252 oiling, then up to 10 additional grid cells may be selected. If GIS maps of shoreline areas known to have incurred heavy oiling by MC252 are not available for the first round of sampling, then it may be necessary to supplement the sample of sites later with both heavily MC 252 oiled sites as well as up to 10 sites receiving no or very light MC 252 oil. Supplemental grid cells in heavily oiled areas and in areas receiving no or very light MC 252 oil will be selected by taking the first grid cells on the GRTS list that are in the desired areas. The number of grid cells needed will be

based on the distribution of exposure levels encountered on the grid cells in the initial sample of approximately 60 and professional judgment to meet the objective of ensuring that some grid cells have both no (or very low) exposure and high exposure levels to better fit a dose-response curve. These supplemental cells will be used in fitting the dose response curve, and will only represent themselves in determining the extent of injury in Stratum C.

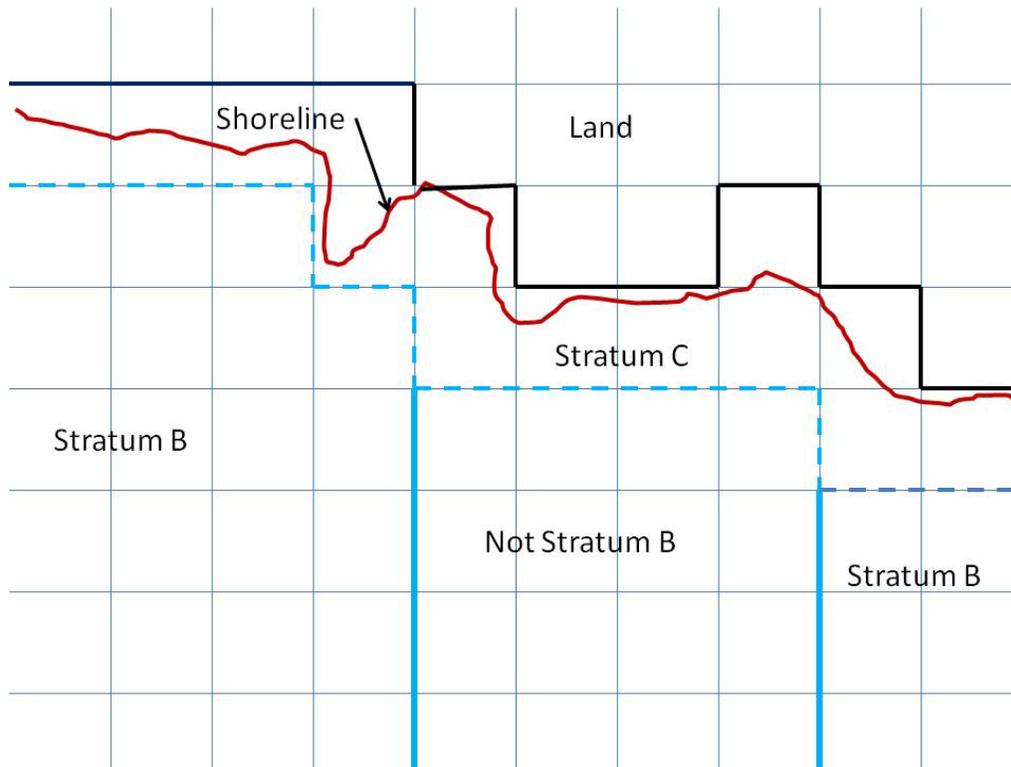


Figure 6. Hypothetical shoreline illustrating 4 ha cells that capture the shoreline (Stratum C), intertidal and subtidal habitat. Also illustrated is the possibility that Stratum B may consist of disjoint subsections.

Cells that cannot be accessed, e.g., private areas with no permission granted, will be labeled “missing data”, and replaced by the next alternate cells on the appropriate GRTS list. The recommended number of sites assumes that the cell has substrate suitable for oyster settlement within it. Cells that contain no oyster habitat based on the site survey (see VI, A.) will be logged as zeros for estimation of the total area of oyster habitat and new cell locations will be generated. Site selection will occur once at the start of the study. Subsequent sampling as defined within the individual SOPs will occur within the same cells (i.e., site selection and verification of existence of oyster habitat within sample cells occurs only once). Figure 7 contains an illustration of a hypothetical reef in Stratum A covered by cells, with one cell selected for sampling.

Initial analyses of data may indicate that sample sizes of, e.g., 60 or 40, are not necessary in some strata of the States or alternatively, 60 or 40 is not enough to provide the necessary information for making statistical inferences on important effect sizes. The advantage of a GRTS list of sample sites is that the sample size can be increased by taking the next few sites on

the list, or decreased by dropping the last few sites on the list, while maintaining spatial balance of the sites.

Considerable information is available on the geographic extent of harvested reefs, unharvested reefs, and other potential oyster habitat in Louisiana (Figure 8), Mississippi, Alabama, and Florida. GIS maps can be used to establish sampling grids and random selection of grid cells can be performed *a priori*.

- **Data needs:** GIS maps of Stratum A and Stratum B oyster areas in Louisiana, Mississippi, Alabama, and Florida. GIS maps of Stratum C in Mississippi, Alabama, and Florida.

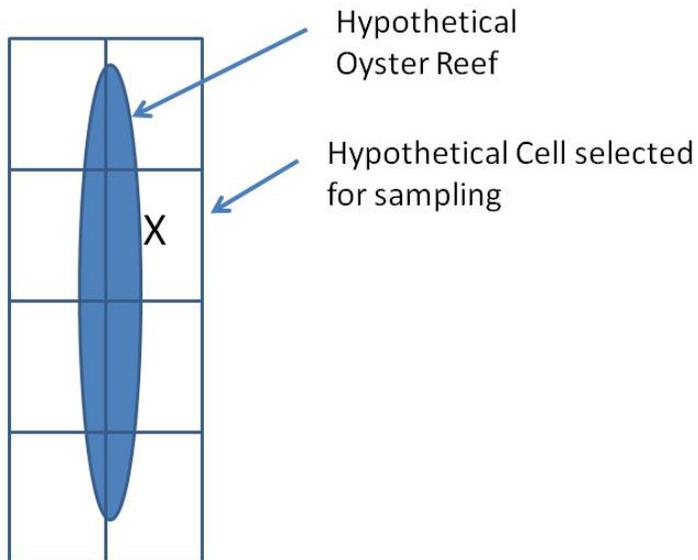
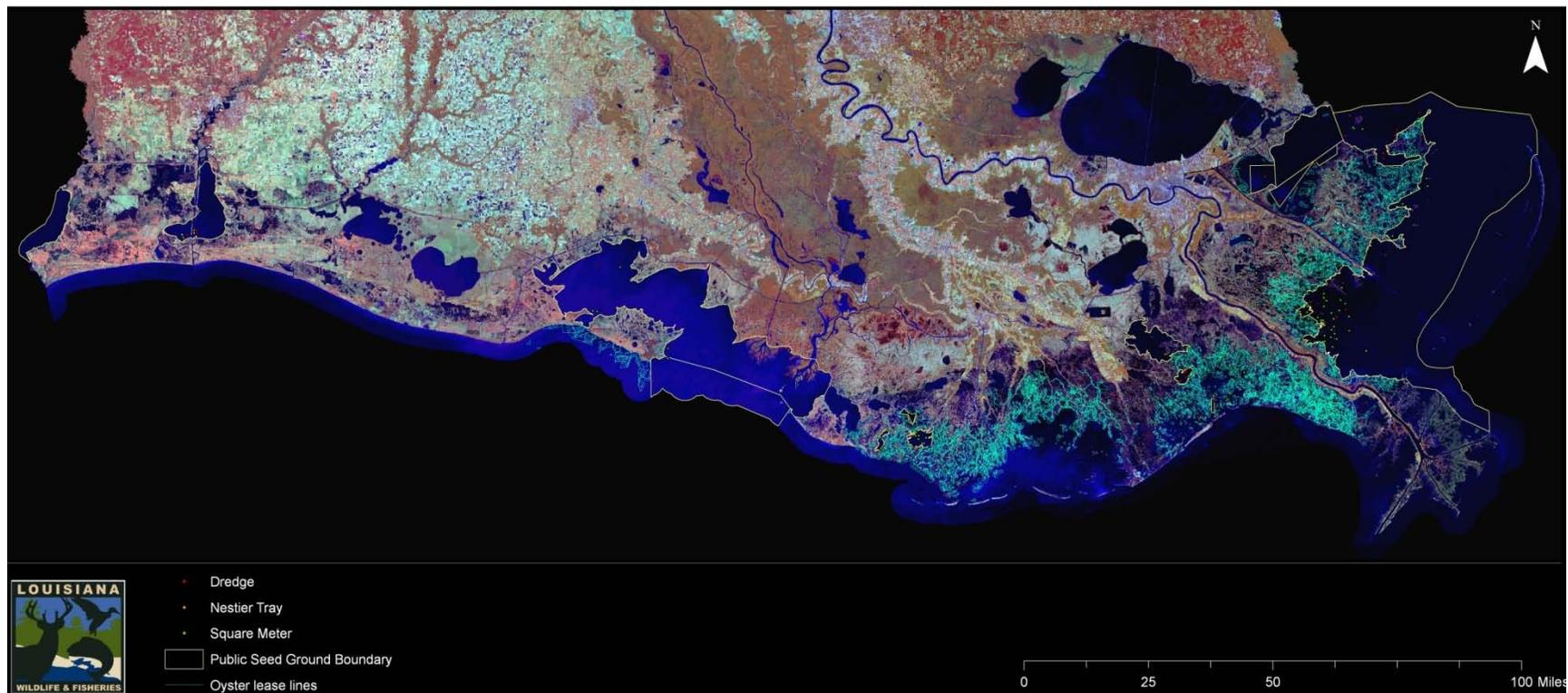


Figure 7. Hypothetical oyster reef covered by 200 m by 200 m cells and one cell selected for sampling.



**Figure 8.** Map of Louisiana public oyster areas and private oyster leases, and the historic sampling sites (by type) located thereon.

### **C. Tier 3.**

NOAA Mussel Watch stations will be sampled in LA, MS, AL and FL by NOAA according to the metrics and protocols specified in this plan (See Figure 9). A brief review and details of the program are included in this section. The Mussel Watch stations data provide a basis for contrast against any potential future impacts by oil from the MC 252 spill.

*Protocols* – In addition to the samples specified in this plan, standard, established Mussel Watch methods/protocols will be repeated quarterly.

- Oysters (organics and petroleum related & metals)
- Oysters (histopathology)
- Sediment (organics & metals)
- Sediment (grain size)
- Sediment (toxicity – P450 assay)

Tissue contaminant samples will be collected biweekly.



**Figure 9.** Musselwatch collection sites in the Gulf of Mexico. (Source [http://www8.nos.noaa.gov/cit/nsandt/download/mw\\_monitoring.aspx](http://www8.nos.noaa.gov/cit/nsandt/download/mw_monitoring.aspx))

#### *Additional Sites in Florida targeting mangrove oysters, *Crassostrea rhizophorae**

Potential Mussel Watch sites in Florida were selected by the Center for Coastal Monitoring and Assessment (CCMA) using the same approach as has been applied to the US coastline in general. Targeted sampling is for bivalves (i.e., mangrove oysters, *Crassostrea rhizophorae*). The mangrove oyster does support some subsistence fishery, especially in certain

locales, but would primarily serve in the traditional role of “Mussel Watch” programs as a sessile, sentinel species used to characterize exposures for an entire habitat.

Spatial distribution of sites was designed to give good geographic coverage with reasonable representation of the general site location (See Figure 10). Site locations are for discussion purposes at this time. Actual site locations will vary based on needs/requests of the oyster working group with final determination by team(s) on the ground.

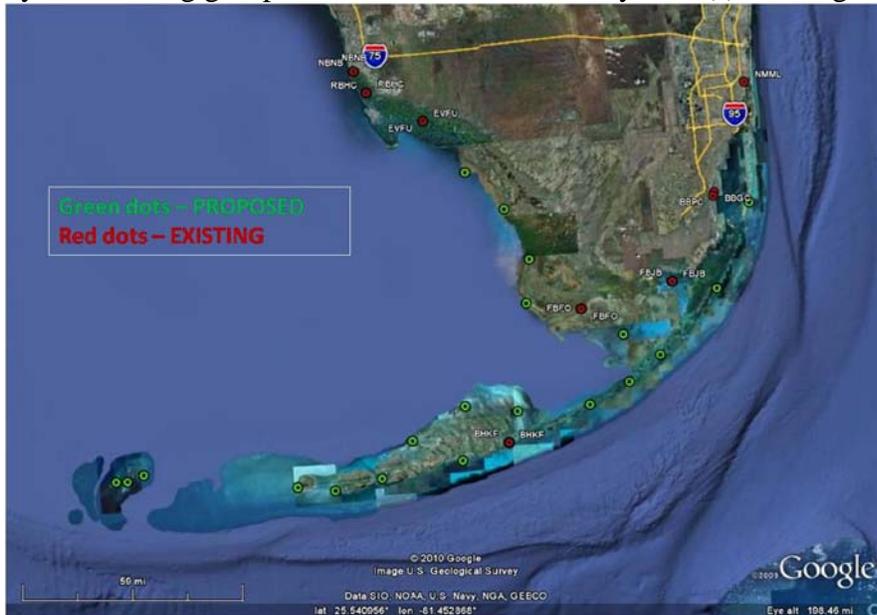


Figure 10. Proposed additional sampling for Mussel Watch stations in Florida.

## V. Site Procedures

1. Navigate to predetermined site.
2. Complete site survey procedures (see section **VI., A**).
3. If oyster substrate is detected, select appropriate subsample location (see **section VI. A**)
4. Set up the camera in accordance with **NRDA Field Photography Guidance** (NRDA\_Field\_Phography\_Guidance.doc, available on the case FTP site). **BE SURE TO READ AND FOLLOW THESE INSTRUCTIONS COMPLETELY.**
5. Set up and use the GPS unit in accordance with case-wide protocols (see **Basic\_GPS\_Skills\_Final\_0223\_2010.doc**, available on the case FTP site). **BE SURE TO READ AND FOLLOW THESE INSTRUCTIONS COMPLETELY.**
  - Perform quadrat sampling (Juvenile/adult oyster SOP see section **VI, B**. The oysters for subsequent analysis will also be included in this sample: Shellfish tissue SOP (see **VI, E.**); Oyster disease SOP (see **VI, H.**); and Gonadal Condition SOP (see **VI, G.**)
6. Collect sediment sample for contaminant analyses (see **VI, F.**)
7. Collect water samples (Larval concentration SOP, see **VI, C.**)
8. Deploy, retrieve and replace settlement plates (Larval settlement **VI, D.**)
9. Complete data forms (see appendices).
10. Navigate to next site (cell).

After completing all field sampling activities for a given day, the field team must take the collected samples, datasheets and electronic information (including photographs and GPS track log) to an appropriate sample processing center.

At this center, the following activities will take place:

- Samples must be appropriately packaged and prepared for shipment to the receiving laboratory(ies).
- **Chain-of-custody** forms must be rigorously completed.
- All data from all field forms should be entered into the appropriate Excel file format (Forms or Flat version) either by the field\_sampler or a data management team member. Once the file is completed, it should be submitted to the\_data management team for incorporation into the database.
- All photographs must be archived, in accordance with the instructions in the **NOAAField Photography Guidance** (NRDA\_Field\_Phography\_Guidance.doc, available on the case FTP site).

- Synchronize the photos with the GPS track in accordance with the instructions in the NOAA ARD-FAST Using GPS-Photo Link instructions (GPSPhotoLink.doc, available on the case FTP site).
- Import the photos into the ORR PhotoLogger database. (This will allow the photos to be uploaded to ERMA.) See the document NOAA PhotoLogger for more information.
- All field data sheets will be scanned and originals stored in a secure location.

Table 5. Summary of sampling procedures, number of sites, replicates, etc.

State	Metric	Method	# of sites					Repl. per site	Est. Samples per event	Freq of sampling	Estimated samples per year	Ref. for SOP & Form
			Tier 1	Tier 2: A	Tier 2: B	Tier 2: C	Tier 3					
<b>Louisiana</b>												
LA	Site Mapping	Chain, pole or side scan	0	60	60	0	0	N = 1	120	Once	120 sites	Sec. VI. A. Form A
	Adult and Juvenile Density	Quadrat or dredge	70	60	60	0	21	N= 8 quadrats	1,688	4/ year (Quarterly)	6,752	Sec. VI. B. Form B
	Oyster Larvae	Water sample	0	60	60	0	0	N = 2	240	12 Biweekly from May – Oct.	2880	Sec. VI. C. Form C
	Oyster Settlement	Settlement plate	0	60	60	0	0	N = 3 plates.	360	12 Biweekly from May – Oct.	4,320	Sec. VI. D. Form D
	Oyster Gondal condition	Oysters	0	60	60	0	21	N = 10 oysters	1,410 oysters	12 Biweekly from May – Oct.	16,920 oysters	Sec. VI. E.
	Tissue contaminant analysis	Oysters	70	60	60	0	21	N = 4 composite samples per grid cell	844	12 biweekly samples plus quarterly samples)	13,504	Sec. VI. F
	Sediment contaminant analysis	Sediment	70	60	60	0	21	N = 4	844	4/ year (Quarterly)	3,376	Sec. VI. G
	Oyster Disease	Oysters	70	60	60	0	21	N = 10 oysters	2,110 oysters	4/ year (Quarterly)	8,440 oysters	Sec. VI. H
<b>Mississippi</b>												
MS	Site Mapping	Chain, pole	0	60	40	40	0	N = 1	140	Once	140	Sec. VI. A.

		or side scan								sites	Form A	
	Adult and Juvenile Density	Quadrat or dredge	25	60	40	40	3	N= 8 quadrats	1344	4/ year (Quarterly)	5,376	Sec. VI. B. Form B
	Oyster Larvae	Water sample	0	60	40	40	0	N = 2	280	12 Biweekly from May – Oct.	3,360	Sec. VI. C. Form C
	Oyster Settlement	Settlement plate	0	60	40	40	0	N = 3 plates.	420	12 Biweekly from May – Oct.	5,040	Sec. VI. D. Form D
	Oyster Gondal condition	Oysters	0	60	40	40	0	N = 10 oysters	1400 oysters	12 Biweekly from May – Oct.	16,800 oysters	Sec. VI. E.
	Tissue contaminant analysis	Oysters	25	60	40	40	3	N = 4 composite samples per grid cell	672	12 biweekly samples plus quarterly samples	10,752	Sec. VI. F
	Sediment contaminant analysis	Sediment	25	60	40	40	3	N = 4	672	4/ year (Quarterly)	2,688	Sec. VI. G
	Oyster Disease	Oysters	25	60	40	40	3	N = 10 oysters	1680 oysters	4/ year (Quarterly)	6,720 oysters	Sec. VI. H
<b>Alabama</b>												
AL	Site Mapping	Chain, pole or side scan	0	60	40	40	0	N = 1	140	Once	140 sites	Sec. VI. A. Form A
	Adult and Juvenile Density	Quadrat or dredge	25	60	40	40	3	N= 8 quadrats	1344	4/ year (Quarterly)	5,376	Sec. VI. B. Form B
	Oyster Larvae	Water sample	0	60	40	40	0	N = 2	280	12 Biweekly from May –	3,360	Sec. VI. C. Form C

	Oyster Settlement	Settlement plate	0	60	40	40	0	N = 3 plates.	420	Oct. 12 Biweekly from May – Oct.	5,040	Sec. VI. D. Form D
	Oyster Gondal condition	Oysters	0	60	40	40	0	N = 10 oysters	1400 oysters	12 Biweekly from May – Oct.	16,800 oysters	Sec. VI. E.
	Tissue contaminant analysis	Oysters	25	60	40	40	3	N = 4 composite samples per grid cell	672	12 biweekly samples plus quarterly samples)	10,752	Sec. VI. F
	Sediment contaminant analysis	Sediment	25	60	40	40	3	N = 4	672	4/ year (Quarterly)	2,688	Sec. VI. G
	Oyster Disease	Oysters	25	60	40	40	3	N = 10 oysters	1680 oysters	4/ year (Quarterly)	6,720 oysters	Sec. VI. H
<b>Florida</b>												
FL	Site Mapping	Chain, pole or side scan	0	60	60	60	0	N = 1	180	Once	180 sites	Sec. VI. A. Form A
	Adult and Juvenile Density	Quadrat or dredge	25	60	60	60	50	N= 8 quadrats	2,040	4/ year (Quarterly)	8,160	Sec. VI. B. Form B
	Oyster Larvae	Water sample	0	60	60	60	0	N = 2	360	12 Biweekly from May – Oct.	4,320	Sec. VI. C. Form C
	Oyster Settlement	Settlement plate	0	60	60	60	0	N = 3 plates.	540	12 Biweekly from May – Oct.	6,480	Sec. VI. D. Form D
	Oyster Gonadal condition	Oysters	0	60	60	60	50	N = 10 oysters	2,300 oysters	12 Biweekly from May –	27,600 oysters	Sec. VI. E.

										Oct.		
	Tissue contaminant analysis	Oysters	25	60	60	60	50	N = 4 compos ite samples per grid cell	1,020	12 biweekly samples plus quarterly samples 4/ year (Quarterly)	16,320	Sec. VI. F
	Sediment contaminant analysis	Sediment	25	60	60	60	50	N = 4	1,020	4/ year (Quarterly)	4,080	Sec. VI. G
	Oyster Disease	Oysters	25	60	60	60	50	N = 10 oysters	2,550 oysters	4/ year (Quarterly)	10,200 oysters	Sec. VI. H

\*number will be determined by existing state information

## **VI. Detailed Standard Operating Procedures (SOPs)**

Synoptic sampling of several parameters is desirable to establish the linkage between oiling and impacts to oyster reefs. Where possible, samples for settled life stages of oysters, larval concentration, larval settlement, oyster condition metrics, and contaminants should be collected at the same sites and times.

### **A. Site mapping - Initial Site Surveys**

#### **(1) Tier 1.**

Site mapping is not required for Tier 1 sites since these locations have been sampled by agencies previously.

#### **(2) Tier 2 Strata A and B**

For selected Tier 2 Stratum A and B cells initial surveys of the area will be mapped using high resolution side-scan sonar that is georeferenced with an accuracy of +/- 1 m (Figure 11, See Allen et al., 2005). The purpose of the site mapping procedures is: (1) to establish percent coverage of oyster habitat within a pre-determined cell and (2) to identify with a high degree of certainty the locations for quadrat selection and subsequent follow up sampling. During post-processing of the side-scan sonar imagery individual oyster reefs will be identified and assigned a number beginning with 1. Sub-sampling within each grid cell (site) will consist of randomly selecting up to eight individual oyster reefs. If a grid cell (site) has 8 individual oyster reefs, all of the reefs will be selected for sub-sampling. If a site has fewer than 8 individual oyster reefs, all reefs will be selected for subsampling, and simple random selection will be used to identify 8 subsampling locations across those reefs within the grid cell. In the event a grid cell (site) is comprised of a single homogeneous oyster reef, simple random selection will be used to select eight points within the grid cell (site). All side-scan sonar imagery will be saved in both raw and post-processed forms. A contact report is the final product for this stage.

If reef material or other oyster habitat is identified, the eight quadrat location will be passed onto the oyster sampling team. If no oyster habitat is found, additional grid cells (sites) will be selected from the GRTS list for future sampling. Additional cells may be added to provide additional data for estimation of the acreage of oyster habitat and to provide data for modeling the effects (if any) of the MC 252 oiling. This assessment will be done after sufficient information is collected on the location of oiling along the Gulf Coast.

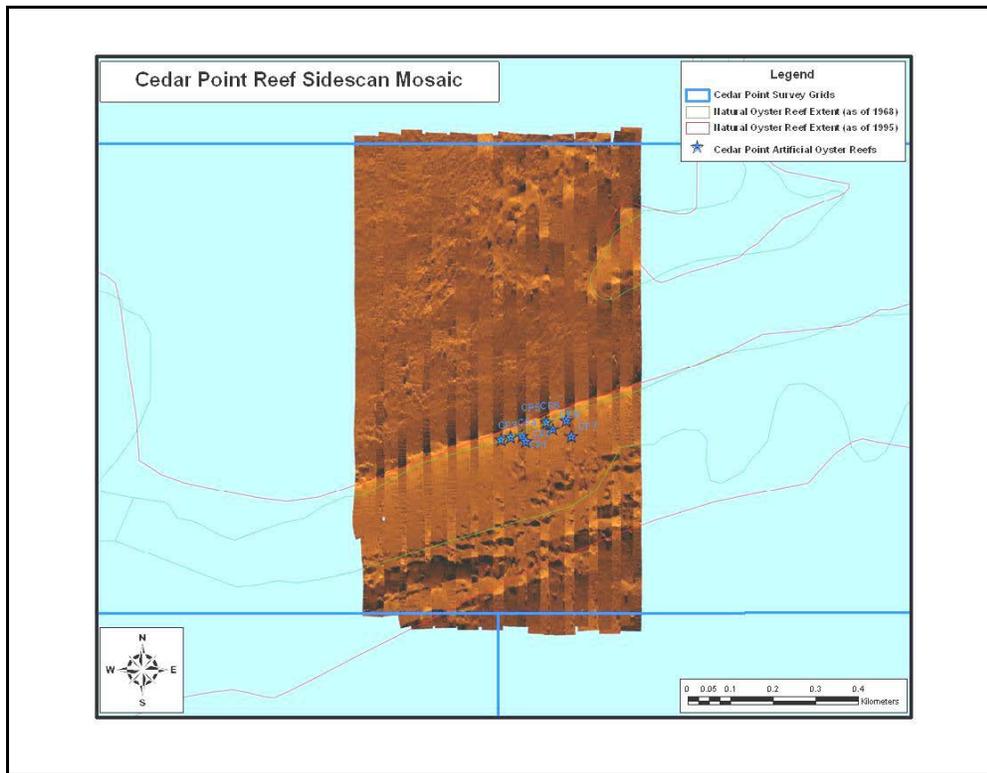


Figure 11 . A side scanned image of oyster reef in Mobile Bay, Alabama.

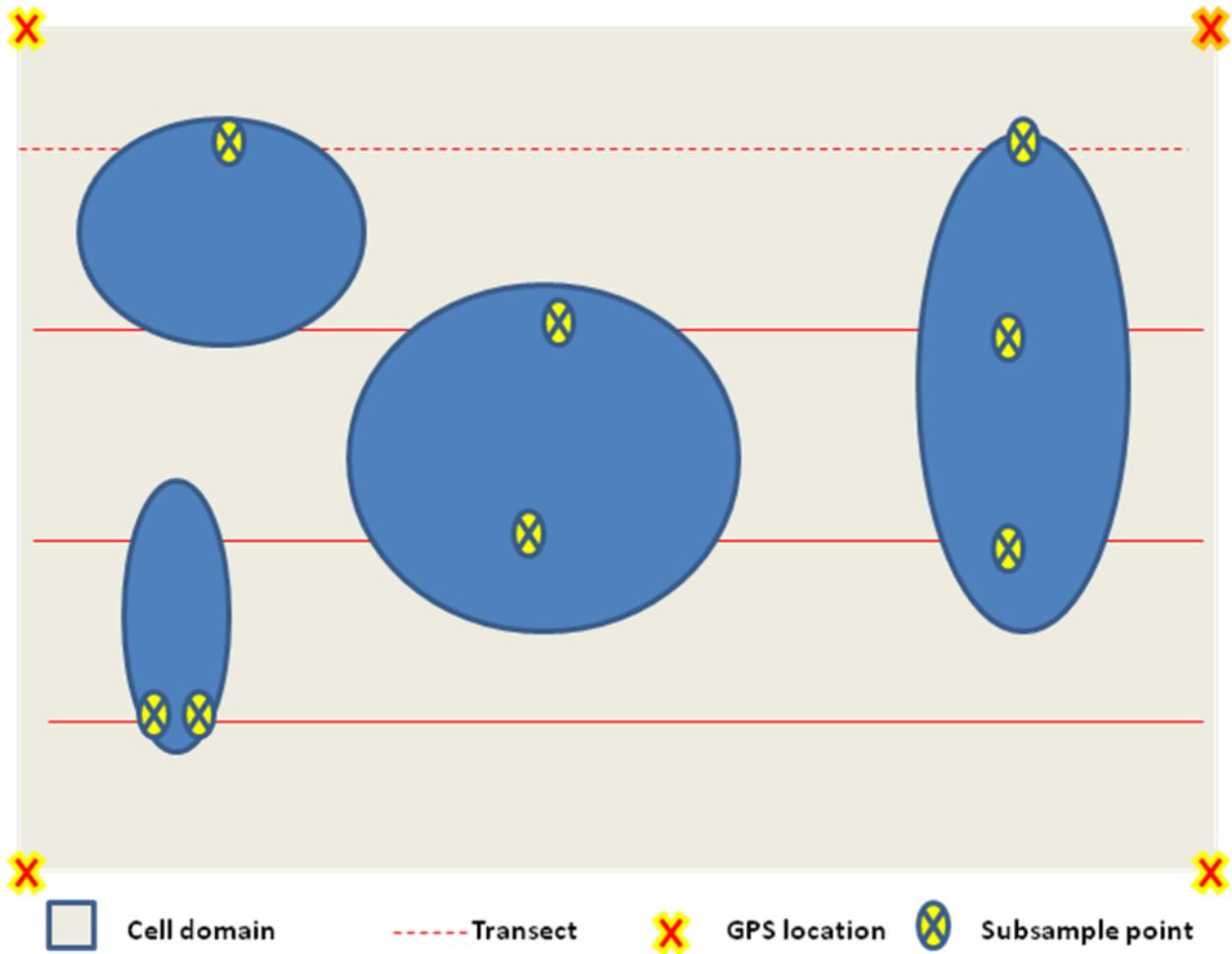
### (3) Tier 2 Stratum C

For Tier 2 Stratum C sites (intertidal or shallow subtidal reefs (0 – 1.0 m)), the area should be mapped visually with the aid of a GPS. Four transects will be run across the cell (uniformly spaced with a random starting point for the first transect, a random number between 0 and 50 should be selected prior to the start of the cruise). A 200 m tape shall be unraveled and secured with PVC pole at boat level across the cell (four corners of each cell will be provided for the field crew). Visually inspecting if conditions allow or pulling a chain (1/4 inch galvanized links)/dragging a long bamboo pole (1/2 inch diameter) in turbid waters over the bottom along the tape, allowing the field scientist to feel or hear the ‘tickle’ of the chain on oyster habitat (Figure 9). The start and end position of oyster reef habitat should be recorded on the data sheet for each of the four transects including potentially intertidal and subtidal habitat. Positions should be recorded as distance from the transect start (based on the tape read) or end. If transects produce no positive segments, then no further subsampling will be conducted in the cell. The field party chief should complete the site survey form (**Oyster Reef Sample Form** - see Appendix A) and move on to the next cell. If reef material or other oyster habitat is intercepted by one of the line transects, the cell will be subsampled. The field team leader should complete the site survey form and move to the next metric to be measured according to the SOPs.

Additional cells may be selected from the GRTS list for future sampling and to provide additional data for estimation of the acreage of oyster habitat and to provide data for modeling

the effects (if any) of the MC 252 oiling. This assessment will be done after sufficient information is collected on the location of oiling along the Gulf Coast.

Subsample points will be located along the line segments of transects that intercept reefs, and other potential oyster habitat (see Figure 12). An attempt will be made to obtain 2 subsamples for density and size-class measurements along these line intercepts. The oyster habitat studies will utilize a  $\frac{1}{4}$  m<sup>2</sup> quadrat (0.5 m x 0.5 m) made of  $\frac{1}{2}$  diameter PVC lengths (See SOP in Section B). If there are more than two locations where a transect intersects oyster habitat in either subtidal or intertidal habitat, then randomly select two segments, e.g., using numbers written on poker chips, selecting two at random. Determine the mid-points of segments selected, and implement the standard operating procedures for sample and data collection. If there is only one location along a transect that intercepts a reef, then divide the segment into two equal length sections, and sample one quadrat on each side. When sampling intertidal habitat, quadrats will be placed with one side directly along the transect and randomly located along the length of the transect by randomly selecting a number between the start and endpoints of the reefs.



**Figure 12.** Hypothetical grid cell with four reefs intersected by 4 transect lines. Eight subsamples are collected along the transects.

## B. Juvenile and Adult Oysters (Settled Life Stages)

### Field Sampling

Samplers should complete the **Oyster Reef Sample Form** (Appendix C). A unique sample code or number should be given to each sample and prominently marked in the upper right corner according to the Oyster Sample Naming Convention (see Appendix A). Sample codes should be recorded in the **Oyster Reef Sample Form** datasheet (see Appendix C) and also in the **NRDA Sample Collection Form – Tissue/Wrack** (available on the case FTP site).

#### 1. Site Description

- Measure / Record:
  - o Site name (general geographic location or established sampling area)
  - o Cell number
  - o Transect number
  - o Time of day and date.
  - o Tidal depth (intertidal or subtidal)
    - If subtidal, estimate the depth at the time of sampling.
  - o Describe reef conditions – recent harvest, oiling, covered in mud, fouled, etc.

#### 2. Physical/Chemical Parameters

- Measure and record:
  - o Bottom and surface salinity
  - o Bottom and surface water temperature
  - o Bottom dissolved oxygen (at subtidal sites only)
  - o Ambient air temperature
  - o Weather conditions
  - o Oiled condition (None, Sheen, Scattered Deposits, Surface substantially covered, Surface completely covered or Deep Deposits).

#### 3. Site (cell) corners

- Record the GPS coordinates of each corner of the sample gridcell, as well as the coordinates of the center point of the cell

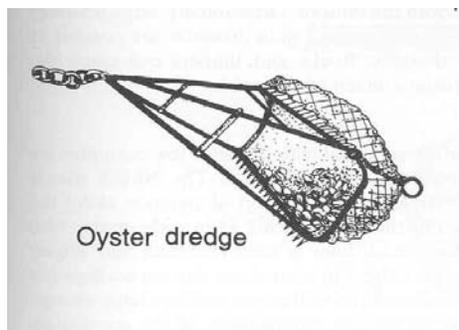
After completing the above steps, move on to oyster sampling within the gridcell in accordance with the sampling methods below.

#### 4. Oyster Sampling

Indicate whether the sampling used quadrat based sampling (record size) or dredges

- For harvesting via SCUBA / quadrats
  - o Determine coordinates via GPS. Ensure that they fall atop reefs. Confirm that you are above the top of the reef using a pole if necessary.

- Place PVC frame directly at arm's length at a random spot at the coordinates. Do not favor abundant areas.
  - Using tools when applicable, harvest all oysters 3-4 cm down into the reef. You should not have to dig into the mud.
  - Place animals in a burlap sack.
  - Gently agitate the sack to remove excessive mud or debris.
  - Close sack.
  - Return to surface and hand sack to team member.
  - Place the burlap sack in plastic bag.
  - Samples should be tagged with an external (flagging tape with permanent marker) and internal tag that prominently denotes sample code.
  - The sample code should be constructed of the location ID, date, matrix, sample team number, and sample number along with information regarding sample type (for details, see the Oyster Sample ID Naming Convention, Appendix XX).
  - Hold animals on ice until delivered to intake team.
- For dredge harvesting
- A standard commercial oyster dredge with 1.4 m tooth bar, 10 cm tooth depth, and 5 cm diameter bag rings should be used.
  - Deploy dredge from the beam of the vessel.
  - Drag dredge across the surface of the substrate for 30 seconds, and target 25 M transects.
  - Record exact start and stop positions using a GPS.
  - Collect up to replicate dredge samples at sites chosen for quadrat sampling.
  - Place animals in a burlap sack.
  - Gently agitate the sack to remove excessive mud or debris.
  - Close sack.
  - Place the burlap sack in plastic bag.
  - Samples should be tagged with an external (flagging tape with permanent marker) and internal tag that prominently denotes sample code.
  - The sample code should be constructed of the location ID, date, matrix, sample team number, and sample number along with information regarding sample type (for details, see the Oyster Sample ID Naming Convention, Appendix XX).
  - Hold animals on ice until delivered to intake team.



### *5. Larvae Sampling*

See separate “SOP for Larval Concentrations”

### *6. Larval Settlement*

See separate “SOP for Larval Settlement”

### *7. Photographs*

See **NRDA Field Photography Guidance** (NRDA\_Field\_Photography\_Guidance.doc, available on the case FTP site) for camera preparation and set-up prior to going into the field. –

- Photograph the operating GPS screen showing the date and time to synchronize the photos with the GPS track.
- Photograph site to describe oiling conditions.
- Collect a close-up photo of the reef showing individual oysters
- Photograph the entire reef.
  
- **DO NOT DELETE ANY PHOTOS**
  
- Document the pictures taken on the Oyster Reef Sampling Form
- Additionally, complete the NOAA NRDA Trustees Sampler Photo Logger form

### *8. Collection and Disposition*

The individual who collected the sample should be noted on the field data form. If more than one person is involved, list the field party leader and the person who entered the data (if different). The final disposition of the sample should also be noted with an explanation of the amount of oysters retained for further analyses and the type of analyses (e.g., disease, histological analyses, contaminant). SOPs for these additional analyses are given below. For each replicate quadrat, a sediment sample should be collected from within the reef matrix or within 5 m of the reef (please see separate SOP describing how the sediment sample should be collected). Samples for tissue concentrations should also be collected and can consist of the same sample used to gather biological data (i.e., length frequency, etc.) if proper handling procedures (i.e. wrap oysters individually in aluminum foil, double bag, place on ice, etc.) are followed during and after sample processing.

### **Lab Processing**

#### *9. Sample Processing: Abundance*

Samples will be brought to a non-field location for processing. Samples should be kept in seawater or in a cooler with ice. Samples should be processed within 12-24 hours to ensure accurate characterization of live and dead oysters.

Regardless of sample method, both live and dead oysters should be enumerated by size category.

- Classify oysters by size:
  - o spat (less than 1 inch [25 mm] shell height),
  - o seed or juvenile oysters (between 1 and 3 inches [25 – 75 mm]),
  - o market size or “legal” oysters (> 3 inches [75 mm] shell height).
  
- Measure shell height (SH).
  - o Use calipers to measure the distance from the umbo (small tapered end of the oyster) to the maximum limit of the shell.
  - o Measure dead oysters in the same way.
    - Dead oysters are oysters that have no living tissue but are still in their articulated form (i.e., the shells are still hinged but no living oyster tissues is present also called “boxes”). These oysters will often appear opened or “gaped”.
  
- Identify and enumerate associated biota.
  - o Identifications and counts should be entered on separate lines under the “Other Species” category on the Oyster Reef Sample Form. Fauna will be preserved for potential future analysis.

#### 10. Sample Processing: Biomass

- Weigh living material:
  - o Separate live material (animal) from shell.
  - o Weighed in aggregate by size category.
  - o Similarly, dead oysters should be weighed by category. Finally, associated species should be identified and weighed by taxon.
  - o What is done in the case of a dead oyster still with tissue?

#### *Equipment List*

- Random number table
- 2 PVC quadrats, dredge
- 3 sets of calipers
- 2 10-m long field measuring tapes (or laser range finder)
- Spring scales (0-10g, 10-100g, 100 – 1000g, and 0-10 kg)
- Large 1 gallon Ziploc bags to separate subsamples for further analyses.
- Digital camera with extra batteries

- GPS with extra batteries
- Nitrile gloves (size M and L)
- Small shovel / tool for separating oysters
- Waterproof data sheets (chain-of-custody, sample tracking, photo log, oyster reef sample form)
- Waterproof labels or tags
- Waterproof pens
- Flagging tape for external tags
- Onion bags or burlap sacks for sample storage.
- Plastic contractor-grade construction bags
- YSI multimeter for DO, salinity

## C. SOP for Larval Concentrations

### 1. Objectives:

- (a) Determine presence/absence of oyster larvae in the water column at each site.
- (b) Quantify the abundance of bivalve veliger larvae (Fig. 2) in the water column.

### 2. Sample collection

(a) Collect fixed volume of ambient water. A water sampler should be used with a fixed volume that can be remotely triggered and samples at least 1 L of water (Niskin or LaMotte water samplers); alternatively, samples can be collected by divers, if present.. Five total 1L samples should be collected - two surface, one mid-depth and one near bottom.

#### (b) Field Procedure

i. Lower the sampler into the water column and allow to remain in place for 1 minute. The device should then be triggered to close. Record the calibration time in the field book.

iv. Collect identical samples and label it for larvae counts.

v. Move the boat a random distance within the 200 x 200 m grid cell. This can be accomplished by choosing a random number between 1 and 200 from a random number table.

#### (c) Intake Laboratory Procedure

Prior to shipment to a laboratory for quantification, the samples should be filtered and preserved within XX hours of collection, using the following steps:

i. Pour the sample from the 50 micron sieve and use the squirt bottle full of filtered water to rinse down contents of sieve into labeled 50 mL Falcon tube. This sample should be labeled for DNA analysis.

ii. Add 70% ethanol. Even better is saline/ethanol – add to about 20 ppt. – when the plankton lyse, some DNA is lost to solution and is harder to recover, but also other species interferes with desired DNA.

### 3. Laboratory analysis

(a) Lab (Larval Probe Analysis). Because oyster larvae are impossible to distinguish morphologically from other bivalve larvae during analysis, the sample should be shipped to a lab capable of DNA based identification of *Crassostrea virginica*.

(b) Lab counts of bivalve larvae. DNA analysis confirms the presence of *C. virginica* but may not provide a quantification of larval concentrations. Thus, traditional counts of bivalve larvae should be performed (Figure 14).



Figure 14. Oyster bivalve larvae under 20x magnification.

- i. The sample should be poured into a large calibrated beaker and raised to 200 ml volume with distilled water. The exact volume of sample should be recorded.
- ii. A Stimpel pipette should be used to collect a 5 or 10 ml aliquot of the sample. Stir the beaker vigorously prior to collecting the aliquot.
- iii. Place the aliquot in a clear petri dish that is subdivided into four chambers.
- iv. Bivalve larvae in each section should be quantified and recorded on the data sheet. Entire sample should be examine under a dissecting scope at 50x magnification.
- v. Extrapolate the subsample measurement to the entire volume of the samples. Divide total volume of sample analyzed in the lab by subsample volume and multiply subsample count by that number. For example  $200\text{ml}/5\text{ ml} = 40$ ,  $40 * 6\text{ bivalve veligers} = 240$  bivalves per 5L (original sample volume) or 48 bivalve larvae per L.
- vi. Return the content of the petri dish to the original sample and repeat the aliquot procedure two additional times.
- vii. Average the three measurements and record the average on the data sheet.

#### Field Materials Needed

- Water sampler (5 L volume)
- 50 micron sieve
- 30 x 50mL Falcon tubes
- Squirt bottles
- Field book
- 70% Ethanol

## D. SOP for Larval Settlement

### *Site Selection for Oyster Larval Surveys*

Oyster larvae will be sampled and collected in each sampled cell during quadrat subsampling for abundance and size class frequency.

### *Spat Sampling Methods*

Spat settlement. A  $\frac{1}{4}$  m<sup>2</sup> quadrat (0.5 m x 0.5m) made of cement board or other appropriate material will be placed at each subsample location within each site. These boards will be collected at a future point in time to help evaluate settlement rates of spat.

#### 1. Objectives

Quantify settlement and early survivorship (recruitment) of oyster spat.

#### 2. Materials needed

Standardize plates can be made from concrete backer board or tiles. Cut plates in 12 x 12 cm squares using a low speed saw. The inner 100 cm<sup>2</sup> will be used to enumerate settlers.

Three settlement plates should be connected to a crab trap via cable ties (4 small  $\frac{1}{2}$  inch holes should be pre-drilled into the corners). (Figure 15).

Plates should be attached to the top and a surface buoy attached.

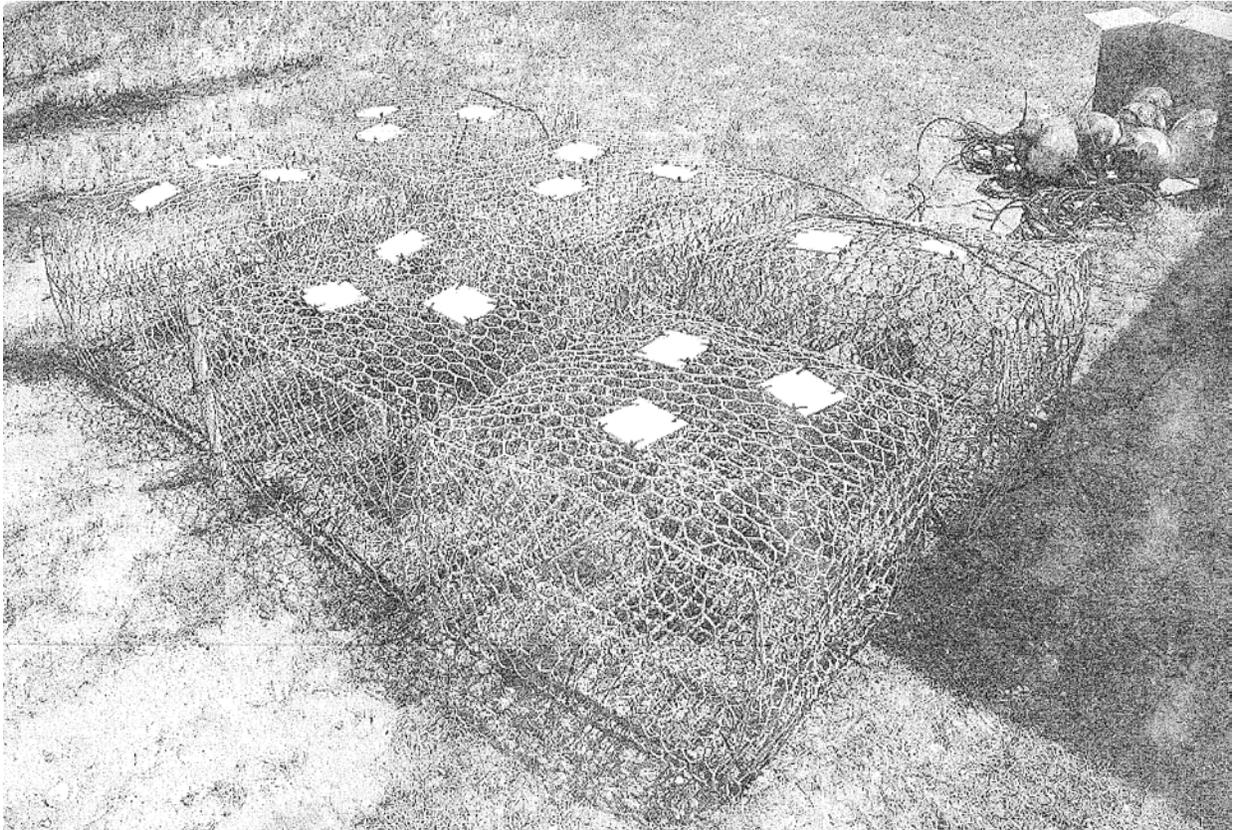
Record exact GPS position of deployment.

#### 3. Field procedures

- i. Two sets of three spat settlement plates should be placed at each site (cell) in the event that one set is lost during the deployment period.
- ii. Plates should be removed and replaced every two to three weeks.
- iii. Plates should be deployed on a crab trap near oyster substrate in a horizontal position.
- iv. Retrieved plates can be stored on ice and taken to the intake laboratory.

#### 4. Lab procedures

1. Plates should be preserved in ethanol after 48 hours at the intake laboratory.
2. Oysters on plates should be enumerated under 10x magnification and both live spats and spat scar (predated spat) should be enumerated.



**Figure 15.** Settlement plates attached to crab pot or trap. Photo courtesy of Jason Herrmann, AMRD.

#### E. SOP for Gonadal Condition

When histological examination is not possible, gonadal condition should be determined.

1. Objectives.

Determine the reproductive condition of oysters at each sampling site. These data can then be compared with larval supply and settlement data to determine potential impact of oil contamination on recruitment of oysters.

2. Field procedures.

- i. Collect 10 market-sized oysters (>74mm) from each site for determination of condition index (CI), gonadal index (GI) and sex. If 10 oysters are not available from quadrat sampling, collect additional animals via SCUBA or dredge sampling.
- ii. Place oyster in a pre-labeled bag and place in a cooler with ice.

3. Lab procedures (within 24 hours)

- i. Select 10 market-sized oysters from the sample, and wash, scrap and scrub to remove mud and attached biota.
- ii. Measure (to the nearest mm) the length (umbo-to-bill) of each oyster.
- iii. Remove and retain the right valve.
- iv. Measure (to the nearest 0.1 mm) adductor muscle length with Vernier calipers.
- v. Detach the left valve from the adductor muscle, and combine with the right valve; matched valves are blotted dry and weighed.
- vi. Blot and weigh (to the nearest 0.1 g) oyster meat to obtain wet weight.
- vii. Bisect the oyster, measure (to nearest 0.1 mm using Vernier calipers) the width of the gonad and blot gonadal material onto the slide for determination of sex. (As a response to stress, oysters may resorb gonadal material or females may revert to the energetically less demanding life of the male.)
- viii. CI is determined as the (blotted) wet weight of the oyster meat divided by (blotted) shell weight.
- ix. GI index is measured as the width of the gonad, standardized by dividing gonadal width by adductor muscle length.
- x. Sex is determined by bisecting the oyster at the plane of the gills and labial palps, and blotting gonadal material on a glass slide for microscopic examination (Soniati and Ray,

1985). Sex is determined as male (motile sperm), female (eggs), undifferentiated (unknown), and both, or hermaphroditic, and expressed as a population statistic, percent female.

These laboratory techniques are non-destructive to the oyster tissue and are potentially available to collaborative studies which measure the hydrocarbon concentration of oyster meats. The objective of this research is to assess differences between impacted and un-impacted sites in recruitment, size-specific mortality, percent female, and oyster condition (CI) and reproductive state (GI).

**F. SOP for Tissue Collection for Contaminant Analyses (Based on Florida SERT Natural Resource Damage Assessment and NOAA Mussel watch protocols).**

**Use NRDA Tissue wrack\_sample\_form\_5.15.10.pdf and NRDA Chain of Custody form\_5.15.10.**

1. Sampling Objectives

- (a) To document extent and duration of the area exposed to the spilled material. Bivalves uptake oil quickly, depurate them slowly, and can be used as “composite” samplers.
- (b) To maintain the integrity the sample(s) during sampling, transport, and storage.

2. Sample Size and pre-sampling activity

- (a) 30 g wet weight (composite of ~20 individual organisms) obtained from the quadrat sample.
- (b) Clean dredges, knives, etc. between samples. If no oil is visible wash in ambient water. If the equipment was obviously contaminated, rinse with Alconox solution. Collect rinsate for proper disposal.
- (c) Take relevant photos at all sites before sampling.

3. Sample Collection Methods

- (b) Collect primarily live animals (shells intact and tightly closed). Attached organisms are pried away from the substrate with a knife, trowel, etc. Infaunal samples should be rinsed with clean site water to remove sediment. Note the condition of dead animals if collected.
- (c) The sampler handling the shellfish should 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.
- (d) Composite samples are recommended to provide enough sample weight to meet detection limit objectives and to average out the variations at a location among individual organisms.
- (e) Individuals should be the same shell (or body) size. Record size range collected or save shells for later measurement. Same size is not as important if only for fingerprinting.
- (f) Shellfish should not be opened in the field to minimize the risk of contamination. Rather, sets of whole organisms are wrapped together in clean aluminum foil.
- (g) Place all individuals of the same species from a site in a certified-clean glass jar (without foil) or double Ziploc bags (with foil).

(h) For bags, the inner bag is labeled with marker pen and a waterproof sample label placed between the two bags. Jars are labeled on an adhesive label and directly on the lid. Use clear tape to protect the label.

(i) 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.

(j) If possible, sample least-oiled areas first, followed by the more contaminated areas to minimize risk of cross-contamination. Avoid sampling from creosoted pilings.

(k) Immediately place all samples in coolers on ice. Ship samples to the laboratory as soon as possible; samples should be received by the lab for processing or freezing within 7 days of collection. If holding samples for several days is unavoidable, samples may be stored frozen before shipping to the laboratory. Consult with [REDACTED] for specific instructions; special shipping will be required to maintain samples in a frozen state until received by the lab.

(l) Use packing material around sample containers to prevent breakage during handling and shipping.

#### 4. Preservation/Holding Times

Immediately place all samples in cooler and keep at 4°C. Freeze as soon as possible.

Please see the Analytical Quality Assurance Plan for the MS Canyon 252 (Deepwater Horizon) Natural Resource Damage Assessment (QAP) for further details on storage and holding times.

#### 5. Labeling, Documentation, and Other Considerations.

(a) On the FTP site, the NRDA Field Sampling Checklist generically summarizes pre- and post-field sampling tasks.

(b) Prepare sample labels as presented in NRDA Data Management Protocol for Field Sampling. If using jars, record the sample number on both the label and lid. IDs on sample labels must be complete and identical to IDs on the chain of custody. Jar labels receive a protective layer of clear tape wrapped around the entire circumference of the container to secure the label and protect the writing.

(c) See the event-specific protocol documents for shipping to designated labs (NRDA Sample Shipping Instructions) and for chain of custody and sampling documentation instructions (NRDA Data Management Protocol for Field Sampling). Tissue sampling log sheets typically record sample number; date/time, location, GPS coordinates, species and tissue type.

(d) Documentation is critical; all field notebooks should be dated, signed, and preserved. If crossing out or correcting any entries, date and initial when making the changes. Original records will be gathered and archived.

(e) Record the presence of oil, weather conditions, etc. in field notes. Record GPS coordinates for each sample.

(f) Take relevant photographs of the sampling locations and sample collection itself if possible. Make sure each photograph or series can later be associated with the corresponding sampling location GPS (see NRDA Field Photography Guidance). Do not delete, open or alter any photos.

(g) All sampling, COC, shipping, GPS and photo files are submitted to [REDACTED] Sampling hotline: [REDACTED]

(h) The labs have received instructions specifying sample processing and analytic methods.

## 6. Analytical Methods

The collected tissue samples should be analyzed in accordance with the MS Canyon 252 QAP. Specific suites of analytes to be measured include:

- Polynuclear Aromatic Hydrocarbons (PAH), including both standard and alkylated PAHs – see full list in Table 1.1a of the QAP, which also specifies the target method detection limits.
- Saturated Hydrocarbons (SHC or AHC). These compounds comprise a major component of crude oils. In fresh oil, they serve as another line of source confirmation. But being straight chain molecules, they are also a preferred carbon source for oil-degrading microbes. As such, they tend to disappear faster than PAHs but do provide information of the weathering state of the oil. Sample prep may require extra steps to remove lipids which may interfere with the analysis.
- Lipid content. Lipid content is defined as the percent of sample tissue extracted and remaining after solvent evaporation. It is used to normalize organic contaminants in tissues, to aid in spatial and temporal comparisons among samples.

### *Equipment List*

- Shovels and/or trowel
- Knife
- Dredges
- Tongs

- Gloves
- Screen (for sieving out sediment)
- Aluminum foil
- Certified-clean glass jars
- Ziploc bags
- Cooler and ice
- Marker pen
- Waterproof sample labels
- Clear tape

PLEASE NOTE: Avoid sources of contamination such as exhaust fumes and engine cooling systems on vessels. Work up-wind of any exhausts. Segregate dirty/clean areas. Lay out clean substrates to work on and replace frequently.

#### Key References

NOAA, 1993. Sampling and analytical methods of the National Status and Trends Program, National Benthic Surveillance and Mussel Watch Projects, 1984-1992. Volumes I-IV, Comprehensive descriptions of trace organic analytical methods. Lauenstein, G.G. and A.Y. Cantillo (eds.). NOAA Tech. Memo NOS ORCA 71, Silver Spring, MD.

**G. SOP for Sediment Collection for Contaminant Analysis (Based on Florida SERT Natural Resource Damage Assessment and NOAA Mussel watch protocols). [The SOP as described below is for the Preassessment Plan. Samples taken during the Phase I Plan were done by LDEQ staff and using LDEQ protocols.]**

**Use NRDA Sample Collection–form-Soil-Sed\_5.15.10.pdf and NRDA Chain of Custody form\_5.15.10.**

1. Sampling Objectives

- (a) To determine the concentration and source of oil compounds in sediments.
- (b) To measure sediment characteristics for interpreting chemical and biological results.

2. Sample Volume by Analytical Method

For hydrocarbons and TOC (combined), a single sample may be placed in two 8 oz glass jars filled  $\frac{3}{4}$ , or in one 16 oz glass jar, filled  $\frac{3}{4}$ .

For grain size, 100 g in a resealable (e.g., Ziploc or Whirlpak) bag or 4 oz jar.

3. Subtidal Sediment Collection Methods

- (a) Sediment should be collected from within or near (within 5m) of the reef.
- (b) All non-disposable sampling gear must be decontaminated before using and between sampling stations. Wash with laboratory-grade detergent and then rinse with clean water. If taking multiple samples at an oiled station, decontaminate sampling equipment between samples.
- (c) When surface slicks are present, avoid contaminating the sampler. Methods to open a sheen may include using a deck hose, disrupting the surface tension with literally one or two drops of kitchen detergent, or swiping with a sorbent pad. Thicker slicks may require deploying the sampler through a floating circle or sorbent boom (deploy collapsed, open on the water surface; use a drop of detergent if an internal sheen persists).
- (d) Lower and retrieve the sampling device at a controlled speed of ~1 foot per second. The device should contact the bottom gently; only its weight or piston mechanism should be used to penetrate the sediment. It is important to minimize disturbance to the surface floc which is likely to contain the oil contaminants.
- (e) On retrieval, inspect the sample to make sure that it meets the following criteria:
  - the sampler is not overfilled; the sediment surface is not pressed against the sampler top.
  - overlying water is present, indicating minimal leakage and subsequent loss of floc.
  - sediment surface is undisturbed, indicating lack of channeling or sample washout.

- the desired penetration depth is achieved (e.g., 4-5 cm for a 2 cm sample).

(f) Siphon or drain off the overlying water in the sampler until the sediment is exposed, paying special attention to retain the surface floc.

(g) Wearing nitrile or other non-contaminating gloves and using any appropriate clean scoop, meticulously collect just the top layer (2 cm), avoiding sediments in contact with the sides or top of the sampler. To avoid cross-contamination, use a clean scoop for each sample.

(h) Onboard a sampling vessel, be aware of contamination sources (exhaust fumes, engine cooling systems, oily surfaces). Work up-wind of any exhausts. Segregate dirty/clean areas. Lay out clean substrates to work on and replace frequently. On each trip, try to sample least-oiled areas first, then the most contaminated areas.

(i) Immediately place all sediment samples in a cooler and keep on ice. Grain size samples should only be refrigerated; hydrocarbon samples can be frozen. Samples should be shipped or delivered to a Sample Intake Center within 2 days.

#### 4. Intertidal Sediment Collection Methods

(a) Sediment should be collected from within or near (within 5m) of the reef.

(b) Photograph the site before sampling.

(c) Wearing nitrile or other non-contaminating gloves and using an appropriate clean utensil (disposable or non-disposable), scoop surface sediments into the sampling jar.

(d) If subsurface samples are required, the shovel or coring device will need decontamination both between stations and between oiled samples. Wash with laboratory-grade detergent and then rinse well with clean water.

(e) Immediately place all sediment samples in a cooler and keep on ice. Grain size samples should only be refrigerated; hydrocarbon samples can be frozen. Samples should be shipped or delivered to a Sample Intake Center within 2 days.

#### 4. Preservation/Holding Times

Immediately place all sediment samples in a cooler and keep at 4°C . Freeze samples for chemical analysis by the end of each day. Refrigerate samples for TOC and grain size (do not freeze). Samples should be shipped or delivered to a Sample Intake Center within 2 days.

Please see the Analytical Quality Assurance Plan for the MS Canyon 252 (Deepwater Horizon) Natural Resource Damage Assessment (QAP) for further details on storage and holding times.

## 5. Labeling, Documentation, and Other Considerations.

(a) On the FTP site, the NRDA Field Sampling Checklist generically summarizes pre- and post-field sampling tasks.

(b) Prepare sample labels as presented in NRDA Data Management Protocol for Field Sampling. If using jars, record the sample number on both the label and lid. IDs on sample labels must be complete and identical to IDs on the chain of custody. Jar labels receive a protective layer of clear tape wrapped around the entire circumference of the container to secure the label and protect the writing. For grain size samples, place a sturdy paper label in indelible ink into the bag and repeat the label on the outside

(c) See the event-specific protocol documents for shipping to designated labs (NRDA Sample Shipping Instructions) and for chain of custody and sampling documentation instructions (NRDA Data Management Protocol for Field Sampling). When and where possible, the Sample Intake Centers should be used to ensure compliance and sample integrity. Sediment sampling log sheets typically record sample number; date/time, location, GPS coordinates, water depth and penetration depth. They may also include surface sediment characteristics: texture, color, biota, debris, sheens, odor, etc.

(d) Documentation is critical; all field notebooks should be dated, signed, and preserved. If crossing out or correcting any entries, date and initial when making the changes. Original records will be gathered and archived.

(e) Record the presence of oil, weather conditions, etc. in field notes. Record GPS coordinates for each sample.

(f) Take relevant photographs of the sampling locations and sample collection itself if possible. Make sure each photograph or series can later be associated with the corresponding sampling location GPS (see NRDA Field Photography Guidance). Do not delete, open or alter any photos.

(g) All sampling, COC, shipping, GPS and photo files are submitted to [REDACTED] Sampling hotline: [REDACTED]

(h) The labs have received instructions specifying sample processing and analytic methods.

## 6. Analytical Methods

The collected sediment samples should be analyzed in accordance with the MS Canyon 252 QAP. Specific suites of analytes to be measured include:

- Polynuclear aromatic hydrocarbons (PAH), including both standard and alkylated PAHs – see full list in Table 1.1a of the QAP, which also specifies the target method detection limits.

- Saturated hydrocarbons (SHC or AHC) - see full list in Table 1.1b of the QAP, which also specifies the target method detection limits. These compounds comprise a major component of crude oils. In fresh oil, they serve as another line of source confirmation. But being straight chain molecules, they are also a preferred carbon source for oil-degrading microbes. As such, they tend to disappear faster than PAHs but do provide information of the weathering state of the oil. Sample prep may require extra steps to remove lipids which may interfere with the analysis.
- Biomarkers (S/T). Sterane/triterpane biomarkers are “fossil” compounds unique to the oil formation that are very resistant to weathering, persisting for decades after some events. These compounds provide a secondary and confirming line of evidence in forensic oil identification.

#### *Equipment List*

- Ponar, modified van Veen grab; Ekman grab; or box dredge.
- Shovel (for intertidal zones)
- Coring device (for intertidal zones)
- Scoops
- Certified-clean glass container with Teflon-lined lid
- Ziploc or Whirl-Pak bags
- Dredges
- Tongs
- Gloves
- Screen (for sieving out sediment)
- Detergent
- Sorbent pad and/or boom
- Cooler and ice

## H. SOP for Oyster Disease Analysis

### Dermo Technique

- Use 10 commercial-size oysters (>75mm)
- Measure shell height (umbo-to-bill distance) to the nearest mm
- Remove the right valve
- Remove a piece of mantle tissue (~6mm<sup>2</sup>) from the right side of the oyster at the anterior margin of the mantle just posterior to the labial palps
- Fortify each tube of fluid thioglycollate (FT) medium (FTM) with 200 units of mycostatin (nystatin) and 200 micrograms of chloromycetin (chloramphenicol) just prior to use (see below for medium preparation)
- Place the tissue in a tube of FTM
- Incubate in the dark at room temperature for a week
- Place the tissue on a glass slide and add 3 drops of diluted Lugol's solution. Flatten the tissue with a blunt probe to get a thin, well-stained preparation. Press a cover slip firmly over the tissue to flatten it more. Remove excess Lugol's with absorbent paper
- Examine stained tissue microscopically at 25X and 100X for brown, blue or black spheres (Ray 1966)
- Rate the level of infection as a disease code number according to the criteria of Craig et al. (1989), where 0 is uninfected and 5 is heavily infected
- Calculate percent infection (PI), weighted prevalence (WP) and infection intensity (II) as:

$$PI = (\text{number of infected oysters} / \text{number of oysters tested}) \times 100$$

$$WP = \text{sum of disease code numbers} / \text{number of oysters tested}$$

$$II = \text{sum of disease code numbers} / \text{number of infected oysters}$$

### Medium preparation

- Rehydrate 29 grams of FTM with 1 liter of distilled water containing 20 grams of NaCl
- Dispense rehydrated medium in 10ml volumes into glass culture tubes and autoclave
- Store sterile tubes of medium in the dark at room temperature until needed

## Literature Cited

Allen, Y.C., C.A. Wilson, H.H. Roberts and J. Supan. 2005. High Resolution Mapping and Classification of Oyster Habitats in Nearshore Louisiana Using Sidescan Sonar. *Estuaries* 28:435-446.

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## **FORMS**

**Appendix A: Oyster Sampling Naming Convention**

**Appendix B: Oyster Habitat site survey Form (complete for each cell or site)**

**Appendix C: Oyster Habitat Sample Form (complete for each subsample, n = 8 per site)**

**Appendix D: Oyster Larvae Sample Form (n =1 per site)**

**Appendix E: Oyster Settlement (n = 1 per site).**

## DRAFT OYSTER SAMPLE ID NAMING CONVENTION

### NOAA NRDA Sample Format:

- **LocationCode – DateCode - Matrix SamplerTeam# Sample#**
  - 6-digit Location code (from maps located on FTP site. These should be the NRDA Grid location code rather than the SCAT location code);
  - 5-digit date: year letter and mmdd (A=2010, B=2011);
  - Matrix letter (T = Tissue);
  - 2 or 3-digit sample team #; and
  - 2-digit sample number.
  
- **EXAMPLE: LAAM24-A0502-T6002**
  - LocationCode = LAAM24;
  - Date = 5/2/2010;
  - Matrix = Tissue;
  - Sample Team # = 60;
  - Sample # = 02

### Additional Information for Oysters:

#### Field Teams

- We will be numbering each sample sequentially. This information will go in the “Sample #” section at the end of the NOAA NRDA required tag. In addition, because all sample types are tissue (i.e., put in “T” for matrix type for all samples), we will add an identifier after the sample number that will indicate the sample type.
  - Q = quadrat sample;
  - L = larval sample; and
  - SP = settlement plate.
  - Examples: **LAAM24-A0502-T6002Q; LAAM24-A0502-T6003L**
  
- All additional information describing the samples will be recorded in the “Sample Notes” field of the NOAA NRDA sample collection forms (see OysterExamples.xls). This additional information differs by sample type.

- Quadrat oysters
  - Cell number (1-12)
  - Type of Cell (Subtidal or Intertidal)
  - Quadrat Number (Quadrat 1-Quadrat 8)
- Larval Samples
  - Cell number (1-12)
  - Type of Cell (Subtidal or Intertidal)
  - Depth (Upper, Middle, or Lower)
- Settlement Plates
  - Cell number (1-12)
  - Type of Cell (Subtidal or Intertidal)

### **Lab Teams**

- **Quadrat subsamples**

- Contaminant subsample
  - Randomly array the quadrats from a cell and select quadrats in this order until four quadrats have been sampled. If insufficient number of oysters in a quadrat, select the next one in order. Make note of any quadrats examined, but not used.
  - Keep original sample name for that quadrat and add “-CT”, e.g., **LAAM24-A0502-T6002Q-CT**
  - Composite across quadrats if necessary (i.e., the cell does not have four quadrats with sufficient number of oysters). In this case, assign the sample the next sequential sample number and indicate in the “Sample Notes” which quadrats the sample is taken from. Also note that this is a composite sample. The “-CT” should still be added to the end of the sample name. GPS coordinates should correspond to the entire cell, rather than a specific quadrat.
- Gonad/Disease subsample
  - Assign the sample the next sequential sample number and indicate in the “Sample Notes” which quadrats the sample came from. Also indicate that

it is a composite. The sample should also have “-GD” at the end, e.g., **LAAM24-A0502-T6002Q-GD**. GPS coordinates should correspond to entire cell, rather than a specific quadrat.

- **Larval samples**

- Retain same sample name; “Sample Notes” field of the NOAA NRDA sample collection forms should indicate which samples are intended for manual counts versus PCR.

Survey Team ID: \_\_\_\_\_

### Oyster Habitat Site Survey [*Print Double-Sided*]

*Please refer to the SOP for a detailed description of the requested metrics by section. One for should be used for each assigned site. Subsequent forms should be used for each metric and subsample*

#### 1. Site Descriptors

Site Name/Cell Number \_\_\_\_\_ Lat: \_\_\_\_\_ Lon: \_\_\_\_\_

Reference MAP # \_\_\_\_\_ and GRID \_\_\_\_\_

Time: \_\_\_\_\_ Date: \_\_\_\_\_ Subsample Number: \_\_\_\_\_

Habitat Setting (check one):  Intertidal  Subtidal (Depth: \_\_\_\_\_)

Overall Reef condition: \_\_\_\_\_

#### 2. Physical/Chemical Parameters

Bottom Salinity: \_\_\_\_\_ Air Temperature: \_\_\_\_\_ Bottom Temperature \_\_\_\_\_

Bottom Dissolved Oxygen: \_\_\_\_\_ Weather Conditions \_\_\_\_\_

Oiled Condition (check one):  none  Sheen  Scattered Deposits \_\_\_\_\_

Surface substantially covered  Surface completely covered \_\_\_\_\_ Deep Deposits

#### 3. Survey

a. Place PVC poles at the four corners of the grid and give GPS coordinates:

NW Corner. Lat \_\_\_\_\_ Long. \_\_\_\_\_

NE Corner. Lat \_\_\_\_\_ Long. \_\_\_\_\_

SW Corner. Lat \_\_\_\_\_ Long. \_\_\_\_\_

SE Corner. Lat \_\_\_\_\_ Long. \_\_\_\_\_

B. Side scan data (complete for subtidal sites)

Start time: \_\_\_\_\_ End Time: \_\_\_\_\_ # of transect painted: \_\_\_\_\_ Frequency: \_\_\_\_\_ hz

Data file names and locations: \_\_\_\_\_

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**B. For intertidal areas: Begin at the NW corner and choose a random number from 0 to 50 m. Proceed due S along the NW to SW line to that random distance down the line. Place a PVC pole at that point, and stretch a 200 m line tape due East and secure the other end of the tape to a PVC Pole. Using a bamboo pole or chain, “feel the bottom” for oyster substrate and mark on map. [Complete Back of Form]**

**C. Space the next 3 transects equal distance to the 200 m end point.**

**D. Identify 8 subsample locations (2 per transect for intertidal, 8 random for side-scan imagery)**

**Trans # ; Distance from origin**

**Indicate start and stop of oyster habitat in m**

1 \_\_\_\_\_ W \_\_\_\_\_ E

2 \_\_\_\_\_ W \_\_\_\_\_ E

3 \_\_\_\_\_ W \_\_\_\_\_ E

4 \_\_\_\_\_ W \_\_\_\_\_ E

**Identify 2 subsample location per transect.**

**Trans 1: A Lat \_\_\_\_\_ A Long. \_\_\_\_\_ B Lat \_\_\_\_\_ B Long. \_\_\_\_\_**

**Trans 2: A Lat \_\_\_\_\_ A Long. \_\_\_\_\_ B Lat \_\_\_\_\_ B Long. \_\_\_\_\_**

**Trans 3: A Lat \_\_\_\_\_ A Long. \_\_\_\_\_ B Lat \_\_\_\_\_ B Long. \_\_\_\_\_**

**Trans 4: A Lat \_\_\_\_\_ A Long. \_\_\_\_\_ B Lat \_\_\_\_\_ B Long. \_\_\_\_\_**

Survey Team ID: \_\_\_\_\_ Location Type: Tier 1, Tier 2 A, Tier 2 B, or Tier 2 C  
(circle one)

### Oyster Reef Sample Form [Print Double-Sided]

*This form is designed to be inclusive of all possible metrics that may be used in later assessments. Samplers should complete sections 1-4 and 7. If time allows section 5 should be completed in that order. If photographs can be taken please complete section 6. Please refer to the SOP for a detailed description of the requested metrics by section. One for should be used for each subsample (i.e. up to 8 form per assigned sampling cell)*

#### 1. Site Descriptors

Site Name/Cell Number \_\_\_\_\_ Lat: \_\_\_\_\_ Lon: \_\_\_\_\_

Reference MAP # \_\_\_\_\_ and GRID \_\_\_\_\_

Time: \_\_\_\_\_ Date: \_\_\_\_\_ Subsample Number: \_\_\_\_\_

Habitat Setting (check one):  Intertidal  Subtidal (Depth: \_\_\_\_\_)

Location of samples with respect to reef: \_\_\_\_\_

Overall Reef condition: \_\_\_\_\_

#### 2. Physical/Chemical Parameters

Bottom Salinity: \_\_\_\_\_ Air Temperature: \_\_\_\_\_ Bottom Temperature \_\_\_\_\_

Bottom Dissolved Oxygen: \_\_\_\_\_ Weather Conditions \_\_\_\_\_

Oiled Condition (check one):  none  Sheen  Scattered Deposits \_\_\_\_\_

Surface substantially covered  Surface completely covered \_\_\_\_\_ Deep Deposits

#### 3. Sampling Methods

Sampling Method (check one):  quadrat (size \_\_\_\_\_)

Dredge (size \_\_\_\_\_; tow duration \_\_\_\_\_)

#### 4. Sampling Results: Abundance

Abundance	< 25 mm SH (Spat)	26 – 74 mm SH (Seed)	> 75 mm (market size)
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Live Oysters			
Dead Oysters (Boxes)			
Other Species (List below)			

Recorder: \_\_\_\_\_

**5. Sampling Results: Biomass**

Wet Weight	< 25 mm SH (Spat)	25 – 75 mm SH (Seed)	> 75 mm (market size)
Live Oysters (shell & meat)			
Oyster Meat Only			
Dead Oyster Shell			
Other Species (List below)			

**6. Photo documentation:**

Close up photo (showing individual oysters): \_\_\_\_\_ *.jpg filename*

Aerial image (showing expanse of reef): \_\_\_\_\_ *.jpg filename*

**7. Collection and Disposition**

Collected by: \_\_\_\_\_

Field Crew Leader: \_\_\_\_\_

Data Entry: \_\_\_\_\_

(Name)

(Agency)

The following subsamples should be retained:

\_\_\_\_\_ Contaminant analyses (subsample ID \_\_\_\_\_) \_\_\_\_\_ # of oysters

*(20 large oysters should be collected)*

\_\_\_\_\_ Disease (Dermo) analyses (subsample ID \_\_\_\_\_) \_\_\_\_\_ # of oysters

*(15 large oysters should be selected)*

\_\_\_\_\_ Gonad analyses (subsample ID \_\_\_\_\_) \_\_\_\_\_ # of oysters

*(15 large oysters should be selected)*

\_\_\_\_\_ Sediment sample (Subsample ID \_\_\_\_\_)

*(two 8 oz glass jars should be filled to three quarters volume)*

Other (Please

Describe): \_\_\_\_\_

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Survey Team ID: \_\_\_\_\_

### Oyster Larvae Sample Form

*This form is designed to be inclusive of all possible metrics that may be used in later assessments. Please refer to the SOP for a detailed description of the requested metrics by section.*

#### 1. Site Descriptors

Site Name/or Cell ID: \_\_\_\_\_ Lat: \_\_\_\_\_ Lon: \_\_\_\_\_

MAP # \_\_\_\_\_ GRID # \_\_\_\_\_

Time: \_\_\_\_\_ Date: \_\_\_\_\_ Replicate Number: \_\_\_\_\_

Habitat Setting (check one):  Intertidal  Subtidal (Depth: \_\_\_\_\_)

Reef size: \_\_\_\_\_ Width \_\_\_\_\_ Length \_\_\_\_\_ Height \_\_\_\_\_

Location of samples with respect to reef: \_\_\_\_\_

Overall Reef condition: \_\_\_\_\_

\_\_\_\_\_

#### 2. Physical/Chemical Parameters

Bottom Salinity: \_\_\_\_\_ Air Temperature: \_\_\_\_\_ Bottom Temperature \_\_\_\_\_

Bottom Dissolved Oxygen: \_\_\_\_\_ Weather Conditions \_\_\_\_\_

Oiled Condition (check one):  none  Sheen  Scattered Deposits \_\_\_\_\_

Surface substantially covered  Surface completely covered  Deep Deposits \_\_\_\_\_

#### 3. Sampling Methods

Water bottle (volume): \_\_\_\_\_ L or Pump sample (volume): \_\_\_\_\_ L

#### 4. Sampling Results: Abundance

Total Volume of Sample: \_\_\_\_\_ (ml);

Abundance	Subsample 1	Subsample 2	Subsample 3
Bivalve Veliger Larvae			

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Total estimate bivalve abundance: \_\_\_\_\_ #/L \_\_\_\_\_ #/L \_\_\_\_\_ #/L

Average estimated concentrations: \_\_\_\_\_ #/L

Divided total volume by subsample volume and multiply subsample count by that number. For example  $1000\text{ml}/5\text{ ml} = 200$ .  $200 * 6\text{ bivalve veligers} = 1200\text{ bivalves per } 1000\text{ ml or } 1200/\text{L}$ .

Recorder: \_\_\_\_\_

Survey Team ID: \_\_\_\_\_

### Oyster Settlement Sample Form

#### 1. Site Descriptors

Site Name/or Cell ID: \_\_\_\_\_ Lat: \_\_\_\_\_ Lon: \_\_\_\_\_

MAP # \_\_\_\_\_ GRID # \_\_\_\_\_

Time: \_\_\_\_\_ Date: \_\_\_\_\_ Replicate Number: \_\_\_\_\_

Habitat Setting (check one): \_\_\_ Intertidal \_\_\_ Subtidal (Depth: \_\_\_\_\_)

Reef size: \_\_\_\_\_ Width \_\_\_\_\_ Length \_\_\_\_\_ Height \_\_\_\_\_

Location of samples with respect to reef: \_\_\_\_\_

Overall Reef condition: \_\_\_\_\_

#### 2. Physical/Chemical Parameters

Bottom Salinity: \_\_\_\_\_ Air Temperature: \_\_\_\_\_ Bottom Temperature \_\_\_\_\_

Bottom Dissolved Oxygen: \_\_\_\_\_ Weather Conditions \_\_\_\_\_

Oiled Condition (check one): \_\_\_\_\_ none \_\_\_\_\_ Sheen \_\_\_\_\_ Scattered Deposits \_\_\_\_\_

Surface substantially covered \_\_\_\_\_ Surface completely covered \_\_\_\_\_ Deep Deposits \_\_\_\_\_

#### 3. Sampling Interval

Date Deployed: \_\_\_\_\_ ; Date retrieved \_\_\_\_\_, # of days \_\_\_\_\_

#### 4. Sampling Results: Abundance

Abundance	Plate 1	Plate 2	Plate 3
Oyster Spat (Live)			
Oyster Spat (Dead or scars)			
Other sessile fauna: Indicate species			
Barnacles:			

Mussels:			

Recorder: \_\_\_\_\_