

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

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As agreed upon by the Trustees and BP, all samples collected for contaminant analysis during the sampling plan described below will be sent to Alpha Analytical Laboratory. 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 BP (or ENTRIX on behalf of BP). The electronic data deliverable (EDD) spreadsheet with pre-validated analytical results, which is a component of the complete LADP, will also be delivered to the secure FTP drop box maintained by the trustees' Data Management Team (DMT). Any preliminary data distributed to the DMT shall also be distributed to LOSCO and to BP (or ENTRIX on behalf of BP). Thereafter, the DMT will validate and perform quality assurance/quality control (QA/QC) procedures on the LADP consistent with the authorized Quality Assurance Project Plan, after which time the validated/QA/QC'd data shall be made available simultaneously to all trustees and BP (or ENTRIX on behalf of BP). Any questions raised on the validated/QA/QC results shall be handled per the procedures in the Quality Assurance Project Plan and the issue and results shall be distributed to all parties. In the interest of maintaining one consistent data set for use by all parties, only the validated/QA/QC'd data set released by the DMT shall be considered the consensus data set. In order to assure reliability of the consensus data and full review by the parties, no party shall publish consensus data until 7 days after such data has been made available to the parties. Also, the LADP shall not be released by the DMT, LOSCO, BP or ENTRIX prior to validation/QA/QC absent a showing of critical operational need. Should any party show a critical operational need for data prior to validation/QA/QC, any released data will be clearly marked "preliminary/unvalidated" and will be made available equally to all trustees and to BP (or ENTRIX on behalf of BP).

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

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

This Amendment modifies the following sections of the Phase I Plan: Approach and Rationale; Data Needs; Site Selection; Estimated Study Cost, and Updated SOPs and field data forms.

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

**Oyster Sampling Plan - Phase I, Amendment 2**

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APPROVED:

*[Signature]* FOR ROLAND GUIDRY  
Louisiana Trustee Representative: \_\_\_\_\_ Date: 2/22/11

*[Signature]*  
BP Representative: \_\_\_\_\_ Date: Feb. 11, 2011

*[Signature]* 2/9/11  
NOAA Trustee Representative  
(on behalf of all other trustees) \_\_\_\_\_ Date: \_\_\_\_\_

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

Amendment 2  
February 3, 2011

**Introduction**

This document amends (Amendment 2) the initial phase of a pre-assessment sampling plan (Phase I Plan) for oysters in the north-central Gulf of Mexico.

The Phase I Plan provides for the collection of ephemeral data on the condition of oysters in the Gulf of Mexico - both in un-oiled areas and oiled areas. Data collected under this amended plan are 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 NRDA process for the MC 252 Spill that is underway pursuant to the OPA. 15 C.F.R. §990.43.

Amendment 2 to this Oyster Plan specifically addresses the following topics covered in the Phase I plan:

- 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 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.

## **I. Approach and rationale**

The following changes have been made to this section of the Phase I Plan:

1. References to the Phase I Plan (including amendments) study duration should say “four months,” not “two months.”
2. The standard operating procedures (SOPs) for Phase I sampling have been attached in full to this Amendment.
3. The text of the fourth paragraph in the Phase I Plan is unchanged; however Table 1 has been updated to reflect changes to the frequency of sampling of individual metrics in the amended Phase 1 Plan.
4. Paragraph five of the Phase I Plan, which describes the designated laboratories for each type of analysis, is amended as follows:

“With the exception of tissue and sediment contaminant analysis, larval counts, and settlement plate analysis, analyses of all metrics either have been or will be conducted at a designated intake laboratory. For samples collected in Louisiana prior to September 12, 2010, the designated intake laboratory was the University of New Orleans; for subsequent samples collected in Louisiana and all samples collected in Mississippi, Alabama and Florida, the designated intake lab was the Center for Ecosystem Based Fishery Management at the Dauphin Island Sea Lab in Dauphin Island, Alabama. Disease and gonad analysis was conducted at the University of New Orleans for all samples. For tissue and sediment samples designated for contaminant analyses, the designated lab was Alpha Analytical Laboratory in Westborough, Massachusetts. The designated lab for samples for larval counts was the Fish & Wildlife Research Institute of the Florida Fish and Wildlife Conservation Commission in St. Petersburg, Florida; and the designated lab for settlement plate samples was the Center for Ecosystem Based Fishery Management at the Dauphin Island Sea Lab in Dauphin Island, Alabama.”

5. The last sentence of this section in the Phase I Plan is replaced with the following: This initial targeted plan includes a maximum of 76 sample sites that includes both shallow and deeper subtidal locations.

## **II. Data needs**

The following changes have been made to this section of the Phase I Plan:

1. First paragraph: The standard operating procedures (SOPs) and field and intake laboratory data forms for Phase I sampling have been attached as Appendices to this Amendment.
2. Third paragraph, first full sentence is replaced with: “During the 2-3 week pelagic duration of oysters, larvae may contact oil, potentially resulting in lethal and sub-lethal effects.”
3. Fourth paragraph, third sentence is replaced with: “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 may be 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).”

4. Fifth and sixth paragraphs (Section C) are replaced with the following:

**“C. Contaminant data in oyster tissue and sediments.** Currently, it appears that oil from the Deepwater Horizon spill affected a large geographic area and at highly variable concentrations. These factors suggest 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), we collected contaminant samples during two rounds of Phase I sampling (Sericano et al., 1996, Hwang et al., 2004).

Sediment samples were collected for contaminant analysis concurrently with oyster quadrat sampling events, according to the protocols outlined in Appendix A, which were derived from NOAA NRDA protocols.”

### III. Health and Safety

This section is unchanged with the exception of the italicized text. We repeat the text here for ease of reference.

- **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 *provided to your team by NOAA NRDA Field Ops staff or the NRDA Oyster Sample Location Coordinator*;
  - 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\_6.22.10.pdf”).<sup>2</sup>
- **If participating in a cruise:** Each cruise may have additional required health and safety procedures, which 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

This Amendment expands previous Phase I sampling as described below:

Initial Phase I efforts were focused on sampling in one unoiled area (Lake Calcasieu) and one oiled area (Barataria Bay) in Louisiana, as described in the original Phase I Plan. Amendment 1 to the Phase I Plan expanded potential sampling locations in oiled areas to include Barataria Bay and Chandeleur Sound. The sampling sites in the potentially oiled areas were selected based on consultation with the Oyster Working Group and consideration of recent oiling observations, such as SCAT data. However, sampling and reconnaissance efforts conducted in the areas identified as oiled generally did not locate sufficient active oyster habitat for sampling and therefore required further modifications to site selection methodology in order to evaluate potential impacts to oysters from MC 252.

Depending on the ultimate results of the Phase I effort, the scale of Phase I may not provide a comprehensive statistical dataset for the reference and oiled areas, and thus it may not be appropriate to draw broad conclusions at this point regarding MC 252 oiling impacts on oyster resources. However, the potentially patchy nature of oyster resource requires a broader based approach to Phase I that includes recently observed oyster habitat across a wider range of areas to enable the NRDA team to more quickly and efficiently generate data to inform the evaluation of both mortality and morbidity metrics.

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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]

The Phase I, Amendment 2 sample sites consist of 4-ha (200 m x 200 m) grid cells spread across Louisiana, Mississippi, Alabama and Florida. The Phase I, Amendment 2 sites are primarily a subset of historically sampled state sites, coupled with additional sites in areas of observed oiling and freshwater diversions. Most of the Louisiana sites and Florida sites are considered Tier 1 sites, which are defined under this plan as sites based on long-term monitoring locations of state agencies. The overall geographic scope of the Phase I plan is well dispersed across the coastal areas of Louisiana and thus expected to capture a range of oiling conditions. In addition, the Louisiana sites were selected to ensure spatial coverage of areas potentially injured by the freshwater diversions undertaken by Louisiana in response to the MC 252 spill. To enhance the likelihood of sampling active or recently active resource, sampling was limited to Tier 1 sites where live oysters or spat have been identified since 2009. In addition, potential exposures to MC 252 oil at these sites were considered based on consultation with the Louisiana Department of Wildlife and Fisheries and consideration of recent oiling observations such as SCAT data. The sites in Mississippi, Alabama, and Florida were chosen based on oiling observations and appropriate reference sites.

Samples were collected at the Phase I, Amendment 2 sites between August and November 2010. Table 2 summarizes the number of sample sites and the maximum number of subsamples that were collected at each site for each metric as part of the Phase I study. We conducted one quadrat and sediment sample event and up to four sampling events for reproductive endpoints, spaced approximately three weeks apart based on logistical constraints (e.g., weather). The first tissue contaminant sampling coincided with the quadrat sample event, and an additional round of contaminant sampling was conducted by dredge in Louisiana, Mississippi, and Alabama, and using hand tongs in Florida as part of the last round of sampling at the Phase I sites.

To assess the potential for finding active oyster habitat at each site, we conducted side-scan sonar mapping of the sites to identify possible oyster reef (following the SOP in Appendix A), followed by the collection of small dredge samples to assess whether live oysters or recently dead oysters are present (see Appendix B for description of oyster habitat). Oysters collected for this purpose during reconnaissance activities were not retained. Further subsampling was only conducted in areas where evidence of active oyster habitat was found in at least one dredge sample.

GIS coordinates for the sample sites (4 ha, 200 m x 200 m grid cells) and contact points were provided to help guide the field crew to the four corners of the corresponding 4 ha study sites. Field crews were given contact points recorded during the side-scan sonar mapping that were arrayed in a random order. Up to eight quadrat contact points and eight sediment contact points were provided for each cell, depending on the mapping results. These contact points excluded areas where dredge samples were collected during reconnaissance. These eight locations were visited in the order in which they were randomly selected until the number of samples in Table 2 were collected or until 2 hours were spent investigating points in a given cell.

## **Louisiana Sampling Sites**

Figures 1 through 4 contain detailed maps of the sampling locations in Louisiana within each individual Coastal Survey Area (CSA). (Note there were no Phase I, Amendment 2 sites in CSA 6.) The majority of these sites are Tier I areas. However, due to the scarcity of Tier I sites in some areas, such as Barataria Bay, we selected a small number of additional samples (13 of the 40 sites are not Tier I sites). These additional sites are in areas of known resource and have been sampled historically by the state of Louisiana, but with less frequency than the Tier I sites (e.g., sites sampled for seafood safety testing). These sites were also selected because they are in areas identified as oiled and/or affected by freshwater diversion.

## **Mississippi Sampling Sites**

Figures 5-7 show the sites sampled in Mississippi. The 14 sampling sites in Mississippi represent known oyster reefs with recent sampling events that have confirmed the presence of oyster resource. Two sites each were selected in Pascagoula and Biloxi Bays. In the Pass Christian area, a sample of 15 sites was drawn. Sampling occurred at the 10 sites shown in Figure 5 (a sample of 15 was drawn so that there would be a pool of additional sites if oyster resource was not located at the first 10 sites). The sites were chosen to represent a cross-section of the oiling continuum, based on oiling data. None of these sites are Tier I sites.

## **Alabama Sampling Sites**

Figure 8 shows the sampling sites selected in Alabama. These 10 sites do not fall under Tier I. These sites represent historically sampled reef where oyster resource has recently been confirmed. Oiling levels were not considered in selecting sampling sites for this state.

## **Florida Sampling Sites**

Figures 9 and 10 illustrate the sampling sites selected in Florida. Nine of the 12 sites in Florida are in Tier I areas. One site in Pensacola Bay was randomly selected among known mapped oyster reefs. The remaining two sites were randomly selected from among reefs that are part of Project Greenshores, a habitat restoration and creation project.<sup>3</sup> These sites are in areas that are most likely to have been affected by oiling.

## **V. Estimated Study Cost**

This Amendment updates the total cost of the Phase I plan with amendments as follows:

Our total estimated budget for implementing the Phase I plan described in this document, including physical measurements, but not chemistry, is \$1,060,931 through the end of April 2011. This estimate includes the costs of collecting, processing, and shipping the samples from

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<sup>3</sup><http://www.dep.state.fl.us/northwest/ecosys/section/greenshores.htm>

Table 2. The cost of Phase I activities conducted prior to this Amendment (i.e., prior to August 9, 2010) are estimated at \$101,750, bringing the total Phase I cost estimate to \$1,162,681 through the end of April 2011 including the original Phase I plan (authorization of \$511,476), Amendment 1 (authorization of \$7,920), and Amendment 2 (an additional authorization of \$643,285). For additional detail concerning the cost estimate, please consult Table 3 and the attached Excel file, "Costmatrix\_Phase\_I\_Plan\_12.6.10.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/Amendment 2 metrics.**

<b>Metric</b>	<b>Proposed Frequency of Sampling</b>
<i>Effect Metrics</i>	
Oyster abundance, by size class and by alive/dead status	One event
Abundance of associated species, by oyster size class	One event
Oyster biomass, by size class and by alive/dead status	One event
Biomass of associated species, by oyster size class	One event
Disease	Up to four events
Gonadal condition	Up to four events
Larval abundance (#/L)	Up to four events (spaced three weeks apart)
Larval settlement	Up to four events (spaced three weeks apart)
<i>Exposure metrics</i>	
Tissue concentrations	Up to two events
Sediment concentrations	One event
Oiling observations (qualitative)	Up to five events

**Table 2. Summary of oyster sampling procedures, maximum number of sites and replicates in Phase I/Amendment 2 Sampling.**

Metric	Method	Potential # of sites				Max. Repl. per site	Est. Samples per event	Freq. of sampling	Total # of samples (estimate)
		LA	MS	FL	AL				
Site Mapping	Side-scan sonar	50	19	12	6	1	87	1	87
Site Resource Confirmation	Dredge	44	Up to 19	12	10	1	Up to 85	1	Up to 85
Adult and Juvenile Density	Quadrat	40	14	12	10	N = 4 quadrats	304	1	304
Oyster Larvae	Water sample	40	14	12	10	N = 5	380	4 (LA) 3 (MS, AL, FL)	1,340
Oyster Settlement	Settlement plate	40	14	12	10	N = 3 plates.	228	4 (LA) 3 (MS, AL, FL)	804
Oyster Gonadal condition and Oyster Disease	Oysters	40	14	12	10	N = 10 oysters	760 oysters	3 (LA) 2 (MS, AL, FL)	1,920 oysters
Oyster Disease	Oysters	40	14	12	10	N = 10 oysters	760 oysters	1	760 oysters
Tissue contaminant analysis	Oysters	40	14	12	10	N = 4 composite samples per grid cell	304	2	608
Sediment Contaminant analysis	Sediment	40	14	12	10	N = 2 composites per grid cell	152	1	152

**Table 3. Costs for the Phase I Oyster Plan, Amendment 2.**

	<b>Louisiana</b>	<b>Mississippi</b>	<b>Alabama</b>	<b>Florida</b>	<b>TOTAL</b>
<b>Side-Scan Sonar</b>	29,590	11,400	3,600	7,200	51,790
<b>Dredging</b>	65,267	28,183	14,833	17,800	126,084
Personnel	35,200	15,200	8,000	9,600	
Boat charges	26,400	11,400	6,000	7,200	
Supplies	3,667	1,583	833	1,000	
<b>Quadrat Sampling</b>	238,400	83,440	59,600	71,520	452,960
Divers	91,200	31,920	22,800	27,360	
Non-Diving Personnel	89,600	31,360	22,400	26,880	
Boat charges	57,600	20,160	14,400	17,280	
<b>Recruitment Sampling</b>	106,800	24,920	17,800	21,360	170,880
Personnel	57,600	13,440	9,600	11,520	
Boat charges	43,200	10,080	7,200	8,640	
Supplies	6,000	1,400	1,000	1,200	
<b>Last Recruitment/Contaminant Sampling</b>	35,600	12,460	8,900	10,680	67,640
Personnel	19,200	6,720	4,800	5,760	
Boat charges	14,400	5,040	3,600	4,320	
Supplies	2,000	700	500	600	
<b>Larval Processing</b>	7,229	3,121	1,643	1,971	13,965
Personnel	5,029	2,171	1,143	1,371	
Supplies	1,100	475	250	300	
Shipping and archive charges	1,100	475	250	300	
<b>Quadrat Processing</b>	59,200	11,013	7,867	9,440	87,520
Personnel	57,600	10,453	7,467	8,960	
Supplies	800	280	200	240	
Shipping and archive charges	800	280	200	240	
<b>Recruitment Processing</b>	46,800	10,920	7,800	9,360	74,880
Personnel	36,000	8,400	6,000	7,200	
Supplies	5,400	1,260	900	1,080	
Shipping and archive charges	5,400	1,260	900	1,080	
<b>Last Recruitment/Contaminant Processing</b>	3,733	1,307	933	1,120	7,093
Personnel	2,133	747	533	640	
Supplies	800	280	200	240	
Shipping and archive charges	800	280	200	240	
<b>Sediment Processing</b>	800	280	200	240	1,520
Supplies	400	140	100	120	
Shipping and archive charges	400	140	100	120	
<b>Cooler Rental</b>	6,600				6,600
<b>TOTAL</b>	600,019	187,045	123,176	150,691	1,060,931
<b>Additional Authorization for Amendment 2<sup>1</sup></b>					\$ 643,285

Figure 1. Phase I/Amendment 2 Oyster Sampling Sites – Louisiana – CSA 7

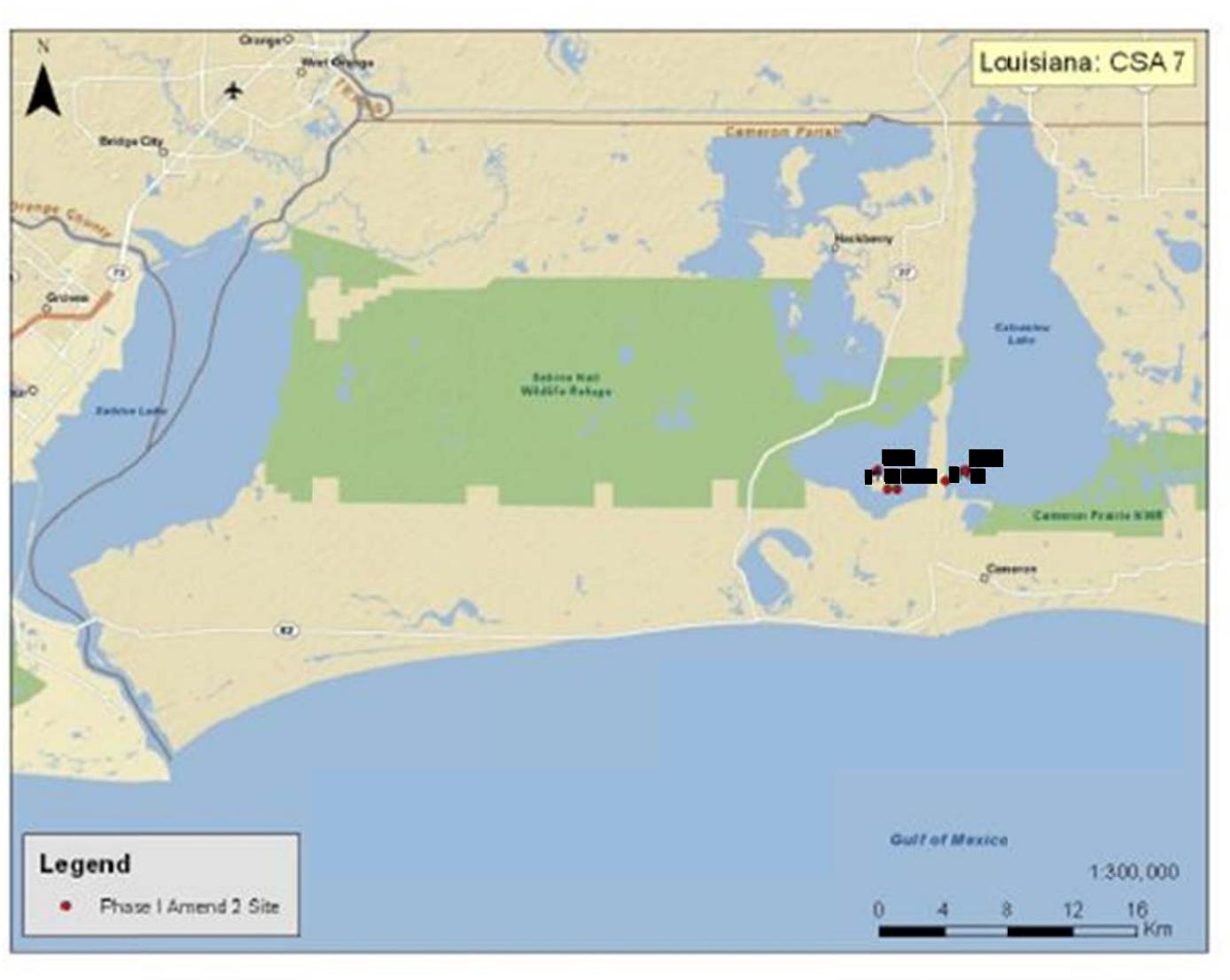


Figure 2. Phase I/Amendment 2 Oyster Sampling Sites – Louisiana – CSA 4 and 5

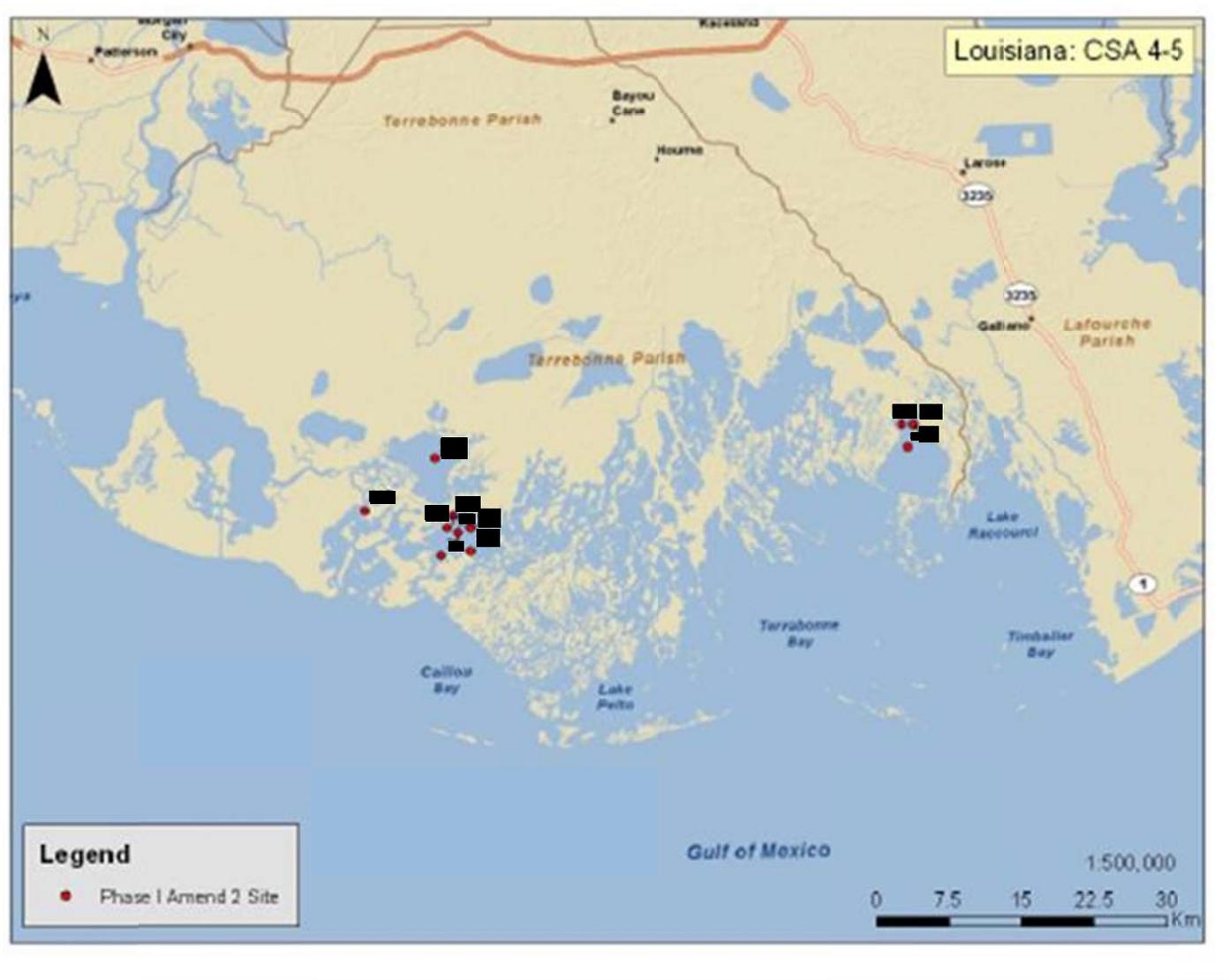






Figure 5. Phase I/Amendment 2 Oyster Sampling Sites – Mississippi – Pass Christian

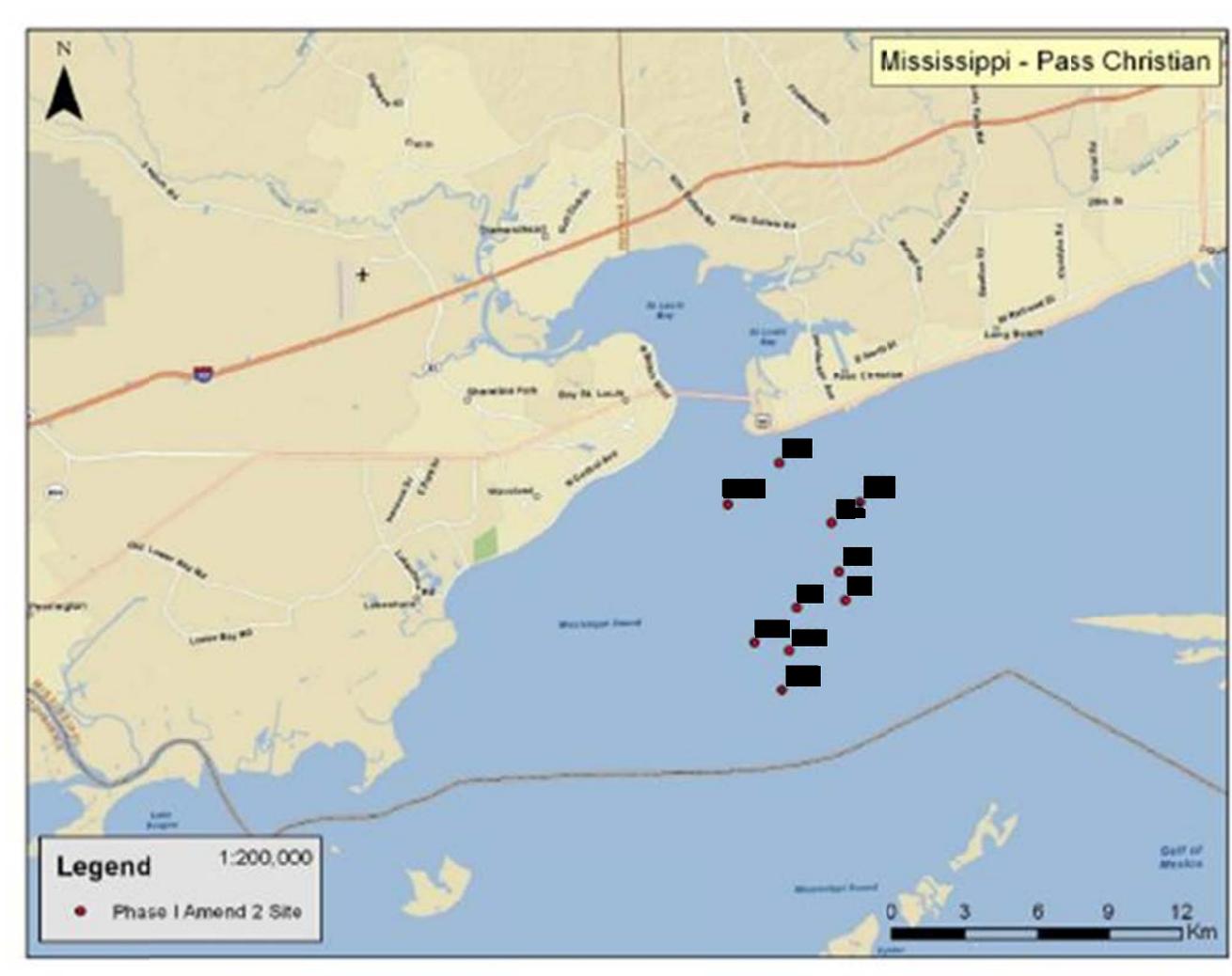


Figure 6. Phase I/Amendment 2 Oyster Sampling Sites – Mississippi – Biloxi



Figure 7. Phase I/Amendment 2 Oyster Sampling Sites – Mississippi – Pascagoula



Figure 8. Phase I/Amendment 2 Oyster Sampling Sites – Alabama

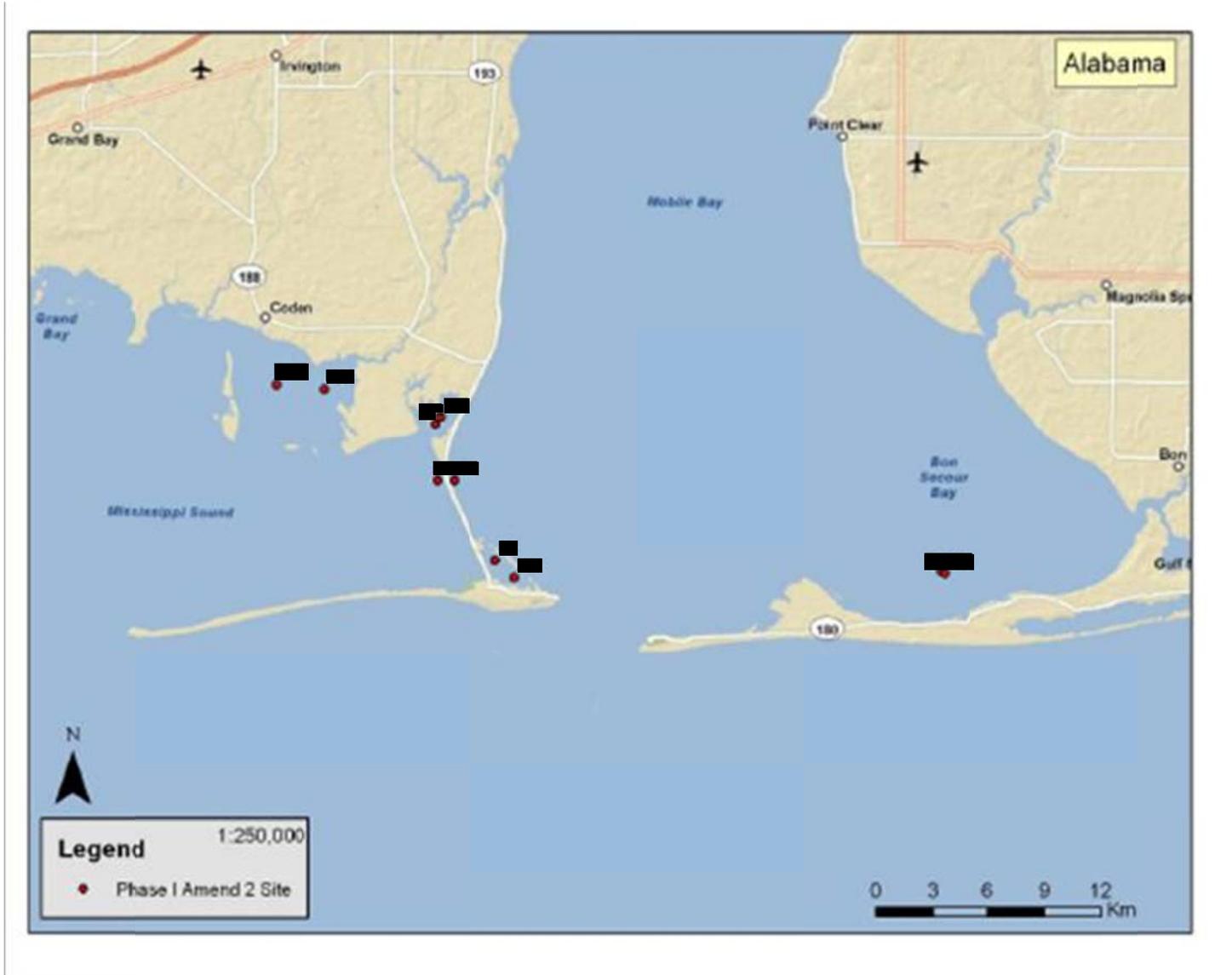


Figure 9. Phase I/Amendment 2 Oyster Sampling Sites – Western Florida

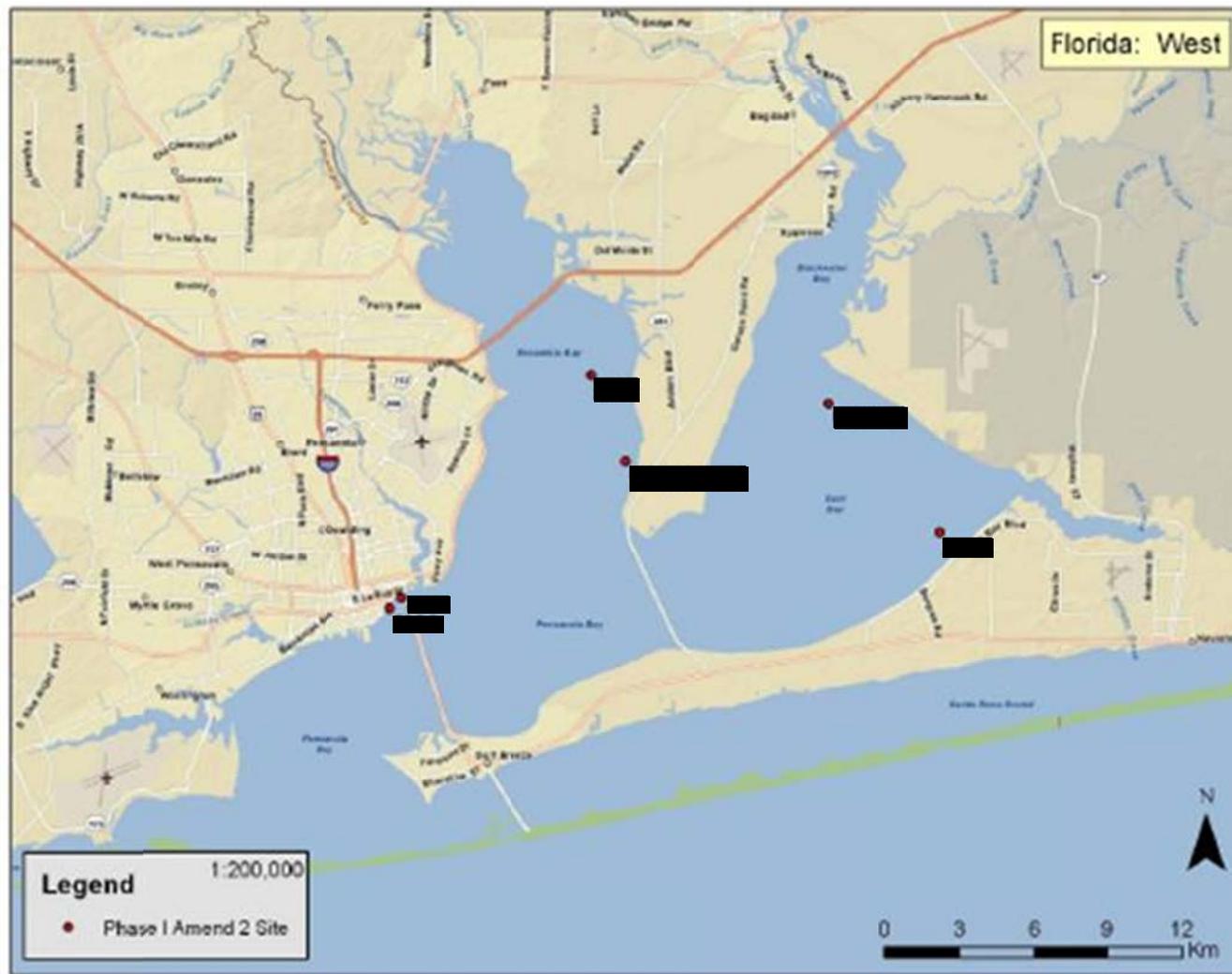
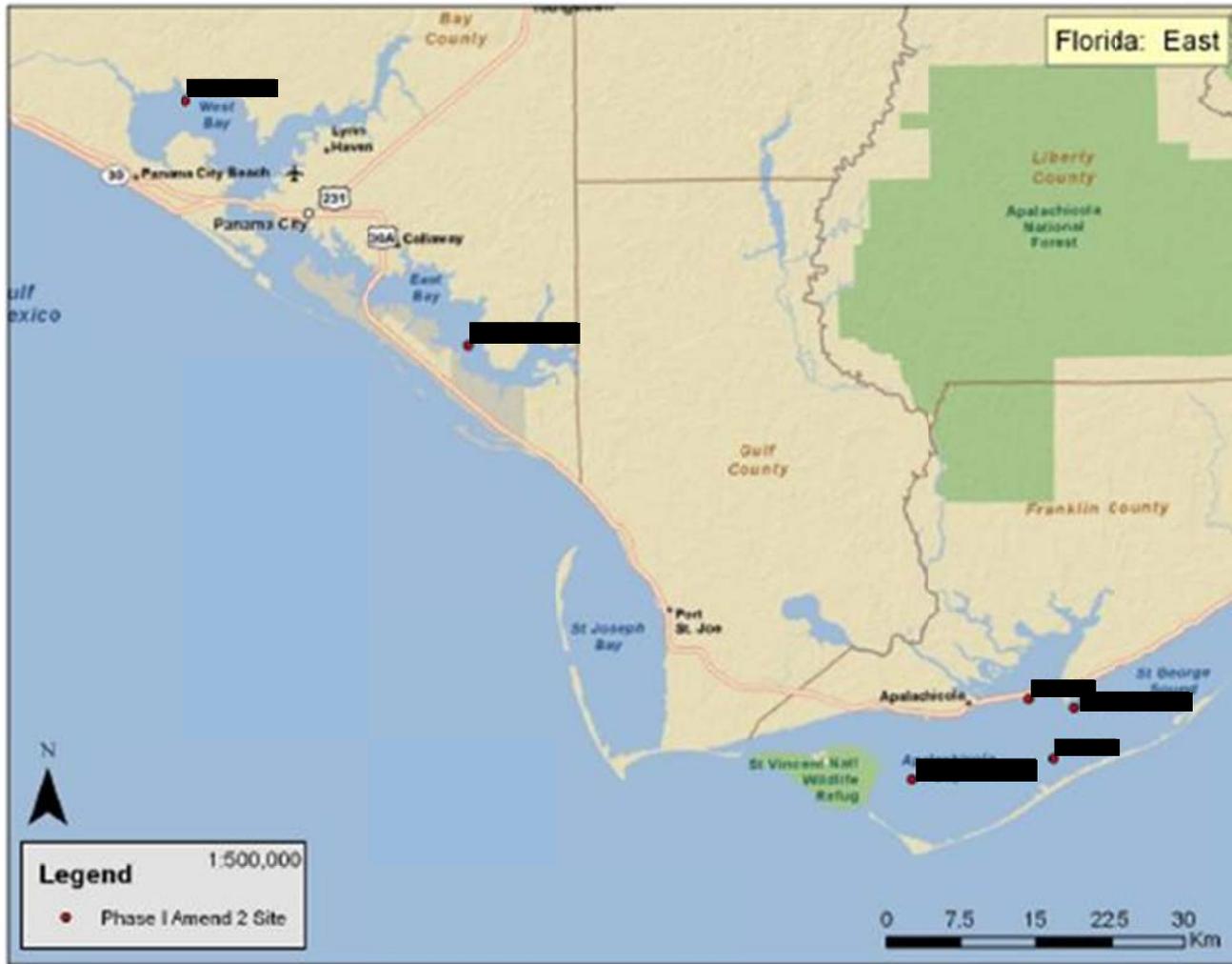


Figure 10. Phase I/Amendment 2 Oyster Sampling Sites – Eastern Florida



## **APPENDICES**

**Appendix A: Standard Operating Procedures for Phase I**

**Appendix B: Oyster Condition Guide**

**Appendix C: Oyster Sample Naming Convention**

**Appendix D: Sampling Forms**

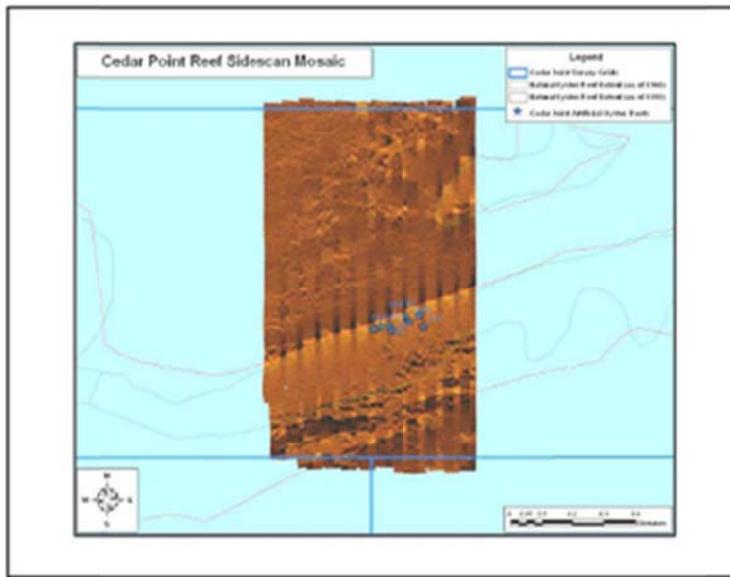
## Appendix A: Detailed Standard Operating Procedures (SOPs)

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

An initial survey of the area will be mapped using high resolution side-scan sonar that is georeferenced with an accuracy of +/- 1 m (Figure A-1, See Allen et al., 2005). The purpose of the site mapping procedures is: (1) to identify with a high degree of certainty the locations for quadrat selection and subsequent follow up sampling and (2) to potentially establish percent coverage of oyster habitat within a pre-determined cell. During post-processing of the side-scan sonar imagery, contact points representing potential oyster reefs will be identified and assigned a number beginning with 1. Potential dredge sample locations for “ground-truthing” the sonar contact points (up to 6) as well as subsample locations for collecting quadrats and sediment samples (up to 8 each) will be selected based on the likely presence of oyster habitat as indicated by the sonar data. These sites will be distributed throughout the 200m x 200 m sample cell across the potential identified reefs; contact points where dredge samples were collected will be excluded from selection for subsampling. Sub-sample locations will be ordered according to simple random selection. 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 quadrat contact points will be passed onto the oyster sampling team. If no oyster habitat is found, these grid cells (sites) will not be sampled.



**Figure A-1.** A side scanned image of oyster reef in Mobile Bay, Alabama.

A 24 inch wide oyster hand dredge will be used to “ground truth” the side scan imagery. Up to four dredge samples per cell will be collected and the presence of live oysters, recently dead oysters, and substrate condition noted. Dredges should be towed for at least 30 seconds in a circular pattern around the contact point generated by the side-scan sonar. If dredging using a hand dredge is not possible (e.g, in Florida waters), alternative methods may be employed. In Florida waters, hand tongs are a suitable substitute for dredging.

### **Quadrat Locations**

Up to eight quadrat locations will be selected for each grid cell from the contact points generated during the side-scan sonar survey. Quadrat locations will be selected based on the likely presence of oyster habitat as indicated by the sonar data; contact points where dredge samples were collected will be excluded from selection for subsampling. The selected subsample points will be ordered according to simple random selection, and the field teams will visit the points in that order to collect samples until either: 1) four quadrat samples have been collected in a given cell; or 2) two hours have elapsed exploring a cell for oyster resource, at which point the field team will move on to the next cell. The oyster habitat studies will utilize either  $\frac{1}{4}$  m<sup>2</sup> (0.5 m x 0.5 m) or 1 m<sup>2</sup> quadrats made of  $\frac{1}{2}$ ” diameter PVC lengths (See SOP in Section B). At a given contact point, the field teams may use cane poles to conduct manual surveillance in the vicinity of the point to ensure that the quadrat sampling frame will intercept oyster reef. The final GIS coordinates of the location of each quadrat sample will be recorded in accordance with NOAA NRDA protocols.

### **Sediment Sampling Locations**

Up to eight sediment sample locations will be selected for each grid cell from the contact points generated during the side-scan sonar survey. These contact points are intended to assist field teams in sediment collection when sampling in the vicinity of quadrat samples proves infeasible due to bottom surface characteristics. Sediment sampling locations will be selected based on the likely presence of sediment as indicated by the sonar data; contact points where dredge samples were collected will be excluded from selection for sediment sampling. The selected sediment sample points will be ordered according to simple random selection, and the field teams will visit the points in that order to collect samples until either: 1) four sediment samples have been collected in a given cell (which will be combined into two composite samples); or 2) two hours have elapsed exploring a cell for oyster resource, at which point the field team will move on to the next cell. Field teams will use the SOPs for sediment sampling as outlined in this Appendix. The final GIS coordinates of the location of each sediment sample will be recorded in accordance with NOAA NRDA protocols.

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

### Field Sampling

Samplers should complete the **Oyster Field Form for Quadrats** (Appendix D). A unique sample code or number should be given to each sample and prominently marked on the form according to the Oyster Sample Naming Convention (see Appendix B). Sample codes should be recorded in the **Oyster Field Form for Quadrats** datasheet (see Appendix D) and also in the **NRDA Sample Collection Form – Tissue/Wrack** (available on the NOAA NRDA site).

#### 1. Site Description

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

#### 2. Physical/Chemical Parameters

- Measure and record:
  - Bottom and surface salinity
  - Bottom and surface water temperature
  - Surface and Bottom dissolved oxygen (at subtidal sites only)
  - Ambient air temperature
  - Weather conditions
  - 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

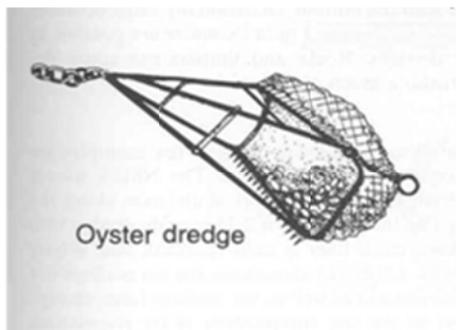
Indicate whether the sampling used quadrat based sampling (record size) or dredge/tongs.

- For harvesting via surface-assisted diving / quadrats
  - 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 ¼ m<sup>2</sup> PVC quadrat frame directly at arm's length at a random spot at the coordinates. Do not favor abundant areas. (Use 1 m<sup>2</sup> quadrats if the team

receives evidence prior to sampling that indicates the site is likely to have little oyster resource.) Make sure to mark the size of the quadrat on the field form for all samples.

- 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 a plastic bag.
  - Samples should be tagged with an external (flagging tape with permanent marker) and internal tag (flagging tape with permanent marker) that prominently denotes sample code.
  - The sample code should be constructed of the location ID, date, matrix, sample team leader code, and sample number along with information regarding sample type (for details, see the Oyster Sample ID Naming Convention, Appendix B).
  - Hold animals on ice until delivered to intake team.
  - If the first two quadrats have little or no live resource switch to the 1 m<sup>2</sup> quadrats and sample the contact points until four quadrats have been collected. Contact points should be skipped (i.e., no data collected) only if there is no evidence of current or past resource (e.g., mud only). Retain all quadrat sample material and submit all quadrat samples to the intake lab. If the first two quadrats have sufficient resource, continue using the ¼ m<sup>2</sup> quadrat. Make sure to mark the size of the quadrat on the field form for all samples.
  - If, after switching to 1 m<sup>2</sup> quadrats, the team fails to collect 10 oysters (>2”) per quadrat on average (i.e., combined less than 40 oysters for the entire cell), then supplement the quadrat samples with dredging or tongs. Limit the period for additional sampling to 2 hours, and make sure to record the coordinates of dredge sampling on the field forms along with any notes. Bag only the live resource from the dredge/tongs for transmission to the lab and label accordingly.
- For dredge harvesting
- Dredge harvesting using a 24 inch wide oyster hand dredge may be used to collect resource for contaminant or disease/gonad samples:
  - Deploy dredge from the beam of the vessel.
  - Drag dredge across the surface of the substrate for 3 minutes in a circular pattern around the contact point
  - Record exact start and stop positions using a GPS.
  - Collect enough replicate dredge samples at sites chosen for quadrat sampling so that the required number of animals for analysis are obtained, if possible.
  - 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 (flagging tape with permanent marker) that prominently denotes sample code.
- The sample code should be constructed of the location ID, date, matrix, sample team leader code, and sample number along with information regarding sample type (for details, see the Oyster Sample ID Naming Convention, Appendix B).
- Hold animals on ice until delivered to intake team.



- In areas where dredging is not possible because of logistical or permit difficulties, oyster tongs may be used to collect oysters.
  - Oyster tongs are generally 2-3 m long and constructed over two rakes welded or bolted together at the center point of the handles. The teeth on the rakes are generally 25 cm long and the head of the rake 1 m in length. The rakes are juxtaposed to form a small basket when closed (local variations on oyster tongs are common and measurements need not be exact).
  - Once at a site, the tongs can be deployed over the side of the boat. Once placed on the bottom the tongs are opened and closed repeatedly to dislodge oyster from a small area.
  - After 6-10 opening and closing events, the tongs are used to collect the dislodged oysters into one grab. The tongs are held closed and the operator withdraws the handles from the water and places the contents on the deck.
  - The entire procedure is repeated until the targeted number of oysters is collected.
  - Four deployments should be made at each site; if no live oysters are collected, the boat should be repositioned 2 m away and the procedure repeated.

## 5. Photographs

See **NRDA Field Photography Guidance** (available on the NOAA NRDA 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

## 6. *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. 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**

#### 7. *Sample Processing: Abundance*

Samples will be brought to a non-field location for processing. Samples should be kept in a cooler with ice. Samples should be processed within 48 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 (between 0.4 and 1 inch [10 - 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.

- 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.

#### 8. Sample Processing: Biomass

- Weigh living material:
  - Weighed in aggregate by size category.
  - Similarly, dead oysters should be weighed by category. Finally, associated species should be identified and weighed by taxon.
  - Dead oyster still with tissue will be classified as dead.

#### *Equipment List*

- i. Random number table
- ii. 2 PVC quadrats, dredge
- iii. 3 sets of calipers
- iv. 2 10-m long field measuring tapes (or laser range finder)
- v. Spring scales (0-10g, 10-100g, 100 – 1000g, and 0-10 kg)
- vi. Large 1 gallon Ziploc bags to separate subsamples for further analyses.
- vii. Digital camera with extra batteries
- viii. GPS with extra batteries
- ix. Nitrile gloves (size M and L)
- x. Small shovel / tool for separating oysters
- xi. Waterproof data sheets (chain-of-custody, sample tracking, photo log, oyster reef sample form)
- xii. Waterproof labels or tags
- xiii. Waterproof pens
- xiv. Flagging tape for external tags
- xv. Onion bags or burlap sacks for sample storage.
- xvi. Plastic contractor-grade construction bags
- xvii. 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 as estimated by QPCR with larval and adult oyster DNA as standards.

(b) Quantify the abundance of total bivalve veliger larvae (non-oyster specific) (Fig. 2) in the water column.

#### Rationale:

The eggs and larvae of many bivalves are indistinguishable using microscopy, but direct counting provides an absolute metric for the number of bivalve larvae in the water column at the time of sampling. There is evidence that sampling through the water column may collect varied life stages: eggs, developing larvae, and presettlement pediveligers (those larvae with a developed foot). In and of itself, this provides a relative index for spatial variability in the plankton, and also a good estimate of oyster larval abundance because oysters can be expected to be a dominant component of the total bivalve larval population. Only pediveligers can reliably be identified visually. QPCR (real-time quantitative polymerase chain reaction) quantifies oyster larvae by measuring the number of gene copies for a specific gene in the same, and relating that measurement back to a standard curve prepared with known concentrations of larvae.

PCR and QPCR are made possible by harnessing the activity of DNA polymerase (commonly referred to as 'taq', after the organism from which it was isolated for this purpose). The reaction occurs at temperatures - ~94, ~60, and ~72 C. The process by which a machine is used to heat and cool the reaction to those temperatures permissive for DNA melting and DNA duplication is known as thermocycling.

QPCR is accepted universally as a means of quantifying microscopic organisms, and finds application in pathogen detection, HIV diagnosis, and estimating larval quantity in other marine mollusks. See for example:

De Faveri, J., Smolowitz, R.M., and Roberts, S.B. 2009. Development and validation of a real-time quantitative PCR assay for the detection and quantification of *Perkinsus marinus* in the eastern oyster, *Crassostrea virginica*. *Journal of Shellfish Research* **28**: 459-464.

Matejusova, I., McKay, P., McBeath, A.J.A., Collet, B., and Snow, M. 2008. Development of a sensitive and controlled real-time RT-PCR assay for viral haemorrhagic septicaemia virus (VHSV) in marine salmonid aquaculture. *Diseases of Aquatic Organisms* **80**: 137-144.

Vadopalas, Brent, Bouma, J.V., Jackels, C.R., and Friedman, C.S. 2006. Application of real-time PCR for simultaneous identification and quantification of larval abalone. *Journal of Experimental Marine Biology and Ecology* **334**: 219-228.

Wight, N.A., Suzuki, J., Vadopalas, B., and Friedman, C.S. 2009. Development and optimization of quantitative PCR assays to aid *Ostrea lurida* Carpenter 1984 restoration efforts. *Journal of Shellfish Research* **28**: 33-41.

## 2. Field Procedure

### a. Materials

- Niskin or LaMotte Water Sampler (fixed volume, remote trigger and 1 L capacity)

- 1-L amber sample bottles
- Waterproof labels
- Bubble wrap bags
- Clear tape
- Sharpie
- Water quality meter and calibration solution
- Distilled water
- PVC pole (to stabilize water sampler)
- Rope
- Coolers
- NRDA Oyster Site Form – Recruitment Sampling

b. Methods

- Label bottles in advance of sampling with NRDA sample code
- Lower sampler into water column at the center of the cell and allow it to remain open for 1 minute; depending on the conditions, the Nisken may need to be secured to a pole to ensure that it reaches the bottom.
- Trigger to close. Record sampling time on the field form
- Collect 5 water samples:
  - o Two approximately 2 inches beneath water surface
  - o Two just above the bottom (touch bottom and raise 3-4 inches)
  - o One mid-water (approximately half-way between top and bottom)
  - o If the water depth is very shallow (< 4ft), collect three bottom samples and two surface samples.
  - o Samples containing more than approximately two tablespoons of sediment should be discarded, and the sample recollected following a distilled water rinse of the sample jar to remove sediment.
- Close lids tightly, record sample time on label, and secure label with clear tape. Note bottom, mid-water or surface in field notes and label/sample name (-LB, - LM, -LS)<sup>4</sup>
- Place jar in bubble wrap bag and store samples on ice.

### 3. Sample Intake Procedure

a. Materials

- 95% Ethanol solution (EtOH). All chemicals must be reagent grade, and water should be filtered and deionized.
- 35-micron sieve (prepare extras in case of rips)
- 250-ml or 500-ml squirt bottles
- 50-mL plastic centrifuge tubes

b. Methods

1. Label one of the top and one of the bottom samples as “direct larval counts”. Label the remaining three tubes (one top, middle, and bottom) as “DNA”.

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<sup>4</sup> (Note: Prior to October 26, 2010, the sample name did not include the depth, and was only labeled with -L).

2. Pour the sample onto the 35-u screen and use the squirt bottle to rinse the contents down to the bottom of the filter.
3. Rinse the sample into a 50-mL centrifuge tube. Ensure that the volume does not exceed 50% of the total tube volume.
4. Add 95% EtOH. Close tube, invert 6 times gently. Store at 4°C.
5. Invert tubes again after 24 h.

#### 4. Laboratory analysis - DNA extraction

##### a. Materials

- Hybridization or drying oven
- Centrifuge with bucket rotor and microcentrifuge rotor
- Qiagen DNEasy kit (Qiagen part # 69506) for Blood and Tissue
- 95% Ethanol (EtOH) Pipettes and tips

##### b. Methods

##### 1. Site preparation

- Turn hybridization oven on and allow warming to 37°C.

##### 2. Follow directions of the DNEasy Kit: All buffer names are used as listed in the extraction kit manual.

1. Centrifuge 50 ml samples @ 4,000 RPM for 5 min
2. Decant as much as possible of the EtOH / NaCl preservative w/o resuspending the pellet
3. Warm the sample to 37°C for 3 hours in a hybridization oven to dry off remaining EtOH
4. Add 180 µl Buffer ATL solution to each sample
5. Vortex on a laboratory microtube shaker (such as Vortex-genie) vigorously for 5-10 seconds or sufficiently long to ensure the pellet is resuspended.
6. Add 20 µl proteinase K (from DNEasy kit, (>600 mAU/ml, solution; Qiagen part # 19131 or #19133) solution to each sample Vortex vigorously 5-10 seconds to ensure pellet is resuspended
7. Bake in hybridization oven for 2-3 hours (56°C), vortexing every hour or until pellet is dissolved and solution is clear
8. Add 400 µl Buffer AL solution to each sample
9. Vortex 5-10 seconds to ensure mixing
10. Incubate at 56°C for 10 min.
11. Add 400 µl 95% ETOH to each sample.
12. Vortex 5-10 seconds
13. Open a sealed DNEasy Mini spin column (filter) in 2 ml collection tube per station.
14. Label lid with sample number. The samples will be transferred multiple times, so proper labeling is essential.
15. Pipette the 0.75 ml of mixture from each 1.5 ml tube into the 2 ml filter on top of a collection tube. Save the tip in the 1.5 ml tube.

16. Centrifuge (hinge side faces center of centrifuge rotor) for 2 minutes at 8000 rpm (6000 x g).
17. Discard flow-through
18. Pipette the remaining 0.75 ml of mixture from each 1.5 ml tube into the 2 ml filter on top of a collection tube.
19. Centrifuge (hinge side faces center of centrifuge rotor) for 2 minutes at 8000 rpm.
20. Discard flow-through
21. Set up new set of 2 ml collection tubes.
22. Remove spin column and place each sample in a new 2 ml collection tube.
23. Discard bottom half of previous collection tube.
24. Add 500 µl Buffer AW1 solution to each sample.
25. Centrifuge for 2 minutes at 8000 rpm (6000 x g).
26. Set up new set of 2 ml collection tubes.
27. Remove spin column and place each sample in a new 2 ml collection tube.
28. Discard bottom half of previous collection tube.
29. Add 500 µl Buffer AW2 solution.
30. Centrifuge for 5 minutes at 14000 rpm (20,000 x g).
31. Set up pre-labeled 1.5 ml tubes.
32. Remove spin column and place each in a labeled 1.5 ml tube.
33. Discard 2 ml collection tube and flow-through.
34. Add 200 µl Buffer AE solution to each sample.
35. Incubate for 1 minute at room temperature.
36. Centrifuge for 2 minutes at 8000 rpm (6000 x g).
37. Remove filter and discard. Keep the 1.5 ml collection tube.
38. Store 50 ul of the DNA extract at 4°C and conduct QPCR within 96 hours.  
Store the remainder of the extract (~ 150 ul) at -80°C for archival purposes.

## 5. Laboratory Analysis - Quantitative PCR Reactions

### a. Materials

- 2X Brilliant II SYBR Green QPCR Master Mix (part # 600828-1) or 2X Brilliant II QPCR Master Mix (part # 600804) with 0.167x SYBR Green dye (diluted from 10,000x stock; Invitrogen S-7567 as per manufacturer's instructions)
- Stratagene MasterMix reference dye.
- For DWH samples use Agilent Technologies MX3000Por MX 3005P quantitative PCR machine:  
<http://www.genomics.agilent.com/CollectionOverview.aspx?PageType=Application&SubPageType=ApplicationOverview&PageID=291>
- Plates or strips & cover caps from stratagene (Agilent 401333 or 401428 & 401425)
- 96-well plates that are suitable for the make of the thermocycler
- Bovine serum albumin (BSA)
- Forward and Reverse *Crassostrea virginica*-specific PCR Primers

b. Methods

1. Prepare master mix for 98 reactions (or number desired per plate):

	Stock Solutions	final concentration	Single reaction	98 reactions
Master Mix	2x	1x	12.5 ul	1225 ul
Forward PCR Primer	25 uM	1 uM	1 ul	98 ul
Reverse PCR Primer	25 uM	1uM	1 ul	98 ul
BSA	2.0 mg/ml	0.52 mg/ml	6.5 ul	637 ul
H2O			1 ul	98 ul
reference dye	25uM	1.0 uM	1 ul	98 ul
Total			23 ul	2254 ul

- Vortex master mix, and spin briefly to collect mastermix.
- Keep mixture on ice until ready for use. Note that SYBR is sensitive to light, so mastermix must be kept covered.

2. Plate setup

- o Column A: larval dilution series (for example: 1, 2, 5, 10 25, 50 100, 1000 larvae)
- o Column B: adult standard curve (10 x 10-fold dilutions)
- o Each plate should include a positive (adult and larval curves) and negative control Each plate will have 1 reaction supplied with 2 ul of the water used in all dilutions but no oyster DNA. A positive reaction in this reaction is indicative of contamination.
- o Each sample should be replicated 2 times
- Place reaction plate onto a clean tray so that it does not touch the benchtop surface, or come directly in contact with ice. This prevents transfer of residue and dirt that add background fluorescence.
- Add 23 ul mastermix to each well, slowly, using the same pipet tip. Do not push air at the end of the pipetting motion. Draw each aliquot from just beneath the meniscus.
- Add 2 ul template (extracted DNA from the sample or standard).
- Cover plate with QPCR cap strips. Centrifuge for 2 min at 1200 RPM to collect the mixture. Vortexing is not needed because the sample will mix during the first incubation.

3. Thermocycling (QPCR) parameters

Cover plate with film. Spin plate down for 2 min at 1200 RPM to collect reaction in the bottom of the plate.

- o 10 min, 95°C (initialization: DNA melt phase)

- 65 cycles of (DNA amplification phase):
  - 30 s, 94°C (denaturation step)
  - 40 s, 58°C (annealing step)
    - (monitor SYBR Fluorescence at the end of each cycle)
  - 45 s, 72°C (extension/elongation step)
- 30 s, 72°C (final elongation)
- Melt cycle
  - Cool sample to 58°C, 30s
  - Melt curve (warm to 95°C at ~ 0.1 °C / 10 s)
  - monitor SYBR Fluorescence at maximum rate available on QPCR machine used (~ 1 x each well / 10s on a 96-well plate, rate will be higher if fewer samples are run)
- Cool to 25°C
- Analysis
  - Samples with multiple peaks should be discarded
  - Samples that deviate +/- 2C from standards should be discarded
  - QPCR plates with positive reaction in the negative control well should be rerun
- Store plate in refrigerator

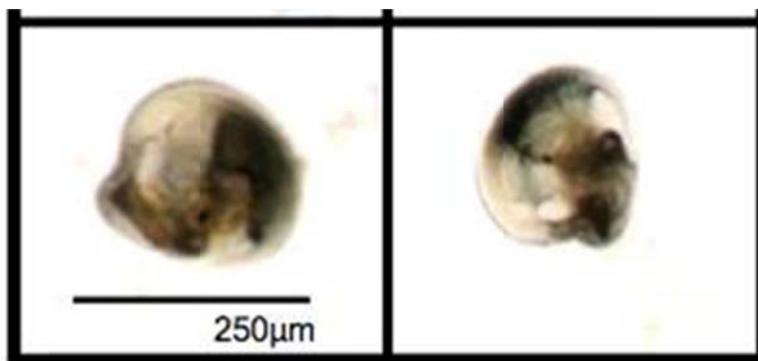
4. Data acquisition

Export output file that contains quantification info and melt curve temperatures.

Include an excel spreadsheet for each plate that contains the NRDA sample names per coordinate, master mix recipe, time started, time finished.

6. Lab counts of total bivalve larvae (non-oyster specific).

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 A-2).



**Figure A-2.** Oyster (*Crassostrea virginica*) bivalve larvae under 20x magnification.

i. If possible, pipette the entire preserved and settled pellet into a 1 ml Sedgewick-Rafter cell (20x50 gridded slide) and count the entire sample. No extrapolations are necessary. If the entire sample fits onto the S-R cell, but larvae are extremely abundant (> 5 per grid cell after the first row), randomly select 4 of the 20 rows to count. The number of rows counted and the total number of larvae counted should be recorded. Because you will have counted 1/5 of the 20 rows, the estimate of the total number of larvae is as follows:

$n * 5 = \text{estimated total larvae} / 1\text{-Liter sample.}$

Return the contents to the 15 ml tube and maintain as evidence. DNA from these samples can be extracted if the DNA sample is lost but the sample is kept preserved.

ii. If the entire settled pellet does not fit into a 1 ml S-R counting slide, it must be subsampled.

a. Record the exact volume of sample.

b. Vortex the sample until the entire settled pellet appears to be in suspension.

c. Invert the sample several times then immediately withdraw a 1-ml sample. Ensure the pipette used has a wide enough opening that it does not clog if large particles, such as copepods or floc, are present. It is critical to pipette this 1-ml accurately.

d. count the sample on a S-R slide as described above. The estimate of the number of larvae per liter will be  $n / (1\text{ml}/\text{total preserved sample ml})$

e. repeat two additional times.

f. Average the three measurements and record the individual estimates and the average on a data sheet.

g. Return the contents to the 15 ml tube and maintain as evidence. DNA from these samples can be extracted if the DNA sample is lost but the sample is kept preserved.

iii. If the sample is very large it can be counted in a 10x10 gridded petri dish.

a. record the exact volume of the sample.

b. Stir the beaker vigorously prior to collecting the aliquot.

c. A Stimpel pipette should be used to collect a 5 or 10 ml aliquot of the sample. Record the exact volume of the subsample.

d. Place the aliquot in a clear petri dish.

e. Bivalve larvae in each section should be quantified and recorded on the data sheet. All sample should be examine under a dissecting scope at 50x magnification.

f. 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  $200\text{ml}/5 \text{ ml} = 40$ ,  $40 * 6 \text{ bivalve veligers} = 240 \text{ bivalves per 1L}$  (original sample volume) or 240 bivalve larvae per L.

g. repeat the subsampling two additional times.

- h. Average the three measurements and record the average on the data sheet.
- i. Return the content of the petri dishes to the original sample and preserve as evidence. DNA from these samples can be extracted if the DNA sample is lost but the sample is kept preserved.

#### Field and Intake Materials Needed

- Niskin or LaMotte Water Sampler (fixed volume, remote trigger and 1 L capacity)
- 1-L sample bottles
- Waterproof labels
- 35 micron sieve (prepare extras in case of rips)
- 50-mL plastic centrifuge tubes
- 250-mL or 500-mL squirt bottles
- Field book
- 95% Ethanol solution. All chemicals must be reagent grade, and water should be filtered and deionized.
- Plastic transfer pipets
- COC forms for larval samples
- Plastic transfer pipets
- COC forms for larval samples

### **D. SOP for Larval Settlement**

#### Spat Sampling Methods

Spat settlement. Settlement plates made of cement board or other appropriate material will be placed at each subsample location within each site. Field teams will return at specified intervals (weather permitting) to attempt to locate and retrieve these boards to help evaluate settlement rates of spat.

#### 1. Objectives

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

#### 2. Materials needed

- Concrete backer board or tiles
- Cable ties
- Ziploc bags (2 gallon size)
- Wire cutters
- Scissors

- Sharpie
- Weatherproof labels
- Crab traps with weight, line, and buoy
- Rope
- Coolers
- PVC poles
- NRDA Oyster Site Form – Recruitment Sampling

Setup:

- 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. Use only the inner 100 cm<sup>2</sup> so as to move away from an edge effect on the plate. Flow around the edge could be more turbulent than natural. It may increase or decrease settlement, but it could introduce variance in settlement unrelated to local conditions.
- Three settlement plates should be connected to a crab trap via cable ties (4 small ½ inch holes should be pre-drilled into the corners). (Figure A-4).
- Attach plates to the top of the cage spaced at least 30cm apart and rough side up. Attach a weight (approximately 5 lb.) via cable tie to the bottom of the trap for stability and attach a surface buoy. Rope should be long enough to account for wind and tidal induced changes in the water level, plus enough length to bring up on the vessel (rope length varies with area).

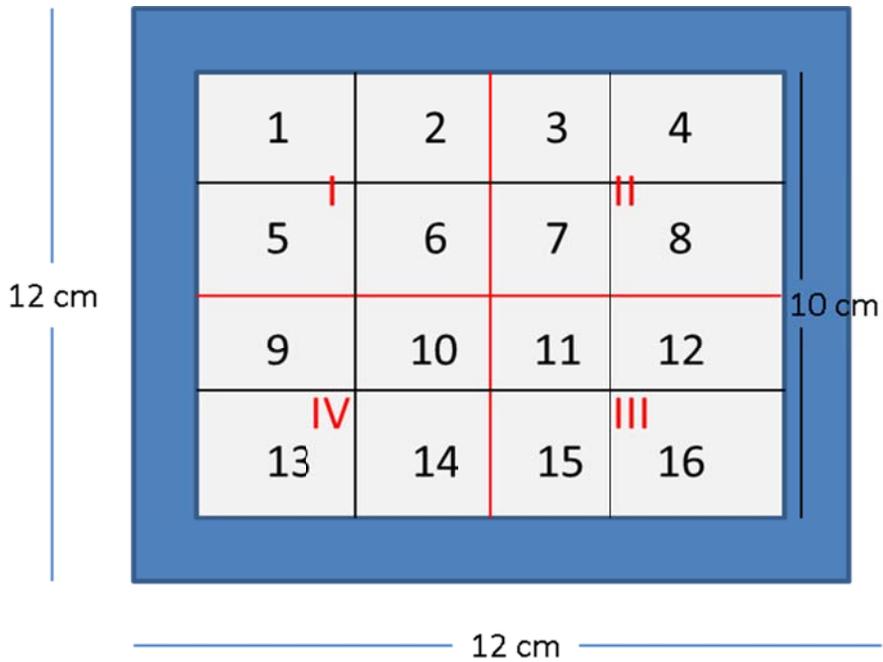
3. Field procedures

- i. Label buoys with identifier that indicates the grid cell ID as well as the corner of the cell (i.e., NE or SW). Identifiers should be written directly on the buoy with a sharpie marker (do not affix a label with the sample ID numbers on duct tape).
- ii. Two sets of three spat settlement plates may be placed at each site (cell) in the event that one set is lost during the deployment period.
- iii. Record exact GPS position of deployment. Crab traps should be deployed at the northeast and southwest corners of the grid cell
- iv. Depth should be checked either with the vessel's depth finder or by lowering a pole or rope over the side. Make sure the amount of rope attached to the pots is appropriate for the site before deploying the pot. 5-10' of rope beyond the depth is ideal.
- v. Remove and replace plates every 21 days (+/- 2 days). If the schedule needs to be adjusted, plates should preferentially be retrieved earlier than scheduled (e.g., approximately two weeks), if weather conditions and personnel availability allow.

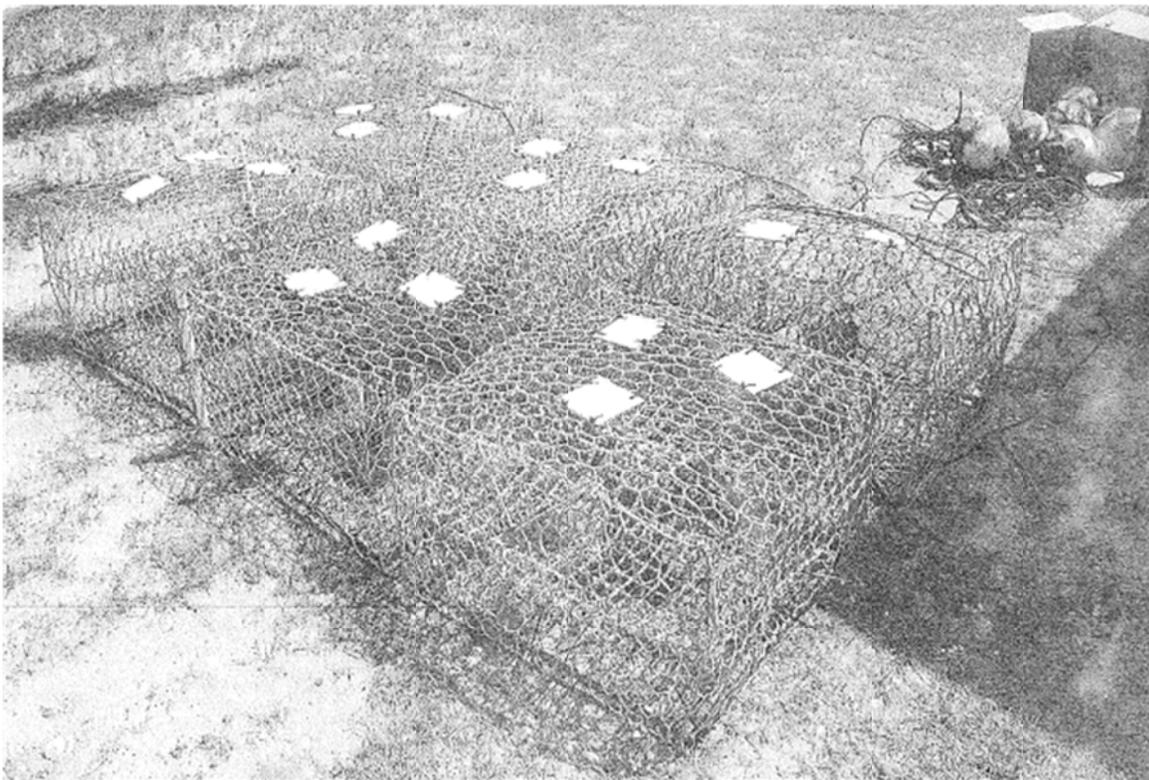
- vi. Deploy plates on a crab trap near oyster substrate in a horizontal position. Where the water is shallow enough and the substrate soft enough, a 10' pvc pole may be planted very near the pot with the GCID and corner (i.e., NE or SW) marked on the pole.
- vii. Retrieve pots and photograph with plates still attached.
- viii. If during retrieval and replacement of settlement plates one trap is missing, do not deploy a replacement trap. If both traps are missing, deploy one replacement trap at either of the coordinates assigned for trap deployment. A trap found exposed during low tide, should be sampled and relocated and at traps located away from their initial deployment site, samples are to be collected and the trap returned to the original coordinates.
- ix. Individually bag and label each retrieved plate; put all three plates into one bag with the sample ID and sample time. Each pot (3 plates bagged individually and then collectively) represents one sample.
- x. Store retrieved plates on ice and take to the intake laboratory. The surface and bottom of the plate should be marked on the plate by etching a B on the bottom side of the plate (side on the trap) with a screw driver or scraping tool. Do not mark the surface side.

#### 4. Lab procedures

- i. Freeze settlement plates until the plates are analyzed.
- ii. Oysters on plates should be enumerated under 10x magnification and both live spats and spat scar (predated spat) should be enumerated.
- iii. The top (surface exposed) of each settlement plate will be examined under a dissecting microscope at 10X magnification. The center area enclosed by a 10 cm x 10 cm frame will be examined for counts. The plates encompass a 12 x 12 cm area and the edges are not examined to minimize the influence of handling damage and hydrodynamic artifacts associated with the edge. For oyster spat, the entire inner 100 cm<sup>2</sup> area is examined and all live oyster spats and recently dead spats (denoted by scars) are enumerated. Other encrusting animals may be enumerated, or the plates may be archived for potential future enumeration of those other encrusting animals. If other encrusting animals are enumerated (e.g., barnacles and serpulid polychaetes), a sub sample is randomly chosen and enumerated. Random selection occurs via a gridded, clear plexiglass overlay placed over the 100 cm<sup>2</sup> inner plate area. If non-oyster encrusting animals are estimated (visually) as >50 individuals a cell chosen to represent ¼ of the plate is enumerated for non-oyster encrusting animals. If non-oyster encrusting animals are estimated (visually) as >100 individuals, a grid representing 1/16 of the plate area is chosen randomly. Random selection occurs by assigning a number to each major grid and using an excel spreadsheet of random numbers from 1 to 4 or 1 to 16.



**Figure A-3.** Settlement plate grid for enumeration



**Figure A-4.** 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 at least 10 market-sized oysters (>74mm) from each site for determination of condition index (CI), gonadal index (GI) and sex.
- ii. Place oyster in a prelabeled bag and place in a cooler with ice.

### 3. Lab procedures (within 72 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.
- 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) 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 unimpacted 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).**

### 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) 20 g wet weight (composite of ~5 individual organisms).
- (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

- (a) 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. If not collecting via quadrat sampling, animals can be collected by hand dredge or hand tongs. Once retrieved via the alternative method, animals should be handled in accordance with the below steps.
- (b) 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.
- (c) 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.
- (d) 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.
- (e) Shellfish should not be opened in the field to minimize the risk of contamination. Rather, sets of whole organisms are collected together in burlap sacks..
- (f) Place all individuals of the same species from a site in a certified-clean glass jar (without foil) or double Ziploc bags (with foil).

(g) 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.

(h) Avoid sources of contamination such as exhaust fumes and engine cooling systems on vessels. Work upwind of any exhausts. Segregate dirty/clean areas. Lay out clean substrates to work on and replace frequently. Take precautions so as not to introduce cross-contamination from oil on boots and shovels.

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

(j) 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.

(k) 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. If warranted, sterane/ triterpane biomarkers may also be quantified.
- 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*

- i. Shovels and/or trowel
- ii. Knife
- iii. Dredges
- iv. Tongs
- v. Gloves (nitrile and knit Kevlar)
- vi. Screen (for sieving out sediment)
- vii. Aluminum foil
- viii. Certified-clean glass jars
- ix. Ziploc bags
- x. Cooler and ice
- xi. Marker pen
- xii. Waterproof sample labels
- xiii. Clear tape

- xiv. Burlap sacks
- xv. Coolers

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.

## **G. SOP for Sediment Collection for Contaminant Analysis.**

### 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) The primary method of collection should be a ponar sampler. If the team is unable to collect valid samples using the ponar, then a diver should collect core samples. If the team is unable to collect core samples, then a diver should collect samples using a horizontal grab (box sampler). Procedures for using the primary method of collection are described below. Procedures for using the alternative methods are described at the end of this SOP (subsection 8).
- (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) For sampling using the ponar, 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.

## 5. 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.

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

(a) The NRDA Field Sampling Checklist generically summarizes pre- and post-field sampling tasks.<sup>5</sup>

(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]

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<sup>5</sup> The NRDA Field Sampling Checklist was originally located on the case's ftp site at [REDACTED] and is currently located on [REDACTED]

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

## 7. 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.

## 8. Alternative methods for Subtidal Collection

### (a) Diver Collected Core Samples

- i. Divers should approach from down-current of the station taking care not to disturb surface sediments. For sites with fine sediments and flocculent layers, great care should be taken to approach the site slowly and using buoyancy to settle to the bottom without disturbing surface materials. Limited visibility may require marking a station with a buoy and divers descend the buoy line to the station.
- ii. Divers will carry core tubes and caps to the bottom in mesh bags or other appropriate container. In multiple cores are needed to collect sufficient sediment for analysis, it is preferred that they be collected at one time.
- iii. Once at the bottom, the diver will locate appropriate substrate for sampling, avoiding areas that are not representative of the station, areas of substantial rock or shellhash. If water is turbid from the divers’ approach, the sampler should move farther up current or wait until the suspended sediments near the sediment surface have settled.
- iv. To collect a sample, the core tube is inserted, sharpened edge first into the sediment. If needed, the core tube may be gently rotated or moved from side-to-side to facilitate insertion. Movement should not cause suspension of the surface sediment. Insert the core tube approximately 6” into the sediment. Less than 6” is acceptable provided the surface layer (upper 2 cm) is not disturbed.

- v. Gently place a cap over the top of the core. The cap should be secure, but does not necessarily need to be inserted completely on the core. Once the top cap is in place, the diver will insert a hand into the sediment under the core tube to hold sediments in the core tube during removal from the sediment. Using both hands, carefully remove the core from the bottom, taking care not to allow movement of the sediment in the core. Once the core is extracted from the bottom, a cap is placed over the bottom the core. The diver returns the core to the surface where the core will be processed. The core must be kept in the vertical position at all times.
- vi. If multiple cores are required, it is best if all core tubes are inserted into the substrate and top caps in place prior to extraction of any of the core tubes. Limited visibility may make this difficult; however, careful systematic placement of cores will help with locating the cores for extraction. When sampling multiple cores, a system should be in place whereby all cores are maintained in the vertical position throughout the sampling process.
- vii. Sample processing may be completed on the sampling vessel or in the laboratory. Clean off any extraneous sediment from the outside of the core tube. Mark the top of the core with a sharpie. To process samples on the sampling vessel, retrieve the cores to a dry area to process the sample. If sediment has been disturbed, the cores should not be processed until surface sediments have settled. Mark the sediment surface with a sharpie. Photograph the core and record the general sediment characteristics including the following:
  - a. general water color
  - b. the presence of any layers,
  - c. general grain size (sand, silt, clay)
  - d. the presence of any major organic matter (ie. shell hash, eelgrass)
  - e. initial sediment depth (cm or inches)
  - f. settling time (if applicable)
  - g. final sediment depth (if applicable)
  - h. final water turbidity (opaque, translucent, transparent, clear, any color)
  - i. Presence or absence of petroleum or tar balls
  - j. Presence or absence of sheen
  - k. Odor and type of odor if present (petroleum, sulfides)
- viii. Decant overlying water from the surface of the sample by removing the top cap and siphoning the overlying water out, without removing surface flocculent layers. Alternatively, a small hole can be drilled into the core tube approximately 0.25" above the final sediment surface (after settling). As mentioned above, turbid water in the sampler may indicate the suspension of recently deposited surface sediments. To prevent loss of surface sediment, allow turbid water to clear prior to decanting and collecting samples. If water does not clear or substantial suspended sediments are lost during the transport of the sample, a new sample should be considered. Once the overlying water has been removed, use a stainless steel spoon or spatula to collect the upper 2 cm of sediment and place in the sampling container or into a stainless steel bowl for compositing.

- ix. In some cases, core samples may be processed in the laboratory. In such cases, place the core tubes vertically in a cooler and pack with ice. Hold samples in a cooler or freezer at approximately 4°C with wet ice or freeze with dry ice.

(b) Diver Collected Horizontal Grab

- i. Divers should approach from down-current of the station taking care not to disturb surface sediments.
- ii. ***Sampling method for cohesive sediments:*** Once the diver is in place, open the cover of the grab sampler. Cut a clean face into the sediment surface by inserting the leading face of the cover vertically into the sediment and then pulling backwards towards the diver (at this point the sampler is upside down; Figure 1). A clean, vertical face should remain. This step should be performed slowly such that adjacent surface sediments are undisturbed.

Invert the sampler so that it is “right-side up” with the handle is facing the diver and the opening of the box facing the freshly cut sediment face. Ensure that the lid is open. Place the front edge of the box at the desired sampling depth. Push the box horizontally into the sediment until the face contacts the back wall of the sampler. Close the lid of the sampler and extract the sample.

For chemistry sampling special care should be taken in transporting the collected sample to the surface. ***Bring to the surface with the open end slightly elevated to prevent water loss at the surface (approximately a 45° angle).*** Rapid movement may cause winnowing of fine-grained surface sediment and loss of recently deposited sediments.

Turbid water in the sampler may indicate the suspension and potential loss of fine particles which represent an important component of chemistry samples. If important for the project objectives, turbid water should be allowed to settle prior to decanting the overlying water and collecting the sample. If substantial amounts of suspended sediment are lost during movement of the sampler, a new sample should be collected.



Figure 1. Using the horizontal grab for sampling cohesive sediments.

- iii. ***Sampling method for non-cohesive sediments:*** For non-cohesive sediments, such as coarse sand, the cut face will not maintain its form. When appropriate, the horizontal grab can be simply inserted into the sediment surface to the desired depth, pushed horizontally until the box is full of sediment, and then the cover rotated in place and the sample removed (Figure 2).

***Bring to the surface with the open end slightly elevated to prevent water loss at the surface (approximately a 45° angle).*** Special care should be taken not to lose fine surface sediments. Rapid movement may cause winnowing of fine-grained surface sediment and loss of recently deposited sediments.

Turbid water in the sampler may indicate the suspension and potential loss of fine particles which represent an important component of chemistry samples. If important for the project objectives, turbid water should be allowed to settle prior to decanting the overlying water and collecting the sample. If substantial amounts of suspended sediment are lost during movement of the sampler, a new sample should be collected.



Figure 2. Using the horizontal grab for sampling uncohesive sediments (such as coarse sand).

- iv. Sample processing is similar for either sampling method. Retrieve to a dry area to process the sample. Decant overlying water from the surface of the sample, avoiding the loss of fine-grained surface sediment. As mentioned above, turbid water in the sampler may indicate the suspension of recently deposited surface sediments. To prevent loss of surface sediment, allow turbid water to clear prior to decanting and collecting samples. If water does not clear or substantial suspended sediments are lost during the transport of the sample, a new sample should be considered. In some cases a different sampling method, such as a diver-collected core, may need to be considered. The field team leader is responsible for deciding whether to attempt additional grabs within the guidelines of the SAP.
- v. Once all surface water has been removed, transfer the sediment with a clean, non-reactive utensil into the appropriate pre-labeled sample container. Hold samples in a cooler or freezer at approximately 4°C with wet ice.

#### *Equipment List*

- x. Ponar sampler
- xi. Coring device and cores
- xii. Horizontal grab (box sampler)
- xiii. Shovel (for intertidal zones)
- xiv. Scoops
- xv. Certified-clean glass container with Teflon-lined lid
- xvi. Ziploc or Whirl-Pak bags
- xvii. Dredges
- xviii. Tongs

- xix. Gloves
- xx. Screen (for sieving out sediment)
- xxi. Detergent
- xxii. Sorbent pad and/or boom
- xxiii. 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

## Appendix B: Oyster Condition Guide

### Live Oyster Indicators

- Emergent structure present
- Sedimentation over habitat low
- Multiple Shells still hinged
- Live intact oysters common
- Live spat common



Live oyster and emergent structure on subtidal oyster reef



Live oyster clumps on sparsely populated live reef



Live oyster and emergent intertidal structure on oyster reef

## Recently Dead or dying

- Emergent structure present
- Sedimentation over reef low to moderate
- Multiple shells hinged in life position with minimal fouling inside shell.
  - *Live oysters extremely rare*
  - *Very low levels of spat*
- Oyster shell still hard and difficult to break in center.



Dead (gaped) oysters with some sedimentation and low and emergent structure

## Relic

- Little emergent structure present
- Sedimentation over reef moderate to high
- No live oysters or spat
- Oyster shell in large pieces (not intact) or easily broken.
- Dead oyster not hinged or if still articulated with heavy fouling inside box.
- Oyster shell is discolored (black) due to anoxic conditions.



Dead (non-hinged) oysters with sedimentation and low and emergent structure

## Appendix C: OYSTER SAMPLE ID NAMING CONVENTION

### NOAA NRDA Sample Format:

- **LocationCode – DateCode - Matrix Leader# 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 or S = Sediment);
  - 2 or 3-digit leader code; and
  - 2-digit sample number.
  
- **EXAMPLE: LAAM24-A0502-TA102**
  - LocationCode = LAAM24;
  - Date = 5/2/2010;
  - Matrix=Tissue;
  - Leader code = A1;
  - 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 there are several different tissue sample types, we will add an identifier after the sample number that will indicate the sample type for tissue samples.
  - Q = quadrat sample;
  - L = larval sample (add LS for surface, LM for mid water column or LB for bottom); and
  - SP = settlement plate.
  - Examples: **LAAM24-A0502-T10302Q; LAAM24-A0502-T10303LS**

- **Dredge Samples**

- Contaminant Sample

- Add “-CT” to the end of the sample name, e.g., **LAAM24-A0502-T10302DR-CT**
- Composite across dredges if necessary. In this case, indicate in the “Sample Notes” which dredges the sample is taken from and how many of each size class were retained from each dredge. GPS coordinates should correspond to the center of the entire cell, rather than a specific dredge.

- Gonad/Disease subsample

- Add “-GD” to the end of the sample name, e.g., **LAAM24-A0502-T10302DR-GD**. GPS coordinates should correspond to the center of the entire cell.

- 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
- Quadrat Number

- Larval Samples

- Cell number
- Depth (Surface, Middle, or Bottom)

- Settlement Plates

- Cell number

- Sediment

- Cell number

## **Lab Teams**

- **Quadrat subsamples**

- Contaminant subsample

- 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-T10302Q-CT**
- Gonad/Disease subsample
  - The sample should have “-GD” at the end, e.g., **LAAM24-A0502-T10302Q-GD**.
- **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.

## **Appendix D: Sample Forms**

The forms in this Appendix represent the various versions of the field forms used during the implementation of the Phase I Amendment 2 plan. Forms changed to accommodate different rounds of sampling, as well as improvements in the forms over time. The various forms are listed below, along with dates of distribution for revised forms:

### **Quadrat Sampling**

- NRDA Oyster Site Form (Quadrat), Version 1.
- NRDA Oyster Site Form (Quadrat), Version 2. September 21, 2010.
- NRDA Oyster Site Form (Quadrat), Version 3. September 29, 2010.

### **Larval, Recruitment, and Dredging Samples**

- NRDA Oyster Site Form (Larval), Version 1.
- NRDA Oyster Site Form – Recruitment Sampling, Version 1 September 16, 2010.
- NRDA Oyster Site Form (Larval), Version 2. September 19, 2010. (Similar to Version 1; used for settlement plate deployment)
- NRDA Oyster Site Form – Recruitment Sampling, Version 2 September 30, 2010.

### **Ground Truthing of Mapped Sites**

- NRDA Oyster Site Form – Dredging, Version 1. (Used for ground truthing with dredge only, not for sample collection)

### **Lab Forms**

- NRDA Oyster Contaminant/Recruitment Form, Version 1.
- NRDA Oyster Quadrat Form, Version 1.

Leader Code: \_\_\_\_\_

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

### NRDA Oyster Site Form

*One form should be used for each assigned site.*

#### 1. Site Descriptors

Site Name \_\_\_\_\_ Cell Number \_\_\_\_\_

Time: \_\_\_\_\_ Date: \_\_\_\_\_

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

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

#### 2. Physical/Chemical Parameters

Air Temperature: \_\_\_\_\_ Surface Temperature: \_\_\_\_\_ Bottom Temperature \_\_\_\_\_

Surface Dissolved Oxygen (%): \_\_\_\_\_ Bottom Dissolved Oxygen (%): \_\_\_\_\_

Surface D.O. (mg/L): \_\_\_\_\_ Bottom D.O. (mg/L): \_\_\_\_\_

Surface Salinity (ppt): \_\_\_\_\_ Bottom Salinity (ppt): \_\_\_\_\_

Weather Conditions \_\_\_\_\_

Oiled Condition (check one):  none  Sheen  Scattered Deposits  
 Surface substantially covered  Surface completely covered  Deep Deposits

#### 3. Site (cell) corners - place markers 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 \_\_\_\_\_

Center Point Lat. \_\_\_\_\_ Long \_\_\_\_\_

#### 4. Quadrat (subsample locations)

Example Sample#: LAAM24-A0502-T6002Q

Q 1 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Time: \_\_\_\_\_

Q 2 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Time: \_\_\_\_\_

Q 3 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Time: \_\_\_\_\_

Q 4 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Time: \_\_\_\_\_

**5. Sediment**

Example Sample#: LAAM24-A0502-S6006

Composite 1 = Composite of Quadrats \_\_\_\_\_ and \_\_\_\_\_ Sample #: \_\_\_\_\_

Time: \_\_\_\_\_ Time: \_\_\_\_\_

Initials of sampler: \_\_\_\_\_ and \_\_\_\_\_

Composite 2 = Composite of Quadrats \_\_\_\_\_ and \_\_\_\_\_ Sample #: \_\_\_\_\_

Time: \_\_\_\_\_ Time: \_\_\_\_\_

Initials of sampler: \_\_\_\_\_ and \_\_\_\_\_

\*\*\*\*\*

Responsible: \_\_\_\_\_ Date \_\_\_\_\_  
Party Rep (Name) (Agency) (Signature)

State Rep: \_\_\_\_\_ Date \_\_\_\_\_  
(Name) (Agency) (Signature)

Federal Rep: \_\_\_\_\_ Date \_\_\_\_\_  
(Name) (Agency) (Signature)

Data Entry: \_\_\_\_\_ Date \_\_\_\_\_  
(Name) (Agency) (Signature)

Leader Code: \_\_\_\_\_

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

### NRDA Oyster Site Form

*One form should be used for each assigned site.*

#### 1. Site Descriptors

Site Name \_\_\_\_\_ Cell Number \_\_\_\_\_

Time: \_\_\_\_\_ Date: \_\_\_\_\_

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

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

#### 2. Physical/Chemical Parameters

Air Temperature: \_\_\_\_\_ Surface Temperature: \_\_\_\_\_ Bottom Temperature \_\_\_\_\_

Surface Dissolved Oxygen (%): \_\_\_\_\_ Bottom Dissolved Oxygen (%): \_\_\_\_\_

Surface D.O. (mg/L): \_\_\_\_\_ Bottom D.O. (mg/L): \_\_\_\_\_

Surface Salinity (ppt): \_\_\_\_\_ Bottom Salinity (ppt): \_\_\_\_\_

Weather Conditions \_\_\_\_\_

Oiled Condition (check one):  none  Sheen  Scattered Deposits  
 Surface substantially covered  Surface completely covered  Deep Deposits

#### 3. Site (cell) corners - place markers 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 \_\_\_\_\_

Center Point Lat. \_\_\_\_\_ Long \_\_\_\_\_

#### 4. Quadrat (subsample locations)

Example Sample#: LAAM24-A0502-T6002Q

Q 1 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_

Time: \_\_\_\_\_ Quadrat Size (check one):  0.25m<sup>2</sup>  1m<sup>2</sup>  Other (Fill in)

Q 2 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_

Time: \_\_\_\_\_ Quadrat Size (check one):  0.25m<sup>2</sup>  1m<sup>2</sup>  Other (Fill in)

Q 3 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_

Time: \_\_\_\_\_ Quadrat Size (check one): \_\_\_\_\_ 0.25m<sup>2</sup> \_\_\_\_\_ 1m<sup>2</sup> \_\_\_\_\_ Other (Fill in)

Q 4 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_

Time: \_\_\_\_\_ Quadrat Size (check one): \_\_\_\_\_ 0.25m<sup>2</sup> \_\_\_\_\_ 1m<sup>2</sup> \_\_\_\_\_ Other (Fill in)

**5. Sediment**

Example Sample#: LAAM24-A0502-S6006

Composite 1 = Composite of Quadrats \_\_\_\_\_ and \_\_\_\_\_ Sample #: \_\_\_\_\_

Time: \_\_\_\_\_ Time: \_\_\_\_\_

Initials of sampler: \_\_\_\_\_ and \_\_\_\_\_

Composite 2 = Composite of Quadrats \_\_\_\_\_ and \_\_\_\_\_ Sample #: \_\_\_\_\_

Time: \_\_\_\_\_ Time: \_\_\_\_\_

Initials of sampler: \_\_\_\_\_ and \_\_\_\_\_

\*\*\*\*\*

Responsible: \_\_\_\_\_ Date \_\_\_\_\_

Party Rep (Name) (Agency) (Signature)

State Rep: \_\_\_\_\_ Date \_\_\_\_\_

(Name) (Agency) (Signature)

Federal Rep: \_\_\_\_\_ Date \_\_\_\_\_

(Name) (Agency) (Signature)

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

(Name) (Agency) (Signature)

Leader Code: \_\_\_\_\_

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

### NRDA Oyster Site Form

*One form should be used for each assigned site.*

#### 1. Site Descriptors

Site Name \_\_\_\_\_ Cell Number \_\_\_\_\_

Time: \_\_\_\_\_ Date: \_\_\_\_\_

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

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

#### 2. Physical/Chemical Parameters

Air Temperature: \_\_\_\_\_ Surface Temperature: \_\_\_\_\_ Bottom Temperature \_\_\_\_\_

Surface Dissolved Oxygen (%): \_\_\_\_\_ Bottom Dissolved Oxygen (%): \_\_\_\_\_

Surface D.O. (mg/L): \_\_\_\_\_ Bottom D.O. (mg/L): \_\_\_\_\_

Surface Salinity (ppt): \_\_\_\_\_ Bottom Salinity (ppt): \_\_\_\_\_

Weather Conditions \_\_\_\_\_

Oiled Condition (check one):  none  Sheen  Scattered Deposits  
 Surface substantially covered  Surface completely covered  Deep Deposits

#### 3. Site (cell) corners - place markers 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 \_\_\_\_\_

Center Point Lat. \_\_\_\_\_ Long \_\_\_\_\_

#### 4. Quadrat (subsample locations)

Example Sample#: LAAM24-A0502-T6002Q

Q 1 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_

Time: \_\_\_\_\_ Quadrat Size (check one):  0.25m<sup>2</sup>  1m<sup>2</sup>  Other (Fill in)

Q 2 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_

Time: \_\_\_\_\_ Quadrat Size (check one):  0.25m<sup>2</sup>  1m<sup>2</sup>  Other (Fill in)

Q 3 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_

Time: \_\_\_\_\_ Quadrat Size (check one): \_\_\_\_\_ 0.25m<sup>2</sup> \_\_\_\_\_ 1m<sup>2</sup> \_\_\_\_\_ Other (Fill in)

Q 4 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_

Time: \_\_\_\_\_ Quadrat Size (check one): \_\_\_\_\_ 0.25m<sup>2</sup> \_\_\_\_\_ 1m<sup>2</sup> \_\_\_\_\_ Other (Fill in)

**5. Dredge (If necessary):**

Example Sample#: LAAM24-A0502-T6003DR

NOTE: A single sample should be collected for each site. The sample should consist of at least 10 adult oysters. Pull up to four dredges until the required volume of oysters is collected.

SAMPLE #: \_\_\_\_\_

*Dredge 1 (If Needed)*

Start of Dredge 1 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

End of Dredge 1= Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

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*Dredge 2 (If Needed)*

Start of Dredge 2 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

End of Dredge 2= Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

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*Dredge 3 (If Needed)*

Start of Dredge 3 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

End of Dredge 3= Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

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*Dredge 4 (If Needed)*

Start of Dredge 4 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

End of Dredge 4 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

\_\_\_\_\_  
\_\_\_\_\_

**5. Sediment**

Example Sample#: LAAM24-A0502-S6006

Composite 1 = Composite of Quadrats \_\_\_\_\_ and \_\_\_\_\_ Sample #: \_\_\_\_\_

Time: \_\_\_\_\_ Time: \_\_\_\_\_

Initials of sampler: \_\_\_\_\_ and \_\_\_\_\_

Composite 2 = Composite of Quadrats \_\_\_\_\_ and \_\_\_\_\_ Sample #: \_\_\_\_\_

Time: \_\_\_\_\_ Time: \_\_\_\_\_

Initials of sampler: \_\_\_\_\_ and \_\_\_\_\_

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Responsible: \_\_\_\_\_ Date \_\_\_\_\_  
Party Rep (Name) (Agency) (Signature)

State Rep: \_\_\_\_\_ Date \_\_\_\_\_  
(Name) (Agency) (Signature)

Federal Rep: \_\_\_\_\_ Date \_\_\_\_\_  
(Name) (Agency) (Signature)

Data Entry: \_\_\_\_\_ Date \_\_\_\_\_  
(Name) (Agency) (Signature)

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

### NRDA Oyster Site Form

*One form should be used for each assigned site.*

#### 1. Site Descriptors

Site Name \_\_\_\_\_ Cell Number \_\_\_\_\_

Time: \_\_\_\_\_ Date: \_\_\_\_\_

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

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

#### 2. Physical/Chemical Parameters

Surface Temperature: \_\_\_\_\_ Air Temperature: \_\_\_\_\_ Bottom Temperature \_\_\_\_\_

Bottom Dissolved Oxygen: \_\_\_\_\_ Surface Salinity: \_\_\_\_\_ Bottom Salinity \_\_\_\_\_

Weather Conditions \_\_\_\_\_

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

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

#### 3. Site (cell) corners - place markers 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 \_\_\_\_\_

Center Point Lat. \_\_\_\_\_ Long \_\_\_\_\_

#### 3. Dredge:

Start of Dredge = Lat \_\_\_\_\_ Long \_\_\_\_\_

End of Dredge = Lat \_\_\_\_\_ Long \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

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**5. Larvae Samples**

Example Sample#: LAAM24-A0502-T6003L

S 1 (Bottom) = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

S 2 (Bottom) = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

S 3 (Middle) = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

S 4 (Top) = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

S 5 (Top) = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

**6. Settlement Plate Deployment**

Coordinates of crab pot 1 = Lat \_\_\_\_\_ Long \_\_\_\_\_

Coordinates of crab pot 2 = Lat \_\_\_\_\_ Long \_\_\_\_\_

\*\*\*\*\*

Field Team Leader: \_\_\_\_\_ Date \_\_\_\_\_  
(Name) (Agency) (Signature)

Collected by: \_\_\_\_\_ Date \_\_\_\_\_  
(Name) (Agency) (Signature)

Data Entry: \_\_\_\_\_ Date \_\_\_\_\_  
(Name) (Agency) (Signature)

Team Leader Code: \_\_\_\_\_

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

**NRDA Oyster Site Form – Recruitment Sampling**

*One form should be used for each assigned site.*

**1. Site Descriptors**

Site Name \_\_\_\_\_ Cell Number \_\_\_\_\_

Time: \_\_\_\_\_ Date: \_\_\_\_\_

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

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

**2. Physical/Chemical Parameters**

Air Temperature: \_\_\_\_\_ Surface Temperature: \_\_\_\_\_ Bottom Temperature \_\_\_\_\_

Surface Dissolved Oxygen: \_\_\_\_\_ Bottom Dissolved Oxygen: \_\_\_\_\_

Surface Salinity: \_\_\_\_\_ Bottom Salinity: \_\_\_\_\_

Weather Conditions \_\_\_\_\_

Oiled Condition (check one):  none  Sheen  Scattered Deposits  
 Surface substantially covered  Surface completely covered  Deep Deposits

**3. Dredge:**

Example Sample#: LAAM24-A0502-T6001DR

NOTE: A single sample should be collected for each site. The sample should consist of at least 10 adult oysters. Pull up to four dredges until the required volume of oysters is collected.

SAMPLE #: \_\_\_\_\_

*Dredge 1*

Start of Dredge 1 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

End of Dredge 1= Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

\_\_\_\_\_  
\_\_\_\_\_

*Dredge 2 (If Needed)*

Start of Dredge 2 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

End of Dredge 2= Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

\_\_\_\_\_  
\_\_\_\_\_

*Dredge 3 (If Needed)*

Start of Dredge 3 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

End of Dredge 3 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

\_\_\_\_\_  
\_\_\_\_\_

*Dredge 4 (If Needed)*

Start of Dredge 4 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

End of Dredge 4 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

\_\_\_\_\_  
\_\_\_\_\_

**4. Settlement Plate Retrieval**

Example Sample#: LAAM24-A0502-T6004SP

*Crab Pot 1*

Corner of Gridcell: \_\_\_\_\_

Sample #'s: \_\_\_\_\_

Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

*Crab Pot 2*

Corner of Gridcell: \_\_\_\_\_

Sample #'s: \_\_\_\_\_

Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

**5. Settlement Plate Deployment**

*Crab Pot 1*

Corner of Gridcell: \_\_\_\_\_

Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

*Crab Pot 2*

Corner of Gridcell: \_\_\_\_\_

Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

**6. Larvae Samples**

Example Sample#: LAAM24-A0502-T6008L

Bottom = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

Time: \_\_\_\_\_ Initials of Sampler \_\_\_\_\_

Bottom = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

Time: \_\_\_\_\_ Initials of Sampler \_\_\_\_\_

Middle = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

Time: \_\_\_\_\_ Initials of Sampler \_\_\_\_\_

Top = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

Time: \_\_\_\_\_ Initials of Sampler \_\_\_\_\_

Top = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

Time: \_\_\_\_\_ Initials of Sampler \_\_\_\_\_

\*\*\*\*\*

Responsible: \_\_\_\_\_ Date \_\_\_\_\_

Party Rep (Name) (Agency) (Signature)

State Rep: \_\_\_\_\_ Date \_\_\_\_\_

(Name) (Agency) (Signature)

Federal Rep: \_\_\_\_\_ Date \_\_\_\_\_

(Name) (Agency) (Signature)

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

(Name) (Agency) (Signature)

Team Leader Code: \_\_\_\_\_

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

**NRDA Oyster Site Form**

*One form should be used for each assigned site.*

**1. Site Descriptors**

Site Name \_\_\_\_\_ Cell Number \_\_\_\_\_

Time: \_\_\_\_\_ Date: \_\_\_\_\_

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

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

**2. Physical/Chemical Parameters**

Air Temperature: \_\_\_\_\_ Surface Temperature: \_\_\_\_\_ Bottom Temperature \_\_\_\_\_

Surface Dissolved Oxygen: \_\_\_\_\_ Bottom Dissolved Oxygen: \_\_\_\_\_

Surface Salinity: \_\_\_\_\_ Bottom Salinity: \_\_\_\_\_

Weather Conditions \_\_\_\_\_

Oiled Condition (check one):  none  Sheen  Scattered Deposits  
 Surface substantially covered  Surface completely covered  Deep Deposits

**3. Dredge:**

Start of Dredge = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

End of Dredge = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**6. Settlement Plate Deployment**

Coordinates of crab pot 1 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

Coordinates of crab pot 2 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

**5. Larvae Samples**

Example Sample#: LAAM24-A0502-T6003L

Bottom = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

Time: \_\_\_\_\_ Initials of Sampler \_\_\_\_\_

Bottom = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

Time: \_\_\_\_\_ Initials of Sampler \_\_\_\_\_

Middle = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

Time: \_\_\_\_\_ Initials of Sampler \_\_\_\_\_

Top = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

Time: \_\_\_\_\_ Initials of Sampler \_\_\_\_\_

Top = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

Time: \_\_\_\_\_ Initials of Sampler \_\_\_\_\_

\*\*\*\*\*

Responsible: \_\_\_\_\_ Date \_\_\_\_\_

Party Rep (Name) (Agency) (Signature)

State Rep: \_\_\_\_\_ Date \_\_\_\_\_

(Name) (Agency) (Signature)

Federal Rep: \_\_\_\_\_ Date \_\_\_\_\_

(Name) (Agency) (Signature)

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

(Name) (Agency) (Signature)

Team Leader Code: \_\_\_\_\_

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

**NRDA Oyster Site Form – Recruitment Sampling**

*One form should be used for each assigned site.*

**1. Site Descriptors**

Site Name \_\_\_\_\_ Cell Number \_\_\_\_\_

Time: \_\_\_\_\_ Date: \_\_\_\_\_

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

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

**2. Physical/Chemical Parameters**

Air Temperature: \_\_\_\_\_ Surface Temperature: \_\_\_\_\_ Bottom Temperature \_\_\_\_\_

Surface Dissolved Oxygen (%): \_\_\_\_\_ Bottom Dissolved Oxygen (%): \_\_\_\_\_

Surface D.O. (mg/L): \_\_\_\_\_ Bottom D.O. (mg/L): \_\_\_\_\_

Surface Salinity (ppt): \_\_\_\_\_ Bottom Salinity (ppt): \_\_\_\_\_

Weather Conditions \_\_\_\_\_

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

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

**3. Dredge:**

Example Sample#: LAAM24-A0502-T6001DR

NOTE: A single sample should be collected for each site. The sample should consist of at least 10 adult oysters. Pull up to four dredges until the required volume of oysters is collected.

SAMPLE #: \_\_\_\_\_

*Dredge 1*

Start of Dredge 1 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

End of Dredge 1= Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

\_\_\_\_\_  
\_\_\_\_\_

*Dredge 2 (If Needed)*

Start of Dredge 2 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

End of Dredge 2= Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

\_\_\_\_\_  
\_\_\_\_\_

*Dredge 3 (If Needed)*

Start of Dredge 3 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

End of Dredge 3= Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

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---

*Dredge 4 (If Needed)*

Start of Dredge 4 = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

End of Dredge 4= Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

---

---

**4. Settlement Plate Retrieval**

Example Sample#: LAAM24-A0502-T6004SP

*Crab Pot 1*

Corner of Gridcell: \_\_\_\_\_

Sample #'s: \_\_\_\_\_

Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

*Crab Pot 2*

Corner of Gridcell: \_\_\_\_\_

Sample #'s: \_\_\_\_\_

Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

**5. Settlement Plate Deployment**

*Crab Pot 1*

Corner of Gridcell: \_\_\_\_\_

Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

*Crab Pot 2*

Corner of Gridcell: \_\_\_\_\_

Lat \_\_\_\_\_ Long \_\_\_\_\_ Time: \_\_\_\_\_

**6. Larvae Samples**

Example Sample#: LAAM24-A0502-T6008L

Bottom = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

Time: \_\_\_\_\_ Initials of Sampler \_\_\_\_\_

Bottom = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

Time: \_\_\_\_\_ Initials of Sampler \_\_\_\_\_

Middle = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

Time: \_\_\_\_\_ Initials of Sampler \_\_\_\_\_

Top = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

Time: \_\_\_\_\_ Initials of Sampler \_\_\_\_\_

Top = Lat \_\_\_\_\_ Long \_\_\_\_\_ Sample #: \_\_\_\_\_ Depth (m): \_\_\_\_\_

Time: \_\_\_\_\_ Initials of Sampler \_\_\_\_\_

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Responsible: \_\_\_\_\_ Date \_\_\_\_\_

Party Rep (Name) (Agency) (Signature)

State Rep: \_\_\_\_\_ Date \_\_\_\_\_

(Name) (Agency) (Signature)

Federal Rep: \_\_\_\_\_ Date \_\_\_\_\_

(Name) (Agency) (Signature)

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

(Name) (Agency) (Signature)

Leader Code: \_\_\_\_\_

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

**NRDA Oyster Site Form - Dredging**

*One form should be used for each assigned site.*

**Site Descriptors**

Site Name \_\_\_\_\_ Cell Number \_\_\_\_\_

Time: \_\_\_\_\_ Date: \_\_\_\_\_

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

**Dredge #1**

Start of Dredge = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time \_\_\_\_\_

End of Dredge = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Dredge #2**

Start of Dredge = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time \_\_\_\_\_

End of Dredge = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Dredge #3**

Start of Dredge = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time \_\_\_\_\_

End of Dredge = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

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**Dredge #4**

Start of Dredge = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time \_\_\_\_\_

End of Dredge = Lat \_\_\_\_\_ Long \_\_\_\_\_ Time \_\_\_\_\_

Description of resource (live, recently dead, dead, etc.) :

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**Site Notes:**



**NRDA Oyster Contaminant/Recruitment Form [Print Double-Sided]**

**1. Site Descriptors**

Site Name \_\_\_\_\_ Cell Number \_\_\_\_\_

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

Time of processing: \_\_\_\_\_ Date: \_\_\_\_\_

**2. Larvae Filtering and Lab Specification**

Filter size: \_\_\_\_\_ (microns)

Sample #

S 1 (Bottom) : \_\_\_\_\_ Analysis (Check One): \_\_\_ Veliger Count \_\_\_ Quantitative PCR

S 2 (Bottom) : \_\_\_\_\_ Analysis (Check One): \_\_\_ Veliger Count \_\_\_ Quantitative PCR

S 3 (Middle) : \_\_\_\_\_ Analysis (Check One): \_\_\_ Veliger Count \_\_\_ Quantitative PCR

S 4 (Top): \_\_\_\_\_ Analysis (Check One): \_\_\_ Veliger Count \_\_\_ Quantitative PCR

S 5 (Top): \_\_\_\_\_ Analysis (Check One): \_\_\_ Veliger Count \_\_\_ Quantitative PCR

**2. Settlement Plates**

Sample #

Plate 1: \_\_\_\_\_ Processed? \_\_\_\_\_

Plate 2: \_\_\_\_\_ Processed? \_\_\_\_\_

Plate 3: \_\_\_\_\_ Processed? \_\_\_\_\_

**3. Contaminant Analysis**

The sample ID will be the sample ID for the contaminant sample with “-CT” added to the end. The sample should consist of 6 large oysters.

Contaminant analysis (sample ID \_\_\_\_\_) \_\_\_\_\_# of oysters

**4. Gonad and Disease Analysis**

The sample ID will be the sample ID for the contaminant sample with “-GD” added to the end. The sample should consist of 10 large oysters.

Disease and Gonad analyses (sample ID \_\_\_\_\_) \_\_\_\_\_# of oysters

Lab Team Leader: \_\_\_\_\_  
(Name) (Agency) (Signature) (Date)

Analyzed By: \_\_\_\_\_  
(Name) (Agency) (Signature) (Date)

Data Entry: \_\_\_\_\_  
(Name) (Agency) (Signature) (Date)

## NRDA Oyster Quadrat Form *[Print Double-Sided]*

### 1. Site Descriptors

Site Name \_\_\_\_\_ Cell Number \_\_\_\_\_

Quadrat #: \_\_\_\_\_ Sample #: \_\_\_\_\_

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

Time of processing: \_\_\_\_\_ Date: \_\_\_\_\_

### 2. Sampling Results: Abundance

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

### 3. 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)			

### 4. The following subsamples should be collected and chain of custody form filled out:

**Contaminant Analysis**

If this quadrat is selected and enough oysters are available from only this quadrat, check the box and fill out the line below. The sample ID will be the sample ID for the quadrat with “-CT” added to the end. Otherwise put in NA and go to next line. The sample should consist of 6 large oysters.

Contaminant analysis (sample ID \_\_\_\_\_) \_\_\_\_\_# of oysters

If the quadrats in the cell do not have enough oysters for contaminant analysis individually, fill out the line below for the number of oysters contributed to the composite (if this quadrat is selected for part of the composite). Put in the new sample ID created for the composite. The sample ID will be a new sample number (next in the sequence) and will have “-CT” added to the end. List all other quadrat sample numbers that will contribute to the composite. If no oysters from the quadrat are selected for contaminant analysis, put NA in the box.

Contaminant analysis (composite sample ID \_\_\_\_\_) \_\_\_\_\_# of oysters contributed

Other quadrats contributing to composite (list): \_\_\_\_\_

**Disease (Dermo) and Gonad Analysis**

Fill out the line below for the number of oysters contributed to the composite. Put in the new sample ID created for the composite. The sample ID will be a new sample number (next in the sequence) and will have “-GD” added to the end. List all other quadrat sample numbers that will contribute to the composite. The sample should consist of 10 large oysters. If no oysters from the quadrat are selected for analysis, put NA in the box.

Disease and Gonad analyses (composite sample ID \_\_\_\_\_) \_\_\_\_\_# of oysters contributed

Other quadrats contributing to composite (list): \_\_\_\_\_

Lab Team Leader: \_\_\_\_\_

(Name) (Agency) (Signature) (Date)

Analyzed By: \_\_\_\_\_

(Name) (Agency) (Signature) (Date)

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

(Name) (Agency) (Signature) (Date)