Mississippi Canyon 252 Oil Spill

NRDA Sampling Plan

Time Lapse Camera and Sediment Trap Retrieval and Redeployment Plan

Deepwater Benthic Communities (Deepwater Coral) Technical Working Group

Final: March 8, 2011

Approval of this work plan is for the purposes of obtaining data for the Natural Resource Damage Assessment. Each Party reserves its right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

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

APPROVED:

Robert W. Ricker — March 8, 2011

Department of Commerce Trustee Representative: Date

BP Representative: March 8, 2011

Date

Time Lapse Camera and Sediment Trap Retrieval and Redeployment Plan

Deepwater Benthic Communities Technical Working Group

Final: March 8, 2011

Proposed Cruise Vessel and Dates

HOS Sweetwater – March 9 – March 13, 2011

1. Background and Objectives:

The Deepwater Horizon (DWH) incident in the northern Gulf of Mexico occurred on April 20, 2010 at a water depth of 1525 meters in Mississippi Canyon Block 252. While some of the oil from DWH incident would be expected to float (average density of 0.849, API 35.2), portions potentially could have moved into offshore and deepwater (> 200 meters) sediments via multiple pathways. In addition, drill cuttings, drill fluids, and other containment fluids may have been released and deposited to the bottom during the blowout.

Preliminary observations and measurements from prior cruises have noted the presence of flocculent material and dense/coarse particles in bottom sediments at several deepwater locations near the DWH well head and within the paths of oil movement predicted by NOAA models. In addition, investigations conducted as part of the Ron Brown Cruise, conducted from October 14th to November 4th, 2010 by the Bureau of Ocean Energy Management, Regulation and Enforcement (BOEMRE) and the National Oceanic and Atmospheric Administriation, Office of Ocean Exploration and Research (NOAA OER), reportedly observed a brown flocculent-like layer covering a hard bottom coral community including corals in the genera *Paramuricea*, *Swiftia*, and *Paragorgia*, located within Mississippi Canyon Block 338 (MC 338). Although this phenomenon has not been documented before, it is unknown whether these materials are naturally occurring or are related to the DWH spill.

On December 11, 2010 as part of the NSF-funded *Atlantis/Alvin* cruise, a time-lapse camera was deployed at the MC 338 site and focused on a coral community covered with the flocculent-like material. The camera is mounted on an elevator platform with two strobes, three batteries, and a junction/control box, and is set to take a digital image of the colony once per hour. The pictures should allow us to follow both the progression of the coral's survival, recovery or death, behavior of the associated brittlestars, and any abrupt event (such as a strong current change or visitation by large predators, such as fish and crabs) that might affect the coral over a two-month time span. Two separate *Alvin* dives (conducted four days apart) documented more than 2 dozen of these corals and their associates with high-definition imagery and confirmed the camera/strobe array to be operational. We calculate that the camera will expend its battery power by mid- to late-February. Therefore, the camera needs to be recovered in order to retrieve the photographic images. The camera recovery team will also use this opportunity to re-locate and photo-

document, in a time-series fashion, the other previously documented coral colonies at the MC 338 site in order to assess their survival and/or recovery at this site; and potentially re-deploy the time-lapse camera.

In addition, the team will retrieve a sediment/larval trap that was deployed on June 23, 2010 at Viosca Knoll 826 (VK 826), as part of an NSF Rapid cruise in order to examine the chemical composition and temporal delivery of particulate matter, and the identification of larval composition (e.g., larvae of coral, tube worms, crustaceans, etc.) and flux. Data from this instrument will complement that collected by two instruments deployed earlier on the Tier 1 Nancy Foster Cruise conducted in late July and early August, as well as two others deployed in September from the Ron Brown as part of the ongoing BOEMRE/NOAA OER study of deep water coral communities. Information provided by analysis of sediment traps may aid in understanding the fate of any hydrocarbon release and dispersion through the deep ocean.

The final objective will be to visit the MC 118 site (approximately 10 nm to the NNW of the Macondo well), where conflicting reports of possible injury to corals exist. During an October/November BOEMRE/NOAA OER cruise, Principal Investigators Chuck Fisher and Tim Shank visited this site and have reported verbally that they observed numerous colonies of gorgonian and hard corals with no visible evidence of recent deleterious impact. Subsequently, in early December during an NSF-funded Alvin cruise, Drs. Samantha Joye and Ian MacDonald report that they observed corals that appeared to be recently impacted in an area of this site not visited by Drs. Fisher and Shank. This plan proposes a single ROV dive to MC118, focusing on the area visited by Drs. MacDonald and Joye to collect new high resolution imagery and examine corals in this area for visual signs of potential impact, and to collect a limited number of soft sediment cores and faunal samples for analysis for the presence of hydrocarbons from the MC252 incident and the assessment of macro- and meiofauna, including identification to the lowest possible taxonomic level and enumeration.

2. Methodology

Below is a narrative with details of the goals, methods, limitations and reasonable expectations for this cruise.

To accomplish the objectives of this cruise, the team will require an ROV with at a minimum:

- Navigation capability
- A manipulator with at least five functions and the capacity to lift at least 50 pounds from the sea floor.
- Sufficient lifting capacity to remove each weight individually and the entire elevator 20 meters away from the site without disturbing the site.

The team will also require a digital still camera of HD video capable of high quality still frames for documentation of the site while on the sea floor. The ROV that is currently being considered for use on this cruise is the ROV Triton XLS.

2.1 Objective 1: Recover a time-lapse camera deployed at Mississippi Canyon 338 on December 11, 2010, redeploy the camera, and re-survey twelve other documented coral colonies at the site.

A time-lapse camera was deployed at MC 338 on December 11, 2010 to take hourly digital photos of a potentially injured coral colony from a distance of three meters. The camera is mounted on an elevator platform with two strobes, three batteries, and a junction/control box. The camera has been confirmed operational, and has an expected battery life that will expire in mid to late February. The primary purpose of this task is to recover, and potentially redeploy, this camera.

The site has been visited on numerous prior dives. In addition, a Sonardyne Homer Probe is mounted on the elevator to facilitate location and recovery on the sea floor. Upon recovery, the photographs will be downloaded, copied and examined, and the camera system will be examined for signs of corrosion and pitting. The camera system will be redeployed if the camera is (1) obtaining adequate pictures of the coral in the frame of view to visualize the flocculent material on the coral, behavior of the associated basket star, and distinguish living coral tissue, and (2) if the entire system is still functional and not showing signs of serious corrosion or pitting that would suggest it may fail during a subsequent deployment. If either of these two conditions is not met, the camera will not be redeployed. Replacement electronics, connectors, releases, housings, strobes, and batteries will be brought on board and used as necessary. If batteries have been shown to have failed, they will be replaced. Assuming that the two conditions above are met, the camera will be redeployed on the same coral colony from the same angle of view, to document further potential temporal changes in coral injury. The redeployed camera will stay on the sea floor at least 1.5 months to be recovered during a subsequent deep benthic community TWG ROV cruise to the area after that time. It is anticipated that it will be recovered as part of either a soft-bottom sediment sampling study or another potential cruise dedicated to investigation of other deep hard bottom communities. We do not envision the need for another dedicated recovery cruise at this time.

Recovery will involve removing two iron plates from a milk crate on the elevator and pulling a rope attached to the release pin to release another set of weights which will leave the system 120 pounds positively buoyant. The ascent rate will be 45 meters per minute and the camera should be recovered by the ship on the surface.

Imagery from the visit to this site last November as part of the BOEMRE/NOAA OER cruise and/or the December NSF Rapid research cruise exists for another 12 colonies at the MC 338 site. In addition to using the ROV to locate and retrieve the time-lapse camera, it will also be used to relocate and re-photograph these previously-visited colonies from similar perspectives to follow their current status. This effort is expected to require approximately 10 to 12 hours of bottom time to image the entire site after release and recovery of the time lapse camera.

Specific Camera Recovery Information:

- MISO-DSPL TL Camera System
- Depth: 1369 meters
- Deployment Heading: 089°

- Sonardyne Homer Probe Frequency Address: 33
- Elevator weight after all weights released: 120 pounds positive
- Current weight on the sea floor: ~50 pounds negative
- On bottom position: Latitude [redacted] Longitude [redacted]

Steps for Recovery:

1. Locate the camera elevator by use of the Homer Probe frequency address 33 and navigation data from previous visits.
2. The site/camera should be approached slowly and only from the West (this gently upward-sloping sea floor area has no obstruction to reach the elevator) (see Figure 1). The location and position of the camera system on the sea floor should be imaged (without any invasive movements toward the fauna east of the camera system). Note whether or not the camera strobes are still flashing approximately every hour at four minutes after the hour.
3. Acquire one of the aft legs of the elevator and slowly manoeuvre backwards away from the site at least 20 meters, and set the mooring back on the sea floor. The elevator should be approximately 50 pounds heavy.
4. Remove the two weights in the black milk crate and place them on the ROV for ballast or place them on the adjacent sea floor.
5. Grasp the red release loop (see Figure 1) tightly and manoeuvre backwards to release the pin and thus the elevator from the sea floor. Note the time of the release on the sea floor.
6. Do not remove the Homer Probe until it is on deck.
7. The likely ascent rate will be approximately 45 meters per minute.

There is a 12" yellow glass float followed by 10 meters of polypropylene line for grappling for recovery, as well as pairings for lifting sections of the mooring.

Upon recovery on deck, the team will photographically document the entire camera system (note the time on deck). A full freshwater rinse of the system will be conducted. The two plugs representing the enabling connections to the strobe and camera will be replaced with dummies. Four Vemco temperature probes were placed on the elevator grating 0.5 meters from the camera lens. The housings were clamped together as pairs, with probe tips extended to the edge of the grating. These temperature probes will be removed and data downloaded as well.

Camera Redeployment:
Redeployment of the camera will necessitate recharging the batteries; a maintenance check; replacement of the strobes and other parts, as necessary; restarting the camera on board ship and deploying the camera from the ship with 100 lbs. of additional weights to assure rapid transit to the sea floor. The camera will then be located using the Homer beacon (and sonar if necessary), its additional descent weights released, and moved into position by the ROV (while it is about 25 pounds negative). During this time, observation of strobe frequency will confirm that the camera is in operation. Finally, two steel plates carried on the ROV (about 17 lbs. each) will be added to the camera/elevator to insure its stability on the sea floor.

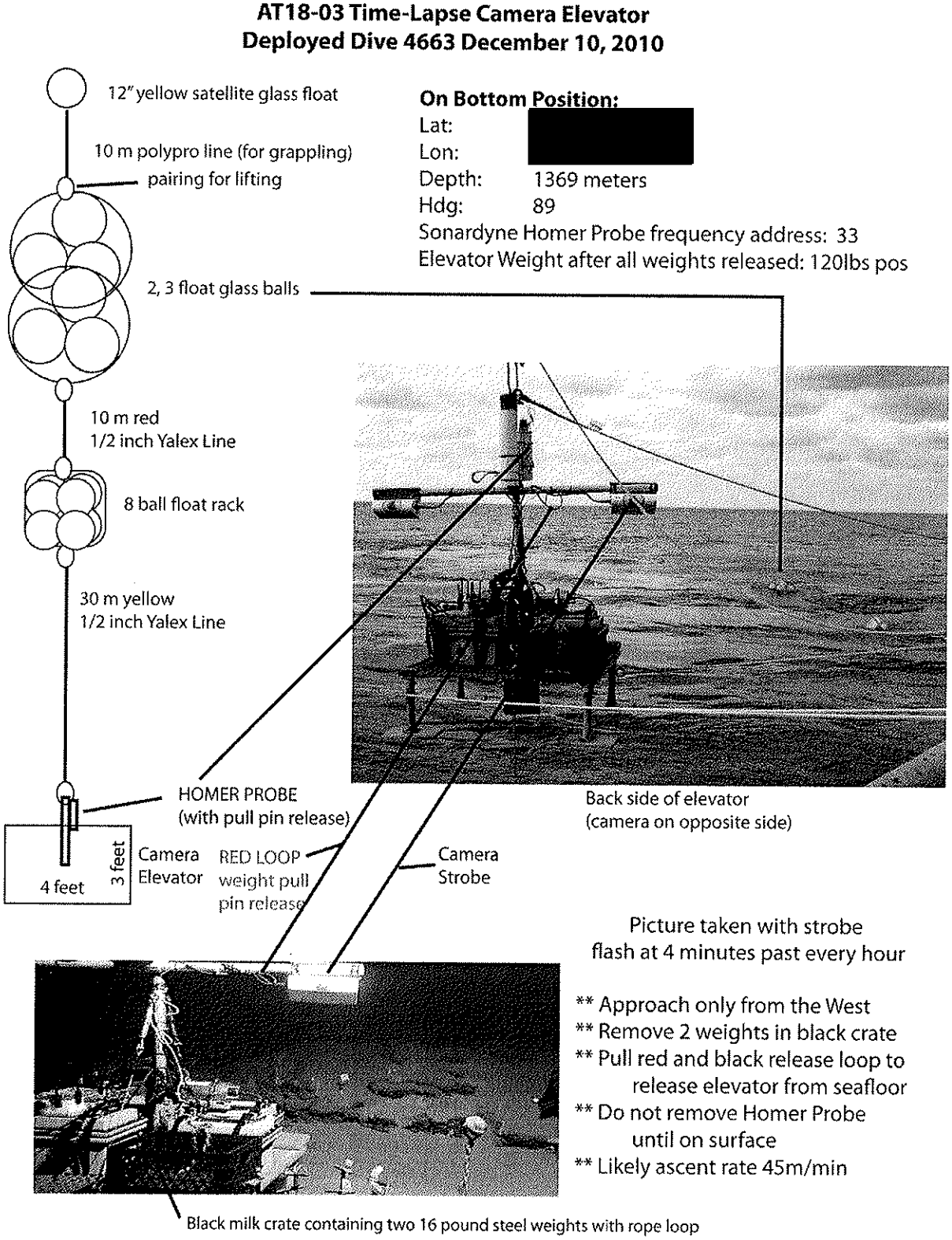

Figure 1. The time-lapse camera elevator diagram, during launch, and on the sea floor.

2.2 Objective 2: Recover a sediment/larval trap deployed on June 23, 2010 at Viosca Knoll 826.

The second objective of this study plan involves the recovery and redeployment of a sediment/larval trap. At this time, it is anticipated that the redeployed sediment trap will be able to be retrieved in a similar manner on a future Deepwater Benthic Communities or Water Column Technical Work Group NRDA cruise, but will not require a dedicated cruise for retrieval. Sediment traps are being used as part of the NRDA effort to document (1) a potential pathway for the release of oil, drilling muds, and dispersant (and their break-down products) to natural resources on the sea floor, and (2) potential impacts to larval species (e.g. coral, tube worms, mollusks, crustaceans, etc.) resulting from exposure to oil, drilling muds, or dispersant, released as part of the DWH incident.

The trap of interest for this study plan was deployed on June 23, 2010 at VK826, as part of a NSF Rapid cruise, but the samples will be handled and analyzed in a manner consistent with sediment traps deployed as part of the NRDA Tier 1 Nancy Foster cruise conducted in late June and early August, 2010 (see below). Steve Manganini from the PARFLUX laboratory (Woods Hole Oceanographic Institute (WHOI), Department of Geology & Geophysics) will oversee recovery of the VK826 sediment-trap mooring at sea. The trap will need to be recovered during daylight. Although it is anticipated that the trap will be called up acoustically, retrieval is being conducted as part of this cruise because the ROV on board will be used as a back-up in case the system does not surface or is fouled on the sea floor.

Specific Trap Recovery Information

- Sediment/Larval Trap Mooring I.D.: GOMEX RR1 VK02
- Depth: 452 meters
- Deployed from RV Cape Hatteras on June 23, 2010
- On bottom position: Latitude [redacted] Longitude [redacted]
- ORE Release SN# 22684
- Interrogate 11KHz
- Reply 12KHz
- Enable 617560
- Disable 617621
- Release 631501

It is recommended that the ship stand off 200 meters to the south of the on bottom position prior to sea floor release of the trap. Through a hydrophone/deck box unit, the mooring will be released acoustically (via a sound wave at a pre-designated frequency) from the sea floor. At 452 meters depth, the mooring should be able to be tracked over the 10 to 15 minute period of ascent, and the ship positioned accordingly for recovery. A single 37" steel sphere is located at the top of the mooring (see Figure 2) and connected by a chain for grappling. There is no light on this mooring and recovery during daylight hours will be required.

Upon recovery on deck, the trap will be placed out of the reach of surface spray and wind and the ship's exhaust system. The sediment trap and sample cups will then be documented with a high-resolution digital camera. Images from many angles, that include the position of the cups upon recovery, the amount of growth on the traps, and the amount of material in the cups, serial numbers, and hardware, including looking down into the trap funnel are required. No photographs will be deleted or manipulated after being taken; and all photographs will be distributed based on data sharing protocols (see below). The cups will then be unscrewed, capped immediately, and denoted with permanent black marker on the cap and side of each cup. The cups will be brought inside the ship and lined up sequentially and images of the full number of cups as well as individually with a ruler for scale close-ups of each cup shall be taken using the same camera as earlier. NRDA labels will then be applied, re-imaged, and pH measured. Black electrical tape and parafilm will be secured around the cap of each cup. The cups will then transferred and stored (under lock) in the 4°C cold room or refrigerator for the duration of the cruise (until shipping). The refrigerator or container in which these cups are placed must be locked.

At the end of the cruise, packs of frozen blue ice will be placed on top of the samples in a large white cooler and then shipped to WHOI under NRDA Chain of Custody procedures for analysis.[1] Prior to and subsequent to analysis, samples will be stored in a locked storage refrigerator within the PARFLUX laboratory at WHOI reserved exclusively for Gulf of Mexico samples.

At WHOI, Mr. Manganini will be responsible for splitting each sample into 10 replicate and representative aliquots for onward processing. Samples will be alliquoted and analyzed according to the following:

- 4/10ths of each sample will be forwarded to Alpha Analytical under Chain of Custody for hydrocarbon fingerprinting analyses.
- 3/10ths of each sample will be analysed biogeochemically at WHOI by CHN analyzer, ICP-OES and ICP-MS. For preliminary biogeochemical characterization, 5-10mg dried subsamples will also be analyzed for organic and inorganic carbon contents as well as nitrogen and biogenic silica and P, Al, Si, Ca, Fe, Mg, Sr, Ba, Ti, P, Mn and Na, using standard methods (Honjo et al. 1995, Eggiman et al. 1980). Analyses will include mass-flux and organic carbon content determinations from a 1/10th sample, the results of which will be forwarded to Alpha Analytical to assist them in their hydrocarbon analyses on these very small samples.
- 3/10ths of each sample will be transferred to Dr. Tim Shank's laboratory in the Biology Department at WHOI, under Chain of Custody, for larval identification.
- Any surplus samples at WHOI will be archived under Chain of Custody in the PARFLUX laboratory.

The mooring hardware (including the trap, current meter, and acoustic release) will be fresh water rinsed and prepared for redeployment of the sediment trap.

[1] See NRDA Field Sampler Data Management Protocol (October 22, 2010 version) for more information.

Sediment Trap Redeployment

Prior to redeployment, the device will be checked for pitting or corrosion, the O-rings will be removed and the exposed portions of the device decontaminated with methanol and a thorough distilled water rinse, O-rings inspected and reinstalled, the batteries and back up 9 V battery changed, and the apparatus reassembled. Immediately before deployment the containers are refilled with preservative and loaded into the rotating plate, after which they are loaded into the device and topped off using the "fill containers" routine and the sample intake spigot. The device is then programmed (to take a sample every 2 weeks for 6 months), starting 24 hours after deployment. After a final diagnostics check using a communications cable attached to a laptop, the dummy plug is placed in the communication port and the system is ready for deployment.

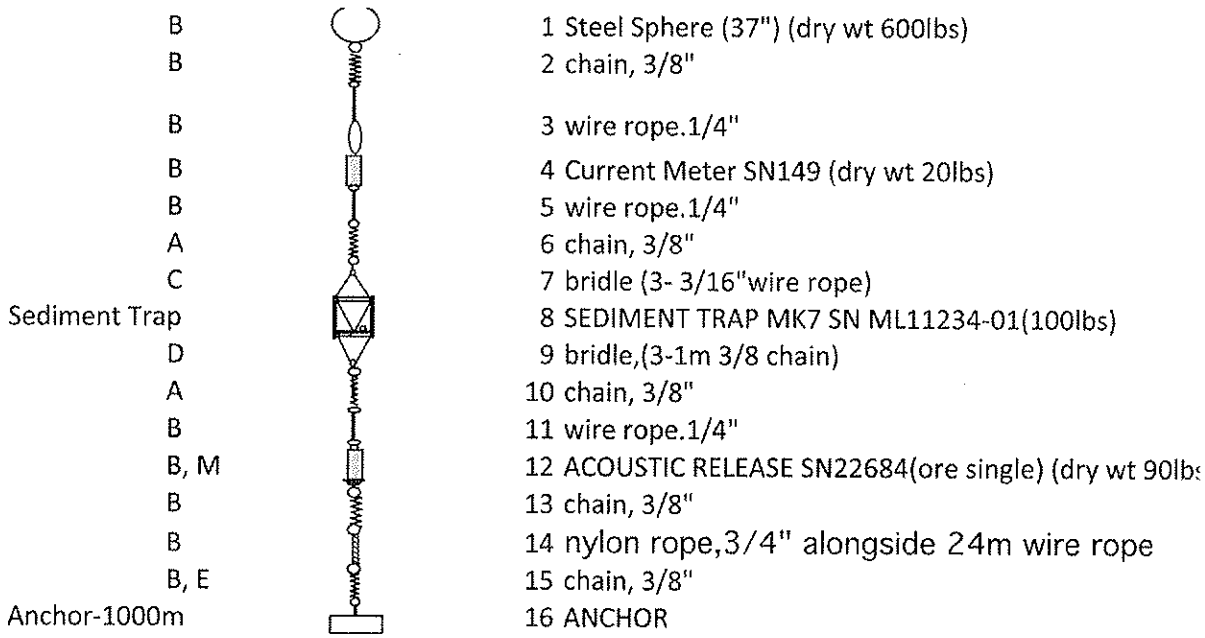

Figure 2. The GOMEX RR1 VK02 Sediment/Larval Trap mooring specifications

2.3 Objective 3a: Visit and Photograph Potentially Injured Corals Located in Mississippi Canyon 118.

To investigate potential injuries to corals at the MC 118 site, the ROV team will conduct a single ROV dive, focusing on the area visited previously by Principal Investigators Ian MacDonald and Samantha Joye. High resolution photographic and video images will be taken of corals during an estimated 12 hours on the sea floor.

2.4 Objective 3b: Physical sampling at Mississippi Canyon 118

In order to quantitatively evaluate the potential presence of hydrocarbons from the MC252 incident and the macro- and meiofauna in the sediment at the MC 118 site, the ROV will be outfitted with (1) standard ROV push cores and quivers for sampling of soft sediment, and (2) quivers equipped with stoppers for faunal sampling. Four cores from each of two locations (to be determined on board by the Principal Investigators, based on the presence of soft sediment

and the potential presence of any flocculent layer or observed impacts) will be collected during a dive: one core for chemical analysis of hydrocarbons; one core for analysis of benthic fauna; one core for grain size analysis; and one extra core to be used as a replicate core should one of the other cores be unsuccessfully obtained. If four cores are successfully obtained, the fourth core will be designated as a replicate core for chemical analysis of hydrocarbons and will be stored in archive unless needed. The ROV will also be equipped to collect up to four biological samples into sealed quivers during each dive, sub-samples from which will be used to genetically identify species and archived for potential analysis of hydrocarbons. Details on sampling protocols are provided in Appendix A.

3. Chain of Custody

All data collected pursuant to this scope of work must adhere to a strict Chain of Custody protocol to ensure the utmost integrity of all data, methods, control and documentation. All data will remain in the documented physical control of the selected contractors at all times. Complete documentation of this Chain of Custody must follow the standard NRDA Chain of Custody for aerial imagery, including acceptance and release signature for this physical control chain.[2] Original copies of all documentation will be provided to the signatories, or their designated representative in accordance with section four below.

4. Data Sharing and Compliance with the Litigation Hold Requirements issued in MDL No. 2179 IN RE: Oil Spill by the Oil Rig "DEEPWATER HORIZON" (E.D. LA 2010):

All data and imagery (including navigation, photographic and video raw data files, instrument data, field logs and documentation) and other electronic data will be saved to an on-board computer, and all data shall be migrated to a dedicated external hard drive. Upon return to port, the vessel Operations Manager shall produce identical copies of the raw and processed electronic media generated during the cruise and deliver one of those copies each to NOAA (or its QA contractor) and to BP/Cardno ENTRIX. Additionally, all non-analytical data, including field reports and data sheets, will be made available to BP/Cardno ENTRIX within a reasonable time after completion of the cruise.

All samples collected for contaminant analysis during the sampling plan, described in section 2.2 and 2.4 above, will be sent to Alpha Analytical. Samples taken for biogeochemical and larval analysis will be sent to Woods Hole Oceanographic Institution. Samples for genetic analysis will be sent to Temple University. Samples taken for biota community analysis will be sent to the U.S. Geological Survey Southeast Ecological Science Center.

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 Cardno ENTRIX behalf of BP). The electronic data deliverable (EDD) spreadsheet with pre-validated analytical results, which is a component of the complete LADP, will also be delivered to the secure FTP drop box maintained by the Trustees' Data Management Team (DMT). Any preliminary data distributed to the DMT shall

[2] Refer to the Work Plan entitled "Technical Specifications and Scope of Work/Services for Aerial Image Acquisition and Image Processing in Support of the MC252 NRDA Process, Fall 2010 Through Spring 2012" (October 6, 2010 version) for more details and for a copy of the COC form.

also be distributed to LOSCO and to BP (or Cardno ENTRIX on behalf of BP). Thereafter, the DMT will validate and perform quality assurance/quality control (QA/QC) procedures on the LADP consistent with the authorized Quality Assurance Project Plan, after which time the validated QA/QC-ed data shall be made available simultaneously to all trustees and BP (or Cardno ENTRIX on behalf of BP). Any questions raised on the validated QA/QC results shall be handled per the procedures in the Quality Assurance Project Plan and the issue and results shall be distributed to all parties. In the interest of maintaining one consistent data set for use by all parties, only the validated QA/QC-ed data set released by the DMT shall be considered the consensus data set. In order to assure reliability of the consensus data and full review by the parties, no party shall publish consensus data until seven days after such data has been made available to the parties. Also, the LADP shall not be released by the DMT, LOSCO, BP or Cardno ENTRIX prior to validation QA/QC absent a showing of critical operational need. Should any party show a critical operational need for data prior to validation/QA/QC, any released data will be clearly marked "preliminary/un-validated" and will be made available equally to all trustees and to BP (or Cardno 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.

5. Milestones and Deliverables:

A cruise report will be submitted within two weeks of the completion of the cruise, documenting the achievement of the cruise goals and preliminary impressions. All photographic documentation will be logged as per NRDA protocol and two copies submitted to the NRDA sample intake team after the cruise. A report of summarizing all findings from the photographic documentation will be delivered within three months. To the extent that other photographic images are used for generating the report and are made available for use in the NRDA, these photographic images will be shared with Cardno ENTRIX. Results of the geochemical and larval analyses conducted at Woods Hole will be delivered within 6 months of recovery of the sediment/larval trap.

6. Key Personnel:
NOTE: All cruise participants are not listed at this time, only the leaders of the effort. We anticipate 24 hour operations and a science party of 12, including 2 BP/Cardno ENTRIX representatives.

- Dr. Chuck Fisher, Pennsylvania State University: Site navigation, coral documentation.

- Dr. Dan Fornari, Woods Hole Oceanographic Institute: Instrument recoveries and data/image/sample downloading

7. Safety Plans:

A request for a ship has been presented to the Vessel Coordination Committee to support this mission. The Hos Sweetwater is the anticipated vessel for this cruise. In addition to complying will all vessel-specific safety protocols, all activities will follow protocols of NOAA's NRDA Field Operations Safety Plan (latest version 13 January 2011). MSDS hazardous materials sheets will be posted, as necessary.

8. Budget:

The Parties acknowledge that this budget is an estimate, and that actual costs may prove to be higher. BP's commitment to fund the costs of this work includes any additional reasonable costs within the scope of this work plan that may arise. The trustees will make a good faith effort to notify BP in advance of any such increased costs.

The field survey costs, miscellaneous costs, and travel costs indicated in Budget Chart # 1 below shall be reimbursed by BP upon receipt of written invoices submitted by the Trustees. The Vessel Costs indicated in Budget Chart # 2 shall be paid directly by BP.

Budget Chart #1.

Salary	
Woods Hole Oceanographic Institution	
• Scientist – [redacted] days @ [redacted]	$6,200
• Scientist – [redacted] days @ [redacted]	$6,200
• Post doctorate/camera technician – [redacted] days @ [redacted]	$1,550
• Post doctorate/camera technician – [redacted] days @ [redacted]	$1,550
Subtotal	**$15,500**
Pennsylvania State University	
• Dr. Chuck Fisher – no salary required	$0
• Student – no salary required	$0
Subtotal	**$0**
Travel	
• [redacted] people @ [redacted] /person	$16,000
Subtotal	**$16,000**
Camera redeployment	
• Rental, reconditioning, shipping, and materials	$27,000
Subtotal	**$27,000**

Sediment Trap redeployment

Mooring hardware acquisition, construction and shipping	$25,000
Geochemical and larval analyses at WHOI	$75,000
Subtotal	**$100,000**

Budget Chart #2

Vessel Costs:	**Cost**
Mobilization Costs	$236,250
Vessel Costs	$600,319
CSA Fleet Mgmt / Shore Support	$105,000
Subtotal	**$941,569**

TOTAL PROJECT BUDGET **$1,100,069**

9. References

Eggimann, D.W., F.T. Manheim, and P.R. Betzer, 1980. Dissolution and analysis of amorphous silica in marine sediments, J. Sed. Pet., 50, 215-225.

Honjo, S., Dymond, J., Collier, R., Manganini, S.J., 1995. Export production of particles to the interior of the equatorial Pacific Ocean during the 1992 EqPac experiment. Deep-Sea Research 42, 831-870.

Appendix A: Sampling Methodology for Push Cores and Biota Collected by ROV

Sediment Sampling Methodology:

Sediment Corer

A sediment coring system will be mounted to the ROV. The sediment coring system consists of core tubes 6.5 cm in diameter (inside diameter) capable of taking samples down to a sediment depth of 10 cm (Figure A1). Twelve core tube quivers will be mounted to the inside of a removable plastic crate that will be mounted on the ROV. Push cores will be secured in eight of these quivers for use on the sea floor and the other four quivers will be reserved for biological samples. Rubber stoppers will be placed in an adjacent plastic crate to seal the biological sample quivers. Once at the seafloor, the ROV deploys one core tube at a time and inserts it into the sediment. Four cores will be taken at each of two stations during the dive to MC 118. The entire operation will be documented on video. Each core tube is outfitted with a floating seal on the upper end (not inserted into the seabed). This floating seal allows water to flow out of the tube during insertion. As the tube is pulled from the seafloor the upper floating seal locks into place due to suction. This prevents loss of the sediment sample during recovery. The core is then retracted to its initial location and sealed at the bottom onto a rubber stopper mounted inside the core quiver. In this manner both ends of the core tube are capped for ascent of the ROV.

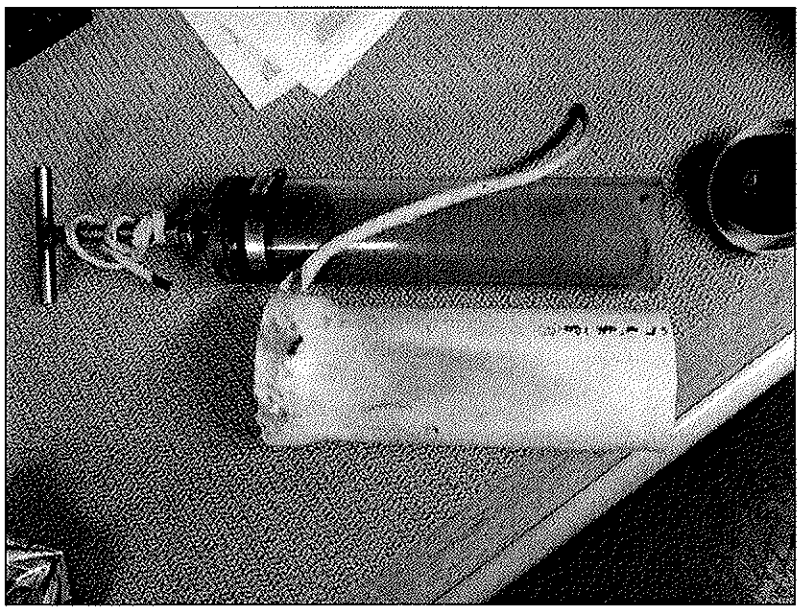

Figure A1. Sediment core tube, floating seal cap (detached), and ROV core mount or holster.

Cores for Chemical Analysis

On deck in the ship's laboratory, biological cores will be extruded using a plunger system (see Sediment Core Extrusion: SOP's, Annex A1). Cross sections of sediment will be sliced from the core at varying depths, based on the analyses anticipated for each core. Specifically, cores will be sectioned at approximately 1 cm to remove the top flocculent layer, then sliced at 2-4cm down (from the original top of the core), followed by a slice at 5-6 cm down (from the original top of the core); targeting obvious color or texture changes for divisions. The remaining

sediment will be divided into segments no larger than 4 cm; this will be dependent upon the total depth of the core and may vary. As necessary, 2 cm sections may be taken from homogeneous layers (see below) based on the length of the core and the discretion of the PIs. Cross sections will be placed in pre-cleaned glass jars and kept frozen until shipment to Alpha Analytical for detailed chemical analyses and fingerprinting. Sample horizons will be clearly labeled and documented in the sample log (see Annex A2). Decontamination procedures (see below) will also be followed (allowing all corers to be utilized interchangeably).

Cores for Biological Analysis

On deck in the ship's laboratory, biological cores will be extruded using a plunger system, as described above for the cores taken for chemical analysis. For biotic community analyses, the top water in the core will be removed using a sterile 60cc syringe, and cores will be sectioned into 1 cm intervals down to a depth consistent with the depth of the third section for chemical analysis (at 5-6 cm down from the original top of the core). Sectioning cores in this way will allow for comparison to cores collected as part of other sampling programs that section cores in wider depth intervals. Each core section and core slicer will be rinsed with filtered (in-line 30-50 um, and 7 um) seawater into new Nalgene HDPE bottle and preserved in formalin. Top water removed from the core will be sieved in a 45 um sieve and sieved material will be rinsed with filtered seawater into the bottle containing the first (top) core section. Sample horizons will be clearly labeled and documented in the sample log (see Annex 2). Decontamination procedures (see below) will also be followed (allowing all corers to be utilized interchangeably). Back in the laboratory, sediment cores will be sieved and macro- and meiofauna will be identified to the lowest possible taxonomic group and enumerated.

Cores for Grain Size Analysis

On deck in the ship's laboratory, cores taken for grain size will extruded in their entirety, sectioned at the same 1 cm depth intervals as cores for biological analysis, their contents placed in pre-cleaned glass jars, and stored at room temperature. Samples will be clearly labeled and documented in the sample log (see Annex A2). Decontamination procedures described below will also be followed (allowing all corers to be utilized interchangeably). Sediment grain size analysis will be by methods described in the MC252 Analytical Quality Assurance Plan Version 2.1.

***ROV Biota Collection Methodology*:**

We will also collect up to four samples of macrofauna encountered by the ROV while surveying the seafloor. The ROV arm will be used to collect any biota samples, which subsequently will be placed in empty core quivers and sealed with rubber stoppers. On deck, two small pieces of tissue (0.1 g) will be removed from each sample for genetic analyses to confirm the species. One subsample for genetic analysis will be preserved in "RNA later" and the other in 100% Ethanol. The remainder will be placed in 500 mL pre-cleaned jars and frozen. Subsamples for genetic analysis will be sent to Temple University and analyzed by Dr. Erik Cordes. Frozen remainder samples will be shipped to Alpha Analytical Laboratories in Mansfield, MA, and archived until such time as they will be analyzed for petroleum fingerprinting (see below).

Sampling Equipment Decontamination:
Decontamination of each core tube will be carried out by washing equipment with liquinox and water on board between uses. Coring equipment and tubes will be rinsed with fresh water from the vessel, and then rinsed with seawater during descent on the ROV to the sampling site. Sampling equipment visibly stained with oil or other hydrophobic material will be further decontaminated before use to minimize cross-contamination. While performing the decontamination procedure, phthalate-free gloves, such as nitrile or butyl rubber, will be worn. Sampling equipment will be decontaminated in the area designated for decontamination. The decontamination procedure will proceed as follows:

- Wash and scrub core tubes with detergent
- Tap water/distilled water rinse
- Tap water/distilled water rinse
- An acetone only rinse or a methanol rinse (solvents must be pesticide grade or better) with an optional hexane rinse if necessary after contact by the equipment with visibly contaminated media that prevents complete decontamination at trace levels using the standard procedure.
    - Used solvents will be recovered, stored on board, and disposed of properly when the cruise vessel returns to land.
- Thorough de-ionized (analyte-free) water rinse (if available; otherwise use distilled water).

All sampling equipment being used to collect samples for polycyclic aromatic hydrocarbons (PAHs), total extractable hydrocarbon (TEH), or volatile organic carbon (VOC) analyses will utilize the methanol rinse.

Laboratory Analysis:
Sediment and biota tissue analysis will be by methods and for analytes described in the MC252 Analytical Quality Assurance Plan Version 2.2, Section 1.0. These are:

- Analysis and reporting for PAHs including alkyl homologues by gas chromatography with low resolution mass spectrometry using selected ion monitoring (GC/MS-SIM). The analytical procedure is based on EPA Method 8270D with the GC and MS operating conditions optimized for separation and sensitivity of the target analytes. Alkyl PAH homologues are quantified using a response factor assigned from the parent PAH compound.
- Analysis and reporting for saturated hydrocarbons by gas chromatography with flame ionization detection (GC/FID) based on EPA Method 8015.

- Acquisition of data by GC/MS-SIM for petroleum biomarkers listed in Tables 1.1e and 1.1f. of the NOAA QAP.

Annex A1. Sediment Core Extrusion: Standard Operating Procedures:

I. Purpose

This section describes the extrusion, sectioning and sample collection of discreet sediment layers after collection with the ROV sediment coring devices. This allows chemical analysis of different layers and isolation of freshly deposited materials from background contaminants.

II. Sampling Technique

During ROV assent, gather necessary deck supplies and begin setup.
Need equipment/supplies:

- Table
- Aluminum foil
- Tongue depressors
- Wide-mouth 500ml sample jars
- Sample photo logs (appendix 1)
- Digital camera
- 5 gallon bucket
- Sediment core plunger
- Nitrile gloves

**Nitrile gloves should be worn at all times throughout sampling procedure

Process and photograph each sediment core completely before moving on to the next corer.

1. Station set up:
   1.1. Cover table surface work area with aluminum foil.
   1.2. Place bucket at end of table for disposal of excess sediment and materials.
   1.3. Make sure the boat is positioned so that the wind is coming from a direction that avoids blowing diesel exhaust into the sampling area.
2. Photograph 1: Entire corer.
   2.1. Each corer has a colored handle (top). This colored handle (top) should be included in the first photograph taken for each sample series.

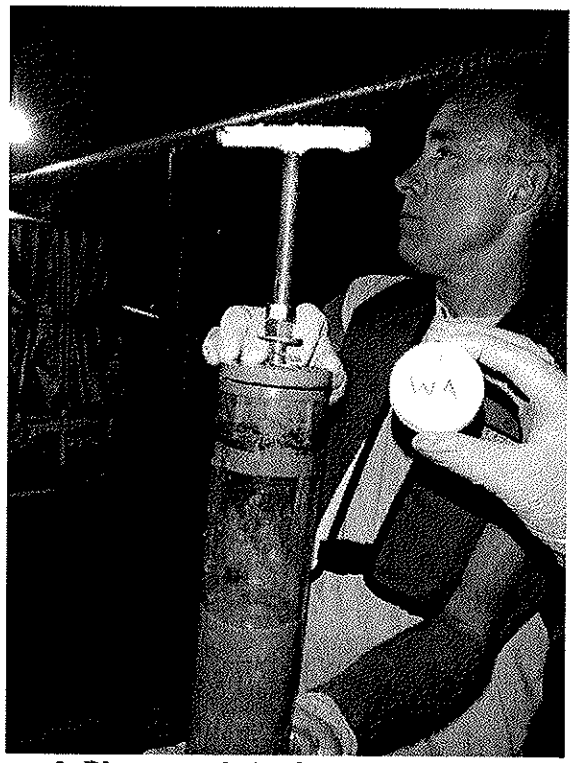

Figure 2. Photograph 1 - Overview of complete corer including colored handle.

3. Photograph 2: Total core length
   3.1. Include cm scale in photograph to record total core length. Record total core length on sample log. (Table 1)

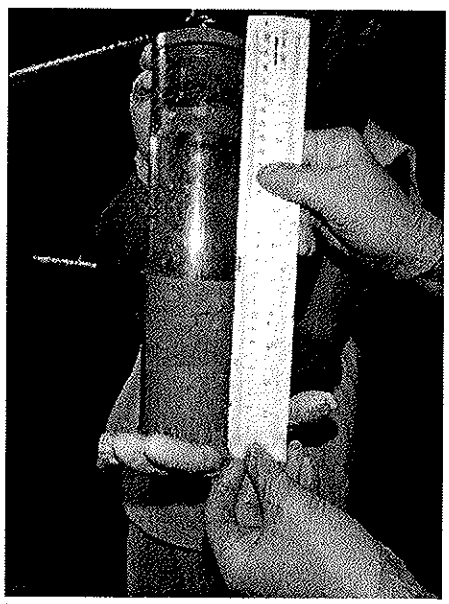

Figure 3. Photograph 2 - cm ruler adjacent to core before extrusion.

4. Remove top:
   4.1. Carefully remove handle of corer (top) while holding the bottom with a gloved hand.
   4.2. Insert plunger into bottom of corer.
      4.2.1. If there is an abundance of fluid on top of the sediment, this may be decanted and disposed of or sampled at the discretion of the lead scientist.
   4.3. Use plunger to slowly push entire contents of corer towards the top.
   4.4. Take another photograph with the cm ruler next to the core pushed up inside the core barrel.
5. Photograph 3: Top layer of sediment prior to collecting sample.
   5.1. Include labeled jar lid in this photograph.
6. **Include labeled lid in photograph for all subsequent layer samples** (i.e. each time a new layer is started).
   6.1. Lid should be labeled with a signifier that identifies the color of the corer (in this case "W" for white) and a letter/number that identifies the layer (here "A" for the topmost sediment layer.)
   6.2. This will help link photographs to the different cores and depths later on during photo processing.

Figure 4. Photograph 3 - Upper layer before extrusion with labeled sample lid.

7. Sediment Sample 1:
   7.1. Use tongue depressor to collect top 1cm of sediment as first sample. In some cases, the top layer might have a "soupy" consistency and may require decanting into the sample jar. Include descriptions of the sample's length, odor, texture, and color on sample log (Table A1).
8. Sample 2:
   8.1. Use the plunger to extrude the next 3 to 4 cm (from top of original core) layer of sediment.
   8.2. Photograph layer with sample jar lid.

8.3. Use tongue depressor to collect sediment into sample jar.

9. Sample 3:
   9.1. Use the plunger to extrude the next 5 to 6 cm (from top of original core) layer of sediment.
   9.2. Photograph layer with sample jar lid.
   9.3. Use tongue depressor to collect sediment into sample jar.
10. Subsequent Samples:
   10.1. The remaining sediment should be divided into segments no larger than 4cm. Any obvious change in sediment type (color, consistency, texture, etc) indicates the need for a new subsample and sample container.
   10.2. In the event that the remaining sediment appears to be homogeneous, extrude the contents onto the aluminum foil and collect the center most 2cm segment as a representative sample (Photograph 4). Discard remaining portions into 5 gallon bucket.

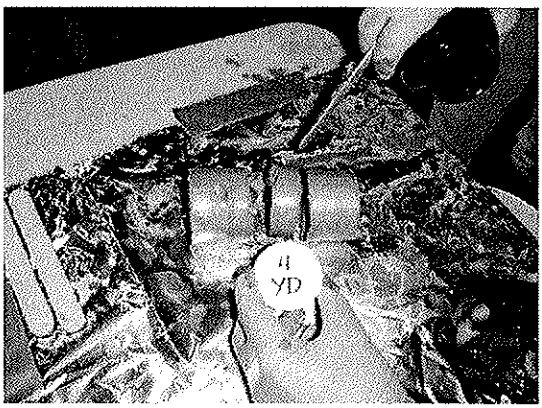

Figure5. Photograph 4 - Extruded core bottom on Al foil for sub-sampling center section of homogeneous bottom layer.

11. Sample jars for sediment chemistry and TOC analyses should be labeled and stored in a freezer once sampling is complete.

Table A1. Sediment core sample log and data sheet

Sediment Core Sample Log

Vessel

Cruise

Station ID

Date

Total length

Depth

Sample Time

Corer Color
(circle one) blue white yellow red

Sample ID	Layer Length	Texture	Odor	Sediment Color	photo number	photo time	comments