

Deepwater Horizon Oil Spill (DWHOS) Water Column Technical Working Group

NRDA Plankton Processing Plan – Analysis of Zooplankton Samples

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Proposed Work Period:

12 months

1.0 Project Description

The purpose of the NRDA Plankton Sample Processing Plan is to establish a protocol for the analysis of plankton samples collected during the natural resource damage assessment (NRDA) associated with the Deepwater Horizon Oil Spill (DWHOS) and to submit associated data to noaanrda.org. The samples to be analyzed under this plan include those collected as part of the cruises listed in Table 1. A tiered approach will be undertaken to assure desired sample prioritization, efficient data flow and effective project management. The tiers are organized hierarchically, and Tier 1 and 2 are broken down into sub-tiers (i.e., 1A, 1B, 1C, 1D and 1E) indicating the prioritization order for analysis. After each 3-month period, all labs involved in processing samples will be responsible for preparing and submitting a standardized quarterly progress report (Attachment 13) that outlines the sample processing and data upload progress during the preceding 3-month period. Prioritization of samples in tiers 3 and 4 is ongoing and subject to change as priorities shift. Details of implementing the prioritization process are provided in Attachment 7.

Data products resulting from analysis of samples from this sample processing plan will include: (i) quarterly progress reports, (ii) electronic data reporting of results of plankton sample analyses upon completion of a sub-tier of samples identified in Attachment 7 (with verbal interim updates on the Water Column TWG calls or by email to the Water Column TWG members, e.g., progress in completing a sub-tier, as they become available), and (iii) an annual, comprehensive data report with summaries of data generated as part of this plan. (The annual data report will not include any analysis, evaluations, or interpretations of the data.) Data products include taxonomic identifications, biomass measurements, counts, and length/width measurements of each component of the plankton samples listed in this plan.

Table 1. Deepwater Horizon Oil Spill – NRDA Water Column Technical Working Group plankton cruises through Summer 2011 (approximately 7407 samples).

Cruise Name	Cruise Type	Dates	Approximate Location of Sampling	Gear Type(s)/ Deployment	Approximate Number of Samples
Gordon Gunter GU-10-04 (60) Fall 2010	SEAMAP/NRDA, Trustee/BP Cooperative	August 24 – September 30, 2010	Gulfwide, from TX border to FLA coast	Bongo (200m & shallow tow for oil droplets), Neuston, 1M MOCNESS (shallow, 130m)	574 samples (Sample processing to be completed summer 2011; so not included in NRDA prioritization scheme.)
Walton Smith 1 Fall 2010	Trustee/BP Cooperative	September 5-17, 2010	Deepwater stations off shelf near wellhead	1 M MOCNESS (Deep 1500M, day/night)	192
Walton Smith 3 Fall 2010	Trustee/BP Cooperative	September 26- October 3, 2010	Deepwater stations off shelf near wellhead	1 M MOCNESS (Deep 1500M, day/night)	96
Cardo ENTRIX Led Plankton Cruises 3& 4 Fall 2010: Nick Skansi, Sarah Bordelon, Meg Skansi	Plan in review; Intended to be Trustee/BP Cooperative [samples in LSU/Sutor custody]	Cruise 3: September 24 – October 4, 2010 Cruise 4: October 15 – October 29, 2010	Shelf and deepwater Stations near wellhead	1 M MOCNESS (Deep 1500M, day/night), Bongo (200m), Neuston	600
Oregon II Winter Survey 2011	Partial Trustee/BP Cooperative	February 16 – March 22, 2011	NRDA Region of Interest (92.5W to 83.5W, 30N to 27N)	Bongo (200m), Neuston, 1M MOCNESS (shallow, 130m)	624
Nick Skansi 8 Winter 2011	Trustee/BP Cooperative	January 7 –April 1, 2011	Deepwater stations off shelf near wellhead	1 M MOCNESS (Deep 1500M, day/night)	640
Nick Skansi 9 Spring 2011	Trustee/BP Cooperative	April 14 – June 30, 2011	Deepwater stations off shelf near wellhead	1 M MOCNESS (Deep 1500M, day/night, shallow 160M day/night)	1176
Bunny Bordelon 6 Spring 2011- Epipelagic Plankton	Trustee/BP Cooperative	April 16 – June 14, 2011	NRDA Region of Interest (92.5W to 83.5W, 30N to 27N)	Bongo (200m & shallow tow to pycnocline), Rectangular Neuston, Manta Neuston (2 nd & 3 rd legs)	468
Walton Smith 4 Spring 2011	Trustee	April 19 – May 28, 2011	Deepwater stations off shelf near wellhead	1 M MOCNESS (Deep 1500M, day/night, shallow 160M)	363

Cruise Name	Cruise Type	Dates	Approximate Location of Sampling	Gear Type(s)/ Deployment	Approximate Number of Samples
Nick Skansi 10 Summer 2011	Trustee/BP Cooperative	July 18 – September 30, 2011	Deepwater stations off shelf near wellhead	day/night 1 M MOCNESS (Deep 1500M, day/night, shallow 160M day/night)	1472
Bunny Bordelon 7 Summer 2011 Epipelagic Plankton	Trustee/BP Cooperative	July 18 – September 30, 2011	NRDA Region of Interest (92.5W to 83.5W, 30N to 27N)	Bongo (200m & shallow tow to pycnocline), Rectangular Neuston, Manta Neuston	500
McArthur II 2,4,6 ISIIS Summer 2011	Trustee/BP Cooperative	Leg 1: June 12 – 30, 2011 Leg 2: July 18 – 31, 2011 Leg 3: August 21 – September 2, 2011	Stations between 90°W - 86°W, and 28°N - 30°N, Offshore Slope to Shelf (potentially 10 stations per leg)	1 M MOCNESS (Shallow 120M, to be used in comparison with ISIIS data)	160
McArthur II 3 – Epipelagic July 2011	Trustee/BP Cooperative	July 6 – 11, 2011	NRDA stations in proximity to wellhead and on shelf to N and NW	1 M MOCNESS (Shallow 160m day/night) Bongo (Deep 200m/Shallow Variable) Rectangular Neuston Manta Neuston	192

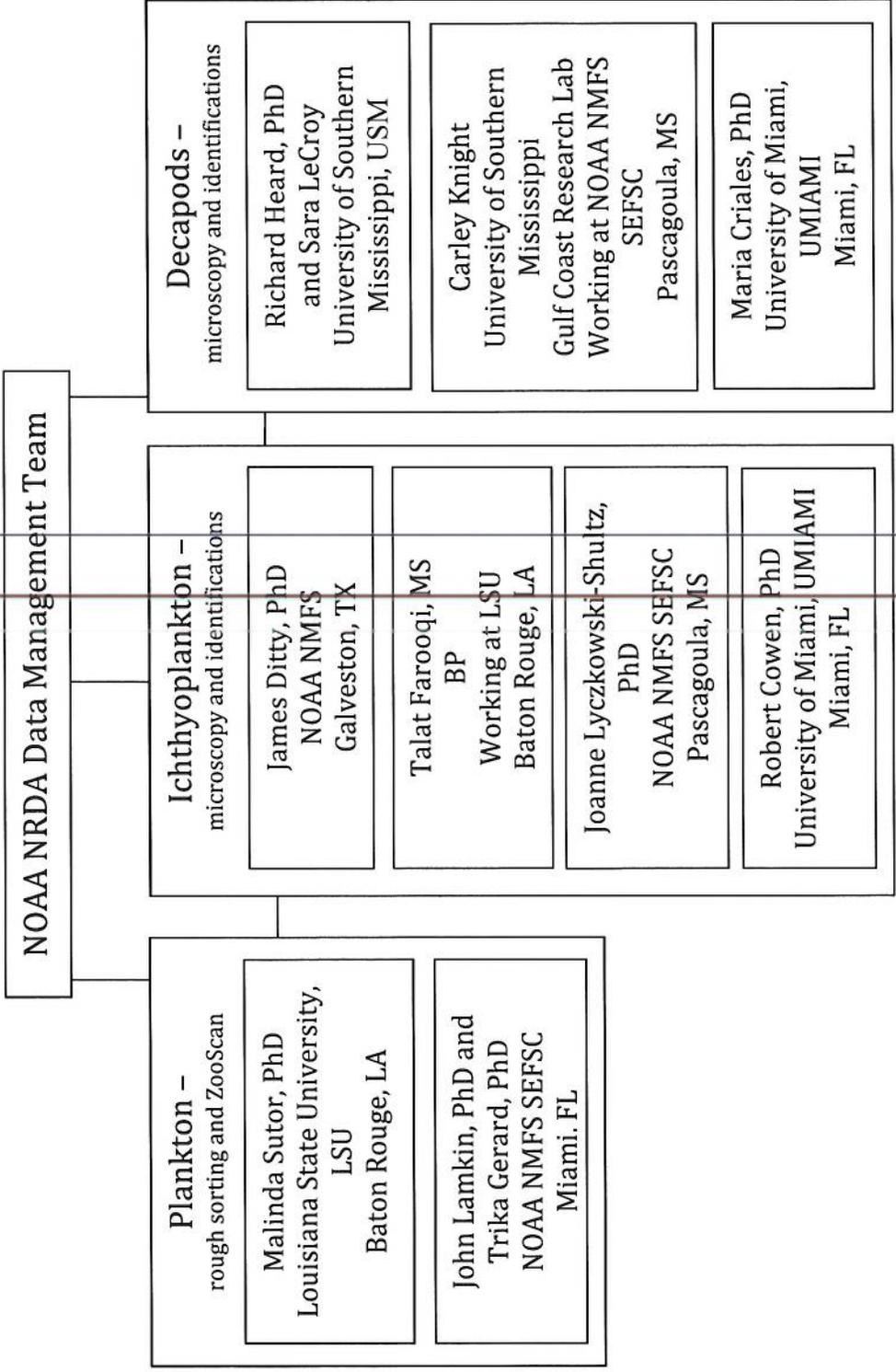


Figure 1. Organization chart of researchers involved in the NRDA Plankton Processing Plan.

2.0 Project Organization and Responsibilities

Although a number of individuals will be involved in processing samples associated with this plan (Figure 1), Malinda Sutor, a NOAA contractor at LSU in Baton Rouge, has chain of custody (COC) of most NRDA samples (i.e., except those collected by the SEAMAP program group of the National Marine Fisheries Service (NMFS), Southeast Fisheries Science Center (SEFSC) and by Robert Cowen of University of Miami, also a NOAA contractor). Those involved in the analysis of samples include Malinda Sutor (LSU), James Ditty from the NOAA lab in Galveston, Texas; Talat Farooqi, a BP contractor located in Baton Rouge, LA; Joanne Lyczkowski-Shultz of the NOAA NMFS SEFSC lab in Pascagoula, MS; John Lamkin and Trika Gerard at the NOAA, NMFS, SEFSC lab in Miami, FL; Robert Cowen and Maria Criales at University of Miami, RSMAS, in Miami, FL; Richard Heard and Sara LeCroy, NOAA contractors at University of Southern Mississippi (USM); and Carley Knight of the University of Southern Mississippi (working with the NOAA NMFS SEFSC lab in Pascagoula). Dr. Sutor will transfer samples under COC to other NOAA and contractor (e.g., University of Miami) labs for analyses. Additional support and plankton specialists may be added at a later time to expedite sample processing as necessary. However, for all analyses, NOAA COC of the samples will be maintained at all times.

Dr. Malinda Sutor (LSU) is responsible for sample coordination with other individuals involved in the project. Dr. Sutor and her Research Associates at LSU will analyze the contents of the plankton samples using the ZooScan machine which employs digital imaging techniques. This will yield taxonomically coarse community composition, abundance, and biomass for both the ichthyoplankton and zooplankton portions of the samples. Ichthyoplankton and larger decapod crustaceans will be sorted at the LSU lab and placed in a series of separate sample jars for further taxonomic analysis using standard microscopy techniques by other taxonomists. Zooplankton will be sorted at the LSU lab and placed in separate sample jars that will be stored under LSU chain of custody until a permanent archive location is identified. Alternate interim storage, if needed, will be under trustee chain of custody and control. Large decapods and other large items/organisms in the samples will be removed, saved separately, and their presence recorded. Splits of the zooplankton samples will be made for further decapod sorting and identification. Sample IDs will be created and tracked for samples that are split into separate jars by having original sample IDs with suffixes added (e.g., Z for zooplankton portion, I for ichthyoplankton portion). Any split samples or new samples created by removal of decapods or other organisms will be labeled with a consistent convention to be determined by the NOAA Miami Lab, LSU, and the NOAA NRDA Data Management Team. The ZooScan will be used on the ichthyoplankton and zooplankton samples.

Dr. James Ditty at the NOAA lab in Galveston, Texas will perform ichthyoplankton microscopy analysis including taxonomic identification, length measurements, enumeration, and performing sample quality control of larval fish identification performed by others. The sorted NRDA ichthyoplankton samples will be sent under NOAA chain-of-custody procedures to the Galveston lab for processing and returned to LSU for short term storage and archive.

Talat Farooqi, a BP contractor, will also perform ichthyoplankton microscopy sample analysis including taxonomic identifications, length measurements and enumerations at the LSU lab, and review data collected by James Ditty and others analyzing ichthyoplankton samples.

Richard Heard and Sara LeCroy, NOAA contractors and decapod taxonomists at USM, will conduct decapod sample analysis and quality control. Dr. Heard's group will also provide expertise with decapod identification and verification of zooplankton ZooScan images as needed. Additional personnel at the USM lab may be identified to assist in the plankton processing efforts. The sorted NRDA decapod samples will be sent under NOAA chain-of-custody procedures to the USM lab for processing and returned to LSU for short term storage and archive.

The NOAA lab in Pascagoula, MS, under the direction of Dr. Joanne Lyczkowski-Shultz, will assist in processing NRDA ichthyoplankton samples following protocols described herein (see analysis sections below and associated Attachments). Carley Knight (USM) will also perform sample analysis for portunid decapods at the NOAA Pascagoula lab on samples under COC of Joanne Lyczkowski-Shultz. The NOAA lab will receive samples under NOAA chain-of-custody procedures from LSU and return the samples to LSU for inclusion in the overall NRDA plankton archive.

Prioritized 2010 SEAMAP plankton samples currently being held in the NOAA Pascagoula lab archive room will be processed at the NOAA Pascagoula lab following the protocols described herein, which also may include conducting a ZooScan of each sample (pending acquisition of a Zooscan). ~~Joanne Lyczkowski-Shultz, the SEAMAP Plankton Unit Leader at the NOAA~~ Pascagoula lab, manages the historical SEAMAP plankton data set and protocols, and the NRDA plankton processing protocols are based (with slight modifications) on the SEAMAP program's work and established procedures. Additional personnel at the NOAA Pascagoula lab may be identified to assist in the plankton processing efforts.

The NOAA lab in Miami, FL, under the direction of Dr. John Lamkin and Dr. Trika Gerard, will receive whole plankton samples under NOAA chain-of-custody procedures from LSU. As will occur in the LSU lab, the NOAA Miami lab will sort samples into ichthyoplankton to be placed in one jar (marked with the suffix "I" after the original sample ID) and zooplankton to be placed in another jar (marked with the suffix "Z" after the original sample ID). Once personnel in the lab are fully trained on the use of the ZooScan, scanning will be done on the ichthyoplankton and zooplankton samples. Prior to scanning with the ZooScan, large decapods and other large items/organisms in the samples will be removed, saved separately and recorded. Splits of the zooplankton samples will be made for further decapod sorting and identification. Any split samples or new samples created by removal of decapods or other organisms will be labeled with a consistent convention to be determined by the NOAA Miami Lab, LSU, and the NOAA NRDA Data Management Team. Dr. Robert Cowen and Dr. Maria Ciales (UMIAMI) will take custody of the ichthyoplankton and decapod samples that come out of the NOAA Miami lab. After ZooScanning of the zooplankton samples, decapod larvae will be sorted from split zooplankton samples for further identification by Dr. Ciales or USM's laboratory (depending on workloads at each laboratory over time). Processed samples will be returned to LSU for inclusion in the overall NRDA plankton archive. However, until the NOAA Miami lab is fully trained on the use of the Zooscan, a digital record of the samples sorted for ichthyoplankton will be scanned using a flatbed scanner in order to get a digital record of the fish larvae. Once the scans of fish larvae are completed, the ichthyoplankton samples will be sent to Dr. Robert Cowen (UMIAMI) and

the samples sorted for zooplankton will be sent back to Dr. Malinda Sutor (LSU) to be scanned and split. For the zooplankton samples that are sent back to Dr. Sutor's laboratory for scanning and further splitting, the large decapods and other large items/organisms will not be removed until the samples are processed at the LSU laboratory.

All ichthyoplankton and decapod experts will consult each other on taxonomic identifications, follow the same processing protocols, and use the same data sheets.

Expert taxonomists will aid as necessary with species identification and/or confirmation and will be selected by the trustees. Such individuals may include Nancy Copley of Woods Hole Oceanographic Institute and others as necessary and may be recommended by either the trustees or BP. Funding from BP for expenses of any expert taxonomist not identified in this sample processing plan is conditioned on BP's approval of the selection prior to initiation of work by the expert(s). All expert taxonomists that are consulted will be referenced in the final data product along with area of expertise, affiliation, and species identified/confirmed.

3.0 Sample Handling, Chain of Custody and Prioritization Procedures

Following standard practices at LSU for zooplankton samples, samples are examined upon arriving at the LSU Baton Rouge laboratory. The label on the lid and the internal label are checked against the sample ID on the COC form and any differences are noted on the COC form. ~~The pH of the sample is measured with a pH probe and buffer (borax) is added to any sample with a pH lower than 7. The pH is measured again after one hour and any additional borax is added to raise the pH to 7.5-8. When borax is added to a sample, the date, time, and initials of the individual that added the borax is noted on the COC form. The samples are then restacked in storage bins and the bins are stored in a locked, ventilated room. Key access to the room with the samples is limited to Malinda Sutor at LSU or a designated individual in her absence.~~

At LSU, Baton Rouge and NOAA Miami, as samples are removed from the storage bins for analysis, the individual removing the samples signs a sample tracking form indicating when the sample(s) were removed, for what purpose, and where they will be located in the lab. Each laboratory is equipped with locking cabinets or a lockable room where the samples will be stored at any time that they are not being actively prepped for analysis. When samples have been processed in the laboratory and then placed back into formalin preservative, their pH will again be checked to ensure it is above 7 and any needed buffer will be added as described above. The samples will then be returned to the original storage bin in the lockable cabinet or storage room to which access is limited to Malinda Sutor/Trika Gerard or a designated individual.

Whole unsorted plankton, ichthyoplankton and decapod samples shipped to NOAA Miami, NOAA Galveston, NOAA Pascagoula, and USM, respectively, will follow NOAA NRDA COC protocols. Once COC has been transferred, all samples will be stored in locked cabinets or rooms at the destination labs. Key access to the room with the samples will be limited to the leads of each lab or a designated individual.

Initially, weekly batches of sorted samples will be sent/transported to each destination lab (i.e., James Ditty at NOAA Galveston, Joanne Lyczkowski-Shultz at NOAA Pascagoula, Richard Heard/Sara LeCroy at USM, and Robert Cowen at UMIAMI Miami). The need for weekly

shipments of samples will be reassessed after the first few months of the program, and maybe increased to biweekly or monthly depending on processing rate and storage capacity of each lab. Once samples arrive at the appropriate destination lab and chain of custody has been established, the sample custodian will maintain a sample-tracking record to track each sample through all stages of laboratory processing within the appropriate laboratory. Once samples have been analyzed, those samples will be returned to Malinda Sutor at LSU Baton Rouge for storage and archiving until a permanent long-term facility can be located.

An inventory of samples under LSU COC has been conducted. Due to the large number of NRDA plankton samples that have been collected to date, priority has been assigned in a tiered approach and is primarily based on season sampled (2010-2011) and geographic region (e.g., central area near wellhead and surrounding area) (see Attachment 7). Sample prioritization will be a cooperative process with NRDA Trustees and BP, and as such the prioritization schedule in Attachment 7 may be modified via amendments in time. In the interim, samples described in tier 1 of Attachment 7 will be processed in the order provided. A projected schedule for completion of samples is below based on assumptions of level-of-effort and start-up times. The prioritization scheme and schedule may be updated as the sample processing ensues, reflecting time required for processing, scheduling needs and findings (i.e., densities and resulting processing needs), and changes and additions of personnel involved in the plankton processing program.

4.0 Laboratory Operations

4.1 Rough Sorting and Plankton Analysis at LSU Baton Rouge and NOAA Miami Laboratory

For each sample, the abundance, size-spectra, and biomass of each taxonomic group will be estimated utilizing digital techniques which analyze scanned images of plankton samples. All of the ichthyoplankton and any large individuals that mechanically interfere with sample splitting (larger jellies, shrimp, crabs, etc.) or are underrepresented in a subsample due to low numbers, will be removed and saved separately (as described below). Additional species identification of ichthyoplankton, decapod crustaceans, and other species of interest will be conducted using standard microscopy techniques with the physical samples.

Before processing samples, the displacement volume of the entire sample will be measured prior to sorting or splitting. Displacement volume of all net samples, including bongo, neuston, manta, and MOCNESS nets, will be measured as is standard SEAMAP practice. The plankton samples will be removed from formalin, rinsed to remove excess preservative, and placed in a sorting tray. All of the ichthyoplankton and any large individuals that would not be amenable to splitting will be removed. These ichthyoplankton and large individuals will be scanned on the ZooScan (Attachment 1) and then placed in two jars, one for ichthyoplankton and one for zooplankton. Scanning the specimens allows them to be measured and creates a permanent digital record of all specimens (which will be saved under chain-of-custody procedures). A split of zooplankton samples will then be made for further decapod sorting and identification. Specimens will then be preserved in 85% ethanol and stored for transfer to a taxonomic expert for identification. Fish eggs will also be sorted out of the entire sample during this step and will be enumerated. Fish eggs will be sorted, counted ($\#/m^3$) and placed into a separate specimen jar (marked with the suffix "E" after the original sample ID) in 85% ethanol for storage and archiving.

The SEAMAP plankton program has used 95% ethanol in the field due to ease of use for technicians at sea (e.g. no mistakes in dilution). In more recent years, certain SEAMAP samples have been preserved in the field using 10% formalin (i.e. one side of the bongos). SEAMAP sorted ichthyoplankton samples have been typically transferred into ethanol (regardless of initial field preservative) as it is better for long term preservation. Historically the SEAMAP program transferred specimens into 70% ethanol which is appropriate for future genetic work (personal communication with Joanne Lyczkowski-Shultz, August 2011). However, it has been found that 70% ethanol becomes somewhat acidic over time and does not preserve otoliths well. Therefore, starting in 2011, larvae, eggs, and zooplankton specimens will be stored and archived in 95% ethanol since all SEAMAP (bulk) plankton samples have always been transferred to and stored in 95% ethanol prior to analysis (sorting) regardless of initial preservative (i.e. formalin or ethanol). This is a decision made by the SEAMAP program to have some consistency between the lab and field protocols. Whole unsorted NRDA samples were mostly preserved in the field using 10% buffered formalin as a primary fixative of the specimens, although certain samples were also initially placed into ethanol (cruise/work plan and gear dependent). For the NRDA samples, it was decided amongst the experts that 85% ethanol was the best option for long term preservation of the sorted ichthyoplankton and decapod samples (regardless of initial preservative). This percentage of ethanol provides the most flexibility with future archiving and ensures that samples will not become acidic over time.

The remainder of the zooplankton sample (after ichthyoplankton, fish eggs, and large individuals have been removed as described above) will then be split sufficiently (e.g., to $\frac{1}{2}$, $\frac{1}{4}$ or $\frac{1}{8}$) to allow for scanning on the ZooScan. Each of the splits will be scanned to create a permanent, digital record of the entire sample (and that record maintained under chain-of-custody procedures). One subsample will then be split further to allow for complete separation of individual organisms and scanned with the ZooScan. At this level, it could be $\frac{1}{32}$ of the entire sample, for example. The lab will then scan three of those samples, and not all 32, for instance. The sample will then be returned to its jar and preserved with 4% buffered formalin for storage and archiving. Smaller mesozooplankton are best preserved long-term in buffered formalin, it prevents individuals from becoming more opaque over time (personal communication with Malinda Sutor, August 2011). The SEAMAP plankton program was originally targeted at larval fish, not other types of plankton, therefore ethanol is the preferred long term preservative. The preservation techniques that are presented here are the preferred methods for preservation of NRDA mesozooplankton (4% buffered formalin) and larval fish and decapods (transfer to 85% ethanol).

The scanned image of well-separated individuals from the most finely split subsample will be processed using the Zooprocess software (Attachment 2). The software identifies each individual plankter in the image, makes a number of length and width measurements, and saves the subsequent cropped image or region of interest (ROI). These data will be further processed using Plankton Identifier (Attachment 3), a software package that identifies the ROIs based on a user-created image library. A trained technician then reviews the automated identifications, corrects and documents errors, and identifies ROIs that need to be examined by an expert for identification. Experienced taxonomists in the laboratory will review these ROIs and identify the organisms or seek verification from expert taxonomists if necessary.

The images of the entire sample will be analyzed for rare organisms using a semi-automated software package called Digitizer (Attachment 4). A technician will examine each image entirely and count and measure all rare taxa using the digitizer software.

The analysis of each sample will result in (i) numbers of individuals in each taxonomic category (identified to lowest possible taxon) and (ii) length measurements (for ichthyoplankton and decapods) for each individual counted. Biomass will be determined from existing allometric length – weight relationships for each taxa. The length measurements of each individual will be converted to biomass using the appropriate equation. Decapods will be counted by stage, and length measurements will be made using both ZooScan and physical measurements as described below.

4.2 Ichthyoplankton and Decapod Identification

For NRDA samples processed under LSU Baton Rouge COC and tracking, initial sorting will occur at the LSU Baton Rouge, NOAA Pascagoula, or NOAA Miami laboratories and follow the protocols outlined in the above section. The ichthyoplankton and decapod samples will then either be processed on site at LSU Baton Rouge (by T. Farooqi) or be sent to other laboratories (NOAA Galveston, NOAA Pascagoula, USM, and UMIAMI Miami) for analysis. Samples will be maintained under NOAA, or NOAA's contractors, COC at all times.

4.3 Ichthyoplankton Analysis

At all labs, microscopy techniques for ichthyoplankton identification and length measurements will be based on the SEAMAP processing protocols used at both the Polish Plankton Sorting and Identification Center and SEFSC (section II, Attachment 8) with minor NRDA modifications as noted in the below text. Aliquoting procedures, especially for large ichthyoplankton samples, outlined in section I of Attachment 8 will be followed when necessary.

Where possible, fishes will be identified to the lowest possible taxon as prescribed by the SEAMAP protocols. Under current SEAMAP sorting and ID protocols the following 8 families of fishes are required to be identified to lowest taxonomic category possible: Clupeidae (e.g., “Sardinella /Harengula” can be used for larvae that belong to one of those two genera but that cannot be reliably distinguished from each other), Sciaenidae, Serranidae, Scombridae, Stromateidae, Mugilidae, Lutjanidae, and Carangidae. However, in practice the larvae of many taxa are routinely identified to lower level when this can be done easily. Members of the Myctophid family will be identified down to genus when possible even though SEAMAP does not require this level of identification for this family. Note that the 8 families listed above may not be numerically dominant in the deeper MOCNESS samples and, as such, deepwater specimens will be identified to at least the family level.

Specimens will be handled with fine insect dissecting forceps to minimize damage to collection material. All specimens will be enumerated and length measurements for all identifiable taxon/groupings in each sample will be made with either an ocular micrometer or digitizing software during the identification step in the process. Undamaged larvae of each taxon will be

measured to the nearest 0.1 mm notochord length (NL) or standard length (SL). Number of specimens per sample measured for length will follow SEAMAP protocols (generally a minimum of 10 for each species or lowest taxonomic group, per sample) (Attachment 8). A minimum and maximum length will be recorded (smallest fish and largest fish in the sample) for each grouping (taking minimum and maximum lengths is a modification of the SEAMAP protocols). The remaining lengths will be taken from randomly selected specimens in the grouping.

As needed to make taxonomic classifications, as prescribed by the SEAMAP protocols, standard taxonomic clearing and staining (C&S) techniques and/or high resolution microscopy will be used to assist with identifications (although unlikely given time constraints). Prior to the application of any such techniques, each specimen will be photographed/imaged so as to preserve a digital record of the specimen (which will be retained under COC and included in data reports to the NOAA NRDA Data Management Team). C&S techniques will follow the overview and protocols established by Helland (Attachment 10). Notes on morphology, meristics, pigmentation and other larval characteristics and/or a silhouette (e.g., tiff or jpg) image(s) of the specimen will be taken prior to initiation of the C&S process to document original characteristics. Images will also be taken of rare and poorly known taxa over a range of sizes that will be used to re-assign, update, and QC identifications. Specimens assigned to a taxon based on a 'best guess' identification will be preceded by the designation 'cf' (i.e., closely follows). ~~Specimens in poor condition, damaged, improperly preserved, etc., will be counted and identified to Order level (i.e., Clupeiformes, Perciformes, Gadiformes, etc), if possible, or placed in an 'Unidentifiable' category. All imagery and associated data will be maintained under COC and included in data reports to the NOAA NRDA Data Management Team.~~

Specimens will be separated and placed into labeled vials by taxon for each sample. Sample identifier or lot number will be written on one side of a label made from waterproof paper and taxon name on the other side. If two or more vials are required to store a given taxon, relevant descriptor information (i.e., vial 1 of 2 or 2 of 2) will also be written on the label. Total number of vials required to house a given taxon will be recorded on the sample identification sheet (e.g., Attachment 5) with the total number of vials for all taxa in the sample summed at the bottom of the sheet. Specimens will be preserved in 85% ethanol. Vials will be sealed with a Polyseal or Teflon-lined cap (or similar type of liner) to minimize loss of preservative.

Information, including taxon name, counts and length measurements will be recorded on a standardized sample identification sheet (Attachment 5). Attachment 5 is a modified version of the typical ichthyoplankton identification data sheet that the SEAMAP program uses (minimum and maximum length fields have been added). This form may be modified, as needed, and all parties will receive an updated version as changes are made. For NRDA data management purposes certain fields may be added to this form including the unique NRDA sample ID# and some other sample identifiers. All parties (Trustee and BP) will be provided with the updated data sheet (and any other data sheets/forms updated or added for the reporting process) for review prior to the use of the data sheet/form. Number of specimens for each taxon will be recorded, but only those identifiable to family-level or below will be measured (i.e., not those in the Unidentified fish category). Collection (i.e., information about the sample) and identification information will be maintained under COC and subsequently entered into an electronic database

maintained by the NOAA NRDA Data Management Team, who will facilitate calculation of densities by taxon, length measurements, depth strata, location, etc. Following QA/QC procedures and levels of review described in Sections 6 and 7 below, these data will be placed on NOAA NRDA.org for distribution to all trustees and BP.

4.4 Decapod Analysis

Decapod crustacean larvae will be identified to the lowest possible taxon. The decapods approximately larger than ~1mm will be analyzed (or in the case of large samples a subsample thereof). This lower limit of the size range may be revisited once sample processing is underway. Size range of decapod larvae to be identified may also be determined by retention on an appropriate mesh size (again to be determined as sample processing is underway). The larval biology and morphology for many of the species, particularly those inhabiting mesopelagic and bathypelagic portions of the water column, are incompletely described (i.e., all larval stages have not been described). Larvae belonging to speciose families such as the commercially important Portunidae (29 species reported from the Gulf of Mexico) and Penaeidae (20 species) will be identified to family initially and genus (when possible). Speciose taxa with morphologically similar larvae such as the Paguridae (45 species) and Munidae (>58 species) will be identified to family initially. Additional effort to identify specimens to more precise taxa will be determined by available time, given the sample prioritization and schedule.

Specimen identification will follow standard techniques to identify early larval stages of decapod crustaceans. Larvae will be handled with foil forceps and/or fine dissecting pins to minimize damage to specimens. Photographic images (jpg or tiff format) taken with a microscope mounted camera and notes on meristic measurements will be made of certain specimens to enhance identification (i.e., well preserved examples of common taxa). Photographic images (which will be retained under COC and included in data reports to the NOAA NRDA Data Management Team) will also be made of rare and poorly known taxa over a range of larval stages and sizes. The images will be used to create a digital catalogue of the larval morphologies of the various species. The identification of specimens assigned with uncertainty to a taxon will be preceded by the designation “cf.” (i.e., closely follows). Damaged specimens or those in poor condition or improperly preserved will be counted and identified to the lowest possible taxon (e.g., Order) or placed in an “Unidentifiable” category.

Each specimen identified will be assigned to a life history stage. Crab larvae will be assigned to zoeal stages (or pre-zoeae and zoeal stages from 1 to stage 7), megalopae, juvenile, or adult stage; some anomuran larvae may also be in a pre-zoea or mysis-zoea stage, some caridean shrimp larvae may also be zoea and postlarvae, and lobsters may also be represented by phylosomae and juveniles or puerulus larvae. Dendrobranchiata shrimp taxa may also have mysis stages in addition to nauplii, protozoeae and postlarvae or decapodid stages. Holoplanktonic shrimps may be represented by all larval, juvenile and adult stages. Adult stages of some natant decapod species (shrimp) and a few reptant species (crabs) may be collected; life history notes such as the reproductive state (e.g., if females are ovigerous) and if spermatophores are attached, will be recorded.

Specimens will be separated and placed into labeled vials by taxon for each sample. Sample identifiers or lot numbers will be recorded on one side of a label of waterproof paper, and the taxon recorded on the other side. If two or more vials are required to store a taxon, relevant descriptor information (e.g., vial 1 of 2, or vial 2 of 2) will be recorded on the label. Total number of vials required to contain each taxon will be recorded on the sample identification sheet (Attachment 5). Specimens will be preserved in 85% ethanol. Vials will be sealed with a Polyseal or Teflon-lined cap (or similar) to minimize loss of preservative.

Information including the taxon name and count by life stage will be recorded on a standardized sample identification sheet. The number of specimens for each taxon will be recorded on a sample identification sheet, but only those identified to family-level or lower will be measured (i.e., not those in the Unidentified category). A minimum of 10 (or all, if a smaller number exists) larvae will be measured to the nearest 0.1 mm for carapace length or total length, as appropriate for the taxon. Measurements will be made with a calibrated ocular micrometer, or with image analysis software (Adobe Photoshop Premier) that simplifies measurements along curved surfaces. Collection and identification information and images will be maintained under COC and entered into a digital database (maintained by the NOAA NRDA Data Management Team) to facilitate calculations of densities by taxon, larval stage, depth strata, location, etc.

5.0 Biomass Determination Protocol

~~Estimation of biomass (dry mass) from Zooscanned plankton samples will be done at the LSU lab using existing length to weight ratios for the different taxa. For taxa in the samples where established length to weight ratios are not available, ratios will be determined using organisms from samples collected during NRDA surveys specifically for the purposes of allometric analysis. All allometric equation parameters, their sources, and results will be documented in the data report.~~

Description of approach:

- The contents of these morphometric samples will be sorted into taxonomic groups using microscopy.
- 100 individuals from each group of interest spanning the normal range of sizes will be measured (on the ZooScan); and then individually weighed to get wet weights and then dried to get dry weights and finally ashed to get ash-free dry weight (carbon weight). Only a few will be digitized at any time to ensure that each individual's length and mass will be associated.
- Regression equations will be derived from the length:dry mass data and used to subsequently determine biomass.

6.0 Quality Assurance and Control Procedures

Quality assurance and quality control measures will be implemented as part of the plankton processing plan. The primary evaluation of precision (as measured by replication of sorting and counting) and accuracy (as measured by correct identification of organisms) will be by comparison of results of sorting, counting and identification of a subset of samples by more than one individual. Proper identification will be further assured by independent identification of a subset of samples by outside experts. All samples, subsamples, identification information, counts and measurements of plankton, associated notes and corrections, and imagery will be

maintained under COC procedures at all times. All labs will conduct a 100% data transcription check upon transfer of sample data from bench sheets to electronic database. The NOAA NRDA Data Management Team will pair field sample metadata with the lab results and determine completeness. Detailed descriptions of these procedures are described below for each component of the plan.

6.1 Zooplankton Image Analysis at LSU Laboratory

Digital analysis techniques provide a unique opportunity for quality control and assurance because the same sample (image) can be independently analyzed by more than one individual and the results compared. The sample is never destroyed and any questions about the analysis can be reviewed by examining the analyzed image. All images will be saved to the computer hard drive and backed up to external hard drives at the end of each day. External hard drives will be stored at an off campus, secure location under Trustee control for submission to the NOAA NRDA Data Management Team as described in the section below titled “Distribution of Laboratory Results”.

There will be weekly meetings with all technicians and Dr. Sutor to review any analysis issues and ensure there is clear communication within the laboratory group about analysis procedures.

~~For the digital analysis, once the computer analysis is complete, a technician will check the computer work and identify any unknowns, to the extent possible. Then, that work will be given to an expert (e.g., Malinda Sutor) to review and identify any remaining unknowns. Training of individuals conducting digital analyses will be documented and records maintained in personnel files (Attachment 15). During the training phase, the analysis will be fully checked to ensure there are no mistakes in any of the identifications for every sample until a technician has five samples in a row with no mistakes. Once it is found that a tech has an error rate of <5%, then every 10th sample will be checked fully. From those 10% (1 in 10) of the samples, the expert (e.g., Malinda Sutor) will randomly select 10% of the samples (1% of all samples) and send them (under COC) to an independent expert (identified by plankton experts involved in this plan or as may be recommended by either the trustees or BP) agreed to by the Trustees to do a full analysis and then compare the results. Additionally, the independent expert(s) will conduct a taxonomic identification check (but not a full independent analysis of the sample) of 5% of the total images for all samples being processed by LSU. Funding from BP for expenses of any independent expert not identified in this sample processing plan is conditioned on BP’s approval of the selection prior to initiation of work by the expert. Notwithstanding any other provision of this plan, the Trustees reserve the right to withhold from BP all information provided by an independent expert not approved by BP (and supporting documentation related to the independent expert’s work).~~

When discrepancies occur, a resolution will be reached by having detailed discussions and providing supporting literature sources and reference material where it exists to justify the decisions being made. Malinda Sutor will be responsible for mediating a resolution and implementing corrective actions. Differences in opinion will be documented in the notes regardless of disposition. All paper documentation will be in ink and any corrections will be performed by crossing out the error and initialing and dating the change. In the case of digital

records, any files that are corrected during the QA/QC process (described below) will be saved as new versions for comparison to the original file and the file name changed to designate the revision (e.g., “[original file name] -- Revision 2”).

Finally, the physical sample will be archived and available to be rescanned or examined microscopically as the process of creating the digital image does not destroy the original sample. As noted above, all digital images will be saved to the computer hard drive, backed up on external drives and stored in a secure location (other than the location where the original digital data is stored) under Trustee chain of custody.

6.2 Ichthyoplankton Sorting

The QA/QC of the rough sorting process (i.e., the removal of fish eggs and larvae) is adapted from the protocols used at the Plankton Sorting and Identification Center in Poland for SEAMAP plankton samples. Once initial training is completed and sorters have demonstrated proficiency at specimen recognition, 10% of the volume of the bulk zooplankton of every 5th sample will be re-examined by another trained sorter. If more than 5% of larvae or 5% of eggs are found not to be sorted out of the bulk zooplankton sample, the entire sample is resorted by the original sorter.

6.3 Ichthyoplankton Identification

During the initial training period, all identified fish larvae will be checked by senior ichthyoplankton experts at the respective laboratories: LSU, Miami, Mississippi, and Galveston. After proficiency in identification is attained, further QA/QC of larval fish identifications will be conducted by the re-examination of only those particularly problematic/poorly known taxa. Either the actual specimens or images of them will be viewed by senior project experts from the various project labs and a consensus identification will be reached.

After initial training period, samples for quality control will be selected based on a random numbers table with additional samples exchanged as needed to ensure inclusion of all depth strata and gear types. Five percent (5%) of the total number of ichthyoplankton samples per sub-tier evaluated by Talat Farooqi or Dr. James Ditty (see Attachment 7) will be exchanged and verified for taxonomic quality control along with sample ID sheets. Similarly, 5 percent of upper water column ichthyoplankton samples per sub-tier processed by the Pascagoula and Miami laboratories (see Attachment 7) will be exchanged and verified for taxonomic quality control. If the identity of a taxon is corrected during QC, the corrected taxon name and specimen length will be recorded along with reason(s) for the correction (i.e., meristics, spination, pigmentation, etc.). If agreement cannot be reached on a given taxon, the specimen will be assigned to the lowest agreed upon taxonomic level. Differences in opinion will be documented in the comments field on the data record form (describing discrepancies and ultimate resolution) regardless of disposition. Specimens re-assigned to a different taxon during quality control will be placed in a properly labeled vial with some identifier (i.e., ‘dot label’ or ‘X’ made with wax pencil) on the vial cap to facilitate locating the vial for specimen re-examination, if necessary. To facilitate processing improvements, a reference collection of images (and/or specimens) of rare and poorly known taxa will be maintained along with collection information and shared between researchers to help ensure uniformity in recognizing identification criteria and to facilitate communication among taxonomists.

6.4 Decapod Identification

Samples for quality control will be selected based on a random numbers table with additional samples exchanged as needed to ensure inclusion of all depth strata and gear types. Five percent of the total number of samples/sub-tier will be exchanged (as whole samples being shipped between labs and/or photographs thereof) and verified by the various decapod taxonomists involved in this plan working at the USM lab, NOAA Pascagoula, and UMIAMI Miami labs for taxonomic quality control. If the identity of a taxon is corrected during QC, the corrected taxon name and specimen length will be recorded on the corresponding lab data record form along with reason(s) for the correction (Attachment 14). If agreement cannot be reached on a given taxon, the specimen will be assigned to the lowest agreed upon taxonomic level. Specimens re-assigned to a different taxon during quality control will be placed in a properly labeled vial with an identifier (i.e., 'dot label' or 'X' made with wax pencil) on the vial cap to facilitate locating the vial for specimen re-examination, if necessary.

6.5 Data Transcriptions

For plankton sorting and ichthyoplankton and decapod identification processes, upon transfer of data from paper data sheets to electronic media, a cross-check of 100% of all transcriptions will take place by an individual other than the individual that entered the original data. This cross-check can take place by individuals in the same lab. The individual conducting the data transcription cross-check and the date it takes place will be documented on the corresponding lab bench sheet. The internal transcription audit is to be performed independent of the person who originally transcribed the bench sheet information into the database and without any intra-lab discussion of determinations prior to the resolution phase of the audit process.

7.0 Distribution of Laboratory Results

Data reporting and distribution will be accomplished as depicted in the data flow diagram (Attachment 12).

Each individual laboratory (e.g., LSU Baton Rouge, NOAA Galveston, USM, NOAA Pascagoula, NOAA Miami, etc.), will coordinate with the NOAA NRDA Data Management Team to be registered for the NOAA NRDA Content Management System [REDACTED]. The NOAA NRDA Content Management System will serve as a repository of information required by NOAA from each lab that is receiving and processing samples. Upon registration, labs will receive training on the NOAA NRDA Content Management System and more detailed instructions on the requirements for each of the categories of documentation, including "Confirmation", "Metadata", "Results", and "Sample Crosswalk."

The NOAA NRDA Data Management Team will coordinate with each lab to determine the appropriate format and required content for each type of data/results. As sample analyses and lab QA/QC processes (per Section 7.1) are completed, labs will upload results, additional metadata and quality control information, and other information determined to be appropriate to support these data (i.e., sample tracking forms, lab data sheets, COC forms, and laboratory logs). Once registered, a designated lab contact will log into the system at periodic intervals (determined by

the lab and NOAA NRDA Data Management Team) to upload the required information to the system.

Upon receipt and inventory of samples, each laboratory shall deliver an inventory and status review of all samples, including all necessary metadata and splitting or composite information, generated as part of this sample processing plan to the NOAA NRDA Data Management Team via the NOAA NRDA Content Management System [REDACTED]. There will be limited accessibility (limited to only lab and NOAA NRDA Data Management Team members) to the data until the full internal data review process has been completed in stepwise intervals, as described in Sections 6 & 7. Data will be identified as to its level of review in the noaanrda.org system and updated (monthly) as the review process proceeds.

There will be three levels of review prior to data release to all parties via noaanrda.org. Throughout the process, any changes made to taxonomic or other information will be documented on bench sheets or forms for recording this information. Imagery will be retained, along with any changes in processing software or results. All of this information will be maintained during all review steps in the process and stored in secure locations under Trustee control and will be provided to all parties as part of the data release process, except that ZooScan images will only be made available to a party upon request.

7.1 The first level of review will be internal lab QA/QC for sorting and identification as described in Section 6.

7.2 The second level will be confirmation/updates of identifications involving consultations among investigators, as described in Sections 6.1, 6.3 and 6.4. The labs will upload biological sample sorting and identification data to the SEAMAP database housed in Pascagoula, MS after this second level of review. The LSU Baton Rouge lab will receive a report of the biological sample sorting/identification data from the SEAMAP database, merge it with the image analysis data, and upload all the data to noaanrda.org.

7.3 The third level of review will be the marrying of the data provided by the labs with the corresponding field information after a 100% transcription verification by the lab providing the data (as described in Section 7.2). The NOAA NRDA Data Management Team will perform a completeness check to ascertain that the laboratory information matches up properly with field sample information and all field information has associated laboratory information.

Once processing of samples from an entire sub-tier (e.g., Tier 1A, Tier 1B, etc., Attachment 7) is completed to the third level of review, the data and supporting information referenced in section 7.0 will be made available to the parties to this agreement either on noaanrda.org or via other means [e.g. portable hard drives, etc.] as determined by the NOAA NRDA Data Management Team. NOAA and the Louisiana Oil Spill Coordinator's Office (LOSCO) on behalf of the State of Louisiana and BP (or Cardno ENTRIX on behalf of BP) will be alerted when these data become available for download on noaanrda.org.

In the interest of maintaining one consistent data set for use by all parties, only the QA/QC'd data set made available on noaanrda.org by the NOAA NRDA Data Management Team 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 14 days after such data have been made available to the parties. The parties agree to review this stipulation under section 8 and consider adjustment to this restriction period as implementation proceeds. Any questions raised on the consensus data set as it was made available to the parties on noaanrda.org shall be handled consistent with the procedures in Section 7.2 of the Deepwater Horizon NRDA Analytical Quality Assurance Plan.

- The trustees and BP shall each designate an individual responsible for raising questions, if any, on the consensus data set.
 - If questions are raised, the two designated individuals will meet to determine the source of the difference and resolve.
 - The questions raised and their resolution shall be distributed to all parties.
 - No changes to the consensus data set will be made if the differences are considered immaterial by both designated individuals, acting on behalf of the parties.
 - If the parties agree that changes to the dataset should be made, the dataset will be updated in accordance with the resolution and reposted with a notation that the dataset has been revised.
 - If the designated individuals do not agree on how to resolve the difference concerning the consensus data set, the designated individuals shall request assistance from the Assessment Managers for the trustees and BP.
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8.0 Adaptive Management

The parties agree to initiate plankton processing as described in this work plan. To address any uncertainty in how the processing will proceed until the labs begin to implement the work plan and to learn in more detail what is involved for each of the various elements, the parties further agree that after the first tier subgroup data are available to all parties, the process will be reviewed, discussed, and modified as appropriate with the agreement of all parties.

Work will continue according to this sample processing plan unless and until modified and agreed to in writing by the parties.

9.0 Progress Reporting Schedule

Progress reports will be submitted quarterly to the NRDA Water Column TWG by each lab and will include two major sections, one describing the status of sample processing in the laboratory and one describing the data uploading progress for the previous three-month reporting period. To the extent practicable, a standardized format will be used for all lab progress reports (Attachment 13). At a minimum, the laboratory operations section of the progress report should include the number of samples analyzed, general location of samples analyzed by station ID, gear and cruise information associated with the samples analyzed, date samples returned to LSU for archiving, operational/logistical issues, and planned activities for next three months. The data uploading section of the progress report should include, at a minimum, when and what data were uploaded to noaanrda.org, when confirmation, metadata, results and sample cross-checks were completed, any operational/logistical issues, and planned activities for next three months. The actual results

of the processing effort will not be summarized in the quarterly progress reports, but rather the status of the processing and data uploading effort. As discussed above, once the three levels of review are completed for a sub-tier, the actual processed data for that sub-tier will be released to noaanrda.org. In addition to the quarterly progress reports compiled by each lab, the NOAA NRDA Data Management Team will prepare and submit status reports after each sub-tier of samples has been identified for release and report the status of data uploading to noaanrda.org.

Table 2 below summarizes the approximate number of samples to be processed by each lab and associated processing task. However, there is considerable uncertainty in the time estimates for processing, as it depends greatly on the densities of animals in the samples and the initial level of experience of the contracted sorter/identifiers. It is assumed that sample processing rates will increase over the duration of the project and that taxonomic identification efficiency (i.e., time required to identify) will improve with time.

Table 2. Approximate number of samples processed by each laboratory at six-month intervals during the next twelve months. Note: Start times for some labs may be later than others, such that the various lab schedules may not be concurrent.

Lab	Processing task	Approximate # of samples per month, during first 6 months	Approximate # of samples per month, after 6 months	Approximate # of samples after 12 months
LSU Baton Rouge	Zooscan	176 (over 4 months)	176 (over 6 months)	1,760
NOAA Pascagoula	Zooscan	-	44	264
NOAA Miami	Zooscan	-	44	264
Subtotal				2,288
NOAA Galveston	Ichthyoplankton Identification	60	80	840
Farooqi – BP working out of LSU Baton Rouge	Ichthyoplankton Identification	44	50	564
NOAA Pascagoula	Ichthyoplankton Identification	77	154	1,386
NOAA Miami and U. Miami	Ichthyoplankton Identification	175	175	2,100
Subtotal				4,890
USM	Decapod Identification	50	100	900
NOAA Pascagoula	Decapod Sorting and Identification	10	10	120
UMIAMI Miami	Decapod Sorting and Identification	50	60	660
Subtotal				2,892

10.0 Budget

The NRDA plankton program includes approximately 7000 samples from summer 2010 through summer 2011. Given the uncertainty in the rate labs can process the samples and the possibility of adding additional resources, we have estimated a first-year budget here, with the expectation that we would update this estimate, the personnel involved, and the time duration of the processing once the work has begun and the scientists have a better feel for the level of effort required per type of sample. The budgets are in Attachments 6 and 11. The total budget for all labs for 12 months of effort is \$4,439,171.

Attachments:

- Attachment 1. ZooScan Description and Specifications
- Attachment 2. Zooprocess Software Description
- Attachment 3. Plankton Identifier Software Description
- Attachment 4. Digitizer Software Description
- Attachment 5. NOAA/NMFS/SEAMAP Ichthyoplankton Data Record Form
- Attachment 6. NOAA/NMFS/SEFSC, Proposal to Sort and Identify Deepwater Horizon Plankton Samples (SEFSC, Pascagoula, MS)
- Attachment 7. DWHOS NRDA Net-Caught Plankton Sampling: Prioritization and Schedule
- Attachment 8. NOAA/NMFS/SEAMAP Ichthyoplankton Sample Processing Protocols
- ~~Attachment 9. NOAA/NMFS/SEAMAP Proposal to Sort and Identify Deepwater Horizon Plankton Samples (SEFSC, Miami, FL)~~
- ~~Attachment 10. Staining and Clearing (C&S) Techniques for Ichthyoplankton~~
- Attachment 11. Budgets by Laboratory.
- Attachment 12. Plankton electronic data flow diagram.
- Attachment 13. Standardized quarterly report template.
- Attachment 14. Decapod data record form.
- Attachment 15. Training record documentation form.

Deepwater Horizon Oil Spill (DWHOS)

Water Column Technical Working Group

NRDA Plankton Processing Plan – Analysis of Zooplankton Samples

Plan Date: January 6, 2012

Approvals

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

BP Approval

Robin Bullock [Signature] 1/10/2012
Printed Name Signature Date

Federal Trustee
Approval

Lisa DePinto [Signature] 1/9/2012
Printed Name Signature Date

Louisiana Approval

KRISTINA DEBOSCHERIE [Signature] 1/27/2012
Printed Name Signature Date
FOR ROLAND GUIPAY