Restoration in Place Strategy for the Deep-sea Soft-Bottom Benthos: Long-term Monitoring to Support Restoration Efforts

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Summary

The Deepwater Horizon (DWH) incident in the northern Gulf of Mexico (GOM) occurred on April 20, 2010 at a water depth of 1525 meters, in Mississippi Canyon Block 252, releasing an estimated 3.19 million barrels of oil over the following 87 days. As part of the Natural Resource Damage Assessment (NRDA) process, a study comprising three field surveys (2010, 2011, and 2014) was conducted to identify effects of the spill on the deep-sea, soft-bottom benthos and sediment quality. Results revealed a zone of severe to moderate impacts on biodiversity linked to the DWH wellhead that persisted through 2014. Thus, an obvious restoration goal for the deep sea is to return biodiversity and other key benthic attributes to normal reference-range conditions. It is hypothesized that burial of the damaged habitat by natural deposition processes will cap the damaged sediment and restore the benthos to background conditions. The obvious question is: how much sediment is needed to cap the DWH contamination, and long will this take? Based on the NRDA studies, 95% of the benthos is within the top 10 cm of sediment. A recent examination of deep-sea sediments in the area of the 1979 Ixtoc spill, found 4 cm of fresh sediment on top of the damaged sediment. Using this rate, it is hypothesized that it will take another 65 years to have a total of 10 cm at the Ixtoc site, which implies it takes about 100 years for deep-sea sediments to recover naturally. Thus, the restoration strategy for deep-sea soft-bottom benthos must be a long-term study to monitor the recovery rate and verify that this assumption is correct.

Now is the time to begin planning specific projects for the open ocean and deep-sea benthos, because the Damage Assessment and Program Restoration (DARP) report is complete and the Open Ocean Restoration activities are being developed. However, two challenges exist: (1) rates of change in the deep sea are very slow, and (2) we know very little about temporal dynamics in the deep sea Gulf of Mexico. Until we understand basic temporal dynamics, it will be difficult, if not impossible, to ascertain if change is a result of recovery, seasonal dynamics, or year-to-year variability. Thus, the proposed sampling strategy includes both a long-term monitoring strategy to measure recovery and a short-tem experiment to identify temporal dynamics. A third component of the strategy is to analyze archived samples of opportunity collected in 2015, 2016, and 2017 during Gulf of Mexico Research Initiative (GOMRI) funded cruises, where analyses of the benthic samples were not funded.

The long-term monitoring study would include sampling 34 NRDA stations bi-annually (every 2 years) until recovery occurs (or for the length of the RESTORE program, which-ever occurs first). The 34 stations consist of 20 moderately and severely impacted sites, and 14 non-impacted sites. Spatial coverage across the treatment categories is necessary as a basis for comparing impacted versus non-impacted areas.

The temporal dynamics experiment would entail quarterly sampling over two years at six stations. Quarterly sampling is necessary to identify if seasonality exists, and a two-year cycle is required to confirm that the patterns are repeatable. Three stations in the heavily impacted zone and three stations from non-impacted zone would be sampled in order to determine recovery based on whether spatial differences between treatments are distinguishable from natural temporal dynamics.

The analysis of archived GOMRI samples will extend the NRDA time series and act as a segue to RESTORE funded monitoring. The GOMRI project was funded to perform the benthic analyses at the Ixtoc oil spill site, but additional samples were collected in the northern GOM near the DWH spill site.

For all three studies, the independent variables to be measured include: benthic macrofauna (taxa richness and total faunal abundance), benthic meiofauna (taxa richness, total faunal abundance, and nematode/harpacticoid ratios); and abiotic environmental variables [total petroleum hydrocarbons (TPH), total polycyclic aromatic hydrocarbons (total PAHs), barium, chromium, lead, zinc, total organic carbon, sediment grain size (% fines), chlorophyll and phaeophytin, and CTD profiles (conductivity, temperature, pH, dissolved oxygen, depth).

The hypothesis-based study design will be analyzed using a combination of analysis of variance (ANOVA) to test for differences in univariate responses, and multivariate analysis to determine patterns in the multivariate community data, and to link biological responses to environmental driver variables. The underlying goal is to assess the persistence of previously observed DWH-related impacts on the benthos and to look for evidence of recovery over time in light of other natural spatial and temporal (including within-year seasonal) variability.

Products will include archived raw data, reports, and "restoration targets" for determining recovery of Gulf of Mexico deep-benthic resources from DWH-related impacts and similar events in the future. Educational components that expose future generations of scientists and others to the value of the RESTORE research will be created at several levels (visiting researchers and teachers, post-doctoral fellows, graduate students, undergraduates, and K-12).

1. Introduction

1.1. Background

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, releasing an estimated 3.19 million barrels of oil over the following three months (DWH Trustees 2016). Oil and gas flowed into the Gulf of Mexico until the wellhead was capped on July 17, 2010. While oil-budget estimates indicate that a majority of the oil had been removed by cleanup operations, other natural mechanisms, or was present at the surface in oil slicks, up to 35% of the hydrocarbons were trapped and transported in persistent deep-sea plumes (Ryerson et al. 2012). There were also dispersants (0.77 million gallons) applied to the wellhead, and this also was injected into the deep sea plume (Kujawinski et al. 2011). It is also likley that significant DWH-derived pollution products could have moved into offshore and deep-water sediments via several potential pathways — e.g., sinking of oil and/or dispersed oil droplets adsorbed onto suspended particles or marine snow, or incorporated into copepod fecal pellets, in either surface or sub-surface layers; onshore-offshore transport of oil-laden particles; sinking of heavier oil by-products resulting from the burning of oil; or settling of oil-mud complexes resulting from the injection of drilling mud during top-kill operations (UAC 2010). In addition, drill cuttings, drill fluids, and other containment fluids commonly used during offshore oil-drilling operations (Neff et al. 1987, Neff 2005) were likely released and deposited to the bottom during the blowout event.

1.2 NRDA Process and Restore

The Oil Pollution Act authorizes certain federal agencies, states, and Indian tribes — collectively known as natural resource trustees — to evaluate the impacts of oil spills, ship groundings, and hazardous substance releases on natural resources.[1] These trustees are responsible for studying the effects of the spill through a process known as Natural Resource Damage Assessment (NRDA). As part of this process, scientists work together to identify potential injuries to natural resources and lost public uses resulting from the spill. After an oil spill or hazardous substance release, response agencies such as the U.S. Environmental Protection Agency or the U.S. Coast Guard clean up the substance and eliminate or reduce risks to human health and the environment. But these efforts may not fully restore injured natural resources or address their lost uses by the public. Through the NRDA process, NOAA and other trustees conduct studies to identify the extent of resource injuries, the best methods for restoring those resources, and the type and amount of restoration required. NOAA conducts the following three steps in an NRDA:

- Preliminary Assessment
- Injury Assessment/Restoration Planning
- Restoration Implementation

The restoration phase implements restoration and monitoring its effectiveness.[2] NOAA and co-trustees have identified the full range of injuries to coastal and marine resources and determined the restoration that must occur. Trustees are now working with the public to select and

[1] http://www.gulfspillrestoration.noaa.gov/assessment/ accessed March 19, 2013.

[2] http://www.gulfspillrestoration.noaa.gov/restoration-planning/gulf-plan accessed 25 April 2017

implement restoration projects for the open ocean.[3] The trustees for the Open Ocean Restoration Area are: U.S. Department of the Interior, National Oceanic and Atmospheric Administration, U.S. Department of Agriculture, and U.S. Environmental Protection Agency. The allocation of Open Ocean Restoration Area Funds are distributed among 10 restoration goals (Table 1).

Table 1. The restoration goals and types for the Open Ocean Restoration Area.

Restoration Type and Goals	Allocation
Replenish and Protect Living Coastal and Marine Resources	
Fish and Water Column Invertebrates	$380,000,000
Early Restoration Fish	$20,000,000
Sturgeon	$15,000,000
Sea Turtles	$55,000,000
Marine Mammals	$55,000,000
Birds	$70,000,000
Mesophotic Reefs and Deep Benthic Habitat	$273,300,000
Provide and Enhance Recreational Opportunities	
Early Restoration of Recreational Loss	$22,397,916
Monitoring, Adaptive Management, Administrative Oversight	
Monitoring and Adaptive Management	$200,000,000
Administrative Oversight and Comprehensive Planning	$150,000,000
Total NRD Funding for Open Ocean Restoration Area	$1,240,697,916

This current project idea is focused towards the goal of restoring "Mesophotic Reefs and Deep Benthic Habitat." It applies however to the deep soft bottom habitat only, and it is proposed that "restoration in place" strategy be adopted, which would require a long-term monitoring strategy.

1.3 Rational for a Restoration in Place Strategy

Restoration in place is recommended because the oil in the deep sea is scattered over a very large area covering as much as 3,200 km^2 (Valentine et al. 2014), but the oil has a very patchy distribution on the seafloor that can vary of scales of 2 – 20 m (Montagna et al. 2016). This vast, patchy distribution, at great depths around 1500 m, means that it is impractical to be able to pick-up or remove the oil that is deposited. However, it is hypothesized that burial of the sea floor habitat by natural deposition processes will cap the damaged sediment and restore the benthos to background conditions. The obvious question is: how much sediment is needed to cap the DWH contamination, and long will this take?

Based on the recent NRDA studies, 95% of the meiofauna and macrofauna in the benthos is within the top 10 cm of sediment (Montagna et al. 2016). Therefore, we can assume that the

[3] http://www.gulfspillrestoration.noaa.gov/restoration-areas/open-ocean accessed 25 April 2017

viable benthic habitat is 10 cm deep, and it will be just a matter of time before fresh material is deposited on the sediment by natural processes and caps the damaged sediment.

A recent examination of deep-sea sediments in the area of the 1979 Ixtoc I spill, found about 3-4 cm of fresh sediment on top of the damaged sediment (Figure 1). Therefore, 34 years

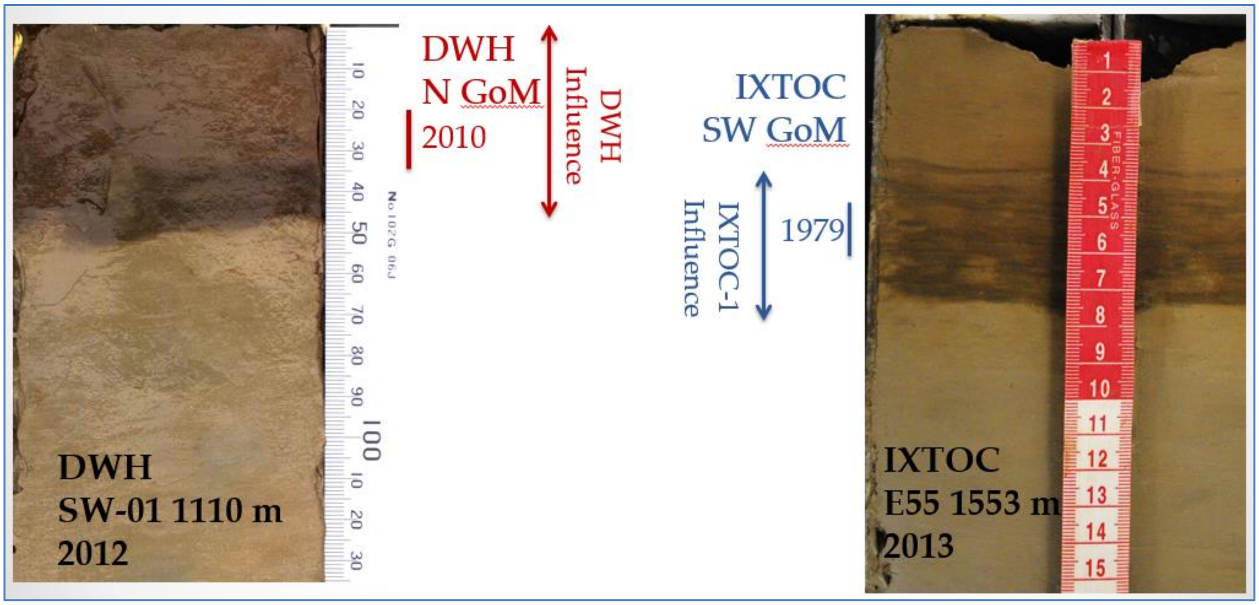

Figure 1. Comparison of Deepwater Horizon 2012 and Ixtoc 2013 sediment profiles.

after the Ixtoc blowout, the sedimentation rate appears to be about 1 mm per year. Using this rate, it will take another 66 years to have a total of 10 cm at the Ixtoc site, which implies it takes about 100 years altogether for deep-sea sediments in the Ixtoc area to recover naturally. Because of the influence of the Mississippi River, it is likely that the deposition rates in the northern Gulf of Mexico will be higher, thus the recovery rates more rapid. Thus, the restoration strategy must be a long-term study to monitor the recovery rate and verify that the assumptions and that this is the correct rate.

Another important aspect of this proposed project is that it is a long-term environmental study. Long-term studies have a disproportionate influence on policymaking and scientific impact than shorter term project (< 4 years) typically being funded (Hughes et al. 2017).

2. Completed Monitoring Work

2.1 Initial Sampling Efforts and Damage Assessment

An assessment study was initiated in May 2011, under the direction of the DWH Natural Resource Damage Assessment (NRDA) Deepwater Benthic Communities Technical Working Group (NRDA Deep Benthic TWG), for the purpose of assessing potential impacts of the DWH oil spill on sediments and resident benthic fauna in deep water (> 200 meters) areas of the Gulf. Key objectives of the study were aimed at completing the analysis of samples from 68 priority stations sampled in September-October 2010 on two DWH Response cruises (*R/V Gyre* and *R/V Ocean Veritas*) and from 38 long-term monitoring sites (including a subset of 34 of the original 68) sampled on a follow-up NRDA cruise in May-June 2011 (Figure 2). Further details are provided in the Deep Benthic TWG Study Plan for Deepwater Sediment Sampling (approved May 2011)[4].

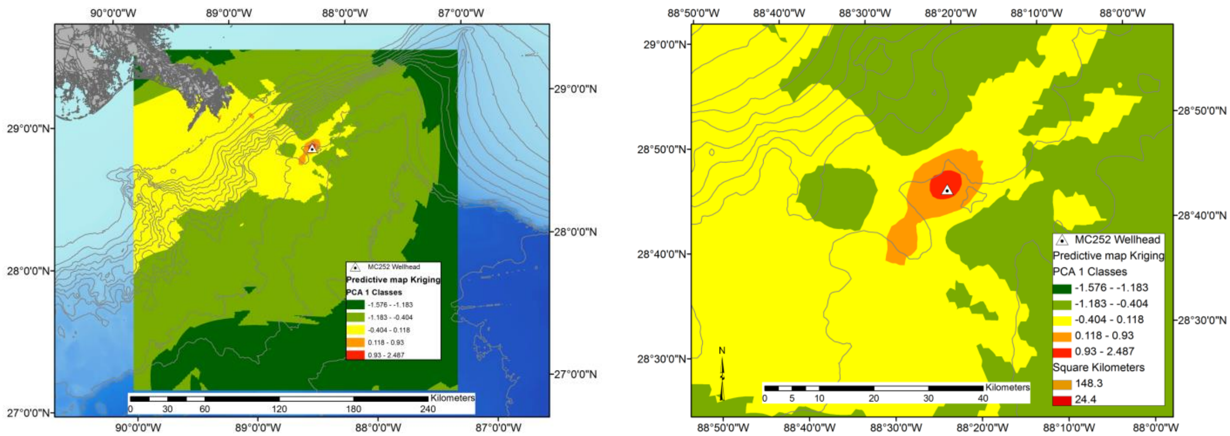

Figure 2. Map of the deep sea benthic footprint of the Deepwater Horizon oil spill where red = severe impact, orange = moderate impacts, yellow = area of uncertainty, light green and green = natural background conditions (Montagna et al. 2013). Left: Map of full area. Right: Map of zoomed in area.

Montagna et al. (2013) provide a summary of results from the initial processing of samples from fall 2010 priority sites. Analyses of the 2010 dataset resulted in first estimates of the oil spill footprint by creating a new combined biotic/abiotic variable with principal components analysis. The first principal components factor (PC1) was indicative of the oil spill impacts and this new variable was mapped in a geographic information system to identify the area of the oil spill footprint. The most severe relative reduction of faunal abundance and diversity extended to 3 km from the wellhead in all directions covering an area about 24 km^2. Moderate impacts were observed up to 17 km towards the southwest and 8.5 km towards the northeast of the wellhead, covering an area 148 km^2. Benthic effects were correlated to total petroleum hydrocarbon, polycyclic aromatic hydrocarbons and barium concentrations, and distance to the wellhead; but not distance to hydrocarbon seeps. Thus, benthic effects are more likely due to the oil spill, and not natural hydrocarbon seepage. The losses of macrofauna were found mainly in surface sediments (Washburn et al. 2017).

Sediment quality benchmarks for assessing risk were derived and the likelihood of impacts to macrofauna and meiofauna communities is low (<20%) at TPH concentrations of less than 606 mg/kg (ppm dry weight) and 700 mg/kg respectively, high (>80%) at concentrations greater than 2144 mg/kg and 2359 mg/kg respectively, and intermediate at concentrations in between (Balthis et al. 2017). Certain macrofauna taxa were found to be bioindicators of change (Washburn et al. 2016), for example Crustacean taxa appeared to be sensitive to the deep-sea blowout. Polychaete taxa varied in their sensitivity, but the family Dorvilleidae, which is often associated with organic

[4] http://www.gulfspillrestoration.noaa.gov/sites/default/files/wp-content/uploads/2011/05/2011_04_07_DEEPWATERBENTHIC_Softbottom_Sediment_la-4-6-2-11.redacted2.pdf

enrichment, was responsible for the largest amount of dissimilarity between stations close and far from the wellhead.

Continuing and more detailed investigations of harpacticoid family-level diversity (Baguley et al. 2015, in prep) suggest a greater spatial extent of the benthic footprint, perhaps 90+ km to the southwest of the wellhead and in the trajectory of the deepwater plume.

2.2 Summary of Follow-up Results: Spring 2011, and Spring 2014

In follow-up cruises in the springs of 2011 and 2014, the sample design was reduced to a total of 34 stations (Figure 3). The stations were divided into two main DWH effects zones as defined by (Montagna et al. 2013, Figure 2): "impact" (zone 1=high impact from the red coded stations, and zone 2=moderate impacts from orange-coded stations versus "non-impact" (zone 3=uncertain impacts from the yellow-coded stations, and zone 4=unlikely impacts from the light green coded stations). For this analysis, there were 20 impact stations and 14 non-impact stations.

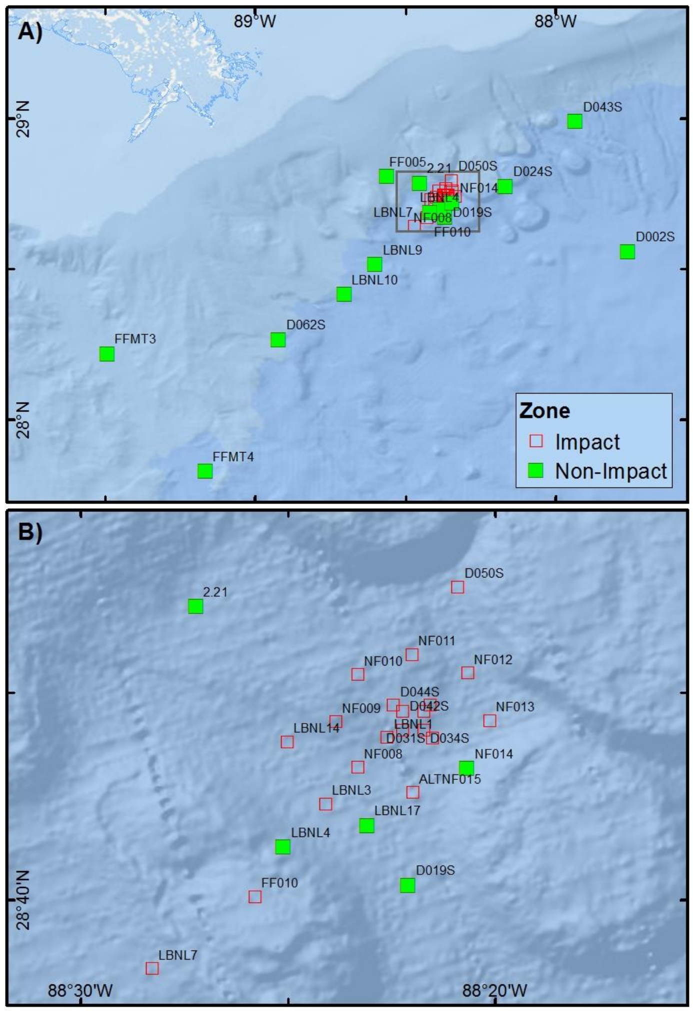

Figure 3. Locations of stations sampled in 2011 and 2013.

Macrobenthic community diversity did not change between 2010 and 2011 cruises, and across impacted vs. non-impacted zones, which suggests that the footprint of benthic impacts in Figure 2 still persisted and that recovery has not yet occurred (Montagna et al. 2017). When all three years were compared, chemical contaminants were significantly different between the two zones (Reuscher et al. 2017). Polycyclic aromatic hydrocarbons averaged 218 mg/kg in the impact zone compared to 14 mg/kg in the non-impact zone. Total petroleum hydrocarbons averaged 1166 mg/g in the impact zone compared to 102 mg/g in the non-impact zone. While there was no difference between zones for meiofauna and macrofauna abundance, community diversity was significantly lower in the impact zone. Meiofauna taxa richness over the three sampling periods averaged 8 taxa/sample in the impact zone, compared to 10 taxa/sample in the non-impact zone; and macrofauna richness averaged 25 taxa/sample in the impact zone compared to 30 taxa/sample in the non-impact zone. The contaminants and diversity metrics had parallel responses for zones over time, suggesting that there was no recovery in these metrics from 2010 to 2014.

The results of all our studies to date indicate that oil originating from the Deepwater Horizon blowout spill reached the seafloor and has had persistent negative impacts on diversity of soft-bottom, deep-sea benthic communities. While there are signs of recovery for some benthic community variables (such as abundance), full recovery had not yet occurred by 2014.

3. Deep-sea Ecosystem Services and Restoration Goals

The Deepwater Horizon accident is the first large-scale deep-sea disturbance event caused by petroleum hydrocarbon production, so implementation of hydrocarbon extraction in the deep sea is now of great concern. In contrast, disturbance caused by deep-sea trawl fishing and mineral extraction has been a major concern for quite a long time. When restoration of the deep sea has been proposed in the past, concern is expressed as to the values of deep-sea ecosystems and what the restoration goals might be. While the deep sea covers nearly 70% of the Earth's surface, we know more about the contours of the surface of the moon than we know about the deep sea, because it is so difficult to sample and explore the deep depths of the world's oceans. Consequently, we know little about the ecosystem services provided by the deep sea that benefit human health and well-being. However, one way to identify ecosystem services is to identify the functional roles of the deep-sea ecosystem in the global ocean system, and which of those roles provide actual benefits to humans.

Contaminants from the Deepwater Horizon spill that ultimately made their way to the seafloor pose risks to soft-bottom benthic fauna living within or in close association with bottom substrates because they are unable to avoid exposure due to their relatively sedentary existence. Potential losses are of concern because these fauna serve vital functional roles in the deep-sea ecosystem. These roles include:

- Sediment bioturbation and stabilization (Thistle 2003).
- Organic matter decomposition and Carbon storage (Gage 2003; Danovaro et al. 2008)
- Nutrient regeneration (Gage 2003; Danovaro et al. 2008).
- Secondary production and energy flow to higher trophic levels (Tenore 1977; Gray 1981; Gage 2003; Danovaro et al. 2008), which includes both ecologically and commercially relevant species.
- Important reservoirs of marine biodiversity with greater species richness than shallow water habitats, and a total global biodiversity that rivals tropical rainforests (Hessler and Sanders 1967; Jumars 1976; Gage 1979; Hecker and Paul 1979; Rex 1981; Rowe et al. 1982; Grassle and Morse-Porteous 1987; Grassle and Maciolek 1992; Blake and Grassle 1994; Baguley et al 2006).

Of the functions listed above, the most important is the high biodiversity. High benthic species diversity has been reported for the Gulf of Mexico with a maximum on the mid to upper continental slope at depths between 1200 to 1600 meters (Tyler 2003; Baguley et al. 2006; Wei and Rowe 2006; Rowe and Kennicutt 2008, 2009; Haedrich et al. 2008; Wei et al. 2010), which coincides with depths of the DWH well site and potential zone of exposure. A recent study by Danovaro et al. (2008) provides evidence linking the loss of benthic biodiversity to an exponential decline in deep-sea ecosystem functioning.

Considering that the primary footprint of the Deepwater Horizon accident is a loss of biodiversity, a major restoration goal for the deep sea should be restoration of biodiversity loss.

Restoration targets for this and other key biological and abiotic attributes will be quantified and tracked for recovery in the proposed study (see section 5 below).

4. Long-Term Monitoring Design Criteria

4.1 Experimental Design Constraints

Evaluation of long-term impacts and the ultimate restoration relies upon the identification of a before vs. after, control vs. impact (BACI) experimental design. The BACI Design – A before vs. after (BA), and control vs. impact (CI) experimental design is necessary to test for significant differences of indicator metrics both before vs. after an event, and at impacted vs. non-impacted locations. This is the most powerful statistical method to determine environmental impacts, and depends upon data collected prior to the environmental change in question both in the region of the impact and at appropriate control sites (Green 1979). In the typical application of the BACI design, sampling occurs prior to an event so that the baseline condition is known for comparison with post-event conditions. In the context of restoration, the "before" baseline condition is the damaged condition observed post-spill; and the "after" condition is the recovery and restoration that will be observed over the long-term. In addition to temporal aspects of the design, there must be a spatial aspect of the design that includes control sites. Controls, or reference sites, are necessary because we must be able to distinguish between changes over time due to the anthropogenic event (i.e., the oil spill) as compared to natural changes (i.e., natural year-to-year variability). Thus a significant interaction between space and time means that something different is happening at the impact site relative to the control site. Thus, the null hypothesis of a BACI design is that there is no significant interaction between BA and CI. In other words: the change in metrics over the long-term is no different from natural change in space and time. As demonstrated in the follow-up studies (section 2.2) we have already identified a series of replicate control and impact stations, which we have labeled "non-impact" and "impact" stations (Figure 3).

4.1. Temporal Variability Constraints

The deep sea is uniformly dark and cold (4 - 5 °C) and relatively isolated from the surface water column. Thus, the dogma of deep-sea research is that the deep sea is a constant, invariant environment. This dogma led Howard Sanders (1968) to propose the stability-time hypothesis to explain the high diversity found in deep-sea environments. While the stability-time hypothesis does not adequately explain all deep-sea diversity patterns, the idea that the deep sea is generally more stable than shallow-water systems over time has not been contested. The influence of the variability of the Mississippi River on the Gulf provides a plausible mechanism for both seasonal and year-to-year changes over time. In addition, there are at least two datasets indicating that stability may not be true in the Gulf of Mexico.

The Deep Gulf of Mexico Benthic Program (DGoMB) (i.e., Rowe and Kennicutt 2009) provides a case study. A total of 43 stations were sampled during the first cruise (May – June 2000); 7 stations were reoccupied during the second cruise (June 2001); and during the third cruise, 2 stations were reoccupied and 5 stations were sampled in the abyssal plain. Rather than use all 66 stations over three years, it is best to use the 7 stations (C7, MT1, MT3, MT6, S36, S41, S42) that were sampled twice (in 2000 and 2001), thus providing a dataset for analysis of temporal change. This is a simple 2-way analysis of variance (ANOVA). So, ANOVA was performed on both meiofauna and macrofauna data that were log-transformed (Table 2). One important finding is that there are differences in meiofauna and macrofauna total abundance between the two years, but there is no significant 'cruise-station' interaction, meaning that change across the area happened in similar ways at all stations. This result indicates that there is year-to-year variability in the Gulf of Mexico deep sea.

Table 2. ANOVA table for differences between cruises (2000 and 2001) and stations (C7, MT1, MT3, MT6, S36, S41, S42) during DGoMB. Five replicates for each cruise-station cell, macrofauna and meiofauna abundance (log transformed).

Source	DF	Pr > F	
		Macrofauna	Meiofauna
Year	1	0.0085	0.0034
Station	6	<0.0001	<0.0001
Year*Station	6	0.2949	0.3770
Error	56		

During the NRDA assessment phase, 34 stations were sampled during the fall 2010, spring 2011, and spring 2014 cruises (Figure 3). If we examine the change for just the 14 far-field stations, then we can determine if there is change over time without regard to the DWH oil spill. Again, differences in abundance and taxa richness among years were found for macrofauna (Table 3, Figure 4). There were no differences for meiofauna abundance among years, but richness was different (Table 3).

Table 3. ANOVA table for differences in macrofauna abundance and diversity between years (2010, 2011, and 2014) and far-field stations during the NRDA process. For macrofauna, there were three replicates for each cruise-station cell, macrofauna abundance Log (nm2 + 1) and species richness (n species/core). For meiofauna, there was one core per station, so there is no interaction.

Source	Macrofauna (Pr > F)			Meiofauna (Pr > F)		
	DF	Abundance	Richness	Df	Abundance	Richness
Year	2	0.0042	0.0001	2	0.5797	0.0003
Station	13	<.0001	<0.0001			
Year*Station	26	0.1323	0.0307			
Error	121			41		

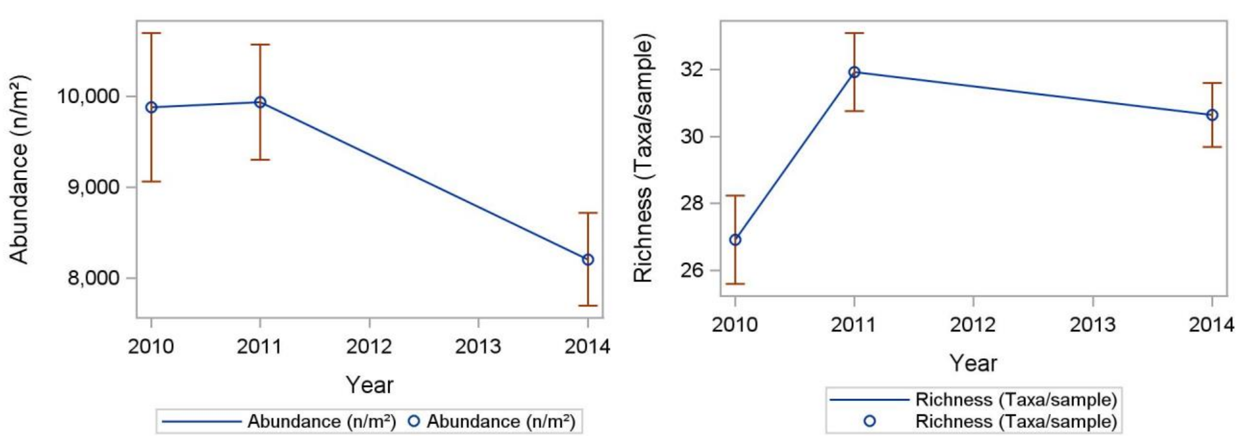

Figure 4. Change in macrofauna abundance and taxa richness over time in the unimpacted stations.

These two studies illustrate an important point. Any long-term monitoring program must be able to distinguish natural year-to-year variability from change due to recovery. In addition, the two cruises in the NRDA study were deployed in fall and spring, so there is also the possibility that the NRDA study results are due to seasonal variability and not year-to-year variability, because we really don't know if there is seasonality in the Gulf of Mexico.

It is easy to hypothesize that both seasonal and year-to-year variability exists in the Gulf of Mexico. Seasonality could be driven by discharge from the Mississippi River, which is higher in spring than at other times of the year. Thus, the surface waters have supplies of nutrients in spring that could lead to spring blooms and thus greater deposition of organic matter in spring, which would fuel increased benthic metabolism and could change benthic structure and function. Year-to-year variability could be driven by any one of three phenomena or a combination of all: inter-annual differences in river discharge (which drives differences in the size of the hypoxic zone on the Louisiana continental shelf); inter-annual variability in the timing, location, and intensity of the loop current; and inter-annual variability in the number, frequency, and strength of tropical storms.

Because it is clear that that we know too little about temporal variability in the Gulf of Mexico deep sea to be certain whether we can distinguish change due to recovery from natural variability, a temporal variability experiment for the study is proposed below. We already have sufficient information to know that inter-annual variability is likely to exist, so the temporal variability component is primarily designed to identify if seasonality occurs. For example, we need to know if we can infer that change from September to May is due to inter-annual or seasonal variability.

4.2. Spatial Variability Constraints

There are two issues about spatial distribution of sampling areas that constrain study design: locations of control sites and the boundary of the impact zone. Both of these issues have been dealt with in the results of the NRDA soft-bottom study (Montagna et al. 2013, Fig. 2). It is clear that we can, and have, identified the reference areas (in green and light green) and the impact areas (in red and orange) during the NRDA phase (Figure 1). There are however, spatial issues to resolve. The main one is the large yellow area that represents a zone of uncertainty. For example, notice there is a large green splotch due west of the impact zone. This splotch was due to one station and the lack of stations to the west of the impact zone into shallower waters. Another concern is that the bottom underneath the full extent of the deep-sea subsurface plume may not have been adequately sampled, which implies that the impact may be much larger and would have been detected with more stations. Whereas the initial analysis based on hydrocarbons, barium, and macrofauna and meiofauna abundance and taxa diversity shows moderate impacts extending only 15 km to the southwest (Figure 2), the analysis of Harpacticoida families indicates that this zone extends 90 km to the southwest (Baguley et al. 2015, in prep).

5. Proposed Long-Term Monitoring Design

The proposed monitoring design consists of three components:

(1) Long-term, bi-annual (i.e, every two years) sampling at 34 stations to test for the persistence of impacts in relation to the DWH wellhead that were observed during previous sampling events in 2010, 2011, and 2014 (Figures 3 and 5). The 34 stations consist of 20 (severe and moderate) impact stations and 14 non-impact stations. Spatial coverage across the three

treatment categories is necessary as a basis for comparing impacted versus non-impacted areas and because it is anticipated that recovery in the moderately damaged zone may occur faster than in the severely damaged zone.

(2) Quarterly sampling over a 2-year period (February, May, August, and November) at six of the above 34 stations in order to provide a basis for evaluating recovery in light of potential natural seasonal changes. The six stations selected for seasonal sampling consist of three of the severely impacted and three of the non-impacted sites.

(3) Analysis of archived macrofauna and meiofauna samples collected during GOMRI-sponsored cruises in the northern Gulf of Mexico would provide a detailed, higher frequency, time series than proposed in the biannual sampling. There were five stations sampled in summers of 2015, 2016, and 2017, of which one was in the impact zone and four were in the non-impact zone (Table 3, Figure 5). These samples were collected as part of the C-IMAGE (The Center for the Integrated Modeling and Analysis of the Gulf Ecosystem) consortium funded by GOMRI. These archived samples are paired with microbial (bacteria and protozoan), biogeochemical, and contaminant samples that have already been analyzed. The benthic invertebrate samples were taken and archived with the hope that they might be analyzed at a later time. These samples would provide a basis of comparison with earlier NRDA samples, and provide a complete picture of the benthic food web effects because there would be samples at every level: bacteria, protozoa, meiofauna, and macrofauna, which provides a unique opportunity to model benthic trophic interactions after the oil spill.

Table 3. List of proposed restoration sampling sites and study components for stations. Designation of impact status is based on results of initial Sep-Oct 2010 sampling (Montagna et al. 2013) and follow-up studies (Montagna et al. 2017, Reuscher et al. 2017); where: I = Impacted, U = Un-Impacted, L = Long-term sampling, E = Experimental sampling, and A = Archived samples from the GOMRI project.

Station	Latitude	Longitude	Depth (m)	Wellhead (km)	Impact	Study Component
2.21	28.784596	-88.453714	1367	10	U	L
ALTNF001	28.734789	-88.370533	1543	1	I	L/E
ALTNF015	28.709925	-88.366436	1607	3	I	L
D002S	28.557089	-87.760689	2389	63	U	L
D019S	28.672706	-88.368517	1656	7	U	L
D024S	28.774570	-88.167545	1697	20	U	L/E
D031S	28.731703	-88.358731	1508	1	I	L
D034S	28.734822	-88.362208	1544	1	I	L
D040S	28.742303	-88.362169	1517	1	I	L/E
D042S	28.742525	-88.370500	1502	1	I	L
D043S	28.989167	-87.934643	1492	51	U	L/A
D044S	28.744919	-88.374242	1493	1	I	L
D050S	28.792450	-88.348483	1432	6	I	L
D062S	28.265647	-88.923322	1303	76	U	L/A
D067S	29.139350	-87.364940	1162	109	U	A
FF005	28.807000	-88.561000	1003	21	U	L
FF010	28.668000	-88.430000	1356	10	I	L
FFMT3	28.218692	-89.491714	1002	125	U	L/E
FFMT4	27.828322	-89.164775	1405	128	U	L/E
LBNL1	28.732000	-88.376800	1578	1	I	L/E
LBNL10	28.415570	-88.704270	1402	49	U	L
LBNL14	28.730175	-88.416986	1535	5	I	L
LBNL17	28.696767	-88.384875	1595	5	U	L
LBNL3	28.705231	-88.401672	1585	5	I	L
LBNL4	28.688081	-88.418439	1422	8	U	L
LBNL7	28.639167	-88.471294	1577	15	I	L
LBNL9	28.514140	-88.600570	1516	34	U	L
NF006MOD	28.745081	-88.359400	1517	1	I	L
NF008	28.720005	-88.388440	1585	3	I	L/A
NF009	28.738219	-88.397370	1489	3	I	L
NF010	28.757164	-88.388669	1439	3	I	L
NF011	28.765306	-88.366883	1449	3	I	L
NF012	28.757853	-88.344461	1520	3	I	L
NF013	28.738786	-88.335619	1567	3	I	L
NF014	28.719603	-88.344700	1579	3	U	L
VK916	29.106744	-87.888737	1132	64	U	A

Table 4. List of proposed GOMRI stations that are comparable to NRDA stations for long-term time series analysis between 2010 and 2017.

GOMRI Station	NRDA Station	NRDA Zone
DSH10	D043S	Non-Impact
SW01	D062S	Non-Impact
PCB06	D067S	Non-Impact
DWH01	NF008	Impact
DSH08	VK916	Non-Impact

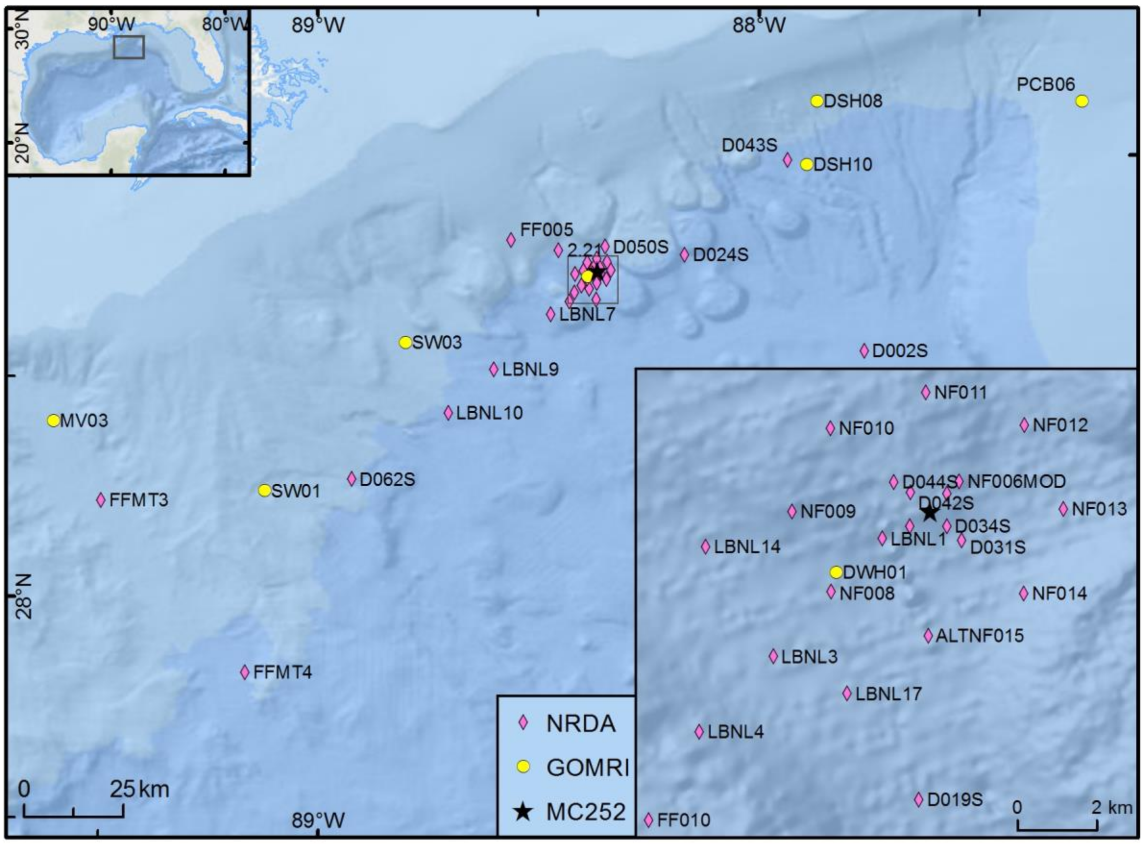

Figure 5. Long-term NRDA and GOMRI station locations.

The following benthic (macrofauna and meiofauna) and abiotic response variables will be sampled synoptically at each of the above sampling sites:

- **Benthic macrofauna:** taxa richness (# taxa to at least family level), diversity (N1), total faunal abundance (#/m^2).
- **Benthic meiofauna:** taxa richness (#major taxa and Harpacticoida to the family level), diversity (N1), total faunal abundance (#/m^2), nematode/harpacticoid ratios.

- **Abiotic environmental variables:** total petroleum hydrocarbons (TPH), total polycylcic aromatic hydrocarbons (total PAHs), trace metals, total organic carbon, total carbon, total nitrogen, sediment carbon and nitrogen stable isotopes, sediment grain size, pigments, CTD profiles (conductivity, temperature, pH, dissolved oxygen, depth).

Methods for the analysis of benthic variables will be consistent with those used in prior related sampling events (September-October 2010, May-June 2011, and May-June 2014; Montagna et al. 2013, Montagna et al. 2017, Reuscher et al. 2017) as well as standard techniques in marine benthic ecology (e.g., Elefteriou and McIntyre 2005). Briefly, macrofaunal samples will be collected and processed in the following manner: (1) three sediment cores (0.01 m^2 each) will be collected from a single multi-core drop at each station; (2) each core will be extruded into five vertical sections (0-2, 2-4, 4-6, 6-8, and 8-10 cm); (3) resulting samples will be preserved in the field in 4% buffered formalin with Rose Bengal, sieved in the laboratory on a 0.3-mm mesh screen, and transferred to 70% ethanol; and (4) animals in each of the above samples will be sorted from remaining sediment and debris under a dissecting microscope, counted, and identified typically to the family level.

Meiofaunal samples will be collected and processed in the following manner: (1) three sediment cores (0.01 m^2) will be collected from a single multi-core drop at each station; (2) six vertical sections (0-1, 1-2, 2-4, 4-6, 6-8, and 8-10 cm) and sub-sampled using a 0.0024 m^2 corer; (3) resulting samples will be treated in the field with 7% $MgCl_2$ as an initial relaxant, fixed in a solution of 4% buffered formalin with Rose Bengal, and sieved subsequently in the laboratory on a 0.045-mm mesh screen; and (4) after sieving, animals in each of the above samples will be extracted from remaining sediment and debris using isopycnic centrifugation in Ludox HS-40 (Burgess 2001), counted, and identified to major taxonomic groups (order level or higher, though harpacticoid copepods will be identified to family level). Specimens will be identified to family or higher taxonomic levels in order to reduce processing time and because many of these deep-sea fauna have not been described previously to the species level. Also, using data from higher taxonomic levels in benthic studies has been shown to depict patterns similar to those using species-level data (Heip et al. 1988, Warwick 1988, Montagna and Harper 1996) and is a much faster, and thus cheaper, process.

Sediment grain-size distribution, water content, carbon (C), nitrogen (N), and total organic carbon (TOC) concentration of sediment will be measured. The sediment grain-size procedures are based upon currently accepted practices in benthic ecology and sedimentology (Folk, 1968; Plumb 1981). The C, N, and TOC methods are based on Hedges and Stern (1984).

Stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ in the sediments will be measured to determine contribution of different sources of C and N in the sediments. The isotopes will be proxy for oil versus marine snow contribution to C and N in sediments. Carbonates will be removed from for δ13C and %C analyses by contact with HCl fumes in a vacuum-enclosed system. Carbon and nitrogen elemental and isotopic compositions are determined using a Costech ECS4010 elemental analyzer (EA) connected to a continuous flow Thermo Delta V Plus isotope ratio mass spectrometer (IRMS) via a Thermo Conflo IV interface (Paul et al. 2007). Solid samples are loaded into a Costech Zero Blank Autosampler and introduced to the EA where they are combusted in an oxidation furnace set at 1000˚C using dynamic flash combustion in helium carrier gas and excess oxygen gas. The gaseous products are carried to a reduction furnace set at 650˚C. N_2 and CO_2 gas are separated in a 3 m GC column (45 ˚C) and introduced to the IRMS via the Conflo IV.

Results are expressed in the δ notation as deviations from standards (Vienna Pee Dee Belemnite for $\delta^{13}C$, N_2 in air for $\delta^{15}N$) following the formula: $\delta^{13}C$, and $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 10^3$, where R is $^{13}C/^{12}C$ and $^{15}N/^{14}N$, respectively.

Photosynthetic pigments will be used as indicators of flux of organic material to sediments. Sediment samples for pigment analysis will be immediately placed in 90% acetone after collection. Chlorophyll a (Chl a) and phaeophytin a (Phae a) concentrations are measured spectrophotometrically or flurometrically by the acidification technique (Lorenzen, 1967) as modified by Montagna and Spies (1985) for sediment samples with oil present.

The analysis of chemical contaminants will focus on concentrations of total petroleum hydrocarbons (TPH), total polycyclic aromatic hydrocarbons (total PAH), and trace metals and will be performed by partnering analytical laboratories (to be determined) using the following or comparable methods: EPA Method 6010C (inductively coupled plasma-atomic emission spectrometry) for analysis of metals; EPA Method 8015 (non-halogenated organics by gas chromatography) for analysis of TPH; EPA Method 8270-SIM (semi-volatile organic compounds by gas chromatography/mass spectrometry with selective ion monitoring) for analysis of total PAHs. Total PAH values will be calculated as the sum of individual PAH analytes (Table 5).

Table 5. Proposed list of analytes to be included in the calculation of Total PAH. CAS# = Chemical Abstract Service Registry Number.

Analyte	CAS#
Naphthalene	91-20-3
C1-Naphthalenes	91-20-3C1
C2-Naphthalenes	91-20-3C2
C3-Naphthalenes	91-20-3C3
C4-Naphthalenes	91-20-3C4
Biphenyl	92-52-4
Dibenzofuran	132-64-9
Acenaphthylene	208-96-8
Acenaphthene	83-32-9
Fluorene	86-73-7
C1-Fluorenes	86-73-7C1
C2-Fluorenes	86-73-7C2
C3-Fluorenes	86-73-7C3
Anthracene	120-12-7
Phenanthrene	85-01-8
C1-Phenanthrenes/Anthracenes	PHENANTHC1
C2-Phenanthrenes/Anthracenes	PHENANTHC2
C3-Phenanthrenes/Anthracenes	PHENANTHC3
C4-Phenanthrenes/Anthracenes	PHENANTHC4
Retene	483-65-8
Dibenzothiophene	132-65-0
C1-Dibenzothiophenes	132-65-0C1
C2-Dibenzothiophenes	132-65-0C2
C3-Dibenzothiophenes	132-65-0C3
C4-Dibenzothiophenes	132-65-0C4
Benzo(b)fluorene	243-17-4
Fluoranthene	206-44-0
Pyrene	129-00-0

Analyte	CAS#
C1-Fluoranthenes/Pyrenes	FLUORPYRC1
C2-Fluoranthenes/Pyrenes	FLUORPYRC2
C3-Fluoranthenes/Pyrenes	FLUORPYRC3
C4-Fluoranthenes/Pyrenes	FLUORPYRC4
Naphthobenzothiophenes	
C1-Naphthobenzothiophenes	NAPBENZOTHIOPC1
C2-Naphthobenzothiophenes	NAPBENZOTHIOPC2
C3-Naphthobenzothiophenes	NAPBENZOTHIOPC3
C4-Naphthobenzothiophenes	NAPBENZOTHIOPC4
Benz[a]anthracene	56-55-3
Chrysene/Triphenylene	218-01-9/217-59-4
C1-Chrysenes	218-01-9C1
C2-Chrysenes	218-01-9C2
C3-Chrysenes	218-01-9C3
C4-Chrysenes	218-01-9C4
Benzo[b]fluoranthene	205-99-2
Benzo[j]fluoranthene/Benzo[k]fluoranthene	205-82-3/207-08-9
Benzo[a]fluoranthene	203-33-8
Benzo[e]pyrene	192-97-2
Benzo[a]pyrene	50-32-8
Perylene	198-55-0
Indeno[1,2,3-cd]pyrene	193-39-5
Dibenz[ah]anthracene/Dibenz[ac]anthracene	53-70-3/215-58-7
Benzo[g,h,i]perylene	191-24-2

Though processed separately, vertical sections of the same core and replicate cores from the same multi-core drop will be combined mathematically for data-analysis purposes. Data from different vertical sections of the same core will be collapsed into a single common species list for the individual core. Data from replicate cores from the same multi-core drop (applies to macrofauna only) will be averaged and reported as per-station means.

Analysis of variance (ANOVA) and other statistical methods will be used to analyze data from the prior (2010 and 2011) and proposed sampling events to test for spatial and temporal patterns discussed above. An underlying goal is to assess the persistence of previously observed DWH-related impacts on the benthos and evidence of recovery over time in light of other natural spatial and temporal (including within-year seasonal) variations. Recovery will be defined as insignificant differences in key biological and abiotic response variables (from above list) between impacted and non-impacted areas and the inability to distinguish such differences from natural temporal variability. Data for non-impacted sites from this and the prior NRDA deep-benthic study also will be used to define normal "reference ranges" in these variables. The reference ranges can then serve as quantitative "restoration targets" to aid in determining recovery of Gulf of Mexico deep-benthic resources from current DWH-related impacts as well as any similar events in the future.

6. Products

Products will include raw data, reports, and quantitative “restoration targets” for determining recovery of Gulf of Mexico deep-benthic resources from DWH-related impacts and similar events in the future. It is also intended to communicate with the scientific community via peer-reviewed journal publications and attendance at scientific conferences.

This long-term RESTORE research program will have great value for future generations of scientists and others. Thus, it is imperative that educational components be created to communicate with a broad group of stakeholders from the public to the technical communities. The education program will be create at several levels including visiting researchers (especially taxonomists), post-doctoral fellows, graduate students, and undergraduates in the technical community; and the public, teachers, and K-12 students at the broader community.

7. Biosketches

This project has a Principal Investigator (PI) and two Co-PI's. Paul Montagna (Texas A&M University-Corpus Christi) is the PI and is in charge of overall project design, implementation, macrofauna and sediment analyses, data analytics, and communication. Jeffery Baguley (University of Nevada-Reno) is Co-PI in charge of meiofauna analyses, and Cynthia Cooksey (NOAA) is Co-PI in charge of field campaigns and logistics. These three have worked together extensively on the soft-bottom benthos NRDA for the Deepwater Horizon accident since 2011. Below are brief biosketches.

Dr. Paul Montagna was named the Endowed Chair for Ecosystem Studies and Modeling at the Harte Research Institute for Gulf of Mexico Studies, Texas A&M University-Corpus Christi in 2006. Previously, he spent 20 years at the University of Texas at Austin, Marine Science Institute where he created the Mission-Aransas National Estuarine Research Reserve. Dr. Montagna has broad expertise on assessing biological and ecological effects of offshore oil and gas exploration and production on continental shelves and the deep-sea, having worked in the Beaufort Sea, Alaska; Santa Barbara Channel and Santa Maria Basin, California; the Gulf of Mexico; and the eastern Atlantic Ocean off West Africa. He has been an invited speaker at many oil and gas meetings. He has performed studies in oil seeps, chemosynthetic habitats, hard-bank reefs, frontier areas, and production areas on the topics of benthic ecology (for both macrofauna and meiofauna communities), genetic structure, population biology, reproduction and settling dynamics, trophic dynamics, food webs, productivity, microbial activity, toxicity, chemical-biological interactions, modeling, statistics and experimental design. From 2011-2016, he led the technical assessment of the effects of the Deepwater Horizon (DwH) blowout on deep-sea soft-bottom benthos communities as part of the Natural Resource Damage Assessment (NRDA) program. He currently continues to study the recovery of deep sea benthos from both the 1979 Ixtoc oil spill and the 2010 Deepwater Horizon oil spill.

Dr. Jeff Baguley has been a faculty member at the University of Nevada-Reno since 2006, where he has maintained an active research program. He is a broadly trained marine benthic ecologist whose research is primarily focused on the Gulf of Mexico deep sea. He received his Ph.D from The University of Texas at Austin under the guidance of Dr. Paul Montagna, and subsequently held a post-doctoral position at The University of South Carolina under the guidance of Dr. Bruce Coull. He has published 22 peer-reviewed manuscripts and has secured $1.96 million in extramural funding. Most recently, Dr. Baguley has served as a member of the Deepwater Horizon Natural Resource Damage Assessment, Deepwater Benthic Technical Working Group. At present, Dr. Baguley has authored or co-authored six peer reviewed manuscripts derived from the Deepwater Horizon project, and has several other manuscripts in preparation for submission. He has a strong record of mentorship having served on 11 graduate committees, mentored four graduate students as the major advisor, mentored 22 undergraduate students in his research lab, supervised 12 research technicians, and mentored a research assistant professor.

Ms. Cynthia (Cindy) Cooksey is currently working in the Habitat Conservation Division of the National Oceanic and Atmospheric Administration (NOAA), National Marine Fisheries Service (NMFS) in Charleston, South Carolina. Cindy is a marine biologist who studies our nation's estuarine and marine ecosystems using an ecosystem-based approach. Cindy's work focuses on living marine resources and the physical characteristics of the environments they inhabit such as grain-size distributions, chemical containments and toxicity of the sediment. Cindy has served as Chief Scientist for numerous oceanographic research operations and small boat, land-based operations. Cindy is originally from Maryland where she received a BA in biology from St. Mary's College of Maryland. She then moved to southern Virginia where she earned an MA in Marine Science from The College of William and Mary, Virginia Institute of Marine Science, studying the reproductive biology of Spanish mackerel. Cindy worked on the DWH NRDA for deep benthic habitats.

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