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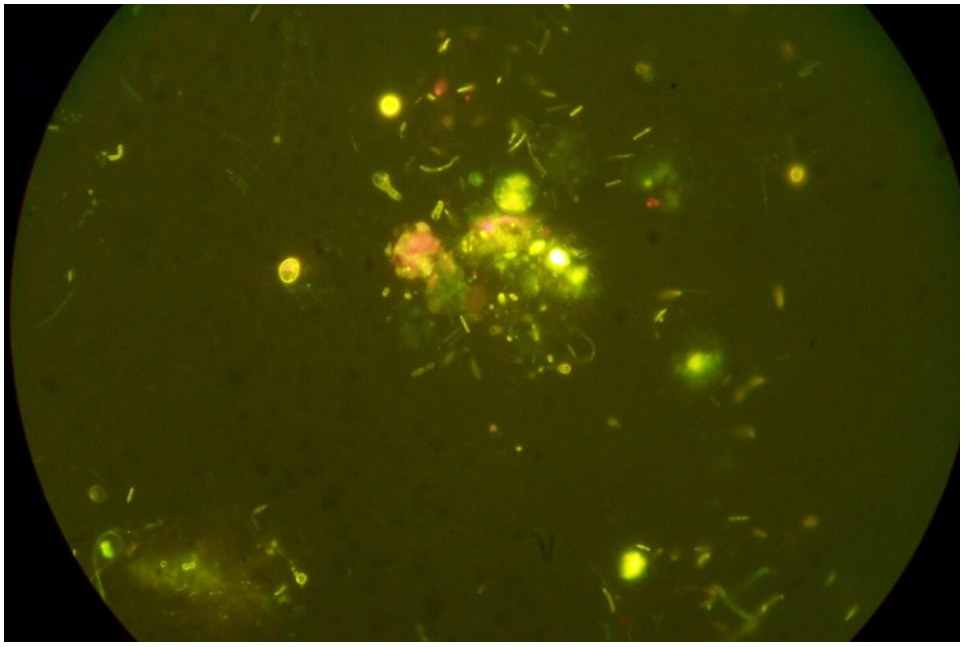
A Survey of Alterations in Microbial Community Diversity in Marine Sediments in Response to Oil from the *Deepwater Horizon* Spill: Northern Gulf of Mexico Shoreline, Texas to Florida

By John T. Lisle



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Front cover image:
Naturally occurring microbial community stained with SYBR Gold® and photographed using epi-fluorescent microscopy at 1250× magnification.

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A Survey of Alterations in Microbial Community Diversity in Marine Sediments in Response to Oil from the *Deepwater Horizon* Spill: Northern Gulf of Mexico Shoreline, Texas to Florida

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Abstract

Microbial community genomic DNA was extracted from sediment samples collected from the northern Gulf of Mexico (NGOM) coast. These samples had a high probability of being impacted by Macondo-1 (M-1) well oil from the *Deepwater Horizon* (DWH) drilling site. The hypothesis for this project was that presence of M-1 oil in coastal sediments would significantly alter the diversity within the microbial communities associated with the impacted sediments. To determine if community-level changes did or did not occur following exposure to M-1 oil, microbial community-diversity fingerprints were generated and compared. Specific sequences within the community's genomic DNA were first amplified using the polymerase chain reaction (PCR) using a primer set that provides possible resolution to the species level. A second nested PCR that was performed on the primary PCR products using a primer set on which a GC-clamp was attached to one of the primers. These nested PCR products were separated using denaturing-gradient gel electrophoresis (DGGE) that resolves the nested PCR products based on sequence

dissimilarities (or similarities), forming a genomic fingerprint of the microbial diversity within the respective samples. Sediment samples with similar fingerprints were grouped and compared to oil-fingerprint data from Rosenbauer and others (2010). The microbial community fingerprints grouped closely when identifying those sites that had been impacted by M-1 oil (N=12) and/or some mixture of M-1 and other oil (N=4), based upon the oil fingerprints. This report represents some of the first information on naturally occurring microbial communities in sediment from shorelines along the NGOM coast. These communities contain microbes capable of degrading oil and related hydrocarbons, making this information relevant to response and recovery of the NGOM from the DWH incident.

Introduction

From April 20 to July 15, 2010, approximately 4.4 million barrels of crude oil from the *Deepwater Horizon* oil rig discharged into the Gulf of Mexico (Crone and Tolstoy, 2010). The oil, classified as Macondo-1, was estimated to cover 68,000 square miles as a surface-water layer (Amos, 2010). The oil poses a health threat to plants and animals that come in contact with it. In response to this spill event, the U.S. Geological Survey (USGS) collected near-surface beach and coastal sediments and tarballs from 41 sites along the coast of Texas, Louisiana, Mississippi, Alabama, and Florida. Sites were selected to include various shoreline types—for example, sandy beaches, wetlands, marshes, and barrier islands. The purpose of this project was to determine if the presence of M-1 oil in the sediments significantly altered the microbial community-diversity structure.

Methods

Sampling

Shoreline sediments were collected from 41 sites that were predicted to have a high probability of being impacted by oil released from the *Deepwater Horizon* oil spill in the Gulf of Mexico. These samples were collected from October 5 to October 14, 2010, along the northern Gulf of Mexico (NGOM) shoreline in Texas, Louisiana, Mississippi, Alabama, and Florida. Replicate samples were collected from selected, but not all, sites. All samples were collected, processed, and shipped as described in the USGS National Field Manual for the Collection of Water-Quality Data (NFM) (*http://pubs.water.usgs.gov/twri9A/*) and Sampling Protocol for Post-Landfall *Deepwater Horizon* Oil Release, Gulf of Mexico, 2010 (*http://pubs.usgs.gov/of/2010/1191/*) (Wilde and others, 2010). This set of manuals includes all of the protocols and methods that ensured sample integrity, consistency, and data reliability for the entire project.

Sample Analyses

All samples were processed and analyzed at the USGS Coastal and Marine Science Center in St. Petersburg, Fla. Samples were kept frozen at -80 °C until processing. Processing included the aseptic subsampling of each sample into sterile dishes with lids. Each subsample was allowed to thaw at room temperature. Once thawed, each subsample was gently and aseptically mixed and an aliquot (approximately 25 grams, g) transferred to a sterile 50-milliliter (mL) tube. The original sample and subsamples were stored at -80 °C until needed for the next step of sample processing.

The sediment subsamples in the 50-mL tubes were used as the sources for DNA extractions. The UltraClean® Soil DNA Kit (MO BIO Laboratories, Solana Beach, Calif.) was used for all DNA extractions, following the recommendations of the manufacturer. The physical disruption step in the process was accomplished using the FastPrep® FP120 (Qbiogene, Inc., Carlsbad, Calif.) at a setting of 5.5 for 30 seconds (s). Approximately 1.0 g of total sediment per sample was extracted, and the purified DNA was suspended in a final volume of 50 microliters (µL) of sterile water.

The primary polymerase chain reaction (PCR) amplifications were accomplished using the 787F (5'-ATTAGATACCCTDTAGTCC-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') primer set (Amann and Ludwig, 2000). The KAPA2G Robust HotStart ReadyMix kit (Kapa Biosystems, Boston, Mass.) was used for the DNA polymerase and master-mix components. The master-mix recipe for the primary PCR reactions (25 µL final volume) consisted of 0.1 µL DNA polymerase, 5.0 µL Buffer A, 5.0 µL Enhancer, 0.5 µL dNTP mixture, 1.0 µL primer 1070F, 1.0 µL primer 1492R, 5.0 µL of DNA substrate, and 7.4 µL sterile water. The thermal cycler (PCR Sprint; Thermo Electron Corp., Waltham, Mass.) program for this primer set was: 30 s at 95 °C; 30 s at 95 °C, 30 sec at 50 °C, 30 s at 72 °C (30 cycles); 1 minute (min) at 72 °C. All primary PCR products were stored at -20 °C.

The nested PCR used primer 1070F (5'-ATGGCTGTCGTCAGCT-3') and 1392Rgc (5'-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCCACGGGCGGTGTGTAC-3') (Ferris and others, 1996; 1997), where the underlined sequence imparts the specificity of the primer and the non-underlined sequence represents the non-annealing GC-tail. The PCR master mix for the nested reactions included (50 µL final volume) 0.2 µL DNA polymerase, 10.0 µL Buffer A, 10.0 µL Enhancer, 1.0 µL dNTP mixture, 2.0 µL primer 1070F, 2.0 µL primer

1392Rgc, 2.0 µL of DNA substrate and 22.8 µL sterile water. The thermal cycler (PCR Sprint; Thermo Electron Corp., Waltham, Mass.) program for this primer set was 30 s at 95°C; 30 s at 95 °C, 30 s at 52 °C, 30 s at 72 °C (30 cycles); 1 min at 72 °C. All nested PCR products were stored at -20 °C.

Denaturing-gradient gels (Muyzer and Smalla, 1998) were double-gradient gels with an acrylamide concentration range of 6 to 12 percent and a denaturant range of 35 to 80 percent [where a 100-percent solution is defined as 40 percent (v/v) formamide plus 7.0 M urea]. All gels and running solutions were made with 1X TAE (0.04 M Tris base, 0.02 M sodium acetate, 1.0 mM EDTA; pH 7.5). A subsample (25 µL) of each nested PCR product was loaded into separate wells of the gel. A set of GC-clamped PCR products was loaded into three lanes for reference standards. Each gel was run at 60 °C at approximately 85 volts (V) for 16 hours (hr). All gels were stained with SYBR Gold (1× final concentration) (Invitrogen, Carlsbad, Calif.) for 30 min and then digitally photographed.

A digital image of each gel was loaded into the nucleic-acid banding-and-fingerprint analysis software, GelCompar II (Applied Maths, Austin, Tex.). The banding pattern or fingerprint from each sample was first normalized and then analyzed for similarity, relative to the standard. The resulting similarity dendrogram was generated using the unweighted-pair-group method with arithmetic averages (UPGMA) and the Dice similarity coefficient.

Results

A considerable effort was used to optimize the PCR conditions for the range of sediment matrices represented by the post-spill samples. PCR inhibitors were a significant obstacle to obtaining a quality primary PCR product. Though a primary and nested PCR product was

obtained for all samples, several of the samples were repeatedly represented as smears on the DGGE gels. These samples were not included in the final similarity analyses. These samples included (sample identification number) Lake Felicity (292046090245400), Grand Pass (300907089144500), AL-4 (301329088003000), St. Andrews (3007290854409000), East Ship Island Beach (301358088533300), Mississippi River at the Gulf Outlet (294108089234500), BLM-1 (301353087561600), AL-5 (301349087541600), East Horn Island Beach (301321088353300), West Horn Island Beach (301425088440600), Lathrop Bayou (300223085260800), and Bay Jimmy (292708089521400). Samples that produced a reliable banding pattern and fingerprint on the DGGE gels are listed in table 1.

Data from the report by Rosenbauer and others (2010) were used to identify sediment sample sites that had been shown to have been contaminated at some level with M-1 oil and (or) a combination of M-1 and other oils. Table 1 denotes those sediment samples that had been contaminated with M-1 oil (Y) or a mixture of oils (M) or that showed no oil contamination (N). In figure 1, those sites contaminated with M-1 oil are highlighted in red and those contaminated by a mixture of oils in blue.

The microbial community-diversity-similarity dendrogram, which is based on the DNA fingerprints, is shown in figure 2. The numerical values at each node in the dendrogram are the similarity index values. The greater the value, the more similar the samples are to the right of that value.

Twelve sediment samples were shown to contain M-1 oil, of which eight produced a usable microbial community fingerprint. All four of the sediment samples that contained M-1 and other oils produced usable fingerprints. Though all of the M-1 (red) contaminated-sediment samples did not cluster tightly, those samples do group together, along with the oil-mixture

(blue) sediment samples (fig. 1). However, there are other samples in which no oil was detected whose microbial community diversity was very similar (as denoted by the similarity-node values >80), if not statistically equivalent, to those from sites where the presence of oil had been confirmed. This confounding result may be due to the fact that microbial communities within the sediment systems of the Gulf of Mexico have been exposed to crude oils for millennia and that some communities contain a relatively cosmopolitan group of microorganisms capable of degrading crude oil without dramatic changes (that is, succession with increase in biomass) in diversity (Hazen and others, 2010). The native microbial community's response to oil from the spill would not have been detected by community fingerprinting of a single grab sample. Another factor to consider when assessing the community-fingerprint data is the sample scale. Chemical and nutrient analyses rely on replicate samples to assign some level of reliability to the resulting data. This task is very difficult for microbial ecologists because the sampling scale is at the level of micrometers. Microbial diversity can be dramatically different between two samples that are collected just centimeters apart. This phenomenon can be seen in the replicate-sample community fingerprints for Galveston Island (N=3) and West Bay (N=2). The diversity within the Galveston Island samples is significant enough to place the three samples in separate groups, or clades, all three of which contained one or more samples that had been identified as being contaminated with oil. The most dramatic example of this is the West Bay samples, where one fingerprint consisted of 4 diagnostic bands, while the other contained 13 and oil was not detected in either sample.

Conclusions

Microbial community DNA was extracted from coastal-sediment samples at locations in the NGOM identified as having a high probability of contamination from the *Deepwater*

Horizon oil spill. Specific sequences within these DNA samples were amplified using PCR and separated using DGGE to produce microbial community fingerprints based on the number and location of DNA bands. Though the community fingerprints from sediment samples that had been shown to contain M-1 oil, or a mixture of M-1 and other oil(s), did group together, there were community fingerprints within these same groupings from sediment samples that had been shown not to be impacted by M-1 or any other type of oil.

Microbial communities respond to perturbations, such as dramatic increases in carbon substrates (for example, crude oil), by systematically degrading those substrates. This degradation process is performed by a succession of microbial species within the existing community that is characterized by increases in biomass of the active species over time. It is this succession of microbial species that can be monitored by determining microbial community fingerprints like those generated in this study. However, a single sample provides only a snapshot of which species are present at a single time point in the community response or degradation process and provides no insight into the rates of crude oil degradation by microbial communities. The collaborations of geochemists and microbial ecologists could provide data on the oil degradation rates and the by-products produced by the microbial activities in the sediments.

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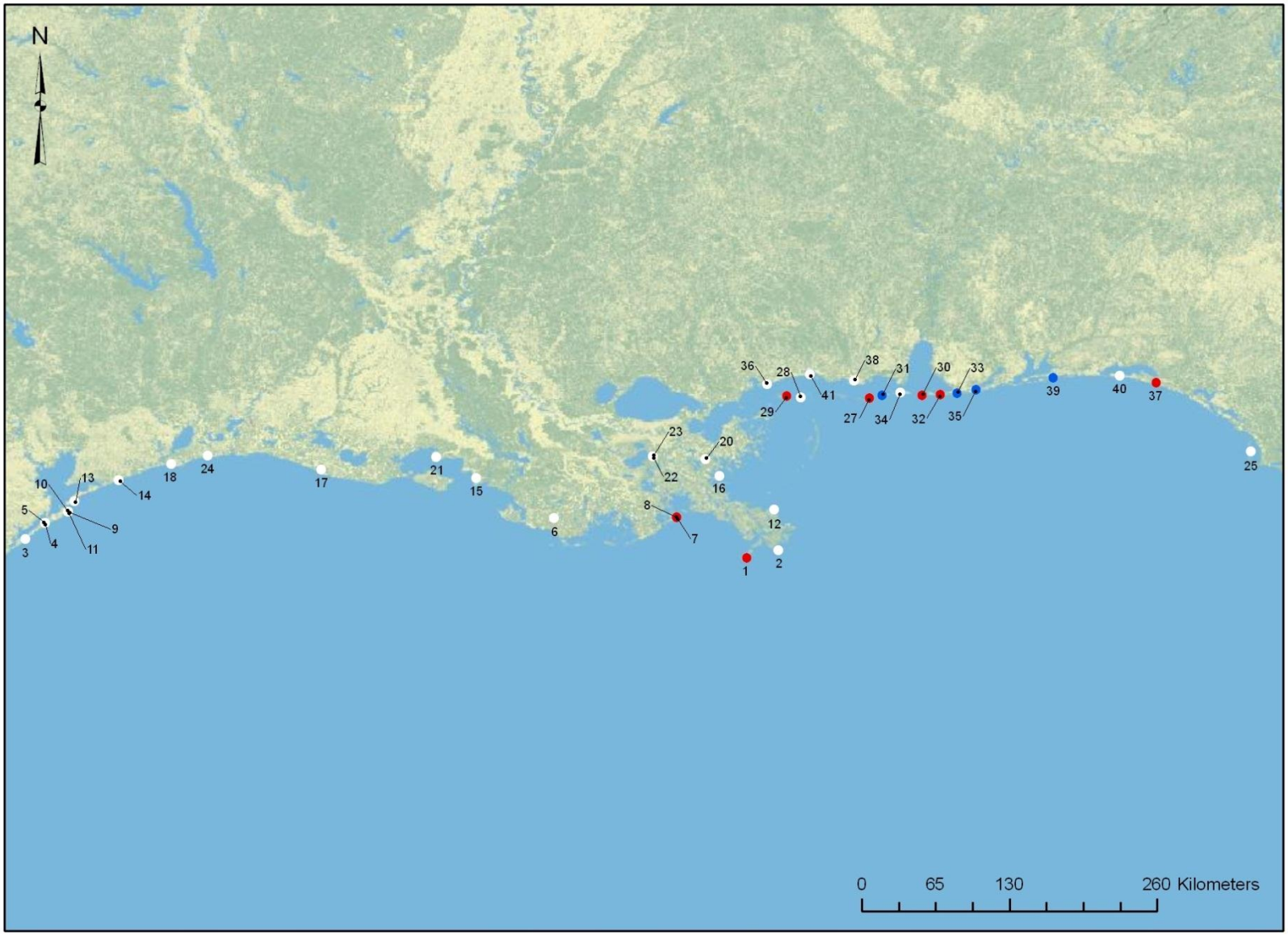

Figure 1. Post-spill sediment sample sites, northern Gulf of Mexico shoreline. ●M-1 oil present; ●M-1 and (or) other oil present; ○no oil present. Site location numbers are listed in table 1.

Figure 2. Post-spill sediment sample similarity dendrogram. Colors are correlated with those in figure 1.

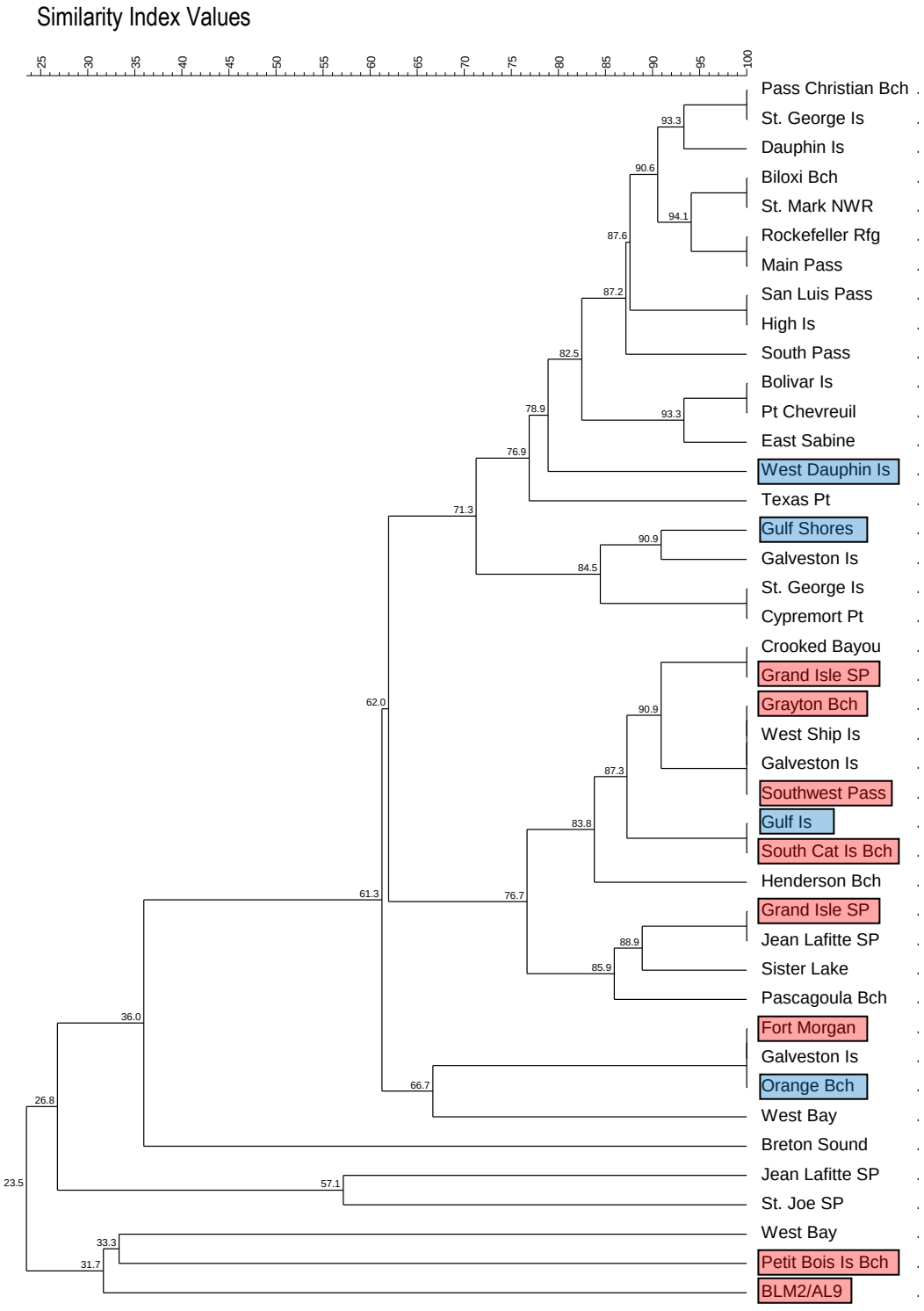

Table 1. *Deepwater Horizon* post-spill sediment samples, northern Gulf of Mexico shoreline.
[Y, yes; M, mixture; N, no]

Map Reference Number	Sample Site Name	Latitude (decimal degrees)	Longitude (decimal degrees)	Sample Site Identification Number	DWH Oil Contamination [1]
1	Southwest Pass	28.93750	-89.39889	285615089235600	Y
2	South Pass	28.99750	-89.14889	285951089085600	N
3	San Luis Pass	29.08667	-95.10861	290512095063101	N
4	West Bay	29.21417	-94.95389	291251094571401	N
5	West Bay	29.21417	-94.95389	291251094571401	N
6	Sister Lake	29.25194	-90.92167	291507090551800	N
7	Grand Isle SP	29.26028	-89.95028	291537089570100	Y
8	Grand Isle SP	29.26028	-89.95028	291537089570100	Y
9	Galveston Island	29.30417	-94.76944	291815094461001	N
10	Galveston Island	29.30417	-94.76944	291815094461001	N
11	Galveston Island	29.30417	-94.76944	291815094461001	N
12	Main Pass	29.32056	-89.18194	291914089105500	N
13	Bolivar Island	29.38833	-94.71917	301448088044000	N
14	High Island	29.55667	-94.36833	293324094220601	N
15	Point Chevreuil	29.57333	-91.53778	293424091321600	N
16	Breton Sound	29.58833	-89.61194	293518089364300	N
17	Rockefeller Rfg	29.63556	-92.76722	293808092460200	N
18	Texas Point	29.68250	-93.95639	294057093572301	N
19	St. George Island	29.69786	-84.76775	294152084460300	N
20	Crooked Bayou	29.72333	-89.72361	294324089432500	N
21	Cypremort Point	29.73500	-91.85361	294406091511300	N
22	Jean Lafitte SP	29.74222	-90.14194	294432090083100	N
23	Jean Lafitte SP	29.74222	-90.14194	294432090083100	N
24	East Sabine	29.74889	-93.66333	294456093394801	N
25	St. Joe SP	29.77917	-85.40853	294645085243000	N
26	St. Mark NWR	30.07419	-84.18044	300427084105000	N
27	Petit Bois Island Beach	30.20222	-88.42667	301208088253600	Y
28	West Ship Island	30.20750	-88.97222	301227088582000	N
29	South Cat Island Beach	30.21917	-89.07972	301309089044700	Y
30	Fort Morgan	30.22493	-88.00833	301341087495200	Y
31	West Dauphin Island	30.22743	-88.32639	301338088193500	M
32	BLM2	30.22881	-87.86721	301343087520200	Y
33	Gulf Shores	30.24131	-87.73026	301428087434900	M
34	Dauphin Island	30.24881	-88.18417	301448088044000	N
35	Orange Beach	30.26909	-87.58165	301608087345400	M
36	Pass Christian Beach	30.31611	-89.23611	301858089141000	N
37	Grayton Beach	30.32406	-86.15506	301926086091800	Y
38	Pascagoula Beach	30.34278	-88.54778	302034088321500	N
39	Gulf Island	30.36239	-86.97017	302144086582100	M
40	Henderson Beach	30.38294	-86.44278	302258086263400	N
41	Biloxi Beach	30.39333	-88.89944	302336088535800	N

[1] Rosenbauer and others (2010)