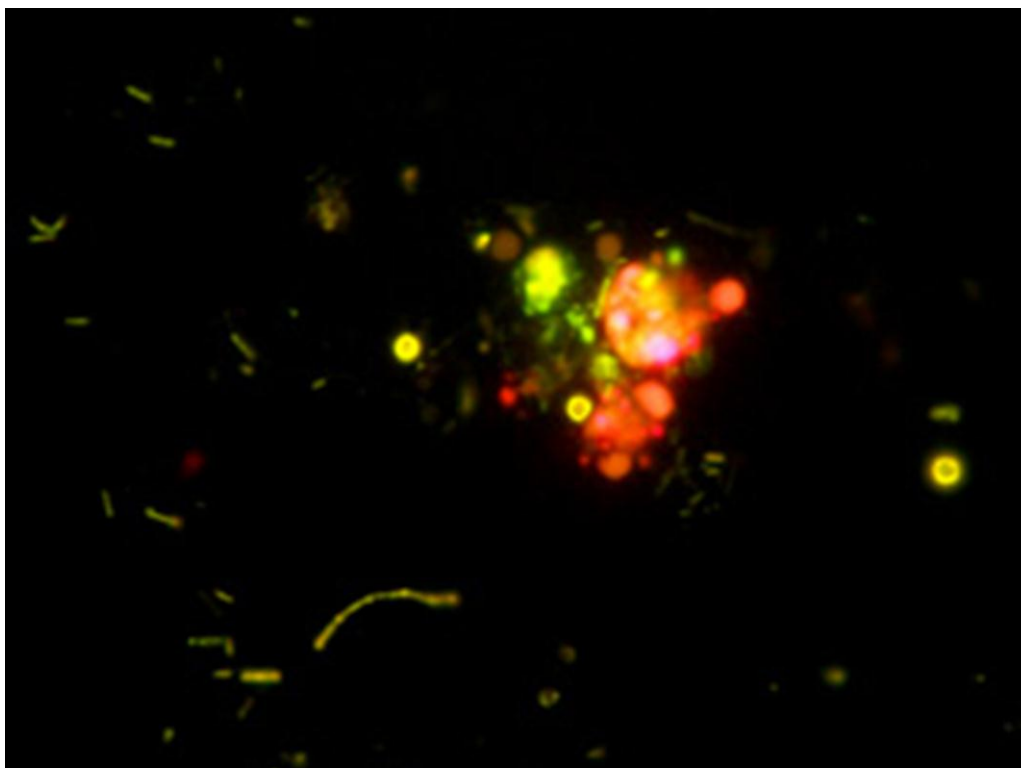




A Preliminary Report to the U.S. Coast Guard, Part 2

A Survey of Microbial Community Diversity in Marine Sediments Impacted by Petroleum Hydrocarbons from the Gulf of Mexico and Atlantic Shorelines, Texas to Florida

By John T. Lisle and Sarah H. Stellick



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Contents

Abstract.....	4
Introduction.....	5
Methods.....	6
Sampling.....	6
Sample Analyses.....	6
Results.....	9
Conclusions.....	11
Acknowledgments.....	12
References Cited.....	12

Figure 1. Sediment sample sites, Gulf of Mexico and Atlantic shorelines. A, Texas to Louisiana; B, Louisiana to Alabama; C, Florida.....	14-17
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Figure 2. Sample-similarity dendrogram.....	18
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Tables

Table 1. Sediment sample site descriptions and locations.....	19
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A Survey of Microbial Community Diversity in Marine Sediments Impacted by Petroleum Hydrocarbons from the Gulf of Mexico and Atlantic Shorelines, Texas to Florida

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Abstract

Microbial community genomic DNA was extracted from sediment samples collected along the Gulf of Mexico and Atlantic coasts from Texas to Florida. Sample sites were identified as being ecologically sensitive and (or) as having high potential of being impacted by Macondo-1 (M-1) well oil from the *Deepwater Horizon* blowout. The diversity within the microbial communities associated with the collected sediments provides a baseline dataset to which microbial community-diversity data from impacted sites could be compared. To determine the microbial community diversity in the samples, genetic fingerprints were generated and compared. Specific sequences within the community genomic DNA were first amplified using the polymerase chain reaction (PCR) with a primer set that provides possible resolution to the species level. A second nested PCR was performed on the primary PCR products using a primer set on which a GC-clamp was attached to one of the primers. The 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. Samples with similar fingerprints were grouped and compared to oil-fingerprint data from the same sites (Rosenbauer and others, 2011). The microbial community fingerprints were generally grouped into sites that had been shown to contain background concentrations of non-*Deepwater Horizon* oil. However, these groupings also included sites where no oil signature was detected. This report represents some of the first information on naturally occurring microbial communities in sediment from shorelines along the Gulf of Mexico and Atlantic coasts from Texas to Florida.

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). In response to this spill and before oil associated with this spill made landfall, the U.S. Geological Survey (USGS) collected near-surface beach and coastal sediments at 63 sites along the coasts of Texas, Louisiana, Mississippi, Alabama, and Florida for microbiological analyses. Sites were selected to include various shoreline types—for example, sandy beaches, wetlands, marshes, and barrier islands. The purpose of this project was to establish the background, or pre-spill, diversity of microbial communities within the sediments.

Methods

Sampling

Shoreline sediments were collected from 63 sites (table 1 and fig. 1) that had been identified as locations with a high potential of being impacted by oil released from the *Deepwater Horizon* spill in the Gulf of Mexico. These sites had not been contaminated with oil from the *Deepwater Horizon* spill at the time of their sampling. Samples were collected from May 7 to June 16, 2010, along the Gulf of Mexico and Atlantic shorelines in Texas, Louisiana, Mississippi, and Alabama and along the Gulf of Mexico and Atlantic shorelines of Florida. Replicate samples were collected from the following sites: TX-51 (3 replicates), LA-23 (2 replicates), LA-31 (3 replicates), MS-38 (2 replicates), MS-45 (4 replicates), FL-6 (3 replicates), FL-7 (2 replicates), FL-11 (3 replicates), FL-16 (2 replicates), and FL-18 (2 replicates). 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/>) (U.S. Geological Survey, variously dated), 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 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 of each primer, 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 s 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'-CGCCCGCCGCGCCCCGCGCCCGGCCGCGCCCCCGCCCCACGGGCGGTGTGTAC-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 μ L final volume) 0.2 μ L DNA polymerase, 10.0 μ L Buffer A, 10.0 μ L Enhancer, 1.0 μ L dNTP mixture, 2.0 μ L of each primer, 2.0 μ L of DNA substrate, and 22.8 μ L 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 molar (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 μ L) 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 \times 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

The microbial community nucleic-acid extraction, PCR amplification, and DGGE procedures produced a reliable community fingerprint for all samples. The microbial

community-diversity dendrogram, based on DNA fingerprint banding-pattern similarities, is shown in figure 2. The numeric value at each node in the branching pattern of the dendrogram is the cophenetic correlation-coefficient value. This value expresses the consistency or reliability of the sequence groupings to the right of the respective values, with 100 indicating an identical match between fingerprints.

Data on the presence or absence of petroleum hydrocarbons in sediments from the same locations were taken from the report by Rosenbauer and others (2011). The data were used to identify sample sites in this study that had and had not been shown to have been contaminated at some level by oil from a non-*Deepwater Horizon* source. Table 1 denotes those samples in which oil and (or) petroleum hydrocarbons of some type were detected (Y), or oil and (or) petroleum hydrocarbons were detected but with a limited biomarker dataset (*), or that showed no oil and (or) petroleum hydrocarbon contamination (N) was present. In figure 1, sites where oil and (or) petroleum hydrocarbons were and were not detected are highlighted in red and white, respectively. Those where oil and (or) petroleum hydrocarbons were detected but with limited biomarkers are highlighted in blue.

Interestingly, 36 (57.1 percent) of the sampled sites were shown to contain a detectable concentration of oil, while another six sites (9.5 percent) contained a limited set of biomarkers for oil. Collectively, 66.7 percent of the samples collected prior to landfall of the *Deepwater Horizon* oil were positive for the presence of oil. Twenty-one samples (33.3 percent) contained no detectable traces of oil of any type. The samples from LA-26, LA-33, LA-34, FL-10 and FL-20 had the most unique community fingerprints, and oil was detected in all of them.

There is a general grouping of sample sites by State based on similar microbial community fingerprints and the presence of non-*Deepwater Horizon* oil (fig. 2). The eastern-

most sample sites from Alabama to Florida that did not have detectable oil showed the most similar community-fingerprint patterns. However, there was no absolute clustering of samples based on the presence or absence of oil. All dendrogram groups contained both sample classifications.

These conflicting observations may be due to microbial communities within the sediment systems of the Gulf of Mexico having been exposed to crude oils for millennia, including the present, and are therefore acclimated for degrading oil at these sites. Kvenvolden and Cooper (2003) estimated the Gulf of Mexico receives approximately 140,000 metric tons ($\sim 1.0 \times 10^6$ barrels) of crude oil per year from natural seeps. Accordingly, microbial communities in the Gulf of Mexico 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; Orcutt and others, 2010).

Another contributing factor to these conflicting observations is that microbial diversity can be dramatically different between two samples that are collected just meters apart. For example, only the community fingerprints from replicate samples TX-51, LA-23, LA-31, MS-38, and MS-45 were similar enough to be closely grouped. The remaining sites at which replicate samples were collected (FL-6, FL-7, FL-11, FL16, FL-18) had community fingerprints, and therefore diversity, that were significantly different within the respective replicate samples. This lack of uniformity in community-fingerprint profiles within replicate sample sets is influenced by, but not limited to, patchiness in the distribution of (1) types and concentrations of nutrients, (2) electron donors and acceptors, and (3) the presence of burrowing macrofauna.

Conclusions

Microbial community DNA was extracted from coastal-sediment samples at locations in the Gulf of Mexico identified as being ecologically sensitive and having a high potential of being contaminated from the *Deepwater Horizon* oil spill. Specific sequences within the DNA samples were amplified using PCR and separated using DGGE to produce microbial community fingerprints based on the number and location of DNA bands. Non-*Deepwater Horizon* oil was detected at 66.7 percent of the sites sampled, indicating oil originating from natural seeps, non-*Deepwater Horizon* spills, and (or) vessel discharges occurring in these areas. The microbial community fingerprints from sediment samples that had been shown to contain some level of oil did generally group together, indicating the oil present in those sediments produced a niche that was predominantly colonized by a community of common microbes. However, there were community fingerprints within these same groupings from sediment samples that had been shown to be free of any oil.

Microbial communities systematically degrade carbon substrates (for example, crude oil) into metabolic by-products and carbon dioxide. This response is controlled by other factors such as, but not limited to, sediment characteristics, temperature, dissolved-oxygen concentrations, nitrogen and phosphorus nutrient concentrations, and phytoplankton blooms. This natural 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. The succession of microbial species 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 oil-degradation rates, by-products produced by the microbial activities in the sediments, and environmental factors that modulate these processes.

Acknowledgments

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Report 2010-1191, 155 p. [<http://pubs.usgs.gov/of/2010/1191/>.]

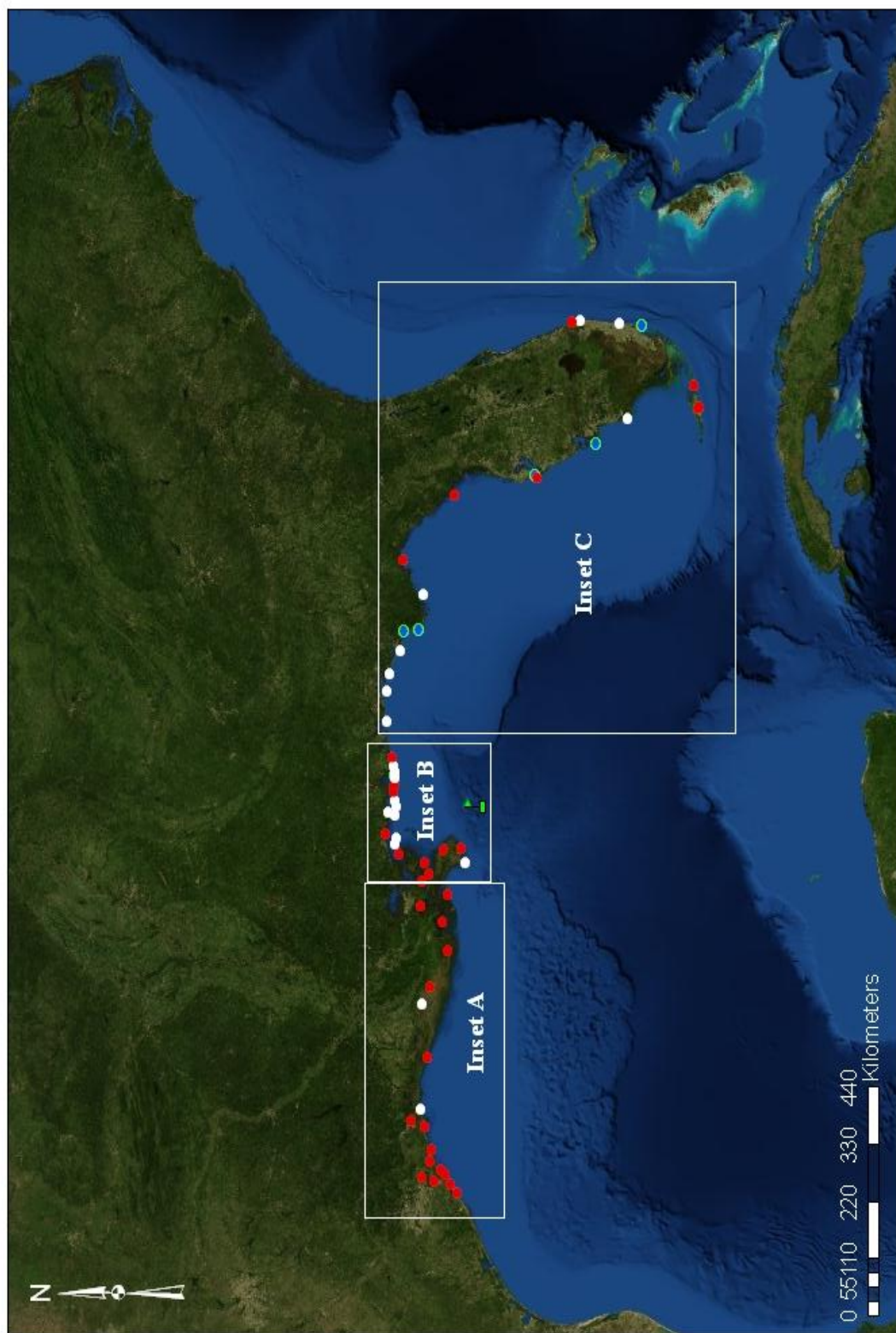


Figure 1. Sediment sample sites, Gulf of Mexico and Atlantic shorelines. ● oil present; ● oil present but with limited biomarker dataset; ○ no oil detected. The *Deepwater Horizon* oil well rig is denoted by the green flag symbol. Site location data are listed in table 1.

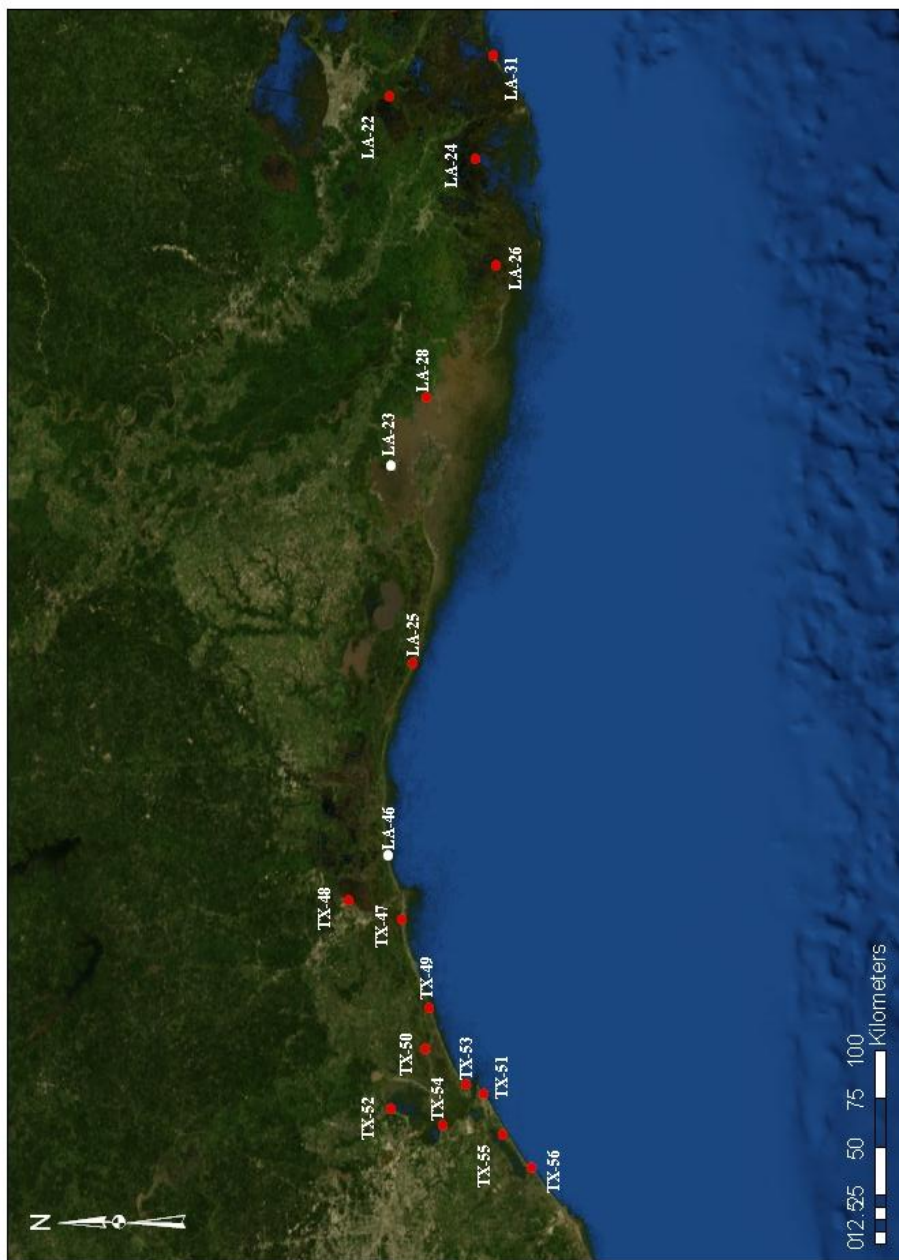


Figure A. Sediment sample sites, Gulf of Mexico and Atlantic shorelines.: Texas to Louisiana. ● oil present; ○ no oil detected. Site location data are listed in table 1.

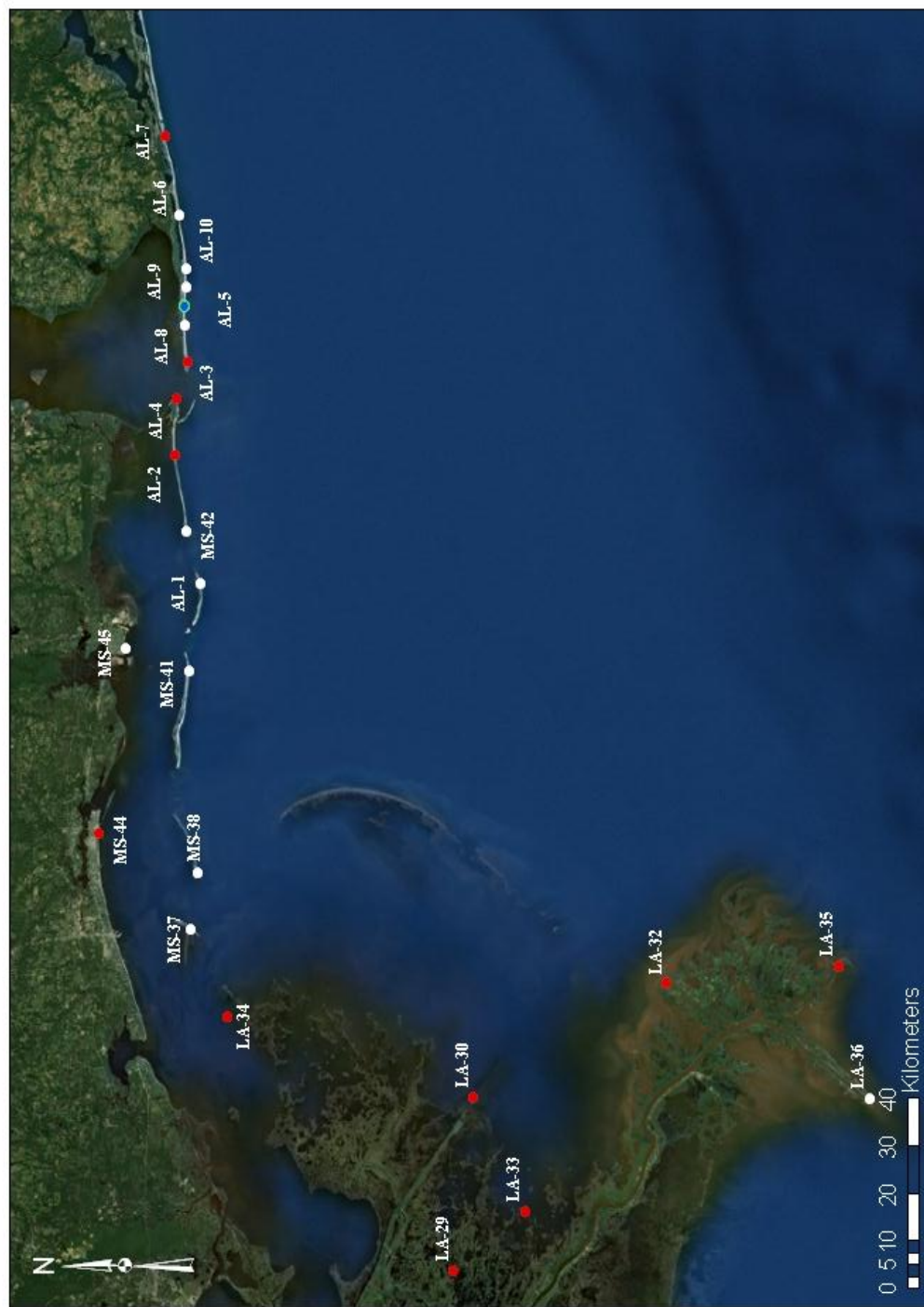


Figure B. Sediment sample sites, Gulf of Mexico and Atlantic shorelines: Louisiana to Alabama. ● oil present; ● oil present but with limited biomarker dataset; ○ no oil detected. Site location data are listed in table 1.

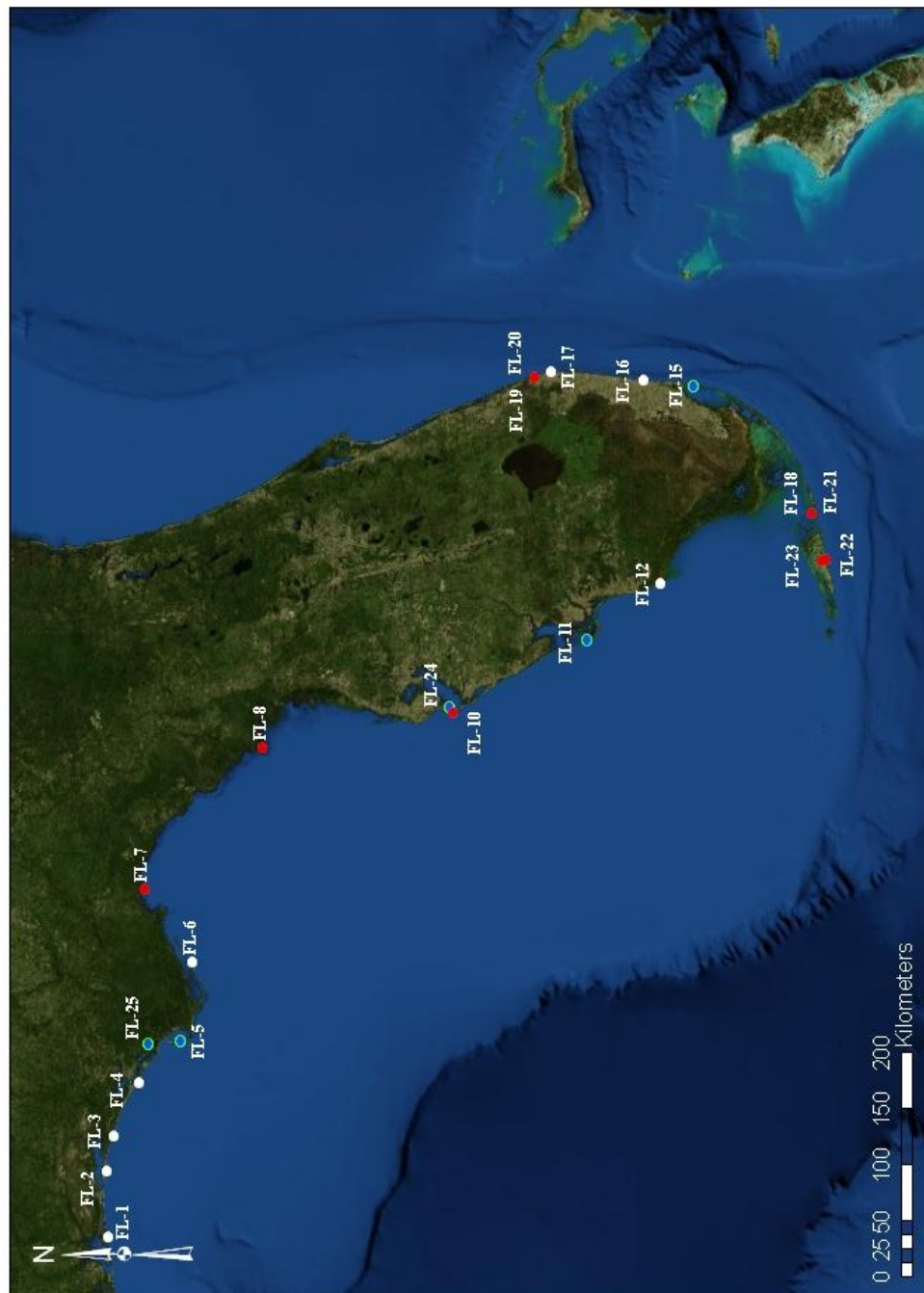


Figure C. Sediment sample sites, Gulf of Mexico and Atlantic shorelines: Florida. ● oil present; ● oil present but with limited biomarker dataset; ○ no oil detected. Site location data are listed in table 1.

Figure 2. Sediment sample similarity dendrogram. Colors are correlated with those in figure 1.

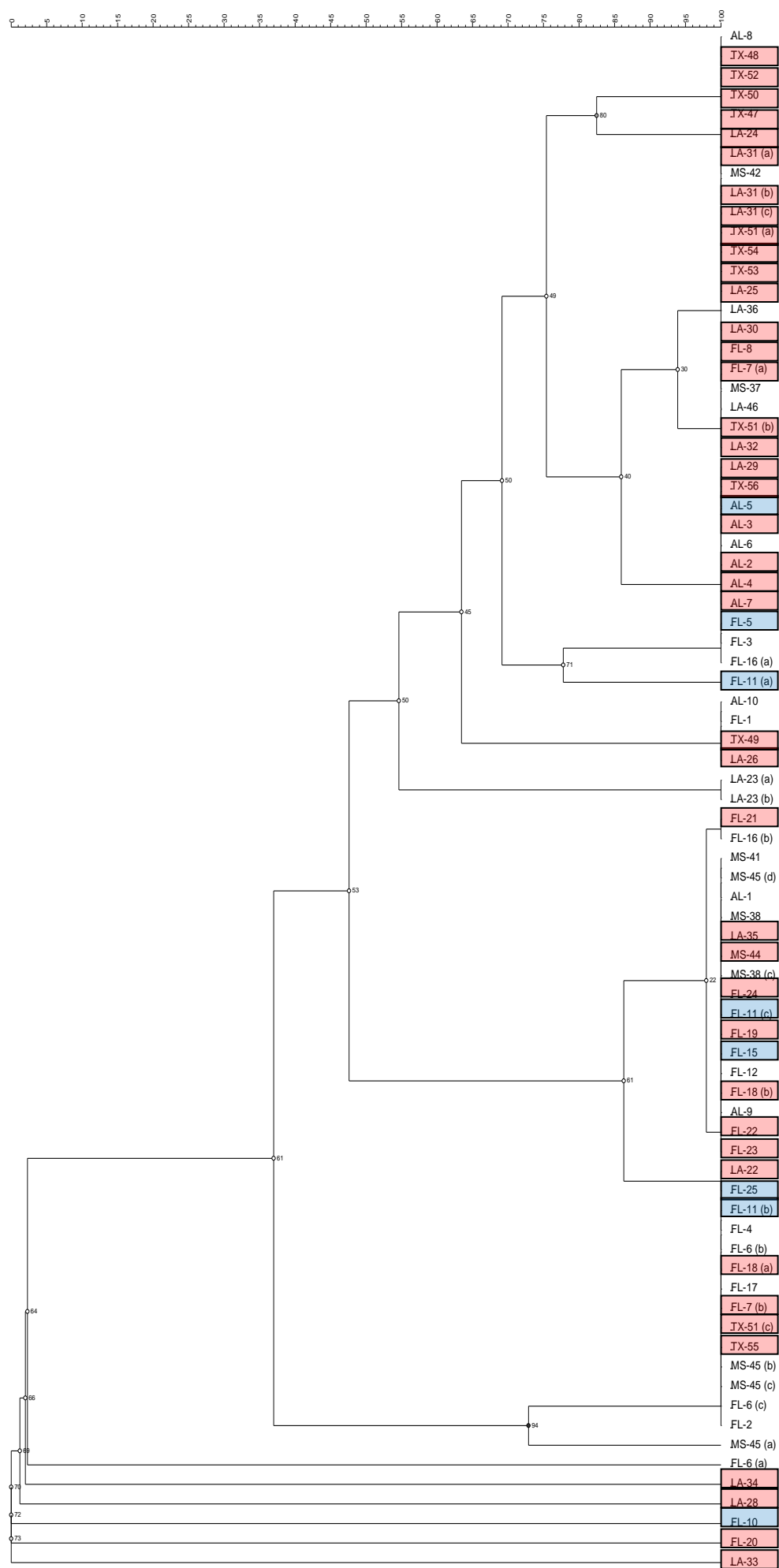


Table 1. Sediment samples, Gulf of Mexico and Atlantic shorelines.
[Y, oil present; *, oil present but with limited biomarker set; N, no oil detected]

Map Site Label	Sample Site Name	Latitude (decimal degrees)	Longitude (decimal degrees)	Sample Date	Oil Present ¹
TX-47	Texas Point	29.682500	-93.956389	5/10/10	Y
TX-48	Sabine Lake	29.928333	-93.871389	5/10/10	Y
TX-49	High Island	29.556667	-94.368333	5/10/10	Y
TX-50	East Bay near Anahuac	29.574722	-94.555833	5/10/10	Y
TX-51	Galveston Island	29.304167	-94.769444	5/10/10	Y
TX-52	Trinity Bay near Beach City	29.735556	-94.836389	5/11/10	Y
TX-53	Bolivar Peninsula	29.388333	-94.719167	5/11/10	Y
TX-54	Galveston Bay near Eagle Point	29.493611	-94.911111	5/11/10	Y
TX-55	West Bay, Galveston Island State Park	29.214167	-94.953889	5/11/10	Y
TX-56	San Luis Pass	29.086667	-95.108611	5/11/10	Y
LA-22	Jean Lafitte National Park	29.742222	-90.141944	5/14/10	Y
LA-23	Cypremort Point	29.735000	-91.853611	5/13/10	N
LA-24	Lake Felicity	29.346111	-90.429167	5/18/10	Y
LA-25	Rockefeller Refuge Beach	29.635556	-92.767222	5/13/10	Y
LA-26	Sister Lake	29.251944	-90.921667	5/17/10	Y
LA-28	Point Chevreuil	29.573333	-91.537778	5/13/10	Y
LA-29	Crooked Bayou	29.723333	-89.723611	5/18/10	Y
LA-30	Mississippi River Gulf Outlet	29.685556	-89.395833	5/7/10	Y
LA-31	Grand Isle Beach State Park	29.260278	-89.950278	5/10/10	Y
LA-32	Mississippi River at Main Pass	29.320556	-89.181944	5/7/10	Y
LA-33	Breton Sound	29.588333	-89.611944	5/7/10	Y
LA-34	Mississippi Sound at Grand Pass	30.151944	-89.245833	5/7/10	Y
LA-35	Mississippi River at South Pass	28.997500	-89.148889	5/7/10	Y
LA-36	Mississippi River at Southwest Pass	28.937500	-89.398889	5/7/10	N
LA-46	East Sabine	29.748889	-93.663333	5/10/10	N
MS-37	South Cat Island Beach	30.219167	-89.079722	5/7/10	N
MS-38	West Ship Island Beach	30.207500	-88.972222	5/7/10	N
MS-41	East Horn Island Beach	30.222500	-88.592500	5/8/10	N
MS-42	Petit Bois Island Beach	30.202222	-88.426667	5/8/10	N
MS-44	Biloxi Beach	30.393333	-88.899444	5/8/10	Y
MS-45	Pascagoula Beach	30.342778	-88.547778	5/8/10	N
AL-1	West Dauphin Island	30.227425	-88.326300	5/8/10	N
AL-2	Dauphin Island	30.248815	-88.184168	5/9/10	Y
AL-3	Dauphin Island	30.246870	-88.077777	5/9/10	Y
AL-4	Fort Morgan	30.224926	-88.008330	5/8/10	Y
AL-5	Fort Morgan	30.230481	-87.904438	5/8/10	*
AL-6	Gulf Shores	30.241314	-87.730265	5/8/10	N
AL-7	Orange Beach	30.269091	-87.581649	5/8/10	Y
AL-8	BLM Tract 1 at Fort Morgan	30.231593	-87.937772	5/24/10	N
AL-9	BLM Tract 2 at Fort Morgan	30.228815	-87.867214	5/24/10	N
AL-10	BLM Tract 3 at Fort Morgan	30.228259	-87.831102	5/24/10	N

Table 1 (cont.). Sediment samples, Gulf of Mexico and Atlantic shorelines.

[Y, oil present; *, oil present but with limited biomarker set; N, no oil detected]

Map Site Label	Sample Site Name	Latitude (decimal degrees)	Longitude	Sample Date	Oil Present ¹
FL-1	Gulf Island National Shores Park near Navarre	30.362389	-86.970167	5/11/10	N
FL-2	Henderson Beach State Park near Destin	30.382944	-86.442778	5/11/10	N
FL-3	Grayton Beach State Park near Seaside	30.324056	-86.155056	5/12/10	N
FL-4	St. Andrews State Park near Panama City	30.124722	-85.736028	5/12/10	N
FL-5	St. Joe Beach State Park near Port St. Joe	29.779167	-85.408528	5/13/10	*
FL-6	St. George Island State Park near East Point	29.697861	-84.767750	5/13/10	N
FL-7	St. Marks National Wildlife Refuge near St. Marks	30.074194	-84.180444	5/18/10	Y
FL-8	Rockefeller Refuge Beach	29.127750	-83.053361	5/18/10	Y
FL-10	Fort DeSoto Park near St. Petersburg	27.624444	-82.738333	5/17/10	*
FL-11	Captiva Island Beach near Captiva Island	26.525639	-82.194222	5/20/10	*
FL-12	Tiger Tail Beach at Marco Island	25.936139	-81.734583	5/21/10	N
FL-15	Bill Baggs Cape near Key Biscayne	25.667417	-80.155528	6/1/10	*
FL-16	Lloyd Beach at Ft. Lauderdale	26.081694	-80.109444	5/26/10	N
FL-17	MacArthur Beach at West Palm Beach	26.822583	-80.038056	5/27/10	N
FL-18	Coco Plum Beach near Marathon	24.729250	-81.169972	5/24/10	Y
FL-19	BLM Tract 1 near Jupiter Inlet	26.956111	-80.081667	6/16/10	Y
FL-20	BLM Tract 2 near Jupiter Inlet	26.956111	-80.081944	6/16/10	Y
FL-21	BLM Tract 1 near Park Key	24.650556	-81.557500	6/9/10	Y
FL-22	BLM Tract 2 near Sugarloaf Key	24.617500	-81.543611	6/9/10	Y
FL-23	BLM Tract 3 near Sugarloaf Key	24.616667	-81.539722	6/9/10	Y
FL-24	BLM Tract 4 at Egmont Key	27.601389	-82.763611	6/14/10	Y
FL-25	BLM Lathrop Bayou near Panama City	30.038944	-85.435472	6/10/10	*

¹ Rosenbauer and others (2011)