Environmental Science & Technology

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Toxicity and Mutagenicity of Gulf of Mexico Waters During and After the Deepwater Horizon Oil Spill

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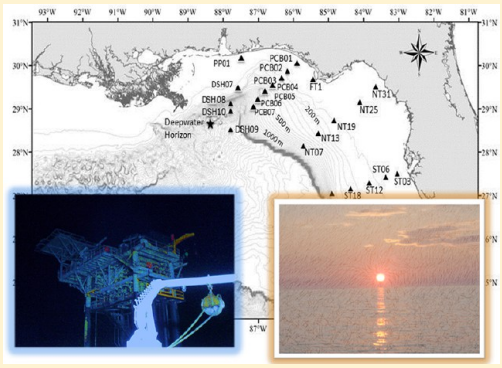
Supporting Information

ABSTRACT: The Deepwater Horizon oil spill is unparalleled among environmental hydrocarbon releases, because of the tremendous volume of oil, the additional contamination by dispersant, and the oceanic depth at which this release occurred. Here, we present data on general toxicity and mutagenicity of upper water column waters and, to a lesser degree, sediment porewater of the Northeastern Gulf of Mexico (NEGOM) and west Florida shelf (WFS) at the time of the Deepwater Horizon oil spill in 2010 and thereafter. During a research cruise in August 2010, analysis of water collected in the NEGOM indicated that samples of 3 of 14 (21%) stations were toxic to bacteria based on the Microtox assay, 4 of 13 (34%) were toxic to phytoplankton via the QwikLite assay, and 6 of 14 (43%) showed DNA damaging activity using the λ-Microscreen Prophage induction assay. The Microtox and Microscreen assays indicated that the degree of toxicity was correlated to total petroleum hydrocarbon concentration. Long-term monitoring of stations on the NEGOM and the WFS was undertaken by 8 and 6 cruises to these areas, respectively. Microtox toxicity was nearly totally absent by December 2010 in the Northeastern Gulf of Mexico (3 of 8 cruises with one positive station). In contrast, QwikLite toxicity assay yielded positives at each cruise, often at multiple stations or depths, indicating the greater sensitivity of the QwikLite assay to environmental factors. The Microscreen mutagenicity assays indicated that certain water column samples overlying the WFS were mutagenic at least 1.5 years after capping the Macondo well. Similarly, sediment porewater samples taken from 1000, 1200, and 1400 m from the slope off the WFS in June 2011 were also highly genotoxic. Our observations are consistent with a portion of the dispersed oil from the Macondo well area advecting to the southeast and upwelling onto the WFS, although other explanations exist. Organisms in contact with these waters might experience DNA damage that could lead to mutation and heritable alterations to the community pangenome. Such mutagenic interactions might not become apparent in higher organisms for years.



INTRODUCTION

The Deepwater Horizon (DWH) well released an estimated 205 million gallons of liquid oil at 1500 m water depth between April 20 and July 15, 2010.[1] In addition, approximately 1.8 million gallons of dispersant (Nalco Corexit 9500A and 9527A) was employed to solubilize the oil into tiny droplets to facilitate bioremediation.[2]

In addition to surface plumes of oil, subsurface plumes were documented to the southwest of the wellhead at 1000−1200 m water depth,[3] and to the northeast, multiple plumes at 400 m and 1000−1200 m water depth were discovered.[4] Although Corexit9500A has not been well studied, other dispersants of the Corexit family have been shown to affect the movement of oil out of the surface into subsurface waters.[5]

Petroleum hydrocarbons in seawater have been shown to be deleterious to a myriad of forms of marine life.[6] As the low molecular weight *n*-alkanes and saturated hydrocarbons are removed and altered by weathering and biodegradation, other petroleum-derived hydrocarbons, including the polycyclic aromatic hydrocarbon (PAH) fraction, remain in the environment and are highly toxic and mutagenic.[7] Subsurface polynuclear aromatic hydrocarbons (PAHs) were found associated with this plume and were speculated to be toxic.[8] Chemical dispersants may also increase the toxicity of the saturated hydrocarbon and PAH fractions,[9] as well as increase the toxicity of weathered crude oil.[10] Dispersants have been shown to break down to products with endocrine disruption activity.[5]

Received: May 9, 2013
Revised: July 31, 2013
Accepted: August 6, 2013
Published: August 6, 2013

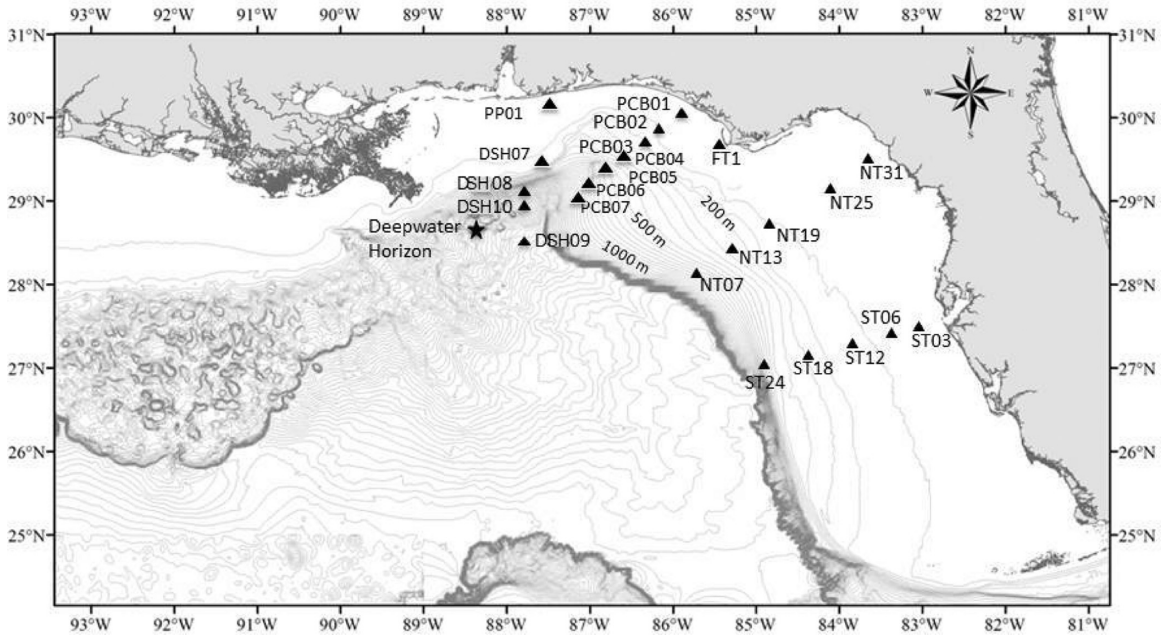


Figure 1. Sampling stations in the Gulf of Mexico. Northeast Gulf of Mexico (NEGOM) stations include PCB01-PCB09, DSH 07-DSH11, and PP01. West Florida Shelf (WFS) stations include ST03-ST24 and NT07-NT31.

Mixtures of weathered crude oil collected from the Gulf of Mexico and Corexit9500 applied to mallard duck eggs resulted in decreased spleen weights in hatchlings compared to controls.[10] Both the crude oil and the dispersant significantly inhibited the reproduction of the earthworm *Caenorhabditis elegans*. Dose-dependent inhibitions of hatched larvae production were observed in worms exposed to both crude oil and dispersant. Importantly, the chemical dispersant Corexit9500A potentiated crude oil effects; dispersant−oil mixture induced more significant effects than oil- or dispersant-alone exposures. While oil-alone exposure and dispersant-alone exposure have none to moderate inhibitory effects on hatched larvae production, respectively, the mixture of dispersant and oil induced much more significant inhibition of offspring production.[11]

Corexit9500A was also shown to result in acute effects on cardiovascular function in rats. Dose-dependent increases in heart rate and blood pressure were observed in rats that inhaled Corexit fumes.[12]

Despite the critical role hydrocarbon degrading bacteria play in the remediation of environmental oil spills, there is not much known about the toxicity of hydrocarbons in general and hydrocarbon−dispersant mixtures specifically on microbial populations in marine environments. There was evidence of efficient hydrocarbon degradation in the surface waters of the Gulf of Mexico in the oil slick, even though there was not a concomitant increase in bacterial biomass.[13] This lack of increase in the microbial population was thought to be due to the phosphate limitation in these waters.

The presence of aerobic microbial communities during all seasons in these nearshore ecosystems suggests that an active and resident microbial community is capable of mineralizing a fraction of petroleum hydrocarbons.[14] The most abundant isolates in oil-contaminated Gulf of Mexico waters were those of *Vibrio*, followed by hydrocarbon-degrading isolates affiliated with *Acinetobacter* and *Marinobacter*.[15] Significant reductions in production and viability of *Acinetobacter* and *Marinobacter* in the presence of the dispersant compared to controls was observed. *Marinobacter* appeared to be the most sensitive to the dispersant, with nearly 100% reduction in viability and production as compared to controls.[15] A study of the hydrocarbon-degrading microbial flora of beach sand found the Gammaproteobacteria (*Alcanivorax, Marinobacter*) and Alphaproteobacteria (Rhodobacteraceae) as key players in oil degradation there.[16] Additionally, a variety of microbial gene functions were enriched in the Deepwater Horizon oil plume, including those for hydrocarbon metabolism and degradation.[17]

Concerned over the potential toxicity of residual hydrocarbons from the Deepwater Horizon Oil release and the potential impact on the Gulf of Mexico pelagic and benthic ecosystems, we undertook two research cruises in 2010 to the eastern and northeastern Gulf of Mexico (NEGOM) and measured toxicity and mutagenicity (i.e., capability to cause DNA damage) of surface and subsurface water samples and the occurrence of petroleum hydrocarbons. An additional 12 cruises were conducted to either the NEGOM or the WFS. Here we show acute toxicity and mutagenicity of 30−50% of the samples collected from surface and subsurface waters taken in the NEGOM during August 2010. We also report an increase in microbial toxicity and genetic toxicity of waters and sediments on the West Florida shelf (WFS) ∼11 months after the sealing of the Macondo Well 262.

EXPERIMENTAL SECTION

Oil and Dispersant. Samples of BP oil (SOB-20100628−047) and dispersant (Corexit EC 9500A lot #SLOE1924AZ) were graciously provided by Dr. Dana Wetzel, Mote Marine Laboratory, Sarasota, FL. To determine the acute impact of the BP oil, dispersant, or mixtures of oil and dispersant, each was added to artificial seawater at concentrations ranging from 10 ppm to 100 ppt for Microtox assays (see below), 0.1 ppm to 1 ppt for QwikLite assays, and 0.1 ppm to 1 ppt for Microscreen assays. LC_{50} were calculated for the Microtox and Microscreen assays while an IC_{50} was calculated for the QwikLite assay.

Sampling Sites. Water samples were collected during 2 process research cruises and 12 monitoring cruises in the Gulf of Mexico (see Tables 1aS and 1bS, Supporting Information). The first process cruise sampled waters over the west Florida shelf (WFS) at stations along northern (NT) and southern (ST) transects aboard the R/V *Bellows* between July 10th and 17th, 2010 (Figure 1). The second cruise sampled waters in the northeastern Gulf of Mexico (NEGOM) to the east of the wellhead and in the vicinity of the DeSoto Canyon aboard the R/V *Weatherbird II* between August 3rd and 13th, 2010 (Stations PCB, FT, and DSH). Water samples were collected using a rosette sampler, equipped with 15 L Niskin bottles.

For monitoring cruise samples, five were conducted on the WFS and seven were conducted in the NEGOM (Table 1bS, Supporting Information, and Figure 1).

Toxicity Assays. Samples taken from the Niskin bottles were deposited in 120 mL EPA approved precleaned sampling bottles. The Microtox microbial toxicity assay (SDI, Inc.) was used to estimate microbial toxicity as per the manufacturer's instructions using *Vibrio fischeri* as the light-emitting organism.. The Acute Toxicity Assay was performed with the 81.9% Screening test selected from the Microtox Omni Software package. Two true replicates from each station were assayed each in duplicate using 2.0 mL of sample and 100 μL of Reagent (that was reconstituted within 3 h of assaying). Both negative (autoclaved and 0.2 μm filtered offshore seawater) and positive controls (0.133 mM phenol) were run during each assay.

The QwikLite toxicity assay (Assure Controls, Inc.) was used as a proxy for phytoplankton toxicity. This assay utilized the light emission from the dinoflagellate *Pyrocystis lunula* as an indication of toxicity of samples. *P. lunula* was grown on a 12:12 light:dark cycle with the light phase ending at 11:00 a.m. and used on assays beginning at 1:00 p.m.[18] Two and a half milliliters of such culture were added to 22.5 mL of sample in subdued light. This mixture (3.25 mL) was added to cuvettes in an Assure Control six position cassette. During each run, a negative control (autoclaved and 0.2 μm filtered offshore seawater) was assayed.

The Microscreen Mutagenicity assay (also formerly termed the Inductest) was performed essentially as described by Rossman et al.[19] The Microscreen assay is based on the known sensitivity of lysogenic bacteria to mutagenic compounds. The assay is performed by incubating a known λ-containing lysogenic strain of *Escherichia coli* (K12) with environmental samples that have the potential to contain mutagenic contamination. To perform the assay, the K12 indicator strain was grown to log-phase in MST broth, measured as an absorbance reading of 0.2−0.3 at a 600 nm wavelength. Five hundred microliters of the sample of interest were then mixed with 4.5 mL of the lysogenic indicator strain. Negative controls consisted of 500 μL of sterile seawater and positive controls were Mitomycin C to a final concentration of 0.2 μg mL^{-1} and mixed with the indicator strain as for the treatments. All treatments were incubated in 15 mL glass test tubes overnight at 37 °C with shaking at 100 rpm. After the overnight incubation, the samples were centrifuged to pellet the cells, and the supernatant was collected for plaque agar overlays. The detector strain, a nonlysogenic strain of *E. coli* (C600), was then grown to log-phase similarly to the indicator strain. The prophage containing supernatants from all of the treatments to the indicator strain were diluted as indicated and mixed with the detector strain, then placed in melted top agar and plated. The plates were incubated at 37 °C overnight, and the plaques on the lawn of the detector strain were enumerated and converted to plaque-forming units (pfu) mL^{-1}. The number of pfu mL^{-1} in the environmental samples was then compared to the positive and negative controls. Mutagenic contamination was indicated by a statistically significant increase in pfu mL^{-1} found between the environmental samples and the spontaneous rate of the negative controls.

Additional Parameters Measured on the Process Cruises Yet Not Performed on the Monitoring Cruises. *In situ Phytoplankton Physiological Status.* DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) is an herbicide that disrupts electron transport between Photosynthesis I and II pathways, where excess electrons are released as fluorescence in the presence of DCMU. Phytoplankton physiological state is assessed based on the ratio between the fluorescence of samples with and without DCMU additions (FDCMU/Fo). Healthy cells emit relatively higher fluorescence compared with cells having impaired photosynthetic activity. This method is considered to be a reliable indicator of photosynthetic capacity of phytoplankton populations when assessing the effects of crude oil.[20,21] Phytoplankton samples were dark adapted for 30 min, then fluorescence was measured before and after the addition of DCMU using a Turner Designs 10 AU. Samples from stations not impacted by oil had FDCMU/Fo ratios >1.5.

Phytoplankton: Simpson Species Diversity Index (D). This biodiversity index takes into account the number of species present, as well as the relative abundance of each species, and is used to determine the sensitivity of an ecosystem to perturbation. $D = 0$ represents no diversity; $D = 1$ represents an infinite diversity. Phytoplankton samples were collected from Niskin bottles and saved in 50 mL brown glass bottles preserved with Lugols solution. Back in the lab, a subsample was placed in a settling chamber and counted within 48 h on an Olympus IX71 inverted microscope. Rarer species were assessed for the entire subsample and abundant species were counted for fewer slide transects. The phytoplankton were a mixed assemblage of diatoms and dinoflagellates. Picoplankton were not included in this analyses.

Hydrocarbon Analyses. Samples were collected via filtration of 20 L of seawater onto precombusted GF-F filters. Filters were stored frozen and lyophilized prior to analysis. Lipids were extracted from dried filters using a Dionex automated solvent extractor (95%dichloromethane, 5% methanol). The resulting total lipid extracts were separated via column chromatography on silica gel columns; the F1 fraction containing the alkanes was eluted with pure hexane. Analysis of the alkanes was performed via splitless injection on a Varian 320 GC-MS with a 15 m × 0.25 mm × 0.25 μm Factor-Four column scanning masses from 50 to 500 AMU. Results shown are total ion chromatograms obtained for each sample.

Excitation−Emission Matrix Spectroscopy (EEMS). Spectra obtained from EEMS can be used to detect hydrocarbons in seawater. Unfiltered seawater was analyzed in a Horiba Fluorolog 2 according to the method of Coble.[22] Data are reported in units of ppb quinine sulfate equivalents. Such methods are useful in comparing the optical properties of dissolved colored organic matter in the oceans.[23]

Profiles of CTD and Bio-optical Properties. The vertical variability of physical and bio-optical properties was determined using several instruments mounted to a CTD (conductivity, temperature, depth) rosette. During the August R/V Weatherbird II cruise, a Seabird Electronics, Inc. model-25 CTD was

used for rosette control, as well as the measurement of pressure, salinity, temperature, and output from several instruments. These instruments included a WETLabs, Inc. "ECO-FL" CDOM fluorometer and an "ECO-FLNTU" chlorophyll fluorescence and turbidity sensor. Fluorescence measurements were validated by comparison with discrete water sample measurements. The backscattering of red light at 117° was converted into a particulate backscatter estimate (as in Figure S1, Supporting Information) by combining the data from the FLNTU with other scattering instruments, which were deployed separately during the cruise.

RESULTS AND DISCUSSION

Laboratory Experiments. Initial experiments were performed to assess the relative toxicity of the Corexit 9500A, the MC252 oil, and mixtures of both using the Microtox, QwikLite, and the Microscreen prophage induction assay (Inductest; Figures 2 and S1, Supporting Information). The Microtox assay

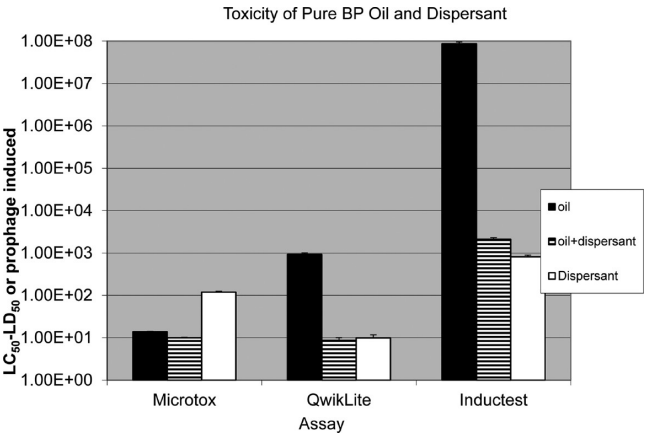


Figure 2. Toxicity of pure BP oil (SOB-20100628−047) and dispersant (Corexit EC 9500A lot #SLOE1924AZ) as determined by the Microtox, QwikLite, and the Microscreen Prophage Induction Assay (Inductest). All values are calculated as a LD_{50} or LC_{50} with the exception of the Inductest, which is prophage produced at a fixed oil or dispersant concentration.

was more sensitive to the oil than the Corexit dispersant when tested separately, while the oil plus dispersant mixture was equally as toxic as the oil alone. In contrast, the Corexit alone was more toxic to the QwikLite dinoflagellate assay and equally toxic in 1:1 oil−dispersant mixtures (Figures 2 and S1, Supporting Information), but less toxic than the oil alone. The Microscreen (Inductest) prophage induction showed a greater sensitivity to the oil alone than the Corexit or Corexit−oil mixture.

July 2010 Research Cruise to the WFS. The first cruise in July 2010 focused on water samples from the WFS (Figure 1), while the second (August 2010) sampled waters in the NEGOM closer to the Deepwater Horizon (DWH) drill site. No indication of surface or subsurface hydrocarbons were detected by either Excitation Emission Matrix Spectroscopy (EEMS) or molecular organic geochemical analyses, using GC/MS, during the July cruise to the WFS (Table S2, Supporting Information). There were no Microscreen or Microtox positive samples in any of the WFS stations. However, water samples from two stations (NT18 and NT25) yielded positive QwikLite results. Although the DWH oil had been flowing for 87 days and was not capped until after the beginning of the cruise, the chemical and biological analyses of water samples from this cruise revealed neither measurable hydrocarbon contamination nor significantly increased Microtox toxicity or mutagenic activity.

August 2010 Cruise to NEGOM. In contrast, petroleum hydrocarbons were encountered and measured during the August 2010 cruise to the east-northeast of the Deepwater Horizon wellhead in the vicinity of the DeSoto Canyon (Figure 1). Table 1 shows the spectrographic and quantitative hydrocarbon data as determined by EEMS and GC/MS, respectively. Elevated concentrations of petroleum hydrocarbons were measured in surface and/or subsurface samples from stations DSH08, DSH09, DSH10, and PCB03, and in subsurface samples only at stations PCB01 and PCB02. Measured concentrations of total petroleum hydrocarbons ranged from 24 to 298 ng/L (or ppb), with higher concentrations in the subsurface samples. No oil slicks or sheen were observed on the surface waters during the August cruise and "tar balls" were nearly absent from our samples.

During the August 2010 cruise, general microbial toxicity analyses indicated that surface waters at stations DSH08, DSH09, and FT1 caused a significant toxicity response with the Microtox assay (10.5−18% inhibition of light emission of negative controls; Table 1). Stations DSH08 (29.1225° N, 87.8687° W) and DSH09 (28.6365° N, 87.8685° W) were 35 and 27 nautical miles (northeast and southeast, respectively) of the DWH well (28.7366° N, 88.3660° W), while FT1 was between PCB03 and the northern transect (NT) transect of the July cruise (Figure 1).

Phytoplankton toxicity as measured by the QwikLite assay was found in the subsurface waters of stations DSH10 (60 m), DSH08 (215 and 275 m), and PCB03 (35 and 50 m). These waters also displayed inhibition of phytoplankton photosynthetic capacity as measured by decreased fluorescence response to the herbicide DCMU (e.g., FDCMU/Fo <1.5; Table 1). Figure S2, Supporting Information, shows a CTD vertical profile at station DSH08, which rendered the greatest degree of toxicity and mutagenicity of all stations in the August 2010 cruise. The subsurface chlorophyll maximum (SCM) was at approximately 100 m depth. The light scattering profile indicated fine particulate material yielded peaks at 215 and 275 m depth, which were possibly from the detection of fine oil-dispersant particles.

EEMS spectra and corresponding hydrocarbon chain length distributions for the same depths at station DSH08 are shown in Figure S3, Supporting Information. Compositionally, surface oil samples at station DSH08 showed lower concentration with a molecular dominance at a carbon chain length of C-22, whereas subsurface oil samples were in higher concentrations but showed a molecular dominance at a higher carbon chain length of C-32 (Figure S3, Supporting Information).

Figure S4, Supporting Information, is a profile of toxicity by Microtox and QwikLite assays, mutagenicity via the Microscreen assay, inhibition of photosynthetic capacity, and fluorescence ratio measurements at DSH08 sampled during the August 2010 cruise. Bacterial toxicity as indicated by the Microtox assay only occurred in surface waters, whereas phytoplankton toxicity via QwikLite was greatest at 275 m, corresponding to the depth of the greatest oil concentration as determined by fluorescence spectroscopy and molecular organic geochemical analyses (GC/MS).

Table 1. Microbial and Phytoplankton Toxicity Response to Waters from the August 2010 Research Cruise to the NE Gulf of Mexico

		Microtox		QwikLite		Microscreen		DCMU				fluorescence ratio (F225/330 nm)
station	depth	% inhibition cont. light emission	range	% inhibition cont. light emission	range	% Increase λ phage abundance	STD	F_{DCMU}/F_o	phyto-plankton >20 μm cells/L	Simpson Species Diversity Index ng/L (or ppb)	Total TPH	QSE
PCB01Surface	2 m	<0	0	<0	0	2.37	0.124	1.76	8733	0.593	ND[a]	ND
PCB01−10 m	10 m	ND		ND		ND		ND	ND	ND		**11.00**
PCB01Bottom	18 m	<0	0	<0	0	−92.58	0.106	1.71	3067	0.340	151	ND
PCB02Surface	2 m	<0	0	5.3	2.65	−21.6	0.1	**0.88**	5333	0.117	ND	ND
PCB02Bottom	25 m	<0	0	<0	0	−15.43	0.36	1.53	2066	0.489	24	**11.08**
PCB03−35 m	35 m	<0	0	21.35	10.7	26.11	0.37	**1.07**	**10333**	0.551	**242**	**13.46**
PCB03−50 m	50 m	<0	0	**54.4**	23.5	−62.9	0.36	**0.68**	**10390**	0.664	**165**	**5.41**
DSH09−3 m	3 m	**11.8**	1.18	1.65	0.825	43.9	0.57	**1.09**	ND	ND	**ND**	**20.23**
DSH09−75 m	75 m	<0	0	11.5	5.7	−43.6	0.19	1.23	ND	ND	**88**	**4.12**
DSH10−Surface	2 m	<0	0	14.4	7.2	**66.17**	0.52	1.17	**333**	**0**	**74**	**4.88**
DSH10−60 m	60 m	<0	0	**28.2**	14.1	**165.6**	1.1	1.15	667	0	ND	**8.02**
DSH10−400 m	400 m′	ND		ND		ND		0.68	2303	0.776	ND	**8.71**
DSH08−Surface	2 m	**18**	3.44	6.2	3.1	**158.9**	0.54	**1.03**	**405 333**	**0.193**	**203**	ND
DSH08−20 m	20 m	ND		ND		ND		ND	ND			0.95
DSH08−215 m	215 m	<0	0	**35**	17.5	**219.7**	1.6	ND			ND	ND
DSH08−275 m	275 m	<0	0	**56.8**	28.4	**283.5**	0.5	ND			**298**	**3.27**
DSH08−1000 m	1000 m	ND	ND	ND		ND		ND			**276**	**2.05**
FT1[b]	2 m	**10.7**	2.25	ND		**234.6**	0.73	1.15			ND	

[a]ND, No data. Bold values indicate statistical significance for toxicity assays only. [b]Sample collected from ship's surface flow-through seawater system.

dx.doi.org/10.1021/es401761h | Environ. Sci. Technol. 2013, 47, 9651−9659

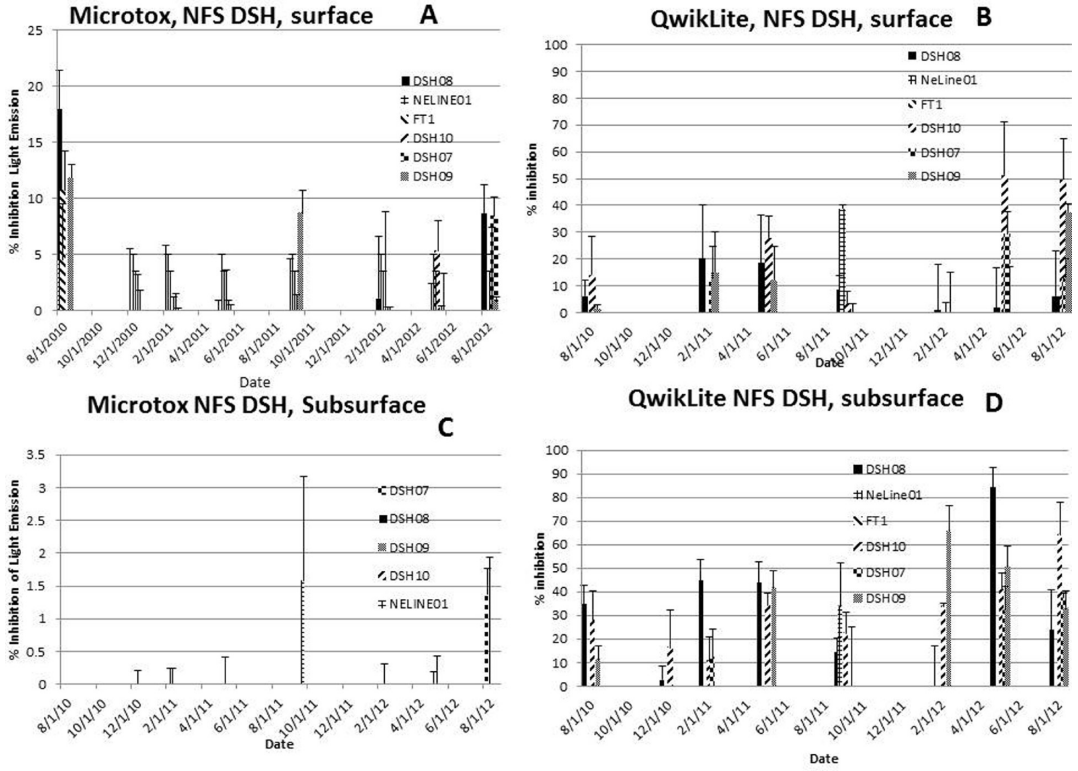


Figure 3. Long-term monitoring of surface waters from the NEGOM including Stations DSH07-DSH 10, NELINE01 and FT1. Error bars are actually ± range of true replicates. Where error bars appear at zero % light inhibition indicates the magnitude of the range between replicates that averaged a zero response. Microtox (A) surface and (C) subsurface samples. Results of QwikLite analyses of (B) surface and (D) subsurface samples.

Even though surface phytoplankton densities were relatively high at this station, the diversity index was low, which is another indication of a perturbed marine community.[24]

All of the August 2010 stations were located in the vicinity of the DeSoto Canyon and station PCB03 represents one of the easternmost locations for water column oil contamination reported. It is also of interest that the QwikLite assay detected toxicity only in subsurface waters, while the Microtox assay only detected a response in surface waters. Tests using pure MC252 oil and Corexit 9500A with both toxicity assays indicated that the Microtox assay was more sensitive to oil than to the dispersant (Corexit), whereas the QwikLite assay was considerably more sensitive to Corexit than to the oil (Figure 2). We surmise that the subsurface oil-contaminated water was most likely solubilized with Corexit, which resulted in enhanced phytoplankton toxicity (QwikLite response). This is in contrast to oil-contaminated surface waters that would not necessarily be bound with dispersant and therefore were more efficiently detected by the Microtox assay.

Mutagenicity testing of these same stations indicated that station DSH10 (3 and 60 m), DSH08 (3, 215, and 275 m), and FT1 (surface) all yielded a DNA damaging response. Thus, organisms in contact with these waters might experience DNA damage that could lead to mutation and heritable alterations to the community pangenome.[6] Such mutagenic interactions might not become apparent in higher organisms for years.[6] For example, the impact of mutations arising from the exposure of fish larvae to contaminated waters might not become fully apparent until these organisms reach adulthood, which for some commercially important species may be three to five years after exposure. Chronic exposure at all levels of the marine food web may result in complex and nonlinear interactions that are difficult to predict at this time.

There was a correlation between the magnitude of microbial toxicity as a function of total petroleum hydrocarbon (TPH) concentration (Figure S5, Supporting Information). The percent increase in prophages in response to DNA damaging agents (Microscreen Assay) was significantly correlated to TPH (Pearson Moment correlation coefficient, $r = 0.675$, $\alpha < 0.05$). The magnitude of the QwikLite decrease in bioluminescence was correlated to TPH concentrations at the 90% CI ($r = 0.601$).

As crude oils biodegrade and weather, there is a rapid loss of the low MW alkane fraction, increasing the proportion of the more refractory higher molecular weight saturated and aromatic hydrocarbon fractions. These fractions include the polynuclear aromatic hydrocarbons (PAHs), which are some of the most mutagenic compounds known.[6] As well as being capable of causing DNA damage, polynuclear aromatic hydrocarbons (e.g., tricyclic fluorene) have been shown to be cardiotoxic to herring embryos.[6]

Microtox and Qwiklite Long-term Monitoring. The results of Microtox and Qwiklite analyses of the waters sampled in the NEGOM between 2010 and 2012 are shown in Figure 3. The surface waters of stations closest to the DWH drill site (DSH8−10, NELINE01 and FT1) yielded the greatest inhibition of *V. fischeri* light emission during the August 2010 sampling (Figure 3A) while the subsurface waters showed none by this assay (Figure 3C). No Microtox toxicity was detected until September 2011 in the surface waters of DSH09 and then again in August of 2012 at station DSH08.

QwikLite analysis of the same Northern Gulf stations indicated some positive stations at all sampling time points in

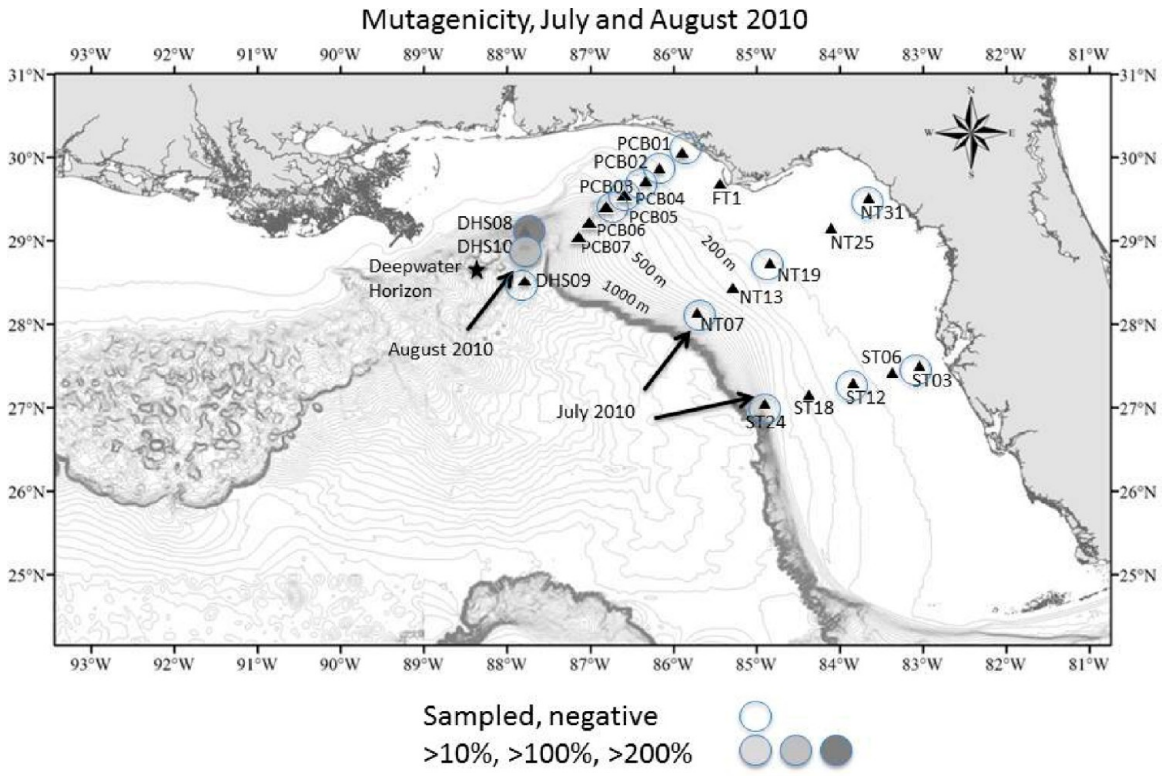


Figure 4. Microscreen mutagenicity assays in July 2010 (WFS NT and ST stations) and August 2010 (NEGOM including DSH and PCB stations). Unfilled circles indicate sampled stations that were negative for mutagenicity. Filled circles indicate Microscreen positive samplings, the degree of fill indicates prophage induction >10%, >100%, or >200% of the negative control.

both surface (Figure 3B) and subsurface (Figure 3D) samples. These observations are consistent with a highly sensitive assay that could be perturbed by many different toxic stimuli, xenobiotics, and other factors to which this assay responds.[25]

The transect of stations from the DWH site to Panama City Beach was used to detect oil movement up the DeSoto Canyon (stations PCB01 through PCB05; Figure S6A, Supporting Information). These waters had no associated toxicity as detected by the Microtox assay until May of 2012 (Station PCB05) and August of 2012 (Stations PCB01 and PCB02). These findings were for surface waters. The subsurface waters were negative for Microtox sensitive material except for station PCB01 at a depth of 18 m (Figure S6C, Supporting Information). QwikLite positive samples were collected at every sampling of the DeSoto Canyon transect (Figure S6B and D, Supporting Information) consistent with greater sensitivity of this assay.

The WFS sampling occurred along two transects, consisting of the Northern Transect (stations labeled NT, three cruises total), and the Southern transect (stations labeled ST, six cruises total). Of the three Northern Transect cruises, two yielded significant Microtox responses, one in May 2011 and the other in March 2012. The May event was characterized by detection of toxicity at Stations NT7,13, 19, and 31 (Figure S7A, Supporting Information). Subsurface toxicity was detected at NT25 (May 2011) and again at this station in August of 2012 (Figure S7C, Supporting Information). QwikLite toxicity of surface (Figure S7B, Supporting Information) and subsurface (Figure S7D, Supporting Information) stations of the WFS Northern transect showed a similar distribution of toxicity except that all three time point samplings yielded positives for at least one station. The difference in these patterns was a QwikLite positive in Northern Transect station NT31 in July 2010.

The Southern Transect of the WFS was characterized by no detectable Microtoxic response until April 2012 (ST12 surface and ST6 subsurface) and June 2012 (ST3, ST6, ST18, and ST24), or up to two years after the Deepwater Horizon disaster (Figure S8A, Supporting Information). QwikLite toxicity in the Southern Transect of the WFS was detected during the June sampling of 2011, or approximately one year before Microtox toxicity was detected in these same waters. The Microtox toxicity results are consistent with a pattern of slow migration of toxicity from the NEGOM to the northern WFS and then to the southern WFS.

Microscreen Mutagenicity Long-term Monitoring. Figure 4 shows the pattern of water column mutagenicity in the WFS in July 2010 and the Northeastern Gulf of Mexico in August 2010. No mutagenicity was detected in any of the stations sampled on the WFS nor the PCB transects of the Northeastern Gulf of Mexico. Mutagenicity was detected in the waters of stations DSH08 and DSH10 but not DSH09. The other stations in the Northeastern Gulf of Mexico were not sampled at this time because of poor weather.

Owing to problems of research vessel availability the WFS was not sampled until June of 2011, at which time mutagenicity was detected at the two outer stations of the Northern Transect (NT07 and NT13; Figure S9, Supporting Information) and one station in the Southern Transect (ST12). Also during this cruise, sediment porewater was collected from the slope at depths of 1000, 1200, and 1400 m not far from ST07 (Figure S9, Supporting Information). These sediments yielded some of the highest prophage induction values of the whole study. Sediments taken on other cruises in different locations were

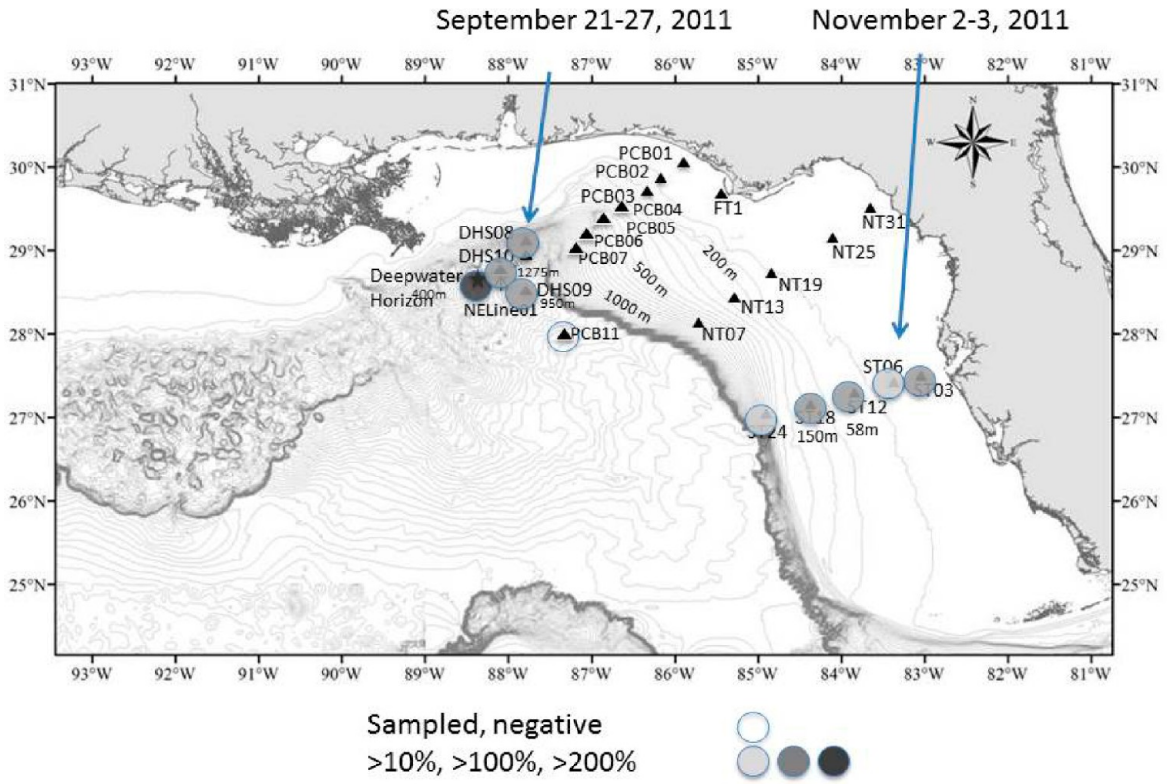


Figure 5. Microscreen mutagenicity assays in September 2011 (NEGOM) and November 2011 (WFS ST stations). Symbols as in Figure 4.

found to be negative, indicating that prophage induction was not simply a result of incubation with porewater (data not shown).

Unfortunately, data for Microscreen mutagenicity assays was only collected through November 2011 (Figure 5). Our results indicate that mutagenic and acutely toxic waters arrived on the WFS after closure of the Macondo 262 well. Our first detection of this toxic/genotoxic water on the WFS was not until June of 2011, our first sampling of this environment since the July 2010 sampling, when no Microtox/Microscreen-positive stations were found. The fact that highly contaminated porewater was obtained on the slope of the WFS corroborates the possible upwelling of dispersed oil onto the shelf.[26]

The tremendous amount of Corexit dispersant released complicates interpretation of the impact of the oil spill using our biological sensors. The ambient microbial population diversity was shown to be altered in response to the Deepwater spill.[15] For example, *Vibrios* were more abundant than α-proteobacteria in Corexit-rich waters. Work with the pure cultures indicated that the Corexit9500 dispersant actually stimulated growth of the nonhydrocarbon-degrading *Vibrios* while the hydrocarbon-degrading bacteria like *Marinobacter* and *Acinetobacter* were greatly inhibited. In our work presented here using *Vibrio fischeri*, we found this organism to be less sensitive to the Corexit dispersant than say phytoplankton in the Gulf. This could mean that use of the dispersant actually inhibits bioremediation by selecting for *Vibrios* and inhibiting ambient HC-degrading bacteria such as *Marinobacter* and *Acinetobacter*.[15]

The lack of visible surface oil slicks during the August 2010 sampling of the NEGOM may have largely been the result of aggressive use of dispersants that reduced the amount of oil that reached the water's surface or rapidly transported oil from the surface to subsurface waters. Our hydrocarbon measurements, using fluorescence spectroscopy and molecular organic geochemical techniques, document that petroleum hydrocarbons were both abundant and widespread in the surface and subsurface waters.

The appearance of toxicity and genetic toxicity in the WFS surface waters and sediment porewater is problematic. The relationship of these observations to the DWH oil spill has yet to be made even though current observations and models suggest that such a fate for the oil was indeed likely. Hopefully studies like this can be useful to environmental managers to alert them to possible outcomes of future catastrophic oil spills in the Gulf.

■ ASSOCIATED CONTENT

Supporting Information

Hydrocarbon analysis results, CTD (conductivity, temperature, depth) profile analysis of station DSH 8, and detailed distribution of toxicity analysis as a function of time in the NEGOM, Northern and Southern transects of the WFS. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by funds from the GOMRI award "C-IMAGE", the University of South Florida Division of

Sponsored Research Foundation grant #94302 to K.D., and the Guy Harvey Ocean Research Foundation to J.H.P. Materials, instrumentation, and reagents for the Microtox Assay were provided by Tracy Weidman, SDIX, and materials for the QwikLite Assay were provided by Brian Bjorndahl of Assure Controls, Inc. We thank J. Wolny for phytoplankton identifications.

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