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Review

A review of oil, dispersed oil and sediment interactions in the aquatic environment: Influence on the fate, transport and remediation of oil spills

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abstract

The 2010 Deepwater Horizon oil spill has spurred significant amounts of researches on fate, transport, and environmental impacts of oil and oil dispersants. This review critically summarizes what is understood to date about the interactions between oil, oil dispersants and sediments, their roles in developing oil spill countermeasures, and how these interactions may change in deepwater environments. Effects of controlling parameters, such as sediment particle size and concentration, organic matter content, oil properties, and salinity on oil–sediment interactions are described in detail. Special attention is placed to the application and effects of oil dispersants on the rate and extent of the interactions between oil and sediment or suspended particulate materials. Various analytical methods are discussed for characterization of oil–sediment interactions. Current knowledge gaps are identified and further research needs are proposed to facilitate sounder assessment of fate and impacts of oil spills in the marine environment.
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Nomenclature

AFM	atomic force microscopy
BTEX	benzene, toluene, ethylbenzene, and xylenes
CLSM	confocal laser scanning microscopy
CMC	critical micelle concentration
DOM	dissolved organic matter
DOR	dispersant to oil ratio
DWH	Deepwater Horizon
ESEM	environmental scanning electron microscopy
FA	fulvic acid
FlowCAM	flow cytometer and imaging system
GC-FID	gas chromatography flame ionized detection
GC–MS	gas chromatography–mass spectrometry
HA	humic acid
HOCs	hydrophobic organic compounds
IFO	Intermediate Fuel Oil
LIF	laser-induced fluorescence
LISST	laser in situ scattering and transmissometry
NCP	National Oil and Hazardous Substances Pollution Contingency Plan
o/w	oil-in-water
OSAs	oil–SPM aggregates
PAHs	polycyclic aromatic hydrocarbons
OSC	on-scene coordinator
PFOS	perfluorooctane sulfonate
RRT	regional response team
SDBS	sodium docecylbenzene sulfonate
SDSS	dioctyl sulfosuccinate
SEM	scanning electron microscopy
SOM	sediment organic matter
SPM	suspended particulate material
TOC	total organic carbon
TPH	total petroleum hydrocarbons
USF	University of South Florida
w/o	water-in-oil

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1. Introduction

Oil may be released into the marine environment from routine or accidental releases as a result of human activities including drilling, manufacturing, storing, transporting, and waste management (NRC, 2003). For example, an offshore oil well blowout or pipe line ruptures can release large amounts of oil into the marine environment. The 2010 Deepwater Horizon (DWH) oil spill released an estimated 4.9 million barrels of South Louisiana sweet crude oil into the Gulf of Mexico, resulting in the largest marine oil spill in U.S. history and perhaps the second largest in the world, after the first Gulf War oil spill from Kuwait (Abbriano et al., 2011; Graham et al., 2010; Hemmer et al., 2011). Oil tankers carrying millions of gallons of oil can also pose a significant threat to the marine environment in the event of ship collisions or grounding (Bandara et al., 2011; Lytle and Peckarsky, 2001). The 1989 Exxon Valdez oil spill discharged 11 million gallons of Alaskan North Slope crude oil through the ruptured hull in Prince William Sound, Alaska, resulting in a contamination of ~ 2000 km of intertidal shorelines within the Sound and the Gulf of Alaska (Bragg et al., 1994). It is noteworthy that most oil spilled into the environment from well blowouts or pipe line ruptures is a mixture of oil and natural gas, and is known as "live oil" for its very high vapor pressures. On the other hand, oil from tankers or some pipelines has been separated from the natural gas, and is called "dead oil" for its low vapor pressure (Reddy et al., 2012).

Application of oil dispersants has been a critical response measure to mitigate impacts of marine oil spill for decades (Franklin and Warner, 2011). Following 1989 Exxon Valdez oil spill, an estimated 5500 gallons of the dispersant known as Corexit 9527 was applied to the oil slick (ADEC, 1993). The 1999 M/V Blue Master Spill resulted in a release of roughly 100 barrels of Intermediate Fuel Oil (IFO) 180 from the M/V Blue Master following a collision with a fishing vessel 55 km south of Galveston, Texas. Twelve hours after the spill, approximate 700 gallons of Corexit 9500 were applied (Kaser, 2001). In 2000, around 2000 barrels of South Louisiana crude oil were discharged into the Gulf of Mexico from a 24 in. pipeline 65 miles south of Houma, Louisiana. In response to the oil spill, 3000 gallons of Corexit 9527 were employed (Stoermer et al., 2001). During the DWH response, BP applied approximately 2.1 million gallons of oil dispersants Corexit 9500 and Corexit 9527, of which 1.4 million gallons of the dispersants were applied at the surface and 0.77 million gallons at the wellhead (Kujawinski et al., 2011).

Generally, oil dispersants are complex mixtures containing three types of chemicals: solvents, additives and surfactants. Solvents are added primarily to promote the dissolution of surfactants, reduce the dispersant's viscosity and affect its solubility in spilled oil. Additives may improve the dissolution of the surfactants into an oil slick and increase the long-term stability of the dispersant (NRC, 2005). Commercial chemical dispersants usually consist of two or more surfactants. These surfactants in fixed ratios can emulsify oil, and hydrocarbon-based solvents can help break up large clumps of high molecular weight, more viscous oil (Kujawinski et al., 2011; Ramachandran et al., 2004). Oil dispersants lower the oil–water interfacial tension, thereby breaking oil slicks into fine droplets and accelerating dispersal into the water column and dissolution of the hydrophobic oil components (NRC, 2005).

Once oil is released into the marine environment, it undergoes complex physical, chemical and biological transformations, including spreading, drifting, dispersion, stranding, and weathering. The important weathering processes include evaporation, dissolution, biodegradation, emulsification (i.e., "mouse" formation), and photo-oxidation (NRC, 2003). Whole oil droplets may be dispersed into the water column while monocyclic compounds (e.g., benzene and alkyl-substituted benzenes) with $\log K_{ow}$ values between 2.1 and 3.7 and selected lower molecular weight, 2–3 ring polycyclic aromatic hydrocarbons (PAHs) with $\log K_{ow}$ values between 3.7 and 4.8 may undergo partial dissolution (NRC, 2005; Payne et al.,

2003). These environmental processes can be strongly affected by interactions between dissolved and dispersed oil components and sediment particles.

Sediments have long been recognized as important vectors in the transport of oil from one environmental compartment (i.e., phase) to another following oil spill events in the aquatic environment. Studies have shown that interactions between oil and sediments play an important role in dispersion and degradation of spilled oil (Muschenheim and Lee, 2002). In nearshore waters, naturally dispersed oil droplets may aggregate readily with suspended particulate material (SPM) such as clay minerals or organic matter to form oil–SPM aggregates (OSAs). Terminologies such as oil–clay flocculation, oil–SPM interactions, oil–mineral aggregation, and oil–fines interactions have been used to describe this natural process (Khelifa et al., 2002, 2005; Muschenheim and Lee, 2002; Omotoso et al., 2002; Owens, 1999; Owens and Lee, 2003; Payne et al., 2003). Interactions between oil and SPM have been documented in several laboratory and field studies. Poirier and Thiel (1941) reported that oil dispersed in a mixture of sediments and seawater settled and was trapped on the bottom by the sediments. Detailed laboratory experiments suggested that mineral–oil interactions may have been instrumental in the natural cleansing of shorelines oiled in Prince William Sound, Alaska, following the Exxon Valdez spill (Bragg and Owens, 1994, 1995; Bragg et al., 1994; Bragg and Yang, 1993; Jahns et al., 1991).

The overall goal of this review is to gauge the state of science pertaining to interactions of physically and chemically dispersed oil with sediments, and how these interactions affect fate and transport of spilled oil. In specific, this review aimed to: (1) elucidate the mechanism governing OSAs formation and the controlling parameters including oil and sediments characteristics, Pickering emulsions, salinity (or ionic strength) and mixing energy, (2) illustrate the effects of environmental conditions, such as sediment organic matter (SOM), mineralogy, temperature, pressure, and salinity, on sorption of dissolved/dispersed oil components onto sediments, (3) interpret effects of deep water conditions and oil dispersants on the oil–sediment interactions, (4) provide an overview of analytical methods for characterization of interactions between dispersed oil and sediments, and (5) discuss future research needs.

2. Interactions between oil and sediments

Oil can be present in water in two physical forms: dispersed oil droplets and dissolved oil components. Likewise, sediments in water can be in the forms of SPM and settled aggregates. Dispersed oil droplets or dissolved oil components can interact with sediments through (1) direct aggregation to form OSAs, and (2) adsorption on or incorporation in the sediment phase (Lee, 2002; Sterling et al., 2005). These interactions can change the fate and transport of oil by removing oil from the aqueous phase, which can be an important component of oil spill countermeasures (Bandara et al., 2011). The formation of OSAs is believed to stabilize the oil droplets and prevent them from re-coalescing, resulting in a reduction of oil droplet size (Ajijolaiya et al., 2006). Smaller oil droplets have larger surface areas and undergo higher rates of biodegradation due to enhanced oil availability. Passow et al. (2012) observed a large marine snow formation in oil-contaminated surface waters of the Gulf of Mexico after the DWH oil spill. One mechanism they suggested was the interaction between oil components with SPM and the associated coagulation. Initially, in situ marine snow remained floating, and within one month of its initial appearance, it disappeared mostly due to settling. Once marine snow lost its buoyancy, its sinking velocity, porosity and excess density were similar to those of miscellaneous aggregates. Bandara et al. (2011) developed a numerical model to simulate oil–sediment interactions and sediment-facilitated oil transport in the nearshore water. The simulations revealed that up to 65% of released oil might be removed from the water column as OSAs, and that the amount of oil partitioned into sediments was 4–5 orders of magnitude smaller than the amount of OSAs formed.

2.1. OSAs formation

2.1.1. Mechanism of OSAs formation

OSAs are aggregates of oil and SPM that form when oil, either as droplets, adsorbed oil or free phase liquid, and solid particles in an aqueous suspension collide and adhere. OSAs formation is postulated to be caused by electrical charge interactions between the particle surfaces and the polar compounds of oil, with the intermediary of cations, acting as electrical bridges (Bandara et al., 2011).

The simplest form of OSAs consists of an oil droplet coated with micrometer-sized solid grains. Using bright-field, UV Epi-fluorescence microscopy and scanning electron microscopy (SEM) techniques, Stoffyn-Egli and Lee (2002) identified three distinct types of OSAs: droplets, solid, and flake aggregates (Fig. 1). Droplet OSAs are the most common type, which are defined as individual micro-sized oil droplets surrounded by solid particles, and sometimes more than one droplet is present to from multi-droplet OSAs (scale of a few l m). Solid aggregates are a mixture of oil and solid bodies of various shapes (scale of tens of l m). Flake aggregates are thin sheets, in which solid and oil particles are arranged in an ordered configuration (scale of up to mm). Another way to classify the OSAs is based on their buoyancy. Omotoso et al. (2002) used confocal laser imaging to analyze the oil–mineral structures and revealed two types of OSAs: (1) negatively buoyant flocs resulting from interactions between low-viscosity oils and hydrophilic minerals (quartz and kaolinite). The minerals stabilize oil droplets in a water-continuous phase (oil dispersed in water), and (2) positively buoyant flocs comprising mineral-stabilized oil droplets and calcite in an oil-continuous phase (water dispersed in oil) (Hou and Shah, 1987).

Typically, OSAs are formed in two consecutive steps (Fig. 2). First, oil film is broken into oil droplets, which can be realized via two types of forces, external disturbing forces produced by the flow field, such as inertia or viscous force, and internal restoring forces of the oil, such as the interfacial tension to sustain the oil droplet shape (Hinze, 1955). The main affecting parameters for this step are the mixing energy, oil viscosity, and interfacial tension between oil and seawater. Second, the oil droplets interact with SPM to form OSAs. This step is actually the interaction between SPM and polar compounds within the oil droplets (Delvigne and Sweeney, 1988). Parameters such as salinity (or ionic strength), oil type and concentration, and sediment type and particle concentration can affect this step.

2.1.2. Factors affecting formation of OSAs

To evaluate the environmental fate, persistence, and potential effects of oil spills, and to assess the effectiveness of oil spill countermeasures, it is desirable to understand the nature and properties of OSAs including the factors that affect formation of OSAs. The key OSAs formation factors include: (1) oil characteristics such as viscosity, droplet size, composition, density, and concentration, (2) the physical properties of the solids such as mineralogy, grain size distribution, organic matter content, density, concentration, and (3) environmental conditions such as temperature, pH, water salinity (or ionic strength), pressure, and hydrodynamic conditions.

Influence of oil characteristics on the formation of OSAs has been investigated (Bragg and Owens, 1994; Khelifa et al., 2002; Le Floch et al., 2002; Omotoso et al., 2002). Viscosity is a major parameter influencing oil interactions with SPM. High viscosity oils are difficult to disperse, and less likely to form aggregates with

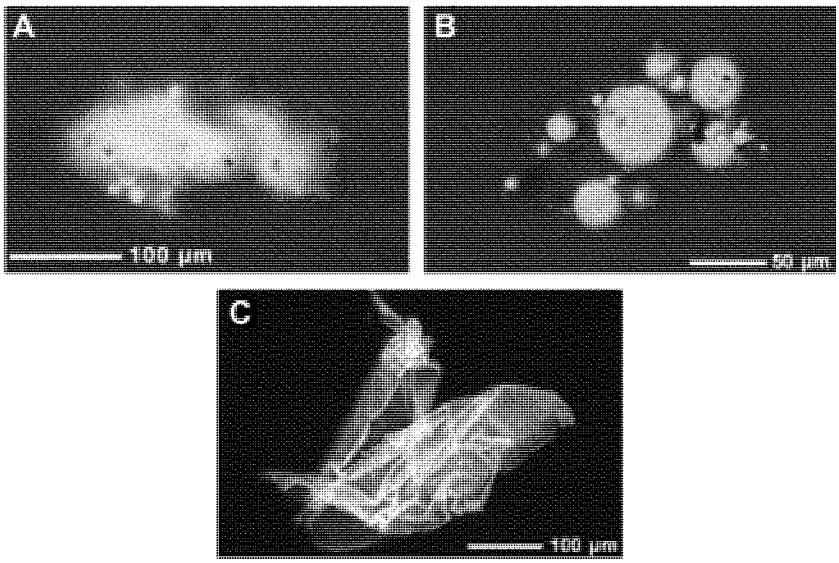

Fig. 1. Three distinct types of OSAs: (A) multiple droplets, (B) solid, and (C) flake aggregates as seen by combined bright-field and UV Epi-fluorescence. Oil appears as bright areas and the mineral particles are dark in these microscopic images (Stoffyn-Egli and Lee, 2002).

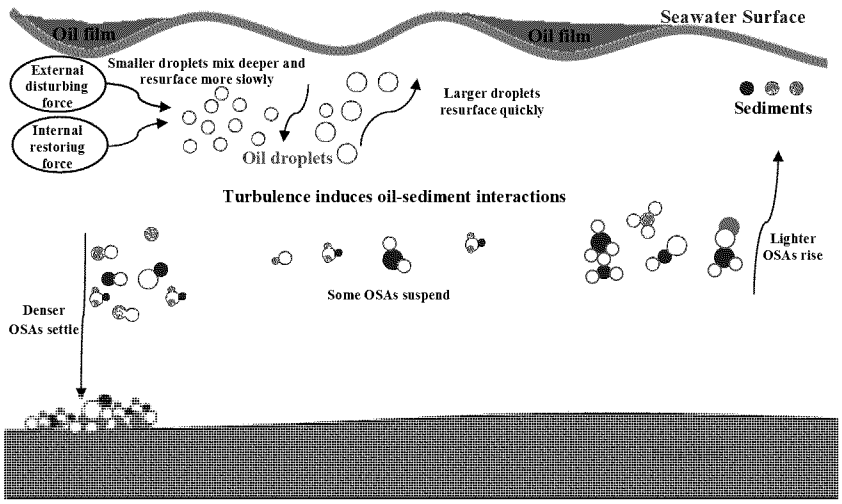

Fig. 2. Formation and movement of various types of OSAs in marine systems.

solids (Le Floch et al., 2002), while low viscosity oils are more favorable for OSAs formation (Khelifa et al., 2002). The viscosity threshold, above which no OSAs will form, was determined to be 9500 mPa-s (Lee et al., 1998). Omotoso et al. (2002) compared settling rates of crude oils of different viscosities with kaolin. Each crude oil had a specific gravity less than 1. They found that OSAs settled less rapidly than oil-free solids in water and that OSAs of highly viscous crude oils settled faster than less-viscous oils. The findings could be explained by the fact that OSAs of more viscous oils have a higher density than OSAs of less-viscous oils.

Bragg and Owens (1994) pointed out that polar hydrocarbons are needed for OSAs to form since polar compounds in the oil droplets enhance the adhesion between oil droplets and sediment particles. The following equation was developed to predict the effects of oil type on the formation of OSAs (Khelifa et al., 2002):

$$\frac{W_0}{W_{ar}} \text{¼ } 0{:}3e^{3{:}23\text{ð}u_d=u_c\text{þ}^{\text{ł}\,0{:}22}} \qquad \text{ð1Þ}$$

where the dimensionless mass concentration W_0 is the ratio between the mass of oil stabilized by OSAs and the initial mass of oil introduced in the system, W_{ar} is the content of asphaltenes and resins in the oil, and u_d and u_c represent the viscosities of oil and water, respectively. Asphaltenes and resins are amphiphilic components in crude oil (Quintero et al., 2009). The polarities of crude oil increase with increasing concentrations of resins and asphaltenes (Guyomarch et al., 2002). For a given viscosity ratio (u_d/u_c), oils with higher W_{ar} are trapped more efficiently in OSAs than those with lower W_{ar} although oils with higher W_{ar} have higher viscosities.

Sediments properties, such as particle size, surface area, concentration and surface chemistry, play an important role in oil–sediment interactions. Poirier and Thiel (1941) pioneered interactions between oil and 11 types of sediments in artificial seawater, and they found kaolin to be the most efficient solid for sedimenting oil among the hydrophilic sediments tested including blue earth silt, marl, diatomaceous earth, decora shale, bentonite, sandy silt, calcareous shale, calcareous silt, oil shale, and humus. Wang et al. (2011) investigated interactions between two different crude oils (Alaska North Slope and Medium South American) and three types of solids (kaolin, modified kaolin, and diatomite). They concluded that the hydrophobicity of the solid phase could

promote the affinity between oil droplets and the solid particles, and facilitate the OSAs formation. Hydrophobic solids (modified kaolin) tended to have more attraction than hydrophilic solids (original kaolin and diatomite). Similarly, Omotoso et al. (2002) reported that hydrophilic solids (e.g., kaolin) interacted with oil to a lesser extent than hydrophobic solids (e.g., calcite).

Zhang et al. (2010) carried out laboratory experiments to study the effects of natural solid properties on the formation of OSAs. They suggested that larger specific surface area or smaller particle sizes of solids favored formation of OSAs. Sun and Zheng (2009) reported that OSAs were readily formed when the sediment particle size was less than 2 l m, and OSAs could be formed when the sediment particle size was smaller than 5 l m, however, when the particle size was larger than 10 l m, formation of OSAs was negligible.

Sediment concentration (i.e., the solid to solution ratio) is another key variable for determining the quantity of oil droplets stabilized by OSAs formation. The ratio between the mass of oil trapped in OSAs and the mass of the oil initially added is referred to as oil trapping efficiency (E):

$$E = \frac{E_{max}\left[\frac{C_s}{C_{S50}}\right]^n}{1 + \left[\frac{C_s}{C_{S50}}\right]^n} \quad (2)$$

where E_{max} is the maximum possible trapping efficiency, C_s is the sediment mass concentration, C_{S50} is the sediment concentration that produces a trapping efficiency of 0.5, and n is a parameter that controls the shape of the curve which is plotted as trapping efficiency versus sediment concentration. Here C_{S50} represents the critical sediment concentration. Below C_{S50}, the stabilization of oil droplets by OSAs decreases rapidly, while above C_{S50}, the stabilization is extensive (Ajijolaiya et al., 2006). Increasing sediment concentration accelerates the process of OSAs formation. Sun et al. (2010) reported that under otherwise identical conditions, the measured E_{max} increased from 23% to 31%, and further to 37% when sediment concentration was increased to 100, 200 and 400 mg/L, respectively. The more sediment grains are available in the suspension, the more OSAs are formed. The process could be accomplished by the formation of a sediment layer on the surface of the droplets (coating) or by trapping one or more droplets in sediment flocs (nesting). It should be noted that the sediment concentrations (100–400 mg/L) are high compared to most natural waters, and extremely high compared to offshore waters under normal conditions. For instance, the SPM concentration in continental shelf waters of Gulf of Mexico was reported to be 0.3–1.8 mg/L (Brooks et al., 1981). In fact, the DWH spill occurred in what is called "blue water" with extremely low sediment loads, and by the time oil reached the more muddy shoreline areas, the oil was fairly viscous and emulsified.

Salinity (or ionic strength) is an important environmental factor in OSAs formation. It affects the flocculation of solid particles and modifies surface properties of oil droplets, thus affecting aggregation between SPM and oil droplets. Bassin and Ichiye (1977) carried out laboratory studies with a smectite-rich marine clay and a South Louisiana light crude oil, and they showed that sufficient salinity was a prerequisite for interactions between oil and clay minerals. Le Floch et al. (2002) tested OSAs formation in the laboratory with diluted seawater (salinity 0–3.5%), and reported that the extent of OSAs formation was not significantly different from that of seawater with salinity in the range of 0.15–0.015%. The precise value of this range depends on oil type and the mineral nature. Below this salinity range, there was a linear decrease in the amount of oil incorporated in OSAs with decreasing salinity, to practically zero in distilled water. Above this salinity threshold, salinity has negligible influence on OSAs formation. They suggested that one mechanism by which salinity affects OSAs formation was through its control on the electrical double layer thickness of oil and mineral particles. As the salinity increases, the electrical double layers on particles surfaces are compressed, which decreases the repulsion potential of particle surfaces and facilitates inter-particle attractions. However, once the double-layer thickness is below a certain threshold value, further increasing salinity has a lesser influence on OSAs formation.

Mixing energy is critical to OSAs formation as it helps to break the oil into small droplets, to suspend sediment particles and oil droplets, and to enhance the collision between oil and sediment (Ma et al., 2008; Wincele et al., 2004). Omotoso et al. (2002) studied effects of mixing energy on the interactions between kaolin and a low-viscosity oil (IFO30) in seawater. Optical micrographs illustrated that the size of the oil droplets decreased with increasing agitation time. Sun et al. (2010) conducted a series of laboratory studies to study the effects of mixing energy on OSAs formation between standard reference materials SRM-1941b and Arabian medium crude oil in artificial seawater. They compared three different shaking rates: 2.0 Hz (to simulate low disturbance of the water surface in the reaction chamber), 2.1 Hz (moderate disturbance), and 2.3 Hz (high disturbance). When the shaking rate increased from 2.0 to 2.3 Hz, the maximum oil trapping efficiency increased from 20% to 42%, and the required shaking time decreased from 3.7 to 0.7 h. It was concluded that increasing mixing energy accelerates the process of OSAs formation.

Wave energy is an important factor for natural removal and cleaning of stranded oil. High energy causes significant sediment motion and flushing of interstitial waters in beach sediments and coastal marshes. These combined processes abrade oil from sediment grains and disperse it in the water column. For example, following the Sea Empress oil spill in 1996, Lee et al. (1997) carried out a surf washing experiment, and observed that stranded oil on the cobble beach was removed as clay–oil flocs when the oiled cobbles were exposed to repeated tidal cycles (Fig. 3). Taking advantage of natural wave mixing energy, a response strategy known as surf-washing has been developed, which increases natural cleaning rates by repeatedly relocating oiled sediments from the high-water mark into the intertidal zone (Lee et al., 1999; Owens, 1999). However, in addition to removing stranded oil from beaches, wave actions may also facilitate spreading of OSAs further into the costal marshes. The DWH event oiled more than 650 miles (1040 km) of the Gulf coastal habitats (Lin and Mendelssohn, 2012). Yet, the role of oil–SPM interactions in the oil transport, which causes either vanishing or spreading of oil, remains unknown.

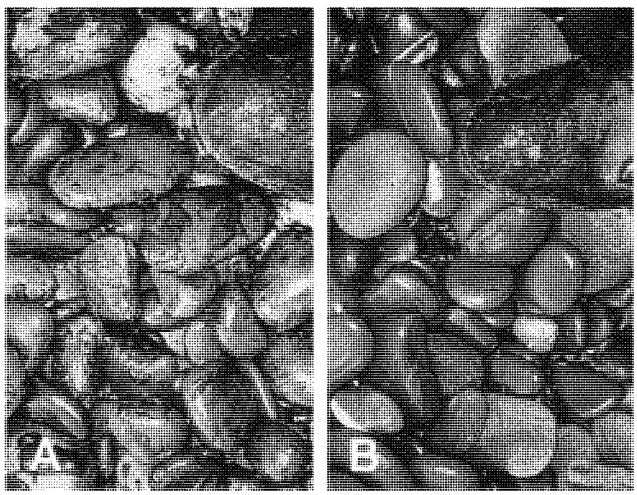

Fig. 3. Cobbles on Amroth Beach before (A) and after (B) surf washing operations to remove stranded oil emulsion (Owens and Lee, 2003).

2.2. The Pickering emulsion and colloids-facilitated stabilization of oil-droplets

When oil droplets are formed and dispersed in water, the oil droplets tend to coalesce to minimize the system energy. However, Pickering (1907) observed that small colloidal particles situated at the oil–water interface were able to prevent the coalescence of oil droplets, i.e., to stabilize the oil–water emulsion. Such colloids-stabilized emulsions are often referred to as Pickering emulsions. As illustrated in Fig. 4, adsorption of colloids (e.g., SPM) to the oil–water interface minimizes the system free energy and forms a rigid barrier acting against coalescence (Aveyard et al., 2003).

The type of emulsion, water-in-oil (w/o) or oil-in-water (o/w), is determined by the three-phase contact angle, h, which establishes the position of the particles relative to the oil–water interface (Binks and Lumsdon, 2000a). Fig. 4A shows h for an ideal, spherical particle at an oil–water interface. The stabilization energy (i.e., the energy of attachment for particles adsorbed at the interface) E_c is given by

$$E_c \text{ ¼ } pr^2 c\delta 1 \text{ } ^{L} \text{ } j\cos hj\text{Þ}^2 \qquad \delta 3\text{Þ}$$

It is evident from Eq. (3) that the best stabilization effect occurs at a contact angle of 90ffl i.e., when the particles are partially wetted by both the water and oil phases; in addition, the hydrophobicity, size, and shape of the particles can impact the stability (Tadros and Vincent, 1983); furthermore, the presence of solvents, surfactants and/or oil dispersants can alter the interfacial tension and surface wettability, and thus, can alter the droplet stability. Hydrophobic particles normally have a contact angle of >90ffland tend to be oil-wetted (i.e., more particle surface is in contact with oil). Thus, w/o emulsions are preferred (Aveyard et al., 2003). In contrast, hydrophilic particles are water-wetted and preferentially stabilize o/w emulsions (Fig. 4B). Extremely hydrophilic or hydrophobic particles are nearly completely wetted by water or oil, and thus no stable emulsions are formed.

Melle et al. (2005) estimated E_c to be $10^7 k_B T$ for a micrometer-sized particle with a contact angle close to 90fflat an oil–water interface and with a c value of around 50 mN/m. Here k_B is the Boltz-mann's constant, and T is the room temperature. The high E_c value indicates that the solid particles in this size range strongly adhere to the oil–water interface and the adsorption is almost irreversible (Aveyard et al., 2003).

Binks and Lumsdon (2000b) investigated the influence of the hydrophobicity of spherical, nanometer-sized silica particles on the type and stability of water–toluene emulsions. The particle surface was varied from hydrophilic to hydrophobic by coating their surface to different extents with a silane reagent. It was found that the emulsions stabilized by the very hydrophilic or very hydrophobic silica particles were large (>100 lm) and unstable to coalescence. However, those stabilized by particles of intermediate hydrophobicity were in sub-micrometer and stable to both sedimentation and coalescence. For spherical silica particles of intermediate hydrophobicity, Binks and Lumsdon (2000a) reported that the oil type can affect the emulsion behavior. For equal volume fractions of oil and water, they found that particles were more hydrophobic at interfaces between water and polar oil (i.e. esters and alcohols), leading to the formation of w/o emulsions; and are more hydrophilic at interfaces of water and non-polar oil (i.e. alkanes), favoring formation of o/w emulsions. The contact angle increased with polarity in going from alkanes through silicone and aromatic oils to esters and alcohols.

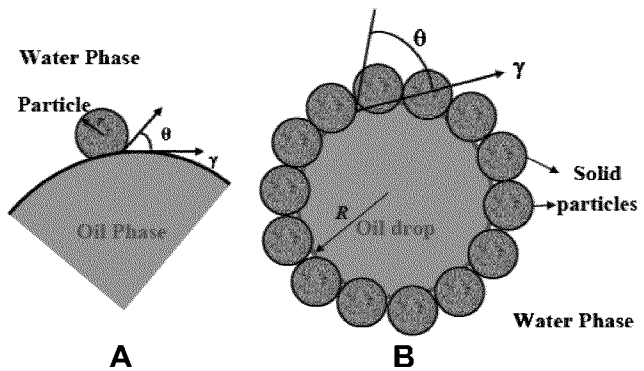

Fig. 4. (A) Position of a spherical particle (radius r) at an oil–water interface with a surface tension c and a contact angle h (measured through the aqueous phase) less than 90ffl and (B) Position of multiple particles adsorbed at an oil–water interface, resulting in a solid-stabilized oil-in-water emulsion (after Aveyard et al., 2003).

2.3. Dissolved oil–sediment interactions

Studies have indicated that the water soluble fraction of oil and their derivatives products are complex mixtures of PAHs, monoaromatic hydrocarbons often referred to as BTEX (benzene, toluene, ethylbenzene, and xylenes), phenols and nitrogen- and sulfur-containing heterocyclic compounds, and heavy metals (Rodrigues et al., 2010; Saeed and Al-Mutairi, 1999). The dissolved oil components in water are considered to be one of the major concerns for both environmental safety and public health, despite of its relatively small fractions (<1% of crude oil) (Saeed and Al-Mutairi, 1999). In marine systems, the fate and transport of dissolved oil components are associated with the process of solubilization, biodegradation, sorption on, and desorption from the sediments and SPM, and dilution into the water column. The vertical transportation of SPM combined with deep-sea blowout makes the dissolved oil components more easily incorporated to the bottom sediments and they can persist for a long time (Gearing et al., 1980; Yamada et al., 2003).

Due to their toxic, mutagenic, carcinogenic and persistent properties, PAHs are one of the principal contaminant classes of environmental concern associated with oil spills, and have been widely studied (Nam et al., 2008). The commonly found PAHs in water phase are the ones with lower numbers of benzene rings (e.g., naphthalene, phenanthrene, fluorene, and pyrene) because of their relatively high solubility. Elevated concentrations of naphthalene, phenanthrene, pyrene, chrysene, and benzo(a)pyrene were observed in the water column during and after the 2010 DWH oil spill (EPA, 2010; Reddy et al., 2012). All these PAHs are included in the list of EPA's 16 priority pollutant PAHs (Bojes and Pope, 2007).

Based on their sources, PAHs are divided into three categories: phytogenic (diagenetic), petrogenic and pyrogenic (Peter, 2003). Phytogenic PAHs are derived from plants, petrogenic PAHs are associated with petroleum, and pyrogenic PAHs result from incomplete combustion of fossil fuels or forest fires. Most environmentally relevant studies have focused on pyrogenic PAHs or parent PAHs comprising 2–6 fused aromatic rings with no alkyl group attached to their ring structures (Readman et al., 2002). Common petroleum contains little amount of parent PAHs compared to their alkylated homologs, i.e. alkylated PAHs. However, incomplete combustion of petroleum can produces large amounts of pyrogenic PAHs (Garrett et al., 2000). Some of the components in oils have many of the same ring structures as the pyrogenic PAH compounds, but the vast majority of PAH compounds in oils also have alkyl groups (mostly methyl groups) attached at various sites on the ring structures. Between one and four or five of these alkyl groups can be attached at various locations around the aromatic rings (structural isomers). These alkyl substituted PAHs are called alkylated homologs of the parent PAH structure or alkylated PAHs,

and each homolog groups can have several structural isomers. Very little is known about the reactive or toxic properties of the alkylated PAH compounds of petrogenic origin.

Yamada et al. (2003) investigated the environmental fate of 38 PAHs existing in the water soluble fraction of heavy residual oil using mesocosms (4 tanks, 5000 L each) simulating natural ecosystems. Gas chromatography–mass spectrometry (GC–MS) analysis results showed that low molecular weight PAHs disappeared in two days dominantly due to bacterial degradation in the systems, while significant portions (10–94%) of high molecular weight PAHs with more than four benzene rings were incorporated into the sediments, which either settled to the bottom or were caught in the sediment trap.

2.3.1. Interactions between PAHs and soil/sediments

In a soil/sediment–water system, the governing parameters affecting the distribution of PAHs are the SOM content and the properties of PAHs. SOM usually exerts the greatest thermodynamic affinity for hydrophobic compounds (White and Pignatello, 1999; Zhao et al., 2001). The sorption behavior of PAHs onto soils/sediments has been investigated extensively during the last several decades. Means et al. (1980) indicated that SOM was a key determination for the sorption of PAHs upon comparing the experimental results with 4 PAHs (pyrene, 7,12-dimethylbenz[a]anthracene, 3-methylcholanthrene, and dibenzanthracene) sorbed onto 14 soil and sediment samples covering a wide range of physicochemical properties (e.g., SOM from 0.1% to 2.4%). The sorption constants for PAHs were observed to be highly correlated to the SOM contents in all cases and independent of other soil properties. And the results also indicated that less soluble PAHs had higher sorption affinity. Similar results were reported by Yu et al. (2006) who monitored 16 PAHs in a PAH-contaminated site in Guiyu, China, and found that individual and total PAH concentrations were significantly related with SOM of sediments.

Humic acid (HA), fulvic acid (FA), and humin are main components of SOM. Pan et al. (2006) investigated the contribution of FA/HA and humin to sorption of phenanthrene and pyrene. The study found that the FA/HA fraction offered much faster sorption rate with an equilibrium time within 48 h, compared to 720 h for humin. Xing (2001) studied the sorption behavior of two model PAHs, naphthalene and phenanthrene, with six kinds of soil HAs. The Freundlich isotherms model was used to fit the nonlinear sorption equilibrium data, and the Freundlich exponents were found to decrease with the increasing aromaticity of the HAs, i.e., more nonlinear, favorable isotherms were observed with more aromatic SOM moieties. Xing (1997) also observed that aged SOM in shale had higher sorption capacity than the young surface soil due to the aromaticity difference. In addition, the mineral surfaces can also serve as HA sorption sites and that explains why mineralogy affects the retention of PAHs (Muller et al., 2007; Murphy et al., 1990).

Temperature, salinity, and dissolved organic matter (DOM) are also potential factors affecting the PAHs sorption to soil/sediments. Researchers have reported an inverse relationship between temperature and sorption coefficient, and have attributed the phenomenon to factors such as the exothermic nature of the adsorption, lowered solubility of PAHs, and alternation of SOM structure at lower temperature (Tremblay et al., 2005; Zhang et al., 2009). Salinity increases uptake of PAHs due to the "salting-out" effect (Tremblay et al., 2005). DOM can be found in pore water of soils and sediments (Haftka et al., 2010). Yu et al. (2011) observed that the presence of DOM in soil systems inhibited sorption of PAHs and increased desorption. In all these cases, water solubility is considered a key factor affecting the sorption behavior of PAHs (Rivas, 2006).

PAHs also compete with each other for the sorption sites on soil/sediments. Zhao et al. (2001) proposed competitive dual-mode kinetic and equilibrium models to fit and predict the competitive sorption and desorption behaviors for co-existing PAHs (phenanthrene, anthracene, and pyrene). In general, the presence of co-solutes accelerated the sorption and desorption rates of each individual PAH, and the competition could influence PAH sorption capacity and the linearity of isotherms. However, these studies did not include the alkylated PAH compounds such as those commonly found in petroleum.

2.3.2. Interactions between PAHs and SPM in marine systems

In the marine environment, SPM acts as an important transport medium for pollutants (Voice and Weber, 1983; Wu and Gschwend, 1986). Because of their hydrophobicity and persistency, PAHs can be adsorbed to these suspended particulates, which in turn affects transport and weathering of the sorbed PAHs (Smith and Levy, 1990). PAHs sorbed in sediments are much less prone to biotic or abiotic degradation, and thus, could persist for decades or even centuries (Gearing et al., 1980). And the horizontal movement could prompt PAHs transport to the open ocean or somewhere away from local contamination origin (Tremblay et al., 2005). The fate and transport of SPM-associated PAHs in natural marine systems are a dynamic and complex process, which involves weathering of PAHs and physical/chemical evolution, and transport of SPM. Guo et al. (2007) surveyed the spatial distribution of PAHs in surface water, SPM, and sediments of several rivers located in some industrial areas in China, and observed that more PAHs existed in the surface water and SPM than in the sediments. The predominant PAHs in the surface water and SPM were 2–3 rings PAHs, while the dominant PAHs in the sediments were 4–6 rings. Luo et al. (2006) observed the same distribution pattern of PAHs in the Pearl River Estuary in China, and identified a positive correlation between PAHs concentration and total organic carbon (TOC) content in the sediments. However, no significant correlations were evident between PAH concentrations and TOC content in SPM samples. The researchers attributed the lack of correlation to the influence of labile organic carbon and microbial activities on SPM. Zhou et al. (1998) studied interactions between fluoranthene and pyrene with SPM in the Humber Estuary, UK. Sorption of these PAHs on SPM were found independent of salinity but decreased with suspended solid concentrations and increased with particulate organic carbon. In general, the source of PAHs, weathering, transport, and properties of SPM and sediments are important factors affecting PAHs–SPM interactions.

2.3.3. PAHs in oil-contaminated systems

Introduction of oil phase into soil/water systems has significant influence on the PAHs distribution. Conventional knowledge and models on the fate and distribution of PAHs in the soil/water systems are often challenged in systems affected by oil spills.

In a lab-scale study, Walter et al. (2000) investigated adsorption and desorption behaviors of 9 selected PAHs with 7 kinds of soils contaminated by oils with different viscosity. A revised equilibrium model was employed to simulate the distribution of PAHs in the four phases: soil, water, adsorbed oil, and emulsified oil (or liquid oil) as shown in Fig. 5. Based on the distribution coefficients, the presence of oil phase in the system drastically decreased the amount of PAHs associated with the soil surface and in the aqueous phase, resulting in more PAHs distributed in the adsorbed and liquid oil phases. The study suggested that the oil content in the system should be considered as a key factor influencing the PAHs distribution.

Traditional sorption models, which are based on SOM partitioning, often underestimate the sediment/soil uptake of PAHs by several orders of magnitude in environmental systems impacted by oil

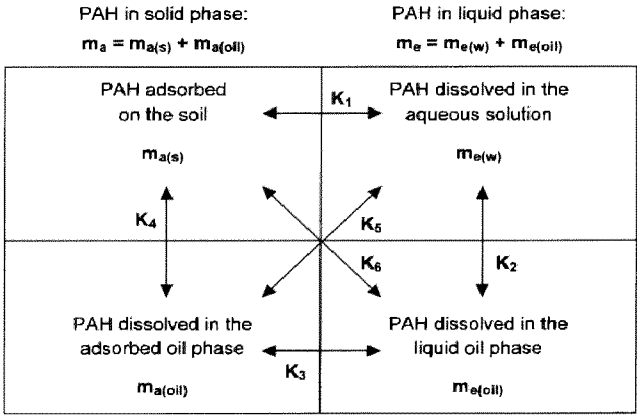

Fig. 5. A conceptualized distribution model of PAHs in an oil-contaminated soil/water system with four distinctive phases (Walter et al., 2000). K_1 – K_6: distribution coefficients of PAHs between the four phases, m_a: mass of PAHs adsorbed on the soil and partitioned in the adsorbed oil phase, $m_{a(oil)}$: mass of PAHs partitioned in the adsorbed oil phase, $m_{a(s)}$: mass of PAHs adsorbed on the soil, m_e: mass of PAHs dissolved in the aqueous solution and in the liquid oil phase, $m_{e(oil)}$: mass of PAHs dissolved in the liquid oil phase, $m_{e(w)}$: mass of PAHs dissolved in the aqueous solution.

spills, in which the oil phase mixes with soil forming unresolved soot-like mixture. Researchers studied distributions of PAHs in San Francisco Bay sediments (Maruya et al., 1996) and Boston Harbor sediments (Maruya et al., 1996; McGroddy and Farrington, 1995), and found that the pore water PAH concentrations were up to two orders of magnitude lower than model-predicted values, which suggested that the soot content in the sediments played an important role in the enhanced PAH uptake. In the case of oil spills, the presence of the oil phase and oil-facilitated PAH uptake should also be considered.

Over the last decade or so, increasing studies have reported the unusual sorption behaviors of condensed carbon phases, i.e., soot and black carbon (Bucheli and Gustafsson, 2000). These condensed carbon particles are highly aromatic residues and are known to play an important role in the sequestration of PAHs (Naes et al., 1998). A large amount of soot was produced during the DWH accident as well as other oil spills due to associated combustion (Azwell et al., 2011). The highly carbonaceous materials were reported to contain over 60% of TOC (Jonker and Koelmans, 2002). Both native and added PAHs were observed to have exceptionally strong affinity for the soot material (Bucheli and Gustafsson, 2000; Jonker and Koelmans, 2001). The carbonaceous materials can provide aromatic flat surfaces (a planar layer on the surface of soot formed by the accumulation of soot-associated PAHs and other planar hydrophobic organic compounds, HOCs), which offer higher affinities toward PAHs than natural SOM (Jonker and Koelmans, 2002). Jonker and Koelmans (2002) also demonstrated that more than 50% of the native PAHs in the soot were trapped within the solid matrix of the soot and therefore sequestered from the aqueous phase. Considering the distinctively stronger sorption affinity of soot, Gustafsson et al. (1997) proposed a revised equilibrium model by considering the soot-carbon as an additional fraction of solid phase as shown below:

$$K_d = f_{oc}K_{oc} + f_{sc}K_{sc} \quad (4)$$

where K_d is the solid-water distribution coefficient of PAHs, f_{oc} is the organic carbon fraction of the solid matrix, and K_{oc} is the organic-carbon-normalized partition coefficient, f_{sc} is the soot-carbon fraction of the solid matrix, and K_{sc} is the soot-carbon-normalized partition coefficient. The modified equation takes into account the soot-enhanced uptake of PAHs and provides a more accurate and mechanistically sounder modeling approach for distribution of PAHs in environmental systems (Bucheli and Gustafsson, 2000).

Weathered oil residues contain numerous PAHs at elevated concentrations (Frysinger et al., 2003), which can be adsorbed on sediments and persist for decades (Reddy et al., 2002). On the other hand, the uptake of persistent oil components by sediments can influence the sediments' sorption behavior of native or oil PAHs (Luthy et al., 1997; Sun and Boyd, 1991). Jonker et al. (2003) tested sediment sorption of various fresh and weathered oils, and studied its effect on the co-sorption of PAHs on the oil-laden sediments. They observed that at relatively low concentrations of oil (100–1000 mg oil/kg sediment), less PAHs were adsorbed to the sediments because of the competitive effects; However, at elevated concentrations of oil (1–100 g oil/kg sediment), the sediment uptake of PAHs was enhanced due to the oil-facilitated swelling of the sediments.

3. Effects of deepwater conditions on oil–sediment interactions

There is no strict definition to deepwater. Considering approximate 10 m of water depth produces a pressure of 1 atm, hydraulic and coastal engineers define the 100 m of water depth as deepwater (Yapa and Li, 1997). However, in the oil industry, drilling depth can often reach 1000 m or more. As a result, the oil industry tends to refer the deepwater/deep-sea to the lower layer in the ocean, i.e., below the thermocline and above the sea-bed at depths of at least 700–1000 m (Camilli et al., 2010; Elordui-Zapatarietxe et al., 2010; Johansen, 2000; Yapa and Li, 1997). Deepwater conditions are characterized with high pressure (usually higher than 16 MPa, even up to 100 Mpa), low temperature (−1 to 4 °C), little or no light, constant salinity (3.5%), sluggish currents (<0.25 knots), and slow sediment accumulation rates (0.1–10 cm per thousands of years) (Glover and Smith, 2003).

The effect of pressure and temperature on solubilization of organic gas has been studied in the lab-scale. King (1969) proposed a modified version of the Henry's law by considering both pressure and temperature effects:

$$f^g = Hx^l \exp\left(\frac{pV^l}{RT}\right) \quad (5)$$

where f^g is the fugacity of a gas in the gas phase, H is Henry's law constant, which is dependent on temperature, x^l is the mole fraction of the dissolved gas in solution, p is the gas pressure, V^l is the partial molar volume of the gas in solution, T is water temperature, and R is universal gas constant. Alaee et al. (1996) observed good fittings with the model for benzene, naphthalene, phenanthrene, and anthracene in distilled water at different temperatures. This equation is useful for studying distribution of volatile or semi-volatile oil compounds in the deepwater environment.

Deep-sea oil plumes were observed in many oil spill cases due to the releasing of oil/gas mixtures from the sea floor (Sauter et al., 2006; Schrope, 2010; Suess et al., 1999). During the 2010 DWH oil spill, an oil plume was reported about 10 miles long, 3 miles wide (16.9 × 4.83 km^2), and up to 400 ft (122 m) in depth (Camilli et al., 2010). Methane was considered the most abundant hydrocarbon released during this accident (Kessler et al., 2011). The dissolution of methane in deepwater plumes was well modeled using Eq. (5) (Zheng et al., 2003). Under deep-sea conditions, especially high pressure and low temperature, methane may be converted to gas hydrates, a "solid state of water resembling ice" (Johansen, 2003). Massive gas hydrate was documented near wellhead, resulting in a gas hydrate zone (Joye et al., 2011). Not only the gas properties will be altered, hydrocarbons beyond C14 can also be formed to a waxy solid phase (Thibodeaux et al., 2011). So there will be gas, gas hydrates, waxy hydrocarbon solid,

sediment, oil, and even more phases co-existing in the plumes. However, detailed information has been lacking on oil–sediment interactions under deep-sea conditions, although effects of temperature on adsorption of hydrocarbons have been well documented. Furthermore, the effects of oil dispersants, especially when introduced at the deep-sea wellhead, on oil distribution are unknown under deep-sea conditions.

4. Effects of oil dispersants on oil–sediment interactions

Oil dispersants are composed of surfactants (surface active agents) dissolved in one or more organic solvents. Commonly used dispersants contain a mixture of two nonionic surfactants, sorbitan monooleate (Span 80) and ethoxylated sorbitan monooleate (Tween 80), together with an anionic surfactant sodium dioctyl sulfosuccinate (SDSS) (Thibodeaux et al., 2011). For example, Corexit 9527 consists of two nonionic surfactants (48%) including ethoxylated sorbitan mono- and trioleates (Tween 80 and Tween 85) and an anionic surfactant (35%) (SDSS) in an aqueous hydrocarbon solvent of ethylene glycol monobutyl ether (17%) (Scelfo and Tjeerdema, 1991). Corexit 9500 contains the same surfactants as Corexit 9527, but it does not contain 2-butoxy ethanol, which is an ingredient in Corexit 9527 (EPA, 2010; George-Ares and Clark, 2000). Table 1 presents the key chemical ingredients in these dispersants. About 0.3 million gallons of Corexit 9527 were applied in the early stage of the DWH oil spill, and later replaced with Corexit 9500 (1.8 million gallons) for the fear of the higher toxicity of the former (Benner et al., 2010; EPA, 2013; Kujawinski et al., 2011), though both dispersants are EPA-approved and by far the most prevalent of all dispersants held in industry stockpiles in the U.S. (NRC, 2005).

Typically, dispersants are sprayed on surface oil slicks to break up oil into small droplets and thus increasing its surface area by lowering the oil/water interfacial tension, and thus, accelerating the dispersal of oil into the water column. When used in the right amount and circumstances, the application of chemical dispersants may promote biodegradation of oil in the water column and eliminate or significantly reduce the impacts of oil on sensitive shorelines and habitats (Lessard and DeMarco, 2000).

The current U.S. environmental regulations require special permissions from the RRT (regional response team) and OSC (on-scene coordinator) for uses of oil dispersants in shallow waters or on beaches. Limited "pre-approved" zones are usually granted for using dispersant countermeasures in waters beyond ┐5 km off the shoreline and at water depths greater than 10 meters (NRC, 2005). More stringent rigorous scientific investigations are needed to decide the suitability of the potential uses of dispersants in nearshore and shallow waters (NRC, 2005). The 2010 DWH oil blowout occurred at about 50 miles off the Louisiana coast and at ┐1500 m below the sea surface. In accordance with the U.S. National Oil and Hazardous Substances Pollution Contingency Plan (NCP), the RRT was authorized to preauthorize the uses of dispersants in the offshore waters (Bejarano et al., 2013).

From the standpoint of dispersant-oil–sediment interactions, the concentration of SPM in undisturbed offshore water is usually low compared to that in nearshore water, which partially accounts for the regulators' caution of nearshore uses. Nonetheless, at least a fraction of the oil dispersants applied offshore and dispersed oil may reach sediments through a number of transport pathways. First, dispersants and dispersed oil can be advectively transported to nearshore sediments via both surface water wave actions, underwater current and dispersed oil plumes; Second, dispersants and dispersed oil can be transported to the bottom sediments via oil-plume settling, and formation and the subsequent settling of marine snow or marine oil snow; And third, re-suspended sediments and drilling mud at the drilling site may harvest considerable amounts of dispersants and dispersed oil. While more robust field data are yet to be collected, a number of field observations following the DWH oil spill support these proposed mechanisms, including the observed large amounts of oil on beaches and in nearshore water, accumulation of oil at the bottom sediments, and migration of massive oil plumes. Our very recent data (Gong et al., 2013) showed that the presence of Corexit 9500 greatly increased sediment uptake of dispersed oil and PAHs. Also, it is noteworthy that as part of the efforts to shut off oil flow into the Gulf during the DWH oil spill, heavy drilling mud was pumped into the crippled blowout preventer on top of the well (Klemas, 2010). As a result, a portion of the oil on the bottom near the wellhead is associated with this use of the drilling mud.

Because of the amphiphilic nature, surfactants can interact with both oil (lipophilic) and water (hydrophilic). When applied to an oil spill, the surfactant molecules diffuse to the oil/water interface, and orient themselves so that the lipophilic tail is attached to the oil phase while the hydrophilic head extends into the water phase, thereby reducing the interfacial surface tension between water and oil. As wave energy is added to chemically dispersed oil at sea, the slick breaks up into small droplets. Fig. 6 depicts the mechanisms of chemical dispersion of surface oil in aquatic systems containing suspended sediment particles. Dispersant/surfactant-facilitated dissolution or solubilization of oil is achieved through formation of surfactant-coated oil droplets or partitioning of oil into the surfactant micelles. The resulting dispersed oil droplets may agglomerate with SPM (Guyomarch et al., 1999, 2002; Khelifa et al., 2008; Li et al., 2007; Mackay and Hossain, 1982; Sterling et al., 2004).

In addition, dispersant/surfactant can be sorbed onto the sediment surface forming so-called "hemimicelles" and "admicelles" (Behrends, 1999; NRC, 2005), which can in turn enhance uptake of hydrophobic compounds through adsolubilization. At low coverage, the sorbed surfactants are principally in the form of monolayers on the sediment surface. Monomers in these surfactant aggregates are viewed as being oriented in such a manner that the hydrophilic groups of the surfactants are next to the surface with the tail groups forming a hydrophobic film in contact with

Table 1
Chemical ingredients for oil dispersants Corexit 9500 and Corexit 9527 after (EPA, 2010).

CAS#	Name
1338-43-8	Sorbitan, mono-(9Z)-9-octadecenoate (Span 80)
9005-65-6	Sorbitan, mono-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) derivs (Tween 80)
9005-70-3	Sorbitan, tri-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) derivs (Tween 85)
577-11-7	Butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (1:1) (SDSS)
29911-28-2	2-Propanol, 1-(2-butoxy-1-methylethoxy)-
64742-47-8	Distillates (petroleum), hydrotreated light
111-76-2	*Ethanol, 2-butoxy-
57-55-6	1,2-Propanediol

* Not included in Corexit 9500.

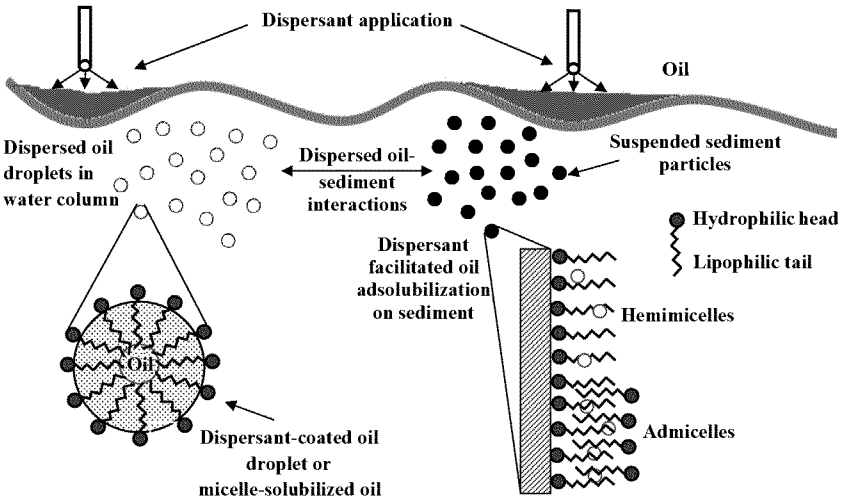

Fig. 6. Mechanism of chemical dispersion of oil. Left: accumulation of dispersant molecules at oil–water interface facilitates formation of fine oil droplets that become entrained in the water column; Right: accumulation of dispersants at sediment–water interface facilitates sorption (adsolubilization) of oil in the solid phase. Alternatively, dispersed oil droplets may also form agglomerates with SPM, which can be stable colloids or re-suspended (temporarily stable) sediment particles.

the aqueous solution. Aggregates of this structure are commonly referred to as hemimicelles (Gangula et al., 2010). At elevated surfactant concentrations, a second layer of surfactant is formed through tail–tail interactions. The bilayered aggregates are termed admicelles. Partitioning of a solute into hemimicelles and admicelles is referred to as adsolubilization (Gangula et al., 2010; Yeskie and Harwell, 1988).

Interactions between chemically dispersed oil and sediments have not been well understood. Mackay and Hossain (1982) conducted one of the earliest exploratory studies of naturally and chemically dispersed oil in the presence of various amounts of sediments and SOM. They found that little actual settling occurred in a turbulent ocean surface layer, and the presence of the dispersant Corexit 9527 resulted in less oil settling to the bottom. The higher the dispersant to oil ratio (DOR), the more the OSAs tended to return to the surface, and the lower the ratio, the more OSAs tended to settle to the bottom. Guyomarch et al. (1999) demonstrated that the formation of OSAs was enhanced in the presence of the chemical dispersant Inipol IP90, thus changing the characteristics of the pollutants. Through large scale wave tank experiments, the researchers showed that 30 min of wave action on a chemically treated oil were sufficient to disperse the oil, and that almost 80% of the oil was trapped as OSAs which settled to the bottom of the tank. Later, Guyomarch et al. (2002) investigated the size distribution of OSAs between clay and chemically dispersed oil. The results showed that aggregate size was correlated to the relative compositions in oil and clay. At clay concentrations <0.2 g/L, the aggregates had a similar size distribution to that of clay without oil at the same salinity, and the aggregates were mainly composed of clay with occasional oil droplets. However, at higher clay concentrations (0.6 and 0.8 g/L), large aggregates containing up to 15 oil droplets in a single cluster were found. The researchers also indicated that the transport behavior of OSAs depended on the size and buoyancy of the aggregates which would vary with salinity, suspended mineral load, and hydrodynamic conditions. Sterling et al. (2004) carried out a series of laboratory experiments in shear reactors and showed that OSAs were formed when artificially weathered Arabian medium crude oil was mixed with Corexit 9500 at a DOR of 1:10 and in the presence of bentonite clay. Li et al. (2007) conducted a wave tank study to investigate the effects of chemical dispersants and mineral fines on the dispersion of oil and the formation of OSAs in natural seawater. Results of UV spectrofluorometry and gas chromatography flame ionized detection (GC-FID) analysis revealed that the combined application of Corexit 9500 and kaolin increased weathered Scotia Shelf Condensate (a light crude oil) dispersion in the water column, and that the presence of the dispersants promoted entrainment of the oil into the bulk aqueous phase and reduced oil and OSAs droplet size. Khelifa et al. (2008) studied behaviors of physically and chemically dispersed oils using the Standard Reference Material 1941b prepared per the National Institute of Standards and Technology, Arabian Medium and South Louisiana crude oils, and Corexit 9500. They concluded that oil sedimentation with the dispersant was 3–5 times higher than that with physically dispersed oil at sediment concentrations of 25 and 50 mg/L.

The application of dispersants may alter the rate and extent of the interactions between oil and sediment. First of all, chemical dispersants tend to produce smaller oil droplets, and increase oil dispersion in the water column. Li et al. (2007) reported that after the same period of mixing time (200 min) and energy dissipation rate in a wave tank, the mass mean diameter of physically dispersed oil droplets was 40 l m measured by a in situ laser scattering and transmissometer (LISSR-100X), while that for the chemically dispersed droplets was 15 l m. GC-FID analysis of total petroleum hydrocarbons (TPH) showed that the addition of Corexit 9500 at a DOR value of 1:50 enhanced oil dispersion into the water column by 68%. The increase in hydrocarbons can be due to the presence of oil droplets in emulsion or increased dissolution of hydrocarbons from the surfaces of the numerous droplets (i.e., surface area effects) (Ramachandran et al., 2004). Li et al. (2009) evaluated the effectiveness of two chemical dispersants Corexit 9500 and SPC 1000 on two crude oils, Medium South American and Alaska North Slope under regular non-breaking and breaking wave conditions in a wave tank facility. The dispersants increased oil concentrations several folds under both wave conditions. The breaking waves created much higher oil concentrations than regular non-breaking waves. In the absence of dispersants, the oil droplet size remained large and variable with a volumetric mean diameter of 150–200 l m under breaking wave conditions. In the presence of dispersants, the size was reduced to ¬ 50 l m. Couillard et al. (2005) reported that the presence of the dispersant Corexit 9500 at a DOR of 1:30 caused a two- to five-fold increase in the concentration of total PAHs and high molecular weight PAHs with three or more benzene rings. The greater number of oil droplets resulted from a higher collision frequency with SPM and thus a greater number of agglomerates. Thermodynamically, a larger

number of smaller oil droplets are more favorably driven to the SPM or sediment phase than a smaller number of larger droplets. For example, based on the TPH analysis, Li et al. (2007) reported that the presence of Corexit 9500 at a DOR of 1:50 increased the light Scotia oil content in the aggregated phase (OSAs) by nearly two times.

Smaller chemically dispersed oil droplets require fewer solid fines in suspension to form OSAs than larger physically dispersed droplets. Khelifa et al. (2008) studied aggregation between physically and chemically dispersed oil droplets and SPM. They found that the sedimentation of oil trapped in OSAs was 3–5 times higher with chemically dispersed oil than with physically dispersed oil at low sediment concentration (25–50 mg/L) based on the TPH measurement in sinking OSAs. The dispersant enhanced OSAs formation was attributed to the fact that chemically dispersed oil droplets were smaller and more abundant. The higher collision frequency with a limited number of sediment particles resulted in more sedimentation of the oil droplets as smaller droplets require less sediment to become negatively buoyant.

As noted above, chemical dispersants tend to produce smaller oil droplets and increase oil dispersion in the water column. Fine droplets on the order of tens of microns tend to remain suspended in the water column, and thus, become widely dispersed in the water column by turbulent diffusion; however, larger oil droplets on the size order of hundreds of microns tend to re-coalesce and resurface unless very strong mixing energy exists to overcome the buoyancy (Li and Garrett, 1998). As a result, the presence of dispersants/surfactants can enhance the formation of OSAs under low agitation energy (Wood et al., 1998).

Because of the amphiphilic nature, dispersants/surfactants can pose two contrasting effects on adsorption of HOCs by sediments (Cheng and Wong, 2006; Pan et al., 2009; Zhang and He, 2011). On the one hand, surfactant can increase the apparent solubility of HOCs, by partitioning in the hydrophobic cores of surfactant micelles, thereby reducing sorption and promoting desorption of HOCs. On the other hand, the dispersant/surfactant itself is subject to sorption onto the solid matrix or sediment including SPM. The sorbed dispersant provides additional sorption capacity for HOCs. Paria and Khilar (2004) reported that surfactants can be adsorbed onto solid matrices through processes such as ion exchange, ion pairing, hydrophobic bonding, and adsorption by polarization of electrons or dispersion forces. The overall effects of surfactants on sorption and desorption of HOCs depend on the extent of these two contrasting factors (Pan et al., 2009). Cheng and Wong (2006) observed that desorption of phenanthrene and pyrene from soil was enhanced in the presence of the nonionic surfactant Tween 80 at concentrations higher than the critical micelle concentration (CMC) of 15 mg/L, however, no significant desorption enhancement was evident when the surfactant concentration was increased from 0 to 7.5 mg/L. Pan et al. (2009) reported that the presence of 4.3 mg/L of an anionic surfactant known as sodium docecylbenzene sulfonate (SDBS) increased sorption of perfluorooctane sulfonate (PFOS) onto natural sediments. At a PFOS equilibrium concentration of 3.2 mg/L, the sorption capacity was increased by 55%. Zhang and He (2011) examined the roles of an anionic surfactant (SDBS) and a nonionic surfactant (Triton X-100) on sorption and desorption of phenanthrene to/from black carbon, and found that the presence of SDBS in the range of 50–200 mg/L (CMC value = 522 mg/L) reduced the sorption capacity and decreased desorption hysteresis. The effect of Triton X-100 (CMC value = 181 mg/L) was concentration-dependent. Phenanthrene sorption was increased when Triton X-100 concentration was lower than 50 mg/L, while the sorption was decreased when the concentration was increased to 150 and 200 mg/L. Yamada et al. (2003) studied effects of a chemical dispersant (Taiho Self Mixing S-7) on the environmental fate of petroleum-derived PAHs in mesocosms designed to mimic natural ecosystems. The results indicated that the addition of the chemical dispersant increased dissolution of the PAHs into the water column, and reduced the sediment-facilitated settling of high molecular weight PAHs (with more than four benzene rings) to the bottom, which was attributed to reduced adsorption of PAHs onto suspended and settling particles due to solubilization by the dispersant.

Chemical dispersants may alter the surface physicochemical properties of oil droplets and, thus, change the bonding forces of oil to sediments. Sorption of chemical dispersants/oil droplets to the sediment mineral surfaces or filling of sediment pores by dispersants/oil may alter the stability or settleability of the sediment particles. While SPMs in the marine waters are typically 2–3 times denser than most crude oils, the buoyancy of OSAs can vary greatly. OSAs associated with light oil components are less likely to settle, while those with heavier oil components settle more easily. Meanwhile, OSAs that are heavier than the crude oil itself may increase the in situ density of oil, and cause more oil dispersed in the water column to settle to the sediment on the seafloor. Following the deepwater dispersant injection at the Macondo wellhead, researchers observed a number of oil plumes (Diercks et al., 2010; Kessler et al., 2011). During May 9–16, 2010, a cruise of opportunity conducted by the R/V Pelican (an oceanographic research vessel) in the vicinity of the DWH site detected subsurface maxima of fluorescence, which were thought to represent subsurface oil plumes (Diercks et al., 2010). The profiles of in situ fluorescence and beam attenuation were characterized with distinct peaks at depths greater than 1000 m, with highest intensities close to the wellhead and decreasing intensities with increasing distance from the wellhead (Diercks et al., 2010). A multiagency report confirmed the deepwater oil plumes did originate from the BP's well based on samples gathered from four separate cruises, and concluded that DWH-MC252 oil did exist in subsurface waters near the well site in addition to the oil observed at the sea surface (JAG, 2010). The report noted that the oil appeared to be chemically dispersed, and indicated that the oil plume first appeared at approximately 1000 m, and then was attenuated between 1300 m and 1400 m deep, with oil concentrations as high as 10 mg/L. Evidence showed that the subsurface plume travelled to the northeast of the wellhead and much farther to the southwest of the blowout, which was consistent with water movement along isobaths (JAG, 2010; Kessler et al., 2011). On July 24, 2010, a team from the University of South Florida (USF) discovered deepwater oil plumes formed by microscopic oil droplets in the northeastern Gulf, and confirmed that the oil plumes came from the BP's well by isotopic and microscopic fingerprinting techniques (Couwels, 2010). Between June 11 and June 21, Valentine et al. (2010) detected elevated C1–C4 hydrocarbon gases between 800 and 1200 m depths using chemical and isotopic surveys. Vertical rosette profiling conducted between June 19 and 28, and in situ mass spectrometer surveys conducted between June 23 and 27 found elevated hydrocarbons between depths of 1100–1200 m and a more diffuse plume between 50 and 500 m (Camilli et al., 2010). Kujawinski et al. (2011) monitored the fate of a key ingredient of the dispersants, the anionic surfactant SDSS, in the Gulf of Mexico deepwater during the active flow and after the flow had ceased. They found that SDSS was sequestered, rather than degraded, in deepwater hydrocarbon plumes at 1000–1200 m water depth and did not reach the surface. The information on the deepwater dispersant fate has provided important constraints on accurate modeling of the deepwater plume and critical geochemical contexts for future toxicological studies. In addition to the oil plumes, accumulation of oil on the seafloor sediments was reported. For instance, following the DWH oil spill, Babcock-Adams et al. (2013) analyzed petroleum biomarkers (hopanes, steranes, terpanes) concentrations in the seafloor sediment samples collected in May and

September 2010. The results showed that concentrations of these biomarkers at impacted sites were 2–4 times higher in September 2010 than in May 2010, indicating that some oil released was deposited subsequently to the seafloor. White et al. (2012) examined 11 deepwater coral communities three to four months after the Macondo well was capped. They reported that healthy coral communities were observed at >20 km from the well, while some coral colonies located 11 km to the southwest of the well showed widespread signs of stress and disease, including excessive mucous production, varying degrees of tissue loss, and retracted polyps. The team observed a brown flocculent material (flocs) coating many of the affected corals, and they showed that the collected "flocs" generated a fingerprint that was unique to the Macondo well. Larson et al. (2013) reported that the surface plankton and other surface materials were deposited on the sea floor at least ten times faster following the BP blowout than normal at more than 40 sites of the Gulf. Chanton et al. (2013) suggested that the oil led to the particles in the water to clump together, causing surface materials to sink to the floor on a massive scale. This underwater rain of oily particles is known as a "dirty blizzard". Hollander et al. (2013) studied sediment cores from the DeSoto Canyon and in the area to the southwest of the DWH blowout site, and indicated that oil interacted with and accumulated in the deep-sea sediments. They proposed several mechanisms accounting for sedimentary oil deposition: (1) direct contact between the sediments and the subsurface oil plumes ("bathtub ring"), (2) intense aggregation and sinking of oil-associated surface particles ("dirty blizzard"), (3) transformation and selective sinking of pyrogenic petroleum components, and (4) flushing of terrestrial-derived components associated with the opening of river flood gates. In addition, they pointed out that the "dirty blizzard" and "dirty bathtub" effects may have sent 30% of the spilled oil to the bottom of the Gulf.

As dispersants can affect sediment uptake of oil, and the buoyancy and settleability of OSAs, SPMs and sediment particles may serve as an important carrier of oil components and facilitate transport of dispersed oil. For instance, Page et al. (2000) compared behaviors of chemically dispersed oil and physically dispersed oil under simulated surf zone conditions in a wave tank facility. Sand was applied to each tank to establish a beach with a prescribed slope of 10 degrees. They found that approximately 49% of the physically dispersed oil in the tanks was sorbed to sediments, and the rest of the oil was removed by the effluent. However, in the presence of the dispersant Corexit 9500 (at a DOR of 1:10), nearly all of the oil was removed by the effluent. Similar results were also observed in a wetland research facility (Page et al., 2002). These findings indicated that the application of a dispersant could reduce residual oil accumulation on beach substrates and greatly alter the transport pathways of oil.

The observed oil plumes, and oil accumulation on the seafloor following the DWH oil spill may be ascribed to, at least in part, the sediment facilitated transport, dispersion and sedimentation. Understanding the dispersant-oil–sediment interactions may facilitate more accurate estimate of transport, budget, mass balance and distribution of dispersed oil in various environmental compartment, which in many cases may account for the "lost oil" but has been largely omitted in current practices.

5. Analytical methods for studying oil–sediment interactions

Many analytical methods have been applied for the characterization of oil/dispersed oil–sediment interactions, such as microscopic analysis of particle morphologies, particle size distribution, and spectroscopic characterization.

5.1. Morphology

Microscopic techniques are generally used to observe the morphological characteristics of OSAs, including transmitted light and UV Epi-fluorescence, confocal laser scanning, and environmental scanning electron microscopy (Stoffyn-Egli and Lee, 2002). Fig. 7 shows selected images filmed by these microscopes, and Table 2 lists salient features of these techniques.

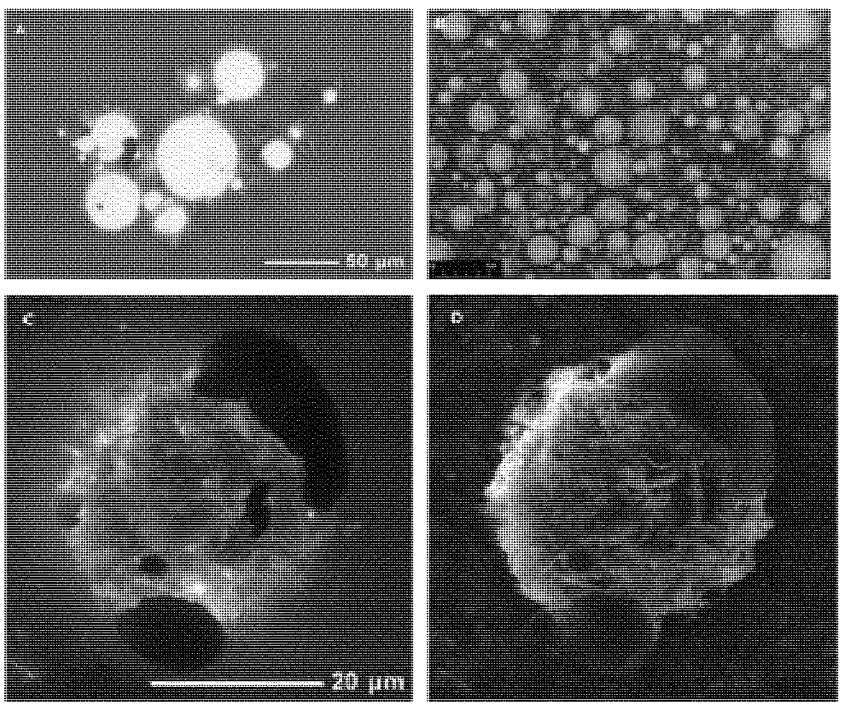

Fig. 7. Microscopic images of OSAs with: (A) UV Epi-microscopy (Khelifa et al., 2005), (B) confocal laser scanning microscopy (Omotoso et al., 2002), (C) environmental scanning microscopy in wet state, and (D) environmental scanning microscopy in dry state (Stoffyn-Egli and Lee, 2002).

Table 2
Salient features of various microscopic techniques (Carter and Shieh, 2010).

Type	Dimension	Advantages	Disadvantages	Remarks
Transmitted light and UV Epi-fluorescence microscopy	2D	Sensitive to oil, simple and fast	Out-of-focus florescence can cause fuzzy image	Recommended for routine OSAs analysis
Confocal laser scanning microscopy	3D	Sensitive to oil, optical slicing capability, sharp image	Long time exposure to intensive laser can cause the change of sample, High cost, time consuming	Internal structure analysis
Environmental scanning electron microscopy	3D	Great magnification	Expensive, need extensive training for operation	External microscopy analysis
Atomic force microscopy	3D	Highest magnification and resolution	Low scanning speed, small single scan image size	Nano-scale surface profile

5.1.1. Transmitted light and UV Epi-fluorescence microscopy

Transmitted light microscopy emits light from a source on the opposite side of the specimen to the objective lens, through which the light reaches a camera or eyepiece where the enlarged image is viewed. For the UV Epi-fluorescence microscopy, the UV light is transmitted to the specimen. The fluorophores on specimen are excited to emit light of longer wavelengths than the UV light, and the fluorescence is selectively passed through a spectral emission filter, and the fluorescent image is then observed.

Transmitted light and UV Epi-fluorescence microscopy have been employed jointly for observing sediment and oil phases of OSAs. Because aromatic hydrocarbons emit strong natural fluorescence when excited by UV light, UV Epi-fluorescence microscopy is sensitive for observing oil components. Moreover, the unwanted, short-wavelength background is eliminated by filtering the spectra of longer wavelength, emitted light (Oheim et al., 2006; White and Errington, 2005). These properties merit the UV Epi-fluorescence microscopy a better method than general optical methods for the OSAs observation. Together with the relatively inexpensive cost, simple sample preparation and analysis procedure, UV Epi-fluorescence microscopy has been recommended for routine OSAs characterization (Stoffyn-Egli and Lee, 2002) to yield information on particle/droplet size, shape, and number (Khelifa et al., 2002, 2005; Lee, 2002; Lee et al., 2003; Stoffyn-Egli and Lee, 2002). Lee et al. (1985) used UV Epi-fluorescence and bright-field transmitted light microscopy to observe chemically dispersed oil droplets associated with particulate materials. Khelifa et al. (2002) used UV Epi-fluorescence microscopy to characterize oil droplets stabilized by mineral particles, and found the concentration of oil droplets decreased with oil viscosity, temperature, and content of asphaltenes-resins, while the oil droplet shape and size were not correlated with oil viscosity.

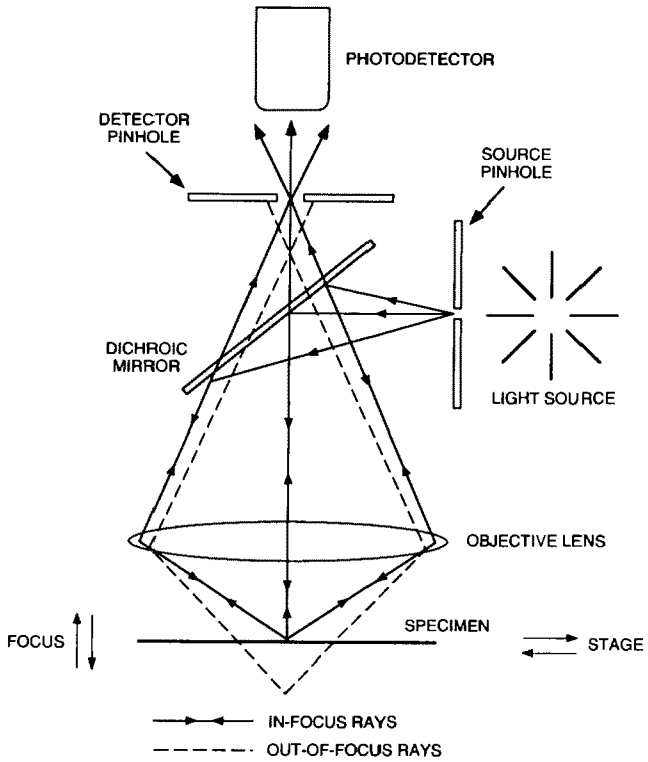

Fig. 8. Ray path in a CLSM (Paddock, 2000).

5.1.2. Confocal laser scanning microscopy

Fig. 8 illustrates the mechanisms of confocal laser scanning microscopy (CLSM). A diffraction-limited light produced by the laser system goes through a pinhole and reflected by a dichromatic mirror, forming a scanning point in the specimen. The emitted fluorescence, scattered and reflected laser light on the specimen are focused by objective lens and goes through dichroic mirror, then pass the detector pinhole and strike the photomultiplier tube detectors. The light signal was transformed into electrical signal and processed by a computer program. With the moving of specimen, the sample is scanned by the laser light point and the image of a certain depth is gradually generated (Minsky, 1988; Paddock, 2000; Patel and McGhee, 2007). CLSM can produce optical images with depth selectivity in samples and observe the interior structures of the particles, and it can give thin (0.5–1.5 l m) section images through fluorescent specimens whose total thickness could be 50 l m or more.

Similar to UV Epi-fluorescence microscopy, CLSM is sensitive to petroleum components, while it gives sharper images than Epi-fluorescence microscopy because of the unique structure of the confocal microscopy. This technique provides noninvasive, direct, and serial optical sectioning of relatively thick samples with minimal sample preparation. However, this microscopy bears with the limitation that it scans the specimens point by point, which is not only time consuming, but also may alter the properties of the samples and cause the image to deviate from its true morphology due to extended exposure to the strong confocal laser. CLSM has been widely used to investigate the structure of OSAs (Omotoso et al., 2002; Zhang et al., 2010). Wang et al. (2011) employed CLSM to image freshly formed OSAs, and observed that more oil droplets were associated with the modified kaolin particles (higher hydrophobicity) than the original kaolin particles. Stoffyn-egli and Lee (2002) employed this technology to investigate the OSAs and found mineral fines could be present within the oil or on the surface of OSAs.

5.1.3. Environmental scanning electron microscopy

Environmental scanning electron microscopy (ESEM) emits an accelerated electron beam from a high vacuum gun area into the

sample chamber, usually the beam is focused and directed on the specimen surface by electromagnetic lenses. When the small focused electron spot (beam) scans on the small specimen area in a raster form, interaction between the beam electrons and sample produces a variety of signal emissions which are collected by the detectors, which are then translated into the images (Cameron and Donald, 1994; Donald, 2003; Thiel et al., 1997). ESEM has greater magnification (100,000) than optical microscopy, it can study samples with the temperature range from 200 to 1000 (McDonald, 2010), and provide detailed three-dimensional and topographical images of the OSAs surface, which makes it a great tool to investigate the morphological properties of OSAs (Stoffyn-Egli and Lee, 2002). Compared with the conventional scanning electron microscopy which only observe dry and electrically insulating materials (Stokes, 2003), ESEM employs differential pumping systems which can transfer electron beam from the gun area with high vacuums to the specimen chamber with high pressures, enabling it to image specimens in the natural state (e.g., wet samples) much faster and easier (Kuo et al., 2007). Stoffyn-Egli and Lee (2002) applied ESEM to imaging dry and wet OSAs, and observed the minerals were coated on the oil droplets, and the relationship between minerals and oil droplets is more visible for dry samples.

5.1.4. Atomic force microscopy

Atomic force microscopy (AFM) is a type of scanning probe microscopy of high resolution. It consists of a cantilever with an extremely sharp tip. The cantilever deflects when the tip interacts with the sample surface during the scanning. The cantilever deflection is typically measured by reflecting a laser spot from the cantilever to an array of photodiodes, and the sample topography was produced according to the cantilever deflection in the scanned sample surface (Cappella and Dietler, 1999). AFM is able to provide nano-scale three-dimensional surface profile, and no special sample treatment is needed, which avoids the artificial change to the final results. However, this method has limited scanning speed and small scanning area (about 150 150 m) as it scans point by point. This method is not typically used to analyze the OSAs formation, thus, it is not discussed in detail in this paper. Tiberg et al. (1999) applied this technique to observe adsorption of nonionic surfactants at the hydrophobic and hydrophilic surfaces such as graphite and silica, and observed either spherical surface micelles or relatively complete bilayers at the hydrophilic surface.

5.2. Particle size distribution

5.2.1. Laser scattering particle size analyzer

Laser scattering can give nominal particle diameters of dispersed particles (Lee et al., 2003). Typically, a dispersed particulate sample is placed in a cuvette or a flow cell (for continuous measurement), which is illuminated by a laser beam. As small particles scatter light at large angles and large particles scatter at small angles, the scattering pattern is recorded and calculated to reveal the particle size distribution. In conventional laser scattering, the particulate sample needs to be transported to the measuring device (Ma et al., 2008), which may disrupt fragile or unstable particle, and make it difficult to measure viscous particles, such as oil. Nonetheless, it is a fast and easy method with good repeatability, and it could analyze particles from the submicron to millimeter scale. The technique has been commonly used to determine the size distribution of particles including sediments and OSAs. Guyomarch et al. (2002) applied this method to investigate OSAs and found the average aggregate size was positively correlated to the oil to mineral ratio. To facilitate the measurement of unstable or viscous particles such as oil droplets and algae cells, a modified approach, known as laser in situ scattering and transmissometry (LISST), has been developed (Ma et al., 2008; Sterling et al., 2004, 2003). LISST can be submersed into solution to analyze the particle size distribution in situ, thus sample transportation is avoided. In addition, it can continuously monitor the particles size online. Li et al. (2008) situated the LISST-100X in a wave tank to measure the dynamic oil-droplet size distribution under the regular non-breaking waves, and found the sizes of physically dispersed oil-droplets were always greater than 200 m while chemically dispersed oil droplets were much smaller (approximate 100 m).

5.2.2. Flow cytometer and imaging system

Flow cytometer and imaging system (FlowCAM) is a combination of flow cytometry and digital imaging system which provides images for data points, and is capable of analyzing particles range from 2 to 1000 m (Sieracki et al., 1998). Flow cytometry is based on the principle of laser diffraction, which can describe the size, fluorescence, and concentration of particles. Meanwhile, the imaging system scans through particles by a high resolution digital camera at each data point (Ma et al., 2008). With the ability to describe fluorescence, FlowCAM is sensitive to OSAs, but it has the similar limitations to conventional laser scatting particle analyzers. Sterling et al. (2004) compared the data acquired with FlowCAM and LISST-100 by measuring spherical calibration beads and emulsified oil droplets, and found that the mean volume-based aggregate diameters measured by FlowCAM were consistently larger than those measured by LISST-100 due to aggregate orientation, but all these methods could provide consistent size distribution results.

5.2.3. Coulter counters

Coulter counters are based on the Coulter principle that when particles go through the orifice under an electrical field, it will produce a change in electrical impedance which is proportional to the particle size. Coulter counters could provide a higher resolution of particle size distribution than LISST and FlowCAM, and it depicts the number of small particles more accurately than LISST (Reynolds et al., 2010). Compared with FlowCAM, Coulter counters have limited ability to characterize particles as it cannot ascertain fluorescence of the particles (Ma et al., 2008). When used for sediment-oil aggregation analysis, Coulter counters generally measure the particle size in the range of 0.4–1200 m (Sterling et al., 2004). Sterling et al., 2004 acquired the 3-D fractal dimension of sediment-oil aggregates of 2.8–240 m by a Coulter counter.

For all the methods discussed above, only LISST can measure the particle size without transporting the sample, which preserves the original particle size and characteristics. In addition, the microscopic imaging techniques, e.g. transmitted light and UV Epi-fluorescence microscopy, can also be used to determine the particle size.

5.3. Other methods

Many other methods have been applied to investigate oil–sediment interactions, such as fluorescence spectroscopy and ellipsometry.

Fluorescence spectroscopy employs a beam of light, generally ultraviolet light, to excite the electrons in molecules of certain species, which makes them to emit light of different wavelengths, then the emission spectra are recorded and the fluorescent wavelengths are analyzed. The excited fluorescence of a component is proportional to its concentration of fluorescent molecules. Based on this principle, the fluorescence spectroscopy quantifies the target component in the samples by measuring the excited fluorescence (Knap et al., 1986). Due to the rapid measuring speed, low cost, and high sensitivity, it has been widely used to estimate crude oil concentrations in natural waters and sediments (Hargrave and

Phillips, 1975; Keizer and Gordon, 1973; Law et al., 1987). This method is matrix sensitive. It has been mostly carried out on solvent extracted samples with the results varying depending on solvent extraction procedures used (Dedomenico et al., 1994). Kepkay et al. (2002) described a UV fluorescence spectroscopy method to directly (without extraction) analyze OSAs in seawater, and confirmed this method could be used to analyze the dispersed/dissolved oil and OSAs in seawater and predict the extent of OSAs formation. Kuo et al. (2007) used time-gated laser-induced fluorescence (LIF) spectroscopy to investigate desorption of pyrene from aged sediment. The results showed this method is superior to conventional methods (e.g., GC–MS) as it can measure the changes in concentration of dissolved pyrene at minute-scale resolutions and at a detection limit of approximately 1 ng/L.

Ellipsometry determines the change of light polarization by measuring the reflection or transmission of a light beam on a surface, and then reveals the properties of samples (thickness and complex refractive index) (Langereis et al., 2009). Electromagnetic radiation is produced by a light source and polarized by a polarizer, and then the radiation goes through a compensator (optional) and reaches the sample. The reflected radiation goes out from the sample with the same angle of the incident light but in a reverse direction, passes the compensator (optional) and second polarizer, and then reaches the detector. The complex reflectance ratios (amplitude ratio W and phase angle D) are measured to indirectly reveal the optical constants and thickness of the sample (Langereis et al., 2009; Tompkins, 1993). Ellipsometry is a non-destructive technique which can accurately represent the thicknesses (ranging from tenths of nm to several l ms) and refractive indices of thin adsorbed layers (e.g., oil or surfactants adsorbed on sediment). It can describe the surface adsorption process by monitoring the adsorbed-layer properties with respect to time.

6. Future research needs

This review reveals some key knowledge gaps in the area of particle, oil, and/or dispersant interactions: (1) interactions between oil dispersants, oil and sediments and various SPMs, (2) how oil dispersants affect sorption/desorption and physical, chemical and biological availabilities of persistent oil components, (3) how dispersant-oil–sediment interactions alter the transport and weathering of oil, and (4) how deepwater conditions (e.g., P = 160 atm, T = 4 ffC, and salinity >3.5%) alter the dispersant performance, and (5) how application of dispersants in deepwater changes the fate and transport of oil. These critical knowledge gaps impose significant constraints and uncertainties in determining fate and transport of spilled oil and in evaluating an oil budget during a spill. These knowledge gaps lead to the puzzling questions, such as "Where does the oil go?", "How oil plumes are formed" and "How does the oil accumulate on the seafloor?".

7. Summary

Understanding the interactions between oil, dispersants and sediments has been considered an important component in developing oil spill countermeasures. Yet, only limited knowledge is available pertaining to sorption and desorption of oil droplets by sediment particles and how dispersants affect the sorption/desorption processes and the formation of OSAs. Understanding of the factors controlling these interactions is crucial for sounder evaluation of the environmental fate and impacts of oil spills and for developing effective remediation strategies. Significant insights have been gained on the effects of sediment particle size and concentration, oil properties, and salinity on the formation and characteristics of OSAs. In addition, the formation mechanisms of Pickering emulsions and controlling parameters on the type of emulsions are reviewed. Effects of SOM and mineralogy on the sorption/incorporation of dissolved oil components onto sediments have also been well investigated. Application of dispersants can reduce the size of oil droplets, change the oil surface physicochemical properties, increase oil dispersion in the water column, and thereby alter the rate and extent of the interactions between oil and sediment. However, detailed information is lacking on how dispersants affect oil–sediment interactions and how such interactions alter the fate, transport and transformations of persistent oil components in the marine environment, in particular, under deepwater conditions.

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Sediments, Section 3: Sediment management at the river basin scale

Research Article

Chronic Toxicity of Unresolved Complex Mixtures (UCM) of Hydrocarbons in Marine Sediments *ff

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Abstract

Background, Aim and Scope. Unresolved complex mixtures (UCM) of hydrocarbons, containing many thousands of compounds which cannot be resolved by conventional gas chromatography (GC), are common contaminants of sediments but little is known of their potential to affect sediment-dwelling organisms. Evidence exists for reduced health status in mussels, arising from aqueous exposure to aromatic UCM components acting through a narcotic mode of action. However, UCM contaminants in sediments may not be sufficiently bioavailable to elicit toxic effects. The aim of our study was therefore to measure the sublethal effects of chronic exposure to model UCM-dominated oils at environmentally realistic concentrations and compare this to effects produced by a UCM containing weathered crude oil. A further aim was to determine which, if any, fractions of the oils were responsible for any observed toxicity.

Materials and Methods. Whole oils were spiked into estuarine sediment to give nominal concentrations of 500 µg g^{-1} dry weight. Juveniles of the estuarine amphipod *Corophium volutator* were exposed to the contaminated sediment for 35 days and their survival, growth rate and reproductive success quantified. Using an effect-directed fractionation approach, the oils were fractionated into aliphatic and two aromatic fractions by open column chromatography and their toxicity assessed by further chronic exposures using juvenile *C. volutator*.

Results. The growth rates of amphipods were reduced following exposure to the oils although this was only statistically significant for the weathered oil; reproductive success was reduced by all oil exposures. Sediment spiked with UCM fractions also caused reduced growth and reproduction but no particular fraction was found to be responsible for the observed toxicity. Survivorship was not affected by any oil or fraction.

Discussion. The study showed that chronic exposure to sediments contaminated by UCM-dominated oils could have population level effects on amphipods. The observed effects could not be explained by hydrocarbons resolved by conventional GC and effects were similar for both UCM-dominated and weathered oils. All of the fractions appeared to contribute to the observed effects; this is in contrast to previous research which had shown that an aliphatic UCM did not cause adverse effects in mussels.

Conclusions. To our knowledge, this is the first study to demonstrate population-level effects arising from exposure to sediments contaminated by realistic environmental concentrations of UCM hydrocarbons. The results are consistent with many compounds, at very low individual concentrations, contributing towards the overall observed toxicity.

Recommendations. Risk assessments of contaminated sediments should take into account the contribution towards the potential for toxic effects from UCM hydrocarbons. Studies into sediment contamination should report both aliphatic and aromatic UCM concentrations to aid risk assessments.

Keywords: *Corophium volutator*; amphipods; UCM; hydrocarbons; toxicity; bioassay-directed fractionation; risk assessment; Tia Juana Pesada; Alaskan North Slope; weathered oil

Introduction

Despite the widespread occurrence of unresolved complex mixtures (UCM) of hydrocarbons in sediments and the fact that they often represent the majority of the petroleum hydrocarbons present (**Table 1**), very little is known about the potential for these sediments to affect sediment-dwelling organisms. A small number of studies have demonstrated that bioaccumulated UCM hydrocarbons can impact the health of mussels, *Mytilus edulis* L [1–5]. These studies suggest that components from the aqueous aromatic fraction, acting via a narcotic mode of action, are responsible for the observed reduction in health status. The UCM hydrocarbons within soils and sediments are resistant to biodegradation and bioremediation [6,7] and can therefore persist for decades [8]. Due to the relatively low bioavailability of sediment-bound UCM hydrocarbons, adverse effects on sediment-dwelling biota are likely to result from chronic exposure and therefore unlikely to be highlighted by standard

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Table 1: Examples of reported UCM and total hydrocarbon (H) concentrations in marine sediments

Location	UCM ($\mu g\ g^{-1}$)	H ($\mu g\ g^{-1}$)	Reference
Argentina – Rio de la Plata Estuary	0–1445[a] 0–206[b]	0–1532[a] 0–207[b]	[33]
Azerbaijan – S. Caspian Sea	36–310[a]	39–315[a]	[34]
Brazil – Todos os Santos Bay	32–156[a] 5–64[b]	33–185[a] 6–67[b]	[35]
Egypt – Alexandria, Eastern Harbour	54–1214[a]	61–1357[a]	[36]
France – Rhone prodelta	76–275[a]	86–302[a]	[37]
Hong Kong – coastline	6–1760[a]	6–1996[a]	[38]
Kuwait – Gulf	28–150[a] 6–80[b]	30–160[a] 7–80[b]	[39]
Spain – Barcelona	488[a]	906[a]	[37]
Spain – Ceuta harbour (N. African coast)	~400–4500	496–6972	[40]
Saudi Arabia Gulf	6–5300[a] 1–1400[b]	9–5500a 1–1400[b]	[39]
Turkey – Bosphorus, Black Sea	4–38[a] 3.6–30[b]	6–44a 6–44[b]	[41]
UK – Mersey estuary	104[a]	ns	[42]
Ukraine – Odessa, Black Sea	78–232[a] 28–63[b]	83–240[a] 30–65[b]	[41]
USA – West Falmouth, Massachusetts	~8000	~8000	[8]

[a] aliphatic UCM
[b] aromatic UCM
ns = not stated

acute sediment tests. In order to test the potential for UCM hydrocarbons to elicit toxic effects, it is preferable to expose sediment-dwelling organisms over a time span sufficient to register meaningful sublethal endpoints such as growth rate and reproductive success.

Corophium volutator Pallas is a sediment-dwelling amphipod that occurs widely in muddy sediments within estuaries of temperate Europe and the east coast of North America [9]. Used as a standard acute sediment toxicity test species in Europe [10], *C. volutator* has now been used to test the chronic toxicity of marine sediments [11,12]. The full life-cycle of *C. volutator* requires over two months to complete and therefore the tests reported herein used a partial life-cycle (≥ 35 days exposure) with sublethal endpoints of growth rate and reproductive success. Sediment contaminated with UCM hydrocarbons contain many thousands of hydrocarbons [13] and investigations into their potential toxic effects are therefore amenable to an effect-directed fractionation approach which aims to reduce the complexity by an iterative process of toxicity testing and fractionation and thus identification of the compounds responsible [14,15]. Sediments contaminated with UCM hydrocarbons often contain toxicants such as polycyclic aromatic hydrocarbons (PAHs), metals, and pesticides e.g. many of the studies cited in Table 1 also report a range of sediment contaminants, thus further complicating toxicity studies into UCM effects. Our study therefore tests the toxicity of two model UCM-dominated oils (**Fig. 1**): in reservoir biodegraded crude oil Tia Juana Pesada (TJP: 98% UCM) and a lube oil Silkolene-150 (SLK: 96% UCM), both of which have been used previously to model UCM contamination [16, 17]. In addition, a crude oil Alaskan North Slope (ANS) was evaporatively weathered and tested for comparative purposes; this also contained a substantial UCM of 87% (see Fig. 1). Further to baseline testing of the whole oils, aliphatic and two aromatic fractions derived from the oils were also subjected to toxicity testing.

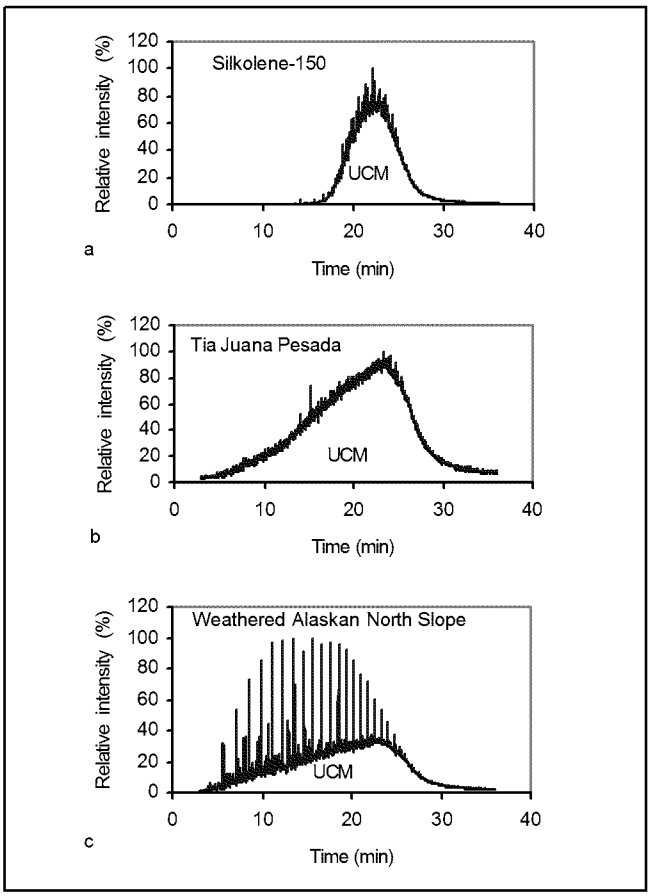

Fig. 1: Gas chromatograms of whole oils showing the unresolved complex mixture (UCM) of hydrocarbons

1 Materials and Methods

1.1 Oils and fractionation

Two UCM-dominated oils were chosen as model UCMs: TJP, a biodegraded crude oil comprised almost entirely of aliphatic and aromatic UCM; and SLK, a lube oil comprised almost entirely of aliphatic UCM when analysed by gas chromatography – flame ionization detection. For comparative purposes, a crude oil, ANS, was weathered by an evaporative method [18] to achieve a 36% loss of mass. The oils were fractionated by open column chromatography adapted from methods described by Sutton et al. [13] and Brack et al. [19]. Aliquots of ca. 1.5 g of oil were adsorbed onto ca. 10 g

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of deactivated alumina (4.5% MilliQ water w/w) by rotary evaporation of the oil and alumina with hexane (20 mL) at 40°C until near dry. The column was packed with alumina (4.5% MilliQ water w/w) over activated silica (ratio 1:1 w/w) and eluted with increasingly polar solvents: 100% hexane (F1 – aliphatic), 90:10 (v/v) hexane:dichloromethane (dcm; F2 – aromatic 1), 100% dcm (F3 – aromatic 2) and 100% methanol (F4 – polar). Elution volumes (× column volumes) were 1.5× for F1 and 2× for F2, F3 & F4. Analysis by GC-MS of column extracts spiked with a mixture of known hydrocarbons showed that the F1 fraction contained only aliphatic compounds and that the F2 fraction contained aromatic hydrocarbons from alkylbenzenes to fluoranthene. Tests were not performed on the negligible F3 fraction of SLK or any of the polar F4 fractions.

1.2 Spiking of sediments

Sieved (300 µm) sediments were spiked with ANS, TJP and SLK to give nominal concentrations of 500 µg g^{-1} (dry wt.) for whole oils based on a method described by Roddie and Thain [10]. For fractionation tests whole oil equivalents were used based on gravimetric analysis of fractions (**Table 2**). Oils were dissolved in dcm as they were not readily soluble in acetone or methanol and spiked (5 mL) onto 20 g aliquots of dry sediments. The spiked sediment was left overnight for the solvent to completely evaporate and then mixed with 320 mL aliquots of wet sediment and 100 mL of 25 psu seawater in wide neck 500 mL glass bottles (Schott). The combined spiked sediments were shaken vigorously by hand, then by orbital shaker at 200 rpm for 3.5 h. The bottles were again vigorously shaken by hand and the slurry from each divided equally between two 2 L Pyrex beakers. Solvent controls were created using 5 mL of dcm as above. Aliquots of sediment were taken for dry:wet weight measurement.

Table 2: Nominal concentrations of whole oils and fractions in sediment (µg g^{-1} dry wt.) used for the spiked-sediment chronic exposure tests with C. volutator. Fraction concentrations correspond to 500 µg g^{-1} (dry wt) of whole oil based on gravimetric analysis

	Concentrations of fractions in sediment (µg g^{-1} dry wt.), and gravimetric analysis of fractions (%)			
Oil	Whole	F1	F2	F3
Silkolene-150	500 (100%)	417 (83%)	77 (15%)	np (<1%)
Tia Juana Pesada	500 (100%)	133 (27%)	141 (28%)	100 (20%)
Alaskan North Slope	500 (100%)	234 (47%)	145 (29%)	60 (12%)

np = not performed

1.3 Sediment Toxicity Tests

1.3.1 Sediment and test organisms

Sediment for all tests (grain size: 33% sand, 67% silt/clay; organic carbon (OC) 3.8%) was collected locally from an intertidal area of the Avon estuary near Aveton Gifford, South Devon, United Kingdom (ordnance survey grid reference: SX 683467). *Corophium volutator* from this location were used for the fractionated oil exposure tests and were supplied by Guernsey Sea Farms (Vale, Guernsey, UK) for the baseline tests with whole oils. Acute 72 h aqueous exposure to a standard toxicant ($CdCl_2$) had shown no differences between the two populations and similar sensitivities to that of reported *C. volutator* populations [20]. Organisms were acclimated to laboratory conditions for a minimum of a week as described by Scarlett et al. [12].

1.3.2 Chronic exposure tests

Chronic sediment exposure tests were performed using *C. volutator* in accordance with that described by Scarlett et al. [12] except that the tests were initiated using 20 juvenile rather than 30 neonate amphipods which allowed the tests to be conducted within a shorter period. In brief: tests were initiated using juvenile *C. volutator* with mean length (excluding antennae) 3.8 mm (standard deviation (sd) = 0.30 mm) and 3.2 mm (sd = 0.50 mm, mean wet weight = 1.580 mg, mean dry weight = 0.129 mg) for whole oil and fractionated oil tests respectively. Nominal oil concentrations are given in Table 2. Exposures were carried out using 160 mL of spiked sieved sediment with 1 L overlying water. Test conditions were 15 ± 1°C, 25 ± 2 psu, pH 8.0 ± 0.3, 12:12 h dark:light period, 80% water exchange once per week 24 h following feeding, with continuous gentle aeration via glass Pasteur pipettes. Five replicate exposures were performed on each of the test sediments including negative and solvent controls. The tests were terminated upon reproduction in all control vessels (35 days and 60 days exposure for whole oil and fractionated oil tests respectively) whereupon the adults and neonates were counted and the growth of adult amphipods measured. For whole oil tests, growth rate was quantified in terms of mean weights (µg day^{-1}) and size classes (subadults <5 mm, mature adults ≥5 mm). For fractionation tests, in addition to growth rate by weight, lengths of all adult amphipods were recorded to provide growth rate in terms of length (mm day^{-1}).

1.4 Statistical analyses

Statistical analyses of results were performed using Statgraphics Plus 5.1. Proportional data were arcsine transformed prior to analysis. Following checks for variance using Cochran's C test, data were analysed by one way analysis of variance (ANOVA). Where there was a significant difference ($P \leq 0.05$) of means, the data were further analysed by the Student-Newman-Keuls test to determine significant differences ($P \leq 0.05$) between treatments.

2 Results

2.1 Whole oil tests

The whole oil sediment exposure test was terminated after 35 days when it was apparent that reproduction had occurred within all control vessels. Throughout the exposure period, normal behaviour was observed within all vessels and water quality measurements were well within acceptable limits. No statistical differences were observed between negative and solvent control treatments for any of the mea-

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Table 3: Effect of 35 days spiked sediment exposure at a nominal concentration of 500 µg g^{-1} dry weight to three oils Silkolene-150, Tia Juana Pesada (TJP) and weathered Alaskan North Slope (ANS) on the mean (standard error) survivorship, growth rate and reproductive success of the amphipod C. volutator. Mean effects compared with combined negative and solvent controls. Values significantly different from solvent control (P ≤0.05) = *

Treatment	Survivorship (%)		Growth rate (µg day^{-1} dry weight)		Offspring/ Survivor		Offspring/ female	
Seawater control	95 (3.2)		23.0 (1.4)		2.26 (0.23)		3.76 (0.35)	
Solvent control	92 (3.7)		21.5 (0.7)		2.52 (0.61)		4.26 (0.86)	
Silkolene-150	90 (3.2)		20.1 (1.1)		0.98 (0.29)	*	1.82 (0.56)	*
Tia Juana Pesada	96 (2.3)		20.3 (1.2)		1.16 (0.35)	*	2.19 (0.59)	*
Alaskan North Slope	92 (4.4)		17.7 (1.3)	*	0.67 (0.21)	*	1.56 (0.48)	*

sured endpoints. Survivorship was very high (≥90%) within all treatments. Juvenile *C. volutator* exposed to all oil exposures had lower growth rates than the negative control organisms but only the weathered ANS was significantly less ($P \leq 0.05$) than the solvent control (**Table 3**). Reproduction was significantly reduced ($P \leq 0.05$) in all oil exposures although this was more pronounced in the ANS treatment (see Table 3).

2.2 Fractionated oils tests

The test was terminated after 60 days upon reproduction within all control vessels. Most of the control vessels had shown evidence of reproduction earlier but in one replicate this was not clear and hence the test was continued until reproduction was confirmed. Normal behaviour was observed within all vessels and water quality measurements were well within acceptable limits. No statistical differences were observed between negative and solvent control treatments for any of the measured endpoints Survivorship was high (mean >80%) within all treatments although one vessel from the ANS F2 treatment had only 65% survival from the starting population of 20 individuals. Growth rates were reduced for organisms exposed to most oil fractions but this was only significant ($P \leq 0.05$) for the TJP aromatic fraction exposed *Corophium* (**Fig. 2**). A summary of the growth rates and reproduction data is provided in the form of notched box and whisker plots (see Fig. 2). Comparisons of each of the oils (fractions grouped) with the controls showed that all three of the oil treatments reduced the growth rate and reproductive success of the amphipods, although the latter was not significant at the 5% level for ANS (**Table 4**).

3 Discussion and Conclusions

Toxicity studies of UCM hydrocarbons have previously been concerned with the short-term effects of an aqueous monoaromatic fraction [3], branched alkylbenzenes [1], alkyltetralins [2] and an extract from UCM-contaminated mussels [5], on the clearance rates of *M. edulis*. Bivalve molluscs are known to bioaccumulate lipophilic compounds as they have little ability to metabolise them [21]. Sediment-associated hydrocarbons on the other hand may be less bioavailable than dissolved compounds. *C. volutator* are closely associated with the sediments in which they live and are reported to deposit feed as well as to filter feed [22]. Hence they interact with contaminants adsorbed to particulates via feeding rather than via the dissolved phase.

The chronic tests revealed that UCM contaminated sediment at concentrations found in the marine environment can indeed elicit sublethal effects on the estuarine amphipod *C. volutator* without affecting survivorship (see Table 3). McGee et al. [23], using a chronic sediment toxicity test with the estuarine amphipod *Leptocheirus plumulosus*, reported that some moderately contaminated sediments from Chesapeake Bay, USA, significantly reduced growth rates and reproductive success of organisms but this was only true of those sediments that had also significantly affected survival. Similarly, Allen et al. [24] reported that a chronic sediment exposure with *C. volutator* did not show any greater sensitivity than the standard acute test. However, the amphipods were not fed during the 28 day exposure and this may have affected their growth in that study. Sediment spiked with oil fractions herein confirmed the baseline toxicity tests with whole oils, with deleterious effects on both growth rates

Table 4: Effect of 60 days spiked sediment exposure of three oils (combined aliphatic F1 and aromatic F2 & F3 fractions), Silkolene-150, Tia Juana Pesada (TJP) and weathered Alaskan North Slope (ANS) on the growth rate and reproductive success of the amphipod C. volutator. Mean effects compared with combined negative and solvent controls. Analysis of variance P values ≤0.05 = *, ≤0.01 = **

	Silkolene-150		Tia Juana Pesada		Alaskan North Slope	
Endpoint	Effect	*P* value	Effect	*P* value	Effect	*P* value
Growth rate (µg day^{-1} dry wt.)	reduction	0.108	Reduction **	0.002	Reduction *	0.024
Growth rate (mm day^{-1})	Reduction **	0.009	Reduction **	0.001	Reduction *	0.024
Offspring $survivor^{-1}$	Reduction **	0.004	Reduction *	0.025	reduction	0.131
Offspring $female^{-1}$	Reduction *	0.010	Reduction *	0.036	reduction	0.144

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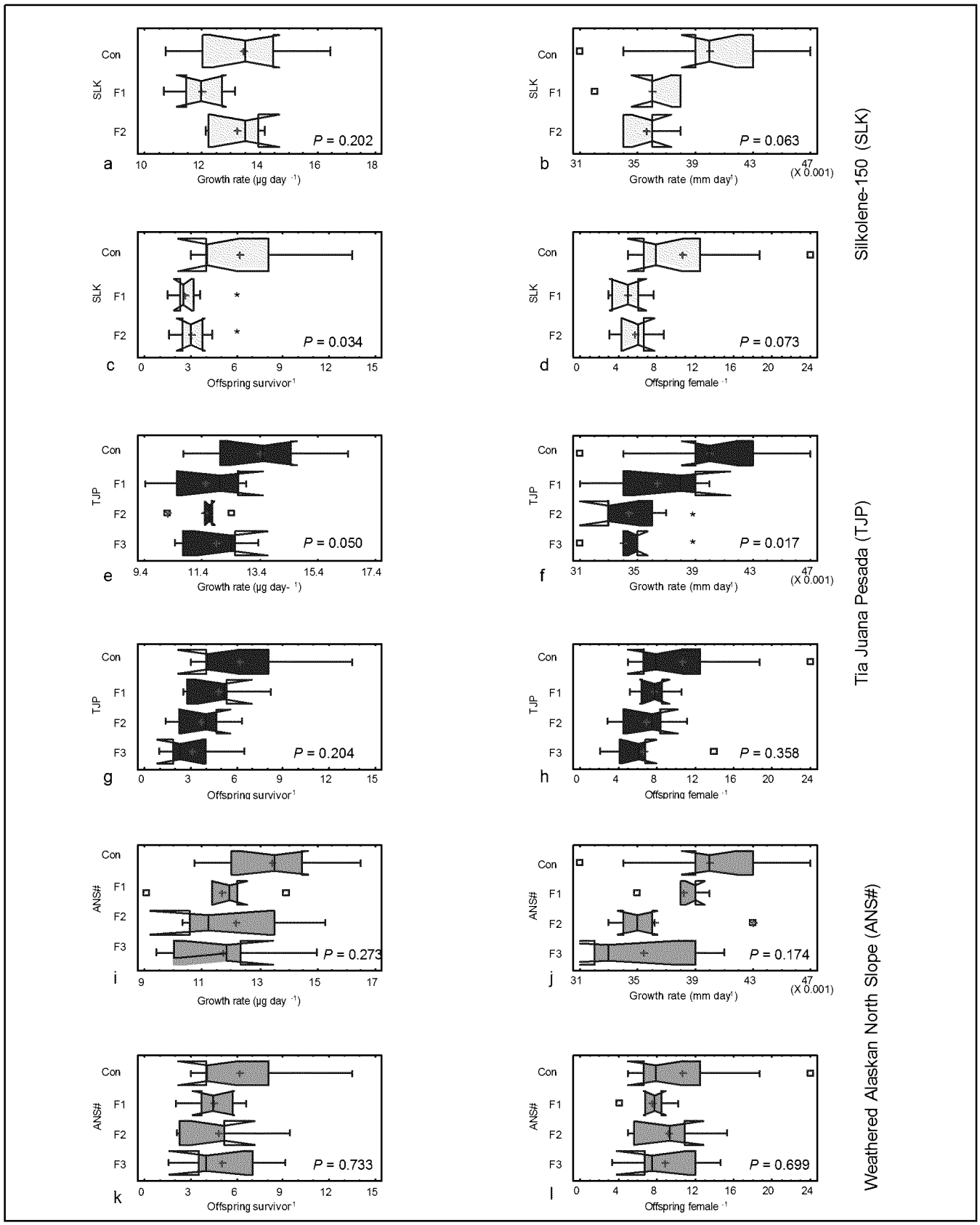

Fig. 2: Box and whisker plots comparing the effects of oil fractions upon growth and reproduction of *C. volutator*. Con = controls, F1 = aliphatic fraction, F2 = aromatic 1 fraction (elution range alkylbenzenes to fluoranthene) and F3 = aromatic 2 fraction. Effects of fractions significantly different ($P \leq 0.05$) to control values identified by *

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and reproductive success (see Table 4). However, unlike the baseline tests where only ANS produced significant reduction in growth rate, all the oils significantly affected the growth of amphipods. Furthermore, significant reductions in reproductive success were not found for all oils as was the case for the baseline study. This was possibly due to the greater size variation within the starting population of the fractionation study.

In contrast to previous studies that suggested only the aromatic fraction of the UCM have deleterious effects [1-5], all of the oil fractions appeared to contribute to the overall toxicity of the oils (see Fig. 2) resulting in very few significant differences (P >0.05) when analysed by ANOVA. Significant reductions were found for both the F1 and F2 fractions of SLK which produced similar effects upon the reproductive success of the amphipods (see Fig 2. c and d). This similarity in effects was unexpected as the aliphatic F1 fraction of SLK had previously been found not to show toxicity in mussels *M. edulis* [25] and aliphatic hydrocarbons in the environment have been assumed to be of low toxicity and low bioavailability. For example, an oil toxicity model by French-McCay [26] was solely based on aromatic hydrocarbon toxicity. Barron et al. [27], in a study of short-term growth and survival of mysids *Mysidopsis bahia* exposed to three oil-derived WAFs, questioned that aromatic hydrocarbons were the primary determinant of toxicity as their results demonstrated that oils with low aromatic content had greater toxicity. One theory of the narcotic mechanism of toxicity suggests that all soluble components of oil bioaccumulated by organisms may contribute to toxicity [28]. Whilst the low solubility of the aliphatic compounds should render them unavailable to the amphipods by this route and therefore not contribute to the overall toxic effect, deposit feeding may provide an alternative route of uptake [22]. It should also be noted that the nominal sediment concentration of the SLK aliphatic fraction was about five times that of the aromatic fraction but produced similar toxic effects. The monitoring of sediment hydrocarbons should therefore consider the aromatic UCM concentration.

The monitoring of hydrocarbon contamination of sediments has routinely focused on resolved PAHs which are known to be highly toxic with mutagenic and carcinogenic effects [29]. The biodegraded crude oil TJP, used in this study, contains very few such resolved compounds (ca. 2%, see Fig. 1) which is similar to the hydrocarbons reported in many of the studies listed in Table 1, but had similar toxic effects (see Tables 3 and 4, Fig. 2) as the weathered ANS oil containing a larger resolved component (ca. 13%). Shelton et al. [30] reported that water-accommodated fractions of biodegraded weathered ANS were toxic to larvae of marine crustaceans whereas the unbiodegraded oil was not toxic, suggesting that degradation products of the hydrocarbons were the main contributors to the observed toxicity rather than the PAHs themselves. The concentration of compounds resolved by conventional GC in the TJP whole oil test sediment was ca. 10 µg g^{-1} (dry wt.), equivalent to ca. 260 µg g^{-1} OC, of which the PAHs represent a small fraction. Swartz [31] reported a consensus threshold effect concentration for total PAH of 290 µg g^{-1} OC although this did not take into account the alkyl-substituted homologues which have been reported to more toxic [32]. Some UCMs accumulated in tissues of mussels with poor health status have now been shown to contain branched alkyl-substituted homologues of mono, di and polycyclic aromatic hydrocarbons [1] and it is therefore likely that all of these compounds contribute to the overall toxic effects.

The results of the sediment exposure tests show that reductions in growth rates and reproductive success of *C. volutator* cannot be attributable to resolved compounds alone or to components from any single fraction. To our knowledge, this is the first study to demonstrate population-level effects arising from exposure to UCM hydrocarbons. Risk assessments of contaminated sediments would benefit from the widespread reporting of both the aliphatic and aromatic UCM concentrations, and from further investigations into the potential sublethal toxic effects arising from exposure to these sediments.

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Oil Spill Dispersant (COREXIT ®EC9500A and EC9527A) Information for Health Professionals

Overview

Dispersants are specially designed oil spill products that are composed of detergent-like surfactants in low toxicity solvents. Dispersants do not remove oil from the water, but instead break the oil slick into small droplets. These droplets disperse into the water and are further broken down by nature. Dispersants also prevent the oil droplets from coming back together and forming another surface slick. Dispersed oil droplets are less likely to stick to birds and other animals, shoreline rocks, and vegetation. Purpose

Purpose

Dispersants can be applied to oil slicks either by aircraft or by ships with spraying equipment. They are also used by injection below water to break up oil before it reaches the surface. The use of dispersants is restricted under the National Oil and Hazardous Substances Pollution Contingency Plan (NCP). Federal and state agencies have agreements establishing areas where rapid decisions on dispersants may be made by the federal on-scene coordinators. Areas outside those designated require additional approval of other agencies identified in the NCP.

Type of Dispersants used on Mississippi Canyon Oil Spill

COREXIT ® 9500 and 9527 are the two types of dispersants currently being used on the Mississippi Canyon Oil Spill.

- Ingredients are not considered to cause chemical sensitization; the dispersants contain proven, biodegradable and low toxicity surfactants.
- The dispersants use an oleophilic solvent delivery system which has shown to be effective in penetrating surfactants.
- Keep the dispersants away from any heat and ignition sources, because the mixture is slightly flammable mixture and combustible.
- Because the specific gravity and density are lower than water, the material should remain at the surface.
- The pH is close to neutral (6.2) with a relatively low vapor pressure at 15.5 mmHg @ 100°F/37.8°C.

Personal Protection Equipment Requirements and COREXIT ® 9500 and 9527

For hand protection, use nitrile or PVC gloves. Wear standard protective clothing (coveralls and boots) and chemical splash goggles. General ventilation is recommended.

Potential Health Threats from COREXIT ® 9500 and 9527 Exposure

Once the dispersant is applied to the oil slick it begins to break down in the environment. In aquatic environments it begins to break down within 16 days. Because of the strict guidelines that must be followed to utilize dispersants it is unlikely that the general public will be exposed to straight product.

The most likely exposure will occur to staff handling and transporting the material, and health effects may include:

- Defatting and drying of the skin and possibly dermatitis, as a result of prolonged exposure.
- Chemical pneumonitis, if aspirated into the lungs. However, ingestion is considered an unlikely route exposure.
- Repeated or excessive exposure to 2-butoxyethanol may cause central nervous system depression, nausea, vomiting, anesthetic or narcotic effects, injury to red blood cells (hemolysis), kidney or the liver, and a metallic taste.
- Respiratory irritation as a result of repeated and prolonged inhalation exposure.
- Eye irritation as a result of repeated and prolonged inhalation exposure.

Treating Exposure to COREXIT ® 9500 and 9527

Skin Contamination. Frequent or prolonged contact with product may cause defatting and drying of the skin, leading to discomfort and dermatitis. Skin contact may aggravate an existing dermatological condition. Immediately wash with soap and water.

Ocular Exposure. Prolonged ocular exposure can result in irritation. No permanent damage is likely to occur. The immediate treatment is to flush the eye with water for 15 minutes.

Ingestion Exposure. Ingestion can cause chemical pneumonitis if the ingested dispersants are aspirated into the lungs (due to vomiting). This is due to the petroleum distillates and aromatic solvents. To treat individuals exposed via ingestion, do not induce vomiting as this may lead to aspiration of the mixture into the lung. If conscious, wash out mouth and give water to drink.

Inhalation Exposure. Repeated or prolonged exposure may irritate the respiratory tract. Remove patient to fresh air and provide oxygen support if needed.

Resources

COREXIT® EC9500A Technical Product Bulletin #D-4 USEPA, Oil Program Center:

http://www.epa.gov/swercepp/web/content/ncp/products/corex950.htm

COREXIT® EC9527A Technical Product Bulletin #D-1 USEPA, Oil Program Center:

http://www.epa.gov/swercepp/web/content/ncp/products/corex952.htm

COREXIT® EC9527A Material Safety Data Sheet (MSDS):

http://www.deepwaterhorizonresponse.com/posted/2931/Master_EC9527A_MSDS.539295.pdf

COREXIT® EC9500A Material Safety Data Sheet (MSDS):

http://www.deepwaterhorizonresponse.com/posted/2931/COREXIT_9500_UsCuEg.539287.pdf

The Mississippi Emergency Management Agency Oil Spill Call Center: 866-920-6362 Poison centers in the gulf region: www.aapcc.org/DNN/ and (800) 222-1222

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From: Mandsager, Kathy
Sent: Thur 3/2/2017 2:05:51 PM
Subject: State-of-Science: Degradation Panel - REPLY REQUESTED
2017.02.28_Degradation and Fate.docx

Dear Degradation and Fate panel members:

Attached you will find the latest version of the document. It seems we should schedule one more call to have a final wrap up discussion on any outstanding issues. Please use this doodle poll (by Monday 3/6) to select a time that ALL of you can participate.

Thank you!

Here is the link for the time zone enabled doodle poll>
http://doodle.com/poll/gh3w3et6mcyqafu4

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NIOSH Interim Information

Reducing Occupational Exposures while Working with Dispersants during the Deepwater Horizon Response

Dispersants are being used in the Deepwater Horizon (Gulf of Mexico) response to remove oil from the surface of water where it can be especially harmful to the environment. The National Institute for Occupational Safety and Health (NIOSH) has prepared this document to guide workers involved in these efforts on ways to protect themselves from potential exposures. This fact sheet will be updated as new information on the types of dispersants being used in the response is received.

Dispersants are usually applied directly to the spilled oil by spraying from an airplane, helicopter, or vessel. Although dispersants are manufactured by many companies and their ingredients may differ, most contain a detergent and a solvent. The solvent allows the detergent to be applied. The detergent helps to break up the oil on the water surface into very small drops. These tiny oil drops are then able to easily mix with the water and be diluted.

Based on the information provided to NIOSH, two dispersants have been used for the Deepwater Horizon response. Both COREXIT® EC9527A and COREXIT 9500 were utilized early in the response, but only the latter product is currently in use. Both products are made by the Nalco Company in Naperville, Illinois. Both products contain 10-30% sulfonic acid salt (detergent) and 1-5% propylene glycol, which are regarded as non-hazardous substances. In addition, COREXIT 9500 contains between 10-30% of petroleum distillates (solvent) and COREXIT EC9527A contains between 30-60% of 2-butoxyethanol (solvent).

Petroleum distillates are a colorless liquid with a gasoline- or kerosene-like odor. They are composed of a mixture of paraffins (C_5 to C_{13}) that may contain a small amount of aromatic hydrocarbons. 2-butoxyethanol, also called butyl cellosolve, is a widely used cleaning agent. The potential human health effects of 2-butoxyethanol have been studied. Dispersants containing 2-butoxyethanol may irritate the skin. 2-butoxyethanol vapors or mists can cause respiratory irritation such as coughing. Several occupational

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exposure limits (OELs) have been established for 2-butoxyethanol. The legally enforceable OEL in the United States is the Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit (PEL). For airborne 2-butoxyethanol the OSHA PEL is 50 parts per million (ppm) for up to a full work shift. The NIOSH Recommended Exposure Limit (REL) for 2-butoxyethanol is 5 ppm, also for up to a full work shift. The NIOSH REL is intended to minimize potential long-term health effects to workers, primarily hemolysis of red blood cells (RBCs). Hemolysis of RBCs has been found in animals exposed to 2-butoxyethanol, but recent data suggests that human RBCs are less susceptible to these effects. Both the OSHA PEL and NIOSH REL contain guidance to minimize skin contact with 2-butoxyethanol.

NIOSH recommends that worker exposures to petroleum distillates, 2-butoxyethanol and similar cleaning agents in dispersants be reduced to prevent harmful respiratory and dermal health effects. Workers can be protected by taking the following steps:

- Mix and load dispersants in well ventilated areas.
- Use automated spraying systems to apply dispersants when available.
- Remain upwind of the mists that are generated if spray systems are manned.
- Wear nitrile gloves during mixing, loading, or spraying of dispersants to prevent skin irritation.
- Wear protective eyewear when mixing, loading, or spraying dispersants.
- Wash hands and any other body parts exposed to dispersants thoroughly with soap and water.
- If personal air monitoring indicates the above steps are not effective at reducing exposures below applicable OELs, then respiratory protection would be needed. Respirators should be used as part of a comprehensive respiratory protection program that includes proper selection, training, and maintenance. The NIOSH respirator topic page at http://www.cdc.gov/niosh/topics/respirators/ provides information for safety and health officers who are designated to establish and conduct such programs.

For more information on oil dispersants:

- NOAA Dispersants: A Guided Tour http://response.restoration.noaa.gov/topic_subtopic_entry.php?RECORD_KEY%28entry_subtopic_topic%29=entry_id,subtopic_id,topic_id&entry_id(entry_subtopic_topic)=155&subtopic_id(entry_subtopic_topic)=8&topic_id(entry_subtopic_topic)=1

For more information on 2-butoxyethanol:

- NIOSH Pocket Guide to Chemical Hazards (http://www.cdc.gov/niosh/npg/)
- NIOSH Topic Page on 2-butoxyethanol (http://www.cdc.gov/niosh/topics/butoxyethanol/)
- New Jersey Hazardous Substance Fact Sheet on 2-butoxyethanol (http://www.cdc.gov/niosh/topics/butoxyethanol/)

For More Information

For more NIOSH information and recommendations for Deepwater Horizon response workers, see http://www.cdc.gov/niosh/topics/oilspillresponse/

To receive NIOSH documents or more information about occupational safety and health topics, contact NIOSH at

1–800–CDC–INFO (1–800–232–4636)
TTY: 1–888–232–6348
E-mail: cdcinfo@cdc.gov

or visit the NIOSH Web site at www.cdc.gov/niosh.

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Dispersants and Seafood Safety
Assessment of the potential impact of COREXIT® oil dispersants on seafood safety

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The April 20, 2010 explosion and subsequent sinking of the Deepwater Horizon oil production platform (DWH) resulted in the largest oil spill in U.S. history. On April 29th, a Spill of National Significance was declared as roughly 53 thousand barrels of oil per day flowed into the Gulf of Mexico (GOM). The U.S. Coast Guard estimated 4.9 million barrels of crude oil escaped before the damaged DWH wellhead was sealed on July 15, 2010 (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling 2010). Oil spill clean-up methods included skimming operations, burning of surface oil, siphoning oil into tankers directly from the wellhead, and the application of chemical dispersants. The first 3 methods mentioned above physically removed spilled oil from GOM waters. The last method, chemical dispersion, distributed insoluble fractions of the oil into the water-column. This was done for 3 reasons: 1) to reduce the exposure of response personnel at-sea to volatile organic compounds emanating from the surface slick; 2) to prevent concentrated surface oil from reaching, and damaging, fragile coastal wetlands, beaches and shoreline ecosystems; and 3) to accelerate the break-down of spilled oil by natural microorganisms in the environment. During oil spill response, the Regional Contingency Plan (RCP) applicable to the GOM (EPA Regions 4 and 6 within the National Response Framework) pre-authorized the use of Nalco Co. (Naperville, IL) oil dispersants Corexit® 9527 and Corexit® 9500 among other pre-approved product formulations. From April 22 to July 19, 2010, an estimated 1.1 million gallons of Corexit® dispersant were applied over approximately 300 square miles of oiled surface waters in the GOM and 771,000 gallons were injected directly into the oil free-flowing from the wellhead 5,100 feet beneath the surface (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling 2010). Corexit® 9527 comprised approximately 215,000 gallons (~11%) of the total dispersant volume applied to the surface oil slick and was discontinued on May 22. The unprecedented volume of chemical dispersants used to combat the DWH oil spill elicited public concerns for the health of responders, coastal communities, marine life, and the safety of seafood from impacted areas of the GOM. This document will address the latter of these concerns.

As part of the federal response to the DWH oil spill, the FDA and NOAA assessed Corexit® dispersant ingredients for toxicity and potential to bioconcentrate in seafood species. With the exception of one proprietary component, the chemical compositions of Corexit® 9527 and Corexit® 9500 were identified in material safety data submitted to EPA

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as required by subpart J of the National Contingency Plan (NCP), Sec. 300.915 for RCP pre-authorization (EPA 2010a; 40 CFR 300.915). As it became apparent that large volumes of dispersants were being used to combat the subsurface and surface oil spill, EPA requested and received from the manufacturer disclosure of the proprietary component (i.e. dioctyl sodium sulfosuccinate, DOSS). Corexit® constituents are listed in Table 1 and described below.

Table 1. From http://www.epa.gov/bpspill/dispersants-qanda.html#list

CASRN	Name	Corexit® 9527	Corexit® 9500
111-76-2	2-Butoxyethanol (ethylene glycol mono-n-butyl ether)	X	n/a
57-55-6	Propylene glycol	X	X
29911-28-2	Dipropylene glycol monobutyl ether	X	X
577-11-7	Dioctyl sodium sulfosuccinate	X	X
64742-47-8	Petroleum distillates, hydrotreated light fraction	n/a	X
1338-43-8	Sorbitan, mono-(9Z)-9-octadecenoate	X	X
9005-65-6	Polyoxy-1,2-ethanediyl derivatives of sorbitan, mono-(9Z)-9-octadecenoate	X	X
9005-70-3	Polyoxy-1,2-ethanediyl derivatives of sorbitan, tri-(9Z)-9-octadecenoate	X	X

The solvent 2-butoxyethanol (CASRN 111-76-2) is a high production glycol ether and constituent of Corexit® 9527. It has other common uses as a solvent and degreaser in industrial, residential, and personal care products including protective surface coatings (spray lacquers and paints), agricultural chemicals, household cleaners, and liquid soaps and cosmetics at concentrations of up to 10%. This compound is approved by FDA as an indirect and direct food additive for use as an antimicrobial agent, defoamer, stabilizer and component of adhesives (21 CFR 175.105(FAP 1B0233); 178.1010(FAP 4A1375); 176.210(FAP 3B0899); 177.1650; 173.315(FAP 5A3079). FDA approval means that the compound is safe for its approved intended uses and the human exposures associated with those uses. 2-butoxyethanol has been well studied. Information is available on metabolism, mechanism of toxicity, pharmacokinetics and impact of exposure on human health. Primary effects are on the hematologic system with secondary renal effects. Acute oral LD_{50} values in a variety of rodent animal models range from 200 to 12,750 mg/kg bw. The Agency for Toxic Substances and Disease Registry (ATSDR, 1998) derived minimal risk levels (MRL[1]) of 0.4 mg/kg bw d^{-1} for human acute-duration (14 d) and 0.07 mg/kg bw d^{-1} for human intermediate-duration (15-364 d) oral exposures. Acute oral exposures of humans to large amounts of 2-butoxyethanol have been shown to cause coma and respiratory depression in addition to hematotoxic effects, although this route of exposure is the least likely for the general population. From quantitative structure-activity relationships, the bioconcentration

[1] An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify target organs(s) of effect or the most sensitive health effects(s) for a specific duration within a given route of exposure. MRLs are based on noncancer health effects only and do not reflect a consideration of carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes.

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factor (BCF) for 2-butoxyethanol has been predicted to be 3, and experimentally estimated in fathead minnows (*Pimephales promelas*) to be 2 (ASTER 1995). The physical-chemical characteristics of 2-butoxyethanol include vapor pressure of 0.88 mm Hg at 25°C (ATSDR 1998), Henry's law constant estimates ranging from 2.08 x 10^{-8} to 3.61 x 10^{-6} atm- m^3/mol at 25°C (HSDB 1995, ASTER 1995), and log octanol-water partition coefficient (log K_{ow}) of 0.83 suggesting a low probability of lipid uptake or bioconcentration in fish (ATSDR, 1998). The half-life of 2-butoxyethanol in surface waters is estimated to be in the range of 7-28 days, and the chemical is reported to be readily biodegraded in aerobic soil and aquatic environments (Howard et al., 1991, HSDB 2010).

Propylene glycol (CASRN 57-55-6) and dipropylene glycol monobutyl ether (CASRN 29911-28-2) are constituents of both Corexit® 9527 and 9500. Dipropylene glycol monobutyl ether is used as a solvent for industrial and residential cleaners/degreasers, paints and plasticizers. Propylene glycol is used in commercial formulations of foods, drugs, cosmetics, and personal care products (e.g. toothpaste, shampoo, mouthwash). Propylene glycol is approved by FDA as a Generally Recognized As Safe (GRAS) ingredient, direct food additive, and indirect food additive for many uses, including as an anticaking agent, antimicrobial agent, antioxidant, color or color adjunct, defoamer, dough strengthener, emulsifier, flavoring agent, formulation aid, humectant, processing aid, solvent, diluent or vehicle stabilizer, thickener or gelling agent, surfactant, and texturizer (21 CFR 175.105(FAP 1B0233, FAP 2B0650); 178.3300; 175.300; 175.320; 177.2420; 177.1680(FAP 5B1808); 184.1666). Propylene glycol ethers as a class are rapidly absorbed and exhibit low acute toxicity by oral exposure and **propylene glycol is infrequently associated with adverse effects.** Acute oral LD_{50} values in a variety of rodent animal models range from 3,000 to 5,000 mg/kg bw for dipropylene glycol monobutyl ether and from18,000 to 46,000 mg/kg bw for propylene glycol (OECD 2003, HSDB 2010). Some acute oral data exist for humans, but the information is limited and includes systemic and neurological effects at high levels of exposure. No MRL for oral exposure to propylene glycol in humans has been derived because data are insufficient (ATSDR, 1997). The FAO/WHO Joint Expert Committee on Food Additives (JECFA) determined 25 mg/kg bw d^{-1} as the acceptable daily intake (ADI) for propylene glycol (FAO/WHO, 1997). Predicted BCFs for dipropylene glycol monobutyl ether and propylene glycol are <10 indicating a low potential for bioconcentration in aquatic food chains (OECD 2003, HSDB 2010). Physical-chemical characteristics include vapor pressures below 1 mm Hg, Henry's law constants ranging from 1.2 – 1.7 x 10^{-8} atm m^3/mol at 25°C, and log K_{ow} from -0.92 to 1.52 suggesting low probability of uptake in lipids or bioconcentration in fish. Propylene glycol and glycol ethers would be expected to be of low to moderate volatility from water and readily biodegradable in aquatic and terrestrial environments with half-lives of 7-28 days (Howard et al., 1991; ATSDR 1997; Staples and Davis 2002; OECD 2003; HSDB 2010).

Petroleum distillates, hydrotreated light fraction (CASRN 64742-47-8) are paraffinic dearomatized, and volatile solvents derived from crude petroleum, and constituents of Corexit® 9500. Production quality of light petroleum distillates is highly variable and common uses include as a solvent for paints, varnishes, polishes, and lubricants, and general purpose cleaners and degreasers. Hydrotreated light petroleum distillates Norpar 13 (food grade aliphatic n-alkanes ranging from nonane to hexadecane: CASRN 94094-93-

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6) were reportedly substituted for 2-butoxyethanol in the formulation of Corexit 9500 (NRC 2005); however, CASRN do not align with that reported by EPA (Table 1). FDA has approved similar food grade odorless light petroleum hydrocarbons as indirect and direct food additives (21 CFR 172.884; 175.105; 176.200; 176.210; 178.3650). $C_8 - C_{20}$ range aliphatic hydrocarbons comprising petroleum distillates, hydrotreated light fraction exhibit low acute oral toxicity. Acute oral LD_{50} values in rodent animal models are greater than 5,000 mg/kg (HSDB 2010). Subchronic toxicity studies suggest the aliphatic hydrocarbon fluids to be relatively non-toxic (EPA 2006). Predicted BCFs range from 60 to 80 and physical-chemical characteristics include vapor pressures below 2.0 mm Hg; Henry's law constant ranging from 0.2 – 3.4 atm-cu m^3/mol, and log K_{ow} from 2.2 to 5.4. Some of the longer chain compounds in the mixture may have a high potential for concentration in aquatic organisms provided they are not volatilized or metabolized. Petroleum distillates are volatile from water and readily biodegradable in days to weeks (EPA 2006, HSDB 2010).

Dioctyl sodium sulfosuccinate (DOSS; CASRN 577-11-7) is an anionic surfactant (butanedioic acid, 2-sulfo-1,4-bis(2-ethylhexyl) ester, sodium salt) listed as a proprietary organic sulfonic acid salt constituent of both Corexit® 9527 and 9500 formulations. Other common uses include wetting and flavoring agent in food, industrial, and cosmetic applications, and a medicinal stool softener in over-the-counter use. FDA has approved this compound as a GRAS ingredient, and as indirect and direct food additives under prescribed conditions of use (21 CFR 73.1; 131.130; 133.124; 133.133; 133.134; 133.162; 133.178; 133.179; 163.117; 168.115; 172.520; 172.808; 172.810; 175.105). DOSS was determined to be essentially non-toxic by oral administration to rodent animal models with acute toxicity ranging from 2.6 to 5.7 g/kg bw. Diarrhea and signs of intestinal irritation were noted. Three-generation reproduction studies in rodent models did not reveal adverse effects; however, parental and weanling weight loss was noted at dose levels above 5 g/kg bw (FAO/WHO 1991a). JECFA determined 0.1 mg/kg bw d^{-1} as the ADI for dioctyl sodium sulfosuccinate (FAO/WHO 1995). Predicted and experimentally derived BCFs are <10, indicating a low potential for bioconcentration and bioaccumulation (Goodrich et al., 1991). Physicochemical characteristics include vapor pressure of 2.17×10^{-11} mm Hg, Henry's law constant 5×10^{-12} atm m^3/mol, and log K_{ow} 6.1. DOSS is essentially nonvolatile from water and several studies have reported >90% biodegradation in 3-12 days at freshwater concentrations of 3.3-12.9 ppm (HSDB 2010).

The nonionic surfactant Span 80 (CASRN 1338-43-8) is a trade name for sorbitan, mono-(9Z)-9-octadecenoate. The nonionic surfactant Tween 80 (CASRN 9005-65-6) is a trade name for a mixture of isomers and congeners of polyoxy-1,2-ethanediyl derivatives of sorbitan, mono-(9Z)-9-octadecenoate. The nonionic surfactant Tween 85 (CASRN 9005-70-3) is a trade name for a mixture of isomers and congeners of polyoxy-1,2-ethanediyl derivatives of sorbitan, tri-(9Z)-9-octadecenoate. Numerous chemical synonyms and trade names exist for these materials. Other common uses for Span and Tween products include as wetting agents, solubilizing agents, or emulsifying agents in cosmetic and personal care products. Span 80 and Tween formulations are widely used in food products, cosmetic and personal care products, oral pharmaceuticals, and parenteral products. They include GRAS direct and indirect food additives commonly known as polysorbates (21 CFR 73.1;

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107.105; 172.515; 172.623; 172.836; 172.838; 172.840; 172.842; 173.310; 173.340; 175.105; 178.340). The National Toxicology Program reviewed Tween 80 (CASRN 9005-65-6) for developmental toxicity in rats and estimated a maternal LOAEL of 500 mg/kg-day based upon an increase in maternal liver weight at this dose. No definitive adverse effects on prenatal development were noted and the developmental NOAEL was projected to be greater than 5,000 mg/kg/day (NTP 1992). JECFA determined 25 mg/kg bw d^{-1} as the ADI for Tween 80 and Span 80 (FAO/WHO, 1973). Predicted BCF/BAF for sorbitan oleates have been estimated from 36 to >300 suggesting potential for bioaccumulation. Physical-chemical characteristics of the sorbitan oleates include vapor pressures less than1 x 10^{-10} mm Hg at 25°C; Henry's law constants <1 x 10^{-10}; and Log K_{ow} from <1 to 6 (KOWWIN v1.54; v1.67; HENRYWIN v3.20; Rorije et al., 1997; EPA 2010c). Sorbitan oleates would not be expected to volatilize from water and are readily biodegradable (HSDB 2010).

The aquatic toxicity of dispersant formulations has been examined and debated over many years. Following extensive reviews in 1989 and 2005, and suggested standardization of testing methods, dispersants in use today are generally reported to be less toxic than formulations prior to 1970 (e.g. NRC 1989, 2005, Fingas 2008). Corexit® dispersants in particular have been well studied with most reports indicating lower aquatic toxicity in direct comparisons with water soluble, water accomodated and chemically dispersed oil fractions (e.g., George-Ares and Clark, 2000; Liu et al., 2006; Judson et al., 2010; Hemmer et al., 2011). Other studies have addressed dispersant influence on the bioavailability and uptake of aromatic and aliphatic constituents of oil by various aquatic species (e.g. Milinkovitch et al., 2011; Jung et al. 2009; Mielbrecht et al., 2005; Fuller et al., 2004; Ramachandran et al., 2004; Cohen et al. 2001; Gagnon et al. 2000; Singer et al., 1998). However, the potential for dispersant constituents themselves to accumulate in aquatic species has received less attention. Several studies investigating uptake and disposition of principal dispersant constituents, ionic and nonionic surfactants, in aquatic species suggest rapid uptake, metabolic conjugation, concentration in liver and bile with transient enterohepatic circulation, and rapid elimination (Alvarez-Munoz et al., 2010; Tolls et al., 2000; Tolls & Sijm 1999; Goodrich et al., 1991; Granmo & Kollberg 1976; Calamari & Marchetti 1973). Distribution of surfactants into muscle tissues was reported to be low order and low BCF values for edible tissues. In one of few studies to assess the uptake and disposition of Corexit® constituent dioctyl sodium sulfosuccinate in aquatic species, Goodrich et al. (1991) noted rapid uptake and concentration in viscera and bile of rainbow trout, at significantly higher levels than uptake in either blood and carcass. BCFs were not determined for viscera or bile due to the lack of steady state conditions. BCFs of 3.47 and 3.78 were derived for blood and carcass compartments, respectively, indicating little bioconcentration in edible tissues.

The initial federal chemical safety assessment of Corexit® dispersants in the context of seafood safety included structure-activity modeling to estimate BCFs for Corexit® constituents. Consistent with existing information, predictive BCFs for Corexit® constituents, other than the nonionic surfactants and light petroleum distillates, fell below 10, suggesting low potentials for bioconcentration and accumulation in edible tissues of aquatic species. The BCFs for light petroleum distillate mixtures ranged from 60 to 80.

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Nonionic surfactant BCFs have been experimentally determined in fathead minnow with an average value of 39.6 and maximum value of 387, suggesting moderate potential for bioconcentration. However, high elimination rate constants indicated that these compounds are rapidly biotransformed *in vivo* are thus unlikely to accumulate in fish (Tolls et al., 2000). Dispersant concentrations in DWH surface applications at sea were estimated by the manufacturer to be approximately 30 μg/L in the area of the oil slick to a depth of 10 meters (Nalco, 2010). Previous research (Georges-Ares & Clark, 2000; NRC 1989) indicates that dispersant would be expected to be rapidly diluted and biodegraded in the ocean environment to levels below detection. Analyses for Corexit® constituents in surface water and sediment samples from the GOM after dispersant applications had ceased did not detect dispersant chemicals above limits of detection (EPA 2010b). However, deep subsurface sampling and analyses detected trace levels (12 ppb) of DOSS entrained in a hydrocarbon plume at 1000-1200 m water depth, suggesting slow to negligible degradation at depth (Kujawinski et al., 2011).

In the development of a method for surveillance of dispersant residues in GOM seafood species, the FDA and NOAA performed controlled exposures of Corexit® 9500 to live Eastern oyster (*Crassostrea virginica*), blue crab (*Callinectes sapidus*), and red snapper (*Lutjanus campechanus*) to generate incurred residues (Benner et al., 2010). DOSS was selected as the indicator compound for potential Corexit® contamination in seafood due to its inclusion in both Corexit® formulations, extremely low volatility, and potential to persist in the environment. The laboratory exposure studies were designed to generate incurred residues in seafood species to support method development and validation under a compressed time schedule, and therefore procedural design was not intended to be sufficient for rigorous pharmacokinetic assessment and environmental relevance. Nevertheless, it was possible to incorporate a limited sampling of uptake and depuration time points in the species exposed.

Performance testing of a rapid extraction procedure and liquid chromatography tandem mass spectrometry (LC-MS/MS) method for DOSS showed an average recovery of 92% and relative standard deviation of 11%. The method limits of detection (LOD) for DOSS in seafood species ranged from 0.003 μg/g (FDA Forensic Chemistry Center) to 0.015 μg/g (NOAA Northwest Fisheries Science Center) and limits of quantitation (LOQ) from 0.010 μg/g to 0.045 μg/g (Flurer et al., 2011). Mean DOSS concentrations in laboratory exposed and depurated oysters exposed to 100 mg/L Corexit® 9500 were 18 and 12 μg/g after 24 and 48 h of exposure, respectively. Levels of DOSS declined by >99%, to 0.023 μg/g within 72 h of depuration in clean water. In crabs exposed to Corexit® 9500 (100 mg/L) for 24 h, mean DOSS level in muscle was 0.9 μg/g, with levels declining to 0.023 μg/g (>97%) within 72 h of depuration in clean water. After 24 h of exposure, mean DOSS concentration in crab hepatopancreas was 11 μg/g, more than 10-fold higher than corresponding muscle tissue. Levels increased initially during the first 24 h of depuration, possibly as a result of the re-distribution of residues within the body during elimination. Levels subsequently declined >95% to 2.4 μg/g after 72 h of depuration. DOSS concentrations in muscle tissues of red snapper exposed to 100 mg/L Corexit® 9500 for 24 h were near or below the LOQ at all sampling times (Benner et al., 2010).

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In retrospective analyses of 393 GOM fisheries re-opening samples collected from June 2010 through March 2011, DOSS was not detected in 116 of 119 samples collected from State waters. Three samples from State waters showed DOSS levels ranging from 0.011 to 0.013 μg/g (FDA 2010a). Of 274 samples analyzed from Federal waters, 12 finfish samples contained DOSS ranging from 0.037 to 0.41 μg/g. Overall, DOSS was detected at or above the LOQ in less than 3.6% (14/393) of the re-opening samples tested and all were below safety thresholds determined for DOSS in finfish (100 μg/g), shrimp and crabs (500 μg/g), and oysters (500 μg/g). Safety thresholds were derived by combining the 2005-2006 National Health and Nutrition Examination Survey (NHANES) 90th percentile finfish, shrimp or crab meal size adjusted by the 90th percentile consumption frequency with the acceptable daily intake for DOSS developed by the World Health Organization, Food and Agriculture Organization, Joint Expert Committee for Food Additives (FAO/WHO 1991a, 1995, FDA 2010b).

Conclusions:

In considering the potential for chemical dispersants to compromise the safety of GOM seafood, initial questions concerned the potential toxicity of dispersant constituents, their concentrations, fate and persistence in the environment, their potential for bioconcentration in seafood species, and their disposition and persistence in seafood species. With the exception of dipropylene glycol monobutyl ether, the constituents of Corexit® dispersants are recognized direct or indirect food additives under prescribed conditions of use. Corexit® dispersants used to treat the DWH oil spill were rapidly and extensively diluted in GOM waters, and environmental concentrations, estimated and measured, were commensurately low when detected. The physical-chemical characteristics and scientific literature indicate that dispersant constituents are susceptible to chemical and biological degradation, and that the potential for bioconcentration and persistence in the edible tissues of seafood species is low. The modeling, experimental, and field assessments performed during the response to the DWH oil spill, as well as the knowledge base accessed through scientific literature, indicated that Corexit® dispersants did not pose a threat to the safety of GOM seafood during or after their use. However, oil spills in different parts of the world differ in the nature and extent of public and environmental health hazards entailed, and consequently response strategies are rarely the same. There are numerous dispersant formulations available to responders, and in development for mitigation of oil spills under varying conditions. Future responders would benefit from a systematic assessment of all dispersant constituents, and their fate in aquatic species. A review of the existing knowledge base for dispersant constituents that are listed on the NCP product schedule should be performed to determine chemical makeup, environmental fate, kinetics, BCF/BAF, and potential human toxicity through direct or indirect exposure in order to identify constituents of potential public health concern. Some level of standardization of experimental designs informed by the needs of risk analysis, and testing methods for toxicologically valid markers of dispersant contamination in aquatic species would benefit risk managers and responders in the event of future oil spills.

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Federal seafood safety response to the Deepwater Horizon oil spill

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Following the 2010 Deepwater Horizon oil spill, petroleum-related compounds and chemical dispersants were detected in the waters of the Gulf of Mexico. As a result, there was concern about the risk to human health through consumption of contaminated seafood in the region. Federal and Gulf Coast State agencies worked together on a sampling plan and analytical protocols to determine whether seafood was safe to eat and acceptable for sale in the marketplace. Sensory and chemical methods were used to measure polycyclic aromatic hydrocarbons (PAHs) and dispersant in >8,000 seafood specimens collected in federal waters of the Gulf. Overall, individual PAHs and the dispersant component dioctyl sodium sulfosuccinate were found in low concentrations or below the limits of quantitation. When detected, the concentrations were at least two orders of magnitude lower than the level of concern for human health risk. Once an area closed to fishing was free of visibly floating oil and all sensory and chemical results for the seafood species within an area met the criteria for reopening, that area was eligible to be reopened. On April 19, 2011 the area around the wellhead was the last area in federal waters to be reopened nearly 1 y after the spill began. However, as of November 9, 2011, some state waters off the Louisiana coast (Barataria Bay and the Delta region) remain closed to fishing.

On April 22, 2010, 2 d after the explosion on the Deepwater Horizon (DWH) drilling platform, the rig collapsed and the wellhead failed. The explosion resulted in the loss of human life and the uncontrolled release of >200 million gallons of Louisiana light crude oil occurring ~5,000 feet below the sea surface. DWH was declared a Spill of National Significance on April 29, 2010 and became the largest oil spill in US history (1). Among the significant human and environmental impacts of the spill, marine fisheries and supporting marine and estuarine ecosystems were subjected to contamination by crude oil, compromising the safety of seafood resources (1). An immediate and coordinated federal and state response ensued to safeguard seafood safety. Federal and state agencies mobilized personnel and resources to begin sampling seafood on April 28, 2010. Federal and state fishery closures were guided by observations of where oil was seen and forecasted to spread on the basis of climatic and hydrographic models (2). Seafood was collected around the periphery of the closed areas and from dockside and seafood market outlets across the Gulf coast and analyzed for oil-spill related contaminants to assess the effectiveness of the fishery closures. When the flow of oil was stopped on July 15, 2010 and the oil began to dissipate, sampling and analyses were conducted to determine whether seafood from previously closed areas was safe for harvest and human consumption. Sampling of reopened areas in federal waters continued through June 2011. We describe how federal agencies, working with the states, developed seafood safety criteria and protocols. In addition, sampling schemes, analyses, and data reporting for seafood safety efforts conducted in federal waters are provided. Results of testing the seafood collected in federal waters are also discussed.

Collaboration Among Federal and State Agencies to Develop a Protocol

The US Food and Drug Administration (FDA) operates a mandatory hazard analysis and critical control point (HACCP) safety program for all fish and fishery products under the provisions of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 et seq.), the Public Health Service Act (42 U.S.C. 201 et seq.), and related regulations. The National Oceanic and Atmospheric Administration (NOAA), under provisions of the Magnuson–Stevens Fishery Conservation and Management Act (16 U.S.C. 1801 et seq.), has the authority to close and open, with concurrence of the FDA, federal waters for seafood harvest. Marine resource and public health agencies of states bordering the Gulf of Mexico retain jurisdiction and responsibility for the health and safety of fish and fishery products within their respective territorial waters (3 miles from coastline except for Florida, which is 9 miles from coastline). Under these guidelines, should an oil spill occur, federal and state agencies, including the NOAA and the FDA, determine whether seafood is at risk for contamination and, if so, when seafood from a previously contaminated area may once again be safe for harvest and human consumption.

As the oil spread and fisheries were closed as a result of the spill, scientists and risk managers from all of the affected state and federal agencies convened to develop a comprehensive protocol to ensure the safety of Gulf seafood before impacted areas could be reopened for harvest for the American public. The NOAA publication titled Managing Seafood Safety After an Oil Spill provides agencies guidance in such situations (3). This guidance and current information from the FDA, the NOAA, the Environmental Protection Agency (EPA), the Centers for Disease Control and Prevention, and counterpart Gulf state agencies were used to establish a unified DWH seafood protocol (4). The DWH seafood safety risk assessment, an integral component of the protocol, was built upon an approach taken by the FDA in 1990 after the Exxon Valdez oil spill in Prince William Sound, Alaska (3). The protocol was implemented, by agreement of all federal and state authorities, in the reopening of commercial and recreational fisheries in both federal and state waters.

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Selection of Toxic Crude Oil Chemical Indicators

Crude oil is composed of a complex mixture of hundreds of compounds. Among them, polycyclic aromatic hydrocarbons (PAHs) are recognized internationally as the oil constituents of greatest human health concern because of their persistence in the environment and their potential for toxic or carcinogenic effects in humans (5). Consequently, 13 PAHs and their alkylated homologs were selected as the appropriate chemical indicators of the human health risk posed by crude oil residues in seafood (see SI Text, List of Polycyclic Aromatic Hydrocarbon (PAH) Chemical Indicators).

Development of Health Risk Assessment

A human health risk assessment of PAHs in seafood was undertaken to develop maximum exposure levels that are believed to be safe or associated with negligible risk. Uncertainty is inherent in any risk assessment process due to interspecies, intraspecies, or high-to-low dose extrapolations required for risk estimation. Various assumptions must be made to extrapolate data from animal or human studies using models to estimate human population risks. Some subpopulations, such as young children, may be at higher risk due to higher seafood consumption per unit body weight. Other subpopulations, such as elderly people with certain diseases, may be more susceptible to PAH effects from seafood consumption due to compromised health (3). However, many of the inputs used in this health risk assessment were derived to be overly protective to account for uncertainty and variability (e.g., upper 95% confidence bound on cancer risk values and inter- and intraspecies uncertainty factors on reference doses for noncarcinogenic PAHs).

Of the 13 PAHs and alkylated homologs selected for critical analysis of potentially impacted seafood, the criteria agreed upon for 7 PAHs with cancer endpoints provided conservative estimates of contamination levels and consumption rates that, if sustained for a period of 5 y, may result in an upper bound consumer lifetime cancer risk of 1×10^{-5}. The agreed-upon criteria for 6 PAHs with noncancer endpoints were derived from EPA reference doses of daily exposure expected to have no significant risk of adverse effect during a lifetime of exposure (4). The EPA reference doses are derived to be protective of the human population, including sensitive subgroups such as children and pregnant women (see http://www.epa.gov/iris/help_gloss.htm for reference value definitions).

Values for event-specific variables were determined for seafood consumption, average body weight, exposure duration, and averaging time. Seafood consumption data specific only to Gulf Coast populations were not available during development of the protocol and therefore the National Health and Nutrition Examination Survey (NHANES) 90th percentile consumption data for national seafood eaters only were used and were adjusted for consumption frequency (6). Meal portion and frequency (16.4 seafood meals per month) were converted to annualized daily equivalents. The average US adult body weight of 80 kg (7) and averaging time of 78 y (8) were assumed. For purposes of risk assessment, average adult body weight may be viewed as an estimate of average lifetime body weight—and averaging time as average life span—of people composing a population. An exposure duration of 5 y was selected in consideration of the nature of the spilled oil (i.e., light crude), physical conditions (e.g., 29.5 °C water temperature), spill location (50 miles offshore), and metabolic capacities of seafood species likely to be impacted.

Sampling Scheme for Federal Waters

At the greatest extent of the oil spill, most state waters extending from Louisiana to the panhandle of Florida were closed to fishing. By June 2, 2010 ~37% (88,522 square miles) of federal waters in the Gulf Exclusive Economic Zone were closed (Fig. 1) (9). Commercial, recreational, and subsistence harvest areas in federal waters that were likely to be in the path of the spilled oil were selected for sample collection in the NOAA National Marine Fisheries Service seafood sampling plan (10). Initially, the survey design for reopening the closed fishing areas was based on selection of random stations in preoil spill nearshore waters along the coastlines of Louisiana, Mississippi, Alabama, Texas, and Florida. However, a more formal survey design was developed and implemented in May 2010 due to the increased area closed to fishing. In this design, the closed region in federal waters was separated into three broad areas on the basis of the extent of cumulative oil inundation from the initial days of the spill. A fourth "perimeter" area was also established to account for uncertainty in the extent of the spill and fish movement and to provide a baseline of samples from preoil conditions for comparison. Each area was further divided into 30 × 30 nautical mile "grids" extending from the state–federal boundary to the outer boundary of the closed area (Fig. 2) (10).

Grids exhibiting heavy cumulative oil inundation were targeted for more intensive sampling than areas with minimal and

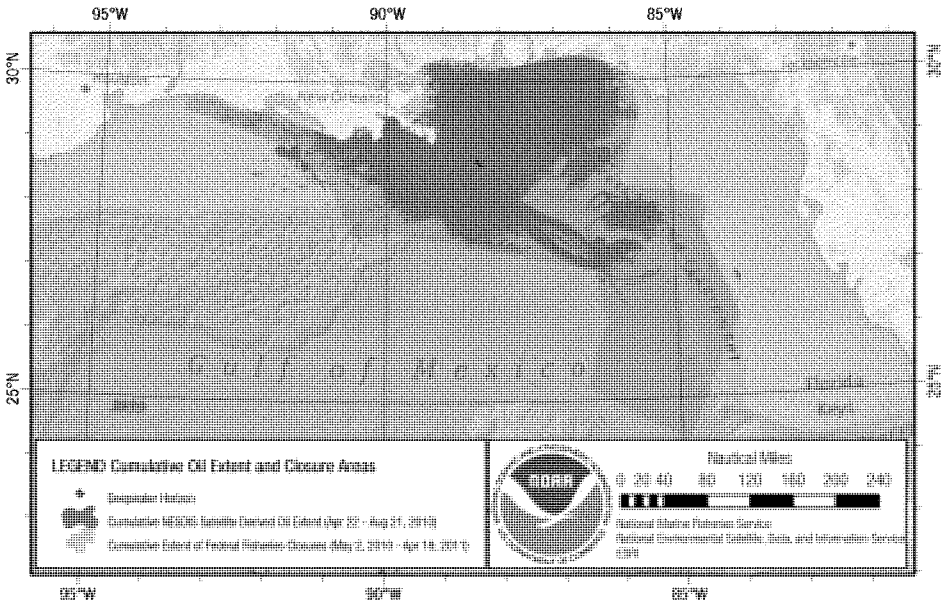

Fig. 1. Gulf of Mexico map showing the extent of oiling that occurred from April 22 through August 21, 2010 after the Deepwater Horizon platform explosion occurred on April 20, 2010. The black-and-white circle indicates the wellhead location. The orange shaded areas show the cumulative National Environmental Satellite, Data and Information Service (NESDIS) satellite footprints of the oil. The yellow polygon overlay shows how the federal fisheries closure areas aligned with the oil distribution.

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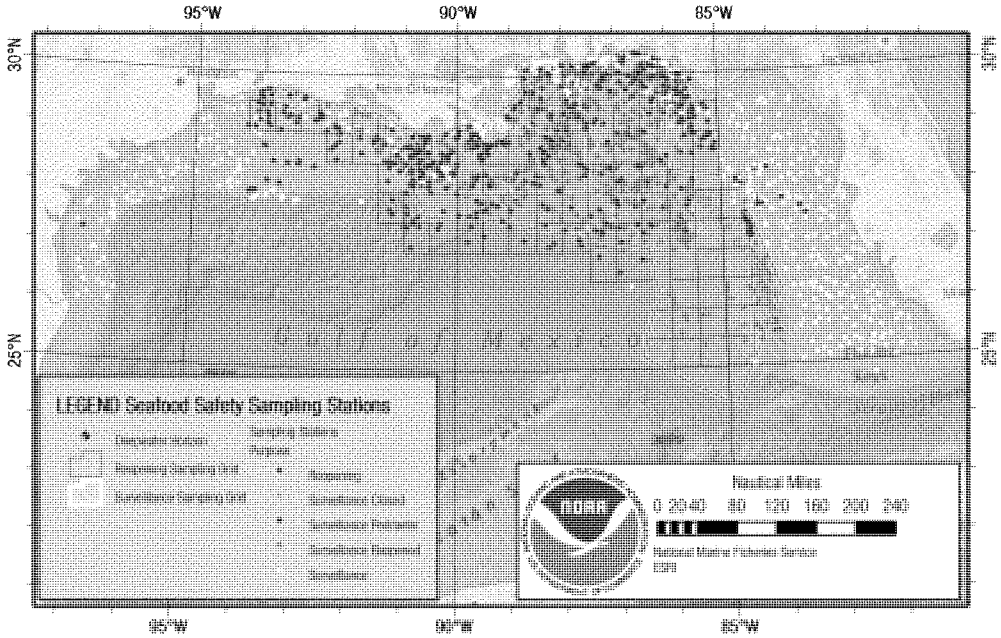

Fig. 2. Gulf of Mexico map showing the constellation of seafood samples collected. The black-and-white circle indicates the wellhead location. The red dots show stations sampled for reopening from June 23, 2010 through March 31, 2011. The yellow, blue, and white dots show stations sampled for surveillance closed, surveillance perimeter, and surveillance, respectively, from April 28, 2010 through January 15, 2011. The orange dots show stations sampled for surveillance of reopened areas from August 13, 2010 through January 9, 2011.

moderate cumulative oil inundation (10). Sampling intensity was measured as the minimum number of stations successfully sampled per grid that then relates to the target sample size. Two stations were successfully sampled in grids with minimal oil inundation whereas five stations were successfully sampled in grids with heavy oil inundation. Stations were generally chosen by random selection for all vessels, with the exception of the pelagic longline sampling, which targets highly migratory species. Due to the amount of area (10–20 miles) covered by the longline gear and the large migratory range of these species within the Gulf, sampling by random stations in a grid was not relevant. For reopening closed areas in federal waters, the number of days needed to collect adequate numbers of representative seafood varied by area, depending upon weather conditions, fishing success, and other factors. Sample collection in some areas was completed within a couple of days whereas in other areas sampling occurred over several weeks (>4 wk in some cases).

NOAA ships and contract vessels were used to collect seafood via trawls, hand lines, and longlines. Nearshore grids were typically sampled for finfish and shrimp, whereas offshore grids (>200 m) were sampled only for finfish (10). A list of key species, including shrimp, snappers, porgies, and groupers, as well as highly migratory finfish such as tuna, dolphin fish, wahoo, jack, and swordfish, was developed on the basis of their importance to commercial and recreational fishing, their prevalence, and their role in the ecosystem (10). Sampling was targeted to collect these key species; however, the species collected varied by area depending on habitat type, depth, and other factors.

By June 2011, >8,000 seafood specimens collected in federal waters had been processed and subjected to sensory testing and chemical analysis. Seafood samples collected at sea were held on ice or frozen onboard vessels and brought to NOAA's National Seafood Inspection Laboratory (NSIL) in Pascagoula, Mississippi for processing. Seafood samples consisted of edible parts of whole fish or groups of individual small shellfish, e.g., shrimp, consisting of at least 227 g composite samples. In general, individual fish specimens of similar type, e.g., red snapper and gray snapper, and the same location were often combined in equal amounts into composite samples for chemical analysis. Compositing was appropriate as the sampling and decision point was to determine if an area could be reopened to fishing.

Materials and Methods

Sampling Scheme for Federal Waters. In response to the DWH oil spill, seafood sampling in the Gulf started on April 28, 2010 and included collection of a number of sample types described in the sampling plan (10). The seafood included surveillance, surveillance-perimeter, surveillance-closed, surveillance-reopened, reopening, and dockside surveillance samples (see definitions in SI Text, Sample Classifications for Federal Waters).

Seafood Collection Criteria for Sensory Testing and Chemical Analyses. The criteria described in the protocol (4) for reopening samples subjected to sensory testing and chemical testing included collecting three to six subsamples per seafood type (e.g., oysters, shrimp, crabs, and finfish) at each sample location (SI Text, Seafood Collection Criteria for Sensory Testing and Chemical Analyses).

Sensory Testing and Chemical Analyses. A panel of seven NOAA- and FDA-trained sensory experts performed sensory testing of seafood samples for abnormal odor or taste (known as "taint"). Samples were sequentially evaluated for raw odor, cooked odor, and cooked flavor (SI Text, Sensory and Chemical Testing).

Seafood PAH analyses were conducted using both a detailed gas chromatography/mass spectrometry (GC/MS) method (11) and a more rapid "screening" liquid chromatography/fluorescence detection (LC-FLD) method (12). Gulf seafood was also analyzed for the dispersant component dioctyl sodium sulfosuccinate (DOSS) using a liquid chromatography/tandem mass spectrometry (LC-MS/MS) method (13, 14) that was developed and validated by the FDA and the NOAA (SI Text, Sensory and Chemical Testing).

Risk Assessment. To establish the safety of seafood following the DWH oil spill, standard FDA and EPA risk assessment methods for carcinogenic and noncarcinogenic PAHs were used.

A toxic equivalency approach was used to estimate the cancer risk for individual PAHs likely to be found in Gulf light crude oil. Tissue levels of carcinogenic PAHs were multiplied by their respective toxicity equivalency factor [relative to benzo(a)pyrene (BaP)] and added to the BaP level to determine the total BaP equivalent concentration. These data were used together with other factors (e.g., body weight, averaging time, seafood consumption rate, and cancer slope factor) to determine the human level of concern (LOC) for each carcinogenic PAH (SI Text, Cancer risk).

Noncancer risks were determined on the basis of the concentrations of anthracene, phenanthrene, fluoranthene, fluorene, naphthalene, and pyrene measured in seafood. Alkylated homologs of naphthalene, fluorene, and anthracene/phenanthrene were summed with the parent compounds and compared with the appropriate toxicity criterion. The LOC values for noncarcinogenic PAHs were estimated using reference dose, body weight, consumption weight, and a conversion factor (SI Text, Noncancer risks).

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Results

Sensory Testing for Seafood from Federal Waters. Federal and state agencies used a tiered strategy to ensure that seafood from the Gulf was safe for consumption. Initially, if oil was visually found on the water surface, a fishery was recommended for closure, which included a precautionary zone around the visible surface sheen. Once no visible floating oil was observed on the sea surface, this strategy combined sensory testing—expected to detect a broad array of potential contaminating chemicals—with sensitive analytical methods that measured chemical contaminants.

For a closed area to be eligible for reopening, the following criteria had to be met (these criteria were based on past oil spill information and provided a high level of confidence that the seafood was not tainted by oil) (4). A minimum of 70% (five of seven) of the expert assessors had to have found no detectable petroleum or dispersant odor or flavor from each subsample. If a subsample failed, then the sample location failed, which in turn failed the grid. Second, if the area passed the sensory test, then samples would undergo chemical analyses. In federal waters 0.16% (6 of 3,810) seafood samples failed sensory testing. This low failure rate was expected because an area was not sampled for reopening testing until there was no visible oil with minimal risk of reoiling.

Chemical Analyses for Seafood from Federal Waters. The GC/MS method measured individual PAHs at low detection levels (<1 ng/g) and provided data on alkylated homologs. The LC-FLD screening method is six times faster than GC/MS and provided reliable measurements of the targeted carcinogenic and noncarcinogenic PAHs in seafood, but did not have the specificity of the GC/MS method to confirm the PAH analyte identity. Thus, if a sample analyzed by screening had a PAH level approaching the LOC for that compound, then the sample was reanalyzed using GC/MS to confirm this finding. Although the LC-FLD method increased laboratory capacity, the method provided limited information on the levels of certain alkylated PAH homologs. Therefore, subsets of seafood samples were selected to be reanalyzed by GC/MS to confirm the PAH results and provide more information on alkylated homologs.

Overall, individual carcinogenic PAHs (Fig. 3) and noncarcinogenic PAHs (Fig. 4) were found at low concentrations or below the limits of quantitation. When detected, the PAH concentrations were at least two orders of magnitude lower than the corresponding LOC for each PAH. Furthermore, DOSS was found only at low levels (ranging from 0.05 to 0.29 μg/g) or below the limits of quantitation. Once all sensory and chemical results for the grids within a seafood harvesting area met the criteria for reopening, that area was eligible to be reopened. For all seafood samples collected in a closure area that passed sensory testing, the concentration of each PAH must be below the FDA LOC as determined using the chemical analytical methods for the closed harvest area to be eligible for reopening (4). All seafood samples collected in federal waters met this criterion established in the protocol (4).

Discussion

No two oil spills are exactly alike and thus sampling protocols and fisheries reopening criteria to ensure seafood safety must be modified from previously established guidelines for each particular event (3). More than 4.9 million barrels of oil were spilled over the course of almost 3 mo before the capping of the DWH wellhead (1), so an immediate and well-coordinated seafood safety response was the highest priority for the NOAA, the FDA, and other responding agencies. Priority issues were (a) closing seafood harvesting areas affected by the spill, (b) developing criteria for reopening areas closed to harvesting, (c) planning for sampling and testing of important commercial and recreational seafood species from the closed areas and from reference areas for spill-related chemicals to determine whether the seafood had been contaminated by spill-related compounds, and (d) conveying the information on testing results to the public and issuing any health advice related to the consumption of seafood potentially contaminated with spill-related chemicals. It was essential that regulators and scientists work together to identify key questions that must be answered and to develop criteria for data that would be collected.

Following the establishment of the protocol (4), a seafood safety sampling plan that contained all relevant information for sampling in federal waters was developed (10). Important

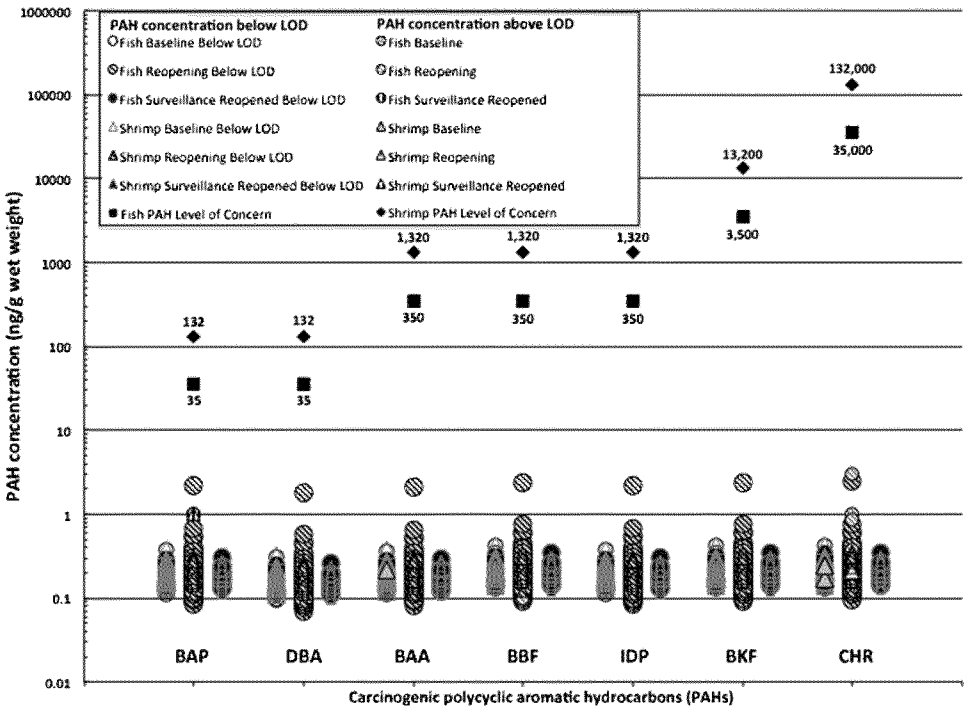

Fig. 3. Concentrations of carcinogenic polycyclic aromatic hydrocarbons (PAHs) measured in seafood collected in federal waters from April 28, 2010 through March 31, 2011 that were tested for reopening, as well as subsets for baseline and surveillance reopened samples. The levels of concern for each PAH are shown as solid diamonds for fish and solid squares for shrimp. Seafood PAH concentrations are shown in three columnar groupings above each abbreviated PAH, with levels for baseline, reopening, and surveillance reopened samples presented in left, center, and right columns, respectively. (Inset) (Left) Circles and triangles indicate samples where the concentrations are below the limit of detection (LOD) of the sensitive gas chromatography/massspectrometer (GC/MS) instrumentation used; (Right) circles and triangles indicate samples where the levels are above the GC/MS LOD. Abbreviations: BAA, benz(a)anthracene; BAP, benzo(a)pyrene; BBF, benzo(b)fluoranthene; BKF, benzo(k)fluoranthene (coelutes with benzo(j)fluoranthene); CHR, chrysene (coelutes with triphenylene); DBA, dibenz(a,c)anthracene + dibenz(a,h)anthracene; IDP, indeno(1,2,3-cd)pyrene.

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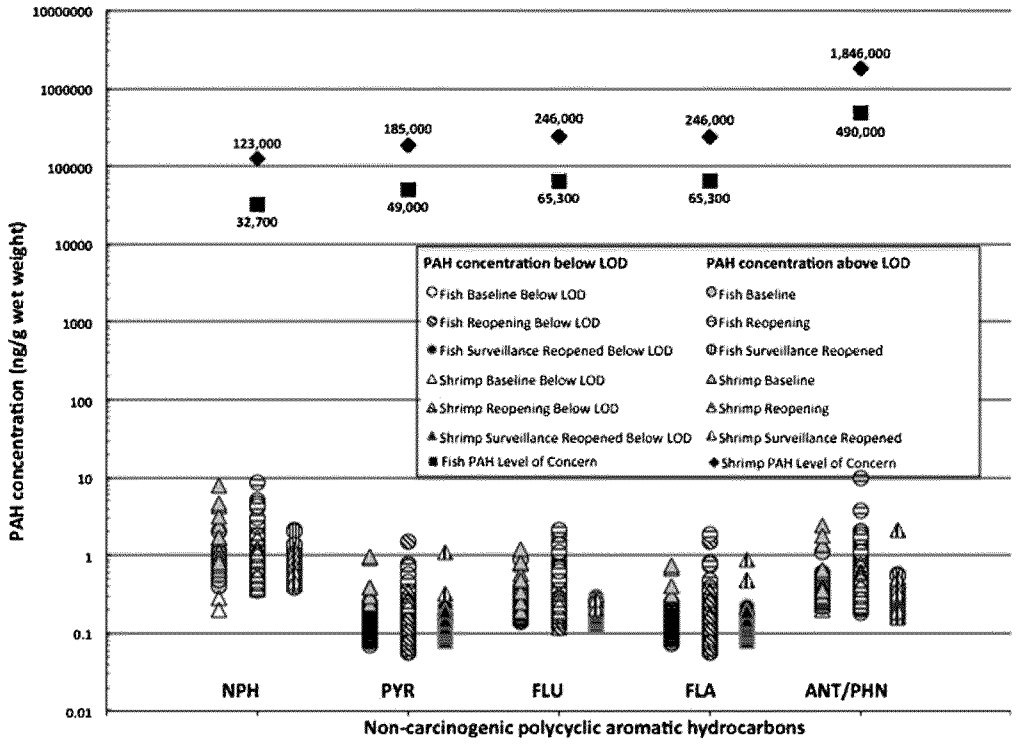

Fig. 4. Concentrations of non-carcinogenic polycyclic aromatic hydrocarbons (PAHs) measured in seafood samples collected in federal waters from April 28, 2010 through March 31, 2011 that were tested for reopening, as well as subsets for baseline and surveillance reopened samples. The levels of concern for each PAH are shown as solid diamonds for fish and solid squares for shrimp. Seafood PAH concentrations are shown in three columnar groupings above each abbreviated PAH, with levels for baseline, reopening, and surveillance reopened samples presented in left, center and right columns, respectively. (Inset) (Left) Circles and triangles indicate samples where the concentrations are below the limit of detection (LOD) of the sensitive gas chromatography/mass spectrometer (GC/MS) instrumentation used; (Right) circles and triangles indicate samples where the levels are above the GC/MS LOD. Abbreviations: ANT/PHN, anthracene + phenanthrene; FLA, fluoranthene; FLU, fluorene; NPH, naphthalene; PYR, pyrene.

commercial and recreational seafood species (including near-shore, reef, and pelagic species) and primary harvesting areas were identified to target sampling in federal waters. The criteria established in the protocol (4) specified the minimum numbers of samples required to be tested for sensory testing and chemical analyses for an area to be considered for reopening. Details on seafood sampling for reopening oil-impacted areas are publicly available online at http://www.fda.gov/Food/ucm217601.htm and information on fishery closure boundaries in federal waters can be found at http://sero.nmfs.noaa.gov/ClosureInformation.htm. In addition, oversampling was recommended so that a portion of each sample could be archived for future studies. Information on seafood sampling, including gear used, species collected, number of individuals sampled, and time and location of collection, was recorded with chain of custody protocols and transferred to a database for access by end users (e.g., federal managers).

Discussions among representatives from responding federal agencies (e.g., NOAA, FDA, and EPA) and the affected Gulf states led to identification of the appropriate compounds to target in analyzing seafood. PAHs were selected as oil-related chemicals of concern due to their toxic and carcinogenic properties as well as their ability to persist in the environment (5). One unusual aspect of the DWH oil spill was the extensive use of dispersant (e.g., Corexit 9500 and Corexit 9527) that was applied onto surface waters as well as applied directly to the waters near the wellhead. DOSS was selected as the best target component of the Corexit dispersant due to its bioactivity, extremely low volatility, and potential to persist in the environment longer than other dispersant components.

Initially, a detailed GC/MS method was used to determine levels of PAHs in seafood but it became apparent soon after testing began that a more rapid method was needed to increase the capacity of the seafood safety program. As a result, a method to rapidly measure PAHs in seafood was developed and validated by the FDA as part of the response to the DWH spill. Because the PAH screening method was much more rapid than the comprehensive GC/MS method, larger numbers of samples were analyzed in less time; however, the GC/MS method remained the standard for measuring PAH levels (including alkylated homologs) in seafood and was also used to confirm PAH screening results.

Published research on the persistence of dispersant chemicals in the environment and their toxicity suggested that potential contamination of seafood would pose a low risk to consumers (see ref. 14 for references). However, public concern about dispersant use led to a collaborative effort between the FDA and the NOAA to develop and validate a rapid, sensitive chemical method for measuring DOSS from the dispersant in a wide array of edible seafood. A description of the chemical method can be found at the Web site http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM231510.pdf, and results of dispersant testing of seafood collected in federal waters as part of the reopening can be obtained at the Web site http://sero.nmfs.noaa.gov/PreviousReopenings.htm. Once the DOSS method was developed and validated, up to 50% of seafood samples previously analyzed for PAHs by GC/MS were subsequently analyzed for DOSS and all subsequent samples for reopening were analyzed for DOSS.

Analyses for spill-related contaminants proceeded in a timely manner, especially considering the large number of samples tested and the nearly 90,000 square miles of federal waters of the Gulf of Mexico that were closed. For example, sensory results were available within 4 h of sample analysis and the chemical data for a sample batch containing 14 field samples were quality assured and finalized within 48 h (LC-FLD PAH results) to 96 h (GC/MS PAH results) postreceipt.

It has been well established in the scientific literature that all teleost fish (modern bony fishes, e.g., grouper, snapper, and tuna) have a well-developed capacity to metabolize and eliminate PAHs and other oil constituents such as aliphatic hydrocarbons (15). Because of this efficient metabolism, there is a very low potential for PAHs to accumulate in muscle and consequently a low potential for transfer of PAHs up the food chain to human consumers. However, efficient PAH metabolism by species other than teleosts is not universal (3). For example, bivalves (e.g., oysters and clams) have a lower capacity to metabolize PAHs, whereas crustaceans such as shrimp have an intermediate metabolic capacity. In the current study, concentrations of individual PAHs measured in seafood collected in federal waters were, in many cases, below the limit of

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quantitation or, when detected, were at least two orders of magnitude lower than the FDA LOC for the particular compound (Figs. 3 and 4). Although scientists were confident that high levels of PAHs would not be found in edible seafood tissues, it was necessary to test seafood to assure the public of its safety. Without an extensive, real-time sampling and analysis protocol, there would be little to no ability to convince the public that Gulf seafood was safe to eat following this major oil spill.

The PAH concentrations determined by various federal and state agencies to be of concern for human health were based on a projected daily level of consumption (grams per day of finfish, mollusks, crabs, and shrimp) and an acceptable risk (e.g., 1:100,000 risk for cancer) for 5 y (SI Text, Cancer risk). For the LOC calculations, to ensure a health protective approach (in addition to using cancer and noncancer values that are designed to be conservative values), the FDA used the 90th percentile of the national consumption data reported in the National Health and Nutrition Examination Survey for fish, shrimp, and shellfish for calculating risk and then adjusted the 90% meal size to account for the number of meals eaten by a 90th percentile consumer to ensure protection for low- through high-level consumers. Nevertheless, some concerns were raised with regard to the seafood consumption rates used by the FDA to calculate the PAH LOCs for the DWH seafood assessment (16). For example, a 2010 Natural Resources Defense Council survey of 547 people living in Louisiana, Alabama, Mississippi, and Florida showed that the average shrimp median daily consumption rate among residents was 3.6 times higher (48 g/d) than the FDA estimated shrimp consumption rate of 13 g/d (17). On the basis of the information collected on PAH levels in various seafood species from the Gulf, risk assessors can calculate the seafood consumption rates that are allowable without being a health concern for Gulf seafood. The Louisiana Department of Wildlife and Fisheries and the Louisiana Department of Health and Hospitals determined that the average consumer could eat ~63 pounds of peeled shrimp, 5 pounds of oyster meat, or 9 pounds of finfish every day for 5 y and have minimal risk of health effects (18). The concentrations of PAHs measured in Gulf seafood were very low or were below the levels of quantitation, and thus these compounds did not appear to pose a health risk, even when higher consumption values are applied to the risk model.

In summary, scientists and regulators from federal and state agencies worked together to develop a seafood sampling plan and analytical protocols to determine whether seafood was safe to eat and acceptable for sale in the marketplace in response to the 2010 DWH oil spill. Approved methods for sensory testing and chemical analyses were used to detect PAHs and the dispersant component DOSS in >8,000 seafood specimens collected in federal waters of the Gulf. Overall, a low percentage (0.16%) of these samples failed sensory testing, which was expected as sampling of an area commenced only when the risk of oil being present was negligible. We also found that the concentrations of individual PAHs and the dispersant component DOSS measured in Gulf seafood were below the limits of quantitation or, when detected, were at least two orders of magnitude lower than the FDA LOC for each compound. Once an area closed to fishing was free of visibly floating oil and all sensory and chemical results for the seafood species within an area met the criteria for reopening, that area was eligible to be reopened. Since July 2010, ~88,500 square miles of federal waters have been reopened, with the area nearest the wellhead being reopened nearly 1 y (April 2011) after the spill began. As of November 9, 2011, a few areas off the Louisiana coast (Barataria Bay and the Delta region), however, remain closed because of residual oil and because the species at risk for exposure, such as oysters, have low metabolic capacity and a higher risk of accumulation of PAHs.

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From: Mandsager, Kathy
Sent: Tue 2/28/2017 7:02:18 PM
Subject: Public Health panel - meeting reminder
Wise et. al 2014 Chemical Dispersants Used in the Gulf of Mexico Oil Crisis are Cytotoxic and Genotoxic to Sperm Whale Skin Cells.pdf
2017.02.24_tox addition for public health dispersant.docx

This is a reminder of our call scheduled for tomorrow (3/1) at 2:00 pm ET. Attached is the latest version of the document (section on toxicity developed by Drs. Fabisiak and Dickey) that we have been discussing.

•□□□□□□□ Please see the blue highlighted areas for the changes that were made on the last call.

•□□□□□□□ This version also has John Wise's suggested edits.

•□□□□□□□ Although I sent it previously, please find attached the Wise manuscript for your review before the call

When it's time, start your meeting.

Join by phone
1-855-244-8681 Call-in toll-free number (US/Canada)
1-650-479-3207 Call-in toll number (US/Canada)

From: Mandsager, Kathy

Sent: Friday, February 24, 2017 3:26 PM

Per today's discussion, please see attached Wise et al 2014 manuscript.

Kathy Mandsager

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Chemical dispersants used in the Gulf of Mexico oil crisis are cytotoxic and genotoxic to sperm whale skin cells

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abstract

The 2010 Deepwater Horizon oil rig explosion in the Gulf of Mexico drew attention to the need for toxicological studies of chemical dispersants. We are still learning the effects these spills had on wildlife. Little is known about the toxicity of these substances in marine mammals. The objective of this study was to determine the toxicity of the two dispersants (Corexit 9500 and 9527). Corexit 9500 and 9527 were both cytotoxic to sperm whale skin fibroblasts. Corexit 9527 was less cytotoxic than 9500. S9 mediated metabolism did not alter cytotoxicity of either dispersant. Both dispersants were genotoxic to sperm whale skin fibroblasts; S9 mediated metabolism increased Corexit 9527 genotoxicity.

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1. Introduction

In 2010, the Deepwater Horizon oil rig exploded unleashing uncontrolled amounts of crude oil into the Gulf of Mexico. To combat the spill, unprecedented amounts of chemical dispersants were used. It is estimated that about 8 million liters (converted from gallons to liters) of dispersants were applied beneath the ocean surface at the depth of the well head leak as well as on the surface (Kujawinski et al., 2011). These agents were applied despite the fact that little was known about their potential toxicological impact to marine mammals; a shortcoming that continues to exist as, in general, most of the toxicology studies that are available are limited to LC50-type acute lethality endpoints in non-mammalian species. Thus, there is wide concern about the potential toxicity of these dispersants for both human and wildlife health.

Two types of dispersants, Corexit 9500 and Corexit 9527, were used to combat the oil spill. Limited data suggests these agents can be toxic, but experimental outcomes were generally limited to lethality. For example, a recent review summarized the available data for the toxicity of the two dispersants and found LC50s for Corexit 9500 in crustaceans, mollusks and fish, and for Corexit 9527 in daphnia, anemones, coral, crustaceans, mollusks, starfish, fish and birds (Wise and Wise, 2011). Remarkably, only one study considered effects in a mammal and reported impacts on body weights and intestinal flora in rats (George et al., as cited in Wise and Wise, 2011). More recently, the impact of dispersants on mammalian model systems have been considered in two human cell culture models. Corexit 9500 and Corexit 9527 induced cytotoxicity and oxidative stress in human HepG2/C3A hepatocytes (Bandele et al., 2012) and induced cytotoxicity in the immortalized human bronchial cell line, BEAS-2B (Shi et al., 2013). These data suggest a significant concern for dispersant toxicity and indicate the need for further study.

One major concern in the Gulf of Mexico is the potential impact of these dispersants on marine mammals. In particular, there are two resident populations of large whale species in the Gulf of Mexico: Sperm whales (Physeter macrocephalus) with a population of about 1600 individuals and Bryde's whales (Balaenoptera edeni) with a population of about 15 individuals (Waring et al.,

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2009, 2010). The loss or impairment of just a few reproductively productive animals in these small populations could result in their ultimate loss from the Gulf. Of note, the sperm whales are known to reside in the areas most affected by the spill (Waring et al., 2009, 2010). Indeed, anecdotal reports indicate that these whales were often observed by workers on the Deepwater Horizon rig and the National Oceanic and Atmospheric Administration (NOAA) and Unified Command received numerous reports of sperm whales swimming in oiled water after the spill (Warren, 2010). Thus, it is highly likely that these animals were exposed to the dispersants. We were unable to locate any data concerning the toxicity of these dispersants in any marine mammals or marine mammal cell cultures. Therefore, to address this concern, we investigated the cytotoxic and genotoxic effects of Corexit 9500 and Corexit 9527 in cultured sperm whale skin cells.

2. Materials and methods

2.1. Materials

All plasticware was purchased from BD Falcon. Dulbecco's Phosphate-Buffered Saline (DPBS), Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12 (DMEM/F-12) and Glutagro supplement were purchased from Corning. Nicotinamide adenine dinucleotide phosphate (NADPH) solutions were purchased from BD Gentest. S9 and fractions were purchased from Celsis In Vitro Technologies. Potassium chloride, demecolcine and sodium chromate were purchased from Sigma/Aldrich. Crystal violet, methanol and acetic acid were purchased from JT Baker. Microscope slides were purchased from Thermo scientific. Giemsa stain was manufactured by Rica Chemical Co. Gurr's buffer, trypsin, penicillin-streptomycin and sodium pyruvate was purchased from GIBCO Invitrogen Corporation. Cosmic calf serum was purchased from Hyclone.

2.2. Cell culture

Primary skin fibroblast cells were obtained from a free ranging sperm whale biopsy that was taken prior to the Deepwater Horizon oil spill as previously described (Wise et al., 2011). Cells were cultured in DMEM/F-12 (50:50 mixture of Modified Eagle's Medium and Ham's F-12) supplemented with 15% Cosmic calf serum, 2 mM l-glutamine, 100 U/ml penicillin, 100 ffig/ml streptomycin, and 0.1 mM sodium pyruvate. Cells were maintained in a humidified incubator with 5% CO_2 at 33 °C (estimated whale body temperature). Cells were allowed to grow to near confluence as a monolayer. They were fed three times a week and expanded weekly (Wise et al., 2011).

2.3. Chemical and S9 fraction preparation

The dispersants used (Corexit EC9500A and Corexit EC9527A) were generously provided by the Nalco Holding Company. Treatment dilutions were prepared under dark conditions using the 100% stock solution and sterile water. Sodium chromate was dissolved in water and was filter sterilized. This was used as a positive control for all experiments.

Although the metabolism of the dispersants is unknown, we also considered phase 1 metabolism to determine if there is a difference in toxicity between the metabolite and the parent compound. S9 fractions were used to induce phase 1 metabolism because fibroblast cells often do not express cytochrome P450 enzymes. S9 fractions were added at the time of treatment, and made using 1× Tris buffer, NADPH regenerating system solution A (451220), NADPH regenerating system solution B (451200), and liver S9 fractions. These were prepared and applied at the time of chemical treatment.

2.4. Cytotoxicity

We used a clonogenic assay based on our published methods to determine the cytotoxicity of each dispersant (Wise et al., 2011). Briefly, cells were seeded into two 6 well tissue culture plates and allowed 48 h to resume normal log phase growth. Then they were treated with either Corexit 9500 or Corexit 9527 for 24 h. All treatment doses were done with and without S9 fractions. After the treatment time cells were reseeded into gelatin coated 100 mm tissue culture dishes at colony forming density. Once adequate cell colonies formed (~2 weeks) dishes were rinsed twice with 1× phosphate-buffered saline (PBS) then fixed in methanol for 20 min. After the methanol was removed the dishes were stained with crystal violet stain for 30 min. Dishes were analyzed for number of cell colonies then averaged per dose and divided by the average number of colonies in the negative control.

2.5. Clastogenicity

We used a chromosomal aberration assay to determine the clastogenicity of each dispersant, based on our published methods (Wise et al., 2011). Briefly, cells were seeded into 100 mm tissue culture dishes for 48 h. Then they were treated with either Corexit 9500 or Corexit 9527 for 24 h. All treatment doses were done with and without S9 fractions. Five hours prior to the end of the treatment period cells were arrested in metaphase using 0.1 g/ml demecolcine. After the full 24 h treatment period, cells were resuspended in a potassium chloride hypotonic solution (KCl) for 17 min then fixed with 3:1 methanol:acetic acid. After two changes of fixative, cells were dropped onto microscope slides and stained with 5% Giemsa stain in Gurr's Buffer. Slides were analyzed for chromosome aberrations in 100 metaphases per treatment concentration according to our published methods (Wise et al., 2011).

2.6. Statistics

The statistical difference between values for cytotoxicity and genotoxicity were evaluated using t-test and multiple regression analysis with dispersant, concentration, and S9 as the three independent variables. No adjustment was made for multiple comparisons, because all of the comparisons were made of a priori substantive interest.

3. Results

3.1. Corexit 9500 and 9527 Cytotoxicity in Sperm Whale Skin Cells

Corexit 9500 induced a concentration dependent increase in cytotoxicity to sperm whale skin cells (Fig. 1). Concentrations of 0.005, 0.025, 0.05 and 0.1% 9500 induced 82, 67, 25 and 3% relative survival, respectively. S9 mediated metabolism did not significantly alter the cytotoxicity of Corexit 9500 (Fig. 1). Concentrations of 0.005, 0.025, 0.05 and 0.1% 9500 with S9 fractions induced 91, 72, 37 and 3% relative survival, respectively.

Corexit 9527 also induced a concentration dependent increase in cytotoxicity (Fig. 2). Concentrations of 0.005, 0.025, 0.05 and 0.1% 9527 induced 86, 79, 70 and 26% relative survival, respectively. S9 mediated metabolism resulted in a similar dose response (Fig. 2). Concentrations of 0.005, 0.025, 0.05 and 0.1% 9527 with S9 fractions induced 86, 77, 70 and 42% relative survival, respectively.

Comparison of Corexit 9500 and Corexit 9527 parent compounds shows that 9500 is more toxic than 9527 (Fig. 3). For

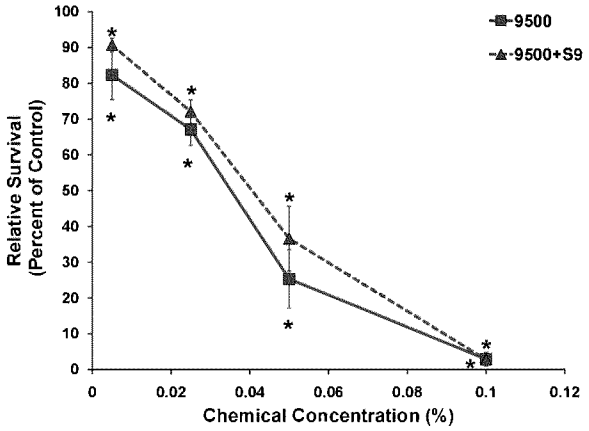

Fig. 1. Corexit 9500 is cytotoxic to sperm whale skin cells. This figure shows that Corexit 9500 was cytotoxic to sperm whale skin cells after a 24 h exposure and S9 mediated metabolism did not alter cytotoxicity (measured as cell survival relative to the control). The overall dose-response is highly significant ($p < 0.0001$). There was no statistical difference observed between 9500 and 9500+S9 ($p = 0.19$). Data represent 3–4 experiments ± the standard error of the mean. Asterisk (*) indicates doses that are significantly different from control ($p < 0.05$).

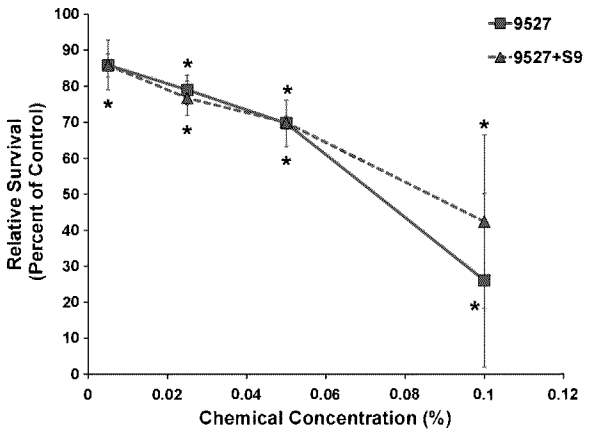

Fig. 2. Corexit 9527 is cytotoxic to sperm whale skin cells. This figure shows Corexit 9527 was cytotoxic to sperm whale skin cells in a concentration dependent manner after a 24 h. Cytotoxicity is measured as cell survival relative to the control. There was no statistical difference observed between 9527 and 9527+S9 ($p > 0.05$). Data represent 3 experiments ± the standard error of the mean. Asterisk (*) indicates doses that are statistically significant from control ($p < 0.05$).

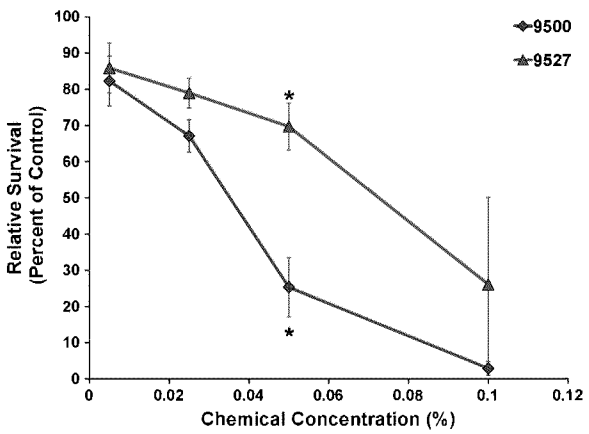

Fig. 3. Corexit 9500 is more cytotoxic than Corexit 9527. This figure compares the cytotoxicity of Corexit 9500 and Corexit 9527 after a 24 h exposure. Sperm whale cells were more sensitive to Corexit 9500 than Corexit 9527 ($p = 0078$). Asterisk (*) indicates doses that were significantly different from each other ($p < 0.05$).

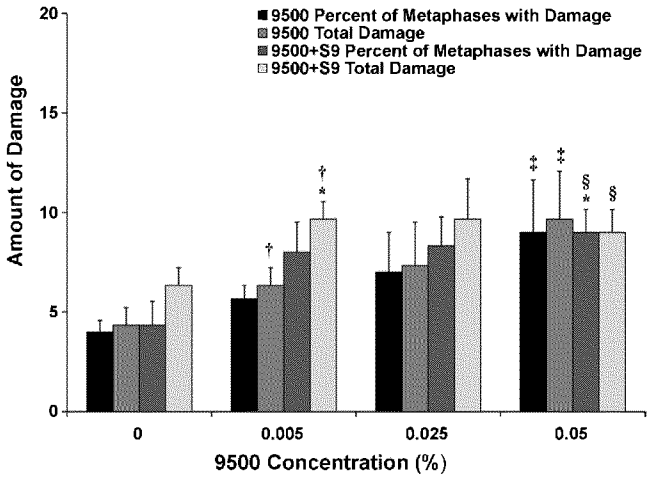

Fig. 4. Corexit 9500 is not strongly genotoxic to sperm whale skin cells. This figure shows Corexit 9500 was not substantially and generally not statistically significantly genotoxic to sperm whale skin cells after a 24 h exposure both with and without S9-mediated metabolism. Data are expressed as the average percent of metaphase with damage and total aberrations in 100 metaphases. Data represent 3 experiments ± the standard error of the mean. Symbol (†) indicates that 9500 is statistically significant from 9500 + S9 ($p < 0.05$). Asterisk (*) indicates doses that are significantly different from control ($p < 0.05$). Symbol (‡) indicates that in one experiment only 68 metaphases could be scored. Symbol (§) indicates that in one experiment only 86 metaphases could be scored.

example, there is a 3-fold increase in toxicity at 0.05% concentration ($p = 0.0002$).

3.2. Corexit 9500 and 9527 clastogenicity in sperm whale skin cells

Corexit 9500 induced a minimal increase in genotoxicity in sperm whale skin cells (Fig. 4). S9 mediated metabolism had no effect on the genotoxicity of Corexit 9500 (Fig. 4). Specifically, concentrations of 0.005, 0.025 and 0.05% 9500 damaged 1.7, 3 and 5.3% of metaphases and induced 2.3, 3 and 5.7 total aberrations per 100 metaphases, respectively (minus the control levels). S9 mediated metabolism damaged 3.7, 4 and 4.7% of metaphases and induced 3.3, 3.3 and 2.7 total aberrations per 100 metaphases, respectively (minus the control levels).

By contrast, Corexit 9527 induced a concentration-dependent increase in genotoxicity after 24 h exposure in sperm whale cells (Fig. 5). Specifically, concentrations of 0.005, 0.025, 0.05 and 0.1% 9527 damaged 3, 4.3, 8 and 10.6% of metaphases and induced 4, 5, 9.3 and 12.7 total aberrations per 100 metaphases, respectively (minus the control values). S9 mediated metabolism increased this effect damaging chromosomes in 4, 8, 11 and 19.5% of metaphases and induced 4, 8.7, 14.3 and 25 total aberrations per 100 metaphases, respectively (Fig. 5; minus the control values). The spectrum of chromosome aberrations for both compounds consisted of mostly chromatid lesions (Table 1).

Comparing of Corexit 9500 and 9527 shows that 9257 is more genotoxic than 9500 (Fig. 6). Corexit 9527 with S9 fractions was the most genotoxic condition, inducing the most total chromosome damage. Corexit 9527 had a higher amount of isochromatid lesions than 9500. Corexit 9500 had a higher amount of dicentric chromosomes and double minutes. The double minutes only occurred in the S9 treated cells. There were few chromatid exchanges in both compounds. Double minutes, dicentrics and chromatid exchanges were not present in any of the controls (Table 1).

4. Discussion

In the aftermath of the Deepwater Horizon explosion, two dispersants Corexit 9500 and Corexit 9527 were sprayed on oil at the

Table 1
Spectrum of chromosome aberrations.[a].

Concentration	Chromatid lesions	Isochromatid lesions	Chromatid exchanges	Rings	Double minutes	Acentric fragments	Dicentrics
Corexit 9500							
0	3	0	0	0	0	0	0
0.005	5	0	1	0	0	0	0
0.025	2	0	0	0	0	0	1
0.05	11	1	0	0	0	0	1
0 + S9	1	0	0	0	4	0	1
0.005 + S9	6	0	0	0	1	0	1
0.025 + S9	5	0	0	0	1	0	0
0.05 + S9	8	1	0	0	0	0	0
Corexit 9527							
0	3	0	0	0	0	0	0
0.005	2	6	0	0	0	0	0
0.025	12	0	0	0	0	0	0
0.05	17	0	1	0	0	0	0
0 + S9	3	4	0	0	0	0	0
0.005 + S9	10	1	0	0	0	0	0
0.025 + S9	20	2	0	0	0	0	0
0.05 + S9	31	0	0	0	0	0	0

surface of the Gulf of Mexico and injected into the wellhead in an effort to reduce the impact of the crude oil as quickly as possible. Little is known about the toxicity and environmental fate of these chemicals and there is significant concern about their potential long term health effects. Our data are the first assessment of Corexit 9500 and Corexit 9257 toxicity in a marine mammal model system and the first data to evaluate the ability of these agents to induce chromosome damage in any species. Our data show that Corexit 9527 is indeed genotoxic to sperm whale skin cells. Such an outcome raises concern about the impact of this agent on reproduction, development and potentially carcinogenesis in marine mammals and in other species.

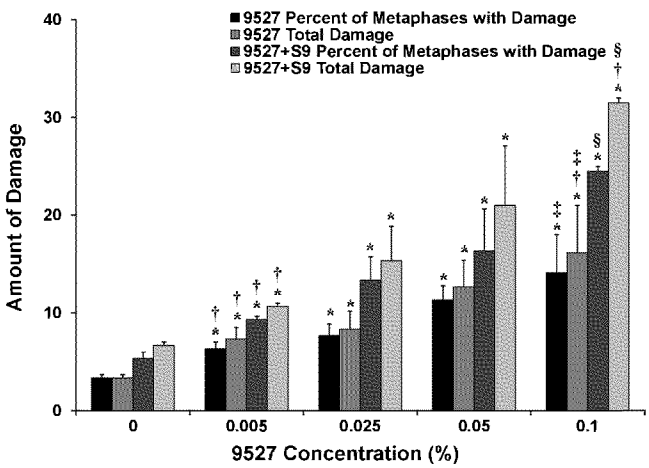

Fig. 5. Corexit 9527 is genotoxic to sperm whale skin cells. This figure shows Corexit 9527 induces a concentration-dependent increase in genotoxicity in sperm whale skin cells after a 24 h exposure. S9 mediated metabolism increases genotoxicity. Data are expressed as the average percent of metaphase with damage and total aberrations in 100 metaphases. The effect for S9 increased with concentration ($p = 0.0005$ for interaction based on percent damage and $p = 0.0003$ for interaction based on total damage). The effect for concentration without S9-mediated metabolism was significant for both percent damage ($p = 0.002$) and for total damage ($p = 0.011$). When evaluated in a multivariate regression and at a concentration of 0.05%, the effect for S9 was significant for both percent damage and total damage ($p < 0.0001$). Data represent 3 experiments ± the standard error of the mean. 0.1% 9500 with and without S9 was not done for the third experiment because at these concentrations not enough metaphases could be obtained due to cell cycle arrest. Asterisk (*) indicates doses that were significantly different from control ($p < 0.05$). Symbol (†) indicates that 9527 was significantly different from 9527 + S9 ($p < 0.05$). Symbol (‡) indicates that in one experiment only 88 could be scored. Symbol (§) indicates that in one experiment only 54 metaphases could be scored respectively.

Our data showed that Corexit 9527 induced an increase in metaphases with chromosome damage and an increase in the total numbers of aberrations in a concentration-dependent manner in sperm whale skin cells. If genotoxicity occurs during essential stages of reproduction or embryogenesis it could cause loss of the offspring or affect individual calf development (El-Makawy et al., 2006; Keshava and Ong, 1999; Nayak et al., 1989). If these outcomes were to affect the ability of an affected individual to survive or reproduce successfully then there could be detrimental effects to the population which is small in number and endangered.

Our genotoxicity results differ from the only other previous report to consider the genotoxic effects of dispersants in mammals. Specifically, the other study reported that, in Fischer 344 rats, a 5 week exposure to Corexit 9527 did not induce hepatic DNA adducts (George et al., 2001). Such an outcome is not inconsistent with our findings, it is simply different. DNA adducts, while a lesion on the DNA, and a genotoxic event, are not necessarily related to chromosomal aberrations. In other words, while an adduct may lead to a chromosome aberration, there are other mechanisms to cause aberrations. Thus, it is reasonable for a chemical mixture like Corexit

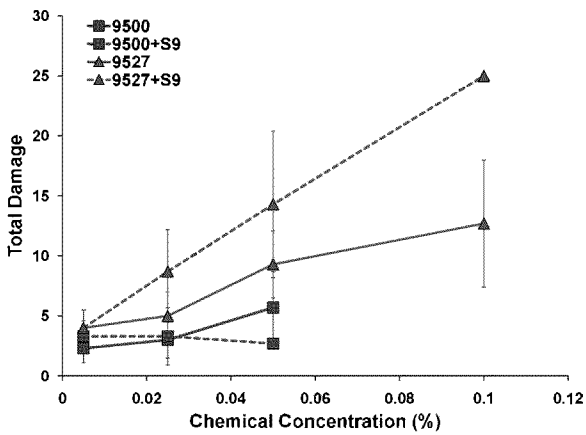

Fig. 6. Corexit 9527 induces more total chromosome damage than Corexit 9500. This figure shows the comparison of the total chromosome damage induced by Corexit 9500 and 9527 with and without S9 fractions after a 24 h exposure. Based on a multiple regression model, there was a significant 3-way interaction involving dispersant, concentration and S9 ($p = 0.023$). When evaluated at a concentration of 0.05 percent, Corexit 9527 was significantly more genotoxic than 9500 with S9 fractions ($p < 0.001$) but not without S9 fractions ($p = 0.33$). Data represent the aberrations in 100 metaphases shown in Figs. 4 and 5 minus their respective negative control levels.

9527 to induce aberrations and not induce adducts. By contrast, Corexit 9500 was only genotoxic at the highest dose 0.05%. At this dose we were unable to score 100 metaphases due to cell cycle arrest for each experiment, so it is unclear if there is a genotoxic effect or another cellular process occurring to arrest the cells. We could not find any literature regarding the genotoxicity of Corexit 9500.

We also found that both Corexit compounds were cytotoxic to sperm whale cells. Extensive cytotoxicity can lead to fibrosis and impair organ function. There are no published reports of Corexit cytotoxicity in whale cells, but our Corexit 9500 cytotoxicity results are consistent with other studies of Corexit 9500 in human cells. Specifically, Corexit cytotoxicity has only been measured in three other studies (Bandele et al., 2012; Shi et al., 2013; Zheng et al., 2014). One study considered bronchial airway cells (BEAS-2B) and used the MTT assay to determine cell viability (Shi et al., 2013). They found Corexit 9500 doses of 0.02 and 0.03% (reported in the study as 200 and 300 ppm) induced 50% and 90% cell loss. These outcomes are similar to our findings, using a clonogenic assay, that 0.025% Corexit 9500 induced 67% cell survival.

The second study measured Corexit 9500 cytotoxicity in human HepG2 hepatocytes using Hoechst 33258 fluorescence (Bandele et al., 2012). In that system, Corexit 9500 doses of 0.02 and 0.03% (inferred from the figure presenting the data) resulted in relative cell viability of 80 and 50%, respectively. Our finding that Corexit 9500 induced 67% cell survival fits right in between those previous outcomes.

The third study used the MTT assay to determine cell viability in 5 different established mammalian cell lines exposed to Corexit 9500, 1 mouse, 1 rat and 3 human cell lines. After being treated with 0.02% Corexit 9500 for 48 h they found a range of about 35–80% cell viability. These data are consistent with the 67% cell survival in sperm whale skin cells that we reported.

Our Corexit 9527 cytotoxicity data was similar to one, but differed from another of these two human cell studies. We found 0.025% Corexit 9527 was not particularly cytotoxic, resulting in 79% relative cell survival. Similarly, 0.02 and 0.03% Corexit 9527 induced 90 and 40% relative cell viability in human HepG2/C3A cells. However, in BEAS-2B cells, 0.02 and 0.03% Corexit 9527 resulted in no cell survival. The underlying explanation for why the sperm whale cells were more resistant then BEAS-2B cells to Corexit 9527 is uncertain, especially when the results for 9500 were similar. The most likely explanation, albeit untested is that 9527 has the additional ingredient of 2-butoxyethanol (Wise and Wise, 2011), thus, the whale cells may be responding differently to this chemical.

One of the concerns raised in both scientific and public circles was to determine which Corexit dispersants was the more toxic of the two. Our data shows the challenge in answering that question in a meaningful way. We found that Corexit 9500 is more cytotoxic than Corexit 9527; however, Corexit 9527 is more genotoxic than 9500. Thus, the answer would be different depending on which outcome is considered. It is curious that 9527 is more genotoxic and less cytotoxic. The underlying mechanisms that explain this difference is uncertain. It might be due to the 2-butoxyethanol in the 9527, or, perhaps, one or more metabolite of 2-butoxyethanol inducing chromosome aberrations while inhibiting cell death pathways. However, studies in rodent and human cells show that 2-butoxyethanol does not induce chromosomal aberrations specifically, and is generally negative in other genotoxic assays (Elliott and Ashby, 1997; NTP, 2000). Of course, it is possible that whale cells may simply respond differently to 2-butoxyethanol than rodent and human cells. Alternatively, it may represent some unanticipated interaction of 2-butoxyehthanol with another Corexit ingredient.

Of course an important, but difficult to address question is to determine how our laboratory exposures relate to actual exposures in the Gulf. There is no known accurate method to measure the amount of whale exposure to dispersants. In the Gulf of Mexico Deepwater Horizon oil spill a final ratio of about 1:63 dispersant to oil was used, i.e. a final concentration of about 1.6% (calculated based on a total applied dispersant amount of approximately 8,000,000 L, reported in Kujawinski et al., 2011 and a total released crude oil amount of 500,000,000 L of crude oil data from Crone and Tolstoy as cited in Joung and Shiller, 2013). Corexit dispersants were sprayed aerially and injected at depth. While some whales may have avoided any exposure, given the locations of the oil and the spraying and the whales, it is highly likely some whales were indeed exposed to dispersants. Thus, the spectrum of dispersant concentrations the Gulf whales might have encountered would have ranged from very high (i.e. 100%) if the whale was directly exposed to the dispersant spray or stream as it entered the gulf; to moderate (i.e. 1–50%) if the whale was exposed as the dispersant became mixed with oil and water; to something much lower (i.e. <0.1%) if the whale was exposed after the dispersant mixture dispersed through the water column or food sources. Given these scenarios and the final ration of dispersant applied, our doses of 0.005, 0.025, 0.05 and 0.1% certainly seem plausible.

In sum, our data show both Corexit 9500 and 9527 are cytotoxic and Corexit 9527 is genotoxic to sperm whale skin cells. Recent reports show that cetaceans in the Gulf of Mexico are suffering from reproductive, respiratory and other health issues in the aftermath of this crisis (Schwacke et al., 2014). Given the DNA damaging potential of the chemicals used in the crisis, care should be taken to monitor the populations for further long term health effects.

Acknowledgements

We would like to thank Louis Falank, Jr. for technical support, Shouping Huang for administrative support and Christy Gianios, Jr. for information technology support. We would also like to thank David Giddings from Nalco for the dispersants. Marine mammal cell line development and use by the Wise Laboratory for Environmental and Genetic Toxicology is performed under NMFS Permit # 16305-00.

This work was supported by the Prince William Sound Regional Citizens Advisory Council (PWSRCAC) and the Maine Center for Toxicology and Environmental Health [955.12.02].

This paper was also developed under GRO Fellowship Assistance Agreement No. [MA-91739301-0] awarded by the U.S. Environmental Protection Agency (EPA). It has not been formally reviewed by EPA. The views expressed in this paper are solely those of the authors, and EPA does not endorse any products or commercial services mentioned in this paper.

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Waring, G.T., Josephson, E., Maze-Foley, K., Rosel, P.E., 2010. "U.S. Atlantic and Gulf of Mexico marine mammal stock assessments. (NOAA Tech Memo NMFS NE 219, 2011; http://www.nmfs.noaa.gov/pr/pdfs/sars/ao2010whsp-gmxn.pdf).

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To: Sharon.Hook@csiro.au[Sharon.Hook@csiro.au]; sarah.allan@noaa.gov[sarah.allan@noaa.gov]; angela_matz@fws.gov[angela_matz@fws.gov]; Barron, Mace[Barron.Mace@epa.gov]; abejarano@researchplanning.com[abejarano@researchplanning.com]; DFrenchMcCay@asascience.com[DFrenchMcCay@asascience.com]; Michel Gielazyn[michel.gielazyn@noaa.gov]; peter.hodson@queensu.ca[peter.hodson@queensu.ca]; rrhopcroft@alaska.edu[rrhopcroft@alaska.edu]; john.incardona@noaa.gov[john.incardona@noaa.gov]; Teri.Rowles@noaa.gov[Teri.Rowles@noaa.gov]; Mathijs.Smit@shell.com[Mathijs.Smit@shell.com]; Sprenger, Mark[Sprenger.Mark@epa.gov]; rstjeerdema@ucdavis.edu[rstjeerdema@ucdavis.edu]; dana@mote.org[dana@mote.org]; john.wise@louisville.edu[john.wise@louisville.edu]; mhziccardi@ucdavis.edu[mhziccardi@ucdavis.edu]; nancy.kinner@unh.edu[nancy.kinner@unh.edu]; doug.helton@noaa.gov[doug.helton@noaa.gov]; gary.shigenaka@noaa.gov[gary.shigenaka@noaa.gov]; Principe, Vanessa[Principe.Vanessa@epa.gov]; Wilson, Gregory[Wilson.Gregory@epa.gov]
From: Mandsager, Kathy
Sent: Tue 2/28/2017 3:56:23 PM
Subject: RE: State-of-Science: Eco-Tox group
2017.02.27_REVISED_Final_EcoToxicity and Sublethal Impacts_pvh_ab_SEA_SH.docx

This is the last call for your final edits and sign-off on this document. If you have not yet reviewed, please use this latest version that may be easier to read. Note yellow highlights where text was altered, missing citations that are still needed, and other panel member comments that need to be addressed.

The deadline for this was 2/27 – your review in a timely manner would be greatly appreciated!

Thank you.

From: Sharon.Hook@csiro.au [mailto:Sharon.Hook@csiro.au]
Sent: Wednesday, February 15, 2017 10:43 PM
To: sarah.allan@noaa.gov; angela_matz@fws.gov
Cc: Mandsager, Kathy <kathy.mandsager@unh.edu>; barron.mace@epa.gov; abejarano@researchplanning.com; DFrenchMcCay@asascience.com; michel.gielazyn@noaa.gov; peter.hodson@queensu.ca; rrhopcroft@alaska.edu; john.incardona@noaa.gov; Teri.Rowles@noaa.gov; Mathijs.Smit@shell.com; Sprenger.Mark@epa.gov; rstjeerdema@ucdavis.edu; dana@mote.org; john.wise@louisville.edu; mhziccardi@ucdavis.edu; Kinner, Nancy <Nancy.Kinner@unh.edu>; doug.helton@noaa.gov; gary.shigenaka@noaa.gov; principe.vanessa@epa.gov; wilson.gregory@epa.gov
Subject: RE: State-of-Science: Eco-Tox group

I've added a few (very minor) edits and comments. I agree with this version.

Sharon

(+61) 2 9710 6839

From: Sarah Allan - NOAA Federal [mailto:sarah.allan@noaa.gov]
Sent: Wednesday, 15 February 2017 9:28 AM
To: Matz, Angela <angela_matz@fws.gov>
Cc: Mandsager, Kathy <kathy.mandsager@unh.edu>; barron.mace@epa.gov; abejarano@researchplanning.com; DFrenchMcCay@asascience.com; michel.gielazyn@noaa.gov; peter.hodson@queensu.ca; Hook, Sharon (O&A, Lucas Heights) <Sharon.Hook@csiro.au>; rrhopcroft@alaska.edu; john.incardona@noaa.gov; Teri.Rowles@noaa.gov; Mathijs.Smit@shell.com; Sprenger.Mark@epa.gov; rstjeerdema@ucdavis.edu; dana@mote.org; john.wise@louisville.edu; mhziccardi@ucdavis.edu; Kinner, Nancy <Nancy.Kinner@unh.edu>; doug.helton@noaa.gov; Gary Shigenaka - NOAA Federal <gary.shigenaka@noaa.gov>; principe.vanessa@epa.gov; wilson.gregory@epa.gov
Subject: Re: State-of-Science: Eco-Tox group

Hi -

I added a few additional edits and citations to the version that Adriana sent.

Thanks,

Sarah

On Tue, Feb 14, 2017 at 11:30 AM, Matz, Angela <angela_matz@fws.gov> wrote:

Thank you, Kathy.

I agree with this version (and subsequent versions with slight edits). Thanks!

Angela

Angela Matz, Ph.D.

Fairbanks Field Office, U.S. Fish and Wildlife Service

c/o AFWCO, 4700 BLM Road Anchorage, AK 99507-2546

angela_matz@fws.gov

Phone: 907-271-2778 Cell: 907--750-8527

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On Fri, Feb 10, 2017 at 11:38 AM, Mandsager, Kathy <kathy.mandsager@unh.edu> wrote:

To the Eco-Tox panel of scientists,

Please find attached a revised "final" version of the document. This version incorporates the many comments, edits and suggestions that were received from the previous version. I have tried to highlight in yellow any text that was changed for your final review. However, there are still a few outstanding issues that need the panel input – please see those in the comments. And please suggest appropriate references as required in several sections.

If you could kindly return any comments and or approval of this document by **Feb 27**, we can get this out on the street for public input by March!

Thank you.

Kathy Mandsager

Coastal Response Research Center

Center for Spills and Environmental Hazards

220 Gregg Hall, 35 Colovos Rd

University of New Hampshire

Durham, NH 03824

603.862.1545

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Sarah E. Allan, PhD
907-271-5146

NOAA Office of Response and Restoration
222 West 7th Ave. Suite 552
Anchorage, AK 99513
~~~~~~~~~~~~~~~~~~~~~~~~~~~~

To: Conmy, Robyn[Conmy.Robyn@epa.gov]; 'fingasmerv@shaw.ca'[fingasmerv@shaw.ca]; 'tchazen@utk.edu'[tchazen@utk.edu]; 'Robert Jones - NOAA Federal'[robert.jones@noaa.gov]; 'Samantha Joye'[mandyjoye@gmail.com]; 'ken.lee@csiro.au'[ken.lee@csiro.au]; 'mbleigh@alaska.edu'[mbleigh@alaska.edu]; 'karl.linden@colorado.edu'[karl.linden@colorado.edu]; 'kmmcfarlin@alaska.edu'[kmmcfarlin@alaska.edu]; msmiles@lsu.edu[msmiles@lsu.edu]; 'thomas.s.coolbaugh@exxonmobil.com'[thomas.s.coolbaugh@exxonmobil.com]; 'Mathijs.Smit@shell.com'[Mathijs.Smit@shell.com]; Sprenger, Mark[Sprenger.Mark@epa.gov]
Cc: Wilson, Gregory[Wilson.Gregory@epa.gov]; Principe, Vanessa[Principe.Vanessa@epa.gov]; 'tracee.nguyen@csiro.au'[tracee.nguyen@csiro.au]; 'Ian Gaudreau'[ipu3@unh.edu]; 'doug.helton@noaa.gov'[doug.helton@noaa.gov]; 'Gary Shigenaka - NOAA Federal'[gary.shigenaka@noaa.gov]
From: Mandsager, Kathy
Sent: Wed 7/8/2015 8:11:58 PM
Subject: RE: Degradation & Fate = document review for next meeting
2015_07_08_Degradation and Fate.docx
Aeppli 2014.pdf
USGS_oil particle interactions and submergence from crude oil spills.pdf

This is a reminder of our call scheduled for tomorrow (Thursday 7/9) beginning at 12ET. (See WebEx details below.)

Attached is an updated copy of our working document as well as 2 publications that require your review.

Thank you!

From: Mandsager, Kathy
Sent: Friday, June 26, 2015 11:39 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate = document review for next meeting

Please find attached the latest version of our document. On our next call, please be prepared to address the comments noted in the text. Since this document is nearing completion, please take the opportunity to read through and offer final edits, comments or approval.

To summarize what is still needed:

1. Robert Jones needs to confirm that he is OK with line 312

2. Please find attached the Aeppli 2014 paper for consideration and discussion if this should be included on line 147

3. If anybody can help us get a pdf of this publication, it would be very helpful:

An integrated model for prediction of oil transport from a deep water blowout
by Spaulding, M.L. (Rhode Island Univ., Narragansett, RI (United States). Ocean Engineering); Bishnoi, P.R. (Calgary Univ., Calgary, AB (Canada). Dept. of Chemical and Petroleum Engineering); Anderson, E.; Isaji, T. (Applied Science Associates Inc., Narragansett, RI (United States))
from Proceedings of the 23. Arctic and Marine Oilspill Program (AMOP) Technical Seminar

From: Mandsager, Kathy
Sent: Monday, June 22, 2015 1:59 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate = schedule next meeting

Please mark your calendar:

This is to confirm our next WebEx meeting on **Thursday, July 9 beginning at 12noon ET**. Please allot 2 hours for this discussion. For your convenience, I will send this information in a calendar request as well.

And we will forward the 'almost final' copy of the document for your review later this week.

You can forward this invitation to others.

Hello,
Nancy Kinner invites you to join this WebEx meeting.

State-of-Science: Degradation & Fate group
Thursday, July 9, 2015
12:00 pm | Eastern Daylight Time (New York, GMT-04:00) | 2 hrs

Join WebEx meeting

Meeting number: 730 289 336

Join by phone
1-855-244-8681 Call-in toll-free number (US/Canada)
1-650-479-3207 Call-in toll number (US/Canada)
Access code: 730 289 336
Global call-in numbers | Toll-free calling restrictions

Can't join the meeting? Contact support.

IMPORTANT NOTICE: Please note that this WebEx service allows audio and other information sent during the session to be recorded, which may be discoverable in a legal matter. By joining this session, you automatically consent to such recordings. If you do not consent to being recorded, discuss your concerns with the host or do not join the session.

From: Mandsager, Kathy
Sent: Friday, June 12, 2015 3:30 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate = schedule next meeting

I would like to schedule another WebEx meeting to continue our discussion of this document. Please use this doodle poll http://doodle.com/38rcrdrbd9kemq3t to select all appropriate 2-hour slots that you can possibly attend. The times are listed in EDT, but should be time-zone enabled for your location. Please submit your options by end of next week (6/19) so we can confirm a date as soon as possible, as I know vacations are underway and we may miss some folks.

Thank you!

From: Mandsager, Kathy
Sent: Wednesday, April 29, 2015 4:42 PM
Subject: RE: Degradation & Fate - next meeting 4/30/2015 at 1 ET

This is a reminder of our WebEx meeting tomorrow. (Login instructions below)

As you review this document, please help us in securing the accurate references. For example, we may have Su et al,., 2013 in our notes (we don't have this paper) but we are wondering if it should really be Sun et al., 2013 (we have this paper). And there may be several papers by Prince but we need to make sure the correct paper is noted accurately in the text.

Also, you might wish to search for your name in case we are looking for specific information from you.

Thank you to each of you for your input into this document!

From: Mandsager, Kathy
Sent: Wednesday, April 22, 2015 1:29 PM
Subject: RE: Degradation & Fate update -COMMENT on document - next meeting 4/30/2015

This is a reminder to submit any comments that you may have as soon as possible. Please mark your calendar for our next (potentially final) meeting for **Thursday 30 April at 1 – 3 ET**. WebEx instructions below.

Degradation & Fate Group: State-of-Science Dispersant in the Arctic
Thursday, April 30, 2015
1:00 pm | Eastern Daylight Time (New York, GMT-04:00) | 2 hrs

Join WebEx meeting

Meeting number: 732 633 609

Join by phone
1-855-244-8681 Call-in toll-free number (US/Canada)
1-650-479-3207 Call-in toll number (US/Canada)

Access code: 732 633 609
Global call-in numbers | Toll-free calling restrictions

From: Mandsager, Kathy
Sent: Monday, April 06, 2015 10:13 AM
Subject: RE: Degradation & Fate update -COMMENT on document

My apologies – I hit send before I was done.

Dear Degradation and Fate Group

Please find attached the latest draft document for your review. We would ask that you review this very carefully and add any comments you may have to the spreadsheet that is also attached. Please especially note any items with yellow highlights, red lettering or with your name noted specifically.

And finally, this is your opportunity (as you review this whole document) to let us know if any topic has been overlooked in our group discussions thus far.

Please return your comments to me (kathy.mandsager@unh.edu) by **Friday 4/17.**

And please submit your times on the doodle poll to secure our next and final call>>
http://doodle.com/pgh4enphe24wqe24

From: Mandsager, Kathy
Sent: Monday, April 06, 2015 10:10 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu';

'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET

We are nearing the conclusion! Please review the edits to this document.

And we would like to schedule a final call to discuss these comments.

Please select all times that you can possibly participate. Doodle poll here>>
http://doodle.com/pgh4enphe24wqe24

From: Mandsager, Kathy
Sent: Tuesday, March 31, 2015 11:14 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET

Additional paper for review. http://ac.els-cdn.com/S0025326X15000855/1-s2.0-S0025326X15000855-main.pdf?_tid=a972873c-d7b7-11e4-9464-00000aab0f27&acdnat=1427814635_2731a355cf42e8b64679699bc221613

From: Mandsager, Kathy
Sent: Monday, March 30, 2015 4:43 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'; Mandsager, Kathy
Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET

This is a reminder of our WebEx meeting tomorrow.

From: Mandsager, Kathy
Sent: Friday, March 20, 2015 6:19 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET

Mark your calendar! This is to confirm our next meeting for **Tuesday, March 31** beginning at 11:00 am ET.

Her are the WebEx directions to join this meeting. Thank you!

Degradation & Fate Group
Tuesday, March 31, 2015
11:00 am | Eastern Daylight Time (New York, GMT-04:00) | 2 hrs

Join WebEx meeting

Meeting number: 734 916 289

Join by phone
1-855-244-8681 Call-in toll-free number (US/Canada)
1-650-479-3207 Call-in toll number (US/Canada)
Access code: 734 916 289
Global call-in numbers | Toll-free calling restrictions

Can't join the meeting? Contact support.

From: Mandsager, Kathy
Sent: Thursday, March 19, 2015 8:31 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION

I greatly appreciate your patience with this process. Please **cancel** the tentative hold on your calendar for tomorrow 3/20. Since we would greatly appreciate a majority of this group to participate in this 2-hour WebEx meeting, I am making another attempt at scheduling this call. Please use this doodle poll (all times noted are ET). Thank you to each of you!

Doodle poll here>> http://doodle.com/2w3gyb3gv4sv7iek

From: Mandsager, Kathy
Sent: Monday, March 16, 2015 4:39 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION

In the spirit of trying to keep everyone informed – there will NOT be a meeting tomorrow at 2

(see request below). I was unable to gain a quorum. Please continue to hold this Friday 3/20 as a potential meeting time. And if you have not yet responded with your availability, please let me know if you could possibly make in on **3/20 beginning at 1pm ET.**

Thank you.

From: Mandsager, Kathy
Sent: Monday, March 16, 2015 9:38 AM
To: 'Ian P Gaudreau'; 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION
Importance: High

While these papers are fresh on your mind, I am offering some dates as soon as possible for our next meeting. Kindly let me know if you can make yourself available for about a 2-hour timeslot at any of the following times:

Tuesday (tomorrow) 3/17 beginning at 2:00 pm ET

Friday 3/20 beginning at 12noon ET

Friday 3/20 beginning at 1:00 pm ET

Friday 3/20 beginning at 2:00 pm ET

Friday 3/20 beginning at 3:00 pm ET

Thursday 3/26 at 10:00 am ET

Monday 3/30 beginning at 2:20 pm ET

A speedy reply will be greatly appreciated. Thank you!

From: Ian P Gaudreau [mailto:ipu3@wildcats.unh.edu]
Sent: Friday, March 13, 2015 10:16 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: Lindsey R Howard; Mandsager, Kathy; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; tracee.nguyen@csiro.au; Ian Gaudreau
Subject: Degradation & Fate update - Official Papers

Hi folks,

Thank you for hanging in there on the conference call Wednesday. We really appreciate all of the hard work you have been doing. The 13 official papers you decided you would review and use as the basis for your comments (e.g. knowns, uncertainties) on the effect of chemical dispersants on oil biodegradation are:

- Venosa & Holder 2007
- Prince et al. 2013
- Prince & Butler 2014
- Mcnaughton et al. 2003
- McFarlin et al. 2014
- Mason et al. 2012
- Lindstrom & Braddock 2002
- Kleindienst et al. 2015
- Chakroborty et al. 2012
- Campo et al. 2013
- Bruheim et al. 1997
- Bruheum et al. 1999
- Baelum et al. 2012

These papers are all located in a folder on BOX entitled "Papers selected by panel - Effect of Chemical Dispersants on Oil Biodegradation". This link can be found here: https://unh.app.box.com/files/0/f/3252405678/Papers_selected_by_panel_-_Effect_of_Chemical_Dispersants_on_Oil_Biodegradation

Kathy will be contacting you to schedule what will hopefully be our final meeting of the Degradation & Fate group.

In preparation for the meeting, please re-read the paper and if you have time, send Kathy the conclusions that you derive from these references regarding what is known and uncertain about the effect of chemical dispersants on the biodegradation of oil, particularly as it applies to the Arctic.

Also, we need your feedback on a paper by Harris et al. 2002 (paper is attached in this email). Would you like us to add this paper to the "Papers selected by panel - Effect of Chemical Dispersants on Oil Biodegradation" folder on BOX? Here is the citation:

Harris, B.C. et al. 2002. Nutrient effects on the biodegradation rates of chemically-dispersed crude oil. *In* Twenty-Fifth Arctic and Marine Oilspill Program (AMOP) Technical Seminar, Nineteenth Technical Seminar on Chemical Spills (TSOCS) and Fourth Biotechnology Solutions for Spills (BIOSS): June 11 to 13, 2002, Westin Calgary Hotel, Calgary, Alberta, Canada: Proceedings. Ottawa, Ont.: Environment Canada. pp. 877-893.

Thanks again for your time and energy. I look forward to talking with you again.

Nancy E. Kinner, Ph.D.

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Professor Civil/Environmental Engineering

UNH Director, Coastal Response Research Center

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Recalcitrance and Degradation of Petroleum Biomarkers upon Abiotic and Biotic Natural Weathering of Deepwater Horizon Oil

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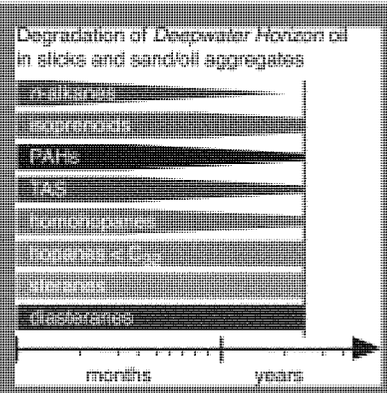
* Supporting Information

Petroleum biomarkers such as hopanoids, steranes, and triaromatic steroids (TAS) are commonly used to investigate the source and fate of petroleum hydrocarbons in the environment based on the premise that these compounds are resistant to biotic and abiotic degradation. To test the validity of this premise in the context of the Deepwater Horizon disaster, we investigated changes to these biomarkers as induced by natural weathering of crude oil discharged from the Macondo Well (MW). For surface slicks collected from May to June in 2010, and other oiled samples collected on beaches in the northern Gulf of Mexico from July 2010 until August 2012, hopanoids with up to 31 carbons as well as steranes and diasteranes were not systematically affected by weathering processes. In contrast, TAS and C_{27}- to C_{35}-homohopanes were depleted in all samples relative to 17α(H),21β(H)-hopane (C_{30}-hopane). Compared to MW oil, C_{35}-homohopanes and TAS were depleted by 18 ± 10% and 36 ± 20%, respectively, in surface slicks collected from May to June 2010, and by 37 ± 9% and 67 ± 10%, respectively, in samples collected along beaches from April 2011 through August 2012. Based on patterns of relative losses of individual compounds, we hypothesize biodegradation and photooxidation as main degradation processes for homohopanes and TAS, respectively. This study highlights that (i) TAS and homohopanes can be degraded within several years following an oil spill, (ii) the use of homohopanes and TAS for oil spill forensics must account for degradation, and (iii) these compounds provide a window to parse biodegradation and photooxidation during advanced stages of oil weathering.



INTRODUCTION

Petroleum biomarkers (i.e., molecular fossils) are commonly used to define the identity of spilled oil[1] and further serve as recalcitrant tracers for weathering studies.[2,3] For example, these compounds have previously been used to study the fate of oil residues in the northern Gulf of Mexico,[4−7] following the explosion of the Deepwater Horizon (DWH) oil rig on April 20, 2010, that led to a release of 6.8 ± 1.7 × 10^8 kg of petroleum hydrocarbons from the Macondo Well (MW).[8,9] While soluble and volatile hydrocarbons were dissolved in the water column or evaporated to the atmosphere, it has been estimated that approximately 10% of the leaked mass formed surface oil slicks that eventually oiled beaches.[9] This amount corresponds to roughly 70,000 t (approximately 500,000 barrels), mostly petroleum hydrocarbons larger than n-pentadecane (n-C_{15}), and is more than twice the estimated release from the 1989 Exxon Valdez oil spill.[10] Given the magnitude of release and the extent of oiled shoreline, reliable fingerprinting methods are clearly needed to distinguish MW oil from other crude oil sources such as natural seeps[11] or accidental releases associated with oil production, transportation, and usage.

Most oil spill fingerprinting relies on the fidelity and variability of hopanoids, steranes and TAS (Figure 1), which are present in crude oils and some of their refined products.[1] The use of these petroleum biomarker compounds for oil spill forensics is an extension of petroleum geochemistry.[12] These compounds are generally assumed to be more recalcitrant toward microbial and abiotic degradation than other saturated (such as n-alkanes, branched alkanes, and isoprenoids) and aromatic compounds (benzene, alkylated congeners of benzene, and polycyclic aromatic hydrocarbons, PAHs).[12,13] In laboratory studies, 17α(H),21β(H)-hopane (I in Figure 1; referred to as "C_{30}-hopane" hereafter) has proven to be remarkably recalcitrant against biodegradation[2,3] and photooxidation.[14] However, biodegradation of C_{30}-hopane and other biomarkers was achieved under aggressive laboratory conditions using aerobic enrichment cultures.[15−17] Degradation of

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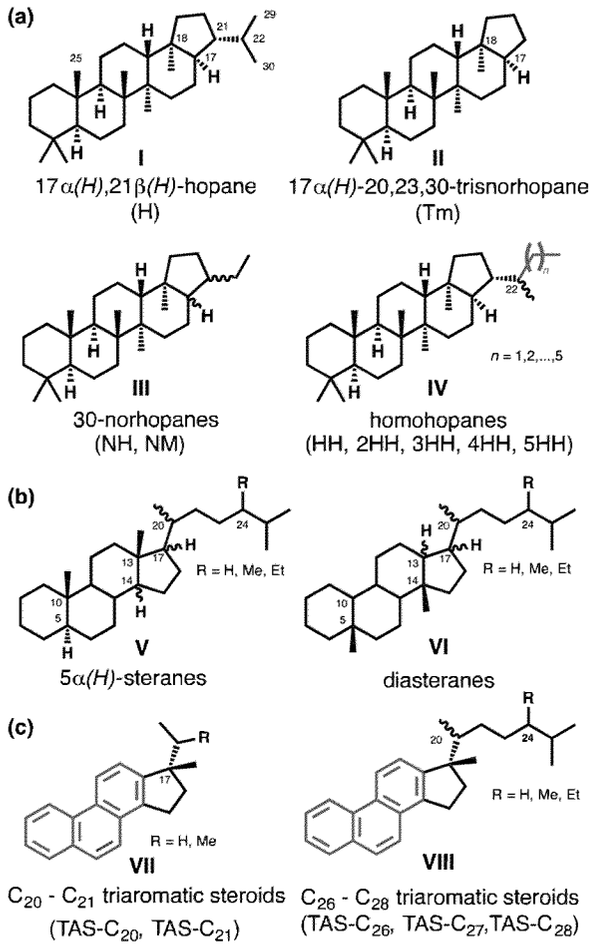

Figure 1. Structures of targeted petroleum biomarkers: (a) hopanoids, (b) steranes and diasteranes, and (c) triaromatic steroids (TAS). See Table S1 for names, abbreviations, and structures of all compounds discussed in this study. The straight alkyl chain of homohopanes and the phenanthrene backbone of TAS compounds (colored in blue) are likely targets of biodegradation and photooxidation, respectively.

biomarker compounds was also observed in several long-term field studies (an overview is given in Figure S1 of the Supporting Information, SI). For example, eight years after an oil spill experiment in a mangrove soil on Guadeloupe (Lesser Antilles), total steranes and hopanes were depleted >25%.[18] Similar observations were made 20 years after an Arctic oil spill experiment,[19,20] 22 years after the Arrow spill on the Nova Scotia coast,[21] 24 years after the Metula spill in the Straight of Magellan,[22] and in oiled soil samples from oil production areas[17] and from natural seeps.[23]

Most of the above-mentioned studies were conducted at latitudes higher than the Gulf of Mexico, and it is of interest to know how the higher average temperatures and intense solar irradiation affected biomarker degradation of MW oil. We previously reported extensive degradation of saturated and aromatic compounds of MW oil within 18 months after the spill in oiled samples collected on beaches, with n-alkanes, isoprenoids, and two-ring PAHs being completely removed in most samples.[5,24] Furthermore, we observed TAS depletion in a limited data set of oil-covered rock samples,[25] and lower amounts of steranes relative to hopanoids in samples recovered from deep-sea corals.[7] This alteration of biomarker compounds in relatively short time scales (months to years) prompted us to investigate systematically abiotic and/or biotic natural degradation of biomarker compounds in MW oil.

The aim of this study was therefore to assess the recalcitrance and degradation of biomarker compounds in MW oil that has undergone natural weathering processes including dissolution, evaporation, biodegradation, and photooxidation. In order to span a large spectrum of weathering, we revisited previously described MW oil, surface slicks, oil-soaked sand, and oil-covered rock samples[4,5,8] and added newer samples collected up to 28 months after the Deepwater Horizon disaster. We used comprehensive two-dimensional comprehensive gas chromatography (GC×GC) for biomarker analysis.[26,27] As noted in recent studies, GC×GC is superior to one-dimensional gas chromatography with mass spectral detection (GC-MS) in terms of analytical resolution, selectivity, and sensitivity[28] and allows for accurate quantification of biomarkers in a complex mixture of petroleum hydrocarbons.[29] Using this technique, we found that hopanoids consisting of 27 to 31 carbon atoms as well as steranes and diasteranes were recalcitrant toward weathering. However, we observed substantial losses of homohopanes with more than 31 carbon atoms as well as of TAS, over 28 months of weathering.

MATERIALS AND METHODS

Samples. A total of 50 oiled samples collected in the Gulf of Mexico during April 2010 through August 2012 were analyzed. This sample set comprises MW oil collected from the well on June 21, 2010,[8] nine surface slicks and oiled floating DWH debris samples collected on the sea surface during the spill,[4,5] and samples washed on beaches (31 oil-soaked sand samples or "sand patties", six oil samples scraped off rocks or "rock scrapings", and four sand-free asphalt pieces), some of which were previously described.[5] Sand patties, rock scrapings, and asphalt pieces were extracted in dichloromethane (DCM) and methanol (90/10 v/v). All samples were dissolved in DCM or DCM/methanol at concentrations of 10−50 mg mL^{-1} for analysis. See Table S3 of the SI for sampling dates and locations.

Beside MW oil and field samples, five reference crude oils were analyzed: (i) a surrogate for MW oil provided by BP, produced from the Marlin Platform on Viosca Knoll lease block 915 (VK915),[30] located 60 km NE of the MW; (ii) the EPA-API reference WP#681 Southern Louisiana Sweet crude (SLSC);[31] (iii) crude oil from Eugene Island lease block 330 (EI330), located 340 km W of the MW; (iv) the petroleum standard reference material (SRM) 1582 from the National Institute of Standards and Technology (NIST), which is a Wilmington crude from the Monterey formation; and (v) cargo oil from the Exxon Valdez (EVC).

Comprehensive Two-Dimensional Gas Chromatography-FID (GC×GC-FID). We used GC×GC-FID methods described in detail previously.[5,32] Briefly, 1 μL of each sample in DCM or DCM/methanol (90/10) was injected in a GC×GC-FID system with a dual stage cryogenic modulator (Leco, Saint Joseph, MI), equipped with a Restek Rtx-1 first-dimension column (60 m, 0.25 mm ID, 0.25 μm film thickness) and a SGE BPX-50 s-dimension column (1.5 m, 0.10 mm ID, 0.10 μm film thickness). The inlet temperature was held at 300 °C. The injection mode was splitless, and the carrier gas was H_2 at a constant flow rate of 1.00 mL min^{-1}. The first oven was programed isothermal at 40 °C for 10 min, 40 to 340 °C at 1.25 °C min^{-1} (held for 5 min). The second oven was programmed

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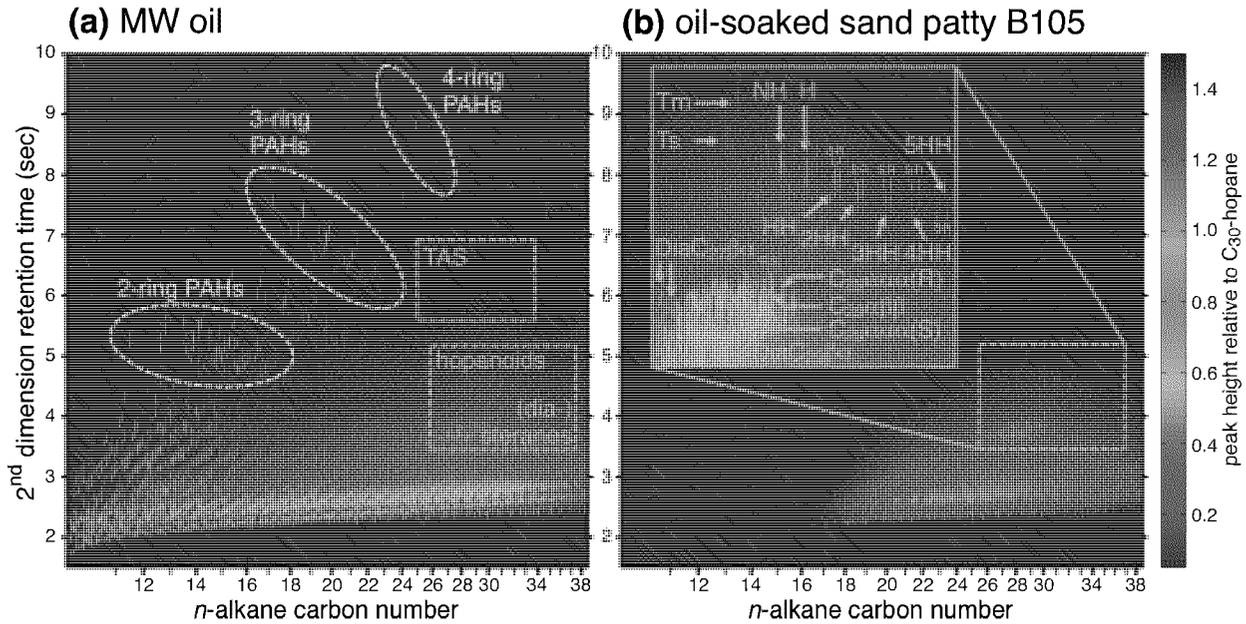

Figure 2. (a) GC×GC-FID chromatograms of MW oil and (b) sand patty B105 collected August 2012 in Gulf Shores, AL. Two biomarker regions (with hopanoids, (dia)steranes, and TAS), as well as PAH elution regions are depicted. Many compounds originally present in MW oil have been removed, but the hopanoid and (dia)sterane region remained largely unaffected. An enlargement of the hopanoid and sterane region with select annotated peaks is given (see Table S1 of the SI for compound names).

as follows: isothermal at 45 °C for 10 min, 45 to 355 °C at 1.29 °C min^{-1} (held for 5 min). The modulation period was 15 s.

Biomarker compounds were identified based on established elution order[27,28] and using authentic standards obtained from Sigma-Aldrich, Chiron, and NIST SRM 2266 (Table S2 of the SI). In addition, GC×GC coupled to time-of-flight mass spectrometry (GC×GC-TOFMS, Leco, Saint Joseph, MI; operated according to ref 33) was used to confirm the identity of compounds based on their mass spectra. See Figure 2 and Figure S2 of the SI for sample GC×GC-FID chromatograms. GC×GC-FID peaks were manually integrated using the ChromaTOF software (Leco). We targeted 17 hopanoids, 13 steranes and diasteranes, and eight TAS indicated in Figure S2 and Table S1 of the SI. We also included several PAHs (phenanthrene, C_1−C_3 phenanthrenes/anthracenes, chrysene, C_1−C_3 chrysene/benz[a]anthracenes), alkanes (n-heptadecane; n-C_{17}, n-octadecane; n-C_{18}), and isoprenoids (pristane and phytane) in our analyses.

Quality control included a linearity test of the FID responses by injecting the NIST SRM 2266 at five concentrations within our operating range. We obtained a linear calibration curve for the seven certified biomarkers present in SRM 2266 (R^2 > 0.999; Table S2 of the SI). Furthermore, all seven compounds had identical response factors (i.e., signal per mass unit biomarker injected), with a relative standard deviation (RSD) of <6%, and these response factors were stable over an investigated instrument run time of 10 days (RSD < 18%; Table S2 of the SI). To investigate the combined analytical uncertainty and the uncertainty introduced by manual integration, we determined 24 ratios of biomarkers peak volumes for eight individual GC×GC-FID injections of the MW oil, measured over a time period of one month (Table 1). For these eight replicates, we obtained RSDs of 2 to 20% for the 24 ratios (average RSD of 11%). Finally, we periodically analyzed solvent blanks, which did not have any detectable compounds, and had the MW oil externally analyzed for biomarkers by GC-MS at GeoMark Research, Ltd. (Houston, TX) and Alpha Analytical Laboratories (Mansfield, MA).

GC-MS Analysis. Biomarkers were also measured using GC-MS for 28 samples. We used an Agilent 6890 GC with a 5973 MSD, 1 µL sample injection (splitless), He as carrier gas (10.5 psi constant pressure), and the following GC temperature program: 4 min at 40 °C and ramped to 320 at 5 °C min^{-1} (held 15 min). The mass traces m/z 191, 217, and 218 were recorded in selected-ion-monitoring mode.

Determination of the Oxygenated Hydrocarbon. As a general weathering indicator, we quantified the fraction of oxygenated hydrocarbons (OxHC) of the oil samples using thin layer chromatography-FID (TLC-FID).[5] Briefly, the samples were spotted on the base of a silica-gel-sintered glass rod and sequentially developed in hexane, toluene, and DCM/methanol (97/3) to different heights on the TLC-rod. FID scanning of the rods using an Iatroscan MK-5 TLC-FID (Iatron, Tokyo, Japan) gave four distinct peaks defined as the saturated, aromatic, $OxHC_1$, and $OxHC_2$ fractions (for the discussion in this study, we combined the two OxHC classes OxHC = $OxHC_1$ + $OxHC_2$). It has been shown that OxHC increased on a relative as well as on an absolute basis (relative to C_{30}-hopane) with progressing natural weathering of MW oil.[5] This absolute increase signifies that OxHC were transformation products of oil degradation and not just preferentially enriched.

Calculations. The fractional loss of a compound X in a sample was calculated by normalizing to C_{30}-hopane (H; compound I in Figure 1) and comparing to the corresponding value in the MW oil, according to eq 1[2,20]

$$Loss_X = 1 - \frac{X_{sample}/H_{sample}}{X_{MW}/H_{MW}} \quad (1)$$

where X_{sample} and H_{sample} are the peak volumes of compound X and C_{30}-hopane, respectively, in the GC×GC-FID chromatogram of a specific sample.

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Table 1. Diagnostic Biomarker Ratios of the MW Oil, Field Samples, and Reference Crude Oils

		MW and surrogate oil[b]		field samples[c]		reference crude oils[d]			
no.	ratio[a]	MW (8 replicates)	VK915 (5 replicates)	surface, sand, rock (n = 46)	asphalt (n = 4)	SLSC (n = 1)	EI330 (n = 1)	NIST (n = 1)	EVC (n = 1)
Hopanoid/Hopanoid Ratios									
1	Ts/Tm	1.4 ± 0.1	1.6 ± 0.1	1.4 ± 0.1	1.0 ± 0.1	0.78	0.93	0.53	0.81
2	Ts/H	0.24 ± 0.02	0.26 ± 0.05	0.27 ± 0.02	0.20 ± 0.02	0.28	0.28	0.10	0.20
3	M/H	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.10 ± 0.02	0.15	0.09	0.15	0.07
4	M/NM	1.4 ± 0.2	1.0 ± 0.1	1.4 ± 0.1	1.1 ± 0.2	0.60	0.82	0.73	1.5
5	HH(R)/HH(S)	0.72 ± 0.02	0.73 ± 0.03	0.72 ± 0.03	0.77 ± 0.06	0.77	0.73	0.74	0.68
6	2HH(S)/H	0.34 ± 0.01	0.29 ± 0.05	0.30 ± 0.03	0.34 ± 0.06	0.20	0.39	0.29	0.42
7	5HH(R)/5HH(S)	0.68 ± 0.08	0.71 ± 0.06	0.67 ± 0.07	0.63 ± 0.01	0.74	0.66	0.76	0.65
8[e]	3HH(R+S)/H	0.43 ± 0.03	0.35 ± 0.05	0.34 ± 0.03	0.41 ± 0.08	0.18	0.43	0.47	0.53
9[e]	5HH(R+S)/H	0.20 ± 0.02	0.15 ± 0.02	0.14 ± 0.02	0.23 ± 0.08	0.07	0.23	0.26	0.40
(Dia)sterane/(Dia)sterane Ratios									
10	$DiaC_{27}\beta\alpha(S)/DiaC_{27}\beta\alpha(R)$	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.3	1.4	0.82	1.5
11	$DiaC_{27}\beta\alpha(R)/DiaC_{29}\beta\alpha(R)$	0.65 ± 0.13	0.82 ± 0.1	0.79 ± 0.06	0.39 ± 0.10	0.71	0.58	0.58	0.47
12	$DiaC_{27}\beta\alpha(R)/C_{29}\alpha\alpha\alpha(S)$	2.0 ± 0.2	2.4 ± 0.2	2.1 ± 0.2	0.62 ± 0.21	2.0	1.2	0.89	2.3
13	$C_{29}\alpha\beta\beta(R+S)/C_{29}\alpha\alpha\alpha(R+S)$	1.7 ± 0.2	1.5 ± 0.1	1.5 ± 0.2	1.8 ± 0.2	1.6	1.8	1.04	2.9
14	$C_{29}\alpha\alpha\alpha(S)/C_{29}\alpha\beta\beta(R+S)$	0.37 ± 0.05	0.29 ± 0.02	0.42 ± 0.05	0.32 ± 0.05	0.35	0.30	0.47	0.14
15	$C_{29}\alpha\alpha\alpha(S)$/sum($\alpha\beta\beta$ steranes)	0.18 ± 0.03	0.14 ± 0.01	0.19 ± 0.02	0.18 ± 0.04	0.17	0.16	0.13	0.08
16	$C_{27}\alpha\beta\beta(S)$/sum($\alpha\beta\beta$ steranes)	0.18 ± 0.03	0.17 ± 0.02	0.21 ± 0.02	0.14 ± 0.02	0.21	0.14	0.10	0.14
(Dia)sterane/C_{30}-Hopane Ratios									
17	$DiaC_{27}\beta\alpha(R)/H$	1.0 ± 0.2	0.70 ± 0.16	1.2 ± 0.2	0.19 ± 0.09	0.44	0.38	0.65	0.36
18	$C_{28}\alpha\beta\beta(R+S)/H$	0.96 ± 0.17	0.75 ± 0.19	1.1 ± 0.1	0.49 ± 0.02	0.40	0.68	3.56	0.54
19	$C_{29}\alpha\alpha\alpha(S)/H$	0.53 ± 0.08	0.30 ± 0.08	0.58 ± 0.06	0.31 ± 0.14	0.22	0.32	0.74	0.16
TAS/TAS Ratios									
20	$TAS\text{-}C_{20}/TAS\text{-}C_{28}(R)$	1.6 ± 0.2	0.97 ± 0.15	1.6 ± 0.3	0.27 ± 0.09	0.43	0.61	0.51	0.92
21	$TAS\text{-}C_{28}(S)/TAS\text{-}C_{28}(R)$	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.2	1.4	1.2	0.98	0.98
22	$TAS\text{-}C_{27}(R)/TAS\text{-}C_{28}(R)$	0.86 ± 0.05	0.83 ± 0.09	0.80 ± 0.07	0.45 ± 0.11	0.87	1.0	1.99	1.2
TAS/C_{30}-Hopane Ratios									
23[e]	$TAS\text{-}C_{20}/H$	0.20 ± 0.03	0.10 ± 0.03	0.08 ± 0.05	0.03 ± 0.01	0.02	0.12	0.14	0.14
24[e]	$TAS\text{-}C_{28}(R)/H$	0.14 ± 0.01	0.12 ± 0.02	0.05 ± 0.02	0.12 ± 0.06	0.05	0.13	0.25	0.13

[a]See Table S1 of the SI for full name of the compounds. [b]Average (±RSD) of 8 measurements of a MW oil and 5 measurements of the surrogate oil VK915. [c]Average (±RSD) of 9 surface slick, 30 oil soaked sand, 6 rock scraping, and 4 sand-free asphalt pieces described in Table S2 of the SI. [d]Reference oils: Southern Louisiana Sweet crude (SLSC), Eugene Island lease block 330 crude oil (EI330), NIST 1582 petroleum standard reference material (NIST), and Exxon Valdez cargo crude oil (EVC). [e]Ratios that are affected by biomarker weathering on the observed time scale.

Diagnostic biomarker ratios given in Table 1 were calculated for all samples and crude oil standards. Principal component analysis of biomarker ratios was performed using the princomp method of the R software package (www.r-project.org).

RESULTS AND DISCUSSION

GC×GC-FID Detection of Biomarker Compounds. Hopanoids, steranes, diasteranes, and TAS were analyzed on GC×GC-FID in nine surface slicks, 31 sand patties (also referred to as surface residual balls, SRBs[34]), and six rock scrapings (Table S3 of the SI). Such samples were previously linked to MW oil.[5] In Figure 2, the elution of petroleum hydrocarbons in the employed GC×GC column configuration is shown. Compounds are separated according to vapor pressure along the x-axis due to the apolar first-dimension column and according to polarity along the y-axis due to the medium-polar second-dimension column. GC×GC allows for a chromatographic separation of individual biomarkers (Figure 2), which would coelute with other petroleum hydrocarbons in traditional one-dimensional GC.[28] See Figure 1 and Table S1 of the SI for structures and names, respectively, of the discussed compounds.

Using two-dimensional chromatographic separation has three major advantages over one-dimensional GC techniques. First, GC×GC allows for superior chromatographic separation of compounds and thus avoids interference. For example, we found unknown compounds in MW oil that coelute in the first dimension with the biomarker Tm (Figure S4). Both compounds yield a fragment at m/z 191. Using one-dimensional GC-MS, the unknown compound and Tm were not separated. Hence, the frequently employed ratio of the biomarkers Ts/Tm determined by GC-MS is lower than Ts/Tm determined by GC×GC. This can be illustrated with MW oil: GC-MS analysis of MW oil gives a ratio of 1.1 (Figure S3, Table S5), while GC×GC analysis allows for chromatographic separation of Tm and the unknown compound, resulting in Ts/Tm of 1.4 (Table 1, Table S5). We observed a similar coelution for several steranes and diasteranes in the m/z 217 ion trace (e.g., for $DiaC_{29}\text{-}\beta\alpha(S)$ and $C_{27}\alpha\beta\beta(R)$; data not shown).

Second, GC×GC-FID allows direct calculation of biomarker ratios without the need to quantify each compound via external standards. This approach is valid because we obtained the same FID response factors for sterane as well as hopane biomarkers (see the Method section and Table S2). We also found a constant FID response factor for a variety of saturated and aromatic hydrocarbons with 10 to 40 carbons (including TAS; data not shown). In contrast, GC-MS response factors are generally variable among different compounds and instruments,

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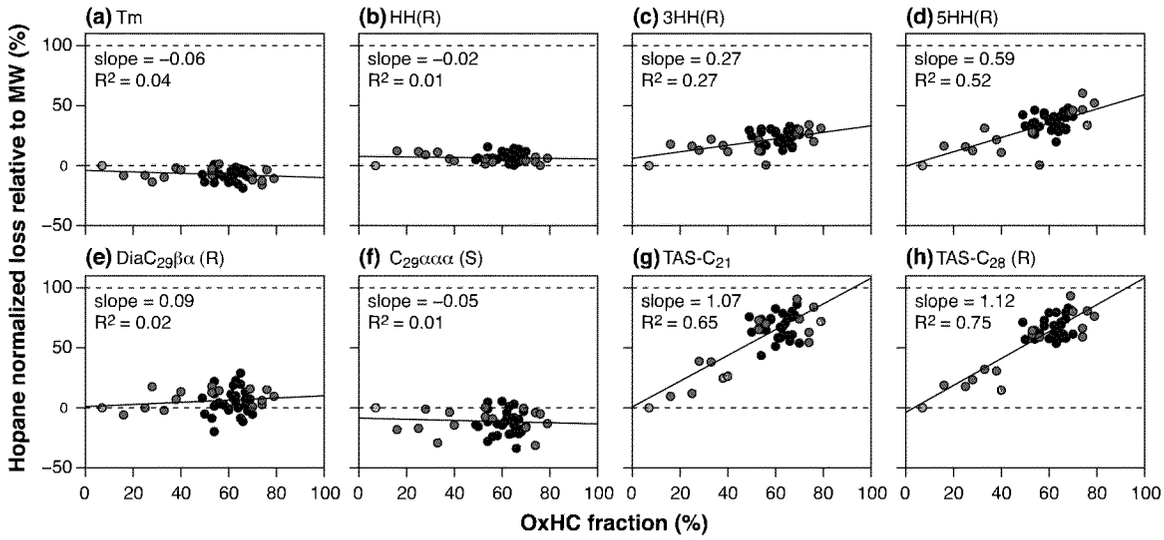

Figure 3. Calculated losses relative to MW (determined by C_{30}-hopane normalization, eq 1) vs degree of weathering, as measured by amount of oxygenated fraction (OxHC). The lower and upper dashed lines represent 0 and 100% loss of compounds relative to MW, respectively. Shown are MW oil (yellow circles), surface slicks (blue circles), sand patties (black circles), and rock scrapings (red circles). The solid lines are linear regressions for the combied data set of all samples (with slopes and R^2 values indicated). (a-d) Hopanoids with ≤ C_{31} were not depleted relative to C_{30}-hopane during natural oil weathering. However, significant degradation of 3HH (C_{33}) through 5HH (C_{35}) was observed. (e-f) No significant trend in loss of diasteranes and steranes was observed. (g-h) With increasing oil weathering, all TAS compounds were depleted relative to C_{30}-hopane, with loss >50% for most sand patties and rock scrapings.

as the MS fragmentation pattern is highly specific for individual compounds and is dependent on the condition of the ionization source.[35] Furthermore, GC-MS biomarker analysis is frequently performed semiquantitatively, i.e., by calculating ratios of mass fragments without external standards for each compounds.[35] Consequently, biomarker ratios determined by GC-MS do not accurately reflect the relative concentrations of the compounds in the sample and are more prone to vary among different laboratories. As example, this issue arose with Ts/Tm in these samples. While Geomark and our GC-MS measurement resulted in Ts/Tm of 1.1 for MW oil, Mulabagal et al. recently reported a ratio of 0.9 (also using GC-MS).[36] Similarly, biomarker analysis by an analytical laboratory using GC/MS without external TAS standards led to an overestimation of TAS concentrations by a factor of eight, as compared to our GC×GC-FID results (data not shown). In contrast, our approach using GC×GC-FID should be more consistent across different users. While we argue that GC×GC-FID provides advantages over GC-MS we are not implying that GC-MS is inaccurate; when standardized protocols are followed for GC-MS analysis of biomarker ratios (such as the "Nordtest method"[37]), round-robin test laboratories routinely determined consistent results.[38]

Third, because GC×GC separates compounds according to two distinct physical properties, the elution space of compounds on the GC×GC plane provides information about their physical properties or structures. For example, the unknown compound that coelutes with Tm in the first dimension elutes very close to C_{28}αααα(S) in GC×GC. Therefore, the unknown compound is expected to have a similar structure or physical properties to this C_{28}-sterane.

To compare biomarker results of GC×GC-FID and GC-MS, we performed GC-MS analysis of hopanoids and (dia)steranes for 24 field samples (Table S5 of the SI). For ratios of hopanoids, we observed similar biomarker ratios with GC-MS and GC×GC-FID. In contrast, the two methods produce remarkably different sterane/hopanoid ratios. Note that it might be counterintuitive to compare GC×GC-FID (rather than GC×GC-TOFMS) to GC-MS. However, due to the superior chromatographic resolution of GC×GC, FID detection is sufficient and better suited for biomarker analysis than TOFMS detection, due to the high sensitivity and the constant response factor of the FID.

Relative Enrichment of Biomarker Compounds During Weathering. We investigated weathering of MW oil on the sea surface as well as on beaches. Natural weathering changed the composition of the spilled oil, as shown in the GC×GC chromatograms of MW oil and a representative oil-soaked sand sample collected on the beach of Gulf Shores, AL more than two years after the onset of the spill (Figure 2). Two robust trends are apparent by visual comparison. First, the oil-soaked sand sample had lost volatile compounds (i.e., having early GC fist-dimension retention times) due to evaporation, including all compounds with less than 15 carbons.[5] Similarly, the more polar compounds (i.e., having late GC second-dimension retention times) were lost to a greater extent than less polar ones due to preferential disolution.[39] For example, we observed a preferential depletion of phenanthrene relative to alkylated (and less polar) phenanthrenes.[5] Second, weathering led to a depletion of specific compound classes, including n-alkanes. In contrast, the (dia)steranes and hopanoids qualitatively appear to be much less affected by weathering, representing a relative enrichment of the biomarker compounds in the weathered oil samples.

Persistence of Hopanoids and Steranes. To investigate quantitatively the persistence of biomarker compounds, we correlated biomarkers in the surface slicks, sand patties, and rock scrapings (Table S3) with their degree of oil weathering. As a proxy for weathering, we used the relative amount of OxHC. We previously reported that this operationally defined oil fraction was a suitable indicator for gauging weathering of MW oil.[5]

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When the C_{30}-hopane normalized compounds (eq 1) are compared to OxHC, their loss due to weathering can be visualized (Figure 3). For hopanoids containing 27 to 31 carbon atoms, such as Tm ($C_{27}H_{46}$) or HH ($C_{31}H_{54}$), no significant loss with weathering was observed for all investigated surface slicks, sand patties, and rock scrapings (Figure 3a-c). Consequently, no weathering-dependent change in Ts/Tm was observed either (Figure S5). Interestingly, such a weathering-dependent Ts/Tm change has previously been observed on longer time scales, e.g., 24 years after an oil spill in the Strait of Magellan,[22] eight years after an artificial oil spill in a Mangrove soil,[18] and in laboratory experiments.[15,16]

Similar to hopanoids ≤ C_{31}, no trend in loss with increasing weathering was observed for steranes and diasteranes (Figure 3e-f). We also did not observe preferential degradation of C_{27} > C_{28} > C_{29} steranes in the investigated samples (Figure S5). In contrast to our study, a susceptibility of steranes over hopanoids as well as a preferential degradation of C_{27} steranes over C_{29} steranes has been observed on time scales of 8 to 24 years (see Figure S1 of the SI for an overview of past studies).[18,19,21,22]

Biodegradation of Homohopanes. In contrast to the persistence of Ts and Tm relative to H, we found preferential losses of homohopanes > C_{31}, such as 3HH ($C_{33}H_{58}$) or 5HH ($C_{35}H_{62}$), relative to C_{30}-hopane (Figure 3d-e). We calculated an average 5HH loss of 18 ± 10%, 35 ± 8%, and 47 ± 9% relative to the MW oil for surface slicks, sand patties, and rock scrapings, respectively (Table S3). In contrast, C_{30}-hopane-normalized HH or 2HH did not change significantly. This preferential degradation of homohopanes with longer alkyl chains is consistent with observations from field studies of salt marshes (on the Strait of Magellan) and mangroves (on Guadeloupe) and has been associated with biodegradation.[18,22] In line with biodegradation, we also observed a preferential degradation of the R vs the S epimer of 3HH, 4HH, and 5HH for heavily degraded samples (i.e., with $OxHC_{tot}$ > 60%; Figure S5).[18,22]

In summary, hopanoids ≤ C_{31}, steranes, and diasteranes present in MW oil appeared to be recalcitrant toward weathering processes in the Gulf of Mexico on the time scale of two years, whereas homohopanes >C_{31} were degraded. The degradation patterns point to microbial activity as being responsible for this degradation. Using hopanoids ≤ C_{31}, steranes, and diasteranes is therefore suitable for fingerprinting weathered MW oil.

Photooxidation of Triaromatic Steranes. We found that TAS were less stable than C_{30}-hopane (Figure 3g-h). An average loss of TAS-C_{28} of 36 ± 20%, 65 ± 9%, and 76 ± 12% was calculated using eq 1 for surface slicks, sand patty, and rock scrapings, respectively (Table S3).

We observed that all investigated TAS were lost to remarkably similar degrees with no preferential removal of individual congeners (Figure 4). For example, the C_{20}/C_{28} TAS ratios for MW oil and surface slick samples were similar (1.6 ± 0.2 and 1.5 ± 0.2, respectively) despite the latter having traveled vertically through the 1.5 km water column and more than 200 km horizontally on the water surface. The variable degree of alkylation of the alkyl side chain of TASs (Figure 1) leads to a range in their molecular weight (260−372 g mol^{-1}), vapor pressure (1.6×10^{-5} to 6.8×10^{-9} Pa), octanol−water partitioning coefficient ($K_{OW} = 1.0 \times 10^{7}$ to 1.8×10^{10}), and aqueous solubility (0.001 to 3.6 μg L^{-1}; see Table S4 of the SI). Therefore, physical processes such as evaporation, sorption, or dissolution are expected to affect different TAS compounds to different degrees. For example, evaporation of TAS was hypothesized to be the main driver for preferential disappearance of TAS-C_{20} and TAS-C_{21} relative to the larger TAS compounds at an arid spill site in Egypt.[40] This is in contrast to our study, where the behavioral similarities of all TASs suggest that dissolution or evaporation were not the main processes responsible for the observed TAS depletion.

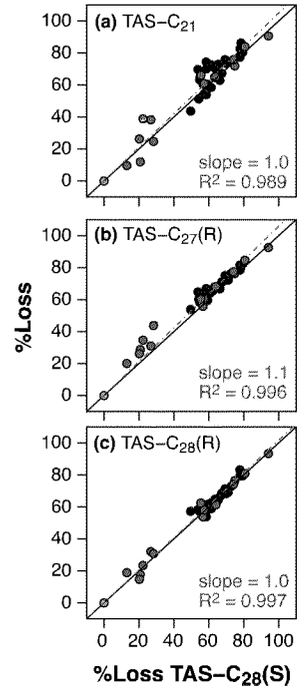

Figure 4. Calculated losses of TAS-C_{21}, TAS-C_{27}(R), and TAS-C_{28}(R) vs losses of TAS-C_{28}(S). Shown are MW oil (yellow circles), surface slicks (blue circles), sand patties (black circles), and rock scrapings (red circles), the 1-to-1-line (solid black line), and the linear regression (blue line, including slope and R^2). Despite differences in aqueous solubility and vapor pressure of 3 orders of magnitude (see Table S3 of the SI) and different stereochemistry, the various TAS compounds are similarly depleted relative to C_{30}-hopane. This suggests that photo-oxidation, rather than evaporation, dissolution, or biodegradation, is responsible for their degradation.

Beside physical processes, biodegradation might be responsible for TAS removal, similar to the above-described biodegradation of homohopanes. In a previous study, TAS biodegradation has been reported under oxic laboratory conditions.[17] TAS-C_{20} and TAS-C_{26} (S) were degraded during a 20-week incubation experiment, while the C_{27} and C_{28} TAS compounds were recalcitrant. In the same study, a preferential depletion of TAS-C_{20} and TAS-C_{26} (S) was observed in soil field samples from a retired oil production area in Ecuador.[17] This preferential depletion was attributed to biodegradation. In contrast to these findings, our results showed no such preferential loss of lower-molecular weight TAS relative to TAS-C_{27} and TAS-C_{28} (Figure 4). This suggests that biodegradation is not the main driver of TAS degradation in our samples.

Finally, this leaves photooxidation as a likely process for TAS degradation. Intuitively, the condensed aromatic backbone of TAS compounds makes them good candidates for photo-oxidation, similar to phenanthrene or other petroleum PAHs (Figure 1).[41,42] Comparing TAS losses to (alkylated)

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phenanthrene and chrysene reveals a similar degradation pattern of PAHs and TAS (Figure S6). Furthermore, abiotic oxidation processes were suggested to be responsible for TAS depletion in naturally weathered bitumens and crude oils from natural seeps.[23] We previously demonstrated that exposing MW oil to sunlight leads to loss of C_{20}- through C_{28}-TAS compounds and attributed this degradation to photooxidation.[25] Although TAS appear to be recalcitrant in reservoirs,[12] we hypothesize that the aromatic structure makes TAS susceptible to photooxidation when exposed to sunlight. Furthermore, we suggest that the diverse physical properties of TAS make this compound class ideal to study oil photochemistry in the environment.

Application: Use of Biomarker Ratios to Fingerprint MW Oil. We found that hopanoids ≤ C_{31}, sterane, and diasterane compounds are recalcitrant toward weathering in the Gulf of Mexico on the investigated time scale. We therefore conclude that the use of diagnostic ratios of these biomarkers is valid to distinguish MW oil from dissimilar sources. We determined a set of biomarker ratios in field samples and compared it to corresponding ratios of MW oil (Figure 5).

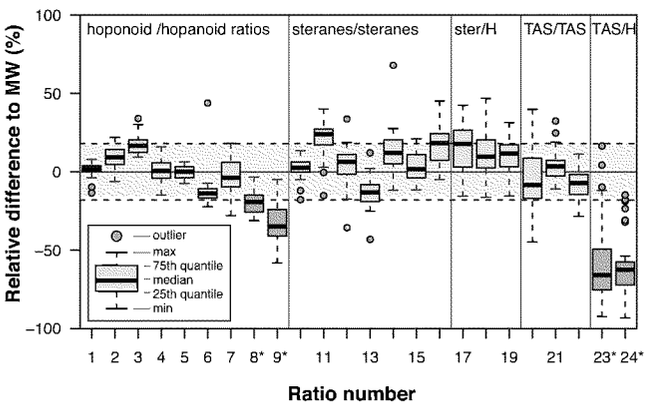

Figure 5. Fingerprinting weathered oil samples using biomarker ratios. Box plot including all 24 biomarker ratios given in Table 1 for the surface slick, sand patty, and rock scraping samples. Given are the relative differences to the ratios of MW oil; the gray shaded area marks the ±20% analytical uncertainty, which is the determined maximal RSD for MW (see the Method section). Ratios with magenta boxes and marked with as asterisk are affected by oil weathering, limiting their utility for fingerprinting MW oil.

Thereby we used diagnostic ratios in Table 1, which are commonly used in oil fingerprinting.[1] Most ratios determined for samples lie within ±20% (the maximum RSD of ratios determined for eight replicates of MW oil) of the MW oil ratios, which implies a match with MW oil. However, for ratios 8, 9, 23, and 24 most samples are outside the MW range. This is because these ratios include 3HH, 5HH, and TAS, which are susceptible to degradation.

In order to validate MW as the source of the surface slicks, sand patties, and rock scrapings, we used principal component analysis (PCA).[43,44] Thereby we excluded weathering-affected ratios 8, 9, 23, and 24. We included four sand-free asphalt samples collected on Grand Isle, LA, Elmer's Island, LA, and Dauphin Island, AL in July 2011 (Figure S8), as well as five reference crude oils. As can be seen from a PCA plot (Figure S9), MW oil, surface slicks, sand patties, and rock scrapings group together, while the asphalt samples are located in a different PCA space. As can be seen in Table 1, the most distinctive feature of the asphalt biomarker ratio was the much lower sterane/hopane ratio (e.g., Ratio 17: 0.2 ± 0.1) compared to the MW oil (1.0 ± 0.2). The nonmatch was also confirmed by oleanane, a compound that was detected in the asphalt samples but not in MW oil (Figure S7). Oleanane is a biomarker for flowering plants, which can be often used to date oil in a reservoir.[45,46]

We tested the sensitivity of the chosen biomarkers to distinguish MW oil from other crude oils. The oil VK915, which is from the Marlin platform on the VK lease block 915 (60 km NE of MW), matches MW relatively well (Table 1 and Figure S9). VK915 was found to be physically, chemically, and toxicologically similar to the MW oil and was therefore chosen by BP as a MW surrogate oil.[30] Similarity in biomarker ratios of oils from deep wells in the Viosca Knoll and Mississippi Canyon Blocks have previously been reported.[47] In contrast, the biomarker ratios from other crude oils that have origin in the Gulf of Mexico region, such as the SLSC (EPA standard from a shallow-water platform) and oil from Eugene Island Block 330 (EI330; 340 km W of MW), are different from MW oil. As expected, Exxon Valdez crude (EVC) and NIST SRM 1582 oils also did not match MW, as these oils have very different origins (Alaska north slope and Monterey formation, respectively).

Next, we explored the use of TAS biomarker ratios to fingerprint weathered oil samples. Some TAS/TAS ratios in our samples were suitable to distinguish asphalt samples from weathered MW samples (e.g., Ratios 21 and 22; Table 1); this can be attributed to the fact that all TAS compounds were lost to a similar extent, thus preserving TAS/TAS ratios. However, ratios of TAS and C_{30}-hopane produced false-negative results for weathered MW samples (e.g., Ratios 23 and 24; see Figure 5). This is due to the weathering-induced depletion of the TAS relative to hopanoids. The utility of TAS biomarker ratios is also limited because TAS are significantly depleted for sand patties and rock scrapings (67 ± 10% for TAS-C_{28}), causing analytical challenges for the detection and quantification of TAS.

Similar to the TAS biomarker, C_{30}-hopane normalized 5HH (Ratio 9) can lead to false-negative results (Table 1). Nevertheless, 5HH(R)/5HH(S) (Ratio 7) was found to be suitable, since we observed only a slight preference of R over S epimer degradation (Figure S5). However, anticipating that ongoing oil weathering will accentuate degradation of homohopanes > C_{31}, we caution against reliance on homohopane ratios for fingerprinting MW oil in the future.

Implications. Several aspects of this study inform the use of biomarkers in oil spill forensics. First, our investigations show that most biomarkers are suitable to fingerprint MW oil samples on the investigated time frame of 28 months postspill. Even in severely weathered MW oil that was exposed to the elements in the Gulf of Mexico for more than two years, we did not see any indication for degradation of hopanoids ≤ C_{31}, steranes, or diasteranes. In a recent study, we also found no degradation of biomarker compounds in MW oil that was trapped in a cofferdam structure on the seafloor for more than two years.[29] However, it is known from studies on longer time scales (decades) that steranes[20] as well as hopanoids[22] may eventually become susceptible toward biodegradation (Figure S1). Also note that our study focused on surface slicks and oil samples collected from beaches (sand patties, rock scrapings). Other environments might exhibit different degradation patterns. For example, a depletion of steranes relative to

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hopanoids has been hypothesized for MW oil that impacted deep-sea corrals,[7] or anaerobic degradation pathways in salt marshes might lead to a different degradation pattern than reported here for surface slicks, sand patties, and rock scrapings.

Second, we found that some biomarker compounds were susceptible to biodegradation and photooxidation. In the investigated sand patties and rock scrapings, we observed an average of depletion of 37 ± 9% for 5HH and 67 ± 10% for TAS. We therefore caution against the use of TAS and homohopanes > C_{31} for fingerprinting weathered oil in the environment.

Third, the differences in biomarker degradation can be used in order to track petroleum degradation in the environment. Commonly used biodegradation indices rely on n-alkanes and isoprenoids or PAHs to assess biodegradation. As these compounds are not detectable or highly depleted in the majority of the investigated MW oil samples, it is difficult to assess the progress of biodegradation for these samples. In contrast, comparing the disappearance of more recalcitrant compounds such as homohopanes or TAS provides a window to identify and quantify active processes at advanced stages of weathering. We recently presented a similar concept based on other recalcitrant compounds (e.g., cyclic and acyclic isoprenoids).[24]

Finally, GC×GC-FID was an excellent tool to investigate the described dynamics of biomarkers. Although GC-MS also proved useful for differentiating MW oil from non-WM oil, the increased chromatographic resolution, the elimination of interference due to coelution, and the robust quantification by FID proved to be critical advantages of GC×GC-FID over GC-MS for this work.

ASSOCIATED CONTENT

* Supporting Information

Supporting tables (Tables S1−S5) and figures (Figures S1−S9), as noted in the text, are provided. 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.

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NOTE ADDED AFTER ASAP PUBLICATION

The version published on June 2, 2014 needed revisions to Figure 2 and Figure S2 in the Supporting Information. The revised version was reposted on June 5, 2014.

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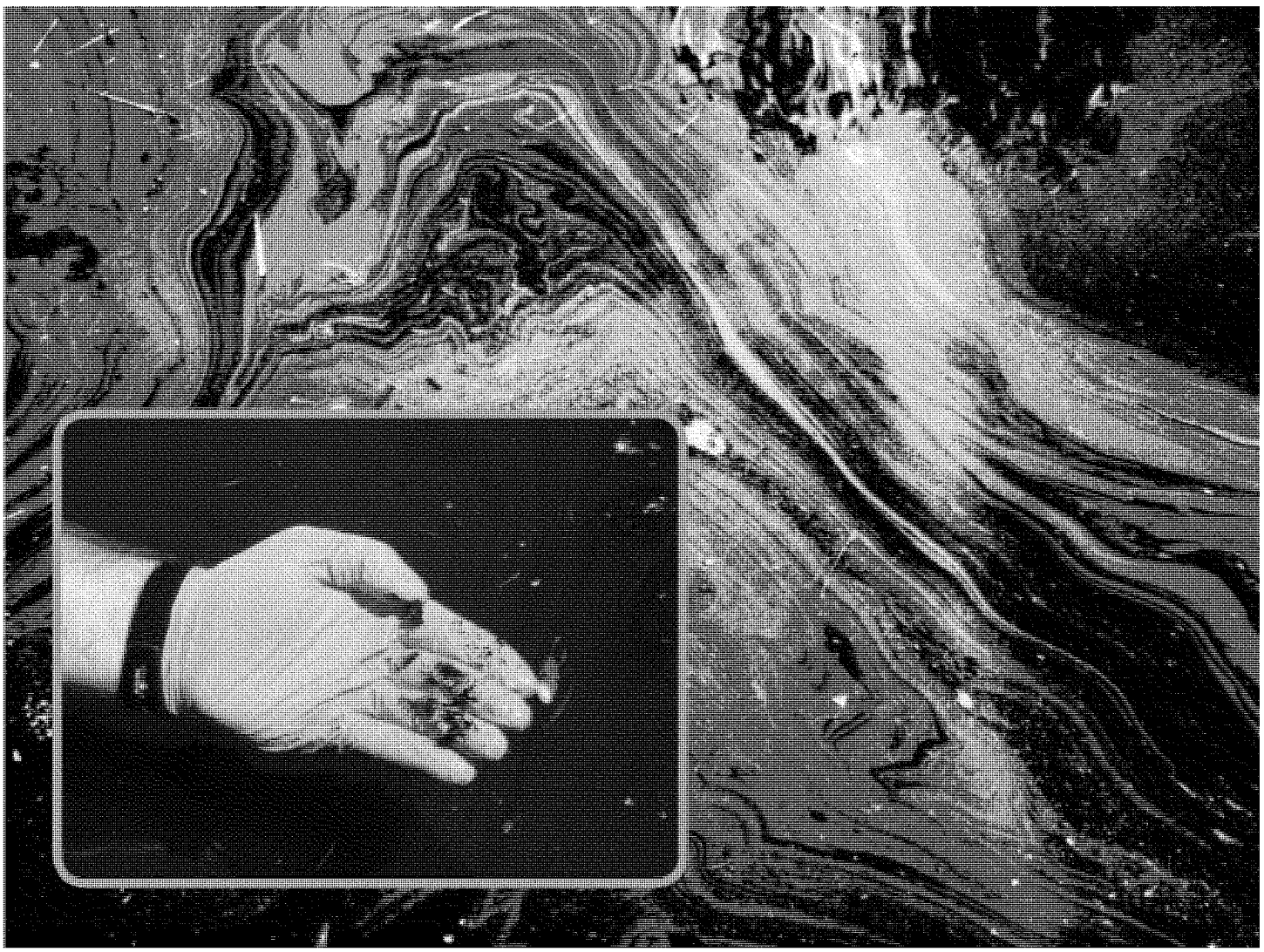
Oil-Particle Interactions and Submergence from Crude Oil Spills in Marine and Freshwater Environments—Review of the Science and Future Science Needs



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U.S. Department of the Interior
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ConversionFactors

SI to Inch/Pound

Multiply	By	To obtain
	Length	
micrometer (□m)	0.000039	inch (in.)
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
	Area	
square meter (m^2)	0.0002471	acre
square kilometer (km^2)	247.1	acre
square centimeter (cm^2)	0.001076	square foot (ft^2)
square meter (m^2)	10.76	square foot (ft^2)
square kilometer (km^2)	0.3861	square mile (mi^2)
	Volume	
liters (l)	0.0063	Barrel (petroleum, 1 barrel = 42 gal)
liters (l)	0.2642	gallon (gal)
cubic meter (m^3)	6.290	barrel (petroleum, 1 barrel = 42 gal)
cubic meter (m^3)	35.31	cubic foot (ft^3)
cubic meter (m^3)	1.308	cubic yard (yd^3)
	Flow rate	
meter per second (m/s)	3.281	foot per second (ft/s)
cubic meter per second (m^3/s)	35.31	cubic feet per second (ft^3/s)
	Density	
gram per cubic centimeter (g/cm^3)	62.4220	pound per cubic foot (lb/ft^3)
	Stress	
pascal (Pa)	0.000145	pound per square inch (psi)
	Dynamic viscosity	
centipoise (cP)	2.42	pound/foot-hour (lb/ft-hr)
	Energy dissipation rate	
square meters per cubic second (m^2/s^3)	10.76	square feet per cubic second (ft^2/s^3)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows: °F=(1.8×°C)+32

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Oil-Particle Interactions and Submergence from Crude Oil Spills in Marine and Freshwater Environments—Review of the Science and Future Science Needs

By Faith A. Fitzpatrick[1], Michel C. Boufadel[2], Rex Johnson[3], Kenneth Lee[4], Thomas P. Graan[5], Adriana C. Bejarano[6], Zhenduo Zhu[7], David Waterman[7], Daniel M. Capone[8], Earl Hayter[9], Stephen K. Hamilton[10], Timothy Dekker[11], Marcelo H. Garcia[7], Jacob S. Hassan[12]

Abstract

Oil-particle interactions and oil submergence are of much interest to oil spill responders and scientists, especially as transportation of light and heavy crude oils increases in North America's coastal marine and freshwater environments. This report contains an up-to-date review of the state of the science for oil-particle aggregates (OPAs), in terms of their formation and stability which may alter the transport, fate, and toxicity of the residual oil and, hence, its level of ecological risk. Operational considerations—detection, containment, and recovery—are discussed.

Although much is known about oil-particle interactions in coastal marine environments, there remains a need for additional science on methods to detect and quantify the presence of OPAs and to understand their effects on containment and recovery of oil spilled under various temperature regimes and in different aquatic habitats including freshwater environments.

Introduction

Suspended particles affect the fate and transport of spilled oil in aquatic environments (Muschenheim and Lee, 2002; Owens and Lee, 2003; Khelifa and others, 2005a, b, c; Sun and Zheng, 2009; Gong and others, 2013), such as rivers and floodplains, shorelines and beaches along lakes and oceans, coastal and riparian wetlands, and deeper waters of oceans and lakes. The manner in which oil interacts with particles and its eventual transport and fate depend on the physical properties of the oil and the particles, as well as environmental conditions including the geomorphic setting, weather, currents, and vertical mixing of the water column (Lee, and others, 2011a; Lee and others, 2002) (fig. 1).

Combinations of oil and particles have various names, including clay-oil flocculation (Bragg and Yang, 1995), oil-mineral aggregates (Lee and others, 1998) and oil-suspended sediment-aggregates (Khelifa and others, 2002) depending on the type of particle involved in the interaction. The term oil-particle aggregate (OPA) is used in this report because it is the more generic term that includes a wider range of particles containing both mineral sediment and organic matter in association with oil that may be retained in suspension and (or) settled out.

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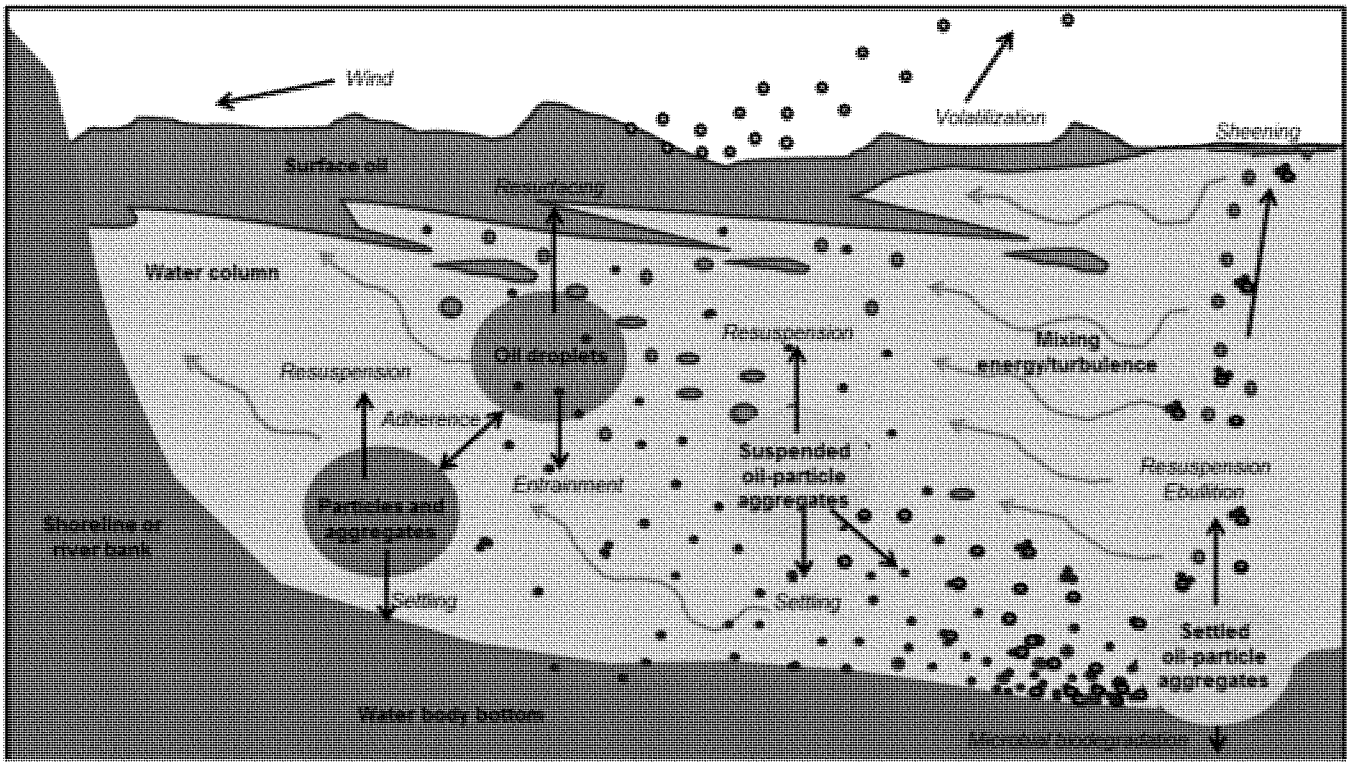

Figure 1. Simplified diagram of the processes and mechanisms leading to oil-particle aggregate formation and breakup in marine and freshwater environments. (modified from Environment Canada, 2013; OPA, oil-particle aggregate).

Traditional clean-up methods based on physical recovery of oil slicks on surface waters, such as booming and skimming, are ineffective for spilled oil once it submerges. Observations of OPA formation associated with the transfer of oil to the benthic environment (Lee, 2002; Payne and others, 2003; Sterling and others, 2005; Passow and others, 2012) have provided the justification for additional scientific studies to understand the processes and characteristics associated with OPA formation and their transport and fate. Results from additional studies will likely influence oil spill response contingency planning and spill response operations that include methodologies for detection, monitoring, recovery, and containment (Bandara and others, 2011; Environment Canada, 2013; Dollhopf and others, 2014; Hansen, 2014).

OPA formation has been correlated with the removal of oil stranded within sediments in freshwater and marine environments by both natural recovery (natural attenuation) and active remediation procedures (the addition of suspended particulate material in the presence of turbulent mixing energy) (Bragg and Yang, 1995; Lee and others, 1996; 1997; Wood and others, 1997; Lee, 2002; Lee and others, 2002; Owens and Lee, 2003). Lee and others (1997; 2003a) note that OPA formation accelerated the removal of stranded oil within the intertidal/surf zone by reducing the adhesive properties of the oil and the tendency of dispersed droplets stabilized by mineral fines to re-coalesce. Thus, either formed naturally or enhanced with addition of clay minerals, the relatively stable OPAs are dispersed more easily in the water column, potentially reducing the oil to concentrations below toxicity threshold limits (Lee and others, 2003a; Lee and others, 2003b) and making the oil more available for biodegradation (Weise and others, 1999; Lee and others, 1996; Lee and others, 1997; Lee and Merlin, 1999; Owen and Lee, 2003). Like chemical dispersants, the exposure pathway is altered

from water surfaces and shorelines to the water column, which transfers the toxicity risks from water fowl and shoreline organisms to planktonic, open water, and benthic species (Venosa and others, 2014).

Submergence can happen to light and heavy oils after they bind to relatively small amounts of particles (mineral sediment or organic matter). This can occur in suspension or while the oil moves along the bottom of a water body, bank, or coastline (Lee and others, 1985; Lee, 2002; Cloutier and others, 2003). The behavior of bitumen from the Canadian tar sands (usually more dense and viscous than traditional heavy oil), and particularly its interaction with particles leading to eventual submergence and accumulation of OPAs in sediments, has recently become a topic of much interest because of the 2010 Enbridge Line 6B pipeline release into the Kalamazoo River when more than 3.2 million liters of diluted bitumen were released into the Kalamazoo River in Michigan (Dollhopf and others, 2011). Response operations for that release began with conventional recovery techniques for floating oil and recovered about 2.9 million liters; however, submerged oil became the focus approximately 1 month into the cleanup and remained the focus through the summer of 2014 (Enbridge Energy, L.P., 2010; Dollhopf and others, 2014). Given the timely research efforts pertaining to the Canada Northern Gateway proposed pipeline, recent studies provided insights on the buoyancy (King and others, 2014), interaction with mineral fines (King and others, 2015), and the ultimate fate of OPAs formed from bitumen (Environment Canada, 2013).

Light crude oil can interact with particles along rivers, as indicated by the 2013 derailment, explosion, and spill of light crude oil in Lac-Mégantic, Quebec when an estimated 100,000 liters of oil spilled into the Chaudière River. A management plan by the Government of Quebec was developed that included recommendations for cleanup of river bottom sediment contaminated with hydrocarbons (Gouvernement du Quebec, Depot Legal, 2014). Laboratory tests at Louisiana State University using E2MS 303 oil from the February 2014 spill of Bakken crude from a barge collision into the lower Mississippi River indicate that the "oil will quickly adhere to suspended solids in the water column, forming unstable emulsions" (Doelling and others, 2014).

More than 15 years ago, before the emergence of concerns about increased pipeline transport of diluted bitumen, the National Coastal Research Council (on behalf of the U.S. Coast Guard) published "Spills of Nonfloating Oils: Risk and Response," a report that included specific recommendations for detection, monitoring, modeling, and recovery of submerged oil, mainly in marine environments (National Research Council, 1999). These recommendations were further tabulated into science needs for detection and monitoring, fate and transport, containment and recovery, and effects and restoration and included specific mention of OPAs (Coastal Response Research Center, 2007). Some areas of science support included developing better sensors to detect OPAs, mapping the extent of OPAs, and determining future resuspension and remobilization of OPAs in bottom sediment. The Coastal Response Research Center (2007) noted that improved characterization of the size, composition, and distribution of particles would enable better forecasting, observation, understanding, and hind casting of OPA behavior for a range of ecological and geomorphic settings.

Fisheries and Oceans Canada has been conducting oil-particle interactions studies with oil and chemically dispersed oils since the mid-1980s. An oil-particle interactions workshop was organized in 2000, and a compendium of papers appeared in the Spill Science and Technology Bulletin in 2003 and 2004 (Lee and Jarvis, 2004). These publications formed the basis for a quickly growing body of literature on the topic of OPAs in both marine and freshwater environments.

Because oil transportation in North America increased greatly on rails, through pipelines, and on barges and vessels as a result of the increased production of Bakken crude in the Williston Basin, North Dakota, and bitumen in the western provinces of Canada (Frittelli and others, 2014; Association of American Railroads, 2014; Committee for a Study of Pipeline Transportation of Diluted Bitumen,

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2013), more attention is being given to submerged oil detection and response techniques in marine and freshwater environments (Coastal Response Research Center, 2007; Hansen, 2014). It is clear that more science is needed on the formation, settling, resuspension, and toxicity of OPAs, and the manner in which these properties vary depending on the nature of the oil and the characteristics of the aquatic environment it enters. Quantification of residual oil following spill response operations needs to account for the fraction of oil associated with suspended/settled particulate material.

Purpose and Scope

The purpose of this report is to describe the state of the science concerning OPAs—their formation, transport, settling, resuspension, and breakup in a variety of geomorphic environments including coastal marine, deep ocean, freshwater lacustrine (Great Lakes), and riverine, and brackish river mouths and harbors. The report contains a synthesis and review of available literature on OPAs from laboratory, experimental tank and flume studies, and some large-scale field experiments. Ongoing studies are described and needs for continuing investigation and new science on OPAs are listed.

Reviewof the Science

The U.S. Geological Survey (USGS) mission is to provide reliable scientific information to describe and understand the Earth; minimize loss of life and property from natural disasters; manage water, biological, energy, and mineral resources; and enhance and protect our quality of life. That mission has involved the USGS in providing science support for oil spill recovery. This review of the science on oil-particle interactions was motivated by questions that have arisen during oil spill response of the Enbridge Line 6B spill in Marshall, Michigan. However, the review is holistic in the sense that it incorporates spills of oils with various properties in freshwater and marine environments, as a result of concerns about spills from the rising quantities of crude oil produced and transported across the Nation. The following questions helped to form the major sections included in this review:

- What environmental conditions lead to the formation of OPAs?
- What is the effectiveness of adding particles to an oil spill for physical dispersion of oil as a spill countermeasure?
- What is the long-term fate and transport of OPAs?
- What are the ecological implications (fate and effects) of OPAs?
- Are there special circumstances for OPAs in cold climates for use as a spill countermeasure or for recovery in icy water?
- What are the operational considerations for recovery of OPAs?

Formation of Oil-Particle Aggregates

Formation of OPAs happens naturally when oil and suspended particles mix in turbulent water (Lee, 2002; Muschenheim and Lee, 2002; Owens and Lee, 2003; Khelifa and others, 2005a; Sun and Zheng, 2009; Gong and others, 2013). An understanding of the exact mechanisms of OPA formation comes mainly from the use of laboratory shaker and wave tanks, including those at the Bedford Institute of Oceanography, Dartmouth, Nova Scotia (Center of Offshore Oil and Gas Environmental Research, 2004), and flume experiments. Results from wave tank and laboratory shaker experiments provide empirical data for models of OPA formation and breakup (Stoffyn-Egli and Lee, 2002; Li and others, 2007; Ma and others, 2008; Wang and others, 2011). However, field observations and data from

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assessment and monitoring activities at spill sites yield information on the timing and range of environmental conditions under which OPAs have formed (Niu and others, 2011).

Major factors affecting the formation of OPA are (1) quantity and viscosity of the oil, interfacial tension of oil-water, and chemical composition of the oil; (2) quantity, type, and surface properties of the particles; (3) magnitude and variability in physical energy of the aquatic environment; (4) temperature; and (5) salinity (Lee, 2002; Khelifa and others, 2002; Payne and others, 2003). Recent tests have focused on the highly viscous diluted bitumen (dilbit) product (Zhao and others, 2014a). In spills of heavy crude oil, particularly bitumen, freshwater environments with fine-grained sediment in the water column and bottom, abundant sunlight, warm temperatures, and strong currents and turbulence create a high potential for oil submerging and ultimately being deposited in the sediments (Silliman, 2014).

The first step to forming OPAs lies with the initial breakup of a slick of oil into oil droplets. Once spilled into a water body with turbulence created by waves or currents, floating oil can break up into droplets and reach a stable droplet size distribution (DSD) relatively quickly, perhaps in minutes to tens of minutes (Zhao and others, 2014b). Smaller droplets are generated when the interfacial tension of oil-water is small and (or) the oil viscosity is small. The interfacial tension of oil-water is more or less constant in the absence of surfactants. However, oil viscosity can increase by orders of magnitude among different types and temperatures of oils (fig. 2). For example, the viscosity of a heavy crude oil or bitumen is at least 1,000 times that of light crude such as a product from the Alaska North Slope or Bakken Formation oil (http://www.etc-cte.ec.gc.ca/databases/oilproperties/Default.aspx). Figure 2 contains a summary of American Petroleum Institute (API) gravity and viscosity for the major categories of crude oil (American Petroleum Institute, 2011). Crude oil densities are usually measured in terms of degrees API gravity. A higher API gravity corresponds with lighter density, with freshwater at 10 degrees API. Crude oils are generally further categorized by their type of hydrocarbon base (paraffinic or naphthenic) and sulfur content (high–sour, low–sweet).

For the common numerical models for the evolution of the DSD, it has been assumed that the interfacial tension is the only force resisting the breakup of droplets (Prince and Blanch, 1990; Tsouris and Tavlarides, 1994), which is not the case for high viscosity oils or for situations when surfactants (in the form of chemical dispersants) are used (Wang and Calabrese, 1986). Alternatively, Delvigne and Sweeney (1988) developed an expression that predicts dispersion, which is based on oil viscosity without any information on the oil-water interfacial tension. Thus, the Delvigne and Sweeney (1988) formula cannot be relied upon to account for the reduction in the interfacial tension when dispersants are used. Recently, Zhao and others (2014b) developed a comprehensive model that accounts for resistance to breakup from both interfacial tension and viscosity of the oil. This model is called VDROP to stress the important role of viscosity. The DSD depends also on the mixing energy, namely the dissipation rate of kinetic energy (National Research Council, 2005; Kaku and others, 2006). Thus, high mixing energy promotes the breakup of droplets (fig. 1). In addition, dilution (as oil mixes with a larger volume of water away from the source) increases the distance between oil droplets and thus minimizes the probability of collision and subsequently the coalescence of oil droplets into larger droplets.

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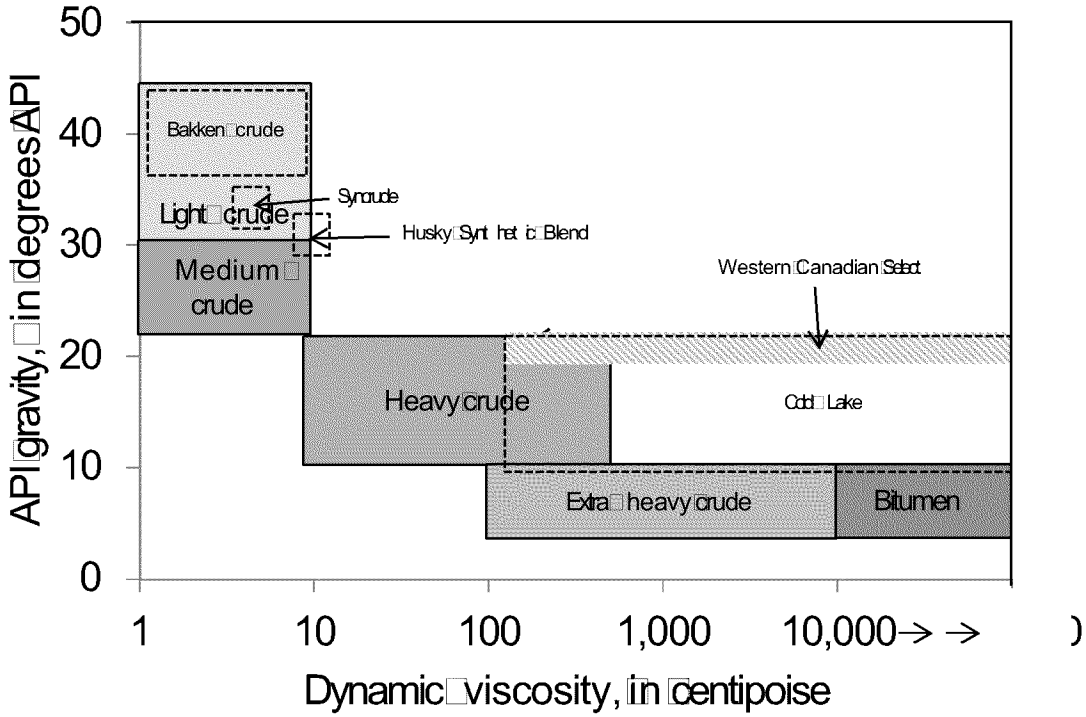

Figure 2. Summary of American Petroleum Institute (API) gravities and dynamic viscosities (at approximately 10-20 degrees Celsius) for major categories of crude oil types and bitumen/oil sands. Overlays are of example crude oils commonly transported in North America. Data summarized from Attanasi and Meyer (2007), American Petroleum Institute (2011), Sia Partners Energy Outlook (2011), Environment Canada's Oil Properties Web Site http://www.etc-cte.ec.gc.ca/databases/oilproperties/Default.aspx, Andrews (2014), Doelling and others (2014), Crude Quality, Inc. (2014); and Enbridge Energy Partners, L.P. (2013).

The size of the oil droplet is a very important factor in the transport and fate of the oil and its interaction with particles. Because large droplets have higher buoyancy than smaller droplets, they tend to float to the water surface, whereas smaller droplets could be driven more easily in the water column as a result of mixing energy (Boufadel and others, 2007). Smaller droplets also have a larger specific area than large droplets, which would enhance dissolution and microbial biodegradation (Lee and others, 1997; Lee and Merlin, 1999; Reddy and others, 2012; Geng and others, 2013).

When particles are attached to oil droplets, they form a type of barrier around the droplet that keeps it from coalescing with other oil droplets (Khelifa and others, 2005a). Droplets with particles result in three main features that contribute to the eventual fate and submergence of droplets: (1) OPA-enhanced stability of the oil droplets that limits coalescence; (2) increased specific density of coated droplets which causes dispersion into a water body and possible settling; and, (3) enhanced microbial degradation.

The actual attachment of oil droplets to particles depends on the viscosity and adhesion properties of the oil droplet and the surface area of the particles, as well as salinity of the water body (Lee, 2002; Khelifa and others, 2005a). There is evidence that salinity increases the formation of OPAs as a result of the reduction of the thickness of the double layer with increased ionic strength of water (Clark, 2009). However, OPA was also observed to form in freshwater (Lee, 2002; Lee and others, 2002). If the droplet size distribution of oil has not reached a steady state prior to interaction with the

particles, the interaction would affect the breakage mechanism of oil droplets. The reduction in the net interfacial tension resulting from attachment of particles would make the oil viscosity the main force resisting oil breakup. In such a case, a model that accounts for oil viscosity in resisting breakup is needed, such as VDROP (Zhao and others, 2014b).

Particle size, amount, and type are important to the formation of OPAs. Clay-sized mineral particles are effective at forming OPAs, especially if the particles are present in large concentrations (Lee and others, 1998; Lee, 2002). However, most natural waters have a range of particle sizes or at least bimodal distributions, and natural particle-size distributions also may vary with the amount of mixing resulting from waves or currents (Boufadel and others, 2007). The particles may be smaller than the oil droplet and form a coating, or they may be larger or the same size for an aggregate. In either droplet type (fig. 3A), the attachment of the oil droplet and particle reduces the interfacial tension of the OPA with the water, and thus the particles have an overall effect similar to surfactants (Lee, 2002). Oil also may attach to mineral or organic matter (Lee, 2002). Phytoplankton can readily form oil-organic aggregates in laboratory tests (Lee and others, 1985). Particle concentrations can be relatively low and still form OPAs (Lee and Stoffyn-Egli, 2001; Rymell, 2009); Khelifa and others (2002) note that, in laboratory shaker experiments with seawater, mineral concentrations as low as 100 milligrams per liter (mg/L) and moderate shaking resulted in OPA formation with various types of oil.

The shape and makeup of OPAs can take multiple forms. Methods used to study the shape and makeup of OPAs, which are generally less than 1 millimeter (mm), involve instrumentation such as combined transmitted light/ultraviolet (UV) light epi-fluorescence microscopy, confocal scanning laser microscopy, and environmental scanning electron microscopy (Stoffy-Egli and Lee, 2002; Lee and others, 2012). The most common forms of OPAs involve a spherical oil droplet surrounded by particles or multiple spherical droplets in a particle aggregate (Bragg and Owens, 1994; Stoffyn-Egli and Lee, 2002; Khelifa and others, 2005a; Zhang and others, 2010; Lee and others, 2012) (fig. 3A). Spherical shaped OPA indicate that the spilled oil formed droplets before forming aggregates, as described in the previous paragraphs. Stoffyn-Egli and Lee (2002) also found two other types of aggregates in laboratory experiments—solid and flake types (figs. 3B and 3C)—that do not necessarily form from dispersed droplets. These additional types are distinguished in that the oil takes on more of the form of the particle or mineral, and the flake type is distinguished by having folds or rolls in a dendritic or feather shape. For all types, the combination of oil and particles can result in a range of specific gravities that are usually similar to, or heavier than, water; thus OPAs can be floating, neutrally buoyant (in suspension), or negatively buoyant (submerging or settling) (Stoffyn-Egli and Lee, 2002). Sometimes the mineral makeup of particles determines their buoyancy, and Omotoso and others (2002) found that low-viscosity oils formed negatively buoyant OPAs with hydrophilic minerals (having a strong affinity for water) but formed positively buoyant OPAs with calcite minerals. Lastly oil-particle interactions can be enhanced by colloidal mechanisms of coagulation of ions (Lee and Stoffy-Egli, 2001), as well as biological activity associated with bacteria and phytoplankton (Passow and others, 2012).

Laboratory simulations of the formation of OPAs in a brackish (salinity of 1.5 parts per thousand) high-energy riverine environment (Rio Desaguadero) were done by mixing sediment-laden water and heavy crude oil from the 2000 OSSA II pipeline spill into the Rio Desaguadero in the Bolivian Altiplano (Lee and others, 2001; 2002). In this spill, a missing oil fraction of 27–37 percent was not recovered; presumably it succumbed to from oil dispersion and enhanced biodegradation caused by the formation of OPAs. The river sediment was rich in smectite clay minerals, which have an affinity for attracting or adsorbing water molecules. Also, the river was in flood stage, with velocities of 2.5 meters per second (m/s) and depths of less than 3 meters (m). Both of these factors may have enhanced OPA formation. The laboratory experiments, which were run at water temperatures of 20–22 degrees Celsius

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(°C), showed that the amount of OPA formation increased with increasing salinity, which is known to cause flocculation of clays. With a combination of fresh OSSA II oil (diluted with a kerosene-range petroleum product), Rio Desaguadero sediment, and solutions using natural seawater and dilution to obtain salinities of 0.35, 0.7, 1.2, 3.5, and 35 parts per thousand (ppt), OPA formation increased with increasing salinity, and at 35 ppt almost all of the oil was taken up in OPA formation (fig. 4). In contrast, a minimal amount of oil was present as OPAs with fresh oil, distilled water, and river sediment, but when the brackish river water was used (1.5 ppt salinity), about 25 percent of the fresh oil formed OPAs.

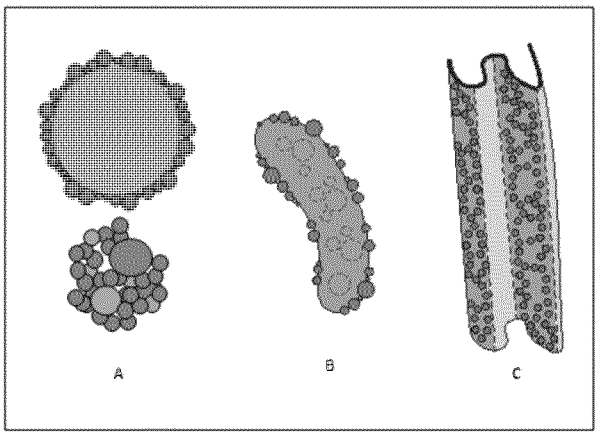

Figure3. Types of oil-particle aggregates: A, single and multiple droplet aggregate, B, solid aggregate of large, usually elongated mass of oil with interior particles (dashed blue circles), and C, flake aggregate of thin membranes of clay aggregates that incorporate oil and fold up (modified from Stoffyn-Egli and Lee, 2002). Blue color represents particles and yellow represents oil.

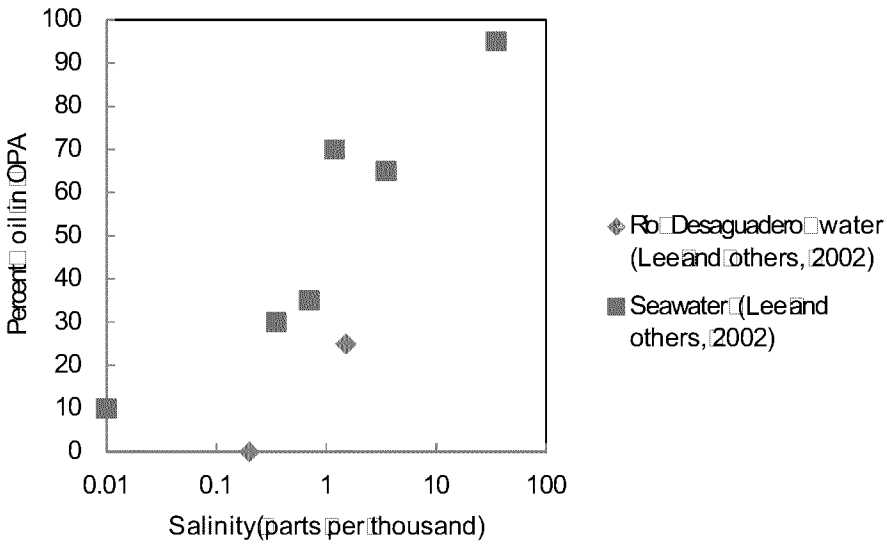

Figure4. Salinity in relation to the percent of oil in oil-particle aggregates (OPAs) from laboratory shaker tests of diluted heavy crude oil form the 2000 OSSA II spill into the Rio Desaguadero in Bolivia and its smectite-rich sediment. (Graph replotted from Lee and others, 2002).

Natural formation and submergence of OPAs occurred in the freshwater, low-gradient environment of the Kalamazoo River after the 2010 Enbridge Line 6B pipeline spill of diluted bitumen into Talmadge Creek, a tributary of the Kalamazoo River near Marshall, Michigan (Dollhopf and others, 2014). The diluent of natural gas condensate (which is used as a solvent in the mixture) volatilized and submergence of the bitumen occurred within a few weeks following the spill (Dollhopf and Durno, 2011; Lee and others, 2012). On the basis of laboratory tests of Cold Lake Blend by Belore (2010) and in an outdoor flume by King and others (2014), the density of the spilled bitumen (mainly Cold Lake Blend) was likely between 0.93 and 0.936 grams per cubic centimeter (g/cm^3) with diluent and 0.981 g/cm^3 after the diluent evaporated. The dynamic viscosity of the Cold Lake Blend in the Belore (2010) laboratory tests ranged from approximately 400 centipoise (cP) with diluent to more than 14,500 cP after evaporation of the diluent. Both the works of Belore (2010) and King and others (2014) were conducted at approximately 15°C.

Some features of the Kalamazoo River likely enhanced the formation, resuspension, and deposition of OPAs. The Enbridge Line 6B pipeline release happened during a flood on the Kalamazoo River with an exceedance probability of 4 percent (for example, a 25-year event) (Hoard and others, 2010) and with a mean velocity of about 1.1 m/s and a mean depth of 1.2 m near the USGS streamgage at Marshall, Michigan (04103500). On the basis of later measurements of suspended sediment at the Marshall streamgage, it can be inferred that, at the time of the spill, the river had relatively low suspended sediment concentrations (less than 100 mg/L) (fig. 5) with suspended particle sizes mainly in the silt-sized range (65–75 percent) (fig. 6). Water temperatures were warm, in the range of 23–25 ° C (Stephen Hamilton, Michigan State University, written commun., 2014). Floodwater increased turbulence in river flows and increased the presence of suspended particulate matter. Additional mixing from flows over two dams may also have played a role, although OPAs and submerged oil accumulated in the first 5 kilometers (km) of river length, between the spill source and the first dam. Aggressive sediment agitation techniques (raking, flushing, aeration, and skimming the river bottom physically or with water jets) were conducted in 2011 to liberate submerged oil as recoverable sheen in 2011 (Enbridge Energy L.P., 2011b; Dollhopf and others, 2014), potentially contributing to further OPA formation and transport of OPAs to downstream reaches (Lee and others, 2012). Lee and others (2012) found that oil from the pipeline spill readily formed OPAs when mixed with Kalamazoo River sediment in laboratory tests (fig. 7).

Assuming that the fraction of spilled oil not recovered by conventional techniques was lost to submergence, the bitumen that submerged in the Kalamazoo River was greater than 300,000 liters, which is around 10 percent of the spilled oil. This is based on the Enbridge Energy, L.P., reported spilled amount of 3.2 million liters and recovered amount of 2.9 million liters after the first year (U.S. Environmental Protection Agency, 2011). Although the volume of oil released and remaining in the river are not finalized at the time of this writing (January 2015), the estimated percentage is similar to that found by Lee and others (2002) for distilled water and brackish water of the Rio Desaguadero (fig. 4). Even though OPA formation in freshwater may be less than that in seawater, the persistent residual submerged oil and oiled sediment in the Kalamazoo River resulted in a protracted cleanup that ultimately required dredging and has accounted for a major share of the cleanup costs, which have surpassed $1.2 billion (Dollhopf and others, 2014).

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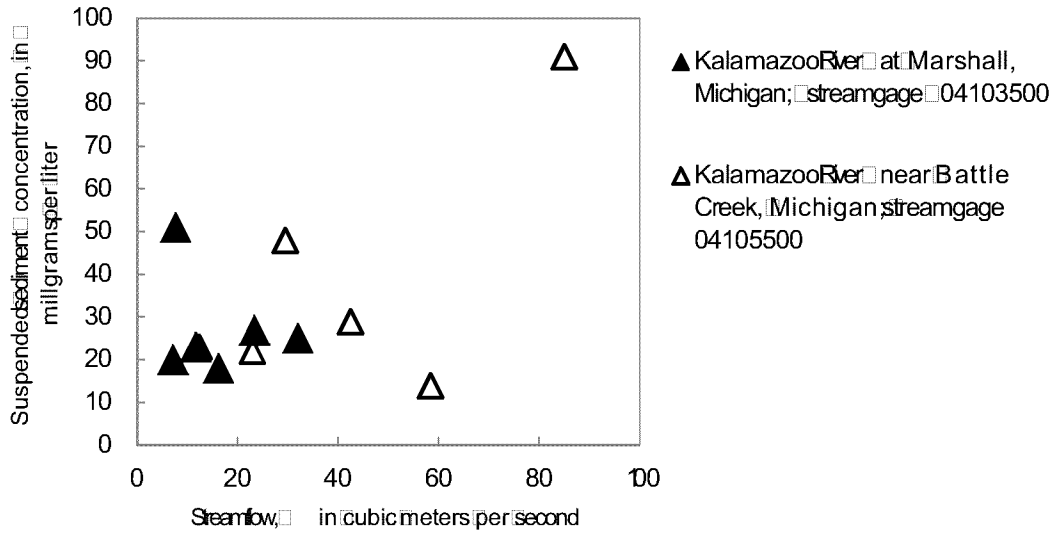

Figure 5. Streamflow in relation to suspended sediment concentration in the Kalamazoo River, Michigan, August 2012-March 2014. Streamflow during the pipeline release on July 28, 2010, during a receding flood event with a 4-percent exceedance probability, was about 34 cubic meters per second (m^3/s) at Marshall, Michigan and 85 m^3/s near Battle Creek, Michigan. The Kalamazoo River is generally a suspended sediment supply-limited system, shown by the overall low concentrations over the entire flow range at both streamgages.

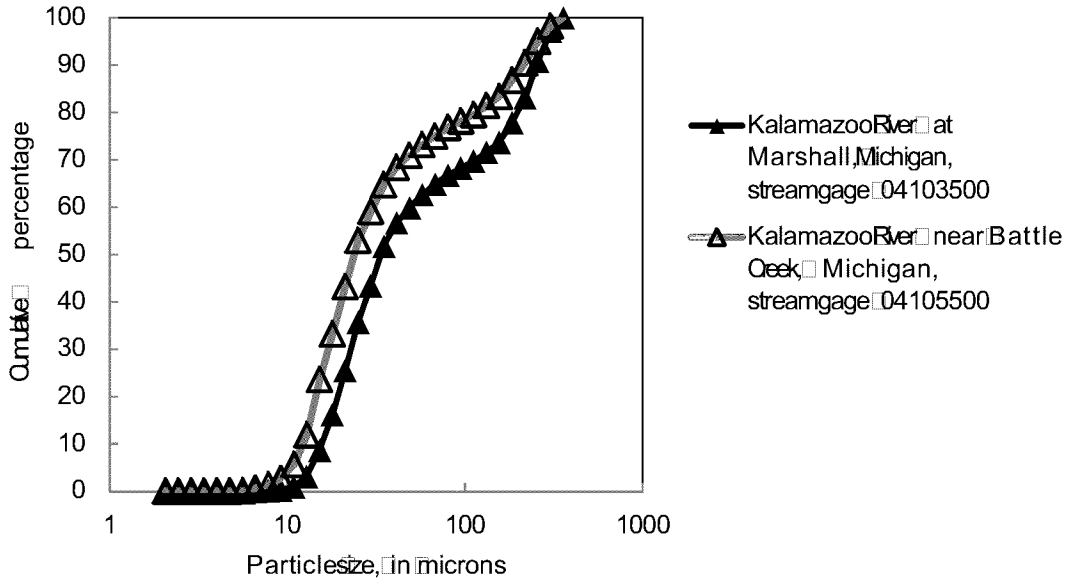

Figure 6. Cumulative particle-size distribution for suspended sediment collected April 22, 2013, during flows of 32 cubic meters per second (m^3/s) at Marshall, Michigan, and 85 m^3/s near Battle Creek, Michigan. Most of the suspended sediment was in the silt-size class—about 65 percent at Marshall and 75 percent near Battle Creek. Samples analyzed with portable laser in-situ scattering and transmissiometry.

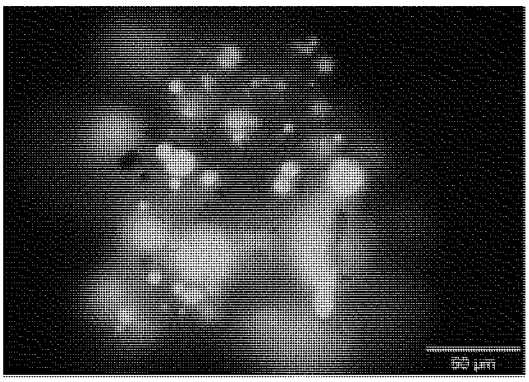

Figure 7. Kalamazoo River sediment spiked with weathered source oil after 48 hours, under ultraviolet-epifluorescence microscopy at 320 times magnification. (from Lee and others, 2012).

Some additional features of the Kalamazoo River may have been important factors in OPA formation, transport, and deposition. The floodplain of the Kalamazoo River has abundant wetlands, thus suspended and bottom sediments have relatively high organic matter content, on the order of 20 percent or more. The river is wide (width/depth ratios of 40) and has an average gradient of 0.06 percent in the spill-affected reach. Deposition of OPAs occurred along channel margins, backwaters, side channels, and oxbows, and in impoundments throughout the entire 61- km stretch of the river affected by the oil spill. Surveys over time provided evidence for resuspension and resettling of OPAs in downstream areas, presumably during post spill floods (Dollhopf and others, 2014).

Oil-Particle Aggregates as a Natural Physical Dispersant

Enhancing physical dispersion of spilled oil through the addition of particulate matter is one of several techniques that have been used for spill cleanup and to prevent oiling of marine coastal areas (Zhang and others, 2010). The oil would be dispersed into small droplets by turbulent mixing from waves or currents and subsequently mixed with mineral and organic particles in the water column. The aggregation of the oil with particles to form OPAs and their subsequent physical dispersion by natural processes would reduce the bioavailability and toxicity of the residual oil to aquatic organisms in the vicinity of the spill. Furthermore, as the activity of oil degrading bacteria is focused at the oil water interface, the formation of small oil droplets enhances microbial biodegradation (Lee and others, 2002; 2009). Petroleum hydrocarbons are not new to the environment because of natural sources such as seepage from oil bearing rocks and biological production by plants and animals. Thus, indigenous oil degrading bacteria are readily available in many aquatic ecosystems (Atlas and Hazen, 2011).

OPA formation has been reported to be a significant contributor to the natural cleansing mechanisms observed during the Baffin Island Oil Spill (BIOS) project and recovery following the Exxon Valdez oil spill incident in 1989 (Bragg and Owens, 1994; Bragg and Yang, 1995). Studies have shown that microorganisms (namely bacteria and archea) within ocean floor sediments in the proximity of natural oil seep sites, such as Scott Inlet, Baffin Island, and the Gulf of Mexico have adapted to utilize oil droplets as a carbon and energy source (Grant and others, 1986; Atlas and Hazen, 2011). The current scientific consensus is that a considerable portion of the oil spilled in the Gulf of Mexico from the Deepwater Horizon incident has been degraded by indigenous bacteria (Atlas and Hazen, 2011; National Research Council, 2013). Considering the magnitude of the spill, Edward Owens (Polaris Applied Science, Inc., oral commun., 2014) has hypothesized that the volume of oil affecting the Gulf of Mexico shoreline was much less than expected, because of the interaction of dispersed oil and surface oil slicks with the naturally high concentration of particulate suspended material near the coast.

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Boufadel et al. (2014) conducted a comparison between the DWH and the Exxon Valdez spills, and based on calibrated modeling, they estimated that around the same mass of oil reached the shorelines from the two spills (around 20,000 tons). But the percentages to the total mass of oil were around 5 percent and 50 percent for the DWH and Exxon Valdez, respectively. For the 1989 Exxon Valdez oil spill, much of the oil along shorelines was depleted (Atlas and Hazen, 2011), except in some beaches with anoxic conditions (Boufadel and others, 2010; Li and Boufadel, 2010).

With natural particulate matter readily available in a coastal marine or lacustrine environment, and especially along river systems, OPA formation must be considered a natural process that enhances the physical dispersion of oil. Indeed, expanding on this hypothesis, Lee and others (2009) suggested that active enhancement of OPA production as a "physical" means to promote oil dispersion, could be an alternative to the use of chemical dispersants that may be potentially toxic in their own right. However, prescribed sinking of spilled oil, or the use of sinking agents, is currently prohibited by U.S. Environmental Protection Agency (EPA) because of the potential risks of acute and chronic toxic effects on benthic organisms and possibly less biodegradation once the oil is deposited (U.S. Environmental Protection Agency, 1993; also 40 C.F.R. §§ 300.310(b), 300.910(e)).

Laboratory experiments have expanded on the knowledge base of the manner in which OPAs facilitate physical dispersion in both seawater and freshwater. For seawater, simulations of coastal environments by Li and others (2007) in wave tank experiments found that chemical dispersants and the addition of fine mineral particles, alone and in combination, enhanced the dispersion of light crude oil in the water column and increased the number of OPAs formed. Similarly, results from wave tank studies of light crude oil with seawater (Lee and others, 2008) indicate that chemical dispersants enhanced OPA formation by transferring oil from a slick floating on the water surface into oil droplets in the water column that more easily interacted with suspended particles. Zhang and others (2010) considered three crude oils (Mesa, Alaska North Slope, and Heidrun) with specific gravities from 0.8746 to 0.9058 g/cm^3 (at 22°C). They conducted laboratory experiments in seawater to evaluate the combined effects of three factors—chemical dispersants, mixing energy, and mineral types—on OPA formation. They also discussed the usage of OPA as a response technique. They found that hydrophobicity (aversion toward water), particle size, and specific surface area of minerals are key factors, which is consistent with other findings in the literature. Slightly hydrophobic particles enhanced formation of OPAs by promoting the attachment of mineral particles to oil. However, highly hydrophobic particles clumped together and did not interact with the oil. Therefore, there was an optimum range of hydrophobicity for maximum OPA formation. If minerals were hydrophilic, the OPAs were generally spherical. Hydrophobic minerals formed irregularly shaped OPAs. The OPAs were larger for hydrophobic particles than for hydrophilic minerals. Chemical dispersants when added became the overriding factor affecting OPA formation because of their stabilizing effect on oil droplets. High mixing energy enhanced dispersion of oil into the water column to form droplets and small-sized OPAs. The Zhang and others (2010) study concluded that in areas of low mixing energy, a chemical dispersant might be needed with a co-application of fine mineral particles to form OPAs.

Laboratory studies in freshwater using automated shaker tests of mixtures of kaolinite clay-sized particles and heavy and intermediate fuel oils (viscosities of 3,900 and 1,350 cP, respectively) were conducted by Perez and others (2014) to simulate of the interaction of oil slicks and suspended sediment in steep, turbulent rivers. Using a spectrophotometer for oil measurement, the amount of oil entrained by sediment was observed to be moderate for heavy fuel oils at wave heights of 2.5 and 7 cm and kaolinite concentrations of up to 16,000 parts per million (ppm). They predicted that a surface slick of 1,000 kg of IFO across a river width of 10 m would result in about 8 percent of the oil entrained in OPA over a 1 km length. However, there was a large variability in the data, and thus these results still need

confirmation of scaling factors using either large scale experiments or with computational fluid dynamics models.

Khelifa and others (2005b) and Niu and others (2010, 2011) developed predictive models to estimate the contribution of OPAs to the dispersal of spilled oil and their potential to cause secondary detrimental effects associated with physical inhibition (that is smothering of benthic organisms) or toxicity. Factors considered in these models include the calculation of the maximum size of droplets, prediction of oil droplet formation from a slick, prediction of sediment aggregate formation, and the calculation of the density of the resultant oil-sediment aggregate. Inputs to the models include environmental conditions, oil properties, and concentration and particle-size distribution of suspended sediment. In a model sensitivity analysis of five crude oils (Hibernia, Louisiana, Prudhoe Bay, Arabian Light, and Alaska North Slope), with a range of densities from 25 to 37 degrees API and dynamic viscosities from 8 to 68 cP, the kinetic energy dissipation rate was varied from 10^{-3} to 10^{2} square meters per cubic second (m^2/s^3) with a sediment particle size of 3 micrometers (□m) and concentration of 250 mg/L; the study showed that OPA formation is strongly dependent on the oil-water interfacial tension and kinetic energy dissipation rate. The OPA contribution to oil dispersion increased when energy dissipation rates were about 1 watt/kg or higher. High turbulence in surf zones or in rivers should therefore be conducive to higher rates of OPA formation.

Transpot and Fate of Oil-Particle Aggregates

As described in the section "Oil-Particle Aggregates as a Natural Physical Dispersant", formation of OPAs changes the fate and transport of oil by potentially changing its rate of horizontal and vertical transport, and biodegradation and levels of bioavailability, which ultimately influence the ecological effects of OPAs. Because of the range of variance in physical, chemical, and biological conditions between sites (for example, types of suspended particulate organic/inorganic material, type of oil, mixing energy) differences in the transport and fate have been observed between marine and freshwater environments. In the coastal marine environment, the formation of OPAs has been found to improve removal of stranded oil from low-energy intertidal environments and is considered to be a natural self-cleansing process that enhances recovery rates following a spill (Lee, 2002). In contrast, in lowland rivers with gentle gradients, naturally formed OPAs can lengthen oil spill cleanup times and require deployment of less conventional and more costly sediment remedial measures (Dollhopf and others, 2014; Gouvernement du Quebec, Depot Legal, 2014). For example, in the 2010 Enbridge Line 6B spill of diluted bitumen into the Kalamazoo River, approximately 100 hectares (250 acres) of oiled sediment remaining in impounded sections of the river was removed by dredging and excavation during 2013–14, in response to persistent sheening problems (Dollhopf and Durno, 2011; Dollhopf and others, 2014). Oil globules and OPAs of various sizes, up to a few mm in size, were resuspended during floods, released upon mechanical agitation or physical disturbance of the sediment, and liberated by gas bubbles rising to the surface in a process called ebullition (as happens naturally when methane is produced in freshwater sediments) (fig. 1). Similarly, following the Lac-Mégantic light crude train spill in the Chaudière River, Quebec, Canada, oiled sediments created a challenge to clean-up operations downstream from the spill site over a 30 km reach of the Chaudière River (Gouvernement du Quebec, Depot Legal, 2014).

Modeling the transport and fate of OPAs in riverine systems requires integration of hydrodynamic, sediment transport, and contaminant fate and transport models (Dollhopf and others, 2014; Niu and others, 2010, 2011), while employing some of the same guidelines used for developing conceptual and mathematical models of fate and transport of contaminated sediment at hazardous waste sites (U.S. Environmental Protection Agency, 2005). Simplified fate and transport studies were done for

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the 2010 Kalamazoo River spill by considering OPAs in a steady state of physical properties, including size, concentration, specific gravity, erosion rates, and settling velocities, as well as hydrocarbon content (Dollhopf and others, 2014). More complex models, accounting for advection/diffusion, settling, resuspension, and breakup characteristics, are being considered for freshwater (riverine and deep-water settings) and marine environments (Lee and others, 2011a; Niu and others, 2011, 2014) and can be built from simpler models that simulate oil slicks (Weaver, 2004).

For the Enbridge Line 6B diluted bitumen spill into the Kalamazoo River, a 2-dimensional (2-D) Environmental Fluid Dynamics Code (EFDC) model was constructed to determine areas of the river prone to resuspension and deposition of submerged oil and oiled sediment under different flow conditions (Hamrick, 1992; Enbridge Energy L.P., 2012a; Dollhopf and others, 2014). Initially, OPAs were assumed to behave similarly to silt-sized particles because the OPAs accumulated in depositional areas and impounded sections of the river with organic- and silt-rich soft sediment. To account for differences in behavior, the model was updated with a new algorithm for OPAs that was incorporated into the sediment transport code of the SNL-EFDC model—a modified version of the original EFDC code developed and maintained by Sandia National Laboratory (James and others, 2005; Thanh and others, 2008). This version of the EFDC model incorporates a custom sediment transport sub-model that is based on the SEDZLJ model algorithms developed by Craig Jones and Wilbert Lick at the University of California – Santa Barbara (Jones and Lick, 2001). The OPA algorithm includes particle classes for representing two types of OPA—a sediment coating on an oil droplet and oil droplets in a particle-dominated aggregate. This algorithm and its application to EFDC and SEDZLJ are under development by the U.S. Army Corps of Engineers Engineering Research and Development Center at the time of this writing (January 2015). Hydrodynamic model results of velocity and horizontal bed shear stress from 2-D and 3-dimensional (3-D) EFDC model simulations of various flows and containment situations on the Kalamazoo River helped to target areas of the 61 km of oil-affected Kalamazoo River where submerged oil and OPAs were subject to resuspension and downriver migration during high flows (Dollhopf and others, 2014; Enbridge Energy L.P., 2012a).

Erosion rates of soft sediment became an important parameter in the modeling because these areas tended to have moderate/heavy oiling, and some of the areas in impoundments switched from depositional to erosional during high flows. The soft sediment was cohesive, and onsite sedflume tests were performed by the U.S. Army Corps of Engineers to parameterize the sediment transport and OPA algorithms in the EFDC models (Perkey and others, 2014). Critical shear stresses of soft sediment with moderate/heavy oiling ranged from 0.1 Pascals (Pa) at the surface to 1.0 Pa at 20 cm beneath the surface (Perkey and others, 2014).

Another approach for fate and transport modeling in rivers is a Lagrangian approach, also known as particle tracking. This approach has been used by the Ven Te Chow Hydrosystems Laboratory at the University of Illinois for the Kalamazoo River and builds on the existing EFDC-based hydrodynamic model.

For impoundments with accumulations of thick fine-grained sediment, the process of bubble formation and release from sediments (ebullition) is likely to be an important mechanism for resuspending OPAs in the water column and releasing oil as sheen on the water surface. Spontaneous releases of oil globules and floating OPAs have been observed regularly in the impounded sections of the Kalamazoo River during 2011–14, resulting in oil sheens at the water surface (Dollhopf and others, 2014). The impoundments had generally 0.5 to 4-m thick accumulations of fine-grained organic-rich sediment which, under anaerobic conditions, allows for bacterial generation of methane (McLinn and Stolzenburg, 2009). These bubbles could rise based on their size and buoyancy, and their release from the sediments could be enhanced by disturbances such as fish and boats movement, falling water levels,

and dropping barometric pressures. Methane production and ebullition are enhanced by warm summer water temperatures (as high as 28°C) that promote bacterial activity and reduce the solubility of methane in sediment porewaters.

Ecological Risk and Toxicity of OPAs and Oiled Sediment

The aquatic toxicity of OPAs is of interest, whether the OPAs formed through addition of minerals as a dispersant or from association of oil droplets with suspended inorganic and organic particles naturally present in a water body after the spill. Once oil droplets are aggregated with particles and submerge, associated contaminants, especially polycyclic aromatic hydrocarbons (PAHs), become a potential problem to suspended and benthic aquatic organisms, including plankton, zooplankton, invertebrates, mussels, and clams, and any higher level organisms that consume them (Long and others, 1998; MacDonald and others, 2000; Passow and others, 2012; Almeda and others, 2013). On the other hand, as illustrated during field studies, oil bound up in OPAs may be diluted to below toxicity threshold limits (Lee and others, 2003b) and may become more available for biodegradation (Lee and others, 1997; Lee and Merlin, 1999). An important ecological consideration is closely linked to the turbulent energy of the environment with more risk associated with submerged OPAs in depositional or low energy environments. Freshwater depositional environments are common in low-gradient rivers, river mouths and harbors, impoundments, ponds, lakes, and wetlands. Low-energy marine environments are likely similar (Niu and others, 2011). Thus, a major factor in ecological risk is whether the OPAs are physically diluted in suspension or concentrated in deposition. The added context of water depth and the geographic extent are also important. However, oil droplets and OPAs have a size range similar to that of planktonic food sources for zooplankton, and biogeochemical processing by organisms could increase or decrease toxicity within different components of the aquatic food web (Passow and others, 2012; Almeda and others, 2013).

Data are sparse on the specific toxicity of OPAs in marine and freshwater environments but there are a few studies to draw upon. Qualitative analysis indicated that residual oil was in a highly biodegraded (that is, less toxic) state in suspended particulate material associated with OPA formation from surf washing operations during the Sea Empress Spill in the United Kingdom (Lee and others, 1997). In terms of the application of bioassays, samples recovered from sediment traps deployed in the immediate area off an experimentally oiled beach site in Svalbard, Norway, remediated by the enhancement of OPA formation by surf-washing, were found to be within Environment Canada's acceptable regulatory limits for the disposal of dredge spoils (Lee and others, 2003a; Lee and others, 2003b).

For freshwater riverine environments, limited acute toxicity testing was done for oiled sediment in the Kalamazoo River after the Line 6B oil release (Bejarano and others, 2012). As part of a Net Environmental Benefits Analysis, effects on aquatic organisms from weathered oil were assessed in laboratory acute toxicity studies of seven sediment samples collected from oil-affected backwater habitats along the Kalamazoo River in February 2012, about 19 months post-spill (Bejarano and others, 2012). Ten-day whole sediment toxicity tests using *Chironomus dilutus* (a species of midge) and *Hyalella azteca* (an amphipod crustacean) were performed by the Great Lakes Environmental Center, Inc., and included survival, growth and biomass as the toxicity endpoints (Great Lakes Environmental Center, 2012). Results from the toxicity tests indicated that *Chironomus dilutus* were more sensitive to oiled sediment (and presumably OPAs) than *Hyalella azteca* but that all samples exceeded the minimum survival (70 percent) and growth (0.48 mg ash-free dry weight at test termination) criteria for acceptable controls for the *C. dilutus* tests (Great Lakes Environmental Center, 2012).

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Chemical analyses (PAHs, total extractable hydrocarbons) and sediment characterization (total organic carbon and sediment composition) were performed on a subset of the above described Kalamazoo River sediment toxicity samples (Bejarano and others, 2012; Great Lakes Environmental Center, 2012). Potential adverse acute and chronic effects on benthic organisms were evaluated using the Equilibrium Sediment Benchmark Toxic Unit Approach (U.S. Environmental Protection Agency, 2003) for sediment PAH data from the same samples used for the bioassays. The results from these analyses indicate that sediment from two heavily oiled sites and one lightly oiled site may pose acute and chronic risks to benthic fauna (*Chironomus dilutus* and *Hyalella azteca*). However, further analyses of the toxicity results in the context of several other sediment characteristics (chemical and physical) showed that unrelated variables, such as percentage of silt in the sediment sample, may have affected survival (table 1). On the basis of the weight of evidence approach and additional risk metrics, it is possible that residual oil from the Enbridge Line 6B oil spill at two heavily and one lightly oiled area may pose some risks to benthic receptors. Chronic toxicity effects from the Enbridge Line 6B residual oil remain unknown at the time of this writing (January 2015).

Table 1. Nonparametric Spearman's Rho correlation coefficients between acute sediment toxicity test results and analytical variables using Chironomus dilutus and Hyalella azteca. Highlighted cells indicate a statistically significant correlation at α=0.05. Negative correlation coefficients indicate that survival, growth, and biomass were reduced in sediment with higher concentrations of low molecular weight polyaromatic hydrocarbons (PAHs) and total extractable hydrocarbons (TEHs) (from Bejarano and others, 2012).

[LMW, low molecular weight; HMW, high molecular weight; µg/kg, micrograms per kilogram; mg/kg, milligrams per kilogram; TPAH, total polyaromatic hydrocarbons; TOC, total organic carbon; %, percent]

Variables	*Chironomus dilutus*			*Hyalella azteca*		
	Survival	Growth	Biomass	Survival	Growth	Biomass
Sum LMW-PAH (µg/kg)*	-0.53	-0.47	-0.51	-0.44	-0.18	-0.17
Sum HMW-PAH (µg/kg)*	-0.45	-0.36	-0.40	-0.36	-0.20	-0.17
TPAH (µg/kg)	-0.49	-0.43	-0.47	-0.39	-0.22	-0.21
TEH (mg/kg)**	-0.52	-0.53	-0.54	-0.47	-0.29	-0.39
%TOC	-0.67	-0.35	-0.42	0.04	-0.65	-0.65
% Gravel	-0.08	0.02	0.03	0.01	0.07	0.18
% Sand	0.56	0.40	0.41	0.23	0.41	0.54
% Silt	-0.54	-0.52	-0.52	-0.36	-0.44	-0.55
% Clay	-0.53	-0.21	-0.25	-0.13	-0.41	-0.53

*Low molecular weight (LMW) PAHs include Naphthalene to Benzo(b)fluorine (38 analytes), whereas high molecular weight (HMW) PAHs include Fluoranthene to Benzo[g,h,i]perylene (26 analytes).

** Total Extractable Hydrocarbons (C9-C44; TEH).

Summaries of the physical effects from burial of benthic organisms by deposited OPAs can be found in available literature on burial effects from agitation dredging (Chapman, 2012) and on sediment deposition in streams (Waters, 1995). Aquatic organisms most likely affected by burial are fish eggs, larvae, and fry; sessile filter feeders such as mussels; and macrophytes (Chapman, 2012; Morton, 1977; and studies cited in Kaplan and others, 1974; Erftemeijer and Lewis, 2006). For marine benthic organisms, recommendations have been made to limit disposed sediment to 15 cm, but species survival is highly variable, with some species destroyed by as little as 5 cm (OSPAR, 2008) and fish eggs by as little as a few mm (Berry and others 2003; see other citations in Chapman, 2012). Deposition effects vary by species requirements, extent and spatial connection of habitat types, and size of the waterbody.

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Effects of Ice in Northern Climates on OPA Formation and Spill Response

Most of the cold-condition studies that have been conducted have looked at the behavior of spilled oil and not how the oil interacted with particles to form OPAs (Fingas and Hollebone, 2003; Lee and others, 2011b; Wang and others, 2013) under controlled laboratory and pilot-scale tank studies. Lee and others (2011b) summarize findings from laboratory studies funded by the Canadian Coast Guard to elucidate the potential significance of OPA formation under cold climatic conditions that included ice (Cloutier and others, 2005; Khelifa and others, 2005c). These studies showed that OPAs can form quickly with strong turbulence, within the first 10 minutes of a spill. Within 40 minutes, most of the oil was converted to OPAs. Brackish water (18 ppt) with slush and broken ice had similar results with strong turbulence. Most particles were less than 1 mm in diameter and about 50 percent of the oil was physically dispersed within 30 minutes (Blouin and Lee, 2007; Cloutier and Doyan, 2008).

Field trials in the St. Lawrence River estuary near Matane, Quebec were conducted during the winter to further evaluate the feasibility of enhanced OPA formation as a spill response countermeasure in ice-infested waters (Lee, Li and others, 2011b). An icebreaker's propeller was used to generate strong turbulence during this exercise to facilitate the formation of OPAs with experimentally released fuel oil sprayed with slurry of fine-grained chalk using the fire hose system on board the ship. Visual observations and results of laser particle size analysis (LISST) indicate that fuel oil physically dispersed as OPA into the water column by this experiment did not readily reform a surface slick. Without the addition of mineral fines and consequential OPA formation, the fuel oil resurfaced within minutes and was difficult to recover because of interference by large ice blocks. Half of the total petroleum hydrocarbons in recovered samples biodegraded after 56 days incubation at a temperature of 0.5 ^{o}C, which is most likely because of microbes in the water that were well adapted to their surrounding environment, despite the low temperatures.

Although not specific to OPAs, Belore (2010) provided a detailed description of simulations of oil and condensate spills over a range of expected temperatures at the marine trans-shipment terminal and confined channel assessment area (CCAA) of concern for syncrude synthetic light oil, Condensate Blend (CRW), Cold Lake bitumen diluted with condensate, and MacKay River heavy bitumen diluted with Suncor synthetic light oil (MKH). The simulations included a hypothetical marine terminal spill and three hypothetical tanker spills for Emilia Island, Principe Channel, and Wright Sounds for spring, summer, fall, and winter conditions. The tests did not consider interactions between oil and particulate matter, but they provided a thorough set of physical properties and behaviors for these oils including density, viscosity, interfacial tension, pour point, flash point, evaporation, emulsion formation, and oil adhesion.

The formation of OPAs and its effect on promoting dispersion of spilled residual oil may vary because of changes in the properties of the oil, water, particle type, temperature, and extent of ice cover. For example, the viscosity of diluted Cold Lake Blend ranges over an order of magnitude from summer water temperatures (for example, 393.2 square millimeters per second (mm^2/s) at 15 ^{o}C) to winter water temperatures (1437.8 mm^2/s at 1 ^{o}C) (Belore, 2010).

Biodegradation and toxicity need more research because potential exposure and biological activity are affected by temperature. Recent results by McFarlin and others (2014) found that oil degrading microorganisms were present in surface, middle, and deep water samples from the Arctic Ocean and that oil biodegradation potential exists for offshore Arctic environments; however, this study did not specifically look at biodegradation of OPAs.

Researchers from the Institute of Northern Engineering–University of Alaska Fairbanks and NewFields LLC have conducted several studies of the toxicity of physically and chemically dispersed

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oil in arctic environments (McFarlin and others, 2011) that provide some insights into OPA toxicity. In one study, the toxicity of physically versus chemically dispersed oil to selected arctic species representative of the Beaufort and Chukchi Seas was examined at typical arctic water temperatures of 2 ^{o}C (McFarlin and others, 2011). Using fresh Alaska North Slope oil, physically dispersed under increased mixing energy in a laboratory, spiked exposure toxicity testing was performed for three arctic species: *Calanus glacialis* (copepod), *Boreogadus saida* (arctic cod), and *Myoxocephalus sp.* (sculpin). When subjected to physically dispersed oil, mean lethal concentration (LC_{50}) values were lower by 3.3 and 3.7 mg/L total petroleum hydrocarbons (TPH) for arctic cod and early season copepods, than the corresponding values in the presence of chemically dispersed oil of 55 mg/L and 22 mg/L, respectively. With the exception of this case, toxicity effects for arctic species from physically dispersed oil were found to be generally no better or worse than for temperate species and warm water temperatures.

In conclusion, the toxicity of suspended and deposited OPAs requires further investigation. OPAs that submerge and accumulate in depositional areas have chemical and physical ecological risks associated with them, either because of toxicity from high concentrations of hydrocarbons, or physical damage, which can result from the smothering and burial of benthic organisms. The appropriate application of laboratory tests to different geographic areas with a range of water depths and water currents needs further consideration.

Operational Considerations

As described in preceding sections, oil can submerge, and OPAs can form rapidly after a spill of light and heavy crude oils, given the right environmental conditions. Key environmental factors that increase the probability of submergence for bitumen spills include (Silliman, 2014):

- Low salinity resulting in relatively low water density,
- Particles that have relatively high roughness in their surface area or are porous,
- High turbidity or high suspended sediment concentrations,
- Exposure to sunlight (UV radiation),
- Strong currents and mixing energy, and
- High temperatures.

Silliman (2014) concludes that if one of these factors is present, then emergency response personnel should equip themselves with response tactics for submerged oil. The following sections describe the state of the science for detection, containment, and recovery of submerged oil, especially submerged OPAs.

Detection

OPAs can be detected by direct observation using specialized microscopy techniques that enable the visualization of oil. On the basis of the strong natural fluorescence of aromatic hydrocarbons and chlorophyll when excited by UV light, Lee and others (1985) devised a bright field transmitted light/UV epi-fluorescence illumination technique that enabled the observation of interaction between chemically dispersed oil droplets and phytoplankton. This technique was subsequently refined and used in a routine manner to characterize and quantify OPAs (Stoffyn-Egli and Lee, 2002; Lee and others, 2003a; Ma and others, 2008). Detailed investigations of the surface and internal structure of OPAs have been conducted by the application of confocal laser scanning microscopy (Stoffyn-Egli and Lee, 2002; Zhang and others, 2010; Wang and others, 2011). Environmental scanning electron microscopy (ESEM) was used to produce high resolution 3-D topographical images of OPA surfaces in their natural state to

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confirm the existence of "droplet" OPA composed of oil droplets that were stabilized by mineral fines on their surface (Stoffyn-Egli and Lee, 2002). The U.S. Coast Guard's Research and Development Center recently specified requirements for submerged oil detection systems that included 80 percent detection probability, 1-m radius of detection, real-time results, reasonable setup time, accommodation of 1.5-m (5-ft) seas and 0.8 m/s (1.5 knot) currents, and coverage of 1.6 km^2 (1 mi^2) in a 12-hour shift (Hansen, 2014). Established on-site mass spectrometer systems to monitor oil in the water column may not be effective if the oil occurs as OPAs or has already settled out. This was the case in the oil-affected reach of the Kalamazoo River.

Laser fluorometers offer the potential for detection of oil in oil-sediment mixtures and were successful in detecting residual oil in pore water in beach deposits along the coast of Taean, South Korea, following the *Hebei Spirit* oil spill (Kim and others, 2010). In addition, trials with submersible fluorometers with an excitation wavelength of 120-325 nanometers (nm) and emission wavelengths of 410-600 nm met with some success for the Kalamazoo River when the OPAs were in suspension during in situ tests of erosion characteristics of deposited oiled sediment from the 2010 Enbridge Line 6B oil release. A limited number of samples collected at the time the OPAs were in suspension were analyzed at the USGS Wisconsin Water Science Center for both absorbance and fluorescence excitation-emission matrices using an Aqualog instrument (Peter Lenaker, U.S. Geological Survey, written commun., 2013). However, once the OPAs are submerged and mixed with bottom sediment the fluorescent properties of the oil are masked by other particles (Lee and others, 2012). As a result of these short-falls identified with current methodologies for oil spill detection in aquatic environments, Hansen (2014) stressed the importance of simultaneous use of multiple systems.

Detection of OPAs by sonar can be difficult because the detectable sonar signature associated with pure oil is diminished when it is mixed with suspended and bottom sediments. Furthermore, particle size is a factor; oil associated with fine-grained (silt and clay-sized) organic-rich particles is difficult to detect by sonar (Hansen, 2014; authors' experience on the Kalamazoo River cleanup).

A novel sediment poling technique was developed for detecting and assessing the spatial distribution of submerged oil and oiled sediment in the Kalamazoo River; it was adapted from studies of contaminated sediment (David Richardson, Tetra Tech, Inc., oral commun., 2011). This became the primary submerged oil assessment tool used in the Kalamazoo River cleanup (Enbridge Energy, L.P., 2013a; Dollhopf and others, 2014). The sediment was agitated using a graduated aluminum pole with a 20-cm-diameter metal disc on the submerged end. If submerged oil was present in the sediment, the agitation action liberated oil from the sediment, allowing it to float to the water surface. The percent coverage of oil sheen and number of globules at the water's surface within 1 m^2 were observed and categorized as none, light, moderate, or heavy according to the field observation submerged oil flowchart (fig. 8). Thickness of soft sediment in depositional areas could be estimated by quantifying the difference between a 1- and 2-hand push of the graduated pole into the sediment. This procedure was used to map the relative concentration and extent of oiled areas to depositional areas of the river with soft sediment (silt and organic matter) accumulations. From 2010 to 2014, over 20,000 poling points were assessed throughout the affected river system. Global positioning system (GPS) coordinates and field observations were recorded upon poling and managed in a geographic information system, which allowed for detailed mapping of sheening as well as bathymetry, substrate, and flow velocity. Some limitations of the method include the need for water temperatures generally greater than 15°C for consistent categorization, velocities less than about 0.3 m/s (otherwise the sheen and globs are swept too quickly downstream), and water depths of generally less than 3 m. At greater water depths, it is unknown whether the liberated oil can rise the full distance to the water surface, resettles, or is transported downstream.

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For the Kalamazoo River spill, multiple rounds of sediment cores were collected, and two types of in situ suspended sediment samplers were deployed for monitoring and assessment (Enbridge Energy, L.P., 2011a). About 25 in situ suspended sediment samplers (Phillips and others, 2000) were placed along the 61-km stretch of the affected river to collect any submerged oil and oiled sediment in suspension in the water column that was being transported over a range of flows including during cleanup activities. In addition, about 70 suspended sediment settling jars, also known as sediment traps (Thomas and others, 2007), were placed in depositional areas of the Kalamazoo River with little to no flow to collect particles that were recently in suspension and settled to the river bottom. These jars were similar to column sampling devices used in lacustrine environments for particulate resuspension and chemical fluxes with ponded water or multiple flow directions, such as wave action and longshore currents (Eadie and others, 1984; Murdoch and MacKnight, 1994).

Initially in the cores, globs of submerged oil from the Kalamazoo River Line 6B spill fluoresced under UV light, making it possible to identify the presence and depth of oil in the cores. In 2012, 2 years after the spill, it became necessary to positively identify that the oil in river sediment originated from the spilled oil and not from a previous spill or other background hydrocarbons (Dollhopf and others, 2014). It was at this time that the oil in the sediment cores was becoming more difficult to visually quantify using UV-fluorescence because of dilution, dispersion, and quenching of oil droplets within the sediment matrix (Lee and others, 2012). It then became necessary to identify a chemical fingerprint of the Line 6B oil using forensic oil chemistry techniques that distinguished a signature of triaromatic stearene biomarkers in Line 6B oil that was unique from other oil contaminants present in Kalamazoo River sediment before the spill (G. Douglas, NewFields, written commun., 2014; Dollhopf and others, 2014). This technique was used to quantify the remaining Line 6B oil concentrations in all three types of sediment samples collected in the Kalamazoo River—sediment cores, in situ suspended sediment samples, and recently deposited samples.

Containment

Conventional containment strategies for floating oil do not address the submergence, potential resuspension, and subsurface transport and redistribution of OPAs. OPAs will easily pass underneath a surface boom. Subsurface booms and silt curtains are more effective. An example of the equipment used to keep submerged oil and oiled sediment from the 2010 Line 6B spill from migrating farther downstream in the Kalamazoo River and into Morrow Lake is shown in figure 9A. The top curtain contains a boom similar to conventional setups to trap floating oil. The bottom curtain is meant to cut off bottom currents and promote deposition. This type of containment curtain is oriented at an angle to river currents to maximize settling of OPAs while limiting the chance for new areas of scour (fig. 9B) (Enbridge Energy, L.P., 2012b, 2013b).

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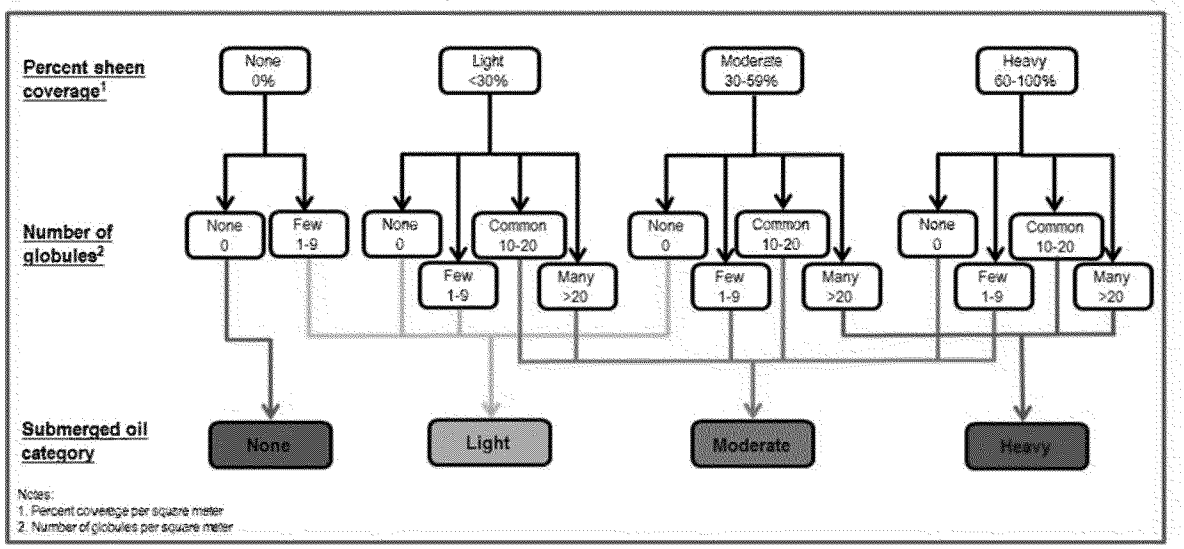


Figure8. Flowchart used for field observations of submerged oil during poling assessments. (from Enbridge, 2013a; %, percent; <, less than; >, greater than).

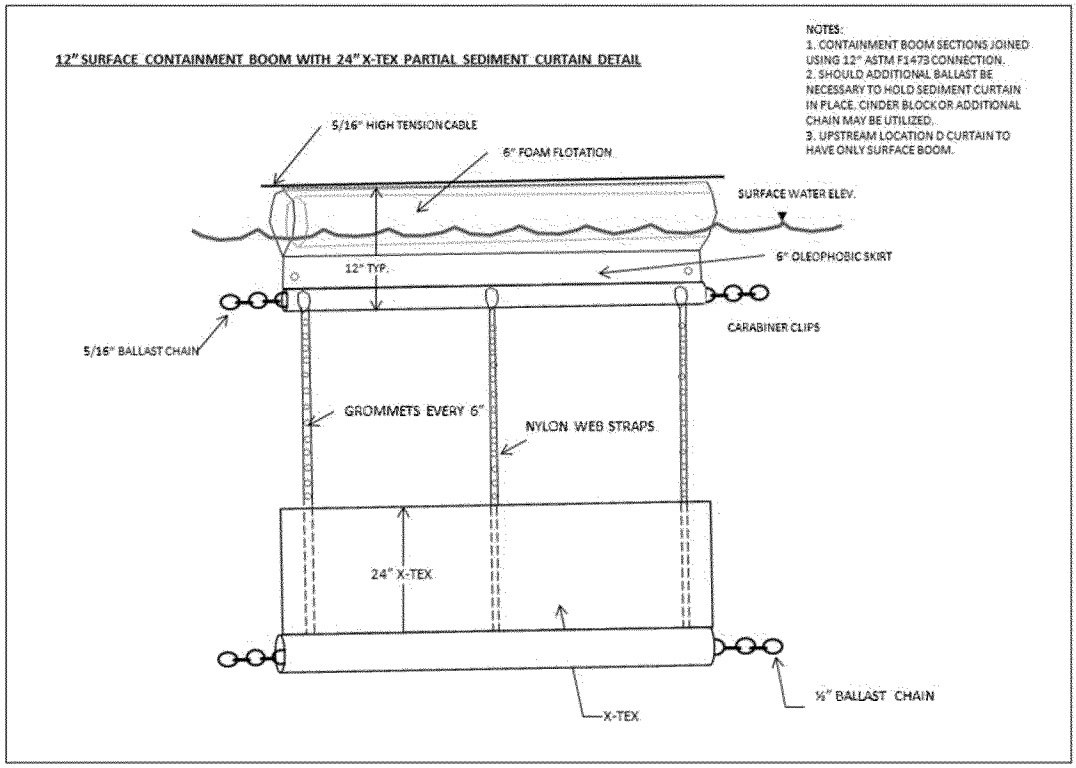
Recovery

Recovery techniques for submerged oil and OPAs in freshwater and marine environments are still in the development phase. In a recent document on the fate and transport of potentially spilled oil associated with the proposed Energy East Pipeline Project from Alberta to New Brunswick the following recovery equipment and techniques were recommended by Energy East Pipeline Ltd. (2014): specialized nets, bottom booms, dams and underflow weir dams, dredging, manual recovery, and air injection. Challenges of oil recovery in cold climates, whether in marine or freshwater environments, involve accounting for the following variables: presence of ice, air and water temperatures, remote locations, and low solar radiation (Lee, Li and others, 2011a, b).

Early on in spills, weighted sorbent materials can be dragged along the sea floor or draped along a river bottom to capture submerged oil and OPAs (Hansen, 2014; Enbridge Energy, L.P., 2010). Subsurface sorbent pom-poms (Pister and others, 2009) have been used for oil in suspension. Where oil in deposited OPAs remains at concentrations that cause concerns for benthic organisms or excessive sheening problems, dredging may be necessary (Dollhopf and others, 2014; Gouvernement du Quebec, Depot Legal, 2014).

Enbridge used a combination of agitation toolbox techniques and sheen sweeps in contained areas of the Kalamazoo River, but sheening problems continued in depositional areas after these techniques were used, leading to the adoption of dredging as the final solution (Enbridge Energy, L.P., 2010; Dollhopf and others, 2014). Agitation toolbox techniques used on Kalamazoo River bottom sediment included mechanical agitation through raking, hand-held tillers, and chain drags, along with hydraulic agitation using hand-held water jets arranged as a single wand or on a rotating head (known as stingers) and vessel-mounted or dragged spreader bars with multiple water jets (Enbridge Energy L.P., 2011b). Conventional oil skimming techniques and sheen sweeps were used in response operations in the Kalamazoo River cleanup during periods of spontaneous release of oil globules from depositional areas of the river.

A



B

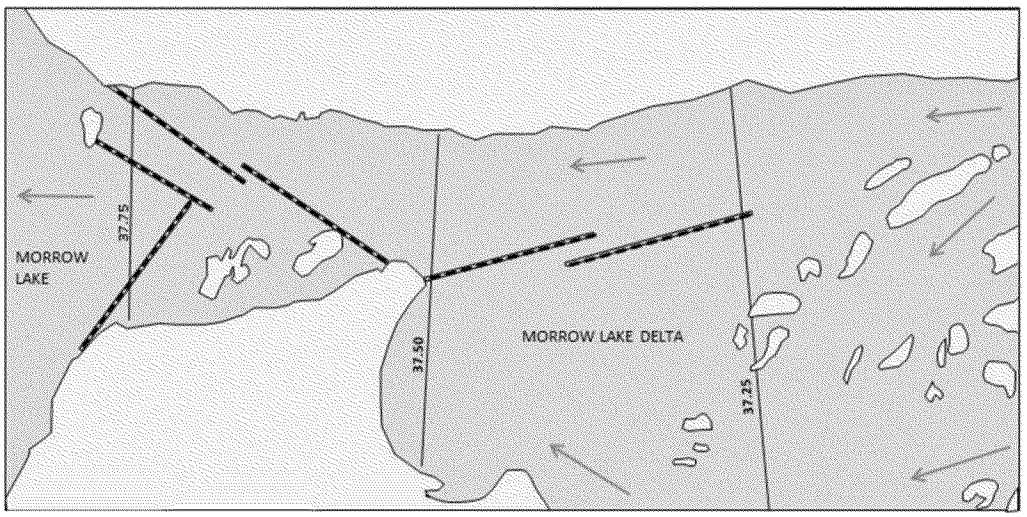


Figure9. A, Diagram of subsurface curtain boom, and B, map of containment boom locations for submerged oil in Morrow Lake Delta from river miles 37.25 to 37.75, Kalamazoo River, Michigan related to the Enbridge Line 6B oil release. Blue arrows on map show general flow direction from right to left. Bold dashed lines are containment boom locations. Redrawn diagrams from Enbridge Energy, L.P. (2013b) containment permits.

Some of the methods that have been used for OPAs in suspension and on the bottom along shorelines include (Pister and others, 2009)

- Weighted sorbent materials (for example, plastic pom-poms),
- Dredging,
- Natural attenuation,
- Vacuum truck (limited by access),
- Small portable submersible pumps,
- Debris removal and wrack cleaning,
- Sediment reworking,
- Flooding, and
- Low and high pressure flushing (ambient water) (may leave a significant quantity of oil that requires additional effort).

Techniques that were generally not recommended for marine shorelines and beaches include (Pister and others, 2009)

- Offshore barriers and berms,
- Mechanical oil removal,
- Vegetation removal,
- Low and high pressure flushing (hot water),
- Steam cleaning,
- Sand blasting,
- Solidifiers,
- Shoreline cleaning agents, and
- Natural microbe seeding.

Unfortunately, there is not a simple operational endpoint for spill clean-up operations when it comes to residual OPAs. Remediation for each spill, whether in marine or freshwater environments, can benefit by the development of a Net Environmental Benefit Analysis, which weighs the benefits and drawbacks of leaving oil in place rather than causing further physical damage to aquatic habitats by aggressive removal techniques such as dredging or agitation (Efroymson and others, 2003; Rayburn and others, 2004; Bejarano and others, 2012). It cannot be automatically assumed that in all situations the oil concentrations in deposited OPAs will fall below toxic concentrations during a short period of time as a result of natural attenuation (dilution and biodegradation). Furthermore, it is important to note that the biodegradation of residual oil associated OPA is disadvantaged under anaerobic conditions that form after burial (Lee, 2000). However, depending on the spatial extent of the OPAs, the environmental setting, or presence of sensitive habitat, oil concentrations in OPAs may be adequately diluted to warrant no recovery and allow natural attenuation to happen (Lee and others, 2011a; Bejarano and others, 2012; Fitzpatrick and others, 2013).

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Future Science Needs

This report has summarized the state of knowledge regarding the formation of OPAs in natural waters, their eventual transport and fate, and considerations for cleanup of oil spills. The existing knowledge base is insufficient but rapidly growing with recent observations from large spills in freshwater and brackish riverine environments, additional laboratory tests and modeling, and studies of environmental effects of proposed new and expanding pipeline systems in North America carrying bitumen products (http://nas-sites.org/dilbit/). Specific science needs for submerged oil and OPAs are listed below.

- Laboratory experiments of resuspension and breakup of OPAs.
- Updated and new models and simulations of fate and transport of oil and OPAs in freshwater and cold climate environments with a range of oil and sediment types.
- Quantitative monitoring and mapping of large areas of OPAs in water depths greater than 3 m.
- Refinement of the Kalamazoo River poling technique with deployment of a fluorometer in the plume of sediment OPAs resuspended by the poling agitation.
- Monitoring and assessment of transport and fate of spreading oil in ice and below ice.
- Field trials in cold climates. More study is needed using realistic field trials or field observations of OPA formation during oil spills. Especially needed are studies of freshwater environments in and around the Great Lakes coastal environments and river mouths.
- Investigation of potential effects on benthic invertebrate communities from residual oil and OPAs in depositional environments including burial and smothering as well as hydrocarbon toxicity in marine, freshwater, and cold-climate environments.
- Investigation of OPA toxicity and physical effects on habitat. Not enough is known yet about the toxicity of OPAs, especially chronic toxicity and routes of exposure, and application of laboratory results to specific aquatic habitats. Data on the potential negative effects of augmented natural dispersion on burial and smothering of benthic organisms is needed, especially for freshwater environments.
- Vulnerability analyses of critical habitats.
- Incorporation of OPA properties into hydrodynamic and sediment transport models.
- Post-spill monitoring and assessment techniques.
- Operation endpoints—monitoring protocols to determine how much cleanup is enough and the manner in which natural attenuation may ameliorate effects in the future.

Summary and Conclusions

Studies of the formation of oil-particle aggregates (OPAs), and related behavior, fate, and toxicity in a wide variety of environments, including freshwater rivers, are of continued interest to researchers as transportation of light and heavy crude oils continues to increase across North America. This report contains an up-to-date review of the state of the science for OPAs from available literature, in terms of formation and stability, use as a physical dispersant, transport and fate, toxicity, behavior in cold climates, operational considerations, and future science needs. Although much is known about OPAs, there remains a good deal of science to be learned, especially in terms of laboratory experiments,

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flume studies, toxicity and habitat effects, field exercises, and modeling at a range of spatial and temporal scales.

Questions that were investigated for this report, along with brief answers developed from the available literature are listed here.

- What is the effectiveness of adding particles to an oil spill for physical dispersion of oil as a spill countermeasure? The effectiveness varies with oil and particle properties and the salinity of water, but in general the addition of particles will almost always result in some OPA formation. The particles act to stabilize oil droplets and prevent them from re-coalescing at the water surface into an oil slick. Some potentially negative consequences occur when OPAs settle to the bottom possibly causing issues with protracted and increased cleanup costs, and loss of habitat to benthic organisms from smothering and burial. The toxicity of OPAs compared to oil droplets in the water column varies or is not known. Because of these negative effects, prescribed sinking of spilled oil, or the use of sinking agents, is prohibited by U.S. Environmental Protection Agency.
- What is the long-term fate and transport of OPAs? Particle interactions tend to stabilize the oil droplets. OPAs can be resuspended when currents or mixing energy increases. OPAs can also be released to the water surface through the process of bubble formation and release from sediments (ebullition) for years following a spill.
- What are the ecological implications of OPAs? Ecological implications have to do with whether the OPAs stay in suspension or settle out and the geographic extent and water depth of the aquatic habitat. If OPAs stay in suspension, then microbial degradation would be likely increased. If OPAs settle out, ecological effects would be likely from toxicity and physical smothering.
- What are the operational considerations for recovery of OPAs? Containment and recovery of submerged OPAs or OPAs in riverine and marine environments require different techniques than those used for floating oil, and a familiarity with hydraulics of sediment transport is helpful.
- Are there special circumstances for OPAs in cold climates? OPAs form readily in cold climates and the addition of particles likely acts as a physical dispersant similar to warm climates. The additional difficulty of using conventional skimming techniques for floating oil where there is broken ice makes physical dispersion a more attractive option. However, habitat loss, burial, smothering, and toxicity effects from submerged OPAs need to be considered, especially for shallow freshwater environments.

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USGS

Fitzpatrick and others—Oil-Particle Interactions and Submergence from Crude Oil Spills in Marine and Freshwater Environments—Review of the Science and Future Science Needs—Open-File Report 2015–1076

ISSN 2331-1258
http://dx.doi.org/10.3133/ofr20151076

ED_001324_00007073-00044

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From: Hazen, Terry C (Terry)
Sent: Thur 4/30/2015 5:58:03 PM
Subject: Re: Degradation & Fate - next meeting 4/30/2015 at 1 ET
2015King_etal_ARMS.pdf

On Apr 29, 2015, at 4:42 PM, Mandsager, Kathy <kathy.mandsager@unh.edu> wrote:

This is a reminder of our WebEx meeting tomorrow. (Login instructions below)

As you review this document, please help us in securing the accurate references. For example, we may have Su et al,., 2013 in our notes (we don???t have this paper) but we are wondering if it should really be Sun et al., 2013 (we have this paper). And there may be several papers by Prince but we need to make sure the correct paper is noted accurately in the text.

Also, you might wish to search for your name in case we are looking for specific information from you.

Thank you to each of you for your input into this document!

From: Mandsager, Kathy
Sent: Wednesday, April 22, 2015 1:29 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'

Subject: RE: Degradation & Fate update -COMMENT on document - next meeting 4/30/2015

This is a reminder to submit any comments that you may have as soon as possible. Please mark your calendar for our next (potentially final) meeting for **Thursday 30 April at 1 ??? 3 ET**. WebEx instructions below.

Degradation & Fate Group: State-of-Science Dispersant in the Arctic
Thursday, April 30, 2015
1:00 pm | Eastern Daylight Time (New York, GMT-04:00) | 2 hrs

Join WebEx meeting

Meeting number: 732 633 609

Join by phone
1-855-244-8681 Call-in toll-free number (US/Canada)
1-650-479-3207 Call-in toll number (US/Canada)
Access code: 732 633 609
Global call-in numbers | Toll-free calling restrictions

From: Mandsager, Kathy
Sent: Monday, April 06, 2015 10:13 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu';

'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate update -COMMENT on document

My apologies ??? I hit send before I was done.

Dear Degradation and Fate Group

Please find attached the latest draft document for your review. We would ask that you review this very carefully and add any comments you may have to the spreadsheet that is also attached. Please especially note any items with yellow highlights, red lettering or with your name noted specifically.

And finally, this is your opportunity (as you review this whole document) to let us know if any topic has been overlooked in our group discussions thus far.

Please return your comments to me (kathy.mandsager@unh.edu) by **Friday 4/17.**

And please submit your times on the doodle poll to secure our next and final call>>
http://doodle.com/pgh4enphe24wqe24

From: Mandsager, Kathy
Sent: Monday, April 06, 2015 10:10 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'

Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET

We are nearing the conclusion! Please review the edits to this document.

And we would like to schedule a final call to discuss these comments.

Please select all times that you can possibly participate. Doodle poll here>>
http://doodle.com/pgh4enphe24wqe24

From: Mandsager, Kathy
Sent: Tuesday, March 31, 2015 11:14 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET

Additional paper for review. http://ac.els-cdn.com/S0025326X15000855/1-s2.0-S0025326X15000855-main.pdf?_tid=a972873c-d7b7-11e4-9464-00000aab0f27&acdnat=1427814635_2731a355cf42e8b64679699bc221613

From: Mandsager, Kathy
Sent: Monday, March 30, 2015 4:43 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'; Mandsager, Kathy
Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET

This is a reminder of our WebEx meeting tomorrow.

From: Mandsager, Kathy
Sent: Friday, March 20, 2015 6:19 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal';

'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET

Mark your calendar! This is to confirm our next meeting for **Tuesday, March 31** beginning at 11:00 am ET.

Her are the WebEx directions to join this meeting. Thank you!

Degradation & Fate Group
Tuesday, March 31, 2015
11:00 am | Eastern Daylight Time (New York, GMT-04:00) | 2 hrs

Join WebEx meeting

Meeting number: 734 916 289

Join by phone
1-855-244-8681 Call-in toll-free number (US/Canada)
1-650-479-3207 Call-in toll number (US/Canada)
Access code: 734 916 289
Global call-in numbers | Toll-free calling restrictions

Can't join the meeting? Contact support.

From: Mandsager, Kathy
Sent: Thursday, March 19, 2015 8:31 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION

I greatly appreciate your patience with this process. Please **cancel** the tentative hold on your calendar for tomorrow 3/20. Since we would greatly appreciate a majority of this group to participate in this 2-hour WebEx meeting, I am making another attempt at scheduling this call. Please use this doodle poll (all times noted are ET). Thank you to each of you!

Doodle poll here>> http://doodle.com/2w3gyb3gv4sv7iek

From: Mandsager, Kathy
Sent: Monday, March 16, 2015 4:39 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION

In the spirit of trying to keep everyone informed ??? there will NOT be a meeting tomorrow at 2 (see request below). I was unable to gain a quorum. Please continue to hold this Friday 3/20 as a potential meeting time. And if you have not yet responded with your

availability, please let me know if you could possibly make in on **3/20 beginning at 1pm ET.**

Thank you.

From: Mandsager, Kathy
Sent: Monday, March 16, 2015 9:38 AM
To: 'Ian P Gaudreau'; 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION
Importance: High

While these papers are fresh on your mind, I am offering some dates as soon as possible for our next meeting. Kindly let me know if you can make yourself available for about a 2-hour timeslot at any of the following times:

Tuesday (tomorrow) 3/17 beginning at 2:00 pm ET

Friday 3/20 beginning at 12noon ET

Friday 3/20 beginning at 1:00 pm ET

Friday 3/20 beginning at 2:00 pm ET

Friday 3/20 beginning at 3:00 pm ET

Thursday 3/26 at 10:00 am ET

Monday 3/30 beginning at 2:20 pm ET

A speedy reply will be greatly appreciated. Thank you!

From: Ian P Gaudreau [mailto:ipu3@wildcats.unh.edu]
Sent: Friday, March 13, 2015 10:16 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: Lindsey R Howard; Mandsager, Kathy; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; tracee.nguyen@csiro.au; Ian Gaudreau
Subject: Degradation & Fate update - Official Papers

Hi folks,

Thank you for hanging in there on the conference call Wednesday. We really appreciate all of the hard work you have been doing. The 13 official papers you decided you would review and use as the basis for your comments (e.g. knowns, uncertainties) on the effect of chemical dispersants on oil biodegradation are:

- Venosa & Holder 2007
- Prince et al. 2013
- Prince & Butler 2014
- Mcnaughton et al. 2003
- McFarlin et al. 2014
- Mason et al. 2012
- Lindstrom & Braddock 2002
- Kleindienst et al. 2015
- Chakroborty et al. 2012
- Campo et al. 2013
- Bruheim et al. 1997
- Bruheum et al. 1999
- Baelum et al. 2012

These papers are all located in a folder on BOX entitled ???Papers selected by panel - Effect of Chemical Dispersants on Oil Biodegradation???. This link can be found here:
https://unh.app.box.com/files/0/f/3252405678/Papers_selected_by_panel_-_Effect_of_Chemical_Dispersants_on_Oil_Biodegradation

Kathy will be contacting you to schedule what will hopefully be our final meeting of the Degradation & Fate group.

In preparation for the meeting, please re-read the paper and if you have time, send Kathy the conclusions that you derive from these references regarding what is known and uncertain about the effect of chemical dispersants on the biodegradation of oil, particularly as it applies to the Arctic.

Also, we need your feedback on a paper by Harris et al. 2002 (paper is attached in this email). Would you like us to add this paper to the ???Papers selected by panel - Effect of Chemical Dispersants on Oil Biodegradation??? folder on BOX? Here is the citation:

Harris, B.C. et al. 2002. Nutrient effects on the biodegradation rates of chemically-dispersed crude oil. *In* Twenty-Fifth Arctic and Marine Oilspill Program (AMOP) Technical Seminar, Nineteenth Technical Seminar on Chemical Spills (TSOCS) and Fourth Biotechnology Solutions for Spills (BIOSS): June 11 to 13, 2002, Westin Calgary Hotel, Calgary, Alberta, Canada: Proceedings. Ottawa, Ont.: Environment Canada. pp. 877-893.

Thanks again for your time and energy. I look forward to talking with you again.??????

Nancy E. Kinner, Ph.D.

University Professor

Professor Civil/Environmental Engineering

UNH Director, Coastal Response Research Center

Director, Center for Spills in the Environment

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<2015.04.29_Degradation and Fate_updated.docx>

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Microbial Responses to the *Deepwater Horizon* Oil Spill: From Coastal Wetlands to the Deep Sea

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Keywords

Gammaproteobacteria, hydrocarbon degradation, methane, bacterioplankton, metagenomics

Abstract

The *Deepwater Horizon* oil spill in the northern Gulf of Mexico represents the largest marine accidental oil spill in history. It is distinguished from past spills in that it occurred at the greatest depth (1,500 m), the amount of hydrocarbon gas (mostly methane) lost was equivalent to the mass of crude oil released, and dispersants were used for the first time in the deep sea in an attempt to remediate the spill. The spill is also unique in that it has been characterized with an unprecedented level of resolution using next-generation sequencing technologies, especially for the ubiquitous hydrocarbon-degrading microbial communities that appeared largely to consume the gases and to degrade a significant fraction of the petroleum. Results have shown an unexpectedly rapid response of deep-sea Gammaproteobacteria to oil and gas and documented a distinct succession correlated with the control of the oil flow and well shut-in. Similar successional events, also involving Gammaproteobacteria, have been observed in nearshore systems as well.

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1. INTRODUCTION

nGoM: northern Gulf of Mexico

DWH: *Deepwater Horizon*

PAH: polycyclic aromatic hydrocarbon

On the night of April 20, 2010, a tragic series of events in the northern Gulf of Mexico (nGoM) led to a blowout of the Macondo oil well in Mississippi Canyon Block 252 (MC-252) (Norse & Amos 2010, Lubchenco et al. 2012). A gas explosion ensued that killed 11 members of the *Deepwater Horizon* (DWH) drilling crew and injured 17 others. The blowout released an uncontrolled, buoyant, pressurized, and heated stream of crude oil and hydrocarbon gases from a depth of approximately 1,500 m—the greatest depth at which a catastrophic well failure has occurred to date. Oil flow rates from the well have been estimated at up to 9,900 m^3 d^{-1} (62,000 barrels d^{-1}), with a total crude oil release of some 650,000–780,000 m^3 (4.1–4.9 million barrels) over an 87-day period (Crone & Tolstoy 2010, McNutt et al. 2011, Reddy et al. 2012). This spill exceeded by a factor of two or more all but the Ixtoc I oil spill, which occurred at a much shallower depth (50 m) in the southern Gulf of Mexico (the Bay of Campeche) and released approximately 30% less oil than the MC-252 well (Jernelov 2010).

Approximately 1.7–3.1 × 10^8 kg of natural gas (C_1–C_5, mostly methane) was also released during the discharge; this is comparable to the mass of liquid oil released and was a distinguishing feature of the spill. The MC-252 spill also differed from others in the extensive use of chemical dispersants: A total of 7,000 m^3 (approximately 2.1 × 10^6 gallons) of dispersants, largely Corexit EC9500A and EC9527A, was injected directly into the oil plume at the wellhead or sprayed onto surface slicks to enhance solubility and promote bacterial remediation (Lubchenco et al. 2012). Subsurface use of Corexit, which accounted for approximately 37% of the total use, had not been attempted previously, and its efficacy remains uncertain.

From the discharge source at 1,500 m, oil, gas, and dispersants rose through the water column, entraining seawater and undergoing a kinetic fractionation process that altered the plume's composition with increasing distance from the wellhead (Valentine et al. 2012). Droplets of oil and associated dispersants were found in several size classes. Neutrally buoyant droplets 10–60 μm in diameter formed an extensive, regional-scale plume at depths mostly between 1,000 and 1,200 m (Camilli et al. 2010, Valentine et al. 2012, Spier et al. 2013). However, at various times, transient currents were also observed to entrain oil and gas droplets at four other depths (Spier et al. 2013). The deep plume was characterized by elevated concentrations of polycyclic aromatic hydrocarbons (PAHs), intermediate-length alkanes, methane, and other gases. Relative to adjacent, unaffected water masses, the plume was also characterized by lower oxygen concentrations (or oxygen anomalies), altered bacterioplankton community composition, and elevated bacterial density and activity (see Section 6).

In addition, large, positively buoyant droplets (~300 μm in diameter) with higher proportions of PAHs and heavier hydrocarbons rose to the surface, where they formed thin but extensive surface slicks (Atlas & Hazen 2011). The distinct surface and subsurface environments selected for different microbial populations in response to the released oil, even though Gammaproteobacteria dominated both surface-slick and deepwater plumes. Not surprisingly, the dynamics and impacts of surface and deep oil differed significantly from each other.

In the plume, both hydrocarbon and bacterial dynamics were strongly determined by complex interactions among biological, chemical, and physical processes, the latter of which included advection and mixing as the plume moved laterally from the wellhead (Adcroft et al. 2010, Ryerson et al. 2011, Valentine et al. 2012). Plume movement was not unidirectional, however; rather, it was affected by deep eddies and currents (DeHaan & Sturges 2005) that resulted in what appeared to be at some times a southwestward motion and at other times a sinusoidal movement to the northeast. Model analyses, which were largely consistent with observations, suggested that the plume was advected away from the wellhead but also returned to the wellhead, where it accumulated additional

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oil (Valentine et al. 2012). The circulation pattern of the plume and the reintroduction of oil had profound consequences for the dynamics of plume bacteria and for the degradation of at least some hydrocarbons (see Sections 3 and 4).

Although the amount of oil and gas entrained in subsurface plumes was substantial (approximately 2×10^8 kg), a considerable fraction of the total release (at least 50%) rose to the surface (Ryerson et al. 2012, Spier et al. 2013), including liquid oil and gas that were captured directly and stored (oil) or flared (gas). Once at the surface, the oil formed extensive slicks of varying thicknesses that affected an area of up to 180,000 km^2. The distribution of surface oil was determined by interactions between winds, currents, and eddies that reflected in part the dynamics of the Loop Current (Mezic et al. 2010). Contrary to initial expectations of a westward movement, partially weathered surface oil, including dispersants, moved to the northwest and northeast and washed ashore along the easternmost coast of Texas and much of coastal Louisiana (Mezic et al. 2010). Surface oil also contaminated beaches and wetlands from Mississippi eastward to the western Florida panhandle. Although extensive efforts were made to remove oiled beach sands and clean some wetlands, tar balls continue to appear on beaches, and oil persists in many wetlands both at the soil surface and belowground (Kelly 2013). In addition to the beached oil, an unknown but considerable amount of oil appears to have been buried in shallow nearshore sediments, where it remains susceptible to resuspension and subsequent beaching. (For a discussion of interactions between oil and bacteria in nearshore and wetland systems, see Sections 4 and 5.)

Of the oil released from the MC-252 well, relatively small percentages were collected by skimming or remediated by burning (approximately 3% and 5%, respectively; Lubchenco et al. 2012) (**Figure 1**). Approximately 17% was recovered directly from the wellhead, and 25% evaporated or dissolved (presumably degraded by bacteria). The remaining 50% comprised naturally and chemically dispersed oil phases (16% and 8%, respectively) and residual oil, which consisted of surface and subsurface sheen, tar balls, and oil that washed ashore or was buried. Some of this material (e.g., tar balls and tar mats) was and continues to be removed mechanically, but the fate of most of the residual oil depends on the dynamics and mechanisms of natural attenuation, which largely involves degradation by bacteria. The remainder of this review summarizes observations on bacterial hydrocarbon transformations measured to date and the prospects for future degradation.

2. PRESPILL BACTERIOPLANKTON COMMUNITIES IN THE GULF OF MEXICO

Before the DWH disaster, relatively little was known about nGoM bacterioplankton. Earlier studies had addressed their distribution, abundance, and secondary production but placed little emphasis on diversity. King et al. (2013) used a pyrosequencing-based analysis of 16S rRNA gene amplicons to assess community composition, diversity, and phylogenetic structure in samples taken at 17 nGoM stations approximately one month before the spill.

Principal component and nonmetric multidimensional scaling analyses showed that samples from depths of ≤100 m had similar bacterioplankton compositions, but these communities differed distinctly from those in deeper samples (**Figure 2*a***). Specifically, SAR11 Alphaproteobacteria and Bacteroidetes dominated communities at depths of ≤100 m, which were characterized by Alphaproteobacteria/Gammaproteobacteria ratios of >1.7 (**Figure 2*b***). At depths of >100 m, by contrast, Thaumarchaeota, Firmicutes, and Deltaproteobacteria were more abundant, and Alphaproteobacteria/Gammaproteobacteria ratios were <1 (**Figure 2*b***). The relatively low Alphaproteobacteria/Gammaproteobacteria ratios in deeper waters are notable because Gammaproteobacteria dominated the populations that responded to the spill (e.g., Hazen et al. 2010).

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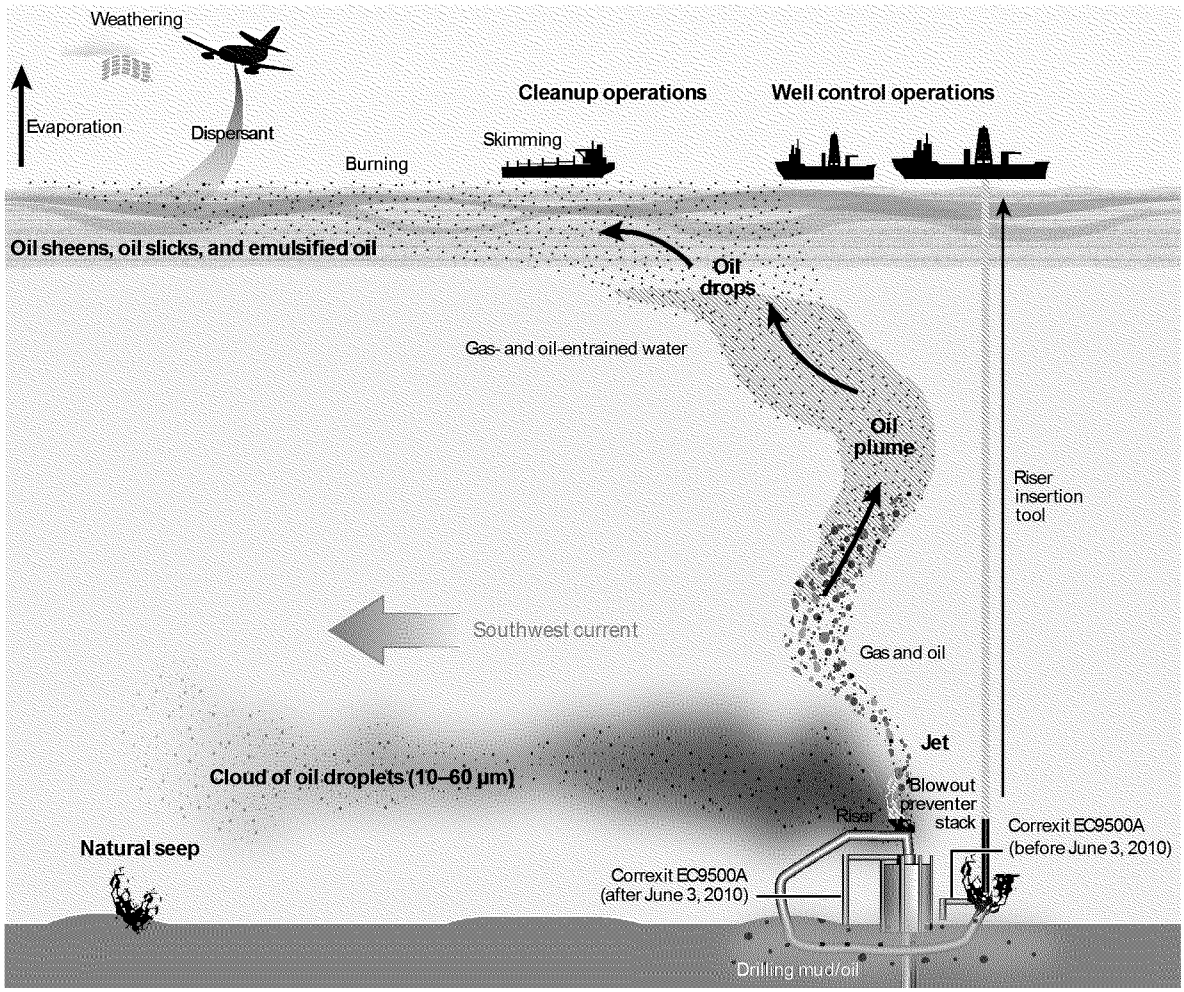

Figure 1

Schematic view of the *Deepwater Horizon* blowout, oil and gas distributions, and cleanup operations. Adapted from Atlas & Hazen (2011).

Given the presence of numerous active hydrocarbon seeps in the nGoM and the magnitude of nGoM oil and gas production and spill potential, the composition and distribution of hydrocarbon oxidizers are of particular interest. Results from 16S rRNA gene sequence analyses revealed 11 known hydrocarbon-oxidizing genera, including *Alcanivorax*, *Cycloclasticus*, *Marinobacter*, *Oleiphilus*, *Oleispira*, *Pseudomonas*, and *Vibrio*, and 13 methanotrophic and methylotrophic genera, including *Methylobacter*, *Methylococcus*, *Methylocystis*, *Methylohalobius*, *Methylonatrum*, *Methylophaga*, *Methylophilus*, *Methylosinus*, and *Methylovorus* (King et al. 2013). Hazen et al. (2010) and Mason et al. (2012) reported that, of these genera, *Colwellia*, *Cycloclasticus*, and an Oceanospirillales taxon related to *Oleispira* were abundant members of the plume community, and Rivers et al. (2013) described evidence for the presence of *Colwellia*, *Cycloclasticus*, *Methylobacter*, and *Methylococcus* based on sequences from a plume transcriptome. Valentine et al. (2010) and Kessler et al. (2011) identified similar taxa in a study of methane and propane respiration within the plume. Gutierrez

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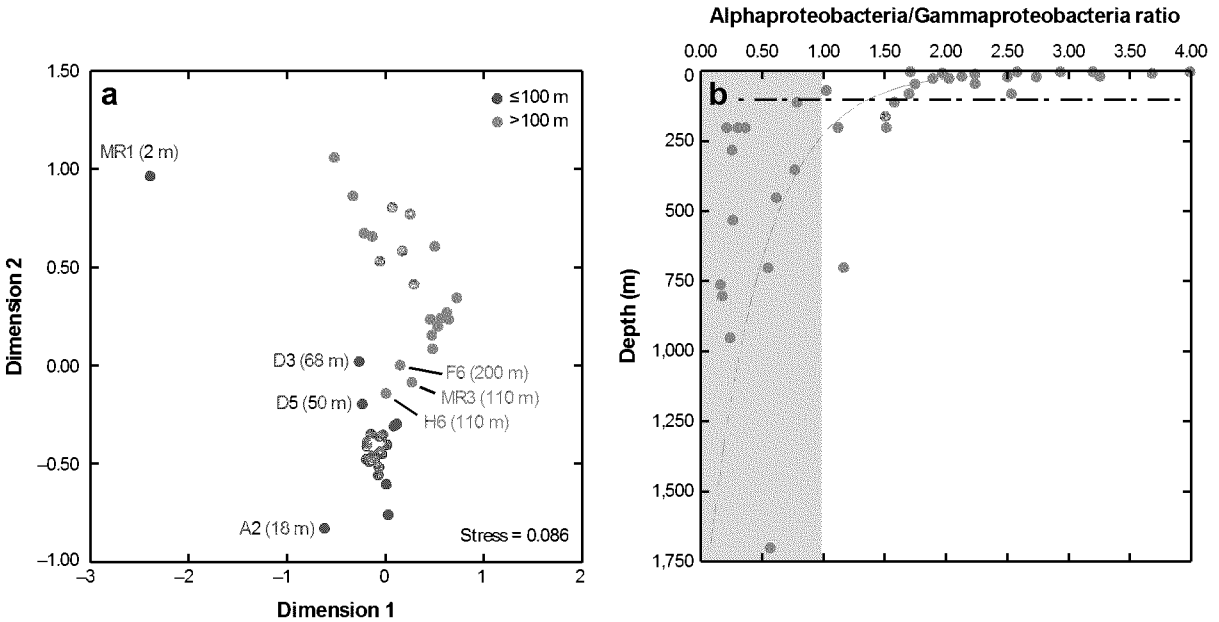

Figure 2

(*a*) Nonmetric multidimensional scaling analysis of northern Gulf of Mexico communities, indicating the structuring of composition by depth. (*b*) Alphaproteobacteria/Gammaproteobacteria ratios with depth. The shaded area indicates ratios less than 1. Adapted from King et al. (2013).

et al. (2013) documented additional taxa, including *Alcanivorax*, *Alteromonas*, *Halomonas*, *Marinobacter*, and *Pseudoalteromonas*, using stable isotope probing and cultivation-based approaches to analyze surface-slick and deepwater plume samples.

Colwellia was unique among the genera identified in the plume in that it was not observed in samples obtained before the spill (King et al. 2013), possibly because its prespill abundances were too low to detect. Regardless, *Colwellia* species responded rapidly to the spill, whereas *Alcanivorax* species—which were observed throughout the nGoM (King et al. 2013, Smith et al. 2013) and which have been regularly associated with hydrocarbon pollution (Harayama et al. 2004, Head et al. 2006)—were not readily detected in plume samples (e.g., Hazen et al. 2010, Mason et al. 2012, Gutierrez et al. 2013, Rivers et al. 2013). This suggests that, although the nGoM harbors a wide range of hydrocarbonoclastic taxa capable of responding to hydrocarbon inputs, the responses of specific groups might vary substantially across time and space.

Smith et al. (2013) provided additional insights into the potential for alkane degradation by analyzing partial sequences for *alkB*, one of the structural genes for alkane hydroxylase. The authors sequenced 401 *alkB* gene clones amplified from genomic extracts obtained from the samples described by King et al. (2013). In contrast to the distinct depth stratification observed using 16S rRNA gene sequences, principal component analyses of alkB operational protein units (OPUs) indicated that community composition did not vary consistently with depth or other major physical-chemical variables. Of 22 distinct OPUs, one was ubiquitous and accounted for 57% of all sequences. This OPU clustered with alkB sequences from known hydrocarbon oxidizers (e.g., *Alcanivorax* and *Marinobacter*) but did not appear to be represented in plume metagenomes or metatranscriptomes (Mason et al. 2012, Rivers et al. 2013). Likewise, none of the less abundant

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OPUs appeared to be represented in the plume community that responded to the spill. Thus, results from *alkB* and 16S rRNA gene sequence analyses indicate that *Alcanivorax* occurs widely within the Gulf of Mexico but might not compete well with other taxa for deepwater hydrocarbons. Variables that affect the hydrocarbon utilization by specific populations and that determine responses to spills remain uncertain.

3. DEGRADATION OF METHANE AND OTHER LIGHT HYDROCARBONS

C_1–C_5 hydrocarbons constituted approximately 24% of the DWH spill by mass, with methane accounting for approximately 60% of the light hydrocarbons (Reddy et al. 2012). Concentration profiles indicted that most of the light hydrocarbons that entered the water column remained at depths of >800 m (Kessler et al. 2011), which explained the low sea–air methane fluxes reported by Yvon-Lewis et al. (2011) from analyses during June 2010.

Several lines of evidence indicated that methanotrophic bacteria substantially oxidized plume methane. Methane concentrations were high and methanotrophic population densities and activities were low in the plume and near the wellhead during the early phases of the spill (e.g., in June 2010) (Hazen et al. 2010, Joye et al. 2011, Kessler et al. 2011), consistent with limited methane oxidation. Although metagenomic and metatranscriptomic data revealed some methanotrophic populations in the plume in late May and early June (Hazen et al. 2010, Rivers et al. 2013), methanotroph abundance appeared to have increased substantially later in the summer (Kessler et al. 2011). Methanotroph blooms characterized by members of the Methylococcaceae expressing particulate methane monooxygenase genes were accompanied by a return of methane concentrations to near-ambient levels, with the decline in methane mirrored in and largely accounting for the plume oxygen anomaly (Kessler et al. 2011). There were initial concerns about long-term and deleterious effects of large methane releases in the deep sea (Joye et al. 2011), but a community of methanotrophs responded robustly and consumed the methane relatively rapidly, with no known adverse impacts.

Other gases (e.g., ethane and propane) were also oxidized rapidly in the plume. Valentine et al. (2010) documented the consumption of both during early stages of microbial responses. They suggested that ethane, propane, and possibly butane supported some of the dominant plume populations, including *Colwellia* and taxa related to *Cycloclasticus*, and that respiration of ethane and propane accounted for up to 70% of the observed June 2010 plume oxygen anomalies. In addition, results from ex situ incubations and stable isotope probing showed that *Colwellia*, and possibly the Oceanospirillales observed in the plume, likely used both ethane and propane (Redmond & Valentine 2012).

These observations were consistent with the results of metagenomic and metatranscriptomic analyses, which showed significant expression of alkane monooxygenase genes that are related most closely to homologs of the particulate methane monooxygenase genes coding for ethane and propane monooxygenases (Mason et al. 2012, Rivers et al. 2013). Rivers et al. (2013) used phylogenetic analyses of particulate monooxygenase sequences to infer putative functions. The sequences clustered into four clades, one of which included particulate methane monooxygenase gene sequences found in methanotrophic Gammaproteobacteria; this is consistent with results from 16S rRNA gene sequence analyses, which showed enrichments of this group (Hazen et al. 2010, Valentine et al. 2010). Two other clades, which accounted for 42% of the monooxygenase reads, contained sequences related most closely to genes coding for ethane and propane monooxygenases, whereas a fourth clade, which accounted for most of the monooxygenase

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reads (50%), contained sequences for which no function could be inferred. The fact that these sequences were relatively highly expressed suggests a role in alkane degradation that should be assessed further.

OTU: operational taxonomic unit

4. HYDROCARBON IMPACTS AT MESOPELAGIC DEPTHS OFFSHORE

Owing to the unique nature of the MC-252 oil spill, considerable attention has been focused on the offshore water column; detailed analyses have been performed of hydrocarbon and dispersant degradation, bacterioplankton community composition and activity, trophic interactions, and connections between the water column and sediment. One somewhat surprising finding from these observations was the robust response of the cold, deepwater bacterial community, which appeared to use hydrocarbons with little or no lag and with no nutrient additions, thereby partially attenuating the contamination (see below for details). Rapid responses were also observed in offshore surface waters, even though they were considered phosphorus limited (Edwards et al. 2011, Ziervogel et al. 2012). In this sense, the MC-252 spill was very different from the *Exxon Valdez* spill, degradation of which appeared to be hindered by the cold conditions in Prince William Sound, Alaska, and for which a variety of bioremediation efforts were attempted (Prince 2010, Atlas & Hazen 2011).

4.1. Hydrocarbon Effects on Bacterial Community Structure Within the Deep-Sea Plume

Hazen et al. (2010) showed that bacterial densities in deep-sea plume samples collected between May 25 and June 2, 2010 (5.5×10^4 cells cm^{-3}), were elevated relative to those in adjacent nonplume samples (2.7×10^4 cells cm^{-3}). Sixteen members of the Gammaproteobacteria were significantly enriched in the plume samples, one of which constituted >90% of 16S rRNA gene sequences recovered from plume DNA extracts (Hazen et al. 2010, Mason et al. 2012). Clone library analysis revealed that the closest cultured relatives of this operational taxonomic unit (OTU) were *Spongiispira norvegica* (95% similarity) and *Oceaniserpentilla haliotidis* (94% similarity), both of which are members of the Oceanospirillales. This same OTU accounted for <3% of sequences in nonplume samples and samples taken in the same area at similar depths one month before the spill (Mason et al. 2012, Dubinsky et al. 2013, King et al. 2013). Many of the other abundant plume sample OTUs were closely related to psychrophilic hydrocarbon degraders (Hazen et al. 2010), which is consistent with in situ temperatures (4–5°C).

Metagenomic and metatranscriptomic analyses revealed both functional potential and community structure (Hazen et al. 2010), in that a single Oceanospirillales OTU was documented as the dominant community member between May 26 and June 3 (Mason et al. 2012), with Oceanospirillales sequences accounting for >60% of all plume metagenomic reads and 49–69% of all plume transcripts. In addition, Rivers et al. (2013) found that 95% of the 16S rRNA gene sequences in their samples were from only four families: Colwelliaceae, Methylococcaceae, Oceanospirillaceae, and Piscirickettsiaceae. Their metatranscriptomic results also indicated that Alteromonadales, Deltaproteobacteria, Pseudomonadales, and SAR86 were active but more limited members of the plume. Results from 16S rRNA gene sequences were consistent with transcript annotations, which revealed relatively few active plume community members; most of the transcripts binned to only six reference genomes. The metatranscriptomic analysis showed that, even though *Colwellia* species were only minor constituents of the 16S rRNA gene libraries from samples collected at this time, they were present and active in the plume, with Alteromonadales comprising 8–11% of recovered transcripts (Mason et al. 2012).

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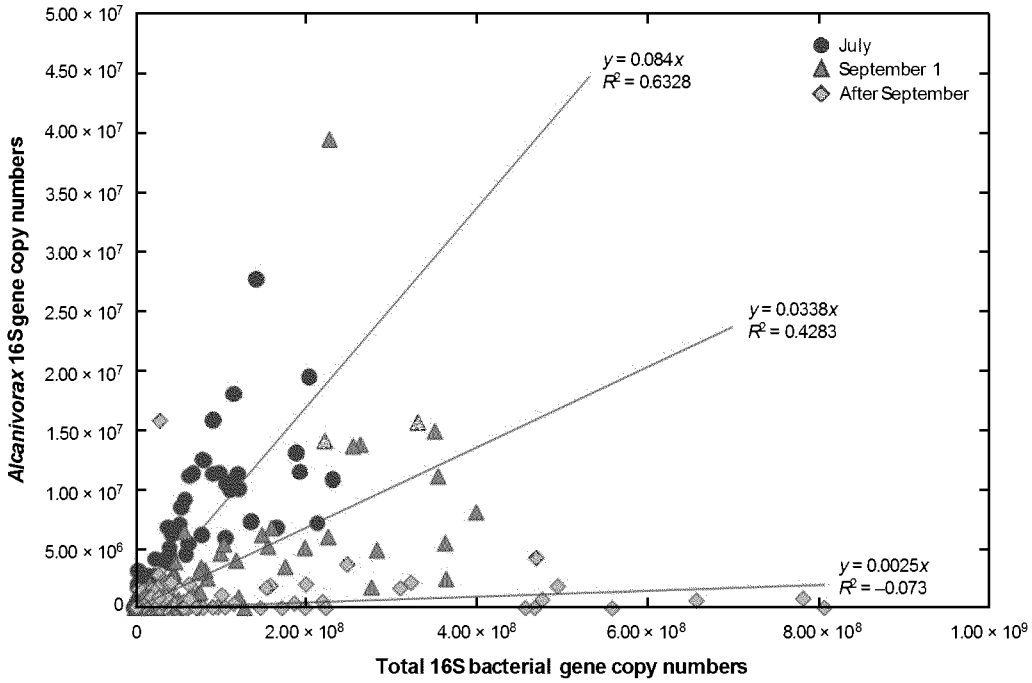

Figure 3

Alcanivorax succession over time plotted as abundance of *Alcanivorax* 16S rRNA gene sequence copy numbers versus total 16S rRNA gene sequence copy numbers for samples collected in summer and fall 2010. Adapted from Kostka et al. (2011).

Samples collected later during the course of the spill (June 2010) revealed a succession in community membership (**Figure3**), with *Colwellia* (Alteromonadales) and *Cycloclasticus* (Thiotrichales) emerging as the dominant genera (Redmond & Valentine 2012). *Colwellia* also increased in abundance during ex situ enrichments of deep-sea water with crude oil at 4°C (Redmond & Valentine 2012). Stable isotope probing revealed that *Colwellia* consumed propane and ethane, whereas *Cycloclasticus* likely consumed benzene-toluene-ethylbenzene-xylene (BTEX) compounds. Results from a later sampling (September 2010) showed additional shifts in plume community composition, with high abundances of methylotrophic bacteria and low abundances of Oceanospirillales, *Colwellia*, and *Cycloclasticus* (Kessler et al. 2011). Although the bacterial community in the plume changed rather dramatically over time and space, the archaeal community remained fairly constant (Redmond & Valentine 2012), even though archaea account for a significant fraction of deep nGoM bacterioplankton (King et al. 2013). This is consistent with the fact that, although some evidence indicates that archaea degrade hydrocarbons (e.g., Al-Mailem et al. 2013), the majority of previous work suggests that many archaeal taxa are either unaffected or adversely affected by oil spills (e.g., Roling et al. 2004, Urakawa et al. 2012).

BTEX: benzene-toluene-ethylbenzene-xylene

To explain microbial community succession in the plume, Valentine et al. (2012) developed a simulation model that combined horizontal circulation and mixing in the deep Gulf of Mexico with hydrocarbon inputs, which fed bacterial metabolism and growth. The model output suggested that as bacteria were exposed to oil, hydrocarbon-degrading taxa bloomed until their preferential substrate was depleted, at which point they declined in abundance and were replaced by bacteria growing on different substrates. The model also indicated that deepwater circulation near the

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MC-252 well resulted in repeated exposures to fresh oil for given parcels of water. Repeated exposure led to increased degradation rates because the standing stock of hydrocarbon degraders was elevated owing to prior exposure.

Changes in the composition and quantity of hydrocarbons over time and space undoubtedly contributed to community succession (Dubinsky et al. 2013). Community structure was correlated with three distinct spill phases: uncontrolled oil flow (April 25–June 4), partial hydrocarbon capture (June 5–July 14), and well shut-in (July 15 onward). During the period of uncontrolled flow, Oceanospirillales and *Pseudomonas* dominated the plume community (Hazen et al. 2010, Mason et al. 2012, Dubinsky et al. 2013). Redmond & Valentine (2012) and Dubinsky et al. (2013) found that, during the period of partial hydrocarbon capture, the community shifted to dominance by *Colwellia*, *Cycloclasticus*, *Pseudoalteromonas*, *Methylomonas*, and *Thalassomonas*. After well shut-in, Flavobacteria (*Tenacibaculum* and *Polaribacter*), Alteromonadaceae, and Rhodobacteraceae dominated the community at plume depths in the persistent dissolved oxygen anomaly (Dubinsky et al. 2013). These groups have been reported to degrade high-molecular-weight organics and dissolved organic matter (DeLong et al. 1993, Kirchman 2002, McCarren et al. 2010, Teeling et al. 2012). Thus, many of the populations that proliferated after well shut-in most likely scavenged organic matter and cell biomass from the decaying hydrocarbon degrader bloom.

4.2. Functional Responses of Deepwater Plume Microbial Communities to Hydrocarbons

Microarray- and sequencing-based approaches revealed dramatic increases in the presence and expression of genes related to hydrocarbon metabolism within the plume (Lu et al. 2012, Mason et al. 2012, Rivers et al. 2013). Microarrays showed that gene diversity was greater within the plume than outside of it and that microbial community functional structure differed significantly between plume and nonplume samples (Lu et al. 2012). Microarray data also showed that 1,600 genes involved in hydrocarbon metabolism, including genes for alkane and PAH degradation, occurred at higher abundances within the plume. In addition, microarray analyses revealed strong shifts in biogeochemical cycling genes, including assimilatory nitrogen reduction, ammonia assimilation, and sulfate reduction (*dsrA/B* and *AprA*) genes that were enriched in plume samples. A large number of cytochrome *c* genes were also observed, suggesting that hydrocarbon-coupled metal reduction may have occurred in the deep-sea plume. Finally, genes encoding proteins involved in organic phosphorus release [exopolyphosphatase (ppx) for inorganic polyphosphate degradation and phytase for phytate degradation] were stimulated in the plume, which might have been a response to phosphorus limitation.

Metagenomic data for the plume during the latter part of the uncontrolled flow period revealed all of the genes required for *n*-alkane degradation and a nearly complete cyclohexane degradation pathway (Mason et al. 2012). Alkane 1-monooxygenase genes (*alkB*) were also identified throughout the water column in samples taken before the spill (Smith et al. 2013) and by microarray analyses of plume samples (Lu et al. 2012). In addition, metatranscriptomic analyses showed that *alkB* sequences accounted for a modest fraction ($>10^{-4}$) of gene transcripts overall (Rivers et al. 2013). Although these genes have been assumed to reflect alkane degradation, some uncertainty exists about their function and substrate specificity (Smith et al. 2013).

Genes for PAH degradation were also enriched within the plume. However, they accounted for a much smaller relative abundance than genes for alkane degradation. This is consistent with the composition of the oil, which contained <2% PAHs (Reddy et al. 2012). These results also suggest that PAHs were not preferentially degraded relative to alkanes with small to intermediate chain lengths.

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Gene transcripts mapping to genes of the dominant Gammaproteobacteria that responded to the spill were enriched by up to two orders of magnitude in the plume relative to samples taken outside of it (Rivers et al. 2013). However, expression analyses based on comparisons of gene and gene transcript abundance indicated that a large number of taxa within the plume were unaffected by the presence of oil, and only a small percentage (5%) appeared to have been inhibited (Rivers et al. 2013). These findings suggested that much of the natural background community existed within the plume at their prebloom levels and that only a few responded positively or negatively.

To better understand the characteristics of the Oceanospirillales that dominated plume samples early during the spill (Hazen et al. 2010), genome sequences were obtained from single cells with >95% 16S rRNA gene similarity to the dominant OTU observed by Mason et al. (2012) and 99% similarity to the Oceanospirillales clone observed by Redmond & Valentine (2012). Based on 16S rRNA gene sequences, the closest cultured representatives to these single cells were *Oleispira antarctica* (Yakimov et al. 2003, Kube et al. 2013) and *Thalassolituus oleivorans* (97% for both). The single-cell genomes encoded cyclohexane degradation and alkane degradation genes similar to those found in metagenomes and metatranscriptomes from plume samples. The single-cell genomes also contained genes for chemotaxis and nutrient acquisition, which suggested that some hydrocarbon degraders sense and swim toward oil droplets. Results from synchrotron radiation-based Fourier-transform infrared spectromicroscopy provided additional support for this possibility (Mason et al. 2012).

4.3. Dispersant Effects

To better understand dispersant effects on oil biodegradation in the Gulf of Mexico and to determine the potential for dispersant degradation, Baelum et al. (2012) assessed enriched microbial communities in water samples amended with high concentrations of oil and dispersants (100 ppm oil and 60 ppm Corexit EC9500A). Uncontaminated water from 1,100 m was treated with a combination of oil, Corexit EC9500A, and iron and incubated at 5°C. Oil degradation was measured as losses of total petroleum hydrocarbons. Twenty-five percent of the dissolved oil was degraded in the oil-only microcosms in the first 5 days, but not much more oil degradation was observed in the following 16 days. By contrast, 60% of the oil was degraded in samples with oil plus dispersant after 21 days. This demonstrated that even at very high concentrations, dispersants likely did not inhibit oil biodegradation in the deep waters of the Gulf of Mexico. Although this study reported only total petroleum hydrocarbon loss, the authors attributed changes predominantly to *n*-alkane degradation.

The fate of dispersants in the nGoM remains a concern because persistent concentrations of dispersant components were found at plume depths after dispersant application had ceased (Oper. Sci. Advis. Team 2010, Kujawinski et al. 2011, Chakraborty et al. 2012). Corexit EC9500A comprises three major components: hydrocarbons (50%), glycols (40%), and the anionic surfactant dioctyl sodium sulfosuccinate (DOSS) (10%). Baelum et al. (2012) showed that the hydrocarbon fraction of Corexit EC9500A degrades rapidly in microcosms with nGoM water from 1,100 m, whereas glycols and DOSS are metabolized very slowly. By contrast, glycols were degraded rapidly by sediment collected near the MC-252 wellhead (Mason et al. 2014). Baelum et al. (2012) also observed the formation at later time points of large flocs in oil- and oil-plus-dispersant-containing microcosms. These flocs were composed of degraded hydrocarbons and macromolecules of microbial origin, such as proteins and extracellular polymeric substances. *Colwellia* constituted more than 70% of the floc community, and Methylococcaceae constituted an additional 16%.

Campo et al. (2013) compared the degradation of dispersed oil in surface water at 25°C with that in water from 1,240 m at 5°C. They used a lower dispersant-to-oil ratio than Baelum et al.

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(2012) (1:25 compared with 1:1.67). Campo et al. (2013) found that >99% of the DOSS was degraded after 8–14 days at 25°C, but at lower temperatures (5°C), there was a 28-day lag before degradation was observed. In the absence of crude oil, 98% of DOSS was degraded at 5°C after 42 days; in the presence of crude oil, only 61% of the DOSS was degraded at 5°C after 42 days. Campo et al. (2013) also observed that alkane degradation rates for South Louisiana crude oil were much faster at 25°C than at 5°C, and that Corexit accelerated degradation at 25°C. However, 7% of the alkanes remained after day 42 of the 5°C incubations, regardless of the presence of Corexit.

By contrast, PAH degradation occurred only after 42 days at 25°C with or without Corexit EC9500A, but began after 14 days at 5°C for dispersed crude oil. Campo et al. (2013) concluded that the difference in behavior between alkanes and PAHs at cold temperatures was due to the solubility of the PAHs and the physical state of the alkanes. These studies indicated that oil biodegradation in the Gulf of Mexico was not inhibited by dispersants and that Corexit EC9500A can increase the extent of oil degradation under some circumstances for some hydrocarbon classes. Furthermore, many of the components of Corexit EC9500A appeared to be relatively labile depending on conditions (i.e., temperature and water column or sediment).

4.4. Hydrocarbon Effects on Deep-Sea Sediment Microbial Communities

Oil from the MC-252 well affected microbial communities in deep-sea sediments (Kimes et al. 2013, Mason et al. 2014). Results from metagenomic analyses revealed increases in the functional repertoires of contaminated sediments relative to those of adjacent sediments. Both hydrocarbon and inorganic nitrogen concentrations were important determinants of community structure for surface sediments, in which the dominant organisms were closely related to uncultured Gammaproteobacteria and *Colwellia* OTUs present in the deepwater plume (Mason et al. 2014). Increases in *Colwellia* could have been due to their very broad substrate range, which includes gaseous hydrocarbons (Redmond & Valentine 2012) and PAHs (Gutierrez et al. 2013).

Genes involved in cycloalkane, aromatic hydrocarbon, and BTEX degradation were significantly enriched in metagenomes from contaminated cores, which included a complete pathway for cyclohexane degradation related most closely to the pathway described from *Brachymonas petroleovorans*, an isolate from a petroleum refinery (Mason et al. 2014). Microcosm incubations with ^{14}C-labeled model substrates also showed that propylene glycol (a component of dispersants) was mineralized rapidly by sediment communities (Mason et al. 2014), whereas alkane degradation was faster than toluene and phenanthrene degradation. Thus, at least some components of dispersed oil appeared to be relatively labile in deep-sea sediments.

Metagenomic analyses also indicated that pathways involved in nitrogen cycling were significantly altered in contaminated samples (Mason et al. 2014). Using both metagenomic and geochemical data, Mason et al. (2014) proposed that nitrogen fixation, together with mineralization, might have resulted in higher ammonium concentrations in oil-impacted sediments, and that nitrate might have been consumed via denitrification. Evidence supporting the latter conclusion was drawn from metagenomic annotations of the major genes for denitrification, which might have been the dominant process for nitrogen loss.

Genes and metabolites involved in anaerobic hydrocarbon degradation were identified in a metagenomic study of cores collected near the MC-252 wellhead (Kimes et al. 2013). Many of the reads from these metagenomes were related to sequences from known anaerobic hydrocarbon degraders, e.g., *Desulfatibacillum alkenivorans* AK-01. Thus, sulfate-reducing, hydrocarbon-degrading Deltaproteobacteria (Kniemeyer et al. 2007, Jaekel et al. 2013) likely responded to hydrocarbon inputs, with consequences for both local nitrogen and sulfur cycling (Kimes et al. 2013).

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5. HYDROCARBON IMPACTS AND MICROBIAL METABOLISM IN THE NEARSHORE WATER COLUMN, SEDIMENTS, AND BEACHES

PB: Pensacola Beach

During the MC-252 discharge, concern for understudied deep-sea habitats led to a surge of research on offshore systems surrounding the wellhead. By contrast, coastal response efforts centered on economically valuable beaches and ecologically valuable salt marshes (Hayworth et al. 2011, Oper. Sci. Advis. Team 2011, Wang & Roberts 2013). MC-252 oil had a pronounced impact on indigenous, benthic microbial communities in intertidal and inland ecosystems, but much less information is available on the responses of planktonic and subtidal microbial communities on the continental shelf (<200-m water depth).

Although microbial responses to oiling in beach ecosystems were documented extensively prior to 2010, these studies were limited by methodological constraints. The DWH spill is the first major event in which next-generation sequencing approaches have been applied to illustrate with high resolution the dramatic changes in the abundance, structure, and metabolic potential of oiled beach and sediment microbial communities (Kostka et al. 2011, Bik et al. 2012, Newton et al. 2013, Lamendella et al. 2014).

A time-series study conducted at municipal Pensacola Beach (PB), Florida, where total petroleum hydrocarbons reached 11,000 mg kg^{-1}, revealed a bloom of bacteria during the first four months after oil came ashore, with the microbial abundance in oiled sands exceeding that in clean sands by one to four orders of magnitude (Kostka et al. 2011). Geochemical evidence confirmed that bacterial degradation of weathered oil took place concurrently with the formation of the bloom (Aeppli et al. 2012, Ruddy et al. 2014). As observed in planktonic ecosystems offshore (Hazen et al. 2010), Gammaproteobacteria—including many bacterial groups known to degrade hydrocarbons—were enriched in oiled beach sands. The abundance of the known hydrocarbonoclastic groups *Alcanivorax* and *Marinobacter* increased dramatically in response to oil contamination, and a comparison of ribosome abundance in RNA extracts indicated that these groups were active within the bacterial community (**Figure 4**).

Oil acts as a strong selective force on the ecology of indigenous microbial communities in marine ecosystems (Head et al. 2006). For example, it has been hypothesized that nutrient availability

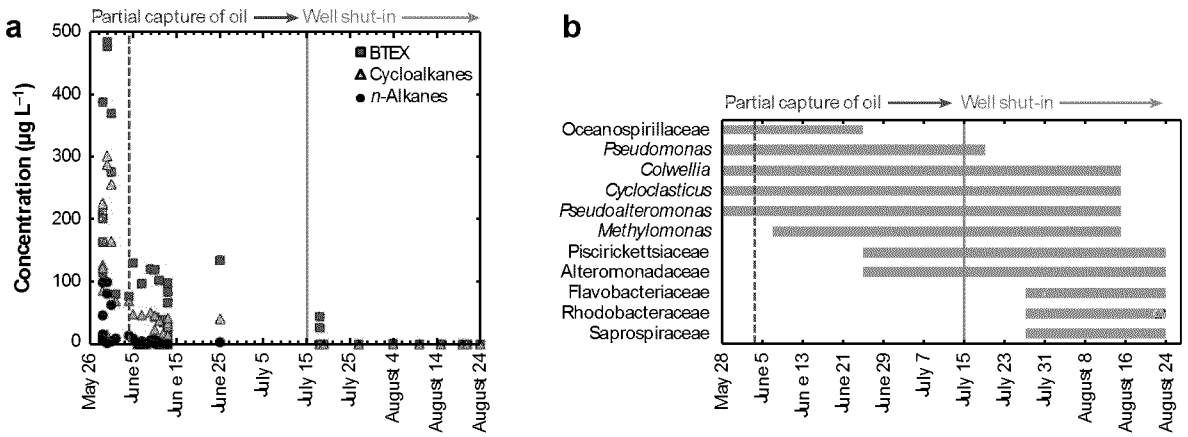

Figure 4

(*a*) Plume hydrocarbon composition [sums of benzene-toluene-ethylbenzene-xylene (BTEX), cycloalkanes, and *n*-alkanes] as a function of sampling date after the initiation of oil discharge. (*b*) Changes in dominant members of plume bacterioplankton communities as a function of sampling date after the initiation of oil discharge. Adapted from Dubinsky et al. (2013).

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controls selection for different strains or ecotypes of *Alcanivorax*, which have been proposed to outcompete other bacteria for hydrocarbons by utilizing both branched- and straight-chain alkane compounds (Head et al. 2006). Analyses of PB sands and sediment have supported the hypothesis that alkane degradation is a species-specific trait, because two distinct *Alcanivorax* phylotypes were observed during the early stages of hydrocarbon degradation. Sequence evidence has also pointed to a succession of microbial populations related to the utilization of different hydrocarbon compound classes. Whereas the Oceanospirillales were dominant in sequence libraries within the first few months after oil came ashore, a shift toward the Alphaproteobacteria (Rhodobacteraceae) and gram-positive (*Bacillus*) groups took place in subsequent months. Thus, microbial community succession occurred in both deep nGoM water column and nearshore systems (see also Section 4). Gammaproteobacteria were prominent in early successional stages in all systems, but the mix of taxa varied considerably. In particular, *Alcanivorax* species were significant members of nearshore communities but minor members of the plume community, suggesting that *Alcanivorax* might be more competitive in benthic rather than planktonic systems.

Studies have examined both the taxonomic and functional diversity of microbial communities in a time series of 12 metagenomes constructed from oiled and clean sands sampled at PB in 2010 and 2011. The succession of microbial populations recorded by 16S rRNA gene amplicon sequencing (Kostka et al. 2011) was in general supported by the metagenome results (Rodriguez-R. et al. 2014). For example, taxonomic diversity largely decreased in oiled sands and then rebounded one year after oil came ashore. An increase in the metabolic potential for petroleum hydrocarbon catabolism and nutrient acquisition was also detected in the metagenomes of oiled sands as a large and statistically significant increase in the relative abundances of genes associated with these pathways. In contrast to the results for taxonomic diversity, the metagenomes revealed an increase in functional diversity in response to disturbance by MC-252 oil, consistent with observations in deep-sea sediments (Mason et al. 2012). Moreover, a functional transition was observed from generalist populations (within four months after oil came ashore) to specialists (a year later, when oil was undetectable), thereby supporting the specialization-disturbance hypothesis (Rodriguez-R. et al. 2014).

Studies conducted at other beaches over the same period in 2010 generally corroborated observations from PB. Lamendella et al. (2014) investigated the impacts of oil contamination on microbial communities at Elmer's Beach in Louisiana. They observed successional changes in the microbial populations in parallel with partial degradation of the oil present and reported elevated abundances of Alpha- and Gammaproteobacteria. Metatranscriptome results revealed that PAH, *n*-alkane, and toluene degradation genes were expressed in oiled sediments (Lamendella et al. 2014). Whereas *Alcanivorax* dominated in oiled sands at PB, *Marinobacter* dominated over the active hydrocarbon-degrading microbial communities at Elmer's Beach. In addition, Newton et al. (2013) searched for microbial indicators of ecosystem disturbance in microbial communities at seven beaches from Mississippi to Florida. Although the presence of oil appeared to be outweighed by the influence of other selective forces on community patterns, the relative abundances of known hydrocarbon degraders (e.g., *Alcanivorax*, *Alteromonas*, and *Marinobacter*) increased in oiled sands, consistent with the results of other studies.

The warm, shallow waters of the nGoM shelf represent a favorable environment for bacterial growth, as evinced by the fact that many of the predominant microbial groups detected in oiled sands by gene sequencing could be cultivated in the laboratory. Isolates were obtained from a variety of hydrocarbon-oxidizing genera (*Alcanivorax*, *Acinetobacter*, *Bacillus*, *Marinobacter*, and *Pseudomonas*), and the cultivation of multiple strains of the same species (*Alcanivorax dieselolei*, *Marinobacter hydrocarbonoclasticus*, and *Marinobacter vinifirmus*) will allow for further testing of the role of genotype in biodegradation (Hamdan & Fulmer 2011, Kostka et al. 2011). Initial physiological testing of oil-degrading strains indicated niche specialization in carbon and major

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nutrient metabolism (Kostka et al. 2011). Whereas *Acinetobacter*, *Marinobacter*, and *Vibrio* strains all utilized a fairly broad range of carbon substrates, the substrate range of *Alcanivorax* was relatively limited, corroborating previous work (Yakimov et al. 2007).

Overholt et al. (2013) further explored the relationship between genotype and metabolic potential by sequencing the genomes of 10 bacterial isolates from oiled PB sands. Based on comparisons of genome assemblies and numbers of hydrocarbon degradation genes in strains of the same genus (*Alcanivorax* or *Marinobacter*), they proposed strain-specific differences in hydrocarbon degradation potential. In addition, they described the genome of the first known *Labrenzia* (Alphaproteobacteria) isolate capable of growth on crude oil. Multiple studies of oil-contaminated marine environments have detected environmental sequences with high sequence identity to *Labrenzia*, and closely related isolates have been shown to degrade hydrocarbons in the laboratory (Lai et al. 2010, Kostka et al. 2011, Al-Awadhi et al. 2012, Liu & Liu 2013). Collectively, these results advance understanding of how native microbial communities respond to crude oil perturbation in beach ecosystems and provide biomarkers for the chemical evolution of oil hydrocarbons during degradation and weathering.

Clearly, hydrocarbons act as a strong selective force on nearshore sedimentary microbial communities in the nGoM. However, in addition to serving as a carbon and energy source, some components of crude oil and dispersants used during response efforts might adversely affect the ecosystem services provided by specific microbial groups. Hamdan & Fulmer (2011) enriched for hydrocarbon-degrading bacteria in oiled sands collected from the Louisiana shoreline closest to the DWH wellhead, using a minimal medium supplemented with hexadecane and the dispersant Corexit EC9500A. Somewhat surprisingly, Corexit was toxic to hydrocarbon-degrading bacteria in a dose-dependent manner. For example, pure cultures of *Marinobacter* were particularly sensitive, showing a nearly 100% reduction in viability as a result of exposure to concentrations likely to be encountered during the DWH spill. In addition, Urakawa et al. (2012) and Radniecki et al. (2013) reported that oil hydrocarbons and Corexit EC9500A inhibit nitrifying bacteria and archaea. However, it should be noted that these studies used levels of Corexit that greatly exceed concentrations that were detected in seawater during DWH response efforts (Kujawinski et al. 2011). Further studies are needed to determine whether toxic effects observed in the laboratory can be extrapolated to responses in situ.

Nearly all previous work on the microbial response to the DWH spill has targeted bacteria and archaea. However, microbial eukaryotes, specifically fungi, are known to degrade hydrocarbons (Head et al. 2006), and fungal communities appeared to thrive at the expense of metazoans in response to oil contamination from the MC-252 discharge (Bik et al. 2012). Bik et al. (2012) also observed a dramatic change in the community structure of microbial eukaryotes in oiled sediments collected from Louisiana and Alabama beaches from May to September 2010.

Relatively few studies have explored the fate of crude oil–derived hydrocarbons or the impacts of the MC-252 discharge on coastal nGoM planktonic ecosystems. Bianchi et al. (2011) examined bacterioplankton in Barataria Bay, Louisiana, in July and September 2010. Although some Barataria Bay salt marshes had been oiled, the authors found no evidence for MC-252 oil in the water column during the sampling periods and no evidence for bacterioplankton community responses to oil; instead, the bacterioplankton community structure appeared to have been determined largely by the massive freshwater diversion through the bay that had been undertaken in an unsuccessful attempt to limit oiling. Likewise, in spite of predictions in the scientific community and news media that MC-252 oil might lead to blooms of pathogenic *Vibrio parahaemolyticus*, no evidence for such blooms was observed, and Smith et al. (2011) showed that coastal isolates had no capacity to grow using PAHs or oil. By contrast, Tao et al. (2011) showed that pathogenic *Vibrio vulnificus* was substantially enriched in tar balls.

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A single study of MC-252 oil impacts on bacterioplankton community structure has been conducted on two surface water and two oil mousse samples collected in the nearshore nGoM off Louisiana (Liu & Liu 2013). As observed in other oiled nGoM environments, Gammaproteobacteria and Alphaproteobacteria (including *Alcanivorax*, *Alteromonas*, a n d*Marinobacter*) dominated the bacterial communities. Consistent with the PB time series, a high relative abundance of *Rhodovulum* and *Stappia*/*Labrenzia* (Rhodobacteraceae) was associated with the more weathered oil components of surface mousse, implicating these genera in the degradation of higher-molecular-weight aliphatic and aromatic compounds. In a study of seawater mesocosms, Ortmann et al. (2012) showed that dispersants employed during DWH response efforts short-circuited the planktonic microbial food web. Amendment of dispersant or dispersed oil resulted in an increase in the biomass of heterotrophic prokaryotes but a significant inhibition of ciliates, suggesting reductions in grazing and in the transfer of carbon to higher trophic levels. However, on the continental shelf, where most nGoM elemental biogeochemical cycling occurs, specific impacts of the DWH spill on the ecology (abundance, distribution, structure, and activity) of in situ planktonic communities remain largely unknown.

By contrast, using Microtox and QwikLite assays, Paul et al. (2013) showed that seawater samples collected in the northeastern Gulf of Mexico in August 2010 were toxic to indicator bacteria and phytoplankton. These assays also indicated that the degree of toxicity was correlated with total petroleum hydrocarbon concentrations. However, Prince & Parkerton (2014) commented that, in the Paul et al. (2013) study, the reported toxicity and mutagenicity in field samples were limited in extent, and the study provided little evidence of toxicity gradients or causality. Furthermore, Prince & Parkerton (2014) pointed out that particulates in the unfiltered water samples tested by Paul et al. (2013) could have confounding effects on bioluminescence attenuation, which is the basis for toxicity detection.

Isotope geochemistry has provided further evidence for the impacts of petroleum hydrocarbons on nGoM planktonic food webs. Carbon isotope ratios in seawater and particulate organic matter indicate the extent to which petrocarbon enters the food web, because petroleum hydrocarbons are depleted in ^{13}C and ^{14}C (radiocarbon) relative to recent photosynthetically derived carbon. In a study conducted in the nGoM near Mobile Bay, Alabama, Graham et al. (2010) determined the stable carbon isotopic composition in filtered samples of small suspended particles (1–200 μm in diameter) and larger particles (i.e, mesozooplankton, 0.2–2 mm in diameter). Carbon isotope depletion was coincident with the arrival of surface oil slicks to the nGoM, whereas terrestrial carbon sources, which also show a depleted carbon isotope signal, were ruled out.

^{14}C provides an even more sensitive indication of oil incorporation into planktonic food webs because crude oil is essentially ^{14}C free. Chanton et al. (2012) observed that ^{14}C was much more depleted relative to dissolved inorganic carbon in samples from the same site visited by Graham et al. (2010), and there was a linear correlation between the ^{14}C and ^{13}C content of particulates. Furthermore, using a carbon isotope mass balance approach, Chanton et al. (2012) concluded that more methane than petroleum was incorporated into the planktonic food web, suggesting that carbon from methanotrophic bacteria enters the food web through the microbial loop.

6. HYDROCARBON IMPACTS AND MICROBIAL METABOLISM IN COASTAL WETLANDS

The coastlines of the nGoM encompass approximately 15.6 million hectares of wetland habitats that provide critical ecosystem services (Corn & Copeland 2010), including support for a large fraction of US seafood production (Engle 2011, Gulf Coast Ecosyst. Restor. Task Force 2011). Thus, the impacts of oiling remain a major concern. Unlike the more uniform conditions of

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shelf and mesopelagic waters, coastal wetlands experience highly variable physical and chemical conditions that alter biogeochemical patterns, primarily through changes in oxidation-reduction regimes (Mitsch et al. 2009). Hydrologic regimes also affect oxygen availability, nutrient levels, salinity, pH, and temperature, which in turn affect patterns and rates of microbial metabolism and hydrocarbon degradation. In this context, it is notable that a large fraction of wetland soil is anoxic, because hydrocarbon degradation proceeds at a much faster rate under oxic conditions than it does under anoxic conditions.

Although various forms of oil (e.g., mousse and tar balls) heavily affected numerous nGoM marshes, surprisingly little is known about microbial responses to the impact in these areas. We consider here the effects of oiling on saline, brackish, and freshwater marshes of the nGoM dominated by *Spartina alterniflora*, *Juncus roemarianus*, *Avicennia germinans*, *Phragmites australis*, and *Sagittaria lancifolia* (Beazley et al. 2012, Mendelssohn et al. 2012, Natter et al. 2012, Tao & Yu 2013).

The DWH spill affected nGoM wetlands from Louisiana to Florida, with most of the impacts occurring in Louisiana (60.6% of the total; oiled shorelines in Florida, Mississippi, and Alabama accounted for 16.1%, 14.6%, and 8.7% of the total, respectively). Approximately 690 km of marsh shoreline was oiled, with more than 280 km (41%) receiving heavy or moderate oiling (Michel et al. 2013). In contrast to the *Exxon Valdez* and Ixtoc I spills in other regions, oil arriving in nGoM wetlands was extensively weathered by a combination of physical, chemical, and biological processes (Beazley et al. 2012, Mendelssohn et al. 2012, Reddy et al. 2012, Anderson 2013). Weathered oil, mousse, tar mats, and tar balls reported at multiple locations in Alabama, Florida, Louisiana, and Mississippi were characterized by increases in heavier (less volatile) oil fractions (Beazley et al. 2012, Natter et al. 2012). For example, tar mats and tar balls collected from Point aux Pins, Alabama, and Bay Jimmy, Louisiana, contained increased relative abundances of long-chain hydrocarbons (elevated C_{17}–C_{30} and elevated C_{23}–C_{35}, respectively) (Beazley et al. 2012, Natter et al. 2012). They were also enriched in asphaltenes, resins, and complex PAHs. For example, oil mousse collected from Marsh Point, Mississippi, contained increased abundances of C_{14}–C_{38} alkanes and PAHs dominated by the four-ring chrysene. The ratio of phenanthrene to chrysene (0.1) indicated that physical, chemical, and biological processes degraded the lighter components of the crude oil.

Weathered oil remnants are considered more toxic than unweathered emulsions, and they persist for much longer (i.e., decades) owing to their degradation resistance. Increased toxicity can be attributed in part to the presence in tar balls of environmentally persistent free radicals. For example, Kiruri et al. (2013) reported a known asphaltene radical species and a newly discovered radical species (Kiruri et al. 2013) in Gulf Coast tar balls. These observations are important because in aqueous systems, environmentally persistent free radicals generate potent reactive oxygen species that can cause damage to a wide range of eukaryotic cellular systems. The impacts on bacteria and hydrocarbon degradation are uncertain, however.

The responses of wetland microbial communities to oil contamination depend on numerous factors, including the physical state of the oil (i.e., weathered), temperature, salinity, nutrient and oxygen availability, and presence of hydrocarbonoclastic microbes (Atlas 1991, Venosa & Zhu 2003, Head et al. 2006, DeLaune & Wright 2011). However, what distinguishes coastal wetlands from other aquatic systems is the presence of vegetation, sediment deposition, and recurring inundation by saline and sometimes fresh water, all of which can collectively contribute to either enhanced oil degradation or subsurface oil sequestration (Lin & Mendelssohn 1998, Hester & Mendelssohn 2000, Beazley et al. 2012).

Previous field and mesocosm studies have determined that the effects of nutrient addition on heavy crude oil biodegradation in salt marshes depend on the type of nutrient used, the

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temperature, and the inundation frequency of the site (Wright et al. 1997, Jackson & Pardue 1999). Wright et al. (1997) demonstrated that an average of 56% of oil hydrocarbons were degraded during winter (17–30°C), whereas 72% were degraded in comparable treatments in summer (27–42°C). The increased degradation in summer was naturally attributed to increased microbial metabolism (Leahy & Colwell 1990), but increases in temperature also reduce crude oil viscosity, which can enhance bioavailability and degradation rates. Nonetheless, even in the most active remediation treatments, 30% of the oil remained (Wright et al. 1997). In a more recent study, Tate et al. (2011) showed that nutrient additions had little effect on crude oil degradation in a Louisiana marsh, largely because the background pore water ammonium concentrations were high enough to preclude nitrogen limitation.

Previous studies have also shown that oil can persist for decades in wetlands (Oudot & Chaillan 2010), and results from a recent study by Natter et al. (2012) indicated that elevated levels of organic carbon in soils and pore water are likely to persist in heavily oiled Louisiana marshes. Although high levels of oil contamination were observed in Louisiana salt marsh sediments, oiling was generally restricted to the periphery of the marsh (within 10 m of the shoreline) (Silliman et al. 2012). Lighter hydrocarbons were rapidly degraded by microbes in marsh sediments, whereas heavier fractions persisted (Natter et al. 2012). Natural-abundance ^{14}C analysis of microbial phospholipid fatty acids provided direct evidence for biodegradation and the incorporation of petrocarbon into microbial biomass at impacted sites several months after oil intrusion, when the highest concentrations of oil were present (Mahmoudi et al. 2013). Although relatively minor shifts in overall bacterial community composition occurred in sediments from oiled sites in comparison with reference sites, higher relative abundances of bacterial groups from known hydrocarbon-degrading taxa (Rhodobacterales and Sphingomonadales) were found in impacted sediments (Mahmoudi et al. 2013). Natter et al. (2012) also noted that elevated total organic carbon and dissolved organic carbon were associated with elevated sulfate-reducing bacteria numbers and higher pore water sulfide concentrations, indicating that dissimilatory sulfate reduction likely was coupled directly or indirectly to hydrocarbon degradation.

Hydrocarbon degradation has long been known to occur more rapidly under oxic conditions than it does under anoxic conditions (Ward et al. 1980, Atlas 1981), even though some alternative terminal electron acceptors (e.g., nitrate and sulfate) support the degradation of some hydrocarbons (Kniemeyer et al. 2007, Widdel et al. 2010, Zedelius et al. 2011). The rhizospheres of marsh vegetation, including *Spartina alterniflora*, *Spartina patens*, *Juncus maritimus*, and *Juncus roemarianus* (Lin & Mendelssohn 2009, Ribeiro et al. 2011, Lin & Mendelssohn 2012), can enhance oil degradation by increasing soil aeration and releasing exudates that act as primers (Beazley et al. 2012, Natter et al. 2012, Mahmoudi et al. 2013). Macrofaunal burrows, particularly those of fiddler crabs, can also increase aeration, enhance hydrocarbon biodegradation, and alter bacterial community composition and structure (Gribsholt et al. 2003, Stauffert et al. 2013).

However, at depths below the rooting zone, anoxic conditions prevail, and hydrocarbon turnover depends almost entirely on anaerobic processes. Microcosm studies have shown that mixed electron acceptors, including sulfate and nitrate, significantly enhance anaerobic biodegradation (Boopathy et al. 2012), and Natter et al. (2012) reported moderate increases in sulfate-reducing bacteria numbers in oiled marsh sediment samples obtained from Louisiana. These results are consistent with the fact that numerous sulfate-reducing bacteria degrade hydrocarbons (Bose et al. 2013).

Soil surface samples from wetlands affected by the DWH spill have consistently shown increased relative abundances of bacteria that degrade lighter crude oil components (Beazley et al. 2012, Natter et al. 2012, Mahmoudi et al. 2013). Beazley et al. (2012) reported increases in the relative abundances of hydrocarbon-degrading Proteobacteria, Actinobacteria, and Bacteroidetes

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when oil concentrations were highest in Point aux Pins, Alabama, during June and July 2010, shortly after the period of moderate oiling in May and June. In addition, analyses of salt marsh soils indicated that Gammaproteobacteria abundance increased as oil reached field sites in June and that Actinomycetaceae, Dietziaceae, Rhizobiaceae, Xanthobacteraceae, Nocardioidaceae, Erythrobacteraceae, and Aeromonadaceae increased in relative abundance in June and July. Mortazavi et al. (2013) further reported that alkane and PAH degraders such as *Hydrocarboniphaga*, *Pseudomonas*, and *Pseudoxanthomonas* dominated in oil-amended intertidal sandy sediments collected from Dauphin Island, Alabama, and Horel et al. (2012) reported significant increases in hydrocarbon biodegradation rates following the addition of organic matter to these sediments.

Collectively, these studies show that coastal marshes in Alabama harbored indigenous hydrocarbon-degrading populations that contributed to crude oil degradation and natural attenuation. Similarly, Mahmoudi et al. (2013) observed biodegradation in oil-impacted marsh soils from Barataria Bay, Louisiana, as well as increases in the relative abundances of hydrocarbon-degrading Rhodobacterales and Sphingomonadales. Mahmoudi et al. (2013) also showed that the class Dothideomycetes dominated fungal communities in impacted marshes. Previous reports have shown that members of Dothideomycetes are often enriched in polluted environments and are capable of hydrocarbon degradation. For example, Bik et al. (2012) detected two Dothideomycetes genera, *Cladosporium* and *Alternaria*, in beach sediments impacted by MC-252 oil, indicating an important and little-investigated role of fungal populations in natural attenuation in nGoM coastal marshes.

SUMMARY POINTS

1. Based on high-resolution next-generation sequencing technologies, extensive analyses of northern Gulf of Mexico (nGoM) bacterial communities support the hypothesis that known hydrocarbon-degrading taxa, especially Gammaproteobacteria, are ubiquitous and that their response to oil is largely determined by physical-chemical conditions in the surrounding environment. However, the rapid and robust response of nGoM microbial communities to oil might reflect adaptations to the extensive hydrocarbon seepage that occurs there, raising the possibility that the observed responses might be somewhat unusual and not necessarily applicable to other ocean basins.
2. Evidence from the nGoM indicates that, although oil acts as a selective force to stimulate hydrocarbon-degrading bacteria and generalist heterotrophs, ecosystem services may be adversely affected through the toxic effects of oil and dispersants on specific microbial groups.
3. Substantial and rapid changes occurred in nearshore to deep-sea microbial communities affected by oil from the Macondo well in Mississippi Canyon Block 252. In general, populations of Gammaproteobacteria formed blooms, the composition of which shifted over time and space in an ecological succession as the oil discharge rate was reduced and in response to physical-chemical conditions and the evolution of oil composition.
4. Hydrocarbon degraders in the deep-sea plume were dominated by members of the Oceanospirillales (Gammaproteobacteria) that were related most closely to *Oleispira* and by members of *Colwellia*, *Cycloclasticus*, and several methanotrophs. By contrast, members of the ubiquitous hydrocarbon-degrading *Alcanivorax* genus (also Gammaproteobacteria) appeared to be more abundant in coastal systems and in sea surface oil.

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5. Dissolved propane might have provided a readily metabolized substrate that fueled blooms of the early hydrocarbon-degrading populations, in essence acting as a primer for biodegradation.
6. Dissolved methane was consumed almost entirely by methanotrophs within the water column, thus limiting exchange across the air-sea interface. Methane and oil consumption collectively accounted for a relatively small oxygen anomaly that dissipated over time.
7. Corexit (a dispersant) likely enhanced oil degradation, and its components were degraded as well. However, Corexit may have affected carbon flow pathways in the water column owing to the inhibition of protozoan grazers, and it might also have inhibited some biogeochemically significant bacterial populations.
8. Hydrocarbon-degrading fungi increased in abundance in oiled salt marshes and beach sands, suggesting that they may contribute significantly to hydrocarbon turnover in some systems. However, the potential roles of fungi in oil remediation are largely unknown.

FUTURE ISSUES

1. To what extent do naturally occurring hydrocarbon inputs potentiate the response of benthic and planktonic microbial communities to marine oil spills?
2. How do the impacts of dispersants on microbial communities and biogeochemical processes vary among systems (e.g., water column versus sediment, salt marshes versus deep sea) and with temperature and nutrient availability?
3. What determines the specific composition of microbial communities that respond to hydrocarbon inputs? To what extent do community compositions reflect stochastic as opposed to deterministic processes?
4. To what extent do low-molecular-mass hydrocarbon gases (C_1–C_5) serve as primers for higher-molecular-mass alkane and polycyclic aromatic hydrocarbon degradation? How does the ratio of hydrocarbon gases to crude oil affect the rates and patterns of crude oil degradation?
5. What determines the rates and patterns of hydrocarbon degradation in mousses and emulsions? Are these forms of oil more or less resistant to decomposition, and to what extent do populations of hydrocarbon degraders within them differ from those in oiled water column or sediment systems?

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DISCLOSURE STATEMENT

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Whale-Fall Ecosystems: Recent Insights into Ecology, Paleoecology, and Evolution
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Errata

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To the Eco-Tox panel of scientists,

Please find attached a revised "final" version of the document. This version incorporates the many comments, edits and suggestions that were received from the previous version. I have tried to highlight in yellow any text that was changed for your final review. However, there are still a few outstanding issues that need the panel input – please see those in the comments. And please suggest appropriate references as required in several sections.

If you could kindly return any comments and or approval of this document by **Feb 27**, we can get this out on the street for public input by March!

Thank you.

Kathy Mandsager

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REVIEW ARTICLE: EFFECTS OF OIL POLLUTION, CHEMICALLY TREATED OIL, AND CLEANING ON THE THERMAL BALANCE OF BIRDS

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Abstract

The acute effect of oil pollution on birds is on their thermal balance. Oil adheres to the plumage and causes a reduction in the water repellent properties of the plumage, causing water to penetrate into the plumage to displace the insulating layer of air. The effect of oil on the plumage insulation is dose-dependent. The effect of oiling is greatly enhanced when the oil is spread in the plumage due to preening. In water, plumage oiling may cause the heat loss to exceed the bird's heat production capacity, resulting in hypothermia. If the oiled bird is ashore, with a dry plumage, it may have a normal thermal insulation. Bird species dependent upon feeding in water (such as diving birds) are therefore much more susceptible to the harmful effects of oil pollution than are semi-aquatic species that can feed ashore. It is possible to restore the water-repelling and insulative properties of the plumage by the process of cleaning if all the oil and soap is removed, and if the plumage is completely dry. Chemical treatment of oil has been suggested as a way to reduce the impact of oil spills on avian life. However, very few reports seem to have addressed the effects of chemically treated oil on the thermal balance of birds, and the results from one study actually indicate that oil treated with dispersants may be more harmful to birds than oil. The urgent need for more information about the effects of chemically treated oil on aquatic birds is therefore stressed.

INTRODUCTION

Marine oil pollution affects most marine life, from planktonic organisms and invertebrates (reviewed by Wells & Percy, 1985), and fish (reviewed by Rice, 1985), to higher vertebrates such as marine mammals (see Geraci & St. Aubin, 1990) and seabirds (reviewed by Clark, 1984; Holmes, 1984; Leighton *et al.*, 1985; Hooper *et al.*, 1987). Mainly because of the visual impact, seabirds are probably the most conspicuous casualties of oil pollution at sea.

Following the 'Exxon Valdez' accident, where 260 000 barrels (30 000 metric tons) of crude oil leaked into the Prince William Sound in Alaska in March 1989, 32 345 birds were found dead (Piatt *et al.*, 1990). Experiments on the recovery of dead seabirds suggests that very few oiled bird corpses drift ashore and are found; recovery rates have been reported to vary between 0·3 and 58% (Coulson *et al.*, 1968; Hope Jones *et al.*, 1970; Bibby & Lloyd, 1977; Bibby, 1981). Based on the number of recovered oiled birds after the 'Exxon Valdez' accident, it is therefore assumed that of the estimated total population of 283 000–370 000 seabirds present in April 1989, 100 000–300 000 birds were probably killed in the first few months after the accident. Using the midpoints, these represent 61% of the birds present.

It has been claimed that the oil pollution from the 'Exxon Valdez' is responsible for more than a 50% reduction in the common guillemot (*Uria aalge*) population in the contaminated parts of Prince William Sound (Heinemann, 1993; Nysewander *et al.*, 1993). Declines in the population of Long-tailed ducks (*Clangula hyemalis*) and Scoters (*Melanitta* sp.) in the Baltic (Lemmetyinen, 1966) have also been blamed on oil pollution.

To be able to combat the impact of oil pollution on avian populations, it is essential to have knowledge about its effects on birds. Oil pollution affects birds in several ways either indirectly by killing or contaminating their nutritional sources, such as plankton, invertebrates and fish, or directly via plumage oiling. Numerous experiments have been carried out to study the effects of oil on birds; most of these studies have been concerned with the toxic effects of ingested oil on the physiology of birds, and these have been reviewed in detail by several authors (see for instance Holmes, 1984; Leighton *et al.*, 1985). In contrast, no review articles have focused on the effects that plumage oiling may have on the thermal balance of birds. The present paper therefore reviews the effects that external oiling has been reported to have on aquatic birds.

Efforts have been made to reduce the effects of plumage oiling in birds. It is possible to remove the oil from the plumage of oiled birds using detergents and to restore the water-repellent properties of the oiled plumage (Clark & Gregory, 1971; Naviaux & Pittman, 1973; Randall *et al.*, 1980; see also the review by Clark, 1984). Chemical treatment of oil slicks has been intro-

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duced as a way of reducing the external oiling of birds. Application of chemical dispersants onto a slick reduces the surface tension of the oil-water interface and distributes the oil into a larger volume of the water column (Canevari, 1984). Birds at sea will thereby encounter smaller amounts of petroleum hydrocarbons and the effects are thought to be reduced, ultimately causing a reduction in seabird mortality (Peakall *et al.*, 1987). This paper also discusses the effects of these efforts.

Since, under natural conditions, oil pollution directly affects birds through a combination of toxic effects due to ingestion of oil, and thermoregulatory effects caused by plumage oiling, the major findings from the numerous studies on the effects of ingestion of petroleum oil by birds will shortly be reviewed. For the readers not acquainted with the field of thermoregulation, a short general introduction to this field will be given before elucidating the effects that external oiling may have on the thermal balance of aquatic birds.

EFFECTS OF INGESTED OIL

It has been demonstrated that the ingestion of oil affects reproductive ability (Hartung, 1965; Grau *et al.*, 1977; Holmes *et al.*, 1978*a*; Wotton *et al.*, 1979; Vandergilder & Peterle, 1980; Ainley *et al.*. 1981; Harvey *et al.*, 1981, 1982; Cavanaugh & Holmes, 1982; Gorsline & Holmes, 1982*a*) and may reduce eggshell thickness (Holmes *et al.*, 1978*a*; Vangilder & Peterle, 1980; Harvey *et al.*, 1982). External oiling of eggs may reduce gas conductance through the eggshell (Jenssen & Staurnes, 1989), slow embryonic growth, induce teratogenic malformations and decrease hatchability (Albers, 1977; Szaro & Albers, 1977; Albers, 1978; Hoffman, 1978; Albers, 1979; Coon *et al.*, 1979; Hoffman, 1979*a,b,c*; King & Lefever, 1979; McGill & Richmond, 1979; White *et al.*, 1979; Macko & King, 1980; Szaro *et al.*, 1980; Hoffman & Gay, 1981; Ellenton, 1982; Lewis & Malecki, 1984). In young birds, ingestion of oil has caused a reduction in growth rate in a variety of species (Miller *et al.*, 1978; Szaro *et al.*, 1978; Butler & Lukasiewicz, 1979; Peakall *et al.*, 1980, 1982), impaired osmoregulation (Miller *et al.*, 1978; Peakall *et al.*, 1980, 1983) and changes in intestinal absorption (Crocker *et al.*, 1974, 1975; Eastin & Murray, 1981).

In adult birds, ingestion of oil has been shown to cause changes in hepatic enzyme function (Gorsline *et al.*, 1981; Gorsline & Holmes, 1981, 1982*b*; Jenssen *et al.*, 1990), osmoregulatory abilities (Miller *et al.*, 1976; Holmes *et al.*, 1978*b*), and adrenocortical function and corticosterone levels (Peakall *et al.*, 1981, Rattner & Eastin, 1981; Gorsline & Holmes, 1981, 1982*b,c*; Gorsline, 1982), and the physiological effects of ingested oil have been reported to increase in birds stressed simultaneously by other means (Holmes *et al.*, 1978*b*, 1979). Ingestion of oil has also been reported to cause anemia in birds (Hartung & Hunt, 1966; Szaro *et al.*, 1978; Fry & Lowenstein, 1982; Pattee & Franson, 1982; Leighton *et al.*, 1983). Crude oils from different sources differ in their toxicity to birds (Crocker *et al.*, 1975; Gorman & Simms, 1978; Peakall *et al.*, 1981), and the toxic effects of petroleum hydrocarbons have been attributed to the polycyclic aromatic hydrocarbons (Hoffman, 1979*a,b,c*; Patton & Dieter, 1980; Peakall *et al.*, 1981, 1982).

A general conclusion from toxicity studies seems to be that, in chicks and sub-adult birds, ingestion of relatively small amounts of oil may cause adverse effects on their physiology or even cause death. In contrast, adults are more resistant to the toxic effects of oil, and even though ingestion of oil generally evokes sublethal physiological effects, large amounts of oil seem to be required to cause direct mortality among adult birds.

PRINCIPLES OF THERMOREGULATION

During their adult life, birds maintain a relatively constant body temperature of about 40·5 ± 0·5°C (Calder & King, 1974), independent of variations in the environmental temperature. The relative constancy of their body temperature is indicated by the term 'homeothermic' (*Homoio-*, similar), and since their high body temperature is achieved through production of heat by means of their own oxidative metabolism, these animals are called 'endothermic' (*endon*, within) (Richards, 1973).

The heat balance of any animal is described by the net rate at which a subject generates and exchanges heat with its environment (First Law of Thermodynamics):

$$S = M - (W) - (E) - (C) - (K) - (R)$$

where S is the storage of heat in the body, M is the metabolic energy transformation, W is work, E is evaporative heat transfer, C is convective heat transfer, K is conductive heat transfer, and R is radiant heat exchange.

For an animal to keep its body temperature stable, at a given set-point (the value at which the body temperature is stabilized by the process of thermoregulation), there is no storage of heat in the body. The metabolic energy transformation, also termed 'metabolic rate' (MR), is the rate of transformation of chemical energy into heat (metabolic heat production, H) and mechanical work by aerobic and anaerobic metabolic activities within the organism. If the animal is at rest, no energy is used for mechanical work, and since the energy is released by oxidative reactions, MR equals H, and the heat production of the organism can be computed from the measured oxygen consumption.

The terms $[(E) - (C) - (K) - (R)]$ represent the external components, or the 'avenues' by which heat is exchanged between the animal and its external environment. In an animal at rest, the avenues of heat exchange are via evaporation (E), convection (C), conduction (K), and radiation (R). In practice, it is difficult both to measure and to distinguish between the avenues of heat transfer within the body or between the body and its external environment. As a simplification, the term

'thermal conductance' (C_T) has been introduced (this term is also known as 'total thermal conductance'). C_T is a combined heat transfer coefficient for the entire body, and is thus a measure of the ease with which heat flows from the body core to the surrounding environment. Thermal conductance may be derived from the following equation:

$$C_T = H/(T_B - T_A)$$

in which H is heat production, T_B is body temperature and T_A is the temperature of the surroundings (air and/or water). If the evaporative heat loss is included, the thermal conductance is often referred to as the 'wet' conductance (Aschoff, 1981), whereas if the evaporative heat loss is excluded [$C_T = (H - E)/(T_B - T_A)$], it is often referred to as 'dry' conductance (Whittow, 1986). The reciprocal of the conductance ($1/C_T$) is the total thermal insulation (I) of the animal.

EFFECTS OF EXTERNAL OILING ON THERMOREGULATION

The effect of oil pollution on the thermoregulation of seabirds was first noted by Portier and Raffy (1934). They experimentally oiled (with olive oil, vaseline or petroleum oil) the plumages of three pigeons and a duck (canard), and found that during exposure to low air temperatures, or immersion in water, their body temperature decreased. They concluded that fat or petroleum oil reduces the insulatory properties of the plumage of seabirds, and that oiled seabirds suffer death due to hypothermia. They also concluded that seabirds are particularly susceptible because, when in contact with water, the rate of heat loss rapidly exceeds the heat production capacity.

Later studies have, in addition to examining the effect of external oiling on body temperature, also included other thermoregulatory variables, such as heat production, heat loss and thermal insulation. Several studies have also addressed the dose–response issue.

Whereas Portier and Raffy (1934) examined only the effect of external oiling on body temperature, Hartung (1967) and McEwan and Koelink (1973) in their studies on externally oiled ducks also included measurements of metabolic heat production. They noted that the mechanism responsible for the reduction of the water repellency of the plumage is that the oil adheres to the feathers, so that they become clogged together and matted. Thus, water can penetrate into the plumage and displaces the insulating layer of air, leading to increased heat loss, and a consequent rise in metabolic rate.

The effect of external oiling on the thermoregulation (i.e. on the thermal insulation of the plumage) of ducks has been shown to be dependent upon the amount of water that is absorbed into the plumage (Jenssen & Ekker, 1991*a*). This is governed by the amount of oil to which the birds are exposed (Hartung, 1967; McEwan & Koelink, 1973; Jenssen & Ekker, 1989, 1991*a*), and the extent to which they have spread the oil into their plumage by preening (Jenssen & Ekker, 1991*b*).

Effects in air and water

The effect of plumage-oiling on thermal insulation is also dependent upon whether the oiled birds are in water or on land. The heat loss of oiled eiders (*Somateria mollissima*) in water was 360% higher than normal, and exceeded the peak metabolic rate of the birds. Thus, these oiled birds became hypothermic (Jenssen & Ekker, 1990). Hypothermia (1·5–4°C) has also been reported in oiled jackass penguins (*Spheniscus demersus*) and Cape gannets (*Sula capensis*) swimming in water (Erasmus *et al.*, 1981; Kerley & Erasmus, 1986; Kerley *et al.*, 1987).

When oiled and water-soaked eiders were placed, on land (i.e. in air, rather than water) their heat loss (thermal conductance) and MR were only 57% and 72% respectively, higher than normal (Jenssen & Ekker, 1990). After their plumage had dried, their C_T and MR did not differ significantly from that of normal eiders, and the oiled eiders had no problems in regulating their body temperature at a normal level. However, when the birds were returned to water, their C_T and *MR* increased, and the birds became hypothermic.

The need for the plumage to be dry in order to protect against heat loss has also been demonstrated in another study, where the MR of four water-soaked, cleaned eiders decreased towards a normal level as their plumage dried (Jenssen & Ekker, 1989). Therefore, unless the plumage is so contaminated by a thick oil that it collapses the plumage, even though it contains no water, the thermal insulation capacity of a dry plumage does not seem to be much affected in oiled birds. In a real-life situation it is, however, possible that precipitation and convection due to wind may cause a higher than normal heat loss in oiled birds, even though their plumage is initially dry.

Hartung (1967), McEwan & Koelink (1973) and Lambert *et al.* (1982) have demonstrated increases in the metabolic rate in plumage-oiled mallards (*Anas playvrhynchos*), black ducks (*Anas rupribes*) and scaups (Aythya spp.) ranging from 7 to 100% in air, while Jenssen *et al.* (1985) reported that no metabolic response was found in a naturally oiled common murre (*Uria aalge*). These figures are all within the range of the metabolic response presently reported for oiled eiders in air (Jenssen & Ekker, 1990).

Furthermore, Culik *et al.* (1991) reports that in air, Adélie penguins (*Pygoscelis adeliae*) contaminated by vegetable oil had reduced heart rates (90 versus 98 bpm), and energy expenditure (4·7 versus 5·2 W kg^{-1}) compared to controls. In water, their swimming speed was lower than that of controls (1·6 versus 1·8 m s^{-1}), their heart rate was higher (321 versus 252 bpm), and their *MR* while swimming was 50% higher than in controls (18·8 versus 12·7 W kg^{-1}). Also, their cost of transport in water was 73% higher than that of controls (12·1 versus 7·0 J kg^{-1} m^{-1}). It is reasonable to believe that the vegetable oil that these penguins were contaminated with, excellently mimicked the effects of petroleum oil. Thus, experiments carried out only in air underestimate the effect that oiling may have on the

thermoregulation and energetics of aquatic birds that normally reside both in air and water.

Birds compared to marine mammals

In aquatic mammals that depend upon a water-repellent fur to remain normothermic in water, such as the sea otter (Costa Kooyman, 1982; Siniff *et al.*, 1982; Davis *et al.* 1988), the muskrat (McEwan *et al.*, 1974) and the polar bear (*Ursus maritimus*) (Hurst & Øritsland, 1982; Hurst *et al.*, 1982; Hurst *et al.*, 1991), external oiling reduces the thermal insulation of the fur, and evokes the same physiological responses as described in plumage-contaminated birds. By contrast, external oiling has little or no effect on the heat balance of aquatic mammals that depend upon a sub-dermal layer of blubber for insulation against heat loss. Pelts from harp (*Pagophilus groenlandicus*), bearded (*Erignathus barbatus*), and Weddel (*Leptonychotes weddelli*) seals and California sea lions (*Zalophus californianus*) show little or no change in heat conductance after oiling (Øritsland & Smith, 1975; Kooyman *et al.*, 1977).

Effects on behavioral thermoregulation

Costa and Kooyman (1982) and Davis *et al.* (1988) showed that oiling caused sea otters to increase their activities, such as grooming and swimming, that are energetically demanding. Taking into account that activity of an oiled individual in water could lead to increased heat loss compared to activity on land, this seems to be an inefficient way of thermoregulation, unless the activity is directed towards feeding. In a behavioral study conducted in a large outdoor seawater pool, it was demonstrated that exposure of two common eiders to 2·5 ml of a Statfjord A crude oil—Finasol OSR-5 mixture, caused the birds to increase their preening activity, and to reduce the time spent in water (Ekker *et al.*, 1989). Since the heat loss of oiled birds is less in air than in water (Jenssen & Ekker, 1990), the behavior of spending less time in water can be considered as a thermoregulatory behavior. However, some aquatic birds, e.g. auks, cormorants, and seaducks, have no possibilities of feeding ashore, and they are therefore faced with a choice between starving ashore or entering the sea, where they are forced to increase their energetic expenditure to remain normothermic. If the heat loss exceeds the peak metabolic rate (PMR), they will suffer death from hypothermia.

Effects in different species

Results from the studies of plumage oiling on birds suggest that different species of aquatic birds may respond differently to plumage contamination (Hartung, 1967; McEwan & Koelink, 1973; Jenssen & Ekker, 1991*b*). Although in some of these studies the different magnitude of effects may be a result of different experimental protocols, in Jenssen and Ekker (1991*b*), the species (common eider and mallard) were treated identically, and the results therefore probably reveal a true difference in susceptibility. It was suggested that the difference in susceptibility between mallards and eiders may be due to differences in their plumage structure. The soft and air-filled plumage of the eider probably collapses more readily than the more compact, but less insulating, plumage of mallards. If this suggestion is true, aquatic birds with low minimal values of thermal conductance (C_{Tmin}) are most susceptible to plumage oiling.

In discussing the impact of oil on birds, it is convenient to group aquatic birds into four broad categories based on how they utilize the aquatic environment. These categories may also be functional when discussing comparative aspects of thermoregulation in aquatic birds. Similar categories have recently been presented by MacArthur (1989) for discussing thermophysiological relationships in aquatic mammals.

The first category consists of *semi-aquatic* species, such as swans (*Cygnus*), geese (*Anserinae*), dabbling ducks (*Anatini*) and gulls (*Larinae*), which readily move between water and land, and seek food in both of these environments. The second category consists of *semi-marine* birds that feed at sea, often inshore. These may be plunge divers or surface feeders, but generally they spend a great part of their time ashore or flying. This category is represented by species such as gannets (*Sulidae*), terns (*Sterninae*) and some gulls. The third category, *pelagic* seabirds, include pelagic species that either are surface feeders, plunge divers, or pluck food from the ocean surface while flying. This group is represented by species such as albatrosses (*Diomedeidae*), fulmars, shearwaters, petrels and storm-petrels (*Procellariformes*), and skuas (*Stercorariidae*). The fourth group, *diving* birds, includes penguins (*Sphenisciformes*), loons (*Gaviiformes*), grebes (*Podicpiediformes*), cormorants (*Phalacrocoracidae*), diving ducks (*Aythvinae*), sawbills (*Merginae*), and auks (*Alcidae*), species that spend most of their lives more or less immersed in the aquatic environment. This latter category can be regarded as the most adapted to an aquatic life/environment.

The dependency of different species on the aquatic environment for feeding will be decisive for their chances of surviving plumage oiling. Oiled diving birds may have a normal thermoregulation when they are ashore, but they will be subjected to a large heat loss as they enter the sea for feeding. In contrast, oiled pelagic, semi-aquatic and semi-marine birds may avoid the great heat loss by feeding ashore or reducing their stay in water. According to Piatt *et al.* (1990) more diving species than pelagic species were killed following the 'Exxon Valdez' spill in March 1989.

Additionally, the heat loss from a small oiled, water-soaked, bird would be greater than that from a large bird due to the surface—volume ratio. Thus, from a thermal point of view, small diving birds with low values of C_{Tmin} are most vulnerable to plumage oiling, whilst large semi-aquatic birds are least vulnerable to oiling. This is in accord with the general view of susceptibility of different bird species, based on reports of mortality following oil spills (Leighton *et al.*, 1985; Hunt, 1987).

It is also worthwhile mentioning that the risk of coming into contact with an oil spill at sea is naturally highest for the more aquatic species.

EFFECTS OF INGESTED OIL ON THERMOREGULATION

Oil can be ingested when animals groom or preen their contaminated fur or plumage (Hartung & Hunt, 1966; Hurst *et al.*, 1982), or while feeding on contaminated prey (Clark & Gourney, 1987). Studies on polar bears, rats (*Rattus norwegicus*), common eiders and domestic ducks (*Anas platyrhynchos*) indicate that ingestion of oil may cause hyperthermia or fever (Hurst *et al.*, 1982; Haim *et al.*, 1984; Jenssen & Ekker, 1988, 1990; Jenssen, 1989). Haim *et al.* (1984) suggested that, since no effect of ingested oil was found on the rats' MR, the hyperthermia was caused by increased peripheral vasoconstriction and thus decreased heat loss. This suggestion is supported by the lack of effect on MR in domestic ducks that had developed hyperthermia following oral dosing of Statfjord A crude oil (Jenssen, 1989).

In contrast to the above-cited reports of hypothermia in oil-exposed homeotherms, are the reports of hypothermia in glaucous-winged gulls (*Larus glaucescence*) that had been covered by 150 ml of Marine Bunker fuel for 4–5 days (Hughes *et al.*, 1990), and in Adélie penguins externally contaminated with vegetable oil (Culik *et al.*, 1991). Hughes *et al.* (1990) indicate that a low salt intake observed in the oiled birds could be associated with their low body temperature (T_B), because such a relationship has previously been reported in chicks (Arad & Skaghauge, 1986). Culik *et al.* (1991) also found that the MR of oiled penguins was reduced, and propose that within the thermoneutral zone in air, the MR and thereby T_B may be reduced in oiled penguins in order to limit heat loss.

Butler *et al.* (1986) showed that when measuring MR with doubly labeled water, experimental oil ingestion by Leach's storm petrel (*Oceanodroma leucora*) led to a 25% increase in the metabolic rate. However, when using respiriometry to measure MR, no such increase in MR was detected in birds that had ingested oil. The increased MR found in the doubly labeled experiment may reflect an effect on behavior of the oil-exposed birds.

EFFECTS OF CLEANING OILED BIRDS

It has been shown that the water-repellent and insulative function of oiled birds' plumages can be regained only when all the oil and detergent are removed, and the plumage has dried completely (Clark & Gregory, 1971; Kerley *et al.*, 1987; Jenssen & Ekker, 1988, 1989; see also review by Clark, 1984). Thus, it is possible that the high MR after cleaning of oiled mallards reported by McEwan & Koelink (1973) was caused by oil or detergent residues left in the plumage, or that their plumage was not completely dry.

In contrast to the rapid return of the MR to pre-oiling levels, and restoration of the plumage insulation, in cleaned birds (Jenssen & Ekker, 1988, 1989), Costa & Kooyman (1982) and Davis *et al.* (1988) demonstrated that the MR of oiled sea otters that had been cleaned needed 3–8 days to return to pre-oiling levels. Williams *et al.* (1988) and Davis *et al.* (1988) also demonstrated that cleaned sea otters needed at least 5 days to re-impregnate the unoiled parts of their pelt with hydrophobic waxes, and more than 7 days to re-impregnate the oiled parts. It therefore seems as though hydrophobic waxes are more important for the water-repellent and insulative properties of the fur of aquatic mammals, than is the case for the water-repellency of the plumages of aquatic birds.

The effect of dispersant mixtures on heat production, following contamination with 2·5 ml, was approximately twice that found for eiders not previously contaminated (Jenssen & Ekker, 1991*b*). This could indicate that rehabilitated birds are more sensitive to subsequent plumage contamination than are normal birds. It is possible that this greater susceptibility of the plumage of cleaned birds is the result of preen-gland waxes being removed during the cleaning process. This effect may have contributed to the high mortality rates reported for rehabilitated birds. Frost *et al.* (1976) reported a 1·8% recovery of ringed rehabilitated jackass penguins, compared to less than 1% of normal birds, indicative of an increased mortality in cleaned birds. In laboratory investigations, Swennen (1977) found an annual mortality of 36% in cleaned birds, as compared to 7% in normal birds.

When oiled birds are brought into rehabilitation, their general condition may be poor, and this may affect their chances of surviving the rehabilitation process. Frink (1982) reported that at Tri-State Bird Rescue, Delaware, USA, there was a strong correlation between body weight and mortality, as well as between body temperature and mortality. In groups of diving ducks with a body weight or body temperature at or above the average for their species a 100% release rate was achieved. Females that did not meet this standard showed an 80% mortality, and males a 56% mortality, during the rehabilitation process.

It has been shown that it is possible to reduce the cleaning time by using more efficient cleaning agents, and that different cleaning agents restore the insulative value of the plumage to different degrees (Jenssen & Ekker, 1989). Differences in the efficiency of different detergents to remove oil and to restore the insulatory function of fur was also demonstrated by Williams *et al.* (1988), who cleaned samples of adult sea-otter pelts using different cleaning agents. Thus, by applying more efficient detergents it is possible to reduce the time needed for cleaning oiled animals, but cleaning and rehabilitation of oiled birds are still time and energy consuming, and to be successful, the process requires considerable skill. Furthermore, as demonstrated by Khan and Ryan (1991), toxic effects of oil that birds have ingested while preening their oiled plumage, may permanently disable birds or protract their recovery.

EFFECTS OF EXPOSURE TO OIL-DISPERSANT MIXTURES

Only two experiments (i.e. Lambert *et al.*, 1982; Jenssen & Ekker, 1991*b*) seem to have been performed to study the effects of chemically treated oil on the thermoregulation of birds. The lack of experimental data concerning the effects of chemically treated oil on birds has led to a divergence of opinion as to whether an oil slick at sea should be dispersed, or not, in order to minimize its impact on seabirds (Clark, 1984; Lindstedt-Siva *et al.*, 1984; Peakall *et al.*, 1987).

The effects of contamination by oil-dispersant mixtures have been reported to be qualitatively identical to that of the oil alone; the mixture reduced the water repellency of the plumage, resulting in absorption of water, increased heat loss and metabolic rate (Lambert *et al.*, 1982; Jenssen Ekker, 1991*b*). It has, however, been reported that, as compared to crude oil (Statfjord A), a much smaller volume (1/100) of chemically treated crude oil (Statfjord A/Finasol OSR-5) was required to cause significant effects on plumage insulation and thermoregulation in eiders (Jenssen & Ekker, 1991*b*).

Although the experiments were conducted on a limited number of birds, one may speculate that the reason for the extremely low tolerance for chemically treated oil is that the surfactants in the dispersants more easily adhere to the feather structure, possibly by binding to the hydrophobic waxes in the plumage. This would reduce the surface tension at the feather–water interface and enhance the effects of contamination on the insulative properties of the plumage.

Lambert *et al.* (1982) demonstrated that exposure to 13·2 ml of a mixture of Prudhoe Bay crude oil and the chemical dispersant Corexit 9527, led to a 23% increase in the metabolic rate of mallards. Thus, exposure to other oil-dispersant mixtures also seems to cause a reduction in the plumage insulation. The greater increase in the MR (30–130%) in mallards contaminated by 10 ml of a mixture of Statfjord A crude oil and Finasol OSR-5 (Jenssen & Ekker, 1991*b*), is most likely due to these ducks being in water, whereas the ducks in Lambert *et al.*'s (1982) study were in air, and thus exposed to an environment with a lower thermal strain.

Since chemically treated oil may form a thin film on the sea surface (where birds swim), in a 'worst case' scenario, chemical treatment of an oil slick may expose aquatic birds to less, but more harmful, chemically treated oil. However, this is based on a limited amount of available data, and so is merely speculative. Until more information about the effects of chemically treated oil on aquatic birds is available, any decision on whether to disperse or not, to reduce the impact on birds, will be based on speculation. The urgent need for more information about the effects of chemically treated oil on aquatic birds is therefore stressed.

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Effects of Plumage Contamination with Crude Oil Dispersant Mixtures on Thermoregulation in Common Eiders and Mallards

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Abstract. Thermoregulatory effects of plumage-oiling with Statfjord A crude oil (SACO), or of SACO mixed with the dispersants Finasol OSR-5® or OSR-12® were studied by measuring the rate of metabolic heat production of common eiders (*Somateria mollissima*) and mallards (*Anas platyrhynchos*) residing in water (6.5°C). The study suggests that oil-dispersant mixtures are more potent than the crude oil alone, and that common eiders are more susceptible to crude oil-dispersant mixtures than are mallards. The species difference is probably due to specific differences in plumage structure, *i.e.*, birds possessing an air-filled plumage, with high insulative properties, are probably more vulnerable than species with a plumage which does not offer so much resistance to heat loss. The level of heat production of contaminated mallards was higher on the day following contamination compared to the metabolic rate recorded two–three hours after contamination, presumably because the birds preened the pollutants further into their plumage, enhancing its wettability. Because exposure to very small volumes of chemically treated oil may cause a significant decrease in plumage-insulation, birds should be prevented from coming into contact with chemically treated oilslicks.

Oiling of birds' plumage reduces its water repellent properties and leads to increased heat loss. To conserve their body temperature, birds with oiled plumage must increase their metabolic heat production and energy expenditure (Hartung 1967; McEwan and Koelink 1973; Lambert *et al.* 1982; Jenssen and Ekker 1988, 1989, 1990). If the oiled birds are residing in water, their heat loss may exceed their maximum capacity for heat production, so that the birds develop hypothermia (Erasmus *et al.* 1981; Jenssen and Ekker 1988, 1990).

Plumage-oiling is probably the major cause of the high mortality among seabirds following oilspills at sea. Chemical treatment of oil slicks has been introduced as one way of reducing the high seabird mortality that ensues after oil spillages at sea. Application of chemical dispersants onto a slick reduces the surface tension of the oil-water interface and distributes the oil into a larger volume of the water column (Mackay and Wells 1983). Birds at sea will come into contact with smaller amounts of petroleum hydrocarbons and the effects on the plumage insulation are thought to be reduced, ultimately causing a reduction in seabird mortality.

Only one study concerning the effects of plumage-exposure to oil-dispersant mixtures on the thermoregulation of birds has been published (Lambert *et al.* 1982). In this study, the thermoregulatory effects on mallards (*Anas platyrhynchos*) were examined on birds residing in air (on land). Since the thermal conductivity and heat capacity of water is much higher than that of air, the heat loss of an oiled bird residing at sea is higher than when it is residing ashore (Jenssen and Ekker 1990). Therefore, it is difficult to extrapolate results of Lambert *et al*'s study to a situation where contaminated birds are at sea.

The lack of experimental data concerning the effects of chemically treated oil on birds has lead to a divergence in opinion as to whether or not an oil slick at sea should be dispersed in order to minimize its impact on seabirds (Lindstedt-Siva *et al.* 1984; Clark 1984; Leighton *et al.* 1985; Peakall *et al.* 1987). Greater knowledge of the effects of chemically treated oil on birds is therefore important, so that the right decisions can be made on how to tackle oil spills at sea and to minimize the ecological impact of oil pollution.

The present study reports on the effects on metabolic heat production and thermoregulation caused by contamination of the plumages of mallards and common eiders (*Somateria mollissima*) with crude oil and with chemically treated crude oil.

Materials and Methods

Experimental Birds

Adult mallards (n = 13, mean body weight [BW] = 1.37 ± 0.09 kg) were purchased from a commercial breeding farm (Svanøy Stiftelse, N-6965 Svanøybukt) and common eiders (n = 10, BW = 1.80 ±

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0.09 kg) from the Trondheimsfjord (63°26′N, 10°24′E). The birds were kept at the Department of Zoology, University of Trondheim, with access to an indoor freshwater pool, and air and water temperatures were between 5 and 12°C. The eiders were kept under light conditions corresponding to the natural diurnal cycle, whereas the mallards were kept under a 12D:12L light cycle. The mallards were fed *ad libitum* with commercial breeding pellets. The eiders were force-fed for the first few days after capture, after which blue mussels (*Mytilus edulis*) and mashed fish were available *ad libitum*.

Metabolic Heat Production

The oxygen consumption (VO_2, mL O_2/g/h) of resting ducks was measured in an open-circuit system (Depocas and Hart 1957) during daytime (during the birds activity-phase). After weighing, each bird was placed in a respiration chamber (70 L), filled with 24 L of fresh seawater, such that the bird was floating freely on the water (Jenssen and Ekker 1988). To keep a stable temperature of 5–8°C in both air and water, the respiration chamber was placed in a temperature-controlled chamber. Using a suction pump (Reciprotor 506 R), air was pumped through the respiration-chamber and dried with silica-gel before a fraction of the air was passed into an oxygen analyzer (Servomex Series 1100) to determinate the O_2 tension. The air-flow through the respiration chamber was adjusted so that the oxygen-extraction was less than 1%, and was continuously measured during the experiments, using a calibrated flow-meter (Cole-Parmer Instrument Co., FM034-39ST). Prior to each experiment, the O_2-analyzer was calibrated, using atmospheric air (20.95% O_2) and nitrogen. In addition to a mock experiment made prior to the actual experiments, each bird was allowed to adapt to the experimental conditions for at least 1.5 h before the desired amount of crude oil, or oil-dispersant mixture, was added to the water. In this way, a situation where the duck was swimming into an oil-slick or a dispersed oil-slick was simulated, and each bird served as its own control. Oxygen consumption was calculated according to Withers (1977) and corrected to standard temperature (0°C), pressure (760 mm Hg), and dry conditions. The metabolic rate of heat production (H, Watts/kg) was estimated by assuming a respiratory exchange ratio (RQ) of 0.85 for a post-absorptive animal and an energetic equivalent of 5.571 W/kg pr. mL O_2/g/h. Metabolic heat production was monitored for 30 min prior to, and for at least 180 min after, the oil or the oil-dispersant mixture was added to the respiration chamber.

Temperature Measurements

The air temperature (T_A, °C) inside the respiration chamber was recorded close to the air outlet, and the water temperature (T_W, °C) 10–15 cm below the water surface. The body temperature (T_B) of the birds was measured, prior to the experiments, by inserting a copper-constantan thermocouple (Honneywell, 0.13 mm teflon-coated) down the esophagus.

Thermal Conductance

The thermal conductance (C_T, W/°C m^2) was calculated according to the equation: $C_T = H \cdot BW/(T_B - T_{A/W}) \cdot A$, where A is the surface area of the plumage (m^2) and$T_{A/W}$ is the mean air/water temperature. Thus, since the heat loss from evaporation is not excluded, C_T represents the "wet" thermal conductance according to Aschoff (1981). The surface area of the plumage was calculated according to Meeh's equation: $A = k \cdot M^{2/3}$, which expresses the relationship of body mass (M, kg) to surface area. The plumage-surface of the mallards was calculated using a mass-coefficient (k) of 0.1, whilst the plumage surface of the common eiders was calculated using a k value of 0.097 (Jenssen *et al.* 1989).

Oil and Chemicals

The Finasol OSR-5 was diluted with sea-water at a rate of 1:10, while the Finasol OSR-12 was diluted with seawater at a rate of 1:1. The diluted dispersants were premixed with Statfjord A crude oil (SACO) for 20 min, using a magnetic stirrer (SACO:OSR-5 + seawater = 10:1, SACO:OSR-12 + seawater = 1:1), and the desired volumes (0.025, 0.25 or 2.5 mL) of the mixtures were added to 1 L of sea-water. After stirring for another 20 min, the liter of contaminated sea-water was added to the sea-water in the respiration chamber through a tube, whilst the bird was floating undisturbed on the water surface. The respiration chamber contained 24 L of water, so that by adding 1 L of contaminated water, this gave 0.001, 0.01 and 0.1 mL/L (1, 10, 100 ppm), respectively, of dispersed oil in the water. Statfjord A crude oil was added directly to the 25 L of sea-water in the respiration chamber, through a tube. The mean temperature of the sea water, the dispersants and the dispersed oil was 6.5°C (range 5.0–8.5°C).

Experimental Protocol

Four groups of mallards (each group consisting of three individuals) were exposed to 2.5 or 10.0 mL of one of the two oil-dispersant mixtures, and their metabolic rates subsequently monitored for 180 min to study whether there were any differences in the effects of the two different oil-dispersant mixtures on thermoregulation. Two of the mallards from each of the above groups were placed once again in the respiration chamber on the day after contamination, to find out whether the effect of exposure to the chemically treated oil was modified after the birds had preened their plumages.

One common eider was exposed to 0.025 mL of the SACO/OSR-5 mixture, two other eiders were exposed to 0.25 mL of the same mixture, and two further eiders were exposed to 2.5 mL of this mixture. One eider was exposed to 2.5 mL of the SACO/OSR-12 mixture, and, to elucidate whether the thermoregulatory effect would be modified if the bird was allowed to preen the contaminant into its plumage, this eider was also placed once more in the respiration chamber on the day after contamination.

Three common eiders were exposed to 2.5, 10, and 30 mL of SACO, respectively, to compare the effects following exposure to chemically treated oil, with the effects following exposure to the oil alone.

Two oiled eiders which had been cleaned with the detergent Taski-Profi (A. Sutler, A.G., CH 9542 Münchweilen, Switzerland) (for methods see Jenssen and Ekker 1989) were exposed to 2.5 mL of the oil-dispersant mixtures to assess how cleaned and rehabilitated birds tolerate recontamination. These experiments were conducted 9 and 12 days, respectively, after the birds had been cleaned.

Some of the common eiders and mallards were weighed immediately after the experiments to find to what extent the wetability of the plumage had been affected by the contamination, since the increase in weight would indicate how much water had been absorbed by the plumage.

The data were analyzed by ANOVA (analysis of variance) and subsequent paired and unpaired Student's t-test for groups (Macintosh Plus computer, StatWorks® version 1.2, Cricket Software Inc., Philadelphia, PA). A probability of less than 0.05 was defined significant.

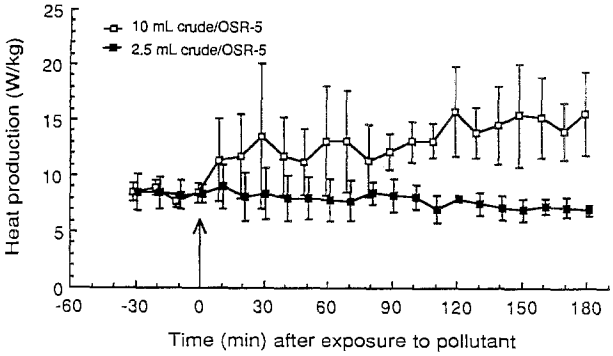

Fig. 1. Metabolic heat production (W/kg ± SD) of mallards (*Anas platyrhynchos*) as a function of time after exposure to 2.5 mL (n = 3) or 10 mL (n = 3) of Statfjord A crude oil mixed with Finasol OSR-5

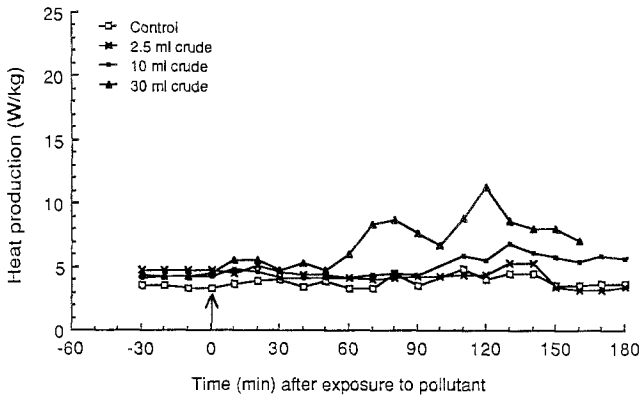

Fig. 2. Metabolic heat production (W/kg) of three common eiders (*Somateria mollissima*), expressed as a function of time after exposure to different doses of Statfjord A crude oil, compared to the values for an unexposed bird as a control

Table 1. Metabolic heat production (W/kg) of mallards residing in water (T_W = 6°C) before, and 2 h after, exposure to Statfjord A crude oil mixed with either Finasol OSR-5 or OSR-12, and the heat production of the same individuals, two h after they were placed in the respiration chamber on the day following contamination

		Metabolic heat production (W/kg)		
Dispersant	Dose (mL)	Before contamination	2 h after contamination	1 day after contamination
Crude/ OSR-5	2.5	7.7	7.9	17.7
	2.5	9.4	8.2	13.3
	10	8.0	14.8	21.2
	10	9.5	12.3	21.6
Crude/ OSR-12	2.5	6.2	6.5	20.5
	2.5	6.5	7.4	23.0
	10	7.1	8.5	22.0
	10	6.2	7.5	10.8

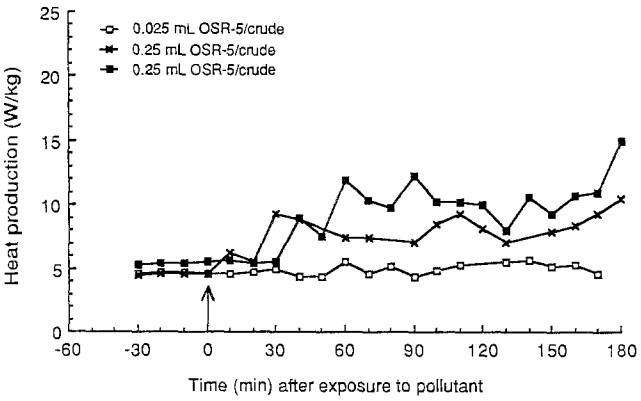

Fig. 3. Metabolic heat production (W/kg) of three common eiders (*Somateria mollissima*) as a function of time after exposure to different doses of Statfjord A crude oil mixed with Finasol OSR-5

Results

The metabolic heat production of common eiders and mallards floating calmly on the water surface in the respiration chamber was 4.3 W/kg (SD = 0.3, n = 10) and 7.2 W/kg (SD = 0.5, n = 13), respectively. The body temperatures of the eiders and mallards were 41.2°C (SD = 0.6, n = 9) and 41.4°C (SD = 0.7, n = 12), respectively, while the thermal conductances were 1.59 W/°C m^2 (SD = 0.37, n = 9) (0.022 mL O_2/g h °C ± 0.005) and 2.28 W/°C m^2 (SD = 0.34, n = 12) (0.036 mL O_2/g h °C ± 0.005), respectively, while resting quietly in 7.0°C (SD = 1.2, n = 9) and 5.6°C (SD = 0.6, n = 12) water, respectively.

Exposure of mallards to 2.5 mL of the two different oil-dispersant mixtures had no significant effect (one-way ANOVA) on heat production. Nor was there any significant increase in the heat production of the birds exposed to 10 mL of the SACO/OSR-12 mixture (one-way ANOVA). Exposure to 10 mL of the SACO/OSR-5 mixture resulted in an immediate increase in the heat production of all three mallards, and their heat production continued to increase until, 90 min after exposure (Figure 1), it was significantly higher than their baseline values (one-way ANOVA and t-test, df = 4, $p < 0.05$). After two to three h, the heat production of these mallards was 30–130% higher than their baseline values.

The heat production values for the mallards prior to contamination, two h after contamination, and two h after they were again placed in the respiration chamber on the day following contamination, are presented in Table 1. On the day following contamination, the heat production of all individuals was higher than on the previous day, two h after contamination (one-way ANOVA and t-test, $p < 0.05$). Thus, although contamination with 2.5 mL of the oil-dispersant mixtures had no effect on heat production two h after contamination, it resulted in a 40–250% increase in heat production on the day after contamination.

Exposure to 2.5 mL SACO had no effect on the heat production of the common eider studied (Figure 2), and the effect on heat production following exposure to 10 and 30 mL SACO was both time and dose related.

Exposure of eiders to 0.025 mL of the SACO/OSR-5 mixture did not have any effect on the metabolic rate (Figure 3). However, exposure to 0.25 mL on the SACO/OSR-5 mixture, caused the heat production of the two eiders to increase by approximately 50–250% (Figure 3), approximately the same thermoregulatory response as exposure to 30 mL of the oil alone (Figure 2). A similar proportional response

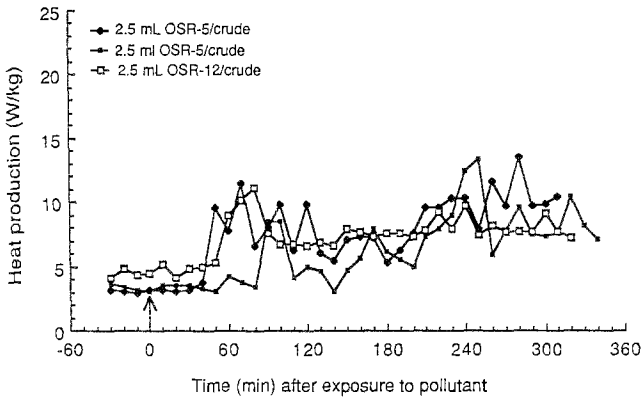

Fig. 4. Metabolic heat production (W/kg) of three common eiders (*Somateria mollissima*) as a function of time after exposure to 2.5 mL Statfjord A crude oil mixed with either Finasol OSR-5 or OSR-12

was also shown by the two eiders contaminated with 2.5 mL of the SACO/OSR-5 mixture, and by the eider contaminated with 2.5 mL of the SACO/OSR-12 mixture (Figure 3). In comparison, exposure to 2.5 mL of the crude oil alone had no effect on the heat balance of the eider (Figure 2).

The effects of exposure to 2.5 mL SACO/OSR-12 were studied in two experiments with one eider, one made on the day of contamination and the other on the following day. Two h after contamination, heat production had increased by approximately 50%, to 6–7 W/kg. When the eider was placed back into the respiration chamber on the day after contamination, its heat production rapidly increased to 18–23 W/kg, 300–400% higher than it's baseline heat production prior to contamination.

Prior to contamination with either 2.5 mL of the SACO/OSR-5 or SACO/OSR-12 mixture, the two eiders which previously had been oiled, cleaned and rehabilitated, had heat production values of 3.6 W/kg and 3.8 W/kg, respectively. Two to three h after contamination, the heat production of the eider exposed to the SACO/OSR-5 mixture had increased to 16–20 W/kg, while that of the eider exposed to the SACO/OSR-12 mixture increased to 11.5–19 W/kg. The thermoregulatory effect following the exposure of the cleaned eiders to the oil-dispersant mixtures was much greater than that observed in eiders exposed to a similar amount of treated oil, but which had not been previously contaminated and cleaned (Figure 4).

Because water had been absorbed by the plumage, the weight of the contaminated birds had increased during the experiments. There was a significant positive correlation ($p < 0.05$) between the amount of water which was absorbed by the plumage (expressed as a % of body weight), and the metabolic heat production of both eiders (H [W/kg] = 5.69 + 0.77*% increase in BW, $r = 0.63$, $n = 11$, $p < 0.05$) and mallards (H [W/kg] = 8.30 + 0.64*% increase in BW, $r = 0.88$, $n = 8$, $p < 0.05$) during the last half-hour before the end of the experiments.

Discussion

External exposure to chemically treated oil had the same qualitative effects as external oil contamination: a reduction in the water-repellent properties of the plumage and a resultant absorption of water by the plumage leading to increased heat loss and a compensatory increase in heat production. However, compared to exposure to oil alone, much smaller amounts of the oil-dispersant mixtures were required to cause the effects. The reason for the higher potency of the chemically treated oil may be that the surfactants in the dispersants more easily adhere to the feather structure, possibly by binding to the hydrophobic waxes (Odham and Stenhagen 1971) which birds preen into their plumage. This would reduce the surface tension at the feather-water interface and enhance the effects of contamination on the insulative properties of the plumage.

Since exposure to 2.5 mL of the oil-dispersant mixture caused a significant increase in the heat production of eiders, whereas mallards did not respond to this amount, the study indicates that different species of aquatic birds may respond differently to plumage contamination. This species effect, which to our knowledge has not been documented previously, is most probably related to the difference in the plumage structure of these two species. The thermal conductance values for the eiders and mallards prior to contamination were 88% and 129%, respectively, of the values predicted using the allometric equation (Aschoff 1981) which relates the thermal conductance (mL O_2 g^{-1} h^{-1} $°C^{-1}$) of non-passerine birds to the body mass (g). The lower than predicted C_T of the eiders is primarily due to their fluffy, air-filled plumage, which provides a good insulation (Jenssen *et al.* 1989). The soft and air-filled plumage of the eider probably collapses more readily than the more compact plumage of the mallard. Thus, birds which have low thermal conductances may be more susceptible to plumage contamination than species with a plumage that does not offer so much resistance to heat loss.

The different effects of the two oil-dispersant mixtures on the thermoregulation of mallards may be due to the differences in the chemical composition of the two dispersants, viz. OSR-5 contains 60% surfactants, while OSR-12 contains only 20% surfactants. If the surfactants are the chemicals that are responsible for the enhanced effects of plumage contamination, then all chemical dispersants containing surfactants may produce a oil-dispersant mixture which is more potent than the crude oil alone.

Previously, Lambert *et al.* (1982) demonstrated that a one-h exposure to 13.2 mL of a mixture of Prudhoe Bay crude oil (PBCO) and the chemical dispersant Corexit 9527, lead to a 23% increase in the metabolic rate of mallards exposed to an air-temperature of −12°C. In the present study, exposure of mallards to 10 mL of the SACO/OSR-5 mixture, at a water temperature of 6°C, resulted in a 30–130% increase in the metabolic rate. Since the thermal conductivity and heat capacity of water is much higher that that of air, the greater effect on the thermoregulation of mallards following contamination with the SACO/OSR-5 mixture, compared to that produced by the PBCO/Corexit mixture (Lambert *et al.* 1982), is probably because our mallards were resting in cold water, not on land, during the experiments. It is therefore not possible to compare the quantitative effect of the oil-Corexit mixture used by Lambert and co-workers, with the effect of the oil-Finasol mixtures used in the present study.

The thermoregulatory effects of plumage-contamination was greater on the day following contamination than imme-

diately after contamination. We have previously found a similar second-day effect following plumage contamination of eiders with SACO alone. Exposure to 10–15 mL of SACO had no immediate effect on heat production, but resulted in a 350% increase in the heat production of eiders which had preened before they were placed in the respiration chamber (Jenssen and Ekker 1990). This increased effect of oil and oil-dispersant mixtures on the day following exposure, is presumably due to preening activity by the birds during the night, whereby contamination is spread over a larger surface and deeper into the plumage. This increases the wetability of the plumage, which thus absorbs a larger volume of water, resulting in an enhanced effect on the thermoregulation of contaminated birds. The long-term thermoregulatory effect of plumage contamination therefore seems to be considerably greater than the immediate effect following the initial contact with crude oil or with chemically treated oil.

The present study also indicates that rehabilitated birds are more sensitive to subsequent plumage contamination than are normal birds. It is possible that such great susceptibility of the plumage of cleaned birds may contribute to the high mortality rates reported for rehabilitated birds (Frost *et al.* 1976; Swennen 1977).

Should Oilspills at Sea Be Treated with Chemical Dispersants in Order to Reduce Their Impact on Bird Life?

The present study was demonstrated that, as regards to their effect on the thermoregulation of eiders, the chemically treated oil was more potent than the crude oil alone. An effect was found on the thermoregulation of eiders swimming in water containing ≥10 parts per million of an oil-dispersant mixture. Even though these results were obtained in laboratory experiments, they strongly indicate that in order to minimize the impact of oilspills on birds, the concentration of the treated oil in a slick needs to be very low by the time it reaches flocks of birds residing at sea. Chemicals such as Finasol are designed to accelerate the natural dispersal rate of oil, and their action on a slick is immediate (McAuliffe *et al.* 1980). The existence of oil-dispersant emulsions on the water surface and in the water-column are (idealistically) short-lived, because the dispersant rapidly mixes with the seawater (Mackay and Wells 1983). It has been demonstrated in field trials that the concentrations of hydrocarbons present in chemically treated slicks are of a magnitude of only 100 to 0.1 mg/L, or even lower (Peakall *et al.* 1987, Lichtenthaler and Daling 1985). Thus, if the slick is effectively dispersed, it is likely that the hydrocarbon concentration in the slick will be reduced to parts per billion levels, or even lower. However, one should also note that dispersants may have a secondary effect, by increasing the surface area of the slick (Peakall *et al.* 1987). In a "worst case" scenario, chemical treatment of an oilslick may therefore increase the risk of exposure of more birds to less, but more harmful, chemically treated oil mixtures. Since the effect of oil-dispersant mixtures on the thermoregulation of seabirds is a function of the amount of the contaminant absorbed by the plumage, the effect is dependent on both the concentration of the pollutants in the water, and on the volume of contaminated water with which the birds come into contact. In the present study, the common eiders were exposed to only 25 L of contaminated sea-water. In a situation in nature, the bird would have been swimming through a much greater volume of contaminated water, and its plumage would then have absorbed a greater amount of the treated oil, and the effect on thermoregulation would be greater.

It was also shown that exposure to the same amounts of oil-dispersant mixtures had a greater effect on the common eider than on the mallard. Thus, different species of birds probably respond differently to plumage contamination. In addition, different oil-dispersant mixtures may have different effects. In order to obtain more reliable guidelines for deciding when an oilspill at sea should be chemically treated, more information about the effects on birds of such treated oil are required. However, until more data are available, one should try to prevent birds from coming into contact with chemically treated oilslicks unless the hydrocarbon concentrations are known to be very low.

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Manuscript received May 8, 1990 and in revised form August 27, 1990.

To: Barron, Mace[Barron.Mace@epa.gov]; Mandsager, Kathy[kathy.mandsager@unh.edu]; sarah.allan@noaa.gov[sarah.allan@noaa.gov]; abejarano@researchplanning.com[abejarano@researchplanning.com]; DFrenchMcCay@asascience.com[DFrenchMcCay@asascience.com]; michel.gielazyn@noaa.gov[michel.gielazyn@noaa.gov]; sharon.hook@csiro.au[sharon.hook@csiro.au]; rrhopcroft@alaska.edu[rrhopcroft@alaska.edu]; john.incardona@noaa.gov[john.incardona@noaa.gov]; Angela_Matz@fws.gov[Angela_Matz@fws.gov]; Teri.Rowles@noaa.gov[Teri.Rowles@noaa.gov]; Mathijs.Smit@shell.com[Mathijs.Smit@shell.com]; Sprenger, Mark[Sprenger.Mark@epa.gov]; rstjeerdema@ucdavis.edu[rstjeerdema@ucdavis.edu]; dana@mote.org[dana@mote.org]; john.wise@louisville.edu[john.wise@louisville.edu]; mhziccardi@ucdavis.edu[mhziccardi@ucdavis.edu]
Cc: nancy.kinner@unh.edu[nancy.kinner@unh.edu]; Gary Shigenaka - NOAA Federal[gary.shigenaka@noaa.gov]; doug.helton@noaa.gov[doug.helton@noaa.gov]; Principe, Vanessa[Principe.Vanessa@epa.gov]; Wilson, Gregory[Wilson.Gregory@epa.gov]
From: Peter Hodson
Sent: Mon 1/23/2017 10:36:01 PM
Subject: RE: State-of-Science: Eco-toxicity and Sublethal Impacts final document review
mace -hodson edits to 2017.01.09_EcoToxicity and Sublethal Impacts.docx

Hi!

I added a few comments on Mace's version (mostly re: order of paragraphs in the fish sections), plus made some small corrections to references.

Peter

Peter Hodson

School of Environmental Studies

Queen's University

Kingston, ON

K7L3N6

613 533 6129

613 539 1423 (cell)

Peter.hodson@queensu.ca

From: Barron, Mace [mailto:Barron.Mace@epa.gov]
Sent: January-21-17 1:45 PM
To: Mandsager, Kathy; sarah.allan@noaa.gov; abejarano@researchplanning.com; DFrenchMcCay@asascience.com; michel.gielazyn@noaa.gov; Peter Hodson; sharon.hook@csiro.au; rrhopcroft@alaska.edu; john.incardona@noaa.gov; Angela_Matz@fws.gov; Teri.Rowles@noaa.gov; Mathijs.Smit@shell.com; Sprenger, Mark; rstjeerdema@ucdavis.edu; dana@mote.org; john.wise@louisville.edu; mhziccardi@ucdavis.edu
Cc: nancy.kinner@unh.edu; Gary Shigenaka - NOAA Federal; doug.helton@noaa.gov; Principe, Vanessa; Wilson, Gregory
Subject: RE: State-of-Science: Eco-toxicity and Sublethal Impacts final document review

Ok, just a few comments in the attached from me.

Wondering if we can move this to manuscript phase soon?

From: Mandsager, Kathy [mailto:kathy.mandsager@unh.edu]
Sent: Friday, January 13, 2017 8:14 AM
To: sarah.allan@noaa.gov; Barron, Mace <Barron.Mace@epa.gov>; abejarano@researchplanning.com; DFrenchMcCay@asascience.com; michel.gielazyn@noaa.gov; peter.hodson@queensu.ca; sharon.hook@csiro.au; rrhopcroft@alaska.edu; john.incardona@noaa.gov; Angela_Matz@fws.gov; Teri.Rowles@noaa.gov; Mathijs.Smit@shell.com; Sprenger, Mark <Sprenger.Mark@epa.gov>; rstjeerdema@ucdavis.edu; dana@mote.org; john.wise@louisville.edu; mhziccardi@ucdavis.edu
Cc: nancy.kinner@unh.edu; Gary Shigenaka - NOAA Federal <gary.shigenaka@noaa.gov>; doug.helton@noaa.gov; Principe, Vanessa <Principe.Vanessa@epa.gov>; Wilson, Gregory <Wilson.Gregory@epa.gov>; Mandsager, Kathy <kathy.mandsager@unh.edu>
Subject: State-of-Science: Eco-toxicity and Sublethal Impacts final document review

Dear Scientific Panel,

We would very much appreciate your review of this final version of the *Eco-toxicity and Sublethal Impacts* document. This document incorporates the edits and suggestions made via email and final discussion.

If you could reply by email with your approval of this document by **Friday, January 27** (2 weeks for this review) that would be greatly appreciated. I will need each of you that participated in this discussion to reply in the affirmative that you approve this document. [The next step is for

this document to be submitted publicly for input.]

Please also confirm that your name and affiliation are listed correctly at the end of this document.

You deserve kudos for this great accomplishment! Thank you very much!

Kathy Mandsager

Coastal Response Research Center

Center for Spills and Environmental Hazards

220 Gregg Hall, 35 Colovos Rd

University of New Hampshire

Durham, NH 03824

603.862.1545

To: Mandsager, Kathy[kathy.mandsager@unh.edu]; sarah.allan@noaa.gov[sarah.allan@noaa.gov]; abejarano@researchplanning.com[abejarano@researchplanning.com]; DFrenchMcCay@asascience.com[DFrenchMcCay@asascience.com]; michel.gielazyn@noaa.gov[michel.gielazyn@noaa.gov]; peter.hodson@queensu.ca[peter.hodson@queensu.ca]; sharon.hook@csiro.au[sharon.hook@csiro.au]; rrhopcroft@alaska.edu[rrhopcroft@alaska.edu]; john.incardona@noaa.gov[john.incardona@noaa.gov]; Angela_Matz@fws.gov[Angela_Matz@fws.gov]; Teri.Rowles@noaa.gov[Teri.Rowles@noaa.gov]; Mathijs.Smit@shell.com[Mathijs.Smit@shell.com]; Sprenger, Mark[Sprenger.Mark@epa.gov]; rstjeerdema@ucdavis.edu[rstjeerdema@ucdavis.edu]; dana@mote.org[dana@mote.org]; john.wise@louisville.edu[john.wise@louisville.edu]; mhziccardi@ucdavis.edu[mhziccardi@ucdavis.edu]
Cc: nancy.kinner@unh.edu[nancy.kinner@unh.edu]; Gary Shigenaka - NOAA Federal[gary.shigenaka@noaa.gov]; doug.helton@noaa.gov[doug.helton@noaa.gov]; Principe, Vanessa[Principe.Vanessa@epa.gov]; Wilson, Gregory[Wilson.Gregory@epa.gov]
From: Barron, Mace
Sent: Sat 1/21/2017 6:44:49 PM
Subject: RE: State-of-Science: Eco-toxicity and Sublethal Impacts final document review
mace edits to 2017.01.09_EcoToxicity and Sublethal Impacts.docx

Ok, just a few comments in the attached from me.

Wondering if we can move this to manuscript phase soon?

From: Mandsager, Kathy [mailto:kathy.mandsager@unh.edu]
Sent: Friday, January 13, 2017 8:14 AM
To: sarah.allan@noaa.gov; Barron, Mace <Barron.Mace@epa.gov>; abejarano@researchplanning.com; DFrenchMcCay@asascience.com; michel.gielazyn@noaa.gov; peter.hodson@queensu.ca; sharon.hook@csiro.au; rrhopcroft@alaska.edu; john.incardona@noaa.gov; Angela_Matz@fws.gov; Teri.Rowles@noaa.gov; Mathijs.Smit@shell.com; Sprenger, Mark <Sprenger.Mark@epa.gov>; rstjeerdema@ucdavis.edu; dana@mote.org; john.wise@louisville.edu; mhziccardi@ucdavis.edu
Cc: nancy.kinner@unh.edu; Gary Shigenaka - NOAA Federal <gary.shigenaka@noaa.gov>; doug.helton@noaa.gov; Principe, Vanessa <Principe.Vanessa@epa.gov>; Wilson, Gregory <Wilson.Gregory@epa.gov>; Mandsager, Kathy <kathy.mandsager@unh.edu>
Subject: State-of-Science: Eco-toxicity and Sublethal Impacts final document review

Dear Scientific Panel,

We would very much appreciate your review of this final version of the *Eco-toxicity and Sublethal Impacts* document. This document incorporates the edits and suggestions made via email and final discussion.

If you could reply by email with your approval of this document by **Friday, January 27** (2 weeks for this review) that would be greatly appreciated. I will need each of you that participated in this discussion to reply in the affirmative that you approve this document. [The next step is for this document to be submitted publicly for input.]

Please also confirm that your name and affiliation are listed correctly at the end of this document.

You deserve kudos for this great accomplishment! Thank you very much!

Kathy Mandsager

Coastal Response Research Center

Center for Spills and Environmental Hazards

220 Gregg Hall, 35 Colovos Rd

University of New Hampshire

Durham, NH 03824

603.862.1545

To: Mandsager, Kathy[kathy.mandsager@unh.edu]
Cc: Conmy, Robyn[Conmy.Robyn@epa.gov]; fingasmerv@shaw.ca[fingasmerv@shaw.ca]; Robert Jones - NOAA Federal[robert.jones@noaa.gov]; Samantha Joye[mandyjoye@gmail.com]; ken.lee@csiro.au[ken.lee@csiro.au]; mbleigh@alaska.edu[mbleigh@alaska.edu]; karl.linden@colorado.edu[karl.linden@colorado.edu]; kmmcfarlin@alaska.edu[kmmcfarlin@alaska.edu]; msmiles@lsu.edu[msmiles@lsu.edu]; thomas.s.coolbaugh@exxonmobil.com[thomas.s.coolbaugh@exxonmobil.com]; Mathijs.Smit@shell.com[Mathijs.Smit@shell.com]; Sprenger, Mark[Sprenger.Mark@epa.gov]; Lindsey R Howard[lrw23@wildcats.unh.edu]; nancy.kinner@unh.edu[nancy.kinner@unh.edu]; Kinner, Peter[Peter.Kinner@unh.edu]; Wilson, Gregory[Wilson.Gregory@epa.gov]; Principe, Vanessa[Principe.Vanessa@epa.gov]; tracee.nguyen@csiro.au[tracee.nguyen@csiro.au]; Ian Gaudreau[ipu3@unh.edu]; doug.helton@noaa.gov[doug.helton@noaa.gov]; Gary Shigenaka - NOAA Federal[gary.shigenaka@noaa.gov]
From: Hazen, Terry C (Terry)
Sent: Thur 4/30/2015 5:31:27 PM
Subject: Re: Degradation & Fate - next meeting 4/30/2015 at 1 ET
OSAT Report_16DEC.pdf

On Apr 29, 2015, at 4:42 PM, Mandsager, Kathy <kathy.mandsager@unh.edu> wrote:

This is a reminder of our WebEx meeting tomorrow. (Login instructions below)

As you review this document, please help us in securing the accurate references. For example, we may have Su et al,., 2013 in our notes (we don't have this paper) but we are wondering if it should really be Sun et al., 2013 (we have this paper). And there may be several papers by Prince but we need to make sure the correct paper is noted accurately in the text.

Also, you might wish to search for your name in case we are looking for specific information from you.

Thank you to each of you for your input into this document!

From: Mandsager, Kathy
Sent: Wednesday, April 22, 2015 1:29 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'

Subject: RE: Degradation & Fate update -COMMENT on document - next meeting 4/30/2015

This is a reminder to submit any comments that you may have as soon as possible. Please mark your calendar for our next (potentially final) meeting for **Thursday 30 April at 1 – 3 ET**. WebEx instructions below.

Degradation & Fate Group: State-of-Science Dispersant in the Arctic
Thursday, April 30, 2015
1:00 pm | Eastern Daylight Time (New York, GMT-04:00) | 2 hrs

Join WebEx meeting

Meeting number: 732 633 609

Join by phone
1-855-244-8681 Call-in toll-free number (US/Canada)
1-650-479-3207 Call-in toll number (US/Canada)
Access code: 732 633 609
Global call-in numbers | Toll-free calling restrictions

From: Mandsager, Kathy
Sent: Monday, April 06, 2015 10:13 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu';

'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate update -COMMENT on document

My apologies – I hit send before I was done.

Dear Degradation and Fate Group

Please find attached the latest draft document for your review. We would ask that you review this very carefully and add any comments you may have to the spreadsheet that is also attached. Please especially note any items with yellow highlights, red lettering or with your name noted specifically.

And finally, this is your opportunity (as you review this whole document) to let us know if any topic has been overlooked in our group discussions thus far.

Please return your comments to me (kathy.mandsager@unh.edu) by **Friday 4/17.**

And please submit your times on the doodle poll to secure our next and final call>>
http://doodle.com/pgh4enphe24wqe24

From: Mandsager, Kathy
Sent: Monday, April 06, 2015 10:10 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'

Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET

We are nearing the conclusion! Please review the edits to this document.

And we would like to schedule a final call to discuss these comments.

Please select all times that you can possibly participate. Doodle poll here>>
http://doodle.com/pgh4enphe24wqe24

From: Mandsager, Kathy
Sent: Tuesday, March 31, 2015 11:14 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET

Additional paper for review. http://ac.els-cdn.com/S0025326X15000855/1-s2.0-S0025326X15000855-main.pdf?_tid=a972873c-d7b7-11e4-9464-00000aab0f27&acdnat=1427814635_2731a355cf42e8b64679699bc221613

From: Mandsager, Kathy
Sent: Monday, March 30, 2015 4:43 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'; Mandsager, Kathy
Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET

This is a reminder of our WebEx meeting tomorrow.

From: Mandsager, Kathy
Sent: Friday, March 20, 2015 6:19 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal';

'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET

Mark your calendar! This is to confirm our next meeting for **Tuesday, March 31** beginning at 11:00 am ET.

Her are the WebEx directions to join this meeting. Thank you!

Degradation & Fate Group
Tuesday, March 31, 2015
11:00 am | Eastern Daylight Time (New York, GMT-04:00) | 2 hrs

Join WebEx meeting

Meeting number: 734 916 289

Join by phone
1-855-244-8681 Call-in toll-free number (US/Canada)
1-650-479-3207 Call-in toll number (US/Canada)
Access code: 734 916 289
Global call-in numbers | Toll-free calling restrictions

Can't join the meeting? Contact support.

From: Mandsager, Kathy
Sent: Thursday, March 19, 2015 8:31 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION

I greatly appreciate your patience with this process. Please **cancel** the tentative hold on your calendar for tomorrow 3/20. Since we would greatly appreciate a majority of this group to participate in this 2-hour WebEx meeting, I am making another attempt at scheduling this call. Please use this doodle poll (all times noted are ET). Thank you to each of you!

Doodle poll here>> http://doodle.com/2w3gyb3gv4sv7iek

From: Mandsager, Kathy
Sent: Monday, March 16, 2015 4:39 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION

In the spirit of trying to keep everyone informed – there will NOT be a meeting tomorrow at 2 (see request below). I was unable to gain a quorum. Please continue to hold this Friday 3/20 as a potential meeting time. And if you have not yet responded with your availability, please let me know if you could possibly make in on **3/20 beginning at 1pm ET.**

Thank you.

From: Mandsager, Kathy
Sent: Monday, March 16, 2015 9:38 AM
To: 'Ian P Gaudreau'; 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION
Importance: High

While these papers are fresh on your mind, I am offering some dates as soon as possible for our next meeting. Kindly let me know if you can make yourself available for about a 2-hour timeslot at any of the following times:

Tuesday (tomorrow) 3/17 beginning at 2:00 pm ET

Friday 3/20 beginning at 12noon ET

Friday 3/20 beginning at 1:00 pm ET

Friday 3/20 beginning at 2:00 pm ET

Friday 3/20 beginning at 3:00 pm ET

Thursday 3/26 at 10:00 am ET

Monday 3/30 beginning at 2:20 pm ET

A speedy reply will be greatly appreciated. Thank you!

From: Ian P Gaudreau [mailto:ipu3@wildcats.unh.edu]

Sent: Friday, March 13, 2015 10:16 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: Lindsey R Howard; Mandsager, Kathy; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; tracee.nguyen@csiro.au; Ian Gaudreau
Subject: Degradation & Fate update - Official Papers

Hi folks,

Thank you for hanging in there on the conference call Wednesday. We really appreciate all of the hard work you have been doing. The 13 official papers you decided you would review and use as the basis for your comments (e.g. knowns, uncertainties) on the effect of chemical dispersants on oil biodegradation are:

- Venosa & Holder 2007
- Prince et al. 2013
- Prince & Butler 2014
- Mcnaughton et al. 2003
- McFarlin et al. 2014
- Mason et al. 2012
- Lindstrom & Braddock 2002
- Kleindienst et al. 2015
- Chakroborty et al. 2012
- Campo et al. 2013
- Bruheim et al. 1997
- Bruheum et al. 1999
- Baelum et al. 2012

These papers are all located in a folder on BOX entitled "Papers selected by panel - Effect of Chemical Dispersants on Oil Biodegradation". This link can be found here: https://unh.app.box.com/files/0/f/3252405678/Papers_selected_by_panel_-_Effect_of_Chemical_Dispersants_on_Oil_Biodegradation

Kathy will be contacting you to schedule what will hopefully be our final meeting of the Degradation & Fate group.

In preparation for the meeting, please re-read the paper and if you have time, send Kathy the conclusions that you derive from these references regarding what is known and uncertain about the effect of chemical dispersants on the biodegradation of oil, particularly as it applies to the Arctic.

Also, we need your feedback on a paper by Harris et al. 2002 (paper is attached in this email). Would you like us to add this paper to the "Papers selected by panel - Effect of Chemical Dispersants on Oil Biodegradation" folder on BOX? Here is the citation:

Harris, B.C. et al. 2002. Nutrient effects on the biodegradation rates of chemically-dispersed crude oil. *In* Twenty-Fifth Arctic and Marine Oilspill Program (AMOP) Technical Seminar, Nineteenth Technical Seminar on Chemical Spills (TSOCS) and Fourth Biotechnology Solutions for Spills (BIOSS): June 11 to 13, 2002, Westin Calgary Hotel, Calgary, Alberta, Canada: Proceedings. Ottawa, Ont.: Environment Canada. pp. 877-893.

Thanks again for your time and energy. I look forward to talking with you again.

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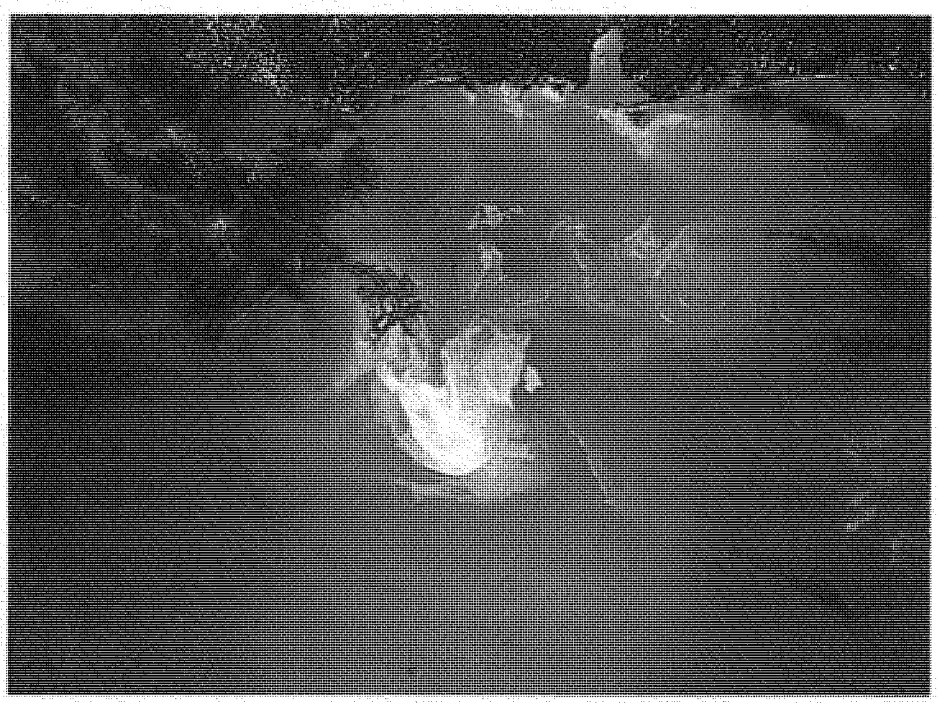
Operational Science Advisory Team (OSAT)

UNIFIED AREA COMMAND



Summary Report for Sub-Sea and Sub-Surface Oil and Dispersant Detection: Sampling and Monitoring

Prepared for

Paul F. Zukunft, RADM, U. S. Coast Guard

Federal On-Scene Coordinator

Deepwater Horizon MC252

December 17, 2010

U.S. Department of Homeland Security
United States Coast Guard

Federal On-scene Coordinator
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16461
17 Dec 2010

MEMORANDUM

From: RADM P. F. Zukunft, USCG
Unified Area Command, New Orleans

To: VADM R.C. Parker, USCG
LANT - 00

Subj: OPERATIONAL SCIENCE ADVISORY TEAM REPORT

On 18 August, 2010 I chartered the Operational Scientific Advisory Team (OSAT) to provide me with situational data and analysis for use in directing the on-going response operations. The team developed a strategy to assimilate available data as well as to identify areas where additional sampling was necessary to guide oil removal activities and assess the presence of dispersants. This sub-sea and sub-surface monitoring assessment effort was comprehensive and culminated in a rigorous set of analytical data.

These data sets were gathered and integrated based upon the 13-step Unified Area Command Strategy promulgated on 18 August 2010. The resulting assessment has guided the oil removal phase of the Deepwater Horizon spill response. This report includes assessment of data from tens of thousands of water and sediment samples taken from over 25 different ocean-going research ships on more than 125 separate cruises and representing over 850 ship-days at sea. This report focuses on information needed to guide response actions and does not draw conclusions about the long-term environmental impacts of the spilled oil. Trustee agencies will continue to study the impacts of this oil spill on the Gulf of Mexico and make assessments under other authorities. The sampling and assessment data in this OSAT report are available to support restoration and research objectives including, but not limited to, the Natural Resource Damage Assessment.

As described in the National Incident Commander's Directive of 13 August, 2010 and the Sub-Sea and Sub-Surface Monitoring Strategy dated 18 August, 2010, the goals of this data collection and analysis were to:

a. Monitor and assess the distribution, concentration, and degradation of the portion of the oil that remains in the water column and/or bottom sediments;
b. Evaluate the distribution of indicators of dispersant or break-down products of dispersants used in oil spill response activities;
c. Identify any additional response requirements that may be necessary to address any remaining sub-surface oil.

In leading the largest pollution response effort in United States history, I established decision points on which to base oil removal actions. These decision points were tied to the following indicators:

a. The presence of oil and/or dispersants. Indicators to evaluate the presence and extent of oil and/or dispersants were established for each zone.

   (1) In nearshore waters, indicators included comparisons of oil and dispersant concentrations to human health and aquatic life benchmarks, fishery closures, and qualitative indications of oil presence. In nearshore sediments, indicators included results of toxicity testing and comparisons to reference locations and pre-impact sampling.
   (2) In the offshore waters, water quality was evaluated using human health and aquatic life benchmarks and fishery closures. Offshore sediment oil and dispersant concentrations were evaluated using aquatic life benchmarks and comparisons to reference locations and pre-impact sampling.
   (3) In the deep water environments, the indicators included dissolved oxygen concentrations, comparisons to aquatic life benchmarks, and fishery closures. In deep water sediments, indicators included comparisons to aquatic life benchmarks and comparisons to sampling at reference locations.

   These indicators were chosen as the basis for the most aggressive oil and dispersant removal activities possible to return the impacted environment to pre-spill condition. Toxicity studies on water and sediment samples were conducted, and will be the subject of a separate toxicity addendum to this report which will be released in early 2011.

b. Whether oil and/or dispersants are "actionable"

   OPA 90 defines a removal action as "containment and removal of oil or a hazardous substance from water and shorelines or the taking of other actions as may be necessary to minimize or mitigate damage to the public health or welfare, including, but not limited to, fish, shellfish, wildlife and public and private property, shorelines and beaches."

c. Whether oil removal actions are feasible and consistent with net environmental benefit.

   (1) The recovery/removal/treatment action does not present an undue safety risk to response personnel.

   (2) The recovery/removal/treatment actions will not cause or increase injury to adjacent habitat or resources.

   (3) The recovery/removal/treatment action will decrease the recovery time of the threatened resource or habitat over natural attenuation of the contaminant.

Based on the robust sampling effort, the expert analysis of the data provided in this report and the decision criteria summarized above, I have determined that there is no actionable oil in the water or sediments of the deep water or offshore zones. Ongoing removal operations will continue where oil remains in nearshore sediments and shorelines.

The Coast Guard was fortunate to have had enormous assistance in this effort from public and private scientists representing the National Oceanographic and Atmospheric Administration, the United States Geological Survey, the Bureau of Ocean Energy Management Regulation and Enforcement, United States Fish and Wildlife Service, the Environmental Protection Agency and numerous academic and oceanographic research institutes and chemical laboratories.

#

Executive Summary

The purpose of this report is to provide the Federal On-Scene Coordinator for the Deepwater Horizon MC252 Spill of National Significance with sufficient information to determine the presence or absence of sub-surface oil and dispersants amenable to removal actions[1] under the provisions of the Clean Water Act, the Oil Pollution Act of 1990, and the National Oil and Hazardous Substances Pollution Contingency Plan.

Response to the Deepwater Horizon MC252 Spill of National Significance (DWH oil spill) resulted in an unprecedented amount of sampling in Gulf of Mexico Waters by multiple federal, state, and academic entities, as well as by BP (a responsible party). To transition sub-surface monitoring out of the response phase of the oil spill, the National Incident Commander (NIC) directed that the Unified Area Command (UAC) develop a comprehensive plan to implement the detection, sampling, and monitoring strategy for sub-surface oil and dispersants, as a removal action in accordance with the Clean Water Act.

The subsequent UAC plans in response to this directive established specific factors to be considered by the Federal On-Scene Coordinator (FOSC) in making the determination about when to transition offshore activities from the emergency response phase to the long term recovery and restoration phase. This decision will be based on: (1) monitoring and assessing the distribution and degradation of the portion of oil that remains in the water column, (2) evaluating the distribution of dispersant indicators used in oil spill response activities, and (3) identifying any additional response requirements that may be necessary to address remaining sub-surface oil.

The Operational Science Advisory Team (OSAT) was formed by the UAC as a small, interagency team (see Appendix H) to assess near real-time data collected by the response relative to specific indicators and to identify sampling gaps as part of an adaptive sampling strategy. This report provides an assessment of the distribution of actionable (i.e. amenable to removal actions) oil and dispersant-related chemicals that remain in the water column and/or bottom sediments and provides a summary of sampling results to inform decision makers on further oil removal operations. Where appropriate, the report also includes results from independent scientific investigations into the DWH oil spill. Assessing non-response questions, including quantitative estimates of remaining oil and the long-term environmental impacts of the DWH oil spill, are beyond the scope of this report. Additional monitoring and assessment efforts will be conducted in accordance with Natural Resources Damage Assessment (NRDA) processes.

[1] The Oil Pollution Act of 1990 (OPA 90) defines a removal action as *containment and removal of oil or a hazardous substance from water and shorelines or the taking of other actions as may be necessary to minimize or mitigate damage to the public health or welfare, including, but not limited to, fish, shellfish, wildlife, and public and private property, shorelines and beaches.*

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Specific Report Indicators

The following specific indicators were established by the UAC to define the presence or absence of potentially actionable oil:

- Qualitative observations of oil.
- Environmental risks associated with oil-related organic compounds.
- Human health risks from exposure to oil-contaminated water.
- Environmental risks related to dispersant component chemicals.
- Fishery closures.
- Toxicity to benthic invertebrates.
- Comparison of analytical chemistry measurements to reference stations and measurements from earlier in the year.
- Indicators of hypoxia in deep water seaward of the continental shelf.

A discussion of uncertainties associated with applying these indicators across the entire response dataset is presented in relevant sections throughout the document and is described in detail in Appendix C.

Key Findings

1. No deposits of liquid-phase[1] MC252 oil were identified in sediments beyond the shoreline.

2. No exceedances of EPA's Human Health benchmark were observed.

3. No exceedances of EPA's dispersant benchmarks were observed.

4. Since 3 August 2010, <1% of water samples and ~1% of sediment s amples exceeded EPA's Aquatic Life benchmarks for polycyclic aromatic hydrocarb ons (PAHs). Analysis of individual samples indicated that none of the water sample exceedances were consistent with MC252. Of the sediment exceedances, only those within 3 km of the wellhead were consistent with MC252.

5. Published research indicates that MC252 oil is weathering and biodegrading under natural conditions. Estimates of weathering and degradation rates vary, precluding the use of simple empirical models to assess the persistence of residual MC252 oil.

6. Of the previously closed fisheries, 87,481 mi^2 (state and federal) have been reopened; 1041 mi^2 around the wellhead remain closed. In addition, $4,213$ mi^2 were closed to royal red shrimping on 24 November.

[1] The presence of liquid-phase oil is highlighted here (as contrasted to oil in the form of tar mats or sediment-entrained oil) for its relevance to decisions regarding recovering oil from the environment

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7. Quantitative results indicate that deposits of drilling mud-entrained oil remain near the wellhead. Seven sediment samples within 3 km of the wellhead collected since 3 August 2010 exceed aquatic life benchmarks for PAHs, with oil concentrations of 2000-5000 parts per million.

8. The study of tar mats in shallow nearshore waters was identified as a sampling gap. The sampling methods previously used did not sufficiently address tar mats. A focused group (OSAT II) has been chartered by the UAC to address this issue.

Based on the analysis of extensive data collected in the nearshore, offshore, and deep-water zones, the OSAT concludes that sampling was adequate to address the presence or absence of sub-surface oil and dispersants (with the exception of the nearshore sampling gap identified in Key Finding 8 above).

Based on the data collected as part of this sampling effort, the OSAT identified sediments within 3 km of the wellhead as one area of concern with respect to the chronic Aquatic Life Benchmarks considered. Some sediment locations near the wellhead have concentrations of oil-related compounds that exceed the aquatic life benchmark, as well as associated quantities of drilling mud, likely from source control efforts. The decision on whether or not this oil is actionable lies with the FOSC. Long-term chronic effects to the benthic environment are being assessed as part of the injury assessment conducted through the Natural Resources Damage Assessment (NRDA) process.

Observations from qualitative programs in the nearshore sampling zone indicate tar mats are present in some sub-tidal nearshore areas. These subsurface tar mats could potentially be remobilized and become a source of shoreline re-oiling. Due to logistical constraints associated with sampling in the shallow nearshore zone, the sub-surface sampling plans did not entirely address this region. Current efforts to address this issue within the UAC include continued monitoring by shoreline assessment teams and the formation of a specialized group at the UAC (OSAT II) to evaluate existing data and make recommendations for any additional sampling.

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Section 1: Introduction

The purpose of this report is to provide the FOSC with sufficient information to determine the presence or absence of sub-surface oil and dispersants amenable to removal actions[1] under the provisions of the Clean Water Act, the Oil Pollution Act of 1990, and the National Oil and Hazardous Substances Pollution Contingency Plan.

During the Deepwater Horizon MC252 Spill of National Significance (DWH oil spill), oil and gas were discharged from the wellhead approximately 5,000 feet below the sea surface for 87 days until the well was successfully capped on 15 July 2010[2]. The last overflight observation of potentially recoverable oil on the ocean surface was made on 3 August. However, both naturally occurring physical processes and the use of dispersants as a response option led to substantial quantities of dissolved and dispersed oil in the sub-surface environment of the Gulf of Mexico ("sub-surface" refers in this report to both the water column and the bottom sediments). Of the total volume[3] approximately 1.4 million barrels were estimated to be naturally (630 k bbl) or chemically (770 k bbl) dispersed, the majority of that amount at the seafloor[4] (US Coast Guard et al., 2010). During the DWH oil spill response, a total of 1.84 million gallons of dispersants were applied both at the surface (1.06 million gallons COREXIT 9500A and COREXIT 9527) and directly at the wellhead on the seafloor (0.78 million gallons COREXIT 9500A) during the DWH oil spill response (National Commission, 2010b). This sub-surface oil and the dispersant compounds presented potential public health and ecological concerns.

Beginning in early May, sampling and monitoring operations were conducted in both surface and sub-surface environments of the Gulf of Mexico to locate any oil and/or dispersant-related chemicals from the DWH oil spill and the associated response. A multitude of state and federal agencies performed pre-impact (the period between the rig explosion and shoreline oiling) sampling and numerous extensive sampling programs continued throughout the response.

On 3 August, the National Incident Commander (NIC) directed that the Unified Area Command (UAC) develop a comprehensive sampling plan to assess the presence of actionable sub-surface oil and dispersant-related chemicals remaining in the environment (hereafter referred to as the NIC directive). In response, the Unified Area Command (UAC) implemented the largest sampling and monitoring program ever conducted during an oil-

[1] The Oil Pollution Act of 1990 (OPA 90) defines a removal action as *containment and removal of oil or a hazardous substance from water and shorelines or the taking of other actions as may be necessary to minimize or mitigate damage to the public health or welfare, including, but not limited to, fish, shellfish, wildlife, and public and private property, shorelines and beaches.* See The Implementation Plan, Section 3 for a more complete description of a removal action.

[2] All subsequent dates in this report are 2010 unless otherwise noted.

[3] The National Incident Command Flow Rate Technical Group estimated that 4.93 million barrels of oil ±10% were released from the well.

[4] Deepwater Horizon MC252 Gulf Incident Oil Budget Government Estimates through August 1, 2010 (Day 104) were generated using an application operated by the USCG and provided by the USGS in cooperation with the NOAA; see USCG, USGS and NOAA (2010).

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spill response. The details of that program were initially set forth by the Federal On-Scene Coordinator (FOSC) in "The Implementation Plan For Sub-Surface Oil and Dispersant Detection, Sampling and Monitoring," 13 September, hereafter referred to as the Implementation Plan (UAC, 2010a). This direction was further superseded by the Operational Annex for Execution and Management of Sub-Sea and Sub-Surface Detection, Sampling, and Monitoring Missions, 1 October, hereafter referred to as the Operational Annex (UAC, 2010b) and the 13 November Strategic Plan For Sub-Sea and Sub-Surface Oil And Dispersant Detection, Sampling, and Monitoring, hereafter referred to as the Strategic Plan (UAC, 2010c). These documents provide critical context to evaluate the data presented in this report. They describe (1) the overall sampling plan and rationale, (2) procedures to enable adaptive additional sampling, (3) approaches to sharing of data, and (4) the role of the Operational Science Advisory Team (OSAT) in the implementation of the sampling plan and production of this report.

During the course of the response, over 17,000 samples were collected for the purpose of environmental review (numerous other samples were collected for a diverse spectrum of applications, including waste characterization, forensic analysis etc.). Sampling and monitoring efforts initiated prior to 3 August 2010 were conducted to inform a number of response decisions. After the NIC directive of 3 August (which approximately coincided with the last visual observations of oil on the ocean surface), the response initiated a comprehensive sampling and monitoring program to locate and identify potentially actionable oil in the sub-surface environment. The transport of oil in the environment can result in highly patchy patterns of deposition to the seafloor. The sampling and monitoring program detailed in the Implementation Plan and the Annex was designed with this in mind using a statistically based approach where practicable for nearshore and offshore areas. The final samples in that program were collected on 23 October. Analytical chemistry and toxicity test results collected by multiple agencies throughout the response were published to Scribe, a data management tool developed by the US Environmental Protection Agency (EPA) for managing environmental data (Appendix B.1). The analytical chemistry data used in this report were retrieved from Scribe on 6 December. Complete results from toxicity tests conducted as part of this response were not available when this report was written. A complete report on toxicity tests will be published at a later date as an addendum to this report. The NOAA National Oceanographic Data Center (NODC) served as the official database and archive for oceanographic data (Appendices B.2, B.6). Data management protocols were established by the Sub-surface Monitoring Unit (SMU) or the data originator (Appendices B.3-B.6).

In mid-August, the Operational Science Advisory Team (OSAT) was formed by the UAC as a small, interagency team to assess near real-time data collected by the response and to identify sampling gaps as part of an adaptive sampling strategy. The team was tasked with making timely recommendations to inform response decisions. Therefore, OSAT recommendations (and this report) include some data that is being finalized and validated. In addition, in attempting to include all analytical data collected by numerous agencies during the response, inconsistencies in methodologies and data reporting led to numerous issues (not all of which were entirely resolved at the time of this report; see Appendix C). These inconsistencies do not affect the conclusions of this report.

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However, the underlying dataset should be considered preliminary and subject to future modification.

The OSAT reviewed 1) the results of the sampling and monitoring efforts conducted by the response to monitor sub-surface oil and dispersants, 2) observations from activities designed to inform other response decisions (e.g. Shoreline Cleanup Assessment Teams [SCAT]), 3) available reference data from sampling programs in existence before the DWH oil spill, and 4) results provided by independent researchers within the academic community. These data were reviewed by the OSAT in order to:

- Inform and guide the adaptive nature of the Implementation Plan (Appendix E.4).
- Make recommendations for filling sampling gaps.
- Compare results to specified indicators.
- Provide an assessment regarding the presence of oil and/or dispersants related chemicals.

This report summarizes the key data reviewed and presents the recommendations and supporting analyses provided by the OSAT to the FOSC.

The indicators used in this report were chosen specifically to assess the presence of oil and dispersant-related compounds and not for assessing long-term ecological impacts in all sub-surface environments. A significant amount of additional work will be necessary to fully evaluate the acute and chronic impacts of this incident. These investigations are currently underway as part of the Natural Resource Damage Assessment (NRDA) process. BP has also established the Gulf Coast Restoration Organization (GCRO) to manage long term response and restoration activities and the Gulf Research Institute (GRI), which will fund independent research to study the impact of the incident on the environment and/or public health in the Gulf of Mexico.

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Section 2: Explanation of Indicators

The Strategic Plan established a suite of sediment and water-quality indicators to determine whether or not oil and/or dispersant-related chemicals have been "detected." These indicators (see Table 2.1) were designed to assess impacts on public health and aquatic life in the immediate context of a removal action. The benchmarks utilized are intended to identify conditions that may pose an immediate threat to the environment; adverse effects (sub-lethal and long-term) could also occur at concentrations lower than these benchmarks.

Table 2.1. Indicators used in the evaluation of sub-surface sampling data (after Table 3.1, The Strategic Plan).

INDICATORS Observations and measurements will be compared to:			
	Nearshore (shoreline to 3 nmi)	**Offshore** (3 nmi to 200 m depth)	**Deep-water** (beyond 200-m depth)
Water	1. Public Health Taskforce Human Health Benchmark for Child Swimmer 2. Fishery closures 3. Observations using qualitative methods (VIPERS,[1] snares, sorbent pads) 4. EPA acute and chronic aquatic benchmarks for PAHs[2] 5. Aquatic benchmarks for dispersant compounds	1. EPA acute and chronic aquatic benchmarks for PAHs 2. Fishery closures 3. Aquatic benchmarks for dispersant compounds	1. Indicators of hypoxia (dissolved oxygen concentration of ≤2.0 mg/L) 2. Fishery closures 3. EPA acute and chronic aquatic benchmarks for PAHs
Sediment	1. EPA acute and chronic sediment benchmarks for PAHs 2. Observations using qualitative methods (ponar grabs) 3. Significant toxicity to benthic invertebrates 4. Concentrations measured at the same station earlier in the year 5. Average concentrations at reference stations	1. EPA acute and chronic sediment benchmarks for PAHs 2. Average concentrations at reference stations	1. EPA acute and chronic sediment benchmarks for PAHs 2. Average concentrations at reference stations

[1] Vessels with Petroleum Ensnaring and Recovery Systems

[2] polycyclic aromatic hydrocarbons

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These indicators do not represent injuries to natural resources under NRDA authorities in Oil Pollution Act of 1990 (OPA 90), which may occur at lower concentrations. The natural resource damage assessment will evaluate the nature and extent of injuries from the DWH oil spill and is still in the process of collecting and analyzing relevant data for this purpose.

The areas of the Gulf of Mexico affected by this incident were divided geographically into three zones, and each zone has specific indicators (Map 7.1). In general, sampling locations from the shoreline out to 3 nautical miles (nmi) are grouped as the "nearshore" sampling zone. Sampling locations between 3 nmi offshore and the 200 m bathymetry contour are grouped as the "offshore" zone. Sampling locations seaward of the 200 m bathymetric contour are grouped as the "deep-water" zone. However, there were some nearshore sampling programs that included stations seaward of the 3 nmi state waters boundary (the state waters boundary is 9 nmi offshore in Florida). These stations are considered as part of the nearshore sampling zone.

Comparisons of sub-surface data to indicators in this report consider both the entire sampling period (from early May onward) and the period after 3 August alone; i.e. the date of the NIC directive to assess remaining sub-surface oil and dispersant compounds in the environment. This date also coincides with the last observations of surface oil by trained aerial observers. Surface oil observed after 3 August is presumed to be due to remobilization of stranded oil or from a source other than MC252.

2.1 Fisheries Closures

During the DWH oil spill, concerns over seafood contamination with oil and dispersant compounds led to a closure of over 88,500 mi^2 in the Gulf of Mexico to fishing on 2 June 2010 (National Marine Fisheries Service [NMFS], 2010). The UAC determined that the status of these fisheries closures was one of the indicators to be used in the evaluation of sub-surface sampling data.

2.2 Concentrations Measured at the Same Station Earlier in the Year

In the nearshore sampling zone, a number of stations were sampled before oil from the DWH oil spill was transported into this zone. Many of these stations were re-sampled in September and/or October. Hence, comparison of results from these pre- and post-impact stations was included as an indicator to be used in the evaluation of sub-surface sampling data for the nearshore zone.

2.3 Reference Stations

Historical sediment chemistry data obtained from several sampling programs (Mussel Watch and MMS[1] studies) were used to provide context for observed concentrations associated with response sediment sampling results. Information from reference stations (Fig. 2.1) was used to provide historical data for comparison. These reference datasets will be discussed and referenced in the subsequent sampling zone sections.

[1] Now the Bureau of Ocean Energy Management, Regulation, and Enforcement (BOEMRE)

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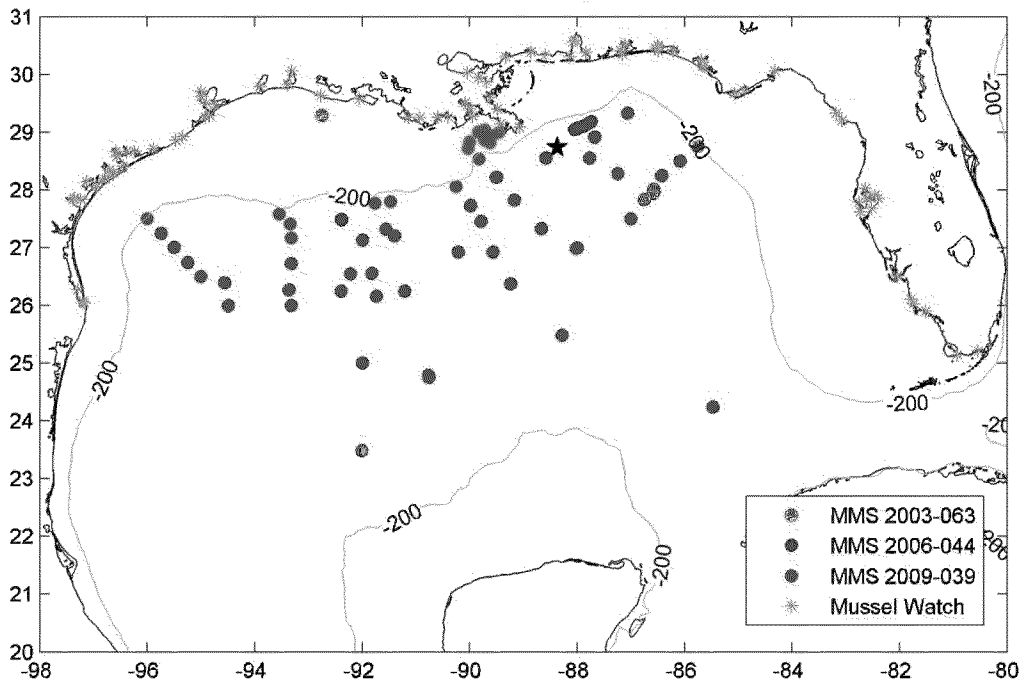
These reference concentrations do not represent "background" co nditions. Concentrations of PAHs in reference samples do not represent clean or pristine conditions, but represent average concentrations in the zone of interest before the spill. Other sources of PAHs, including seeps may be represented in these comparisons.

Figure 2.1. Location of sediment chemistry reference stations for nearshore, offshore, and deep-water zones.



2.4 Qualitative Observations of Oil

The potential presence of oil in nearshore waters was evaluated using snares and sorbent pads deployed along areas of the coast. These qualitative methods were used continuously in some regions and for periodic screening in other areas. Generally, when oil was observed using these qualitative methods, samples were collected for further chemical analyses. The comprehensive extent of these nearshore qualitative programs makes them an important indicator of potential oil contamination in that sampling zone.

2.5 Significant Toxicity

Toxicity tests were conducted on various benthic and pelagic species. Complete results of those tests were not all available at the time this report was produced. An addendum to this report presenting those results will be produced at a later date.

2.6 Human Health Benchmarks

Human health benchmarks developed by the EPA in coordination with the U.S. Department of Health and Human Services are used to assess potential human health risks from

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exposure to oil-contaminated water.[1] These benchmarks are based on potential cancer and non-cancer health risks. Where applicable, the benchmarks account for both skin contact and incidental ingestion of water by a child swimmer, assuming 90 minutes of exposure (or 1 hour per day for 90 days). The benchmark consists of screening levels for a number of compounds, including volatile organic compounds (VOC), polycyclic aromatic hydrocarbons (PAHs), and metals; see Appendix 1.

2.7 Aquatic Life Benchmarks for PAHs

PAHs are among the most toxic and persistent components of oil. PAHs can exhibit both chronic and acute toxicological effects, and several are suspected carcinogens. Accordingly, aquatic life benchmarks for PAHs were selected by the HVAC as "indicators" to be monitored.

The EPA established benchmark levels of concern for PAHs in water and sediment to screen for potential adverse impacts to aquatic life.[2,3] For these benchmarks, a total of 41 oil-related organic compounds are assessed jointly through a mixture approach because they have a cumulative effect on aquatic organisms. These compounds include 7 volatile organic compounds, 16 parent PAHs and 18 alkylated homologues of the parent PAHs. The individual compounds are given potency divisors, which are used in calculating the cumulative toxicity of the mixture of compounds in each sample – hereafter referred to as the acute or chronic aquatic life ratio. Aquatic life benchmarks are established based on a suite of laboratory toxicity evaluations using contaminated water and various species, life stages, endpoints, and exposure durations. Sediment benchmarks are based on equilibrium partitioning theory to calculate dissolved concentrations in interstitial pore water and compare them to the same thresholds intended to protect organisms from water column exposures. The amount of total organic carbon (TOC) in the sediment is factored into the calculation because organic matter binds PAHs reducing their availability. To assess the potential hazard to aquatic organisms, the aquatic life ratio is compared to a hazard index of 1. A value >1 indicates that the sample exceeds concentrations calculated to protect aquatic organisms from acute or chronic effects. Appendix A.3 describes the calculation in more detail and includes a complete list of these compounds with their divisors.

Some samples were analyzed only for the parent PAH compounds (this will be noted where applicable in the following sampling sections). When evaluating a sample in which the alkylated PAHs were not measured, it is necessary to compensate for their contribution by using "alkylation multipliers" (Appendix A.3). See Appendix C.3 for a complete discussion of the development of the multipliers and the uncertainty associated with their use with regard to the response sub-surface data.

In addition, some samples collected early in the response were analyzed for only all 16 parent PAHs. These samples are included in this report but the determination of the

[1] http://www.epa.gov/bpspill/health-benchmarks.html
[2] http://www.epa.gov/bpspill/water-benchmarks.html
[3] http://www.epa.gov/bpspill/sediment-benchmarks.html

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human health benchmark or if they exceeded the aquatic life benchmark. Samples with less than 16 analytes and did not exceed the benchmark, were not included in the total count of non-exceedances for the aquatic life benchmark.

Samples that exceeded EPA aquatic life benchmarks were reviewed to assess the likelihood that contamination resulted from MC252 oil. This was necessary since there are numerous other potential sources for oil related compounds within the Gulf of Mexico. For example, the National Research Council (2003) estimated that 140,000±60,000 tonnes (approximately one million barrels) of petroleum hydrocarbons enter the Gulf of Mexico from natural seeps each year. On an average annual basis the Gulf also receives an additional 10,400 tonnes (approximately 74,000 barrels) of oil spilled from oil production operations, transportation accidents such as tanker leaks, and other sources such as unburned fuel through engines. A three-year study of the source of PAH compounds detected along the Louisiana coast determined that approximately 50% of sources were from petrogenic (crude oil) sources, 36% were from pyrogenic (combustion or engine exhaust) sources, and 14% were from biogenic or diagenetic sources (Iqbal et al., 2008). To assess the source of the oil related compounds in exceedance samples, the OSAT followed methods described by Bence, et al. (1993), Boehm (2006), Overton et al. (2004), Page et al. (1995), and Wang (1999). Appendix D details the methodology for this assessment. If enough information was available, a determination was made that the sample was "consistent" or "inconsistent" with MC252 oil. If examination of the sample yielded insufficient data to make the determination, the sample was included as "consistent" in all plots and tabulations of exceedances, i.e. no distinction was made between samples consistent with MC252 and indeterminate samples.

2.8 Dispersant Benchmarks

A total of 1.84 million gallons of dispersants were applied both at the surface (1.06 million gallons, primarily COREXIT 9500A and some 9527) and directly at the wellhead on the seafloor (0.78 million gallons COREXIT 9500A) during the DWH oil spill response (National Commission, 2010b).

These dispersants break down rapidly in seawater; therefore, water samples were analyzed for individual dispersant constituents. Benchmarks (based on dissolved seawater concentrations) for the individual compounds are used to explain the relevance of measured concentrations of individual compounds, i.e., concentrations above benchmark levels are "levels of concern." These benchmarks are based on available biological effects data and are conservatively designed to protect aquatic life (see Appendix A). Four dispersant constituents were analyzed during the DWH oil spill response:

- 2-Butoxyethanol
- Dipropylene Glycol n-Butyl Ether (DPnB)
- Propylene Glycol
- Dioctylsulfosuccinate (DOSS)

These compounds represent major constituents of COREXIT, including those with known toxicology data, and also those with newly established analytical methods. Each of these

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compounds is also subject to wide commercial use in other products beyond COREXIT. If a benchmark was exceeded, then the potential for other sources of the chemical were also evaluated.

2.9 Hypoxia

Dissolved oxygen was measured in the deepwater zone as part of the sub-surface sampling program beginning in early May. Concern was raised early in the spill that biodegradation of hydrocarbons in the deep dispersed plume would consume dissolved oxygen, potentially decreasing concentrations to levels that could threaten aquatic life (i.e., hypoxia). Hypoxic conditions are generally agreed to occur when dissolved oxygen falls below 2 mg/L (1.4 ml/L).

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Section 3: Nearshore Sampling Results

3.1 Description of Sampling and Monitoring Activities in the Nearshore Zone

The nearshore sampling zone is defined as waters inshore of the 3-nmi state waters boundary (Strategic Plan). Qualitative and quantitative data are analyzed to evaluate the distribution and concentration of 1) any remaining oil and oil-related compounds, 2) dispersant-related chemicals, and 3) any associated by-products in this zone.

A number of qualitative observational programs were designed to identify and delineate sub-surface oil in nearshore areas. Water column detection tactics included snare sentinels, sorbent pads, VIPERS (Vessel with Intrinsic Petroleum Ensnaring and Recovery Systems), and snare drag trawls. The snare sentinel and sorbent pad drop programs provided observations at discrete locations at multiple water column depths. Conversely, the VIPERS and snare drag trawl programs were dynamic, and the observations were reported along a cumulative trawl distance. Ponar [1] samples were used to qualitatively assess the presence or absence of oil in sediment samples. When potential oil was encountered using these investigative methods, samples were collected for further laboratory chemical analysis. These observational programs are described in more detail in the Strategic Plan and in Section 3.4.

Water and sediment samples were collected (Maps 7.2a/b; Fig. 3) in the nearshore zone at multiple sites for quantitative analysis of oil and oil-related compounds, dispersants, or by-products. The main nearshore sampling efforts were conducted by the EPA, the U.S. Geological Survey (USGS), and the Center for Toxicology and Environmental Health (CTEH; a BP contractor), with additional samples provided by other federal and state agencies. The nearshore sampling plan was designed to determine if the spill had contaminated the sediments and surface waters with oil-related products and/or dispersant-related chemicals. A full description of the derivation of the sampling plan can be found in the Strategic Plan (or see Appendix E.4 for a brief description).

[1] A sediment grab sampler

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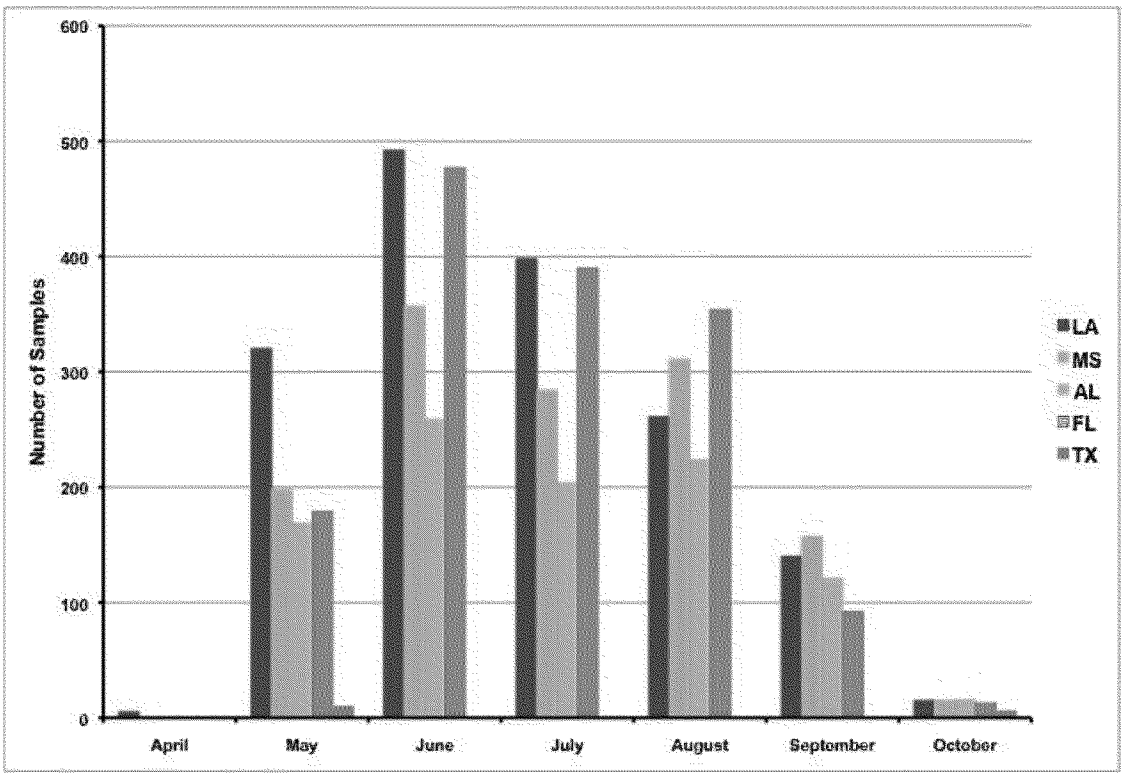
Figure 3.1. Total number of nearshore water samples collected between 30 April and 18 October, plotted by state and month.



3.2 Fisheries Closures

Fishery closures and re-openings in the nearshore zone are controlled by state agencies. States relied on federal and state testing and FDA recommendations to determine whether fisheries should re-open.

Florida[1] – On 16 June, Florida Fish and Wildlife Commission (FL-FWC) closed all fisheries (except oysters) located between the FL-AL state line and Pensacola Beach as a precautionary response to the DWH oil spill. FL-FWC re-opened the finfish fishery on 31 July, the shrimp fishery on 17 August, and all remaining fisheries on 15 September.

Alabama[2] – Alabama Marine Resource Division (AL-MRD) of the Alabama Department of Conservation and Natural Resources (AL-DCNR) closed both recreational and commercial fisheries when visible oil moved into coastal waters within the nearshore zone. On 1 June, state waters south of Dauphin Island between Mobile Pass and the AL-MS state line out to 3 nmi were closed. On 4 June, waters east to the AL-FL state line were also closed. Mississippi Sound waters were closed between Dauphin Island Bridge and AL-MS state line on 10 June. As of 6 September, all commercial and recreational fisheries in AL state waters were re-opened with the exception of public oyster reefs, which were closed for rehabilitation purposes.

[1] http://myfwc.com/OilSpill/index.htm

[2] http://www.dcnr.state.al.us/fishing/saltwater/dh/Timeline.cfm

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Mississippi[1] – Mississippi Department of Marine Resources (MDMR) closed both recreational and commercial fisheries when visible oil moved into coastal waters within the nearshore zone. On 1 June, MDMR closed all recreational and commercial fisheries in state waters in Mississippi Sound as a pre-cautionary measure. By 1 July, MDMR had closed all state coastal waters to both recreational and commercial fisheries. On 30 July, MDMR re-opened all intra-coastal waters to recreational and commercial finfishing and shrimping. By 21 August, all finfish, shrimp, and blue crab fisheries were re-opened. FDA declared oyster tissue from Mississippi state waters safe to eat on 25 August. MDMR opened oyster beds to harvesting in select waters on 8 and 20 November.

Louisiana[2] – Louisiana Department of Wildlife and Fisheries (LA-DWF) began closing fisheries on the 28 – 29 April as precautionary measures to prevent the harvesting of oil-tainted or oiled finfish, shrimp, or crabs. The delineation of fisheries closures in LA was complex due to the geomorphology of the coastline and the numerous back-barrier and open-water bays. Many of the fisheries were closed between May and August. Re-openings also occurred during this period; detailed time-series maps of opening and closures are provided at the LA-DWF website. As of 8 November, 98.5% of state waters have been reopened for commercial fishing.

3.3 Reference Stations and Pre-Impact Data

The OSAT reviewed sample data from the Mussel Watch program to establish reference conditions for oil-related compounds that may have been present prior to the MC252 spill. Since 1986, NOAA's Mussel Watch program has consistently studied and monitored 300 sites around the country for over 100 organic and inorganic pollutants in sediments, oysters, and mussels. The Mussel Watch dataset that the OSAT used for the Gulf coast included data from 1989 - 2007 at 98 unique locations (Fig. 2.1). Only 1 of the 163 total Mussel Watch sediment samples considered exceeded the chronic aquatic life benchmark. This sample was collected offshore of Pensacola and had a classic pyrogenic signature (Appendix D). The chronic aquatic life ratios for Mussel Watch ranged from 1.4×10^{-5} to 1.26, with an average ratio of 0.020.

Pre-impact sediment samples were collected in the nearshore zone at multiple locations before landfall of oil associated with the DWH oil spill. Pre-impact samples refer to samples collected before 15 May for LA and 1 June for AL/MS/FL. These dates approximate the point in time when visible oil reached the shoreline in these different areas. A total of 239 pre-impact sediment samples were collected in April and May, of which 96 samples had detected oil-related organic compounds. The chronic aquatic life ratios for pre-impact data ranged from 0 to 8.5, with an average of 0.13 across all samples. For comparison to post-impact data, an average chronic aquatic life ratio equal to 0.084 is used, which was computed from all Mussel Watch and pre-impact data for the Gulf coast.

[1] http://www.dmr.state.ms.us/

[2] http://www.wlf.louisiana.gov/oilspill/actions

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3.4 Qualitative Observations of Oil

Various qualitative methods were used to determine if oil was present in the sub-surface environment. These activities were conducted by the two primary Incident Command Posts (ICPs) in Mobile, AL and Houma, LA; the two ICPs had areas of responsibility (AORs). With respect to the nearshore sampling zone, the Mobile ICP conducted qualitative sampling programs in MS, AL and FL, whereas the Houma ICP conducted programs in LA.

Several qualitative programs were designed to detect the presence of oil in the water column. In the Mobile AOR, VIPERS trawled 266 nmi along the 3 nmi state waters boundary from Cat Island, Mississippi, to Bay County, Florida between 22 August and 9 September. Only one small tar ball was recovered near Panama City, FL, on 24 August and source characterization analysis indicated it was inconsistent with MC252 (Appendix D).

Sorbent probes were also utilized at 5,880 sites in MS, AL and FL to identify potential oil in the water column. Potential oil presence was noted at six sites in the MS Sound. Follow-up (analytical) water samples collected in these areas did not detect oil. Oil was observed on 4 poms from a total of 108 snare drag trawls along 164 nmi in Breton Sound on 14–15 August. The 4 poms were collected from the same general area, and analytical results indicated weathered MC252 oil equivalent to a total of 10 mg/L of oil. No oil was detected during snare drag trawls conducted in Barataria Bay on 18–30 August.

Between 25 August and 12 September, 148 snare sentinels were deployed along the shorelines of Mississippi, Alabama, and Florida; 16 snares were reported as "very lightly oiled". The snares along with 36 water samples and 5 sediment samples collected near these snares were submitted for analysis. Forensic fingerprinting results from all 16 snare samples were interpreted as "unlikely Macondo (MC252 oil) derived" (Snare Sentinel Summary Report, 25 September). Analyses of Oil Range Organics (OROs), Volatile Organic Compounds (VOCs), Semi-Volatile Organic Compounds (SVOCs), Nickel, and dispersant indicators in water samples were below minimum detection limits. Diesel Range Organics (DROs) were detected in 2 of 36 water samples at concentrations ranging from 49.7 to 56.0 µg/L. Vanadium was present in 31 of 36 water samples at a maximum concentration of 76 µg/L, well below the 5400 µg/L USEPA Human Health Benchmark for a Child Swimmer. Fingerprint analyses determined that 1 of the 5 sediment samples was identified as MC252 oil. The remaining four sediment samples were determined to be "unlikely Macondo (MC252 oil) derived." The Mobile ICP Operations Unit concluded that no recoverable amounts of oil were identified using this procedure.

Within the Houma AOR, 449 locations were occupied using a similar snare sentinel program between 28 July and 2 October (UAC Snare Summary Report, 30 November). Sites spanned the nearshore region from Caillou Bay east to Chandeleur Sound. Oiled surface snares were recovered from four regions: Timbalier Bay, Barataria Bay, Chandeleur Sound and off the Mississippi River Delta. Oiled bottom snares were recovered from Barataria Bay and Timbalier Bay. Out of 6,911 observations, 40 snare sentinel at 32 unique locations were reported as oiled (26 top, 12 middle and 21 bottom). The most recent surface detection was 31 July in Barataria Bay, and the most recent sub-surface detection was 20 September in Barataria Bay. All observations of sub-surface oiling were located off

previously oiled shorelines. Based on these data, the OSAT inferred that future detection of sub-surface MC252 oil by Snare Sentinels would most likely be due to remobilization of oil already present in the nearshore environment (i.e., in known regions of heavily oiled shoreline), and on 18 October recommended phasing out the Louisiana Snare Sentinel program (Appendix F.10).

To assess oil presence in bottom sediments, samples were collected at 2066 sites using a stainless steel Ponar grab sampler in the Mobile AOR. The material was visually inspected for the presence of oil. If oil presence was suspected, then a representative sample was sent for laboratory analysis. Based on visual inspection, 32 sediment samples were sent for laboratory analysis. Three samples collected on the back side of barrier islands near Pensacola, FL contained detectible oil concentrations.

The three sites confirmed to have oil present in sediments by Ponar surveys, are consistent with one of the regions identified by SCAT teams as potentially having submerged tar mats. Early on during the response, the possible formation of tar mats (i.e. submerged sedimented oil) in the nearshore region was recognized (see NOAA-ORR, 2010 for a description of how they form). The OSAT noted that a data gap may exist since the assessment programs were generally unable to monitor in waters shallower than ~10 feet. Submerged tar mats have been identified in some areas from the lower intertidal zone out to the super low tide zone by SCAT teams. Tar mat locations have not been directly observed by SCAT teams using snorkeling equipment. Location of tar mats have also been inferred from observations of shoreline tar ball re-oiling. Currently, SCAT teams are monitoring these known sites and operational clean-ups will occur if shoreline re-oiling is observed.

3.5 Comparison to Human Health Benchmarks

A total of 6090 water samples were considered for comparison to the Human Health Benchmarks. (Table 3.1, Map 7.6). None of samples exceeded the EPA benchmark for human health (child swimmer scenario).

3.6 Comparison to Aquatic Life Benchmarks

Chronic and acute aquatic life ratios were calculated for all samples (water and sediment) from the nearshore zone in which PAH compounds were analyzed. Of 6701 samples, the full suite of analytes was quantified for 511 samples (7.6%). In most of the samples only the parent PAHs were quantified. The OSAT recognized this issue soon after they began reviewing the dataset and made a recommendation that all future samples be analyzed for the complete set of analytes (20 September, Appendix E). Therefore, many of the more recent samples were analyzed for the full suite of PAHs. These samples are the most essential for determining post-impact environmental conditions. However, where only the 16 parent PAHs were reported, mulitpliers were used to account for the missing alkylated PAHs (Appendices A.3, C.3).

A total of 41 nearshore water benchmark exceedances were observed throughout the incident (Maps 7.4a/b. Fewer benchmark exceedances were observed in EPA Region 6 (LA and TX; Fig. 3.2) than in EPA Region 4 (AL, MS, and FL; Fig. 3.3). Of these exceedances, 13

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samples were of indeterminate origin, 19 were considered not co nsistent with MC 252 oil, and 9 were deemed consistent with MC252. No water benchmark exceedances measured after 3 August were consistent with MC252 oil (Table 3.2).

Figure 3.2. Temporal distribution of chronic aquatic life ratios for PAHs in Region 6 (LA and TX) nearshore water samples. Red circles indicate samples that exceeded the chronic aquatic life benchmark and yellow circles indicate exceedance samples that were not consistent with MC252 oil. The blue dotted line corresponds to the aquatic life benchmark of 1. The date tick mark in all figures corresponds to the first of the month.

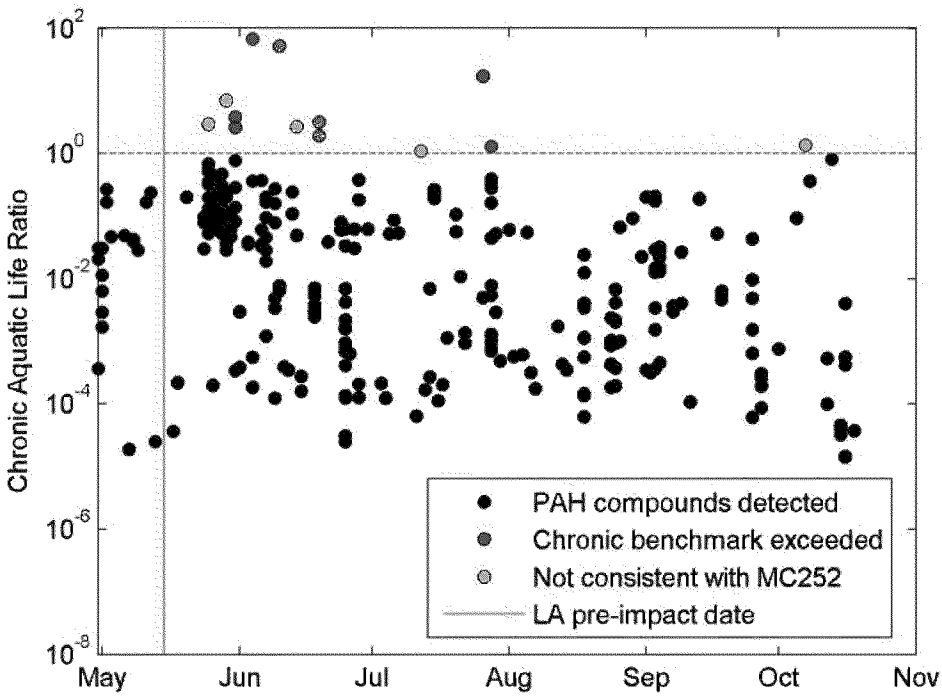
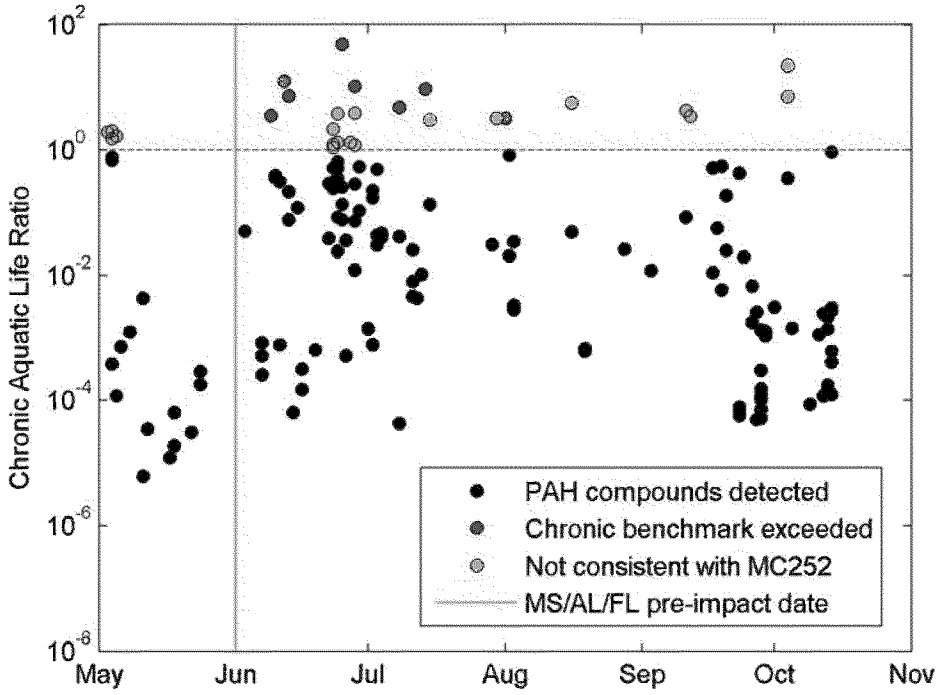


Figure 3.3. Temporal distribution of chronic aquatic life ratios for PAHs in Region 4 (AL, MS, and FL) nearshore water samples. Symbols are the same as in Fig. 3.2.



A total of 24 nearshore sediment benchmark exceedances were observed throughout the incident (Maps 7.5a/b). As with water, fewer sediment benchmark exceedances were observed in EPA Region 6 (TX and LA; Fig. 3.4) than in EPA Region 4 (AL, MS, and FL; Fig. 3.5). In EPA Region 4, some of the samples indicating pre-impact sediment benchmark exceedances did not have enough information to determine oil source since only the 16 parent PAHs were measured. In the absence of additional information, these were included as MC252 exceedances, although they occurred before MC252 oil was observed to have been present in the nearshore zone in this region (red dots prior to 1 June in Fig. 3.5). Of the total exceedances, 9 samples were of indeterminate origin, 11 were considered not consistent with MC252, and 4 were consistent with MC252. All exceedances after 3 August were inconsistent with MC252 oil (Table 3.2).

Chronic aquatic life ratios for sediments were also compared to the average ratio for pre-impact and reference samples. This average ratio, which will be referred to here as the "reference ratio", equaled 0.084 (Section 3.3). After 3 August, all sediment samples collected in both Regions 4 and 6 (Figs. 3.4 and 3.5) had ratios that were, on average, below the reference ratio. By the end of sampling in October, all sediment samples were below the reference ratio, indicating that pre-impact average concentrations had been reached.

Figure 3.4. Temporal distribution of chronic aquatic life ratios for PAHs in Region 6 (LA and TX) nearshore sediment samples. Symbols are the same as in Fig. 3.2. The green line corresponds to the average chronic aquatic life ratio in reference and pre-impact samples.

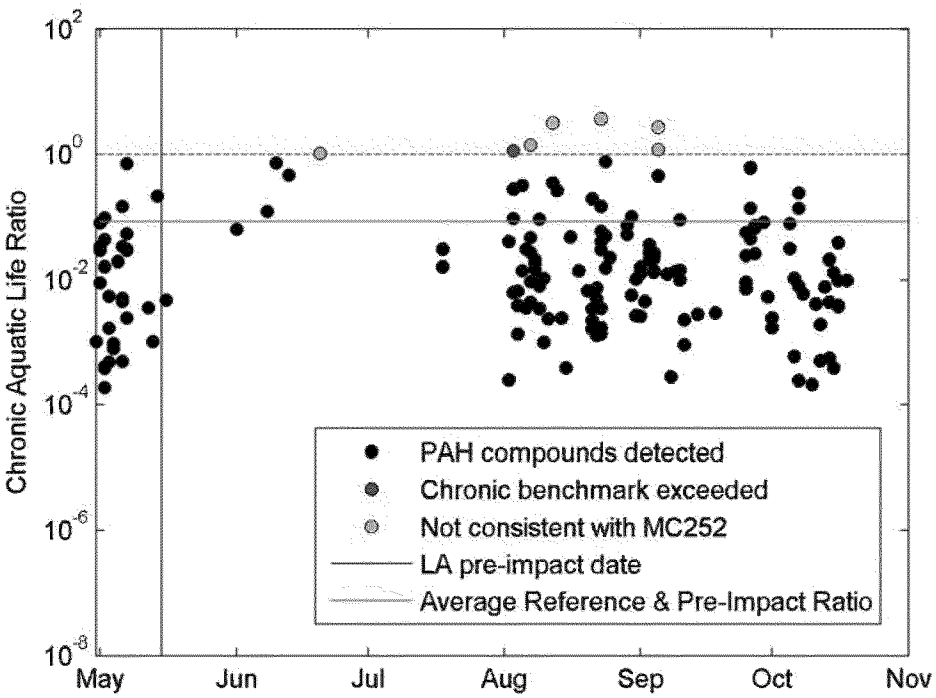


Figure 3.5. Temporal distribution of chronic aquatic life ratios for PAHs in Region 4 (AL, MS, and FL) nearshore sediment samples. Symbols are the same as in Fig. 3.2. The green line corresponds to the average chronic aquatic life ratio in reference and pre-impact samples.

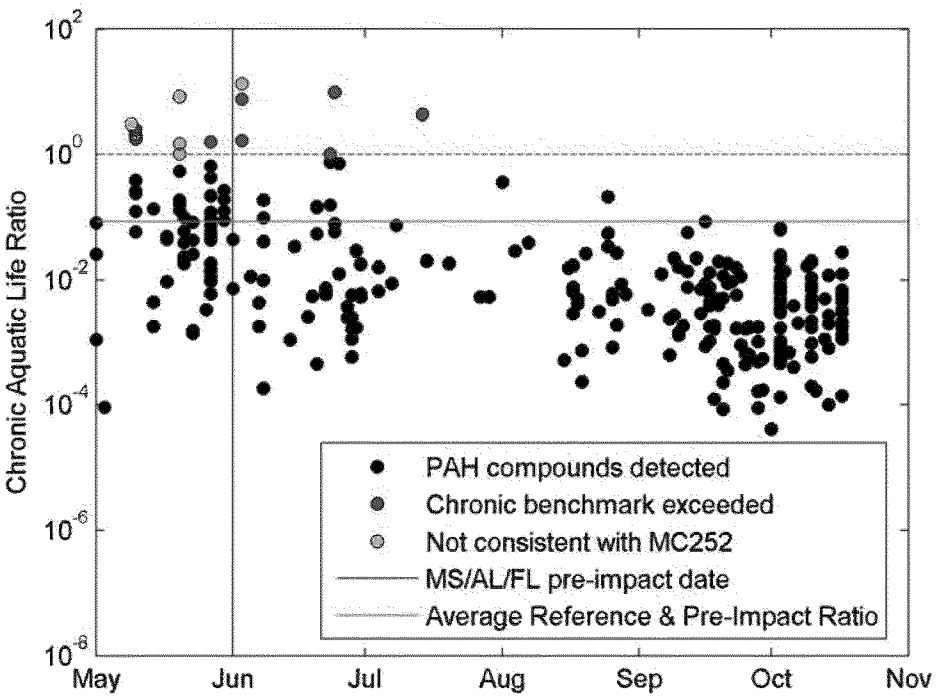


Table 3.1. Summary of water and sediment analytical chemistry results in the nearshore zone. Datasets were compared to human health benchmarks, aquatic life benchmarks, and dispersant screening levels. For each benchmark (or screening level), total numbers of samples that were non-detect, below benchmark, or exceeded the benchmark were computed. The total number of samples is not the same for the human health, aquatic life, or dispersant categories because not all samples were analyzed for all analytes.

Sample Type	Indicator Level	Human Health	Aquatic Life	Dispersant
Water Column	Non-detect	3405	5337	4790
	Below Benchmark	2685	395	60
	Exceed Benchmark	**0(0)***	**41(22)***	**0**
Sediment	Non-detect	n/a	671	406
	Below Benchmark	n/a	441	6
	Exceed Benchmark	**n/a**	**24(13)***	**n/a**
Total Samples		**6090**	**6909**	**5262**
Total Exceedances		**0**	**65(35)**	**0**
Percent of Exceedances		**0%**	**0.94%**	**0%**
Total Exceedances After 3 August		**0**	**11(0)**	**0**

*The number in parentheses refers to exceedances consistent with MC252 oil or indeterminate.

Source characterization of exceedances

Any water or sediment sample that exceeded the aquatic life benchmark was examined in detail to assess the likelihood that the exceedance was due to MC252 oil (Table 3.2; see Appendix D for source characterization process). The samples that were not consistent with MC252 were from pyrogenic sources (combustion related).

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Table 3.2. List of aquatic life benchmark exceedances for water and sediments in the nearshore zone after 3 August. Details are listed for each exceedance including the source of the oil, identified as follows: (1) consistent with MC252 oil, (2) not consistent with MC252 oil, and (3) indeterminate. Highlighted rows indicate sediment samples; non-highlighted rows indicate water samples.

Sample Date	Type of Exceedance	Sample Location		Sample ID	Source
		Lat	Long		
8/7/10	Chronic	29.41648	-90.04369	T007-1331-100807-SD-1	2
8/12/10	Chronic	29.65621	-92.82204	T008-0029-100812-SD-1	2
8/16/10	Acute	29.68642	-84.78892	BCH11-SW-201008	2
8/23/10	Chronic	29.31747	-94.79753	SL-20100823-SR3-002	2
9/5/10	Chronic	29.72568	-88.98950	T001-R678-100905-SD-1	2
9/5/10	Chronic	29.87817	-88.88842	T001-R680-100905-SD-1	2
9/11/10	Acute	30.37534	-88.60680	WPR1-SW-09112010	2
9/12/10	Chronic	30.60264	-87.94630	UpMB-SW	2
10/4/10	Acute	30.31314	-86.12028	SBFL1004SW0103	2
10/4/10	Acute	30.32568	-87.17510	PEL1004SW0103	2
10/7/10	Chronic	28.99191	-89.14442	SW-20101007-USGSLA1-LA-35-003_LLI	2

*Source = "consistent with MC252 oil (1), "not consistent with MC252 oil" (2), "indeterminate" (3)

3.8 Comparison to Dispersant Benchmarks

While dispersants were not used in the nearshore sampling zone as part of the response, there were concerns that dispersant-related chemicals could be transported into the nearshore zone. Sediment and water samples collected in the nearshore zone were analyzed for a number of dispersant-related chemicals, including, but not limited to DPnB, Propylene glycol, and DOSS (Appendix A.1). Between 13 May and 20 October, 4850 water and 412 sediment samples were collected in the nearshore zone (Table 3.1). None of the concentrations of dispersant-related chemicals found in water samples collected during the response exceeded the benchmarks. Only 66 samples (60 water and 6 sediment) had detectable levels of dispersant- related chemicals. DPnB was the most common detectable dispersant-compound and was found in 57 of the 60 water samples; however, concentrations never exceeded 3 □g/L (cf. EPA screening level 1 mg/L). Presence of dispersant-related chemicals in water occurred all along the Gulf coast; however, a majority of the nearshore detects were encountered around LA (Map 7.7). The most recent detect for the water samples was 18 October. Propylene glycol was the only dispersant-related chemical detected in the sediments (Map 7.8). Unfortunately, no benchmark for dispersant indicator compounds in sediment exists, thus the significance of these concentrations is unknown. The most recent detect for sediments occurred in a sample collected on 28 September.

Section 4: Offshore Sampling Results

4.1 Description of Sampling and Monitoring Activities in the Offshore Zone

The offshore sampling zone is defined as the geographic region between the state water's boundary and the 200 m bathymetric contour, which delineates the continental shelf break (Map 7.1). This region was identified as a sampling gap (particularly for sediments) in the Strategic Plan: prior sub-surface sampling had primarily been conducted in the deep-water and nearshore zones.

This zone was targeted for sampling by two main programs (see Map 7.3 for stations with analytical data available for this report). The first sampling program, the Fate of Oil Research Team (FORT) conducted water sampling (CTD rosette) and sediment sampling (grab sampler) on 15 cross-shelf transects from east of the Mississippi River Delta (offshore of Breton Sound) west to Port Arthur, TX (along one of the transects increased west of Houma, LA. FORT resulted in 240 water samples and nearly 60 sediment samples from late August through mid-October.

The second sampling program was the offshore component of the response's sub-surface monitoring program (Operational Annex). This sampling program collected 93 sediment samples. Water sampling at discrete depths was co-located with two of the sediment sites (40 water samples were taken). The sampling strategy identified sampling locations with the highest potential for oil to be found in sediments based on the intersection with the freshwater convergence zone of the Mississippi River, the locations of aerial dispersant applications, and the movement of oil based on currents. Once targeted sample locations were determined, additional statistically based samples were added to ensure statistical rigor (see the Strategic Plan and Operational Annex). Most of the sediment samples were taken with a multi corer. Shallow areas and areas with sand or shell bottom were sampled with a sediment grab or a box corer.

In addition to the response sampling, NOAA also provided samples for analysis that were collected during a cruise for "The Regional Assessment of Ecosystem Condition and Stressor Impacts" along the Northeastern Gulf of Mexico Shelf on the NOAA Ship R/V *Nancy Foster* (August 12–22). This cruise sampled for a variety of parameters, but only the data applicable to the response were analyzed. Included in this dataset were 50 sediment samples taken during the cruise.

At the time of this report approximately 700 water and 250 sediment samples in the offshore sampling zone had been analyzed and published (see Table 4.1 for the sample count breakdown by indicator; Map 7.3 shows the geographic coverage). The distribution of the water sampling with depth is detailed below (Fig 4.1). Most of the sampling was in the upper 50 m of the water column and occurred after 3 August, i.e. as a component of the comprehensive sub-surface sampling efforts stemming from the NIC directive.

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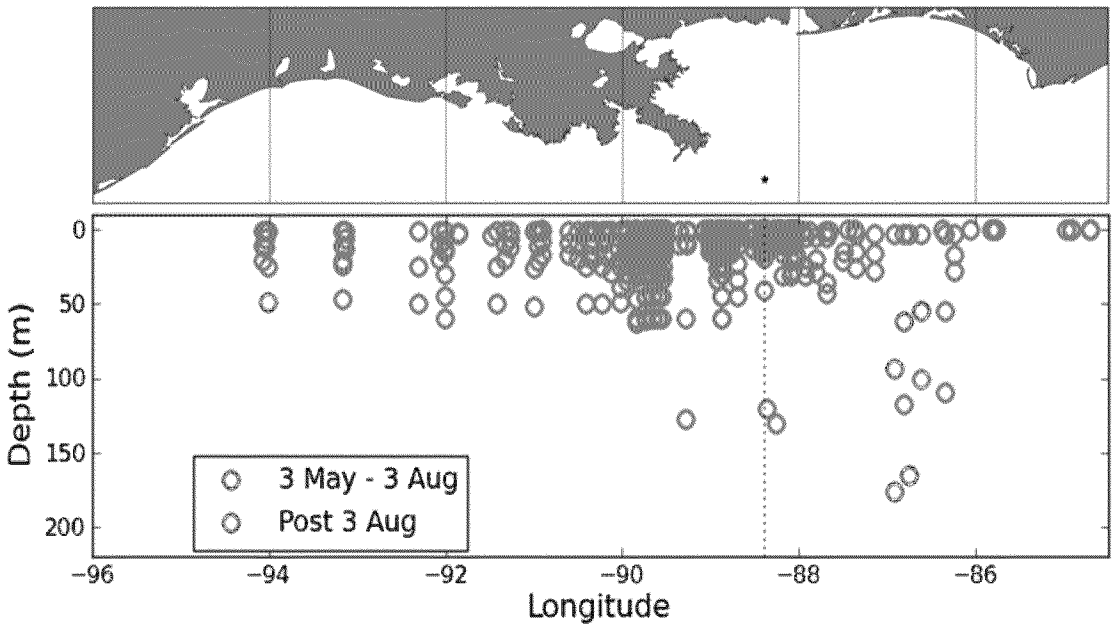
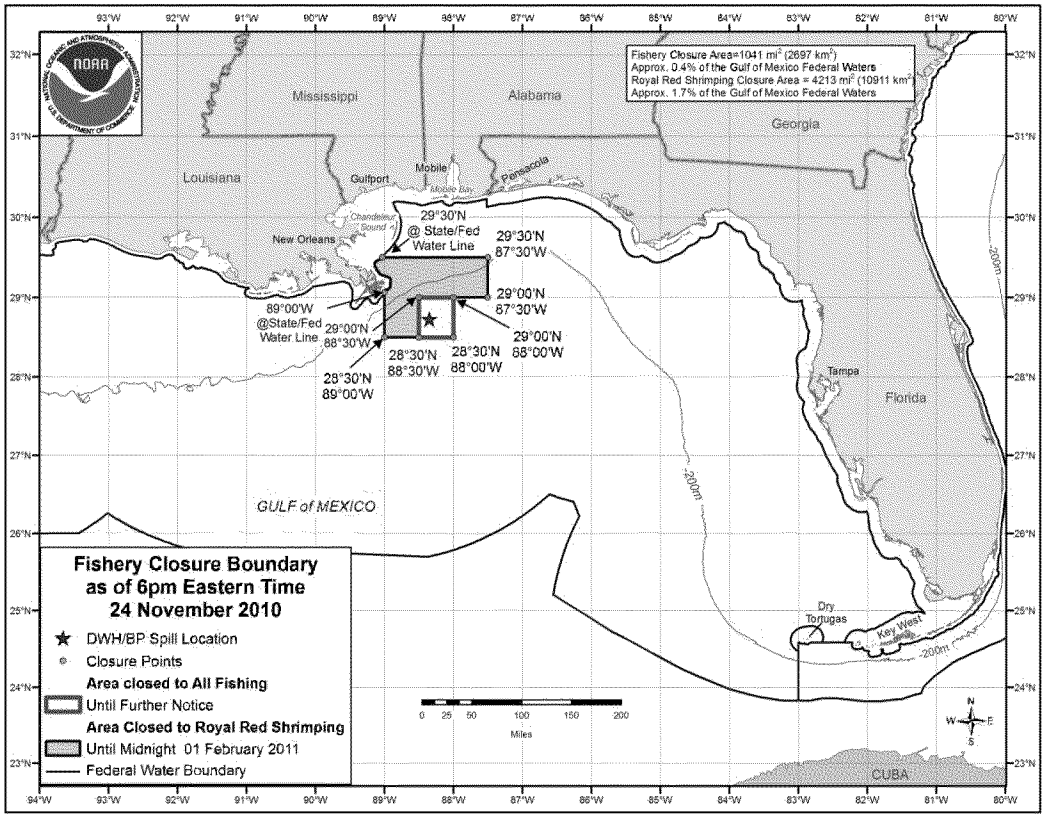
Figure 4.1 Offshore water sample locations as a function of depth. The black dot in the top panel denotes the wellhead location.



4.2 Fisheries Closures

At its peak, 37% of Gulf waters (88,522 mi ^{2}) were closed to fishing on 2 June due to the DWH oil spill. Prior to re-opening, fish samples in all areas underwent sensory testing and analysis for PAH chemical contaminants. After an extensive consultation with the FDA, NOAA re-opened approximately 26,388 mi^2 on 22 July at the southern extent of the federal closed area (Fig. 4.2). Additional shelf areas were re-opened in August and early September, extending from western Louisiana through the Florida panhandle. On 21 September, NOAA re-opened 7,970 mi^2 of Gulf waters to recreational and commerc fishing along the southern boundary of the federal closed area. Most remaining areas were re-opened during October and November as per the order shown in Fig. 5.2. However, on 24 November one of the re-opened areas was closed again when tarballs were found in nets with Royal Red Shrimp. Currently, 4,213 mi ^{2} remain closed to Royal Red shrimping in an area covering portions of the OSAT's offshore and deep zones. Several vessels have been investigating this region further, performing trawls of the seafloor. Findings thus far have been of a few tarballs from several of the gallons, less than 2 inches in diameter. Tarballs were described as not-sticky (in that they did not adhere to the nets). The origin of these tarballs has not yet been determined.

Figure 4.2. A square region around the wellhead remains closed to all fishing (red box), and another region north and west of the wellhead was closed on 24 November to Royal Red shrimping (gray area).



4.3 Reference Stations

An MMS study by Turner et al. (2003) focused on the Louisiana Bight region west of the Mississippi River Delta of the Mississippi River. Concentrations of PAHs in sediment cores ranged from 5.5 ng/g to 1200 ng/g (average 154 ng/g). The acute and chronic aquatic life ratios were calculated for the samples taken from the 5 cm 1 of each core (a TOC value of 1% was used in the calculation). No samples exceeded the acute or chronic aquatic life benchmarks. The average value of the chronic aquatic life ratios from these cores was used as the reference level in the figures below.

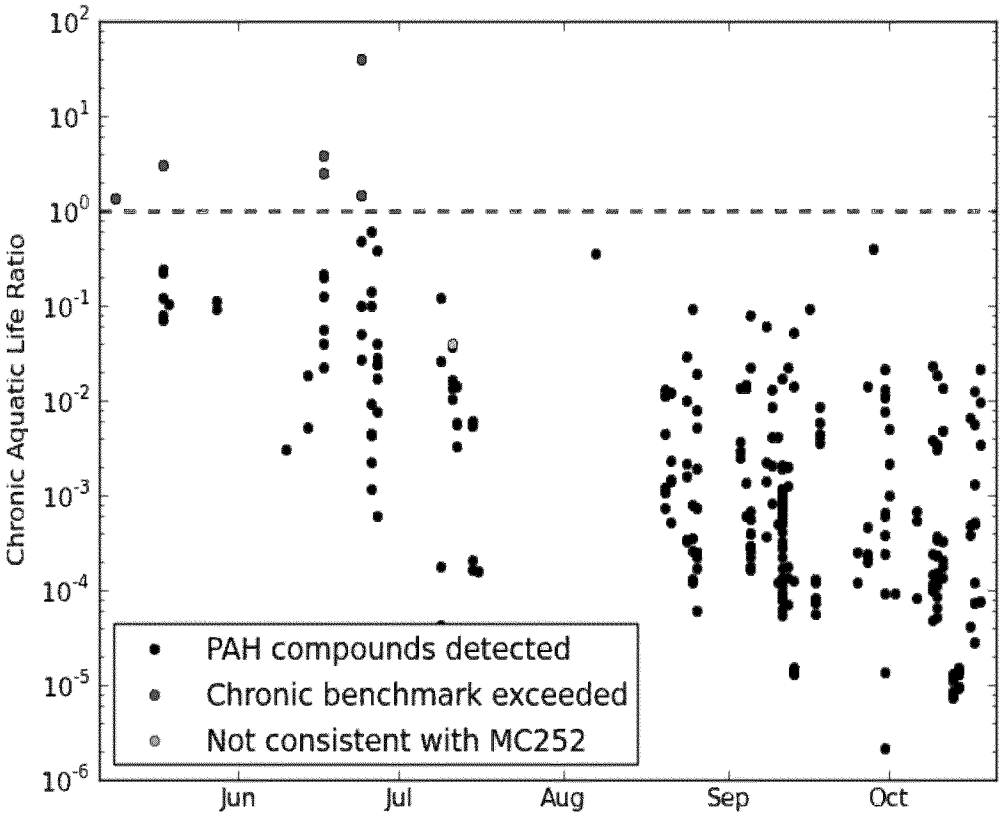
4.4 Qualitative Observations of Oil

Sediment cores and grabs collected in the offshore sampling zone were initially inspected for visual or olfactory indications of oil. These qualitative sensory inspections gave real-time preliminary indications of where oil contamination of sediments might have occurred. These results were used to inform the sampling plan and to make any adaptations to address areas of potential concern. Several cores from stations in the vicinity of the Mississippi River freshwater convergence zone showed qualitative indications of oil. The OSAT recommended additional sediment sampling in this region to ensure adequate coverage in the event that analytical results confirmed presence of MC252 oil in these samples (OSAT Recommendation, 19 October, Appendix F.11).

4.5 Comparison to Aquatic Life Benchmarks

Chronic and acute aquatic life ratios were calculated for all samples in which PAH compounds were analyzed (Maps 7.4a and 7.4b; Table 4.1). In the offshore sampling zone, the majority of the samples (~70% of water samples and 90% of sediment samples) were analyzed for the full suite of analytes (parent PAHs and alkylated PAHs). However, the analyses for the volatile organic compounds (e.g. benzene, toluene, ethylbenzene and xylene) were given a different sample name and it was not possible to match up the samples within Scribe and hence they were not included in the aquatic life ratio calculation (for more information on this problem and its implications see Appendices C.1 and C.5, respectively). Any water or sediment sample that exceeded the aquatic life benchmark was examined in detail to assess the likelihood that the exceedance was due to MC252 oil (see Appendix D for source characterization process).

Figure 4.3 Chronic aquatic life ratio in water samples as a function of time. Red dots above the dashed line exceed the chronic aquatic life benchmark. The six exceedances are near surface (~1 m) samples.



Six water samples in the offshore sampling zone exceeded the chronic aquatic life benchmark. One of these six also exceeded the acute aquatic life benchmark. All six were near-surface samples (~1 m) collected in May and June (Figure 4.3). These samples were collected in association with the Dispersant Effects Project (DEEP;

Appendix C.11) which targeted re gions of observed surface oil b efore and after dispersant application. No samples exceeded the benchmark after 3 August.

In sediment samples, none of the samples exceeded the EPA chronic aquatic life benchmark in the offshore zone (Maps 5a and 5b; Fig. 4.4; Table 4.1). There were very few sediment samples in this zone prior to th e sub-surface sampling efforts that occurred in late-August through October, however, comparison with the average chronic aquatic life ratio calculated from the reference dataset (Section 4.3), indicates most of the samples have ratios that are below this level.

Figure 4.4 Chronic aquatic life ratio in sediment samples as a function of time. There were no exceedances of the chronic aquatic life benchmark. The green line corresponds to the average chronic ratio at offshore reference stations.

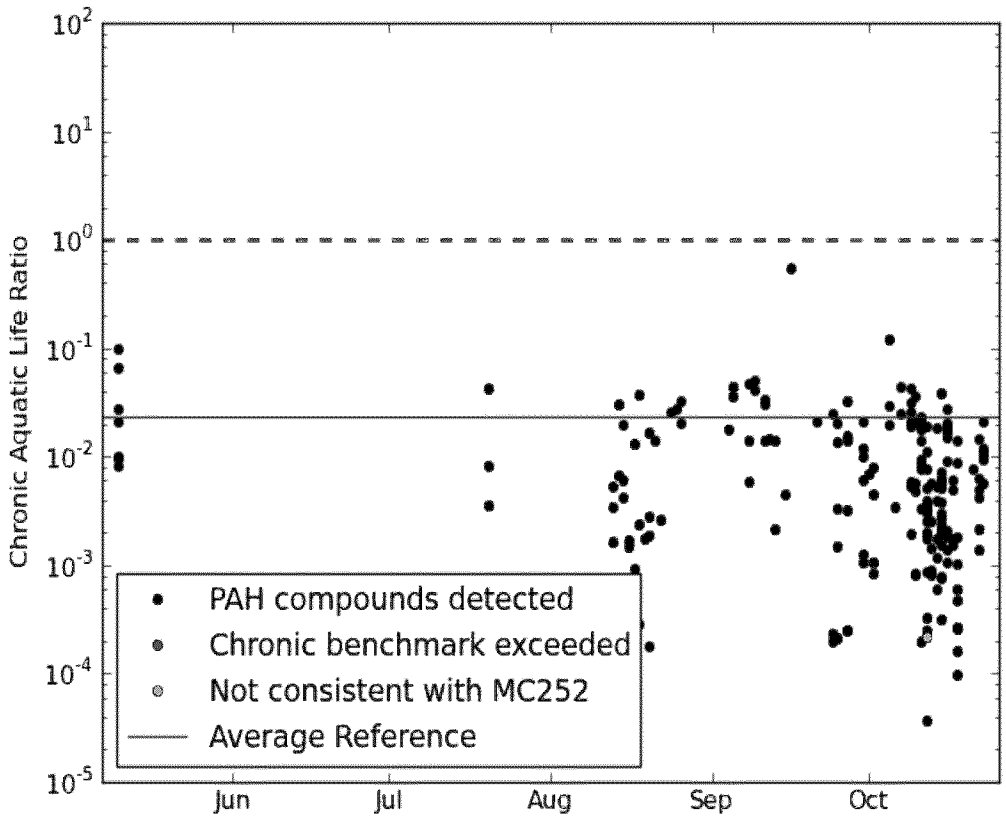


Table 4.1. Summary of water and sediment analytical chemistry results in the offshore zone. Datasets were compared to benchmarks for human health, aquatic life, and dispersant chemicals. For each benchmark, total numbers of samples that were non-detect, below benchmark, or that exceeded the benchmark were computed. The total number of samples is not the same for the human health, aquatic life, or dispersant analyses because not all samples were analyzed for all analytes.

Sample Type	Indicator Level	Human Health	Aquatic Life	Dispersant
Water Column	Non-detect	508	192	241
	Below Benchmark	242	283	199
	Exceed Benchmark	**0(0)***	**6(6)***	**0**
Sediment	Non-detect	n/a	61	242
	Below Benchmark	n/a	207	1
	Exceed Benchmark	**n/a**	**0**	**n/a**
Total Samples		**750**	**749**	**682**
Total Exceedances		**0**	**6(6)**	**0**
Percent of Exceedances		**0%**	**0.80%**	**0%**
Total Exceedances After 3 August		**0**	**0**	**0**

*The # in parantheses refers to exceedances consistent with MC252 oil.

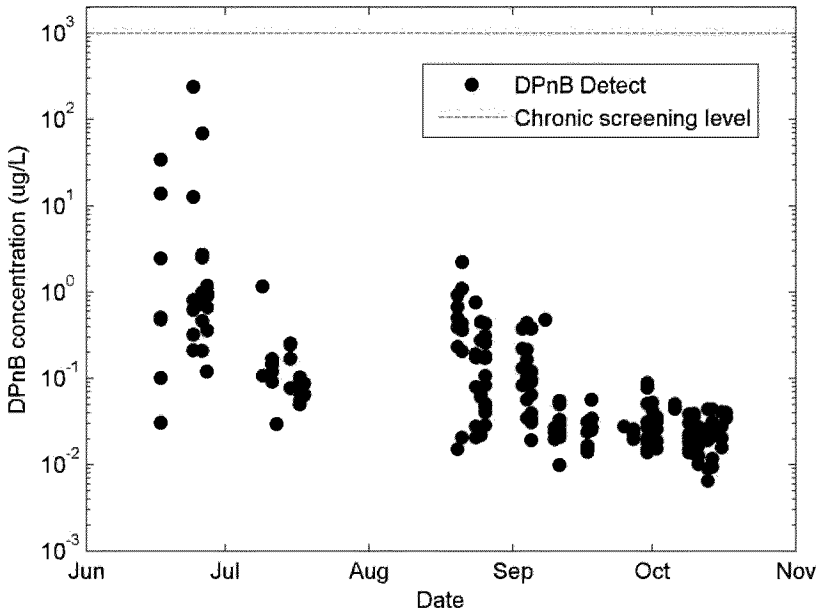
Table 4.2. List of aquatic life benchmark exceedances for water and sediments in the the nearshore zone after 3 August. Details are listed for each exceedance including the source of the oil, identified as follows: (1) consistent with MC252 oil, (2) not consistent with MC252 oil, and (3) indeterminate.

Sample Date	Type of Exceedance	Sample Location	Sample ID	Source
NONE	NONE			

4.6 Comparison to Dispersant Benchmarks

There were no exceedances of the dispersant-related chemicals benchmarks in the offshore sampling zone. Approximately half the samples in the offshore had detections of dispersant-related chemicals in water samples (199/440). Figure 4.5 shows the concentration of DPnB in water samples collected in the offshore zone over the course of the response. In sediment samples, there was only one detect of a dispersant-related chemical out of 243 samples.

Figure 4.5 DPnB concentration over time. Note that no samples exceeded the benchmark.



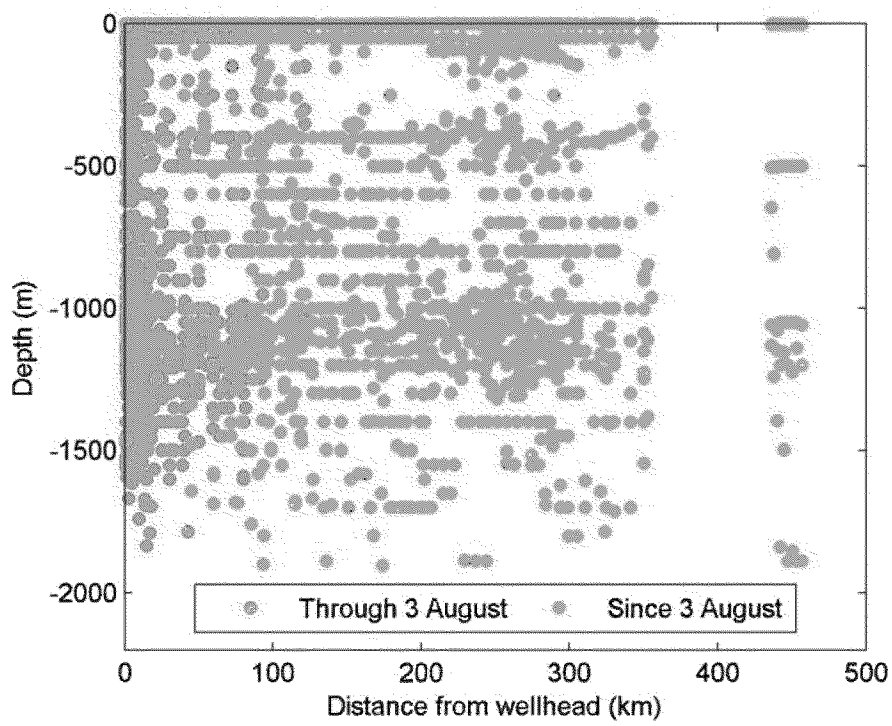
Section 5: Deep-Water Sampling Results

5.1 Description of Sampling and Monitoring Activities in the Deep-water Zone

The deep-water zone is defined as the region seaward of the 200-m bathymetric contour (approximately the continental shelf break; Map 7.1). Beginning in May 2010, extensive water sampling in this zone was conducted by private, federal, and academic scientists.

Water samples were collected by a number of vessels (NOAA, BP contract, and academic) operating both in the vicinity of the wellhead and in the far field (Map 7.3). Conductivity/temperature/depth (CTD) casts—vertical profiles measuring physical and chemical water properties—were conducted by most of the samplin g vessels operating in the deep-water zone. The CTD sensor array typically included a dissolved oxygen sensor and a fluorometer for qualitative screening of hydrocarbon signatures. Water samples collected at discrete depths were analyzed fo r a number of parameters, inclu ding oil chemistry, dispersant indicators, and toxicity to rotifers and luminescent bacteria (data to be presented as a addendum to this report). Analytical data were uploaded to the Scribe database for comparison with water-quality benchmarks for the deep-water zone (Section 2). The distribution of analytical data available in Scribe is shown with respect to depth and distance from the wellhead in Figure 5.1.

Figure 5.1. Distribution of analytical data for deep-water sampling zone. Data spans the period from 9 May through 16 October. Different colors denote sampling through and since 3 August.



Early in the response, observations from vertical profiles within approximately 30 km of the wellhead identified a signature of dispersed oil droplets in a depth range of 1100–1300 m. Much of the subsequent work in the deep-water zone focused on delineation and characterization of this sub-surface plume. From mid-July on, a mission guidance program provided coordination, allowing for the multiple vessels to more efficiently and effectively sample the deep water and to create a 4D-image of dispersed oil in the sub-surface. To coordinate and direct the sampling, mission guidance was kept informed by daily reports from Chief Scientists in the field, less-time data, knowledge of ocean currents, and predicted fate and transport of dispersed/dissolved oil from trajectory models. Further review of data for the sub-surface plume was generally confined to a specific density layer defined by potential density 1027.70–1027.71 kg/m^3.

Sub-surface monitoring, trajectory models, and ocean current data indicated that the overall movement of the sub-surface plume would be primarily along bathymetric contours to the southwest. A significant water-sampling effort effectively characterized the plume as it moved to the southwest; this sampling spanned the along-shelf region from the wellhead out to 500 km to the southwest and 200 km to the northeast. However, sampling was not temporally linked to strong current reversals, particularly to the NE when movement into that region would occur. In late September, after analysis of recent results and comparison with water-quality indicators, the OSAT recommended that further far-field water sampling of the dispersed oil plume was no longer necessary as part of the response (OSAT recommendation 1, 20 September 20, App. F).

As much of the sampling in August and September had focused on delineating this deep plume as it moved to the southwest, observations since July in the vicinity of the wellhead were limited. As part of the Strategic Plan, the OSAT recommended a limited number of stations in the near-field be resampled (20 September, App. F.1). Many of these stations had been sampled very early in the spill and indicated oil presence. These stations were revisited in late September.

At the time of this report, approximately 4000 water and sediment samples from the deep-water zone had been analyzed and published to the Scribe database (Map 7.3; Fig. 5.1; Table 5.1). Due to the evolution of sampling described above, the bulk of the samples from May through early August are in the near-field vicinity of the wellhead, whereas the bulk of the samples from August and September are primarily to the southwest. In September, water sampling in the deep-water zone was limited to the near-field stations mentioned above.

The need for sediment sampling in the deep-water zone was identified as a sampling gap in the Strategic Plan. This sampling was undertaken beginning in late September. Samples were collected using a Mega Corer in the near field around the wellhead, and in the far field toward the northeast and southwest (in the along-shelf direction). Sediment cores (Map 7.3) were analyzed for oil chemistry and microtox toxicity.

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5.2 Fisheries Closures

Many of the fishery closure areas span both the offshore and deep-water zones, and were discussed earlier in Section 4.2. A deep-water fisheries area that has yet to be re-opened is a square region around the wellhead that is approximately 30x30 nmi in size (1041 mi^2) (Fig. 4.2). One factor determining the re-opening of this region is the decontamination of the M/V *Discoverer Enterprise* drillship in deep water, which is currently in process.

5.3 Reference Stations

Reference stations for sediments in the deep-water zone were obtained from two historical MMS studies. The 2009 MMS study (Rowe and Kennicutt, 2009) was part of the Deep Gulf of Mexico Benthos (DGoMB) project and resulted in the collection of sediment samples at 67 different locations, during 2000–2002 (Fig. 2.1). The sediment PAH values were in the range of 0 to 1033 ng/g (mean of 139.5 ng/g). The acute and chronic ratios for comparison to EPA's aquatic life benchmarks were calculated for these samples, and all ratios were well below the benchmark of 1 (ranging from 0.0011 to 0.1881 for the chronic ratio).

The 2006 MMS study (Continental Shelf Assoc., 2006) measured sediment PAHs at 45 locations in four distinct lease blocks (Viosca Knoll Block 916, Garden Banks Block 516, Garden Banks Block 602, and Mississippi Canyon Block 292) during 2000–2002 (Fig. 2.1). Sediment PAH values ranged from 11.8 to 23,840 ng/g (with a mean of 801.5 ng/g). The mean PAH concentration across all deep-water sediment samples was 596 ng/g. Both of the MMS 2006 and 2009 studies measured numerous parameters in addition to sediment PAHs, including metals, meio/macrofauna, bacterial activity, and sediment toxicity.

5.4 Qualitative Observations of Oil

Fluorometers were deployed concurrently with CTD and dissolved oxygen sensors to delineate areas of potential sub-surface oil by detecting the presence of dissolved organic compounds in the water column or very finely dispersed oil droplets (generally <60 µm). The fluorometer does not identify specific compounds or distinguish the sources of organic material. However, the intensity of fluorescence is influenced by the presence of specific compounds and the excitation wavelength of the ultraviolet light source. Therefore, fluorometry is a screening tool that estimates the level of oil in the water, but it is not a standalone method for accurately measuring oil concentration.

Sub-surface monitoring in the near field around the wellhead initially identified the sub-surface plume as a relative increase in background fluorescence between 1100 m and 1300 m, measured with a Chromophoric Dissolved Organic Matter (CDOM) fluorometer. Fluorescence, though not a quantitative measurement of hydrocarbon concentration, was found to correlate very strongly with PAH concentrations in the deep water. The same study measured PAH concentrations in this layer around the well head of up to 189 ppb in May, resulting in large spikes in fluorescence intensity (Dierks et al., 2010). Fluorescence remained a valuable screening tool throughout the response, providing a rapid indication of potential dispersed oil in the water column. After the well was capped on July 15, the fluorometry signal began to attenuate. By September, many vessels began using the Chelsea Aquatracka, which was shown to be more sensitive for MC252 oil.

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The R/V *Pisces* cruise that performed water sampling in late September near the wellhead also detected fluorometric anomalies (signal spike) at a depth consistent with previous observations of the sub-surface plume using an Aquatracka. As per OSAT Recommendation 2 (September 20, App. F.1), the R/V *Pisces* returned to the area to collect water samples for chemistry measurements. As of the writing of this report, the analytical chemistry results from this cruise were still not published to the Scribe database. However, preliminary communication with LSU labs (Scott Miles, pers. comm.) indicate that all PAH samples were below detection limits. Thus, it appears that PAH concentrations have significantly decreased around the wellhead since the previous sampling in mid-July, when exceedances of benchmarks were still being observed.

Response sediment sampling in deep-water zone was primarily conducted using a multi corer on the vessels R/V *Gyre* and R/V *Ocean Veritas*, with a smaller number of samples collected using a grab sampler. In total, for the deep-water zone, qualitative results from multi corer sampling were obtained for 64 sediment samples from the R/V *Gyre* and 56 samples from the R/V *Ocean Veritas*, for a total of 120 samples (Fig. 5.2). These samples were analyzed for qualitative indications of oil, including both visual and olfactory inspection. Based on qualitative indicators, 29% of the 115 deep-water sediment samples showed potential indication of contamination by oil. Note that based on *quantitative* results 6% of the 115 sediment samples exceeded aquatic life benchmarks, as will be discussed in Section 5.7. Qualitative comments of possible oil contamination ranged from "thin layer of oil sheen on supernatant water surface" to "oil in sediment with slight oil smell." Example pictures are shown (Fig. 5.3) of R/V *Gyre* sediment cores for which MC252 oil was confirmed in the sample. A fluffy, brown, flocculated layer was reported in the upper part of some cores, which differs in color from the grayer muds. This flocculated layer likely includes contributions from organic detritus sinking out of surface waters, including for example, algal flocs and zooplankton fecal material. Historical data from MMS (2003) similarly shows an upper flocculated layer in sediment cores in Viosca Knoll, next to Mississippi Canyon.

Qualitative observations from two academic cruises were also reviewed by the OSAT (Fig. 5.2). Note that, as of this report, quantitative sediment results from these academic cruises were not yet available, so qualitative assessments of oil have not been fingerprinted. Dr. Samantha Joye (UGA) shared qualitative results with the response from the R/V *Oceanus* academic cruise (21 August to 16 September; Fig. 5.2). They found indications of oil at 10 of the 14 sites they sampled, with thicker layers of potential oil-entrained sediments found all the way out to 37 km from the wellhead. Dr. Kevin Yeager (USM) was chief scientist on the R/V *Cape Hatteras* academic cruise (10-17 October; Fig. 5.2) that also collected sediment samples. He teleconferenced with the OSAT and discussed qualitative results from their samples. They observed 6 stations within ~20 km of the wellhead with some indication of oil (fingerprinting pending). The two stations with particularly strong indications of oil were within 5 km of the wellhead. All samples indicated that oil was sediment-entrained and not in liquid form (free-phase). Response sampling conducted under the Operational Annex re-visited many of these sampling sites between 22 September and 23 October. Sediment samples collected on these response cruises found MC252 oil present at levels of concern (i.e., levels that exceed aquatic life benchmarks) extending as far as 3 km from the

wellhead (Section 5.7). The aquatic life ratios approach reference levels within ~10 km from the wellhead.

A recent NOAA-BOEMRE cruise on the NOAA Ship *Ronald H. Brown* from 14 October to 4 November noted stressed deep water coral at a location approximately 11 km southwest of the wellhead location. As part of the response Implementation Plan, sediment cores were taken that surrounded both the wellhead location and the stressed coral location. Although there were detectable oil-related compounds in the sediments of that immediate area, concentrations of oil-related compounds in these samples did not exceed aquatic life benchmarks (Fig. 5.2).

Fig 5.2. Location of multi corer sediment samples collected by the response. "Oil present" in the legend refers to positive qualitative (visual and/or olfactory) observations of oil. Qualitative results from two academic cruises (R/V Cape Hatteras and R/V Oceanus) are also shown. Analytical data collected during the response indicate exceedances of aquatic life benchmarks in sediment within approximately 3 km of the wellhead.

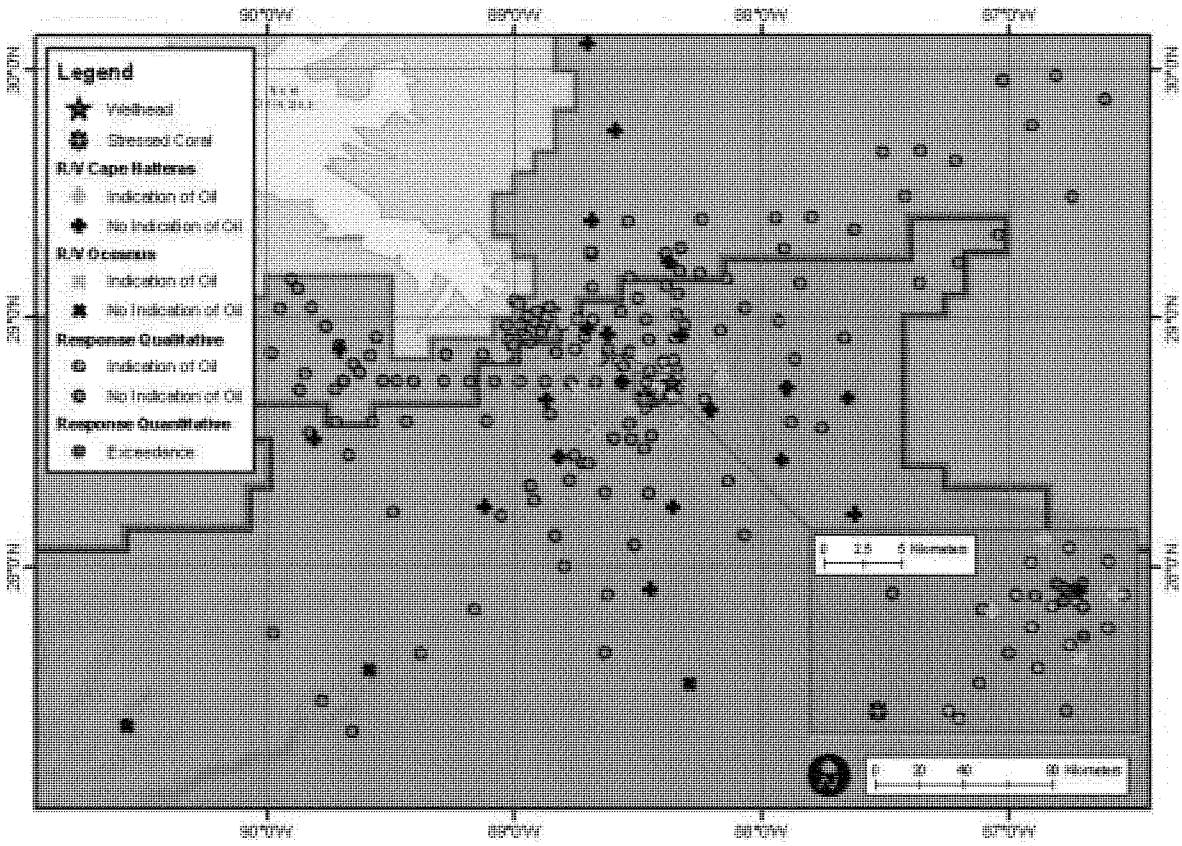
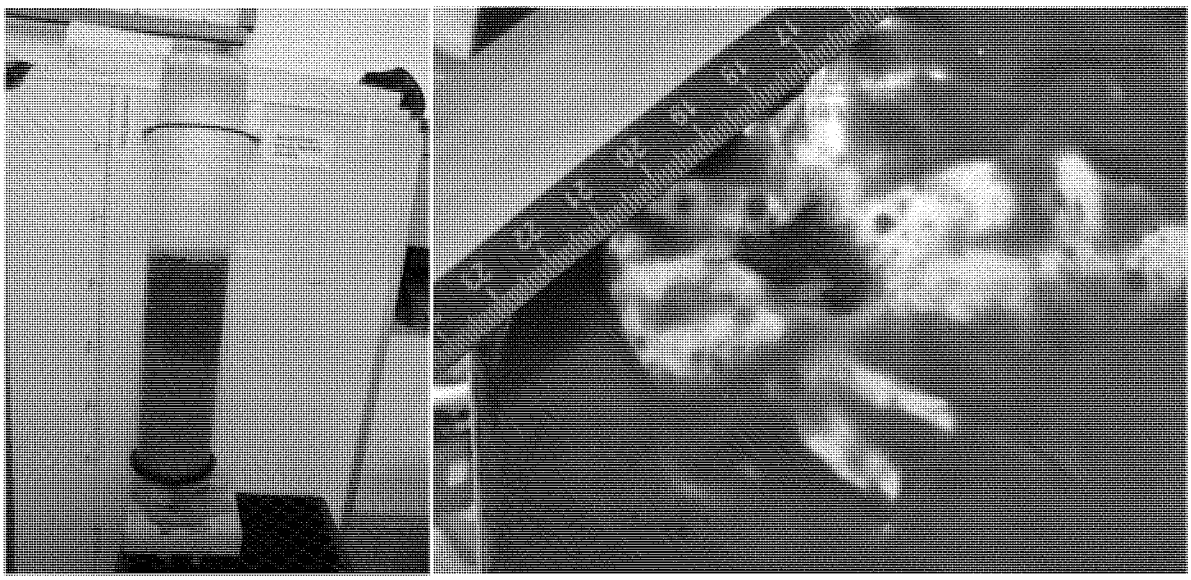


Figure 5.3. Pictures of sediment samples collected by the R/V Gyre near the wellhead. These samples were confirmed to contain MC252 oil. The sample IDs are SE-20101001-GY-ALTNF001-VA-020 (left panel), which shows the sediment depth profile, and SE-20101001-GY-NF006MOD-VA-021 (right panel), which shows extruded sediment (into a plastic bag) containing oil from the top 3 cm of the core.



5.5 Hypoxia

Dissolved oxygen data collected from 8 May to 9 August was analyzed and discussed by the Joint Analysis Group (JAG) (Report 3, 16 August). They reported significant dissolved oxygen depressions relative to background concentrations (minimum dissolved oxygen concentration observed was 3.7 mg/L in late July; depressions ranged from 0.14 mg/L to 3.7 mg/L below background). These dissolved oxygen depressions occurred coincident with relative maxima in fluorescence profiles used to screen for hydrocarbons and extended as far as 80 km from the wellhead. However, as dissolved oxygen concentrations were not approaching hypoxic levels (<2.0 mg/L) and did not appear to be decreasing over time, their analysis indicated that hypoxic conditions would not occur in association with the deep dispersed plume.

From 3 August through early October, numerous vessels sampling the plume responded to mission guidance, i.e., results were reported on dissolved oxygen depressions on a real-time (daily) basis. These measurements were made using an *in situ* oxygen sensor on the CTD (CTD/O_2). The accuracy of these sensors was calculated in the AOML Oxygen Report (2010) which demonstrated that the CTD/O_2 sensor and validation by Winkler's titration method were accurate to within 2%. Response sampling since 3 August resulted in a comprehensive delineation of the plume to the southwest. The minimum dissolved oxygen value measured since 3 August was ~4 mg/L, a depression relative to climatological background of <0.3 mg/L (JAG Oxygen Report, 2010). Stations revisited around the wellhead in October showed no measurable oxygen depressions.

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5.6 Comparisons to Aquatic Life Benchmarks

In the deep-water sampling zone, the majority of the samples (~ 90%) were analyzed for the full suite of analytes (parent PAHs and alkylated PAHs). However, in a subset of these samples (~30%), the analyses for the volatile organic compounds (e.g. benzene, toluene, ethylbenzene and xylene) were given a different sample name and it was not possible to match up the samples within Scribe and hence these compounds were not included in the aquatic life ratio calculation (for more information on this issue and its implications see Appendices C.1 and C.5, respectively). A number of samples (~1000) early in the response were analyzed only for BTEX compounds. Data from each sample that exceeded the benchmark for aquatic life in either the water or the sediment was examined in detail to confirm the validity of the exceedance and to assess the hypothesis that the exceedance was due to MC252 oil (see Appendix D for methodology).

In the deepwater sampling zone there was a total of 70 exceedances of aquatic life benchmarks in water and 7 exceedances in sediment. Total aquatic life benchmark exceedances for PAHs in water and sediment represented ~2% of all samples collected (Table 5.1).

Table 5.1. Summary of water and sediment analytical chemistry results in the deep-water zone. Datasets were compared to benchmarks for human health, aquatic life, and dispersant chemicals. For each benchmark, total numbers of samples that were non-detect, below benchmark, or that exceeded the benchmark were computed. The total number of samples is not the same for the human health, aquatic life, or dispersant analyses because not all samples were analyzed for all analytes.

Sample Type	Indicator Level	Human Health	Aquatic Life	Dispersant
Water Column	Non-detect	4121	2721	3761
	Below Benchmark	673	821	353
	Exceed Benchmark	**0(0)***	**70(63)***	**0**
Sediment	Non-detect	n/a	6	119
	Below Benchmark	n/a	114	1
	Exceed Benchmark	**n/a**	**7(7)***	**n/a**
Total Samples		**4794**	**3739**	**4234**
Total Exceedances		**0**	77(70)	**0**
Percent of Exceedances		**0%**	**2%**	**0%**
Total Exceedances After 3 August		**0**	**7(7)**	**0**

*The # in parentheses refers to exceedances consistent with MC252 oil.

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The chronic aquatic life ratios in water samples decreased with distance from the wellhead (Fig. 5.4). Chronic exceedances potentially associated with MC252 oil were constrained to within approximately 70 km of the wellhead in deep water (Fig. 5.4; Map 7.4a). No exceedances consistent with MC252 oil were observed in the last August 3 (Fig. 5.5; Map 7.4b). The last exceedance consistent with MC252 oil was observed on 22 July at a depth of 1,140 m.

Exceedances of the chronic aquatic life benchmark were generally observed at two depth ranges throughout the water column. The highest aquatic life ratios were associated with surface or near-surface water. Exceedances were also observed in the water column at the depth range from 1100 m to 1300 m (Fig. 5.6), consistent with the qualitative observations of the deep plume of dispersed and dissolved oil.

A total of 16 samples also exceeded the acute toxicity benchmark in the deep-water sampling zone. All of these occurred prior to mid-July.

Figure 5.4. Chronic aquatic life ratios as a function of distance from the wellhead. The horizontal dotted line represents the chronic aquatic life benchmark of 1.

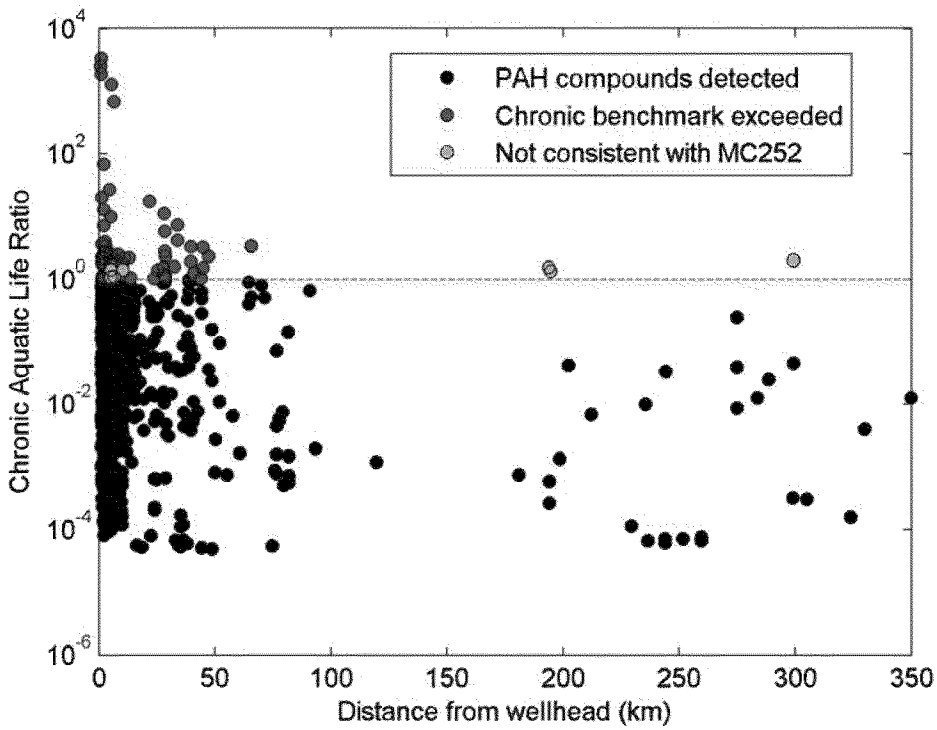


Figure 5.5. Chronic aquatic life ratios as a function of time. The horizontal dotted line represents the chronic aquatic life benchmark of 1.

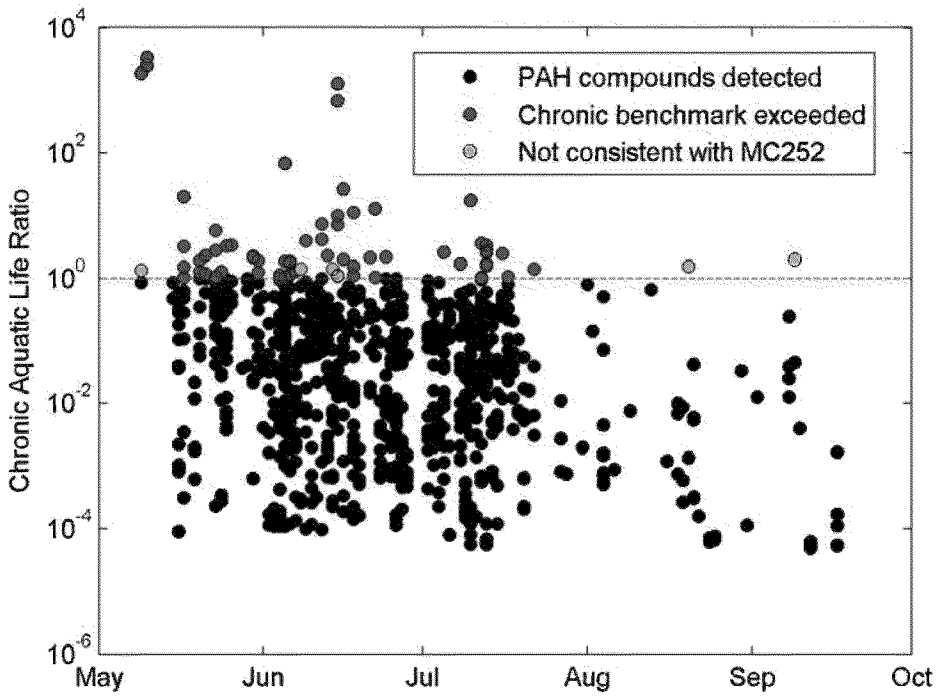
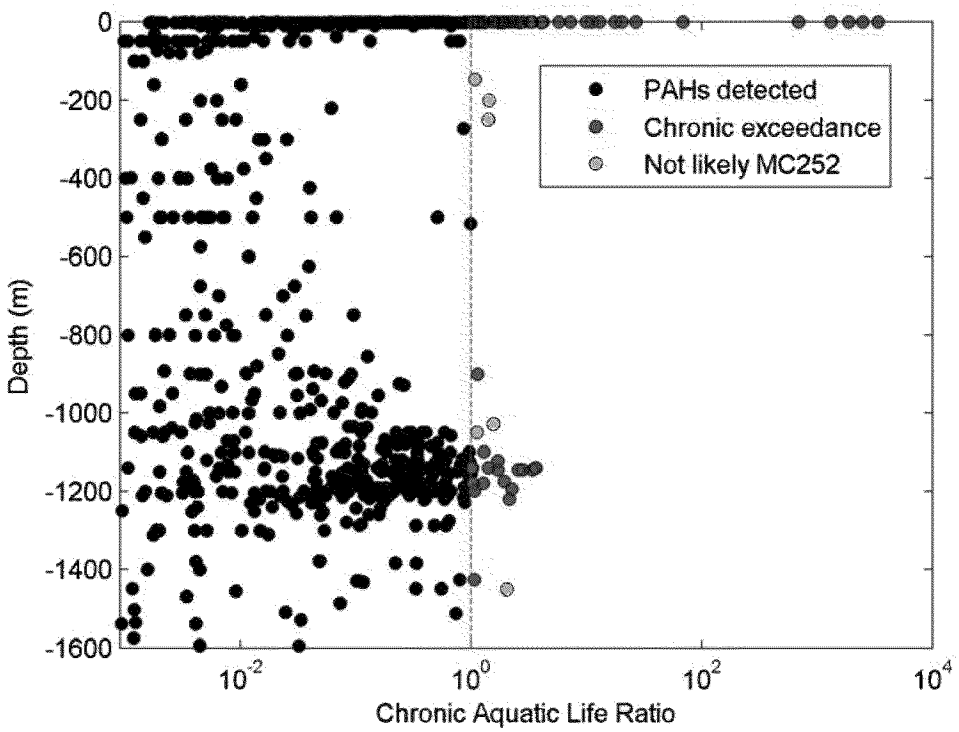
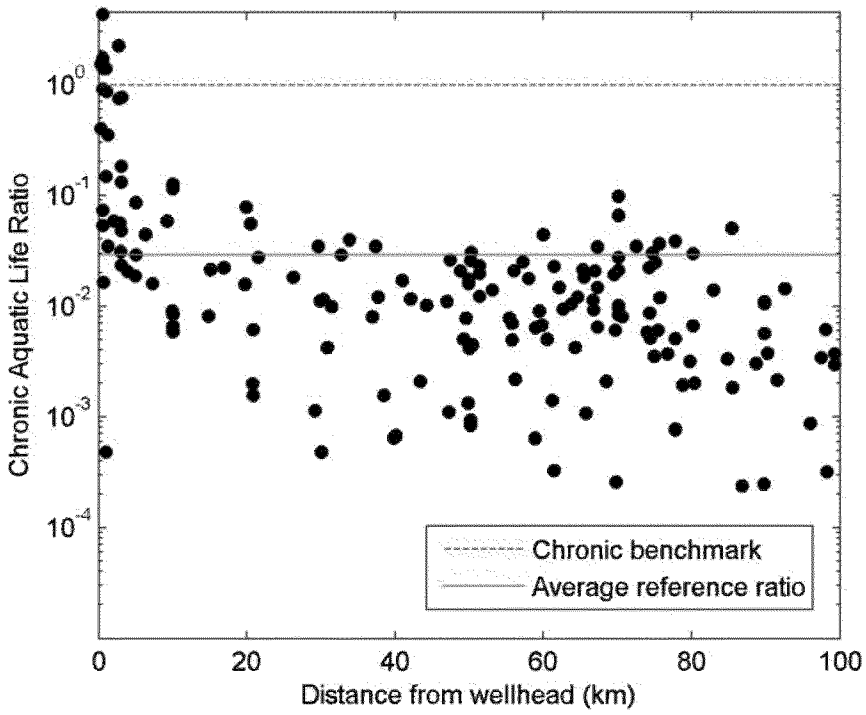


Figure 5.6 Chronic aquatic life ratios as a function of depth. The horizontal dotted line represents the chronic aquatic life benchmark of 1.



All sediment samples collected in the deep-water zone were collected as part of the Operational Annex (Maps 7.5a,b). Samples were compared to EPA's aquatic life benchmarks for sediments. Seven samples were found to exceed the chronic benchmark (Table 5.1; Fig. 5.7). One of the sediment samples also exceeded the acute benchmark. Samples that exceeded sediment benchmarks were all located with in the range of 0.33-2.7 km of the wellhead and were all confirmed to be consistent with MC252 oil (Table 5.2; Figure 5.7). The absolute concentrations of total PAHs were 9,900 ng/g to 28,000 ng/g.

Figure 5.7 Sediment chronic aquatic life ratios as a function of distance from the wellhead. The blue horizontal dotted line represents the chronic aquatic life benchmark. The green horizontal line represents the average chronic ratio at deep-water reference stations.



Chronic aquatic life ratios for sediment samples were also compared to ratios for reference locations in the deep water. These historical, reference studies included two MMS studies (Continental Shelf Assoc., 2006; Rowe and Kennicutt, 2009). The average chronic aquatic life ratio at these deep water reference stations ranged from 0.0011 – 0.33, with an average of 0.029 across all stations. In comparison to the average chronic ratio at reference stations, the chronic ratio for response samples were (on average) at or below the reference value beyond 10 km from the wellhead (Fig. 5.7). Thus, chronic aquatic life exceedances were observed within 3 km of the wellhead, but elevated levels were observed out to 10 km from the wellhead.

Source characterization of exceedances

A vast majority of the samples that exceeded the benchmark were either indeterminate in origin (given lack of data) or were consistent with MC252 oil. A portion of these exceedances were from the DEEP program (Appendix E), all of which were consistent with MC252 oil. The 7 samples that were considered not consistent with MC252 oil fell into one of the following categories: consistent with diesel range oil (likely caused by other sources), consistent with pyrogenic PAHs, or consistent with seep sources (see Appendix D).

The one water sample that was associated with a seep source (SW-20100909-OV19-12) was collected on 9 September in 1450 m of water at a distance of 299 km from the wellhead. The GC/MS Extracted Ion Current Profiles (EICPs) indicated the presence of crude-oil- related hydrocarbons. The abundance and distribution of the triterpanes and steranes are substantially different from MC252 oil, which demonstrates that MC252 oil is likely not the source of the hydrocarbons in this sample. The overall distribution of hydrocarbons suggests that the petroleum in this sample may be related to seep oil. This result was corroborated by preliminary data provided by NOAA's analyses that indicate that this sample is near a known seep area.

The 7 sediment samples that exceeded benchmarks were confirmed to be consistent with MC252 oil based on an analysis of the chromatograms. The samples IDs for these are preceeded by "SE" in Table 5.2. These deep-water samples were all collected within 3 km of the wellhead. There were also indications of drilling mud compounds in these samples based on 3 parameters: (1) higher barium levels than area sediments, (2) the presence of C16 and C18 alpha olefins (synthetic drilling mud additive), and (3) presence of relatively unweathered crude oil. Oiled sediment samples were not weathered to the same extent as oiled water samples in surface waters. For example, SE-20101001-GY-ALTNF001-020 lost its gasoline range organics through C10-11, and SE-20101001-GY-NF006mod-022 lost them through C12. Most of the sediment exceedance samples showed PAH depletion of 4-8% and a few were as high as 57–75%.

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Table 5.2. List of aquatic life benchmark exceedances for water and sediments in the the deep-water zone after 3 August. Details are listed for each exceedance including the source of the oil, identified as follows: (1) consistent with MC252 oil, (2) not consistent with MC252 oil, and (3) indeterminate. Highlighted rows indicate sediment samples; non-highlighted rows indicate water samples.

Sample Date	Type of Exceedance	Sample Location	Sample ID	Source
8/20/2010	Chronic	27.1512, -88.1777	SW-20100820-OV15-004	2
9/9/2010	Chronic	26.8251, -90.5024	SW-20100909-OV19-012	2
10/1/2010[1]	Chronic	28.7347, -88.3705	SE-20101001-GY-ALTNF001-HC-021_BDO	3
10/1/2010	Chronic	28.7347, -88.3705	SE-20101001-GY-ALTNF001-HC-020_BDO	1
10/1/2010	Chronic	28.7451, -88.3591	SE-20101001-GY-NF006MOD-HC-022_BDO	1
10/17/2010	Acute	28.7150, -88.3587	SE-20101017-GY-D0315-HC-050_BDO	1
10/17/2010	Chronic	28.7404, -88.3681	SE-20101017-GY-D0388SW-HC-053_BDO	1
10/17/2010[2]	Chronic	28.7449, -88.3742	SE-20101017-GY-D0445-HC-055	1
10/17/2010	Acute	28.7423, -88.3622	SE-20101017-GY-D0405-HC-052_BDO	1
10/17/2010	Chronic	28.7425, -88.3705	SE-20101017-GY-D0425-HC-054_BDO	1

[1]This sample was removed from consideration because it is a field duplicate.

[2]When the actual TOC is used for this sediment sample (versus an assumed 1%), it becomes an exceedance.

5.7 Comparison to Dispersant Benchmarks

Deep-water samples were analyzed for the dispersant-related che micals 2-Butoxyethanol, DPnB, and Propylene Glycol (Appe ndix A, Table A-1). Screening l evels exist for dispersant compounds in water only.

The dispersant-related chemical measures predominantly in the deep-water zone was DPnB. The benchmark for DPnB in water is 1,000 µg/L (1 ppm). Of the 4114 total samples that were analyzed for dispersants in the water column, 353 samples contained measurable amounts of DPnB. The range in detected DPnB concentr ations was 0.0170 – 113.4 µg/L (mean 4.3 µg/L), with all samples significantly below the chronic screening

level (Fig. 5.8). Concentrations of DPnB decreased with time, with all values less than 5 µg/L by July 30. Peaks in DPnB detects were observed in twotithist layers, at the surface and in the sub-surface (1100 to1 300m; Fig. 5.9) similar to dist ributions of exceedances of the aquatic life benchmark for PAHs.

Figure 5.8. Concentrations of the dispersant DPnB in water samples versus time.

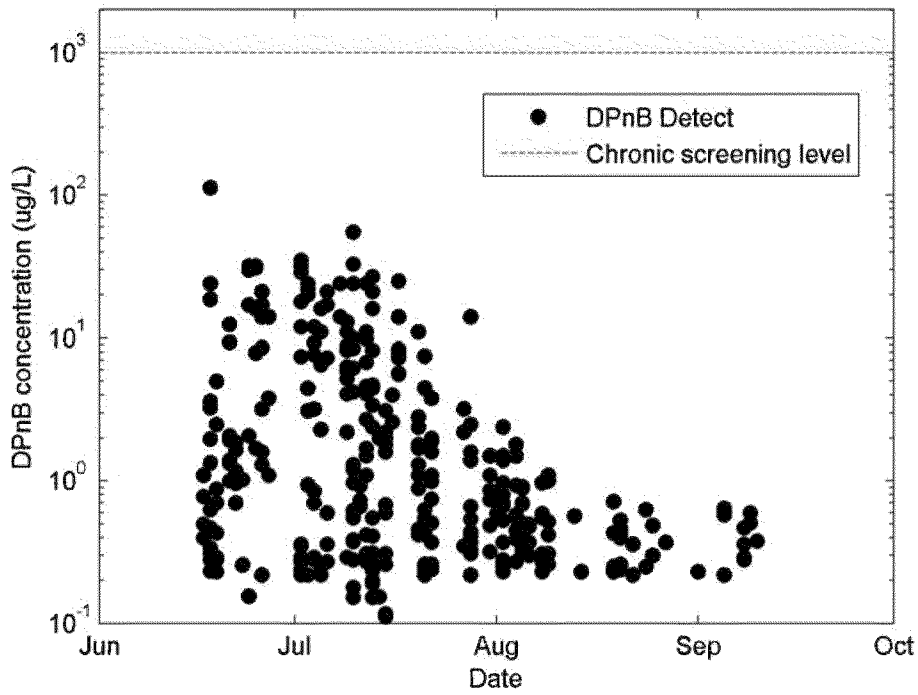
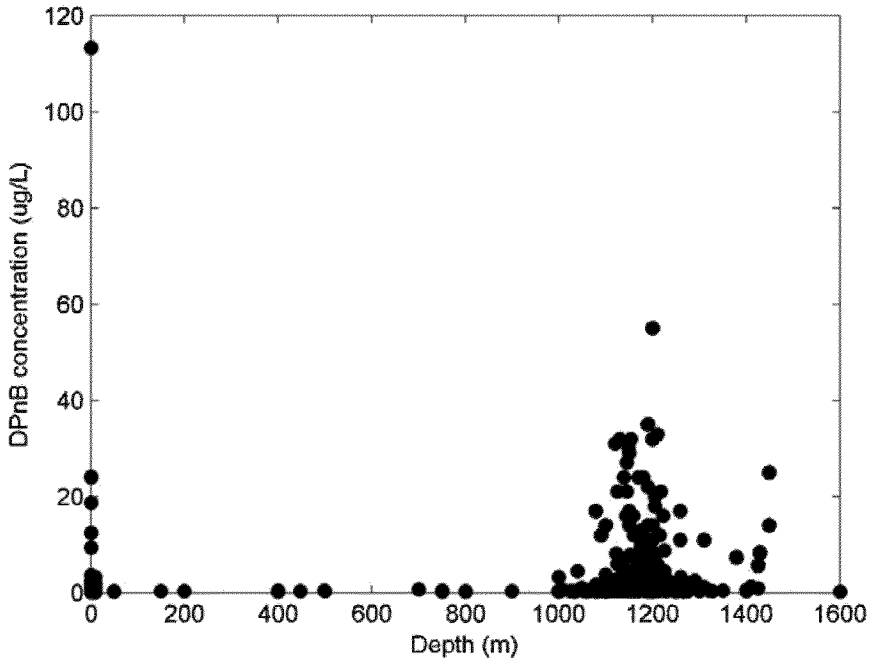


Figure 5.9 Concentrations of DPnB versus depth.



5.8 Biodegradation

Measurements of the microbial community, including both community structure and biodegradation, have been an important topic of research during the DWH oil spill. Many of these studies have been funded through academia, with some supp ort from the response. For example, Dr. David Valentine's (UCSB) microbial sampling on the R/V *Pisces* (9/8/2010 – 9/17/2010) was coordinated with John Kessler's chemistry sampling which is being funded by the Response. However, Valentine's post-cruise labor atory analyses are being funded by multiple sources, and that data will not be available for a couple of months. Several different researchers and their respective science teams have been involved in academic microbial sampling missions, including Dr. Terry Hazen (LBNL), Dr. David Valentine (UCSB), Dr. Andreas Te ske (UNC-Chapel Hill), Dr. Rich ard Camilli (WHOI), and Dr. Samantha Joye (UGA). Much of this work has not yet been published. As well, the JAG Oxygen Report (2010) discusses factors affecting biodegradation of deepwater hydrocarbons and modeled the impact biodegradation could have on the time required to reach hypoxia in the deep sea.

The OSAT reviewed published literature for the DWH oil spill to enhance our understanding of oil biodegradation. Microbial analyses in Hazen et al. (2010) were performed on water samples collected from vertical casts at the wellhead in late May and early June. Hazen's team provided evidence that a large number of bacterial genes involved in hydrocarbon degradation were significantly increase d in sub-surface oil plume samples. Total bacterial densities were also significantly correlated with MC252 alkane concentration in the plume. Hazen's group measured 16S rRNA gen e sequences in bacteria from the plume and found that they were dominated by the order Oceanospirillales in the γ-proteobacteria. The presence of this order was unique to the deep-water plume and this

order was not present above or below it. Hazen's group calculated maximum biodegradation rates based on concentrations of C13-C26 n-alkanes, resulting in half-lives ranging from 1.2 to 6.1 days that were similar for both microcosm and field conditions. Previous laboratory studies using crude oil and dispersants (including COREXIT 9500) showed rapid loss of dispersed oil in the sea by biodegradation (Venosa and Holder, 2007).

Biodegradation rates will necessarily vary as the sub-surface plume ages, and the most labile components of the oil are degraded, leaving behind the recalcitrant fractions. Hazen's rates indicated that the least recalcitrant fraction (alkanes) of the oil would persist for only weeks. The degradation rates for the complete mixture of compounds that characterize MC252 oil have not yet been determined.

Estimates of oil biodegradation have also been made using changes in dissolved oxygen concentrations measured in the sub-surface plume, premised on the bacterial degradation of oil is expected to cause consumption of dissolved oxygen. Camilli et al. (2010) estimated bacterial respiration based on temporal changes in dissolved oxygen concentration in the sub-surface plume. Their work was based on samples collected farther away from the wellhead and at a later date (i.e., late June) compared to the Hazen study. The results of Camilli et al. (2010) suggested that microbial respiration rates within the plume were not appreciably more than 1 μM O_2/day, suggesting that minimal biodegradation was occurring in the remaining plume, which included monoaromatic hydrocarbons, such as BTEX. The JAG Oxygen Report (2010) also estimated biodegradation of oil in the plume using various techniques for estimating biochemical oxygen demand. They estimated a first-order biodegradation rate constant of 0.0054/d as a lower bound on biodegradation estimates. The report estimated the possible time needed to reach hypoxia, assuming no replenishment of dissolved oxygen by mixing with oxygen-rich surrounding waters, ranged from 24 to 74 days. A shorter time of 1.3 days to hypoxia was estimated using using Hazen's high first-order biodegradation rate. Significantly, hypoxia was never observed in deep water during the incident.

In summary, the various microbial and chemical (i.e., oxygen) measurements made in the deep-water plume confirm that biodegradation of oil has been occurring. Estimates of biodegradation rates range from a lower bound of 0.0054/d estimated in the JAG oxygen report, which is likely more representative of the whole oil mixture, to an upper bound estimated by Hazen's group of 0.310/d for alkane biodegradation.

Section 6: Conclusions

The UAC's Operational Science Advisory Team (OSAT) concludes that sampling was adequate to address the presence and distribution of sub-surface oil (with the exception of very shallow nearshore areas where submerged tar mats may be present; discussed below). No deposits of liquid-phase MC252 oil were identified in sediments beyond the shoreline. Based on the analysis of extensive data in the nearshore, offshore, and deep water zones, the OSAT identified two areas of concern with respect to the indicators considered. Sediments exceed the aquatic life benchmark within 3 km of the well head and are above reference levels within 10 km and tar mats exist in some shallow sub-tidal areas near the shore.

Sediment PAH concentrations exceeded the aquatic life benchmark within 3 km (~2 miles) of the wellhead and were above reference concentrations within 10 km. The decision on whether or not this oil is actionable lies with the FOSC. The assessment of acute and long-term chronic effects to the Gulf of Mexico ecosystem is not within the purview of the response and is being addressed as part of the injury assessment conducted through the Natural Resources Damage Assessment (NRDA) process.

Observations from qualitative programs in the nearshore sampling zone indicate tar mats in sub-tidal nearshore areas are another area of concern. The sediment-bound oil within these subsurface tar mats could potentially be remobilized leading to the re-oiling of adjacent or neary-by shorelines. The sub-surface sampling did not entirely address this region due to difficulties with sampling in shallow water. Current efforts to address this issue within the UAC include continued monitoring by shoreline clean up and assessment teams (SCAT) and the formation of a specialized group at the UAC (OSAT II) to evaluate existing data and make recommendations for any additional sampling.

Other than these two areas of concern (sediments near the well head and near shore tar mats) the OSAT found:

- No deposits of liquid-phase MC252 oil in sediments.
- No exceedances of EPA's Human Health benchmark in water.
- No exceedances of EPA's dispersant benchmarks.
- Since 3 August, no exceedances of the aquatic life benchmark for PAHs in water that were consistent with MC252 oil.
- Since 3 August, no exceedances of the aquatic life benchmark for PAHs in sediment beyond 3 km of the wellhead that were consistent with MC252 oil.

The OSAT is confident that the quality of these data is sufficient to address the presence or absence of sub-surface oil and dispersants within the context of an operational emergency response evaluation. However, several factors should be considered in interpreting these findings for any other purpose.

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- Sample detection limits varied. Because some analytes had very high detection limits, the treatment of "below detection" results (calculating them as zeros) has the potential to underestimate concentrations.

- Many of the aquatic life ratios used in the Aquatic Life Benchmark for PAHs are estimates. Since some calculations did not include contribution of volatile organic compounds, ratios may be underestimated in some circumstances. Some analyses did not include alkylated compounds. The use of a multiplier to adjust total concentrations could underestimate or overestimate concentrations.

- Reference concentrations do not represent "background" conditions. Concentrations of PAHs in reference samples do not represent clean or pristine conditions, but represent average concentrations in the zone of interest before the spill. Other sources of PAHs, including seeps, may be represented in these comparisons.

- Many TOC values used in calculating the Aquatic Life Benchmarks for PAHs for sediments are estimates because actual TOC values were often not available. Though a conservative TOC concentration was applied, this could underestimate or overestimate exceedances of sediment quality benchmarks for individual samples.

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Section 7: Maps

Map 7.1: Sampling Zones

Map 7.2a: Sample Locations in Nearshore Zone Houma AOR

Map 7.2b: Sample Locations in Nearshore Zone Mobile AOR

Map 7.3: Sample Locations in Offshore and Deep-Water Zones

Map 7.4a: PAH Aquatic life Benchmark Analyses (Water Column) All Samples

Map 7.4b: PAH Aquatic life Benchmark Analyses (Water Column) Samples post 3 August

Map 7.5a: PAH Aquatic Toxicity Benchmark Analyses (Sediment) All Samples

Map 7.5b: PAH Aquatic Toxicty Benchmark Analyses (Sediment) Samples post 3 August

Map 7.6: Human Health Benchmark Analyses

Map 7.7: Dispersant Benchmark Analyses (Water Column)

Map 7.8: Dispersant Benchmark Analyses (Sediment)

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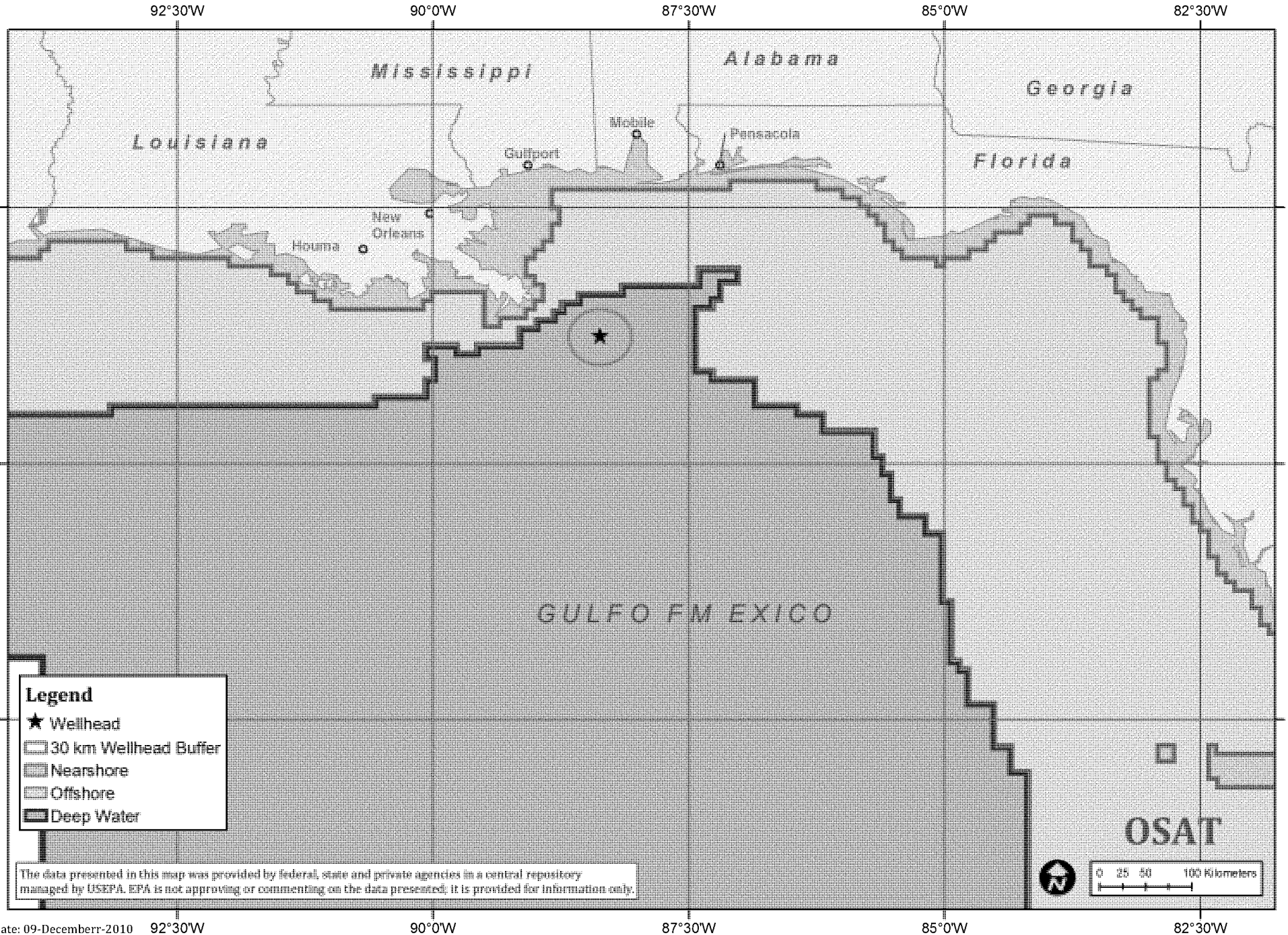
Map 7.1: Sampling Zones

Deepwater Horizon Response, Gulf of Mexico

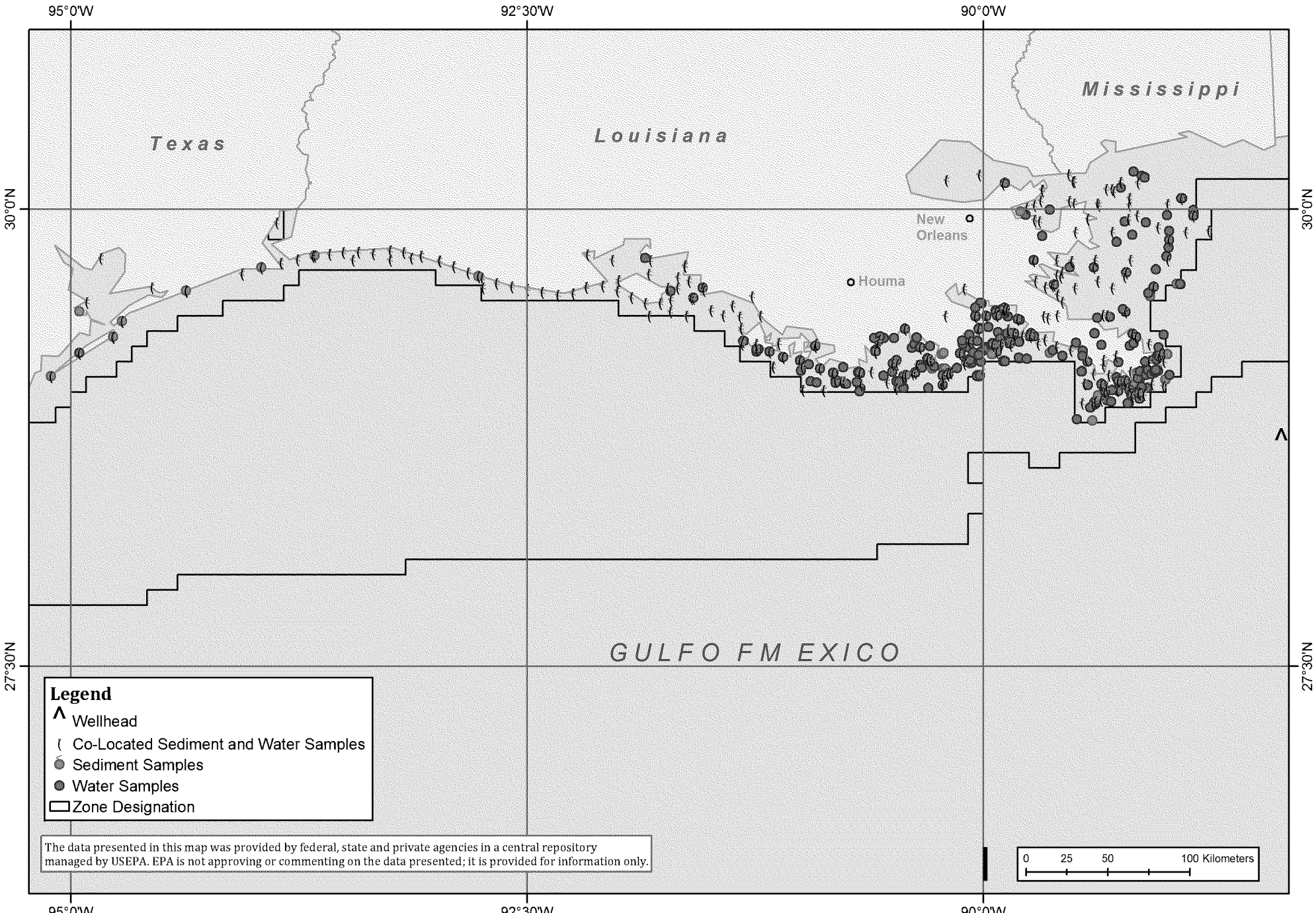


Map Date: 09-Decemberr-2010

Map 7.2a: Nearshore Sample Locations - Houma AOR

Sample Dates: 30 April 2010 to 20 October 2010

Deepwater Horizon Response, Gulf of Mexico

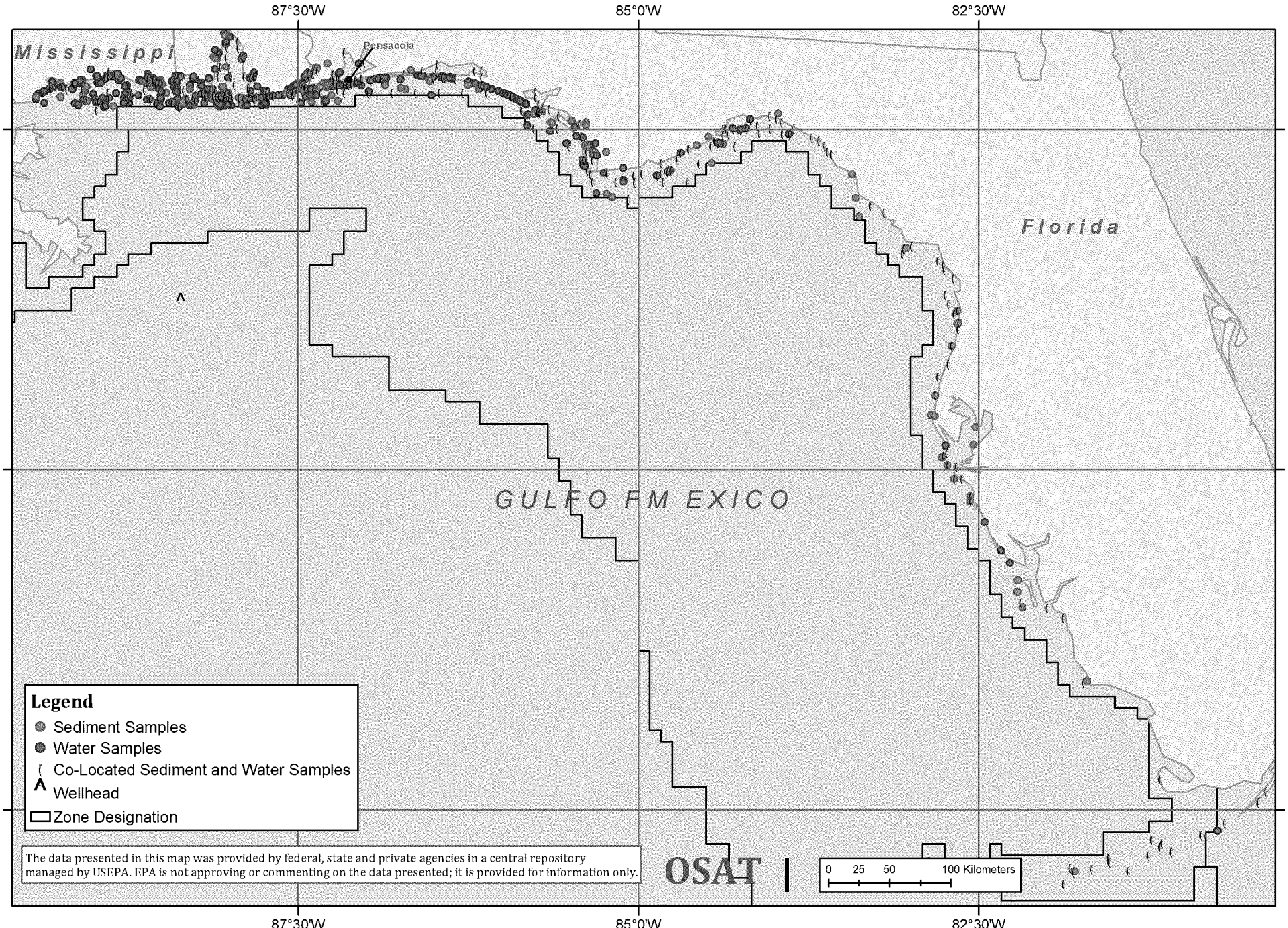


Map Date: 13-December-2010

Map 7.2b: Nearshore Sample Locations - Mobile AOR

Sample Dates: 30 April 2010 to 20 October 2010

Deepwater Horizon Response, Gulf of Mexico



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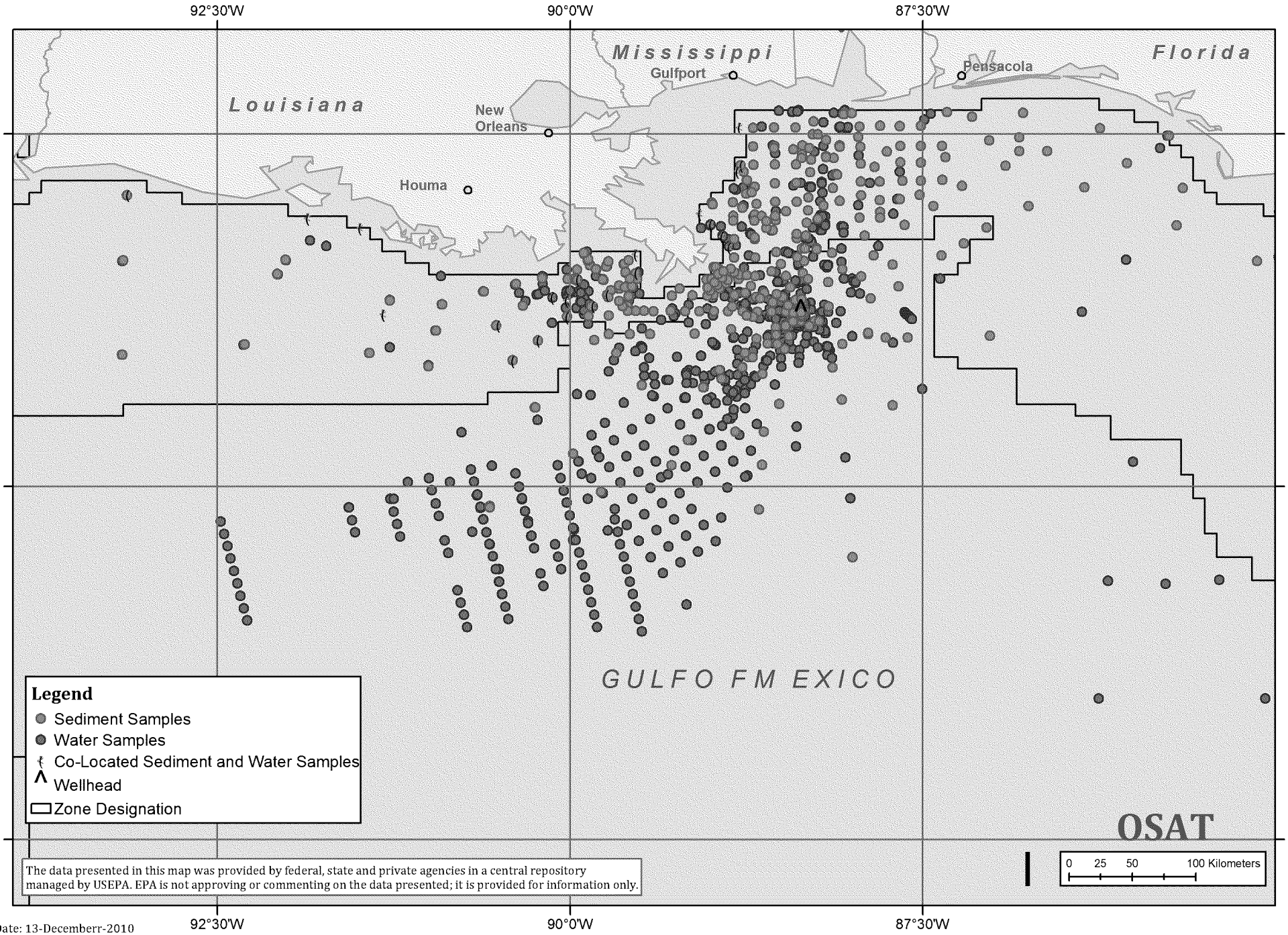
Map Date: 13-December-2010

Map 7.3: Offshore & Deep Water Sample Locations

Sample Dates: 28 April 2010 to 23 October 2010

Deepwater Horizon Response, Gulf of Mexico



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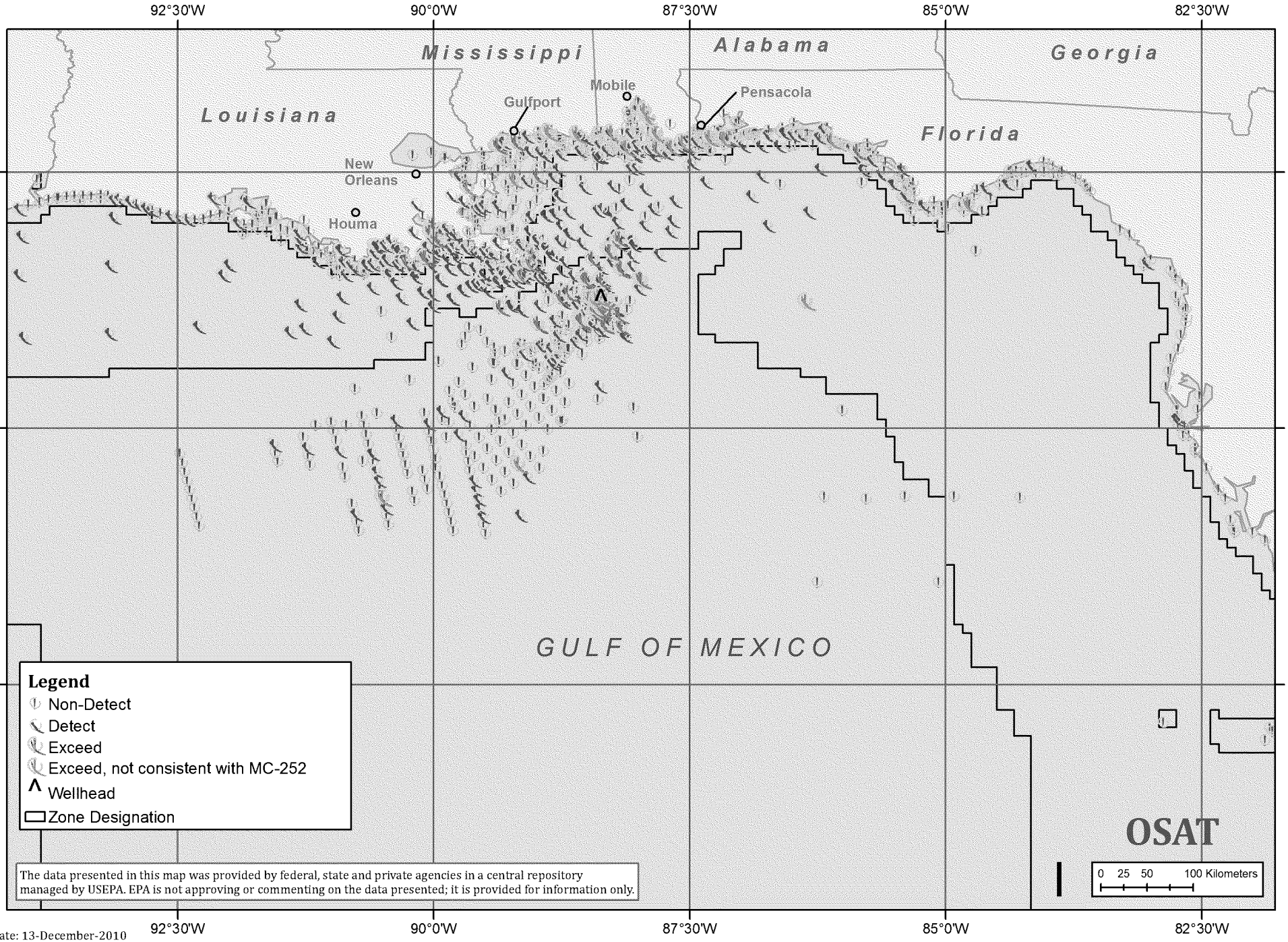
Map Date: 13-Decemberr-2010

Map 7.4a: PAH Aquatic Life Benchmark Analyses - All Water Samples

Sample Dates: 30 April 2010 to 18 October 2010

Deepwater Horizon Response, Gulf of Mexico



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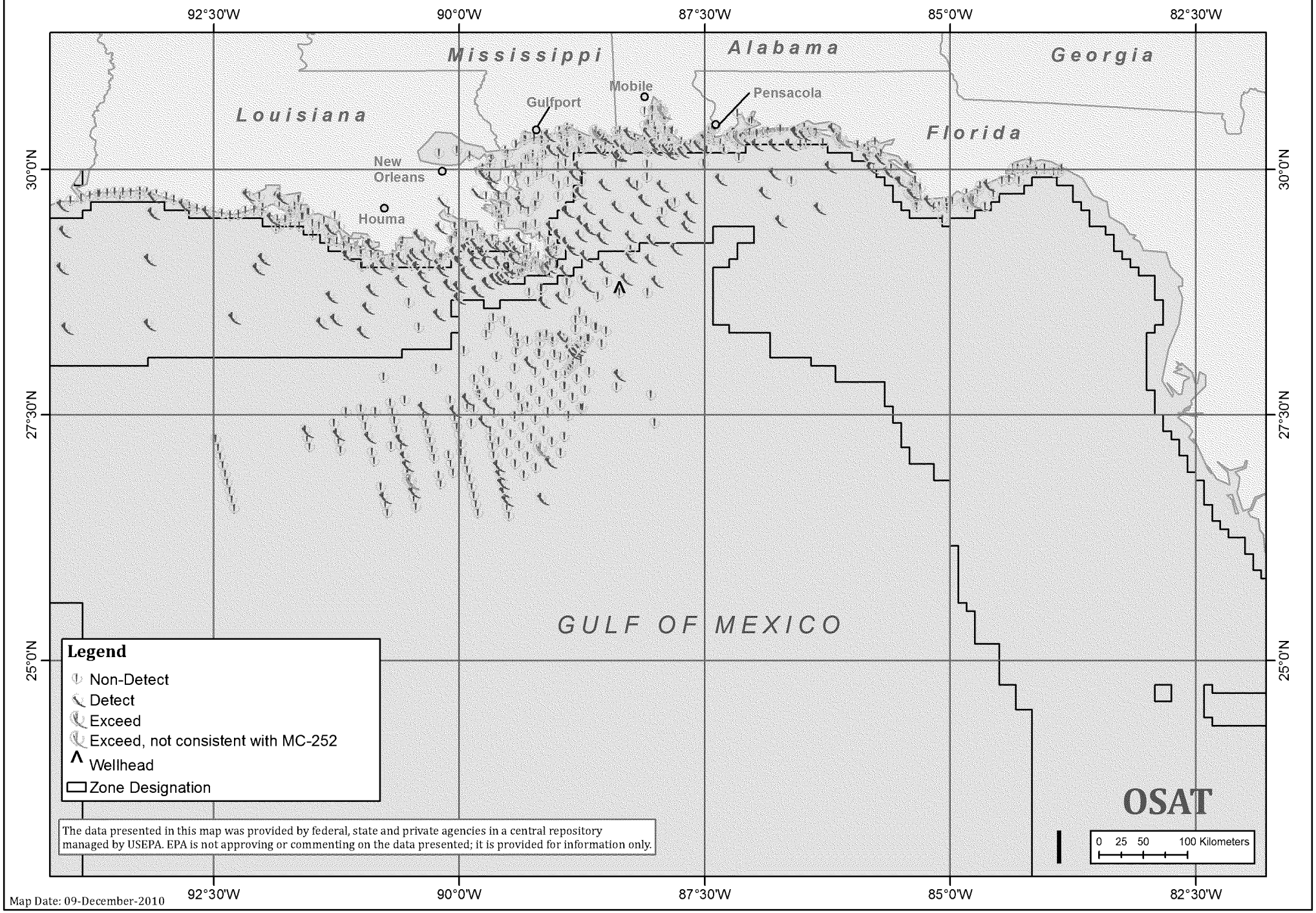
Map Date: 13-December-2010

Map 7.4b: PAH Aquatic Life Benchmark Analyses - All Water Samples Collected After 3 August 2010

Sample Dates: 4 August 2010 to 18 October 2010

Deepwater Horizon Response, Gulf of Mexico

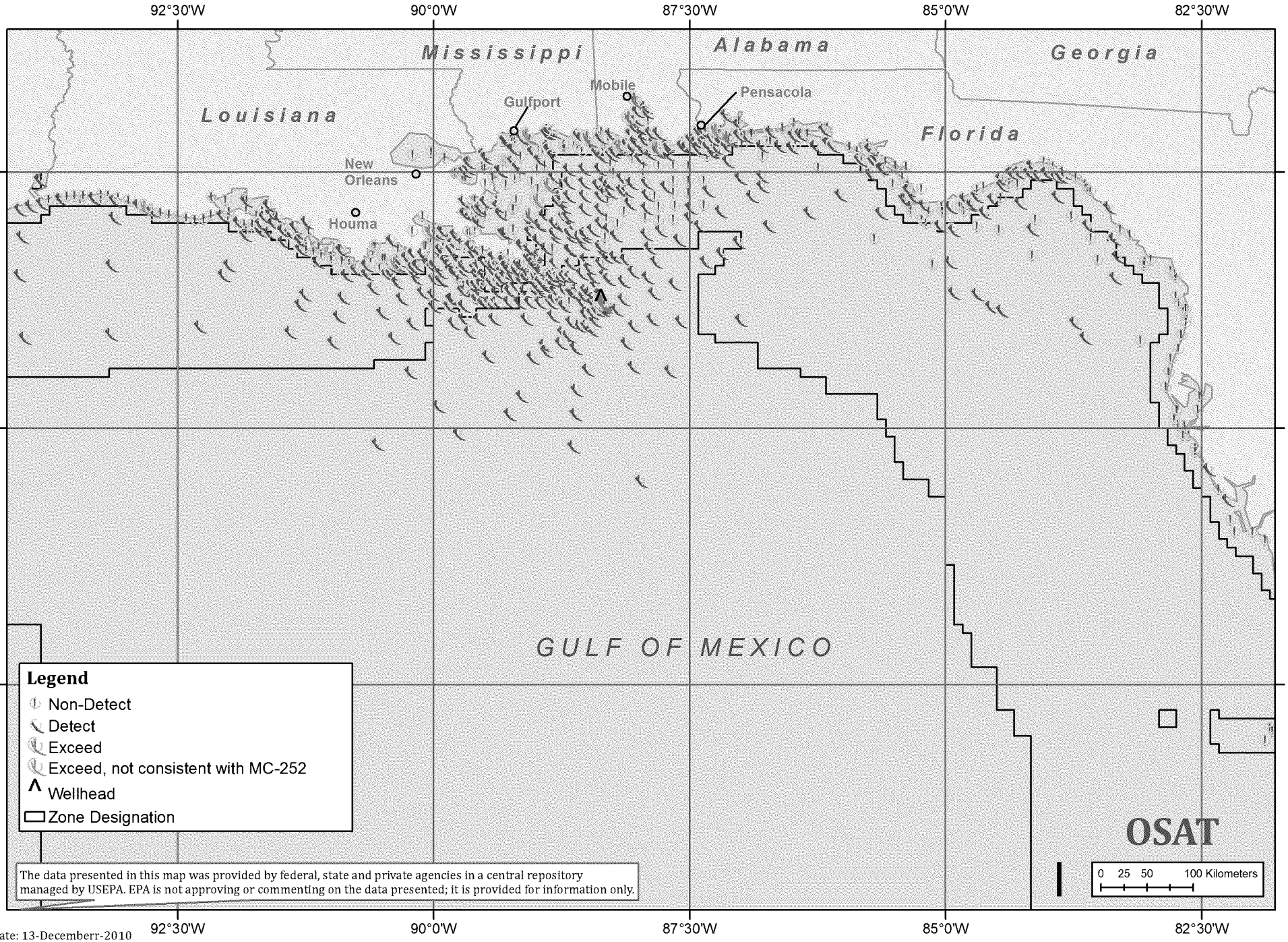


Map Date: 09-December-2010

Map 7.5a: PAH Aquatic Life Benchmark Analyses - All Sediment Samples

Sample Dates: 30 April 2010 to 23 October 2010

Deepwater Horizon Response, Gulf of Mexico



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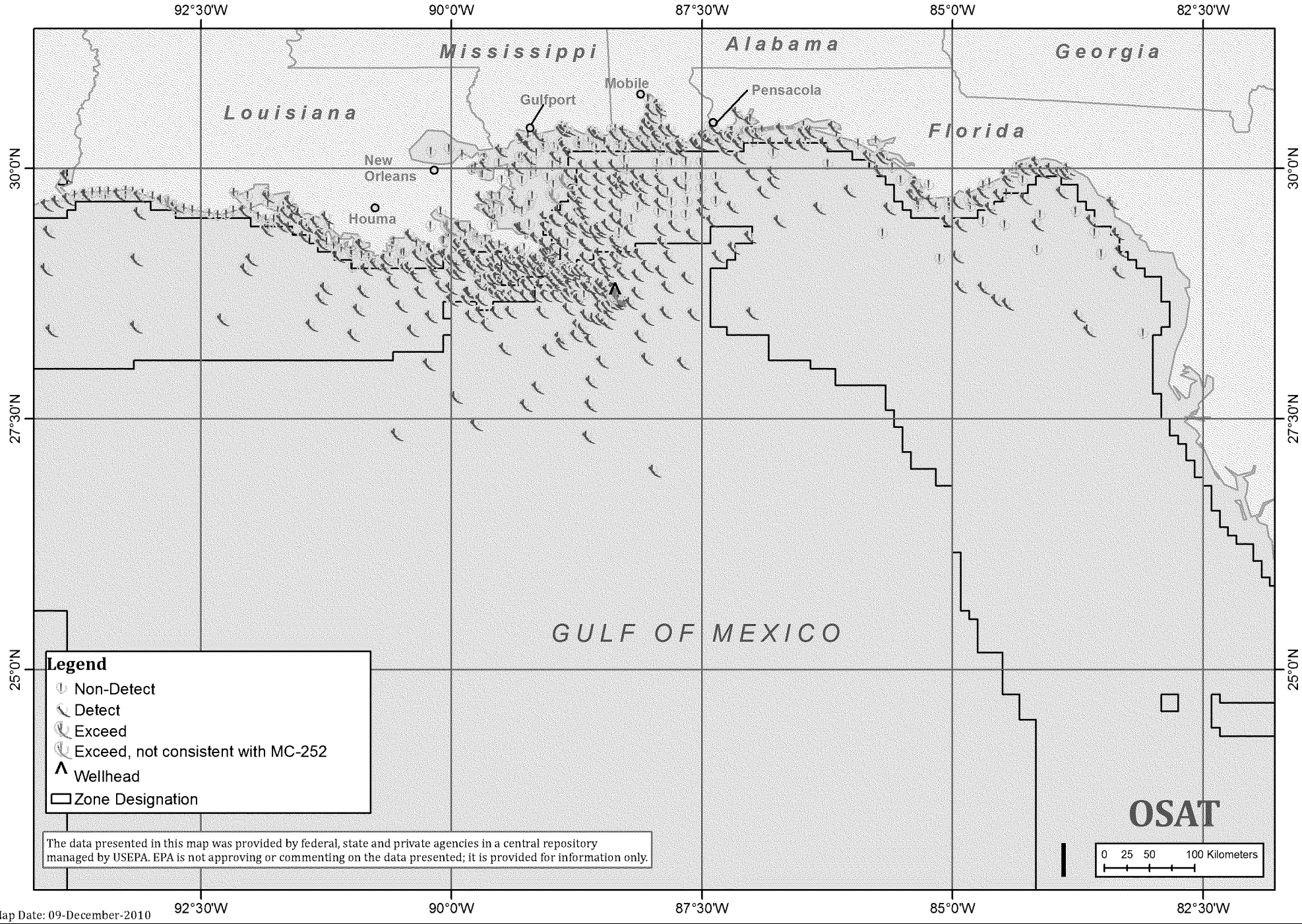
Map Date: 13-Decemberr-2010

Map 7.5b: PAH Aquatic Life Benchmark Analyses - All Sediment Samples Collected After 3 August 2010

Sample Dates: 4 August 2010 to 23 October 2010

Deepwater Horizon Response, Gulf of Mexico

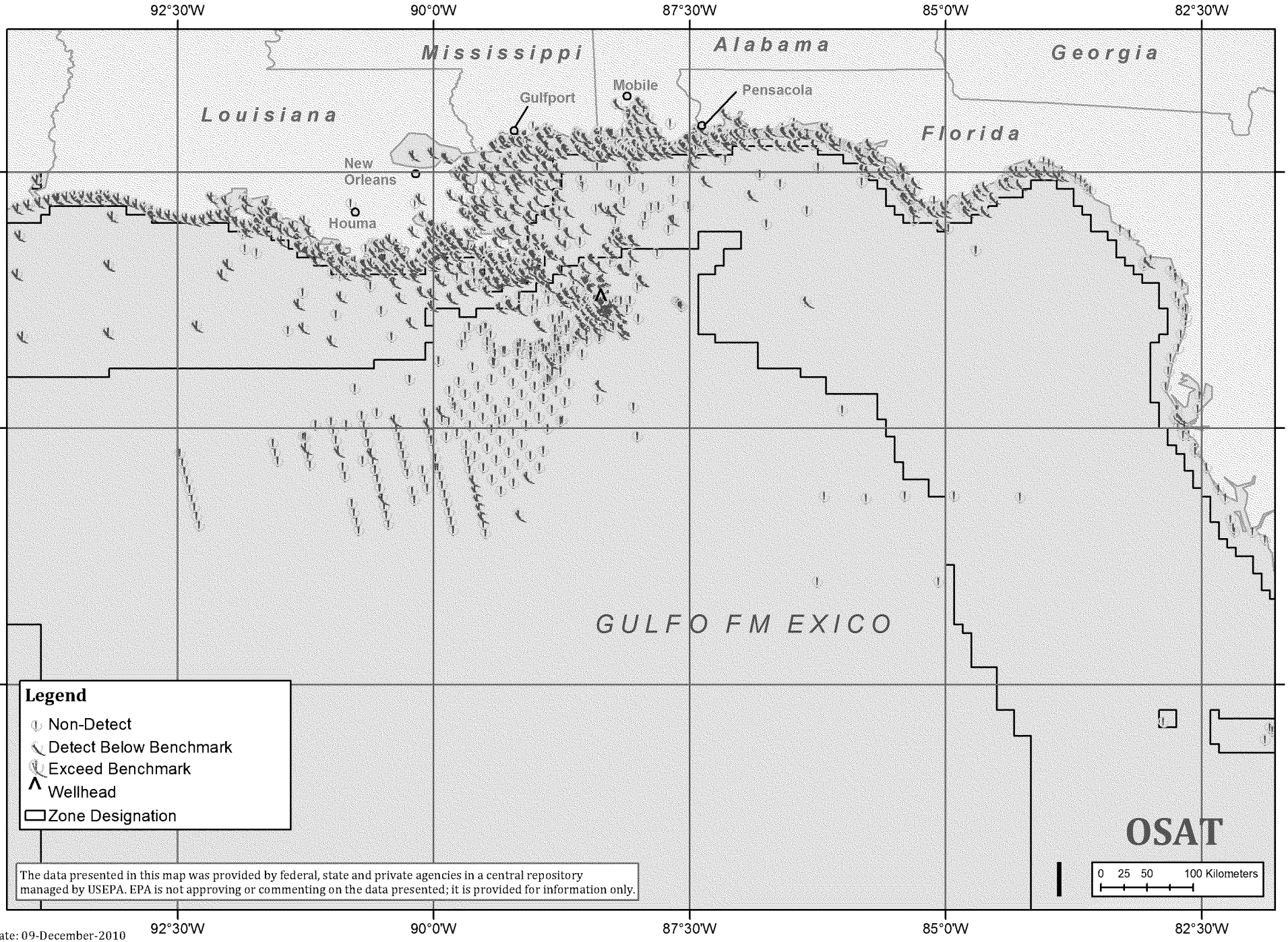


Map Date: 09-December-2010

Map 7.6: Human Health Benchmark Analyses - All Water Samples

Sample Dates: 30 April 2010 to 18 October 2010

Deepwater Horizon Response, Gulf of Mexico



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Map Date: 09-December-2010

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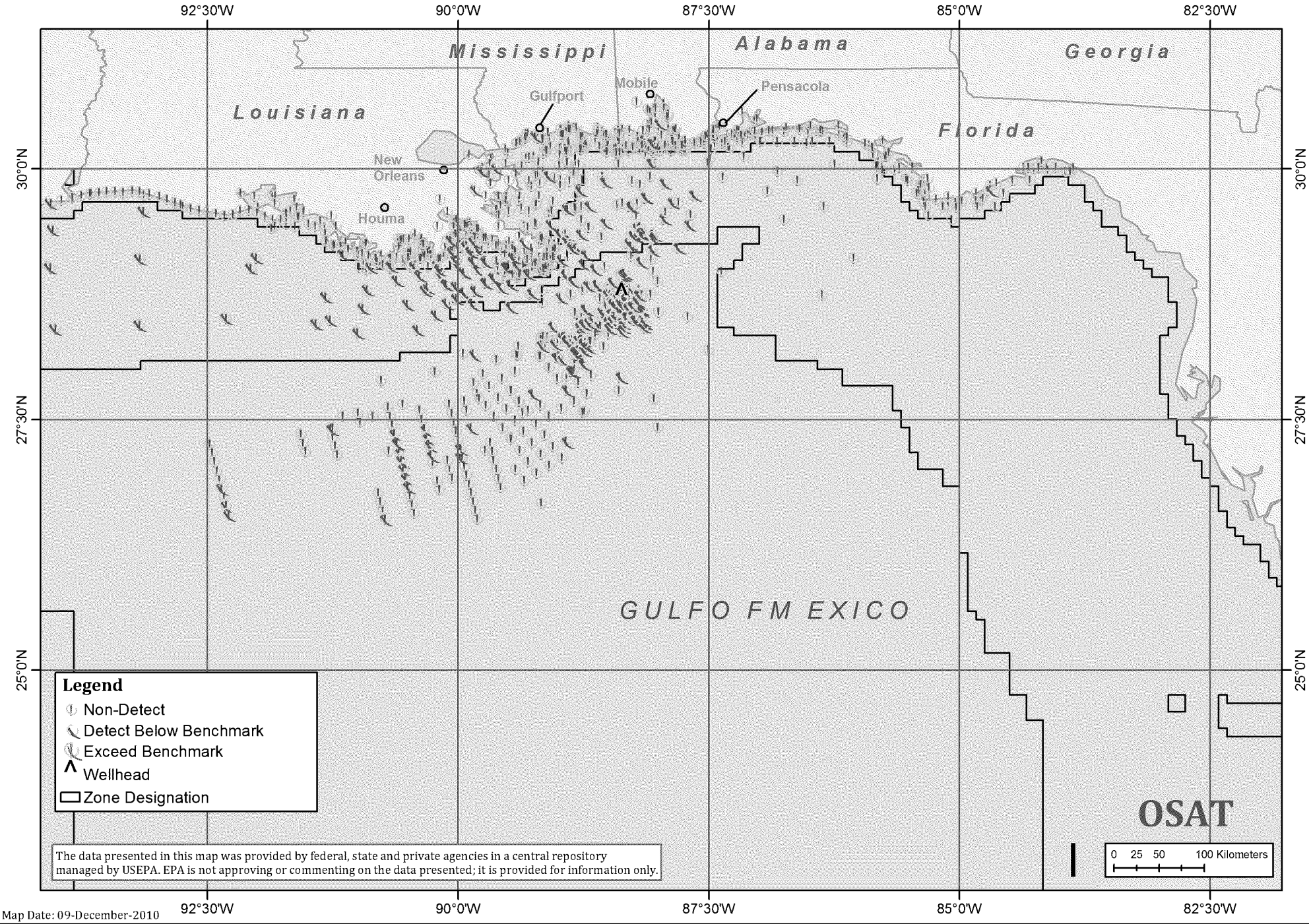
Map 7.7: Dispersant Indicators - All Water Samples

Sample Dates: 5 May 2010 to 18 October 2010

Deepwater Horizon Response, Gulf of Mexico



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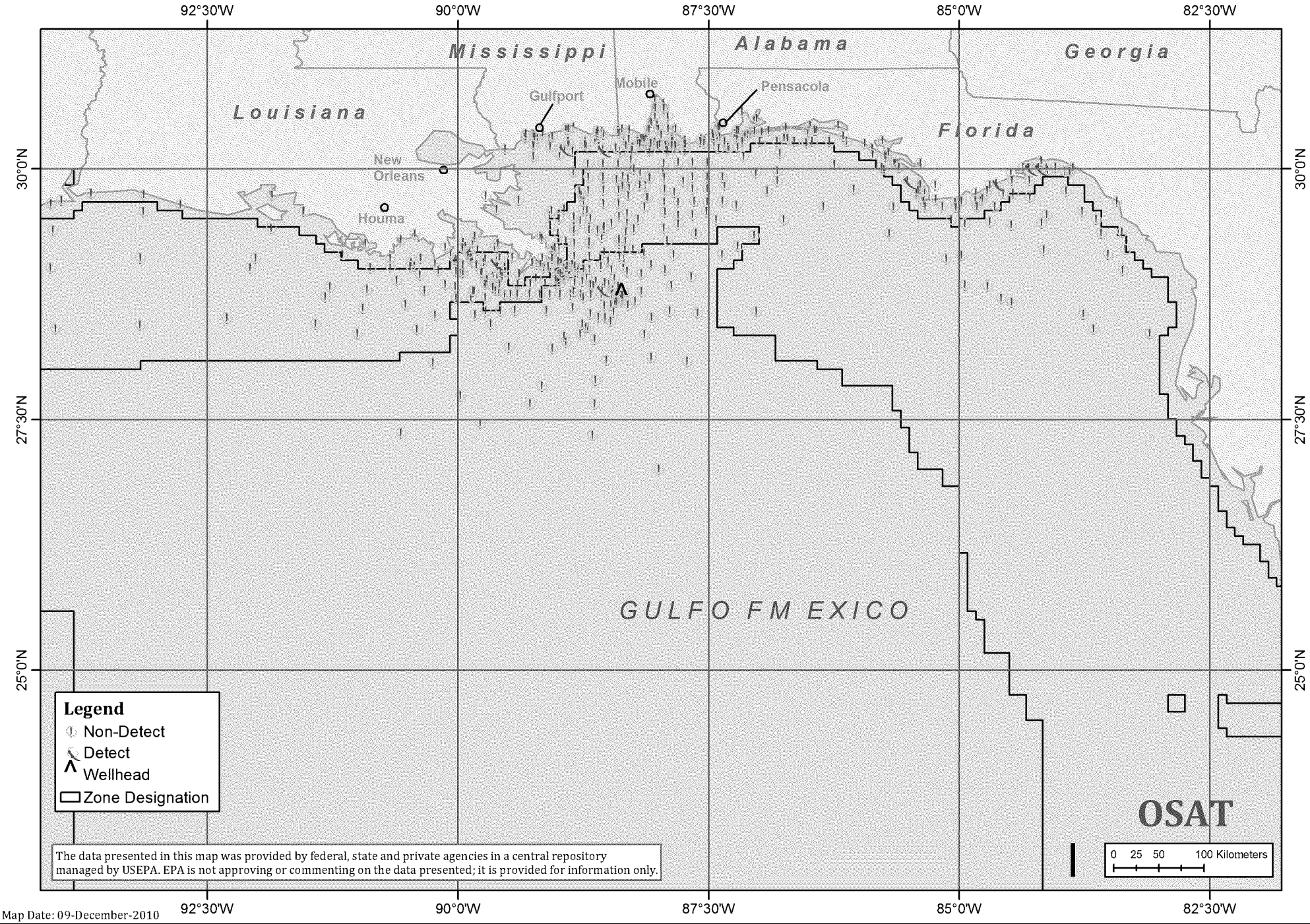
Map Date: 09-December-2010

Map 7.8: Dispersant Indicators - All Sediment Samples

Sample Dates: 5 May 2010 to 23 October 2010

Deepwater Horizon Response, Gulf of Mexico



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Map Date: 09-December-2010

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Appendix A: Benchmark Calculations and Screening Levels

A.1 Dispersant Benchmarks

The EPA has provided benchmarks to aid in the assessment of potential risk associated with dispersant-related chemicals.[1]

Water and sediment samples associated with the DWH oil spill were analyzed to determine if any dispersant-related chemicals could be detected. Some of the chemical analyses used existing EPA methods that EPA modified to enhance the performance of the method for the particular chemical. Other chemicals required the creation of new methods that EPA also developed. Table A-1 summarizes the dispersant-related chemicals measured during the response, the associated analytical method, and the aquatic toxicity benchmarks for these chemicals in water. Currently, there are no benchmarks for dispersant-related chemicals in sediments.

Table A-1. Summary of EPA analytical methods and screening levels for dispersant chemicals in water samples

Compound	CAS Number	EPA Method ID	Technology	Reporting Limits	EPA Aquatic Life Benchmark
Propylene Glycol	57-55-6	EPA SW 846 Modified 8270	Direct Inject GC/MS	500 µg/L	500,000 µg/L
2-Butoxyethanol	111-76-2	EPA R5/6 LC	Direct Inject LC/MS/MS	125 µg/L	165 µg/L
Di(Propylene Glycol) Butyl Ether (DPNB)	29911-28-2	EPA R5/6 LC	Direct Inject LC/MS/MS	1 µg/L	1 mg/L (chronic)[2]
Dioctylsulfosuccinate, sodium salt (DOSS)	577-11-7	EPA RAM-DOSS	LC/MS/MS	20 µg/L	40 µg/L (chronic)

A.2 Human Health Benchmarks

The EPA, in coordination with the U.S. Department of Health and Human Services, developed benchmarks to assess potential human health risks from exposure to oil-contaminated water.[3] Human health benchmarks are based on potential cancer and non-cancer risks associated with exposure to oil-contaminated water in the Gulf (for a child swimmer assuming an exposure of 90 hours). These benchmarks are designed for comparison to water samples only. If a sample exceeds a benchmark value for any of the chemicals listed below, the sample is reported as exceeding the Human Health Benchmark.

Table A-2. Human Health Benchmark values by chemical

CHEMICAL	CAS Number	Human Health Benchmark	Toxicity

[1] http://www.epa.gov/bpspill/dispersant-methods.html

[2] Chronic screening level for DPNB agreed upon by BP and EPA during the DWH response.

[3] http://www.epa.gov/bpspill/health-benchmarks.html

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Volatile Organic Compounds, µg/L			
Benzene	71-43-2	380	C
Cumene	98-82-8	20,000	N
Ethylbenzene	100-41-4	610	C
Total xylene*	108-38-3	18,000	N
Toluene	108-88-3	120,000	N
Semivolatile Organic Compounds, µg/L			
2-Methylnaphthalene	91-57-6	170	N
Polyaromatic Hydrocarbons (PAHs) µg/L			
Naphthalene	91-20-3	1,800	N
Acenaphthene	83-32-9	2,500	N
Fluorene	86-73-7	12,000	N
Anthracene	120-12-7	22,000	N
Fluoranthene	206-44-0	UD	
Pyrene	129-00-0	4,100	N
Benzo(a)anthracene	56-55-3	UD	
Chrysene	218-01-9	UD	
Benzo(b)fluoranthene	205-99-2	UD	
Benzo(a)pyrene	50-32-8	UD	
Benzo(k)fluoranthene	207-08-9	UD	
Indeno(1,2,3-cd)pyrene	193-39-5	UD	
Dibenzo(a,h) anthracene	53-70-3	UD	
Metals, µg/L			
Nickel	7440-02-0	15,000	N
Vanadium	7440-62-2	5,400	N
*total xylene based on the most toxic of the xylenes (m-xylene)			
C - Concentration for generally acceptable cancer risk level			
N - Concentration for generally acceptable exposure for non-cancer endpoint			
UD - Benchmark under development			

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A.3 Aquatic Life Benchmarks for PAHs

The EPA has provided benchmark values to aid in the assessment of potential risk to fish and other marine life that may come into contact with oil present in the water column or sediment. [1,2]

The effects of PAHs are additive across all of the PAH compounds in petroleum. For a single PAH, only a small percentage of the overall effect of the petroleum-contaminated mixture is measured. To determine the overall toxicity of PAHs in water or sediment, the contributions of every individual PAH compound in the petroleum mixture must be included (Table A-3). The effect of the mixture is calculated by adding together the fractional contributions of all components of the mixture. To estimate the total effect of the mixture of PAHs in an oil sample, the concentration of each compound is divided by a "potency divisor" before the ratios are added together (Table A-3). Potency divisors are not chemical-specific benchmarks, but are intermediates used in calculating the aggregate toxicity of the mixture. These potency divisors are used in the calculation to represent the amount of an individual chemical alone that could cause an adverse effect. After division by the acute or chronic potency divisor, the individual components are summed up – if the total is > 1, the sample exceeds the benchmark. Detailed sample calculations are available on the EPA website.[3]

Special consideration for sediment samples

To determine toxicity in sediment from PAHs, it is important to also factor in the amount of total organic carbon (TOC) in the sediment. When organic carbon is present in sediment, PAHs bind to it, making the PAHs less available to aquatic life, thus lessening their toxicity. In order to account for the differences in bioavailability, the dry weight-based PAH concentrations measured in sediment are divided by the TOC concentration.

Special consideration for samples in which alkyated PAHs were not measured

When evaluating a sample in which the alkylated PAHs were not measured, the EPA has devised a method to compensate for their contribution by using "alkylation multipliers". These multipliers account for the unmeasured alkyl compounds that are missed. The multipliers were created based on the reported analyses of a tar ball collected from Dauphin Island and forensically identified to be of MC252 origin. The multipliers were also checked against the published oil composition from the Exxon Valdez spill. These two samples were used by the EPA as they were available and represented two different crude oil types and weathering states. See Appendix C.3 for a discussion of the use of these multipliers within this report.

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[1] http://www.epa.gov/bpspill/water-benchmarks.html

[2] http://www.epa.gov/bpspill/sediment-benchmarks.html

[3] http://www.epa.gov/bpspill/water/explanation-of-pah-benchmark-calculations-20100622.pdf

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Table A-3. Oil related organic compounds, analytical methods, and potency divisors (acute and chronic) used in the cumulative PAH Aquatic Toxicity Benchmark calculation.

PAH Compounds	CAS Number	Alkylation Multiplier	Acute Potency Divisor		Chronic Potency Divisor	
			Water (µg/L)	Sediment (µg/kg C)	Water (µg/L)	Sediment (µg/kg C)
Benzene	71-43-2	N/A	27,000	3,360,000	5,300	660,000
Cyclohexane	110-82-7	N/A	1,900	4,000,000	374	786,000
Ethylbenzene	100-41-4	N/A	4,020	4,930,000	790	970,000
Isopropylbenzene / Cumene	98-82-8	N/A	2,140	5,750,000	420	1,130,000
Total xylene	108-38-3	N/A	3,560	4,980,000	700	980,000
Methylcyclo-hexane	108-87-2	N/A	463	4,960,000	91	976,000
Toluene	108-88-3	N/A	8,140	4,120,000	1,600	810,000
Naphthalene	91-20-3	120	803	1,600,000	193	385,000
C1-Naphthalenes	--	N/A	340	1,850,000	81.7	444,000
C2-Naphthalenes	--	N/A	126	2,120,000	30.2	510,000
C3-Naphthalenes	--	N/A	46.1	2,420,000	11.1	581,000
C4-Naphthalenes	--	N/A	16.9	2,730,000	4.05	657,000
Acenaphthylene	208-96-8	1	1,280	1,880,000	307	452,000
Acenaphthene	83-32-9	1	232	2,040,000	55.8	491,000
Fluorene	86-73-7	14	164	2,240,000	39.3	538,000
C1-Fluorenes	--	N/A	58.1	2,540,000	14	611,000
C2-Fluorenes	--	N/A	22	2,850,000	5.3	686,000
C3-Fluorenes	--	N/A	7.99	3,200,000	1.92	769,000
Phenanthrene	85-01-8	6.8	79.7	2,480,000	19.1	596,000
Anthracene	120-12-7	1	86.1	2,470,000	20.7	594,000

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PAH Compounds	CAS Number	Alkylation Multiplier	Acute Potency Divisor		Chronic Potency Divisor	
			Water (µg/L)	Sediment (µg/kg C)	Water (µg/L)	Sediment (µg/kg C)
C1-Phenanthrenes	--	N/A	31	2,790,000	7.44	670,000
C2-Phenanthrenes	--	N/A	13.3	3,100,000	3.2	746,000
C3-Phenanthrenes	--	N/A	5.24	3,450,000	1.26	829,000
C4-Phenanthrenes	--	N/A	2.33	3,790,000	0.559	912,000
Fluoranthene	206-44-0	1	29.6	2,940,000	7.11	707,000
Pyrene	129-00-0	2.1	42	2,900,000	10.1	697,000
C1-pyrene/ fluoranthenes	--	N/A	20.3	3,200,000	4.89	770,000
Benzo(a) anthracene	56-55-3	1	9.28	3,500,000	2.23	841,000
Chrysene	218-01-9	5	8.49	3,510,000	2.04	844,000
C1-Chrysenes	--	N/A	3.56	3,870,000	0.856	929,000
C2-Chrysenes	--	N/A	2.01	4,200,000	0.483	1,010,000
C3-Chrysenes	--	N/A	0.699	4,620,000	0.168	1,110,000
C4-Chrysenes	--	N/A	0.294	5,030,000	0.0706	1,210,000
Perylene	198-55-0	1	3.75	4,020,000	0.901	967,000
Benzo(b) fluoranthene	205-99-2	1	2.82	4,070,000	0.677	979,000
Benzo(k) fluoranthene	207-08-9	1	2.67	4,080,000	0.642	981,000
Benzo(e)pyrene	192-97-2	N/A	3.75	4,020,000	0.901	967,000
Benzo(a)pyrene	50-32-8	1	3.98	4,020,000	0.957	965,000
Indeno(1,2,3-cd)pyrene	193-39-5	1	1.14	4,620,000	0.275	1,110,000
Dibenz(a,h) anthracene	53-70-3	1	1.17	4,660,000	0.282	1,120,000
Benzo(g,h,i) perylene	191-24-2	1	1.83	4,540,000	0.439	1,090,000

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Appendix B: Data Management

B.1 Scribe

Early in the response, a decision was made to use Scribe as the official database for managing unifying, and distributing analytical sample data. Scribe is a database developed by the USEPA's Environmental Response Team to assist in the process of managing environmental data. Scribe is also managed by the EPA.

Data in Scribe includes all sub-surface water column and sediment samples collected as part of the response for laboratory analysis (i.e. data originating from a number of agencies including Federal and State agencies, as well as BP-contractors).

Scribe captures sampling, observational, and monitoring field data. Examples of Scribe field tasks include soil sampling, water sampling, air sampling and biota sampling. Scribe can import electronic data, including analytical lab result data and sampling location data, such as GPS.

Scribe outputs include labels for collected samples, chain of custody generation, and analytical lab result data reports. Scribe provides a flexible user interface to manage, query, and view all this information. Scribe supports exporting electronic data for user services, such as GIS tools and spreadsheets, so sampling data may be further analyzed and incorporated into report writing and deliverables.

The main Scribe server (Scribe.NET) provides a method of storing and sharing Scribe projects. Using Scribe.NET, Scribe projects can be shared between Scribe desktop clients and/or enterprise Oracle/SQL database clients. Scribe projects are "Published" from the Scribe desktop client, and other desktop/enterprise users "Subscribe" to the published projects. Users can subscribe to individual or multiple projects. Regional or global subscriptions can also be created for sharing entire sets of published projects. Some Scribe functions and limitations are listed:

- Data may be loaded manually or in an electronic file.
- There is no error checking utility to verify that the electronic data have been loaded successfully.
- Sample location coordinates output from a global positioning system (GPS) may be entered electronically.
- Without expert knowledge, it is still possible for the user to complete a sampling event by following logical steps.
- Some knowledge of database functions is required to filter results and set up views.

B.2 NODC

The NOAA National Oceanographic Data Center (NODC) served as the official database and archive for oceanographic data collected aboard vessels. Examples of products that OSAT used from NODC included CTD, fluorescence, and dissolved oxygen profiles. The initial

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repository for oceanographic data from vessels was the NOAA Office of Response & Restoration (OR&R) secure file transfer protocol (SFTP) server. OSAT used a variety of products from this site including, for example, daily ship deliverables with qualitative descriptions of sediment samples. The NOAA OR&R Environmental Response Management Application (ERMA) served as the primary visual UAC. These data are available in ERMA's map and display format. Oil presence or absence observational data are also available in map and display format in ERMA.

B.3 Quality Control, Quality Assurance and Validation

The purpose of data validation was to detect and to correct errors and then to verify that all data values correctly represent the actual conditions at the sampling station. Effective data validation procedures were usually completely independent of the procedures of the initial data collection. Moreover, it was advisable that the individuals responsible for data validation not be directly involved with data collection. Data validation was necessary to identify data with errors, biases, and physically unrealistic values before they were used for identification of exceedances, for analysis, or for modeling. Data validators were charged to validate data generated by the laboratories for chemical analyses of project samples. Data generated for toxicological assessments were reviewed by appropriate science advisors.

In addition to the laboratory quality assurance (QA) reviews, data were evaluated and validated by experienced personnel for the following criteria:

- Conformance with requested testing requirements,
- Completeness,
- Reporting accuracy (including hardcopy of Electronic Data Deliverables),
- Confirming receipt of requested items, and
- Traceability, sensibility, and usability of the data.

In addition to the above criteria, the data validators used guidance from two EPA publications where appropriate and applicable:

- *US EPA Contract Laboratory Program National Functional Guidelines for Superfund Organic Methods Data Review, EPA-540-R-08-01* (June 2008)
- *National Functional Guidelines for Inorganic Data Review* (October 2004).

It should be noted that these guidelines were not completely applicable to the EPA and SW-846 methods used in the response. Consequently, professional judgment was used to evaluate data usability.

Data acquired by subsurface monitoring and sampling efforts were considered validated upon completion of quality assurance (QA) and quality control (QC) procedures. The originating entities that prepared the sampling plans for the data and results, but not those individuals involved in taking the samples, were responsible for QA, QC, and validation. QA/QC procedures begin at the time of data capture (such as sampling procedures shipboard), in laboratory settings, and before data are delivered to Scribe or NODC. The EPA's QA levels and their definitions are listed below:

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Level 1: A verification and validation based only on completeness and compliance of sample receipt condition checks.

Level 2: A verification and validation based on completeness and compliance checks of sample receipt conditions and both sample-related and instrument-related QC results.

Level 3: A verification and validation based on completeness and compliance checks of sample receipt conditions, both sample-related and instrument-related QC results, and recalculation checks.

Level 4: A verification and validation based on completeness and compliance checks of sample receipt conditions, both sample- and instrument-related QC results, recalculation checks, and the review of actual instrument outputs.

One exception to the validation described above is the data analyzed from the early stages of the response at the Louisiana State University (LSU) laboratory in Baton Rouge. These data were not validated to Level 2 because some sample documentation lacked information pertaining to chain of custody or laboratory standard operating procedures for preparation and analyses of samples. These data also had higher than normal method-detection limits than the data sets from commercial laboratories with rigid standard procedures.

B.4 Qualifications on the Data—Lab Qualifiers

The following definitions provide brief explanations of the qualifiers assigned to results in the Scribe database for the Deepwater Horizon response.

B The analyte is found in the associated blank, as well as in the sample. It indicates possible/probable blank contamination and warns the data user to take appropriate action. This flag is used to positively identify a target compound list (TCL) compound. A tentatively identified compound (TIC) found in both the sample and the associated blank receives an "R" flag.

C The sample results are confirmed by other analytical techniques, including analysis of a reference standard.

D This flag identifies all compounds in an analysis at the secondary dilution factor. If a sample or extract is reanalyzed at a higher dilution factor, in the "E" flag, the "DL" suffix is appended to the sample, and all concentration values reported on Form 1 are flagged with the "D" flag.

E This flag identifies compounds whose concentrations exceed the calibration range of the gas chromatograph/mass spectrometry (GC/MS) instrument for that specific analysis.

J The analyte was positively identified, but the associated numerical value is an estimated concentration of the analyte in the sample based on its associated quality measures.

JN A combination of the "J" and "N" qualifiers, this analysis indicates that the analyte is "tentatively identified" and the associated numerical value may not be consistent with the amount actually present in the environmental sample.

N The analysis indicates the presence of an analyte for which presumptive evidence creates a "tentative identification."

R The sample results are rejected due to serious deficiencies in the ability to analyze a sample and meet QC criteria. The presence or absence of the analyte cannot be verified.

U The analyte was analyzed for but not detected above the method detection limit, as defined in the laboratories' Statements of Work.

U* This analyte is considered "not detected" because it was detected in an associated blank at a similar level.

UJ The analyte was analyzed for but not present above the level of the associated value. The associated numerical value may not accurately or precisely represent the concentration necessary to detect the analyte in this sample.

UR Unusable detection limit; analyte may or may not be present in this sample

X The datum is tentatively rejected because project-specific data quality objectives (e.g., for sensitivity, accuracy, or precision) were not met or were not demonstrated. When objectives for sensitivity are not met, the X flag typically indicates that a result (a detection or non-detection) is potentially unusable with respect to an action level (e.g., the result does not demonstrate that a target analyte is actually present in an environmental sample at a concentration above or below a risk-based decision limit).

B.5 Data Flow

During the early period of the response and for most of the response time frame, data flow was controlled by the two primary Incident Command Posts (ICPs) in Houma, LA, and in Mobile, AL. At the ICP level, sampling plans were devised, field procedures defined, analyses outlined, and laboratories selected as needed. Various organizations ran projects for the ICPs and input data to Scribe. Primary projects are listed in Table B-1.

Data tended to flow as follows:

- After collecting individual samples onboard vessels or collecting individual samples in the field onshore, samples were transferred to the Sample Receiving Office (SRO) in Houma or in Mobile. Samples received at the SRO were unpacked, subjected to several QC checks and repacked for shipment to the appropriate laboratory (or laboratories) for analysis.
- After analysis, reports were issued to parties identified on the Analytical Request Form or as otherwise communicated to the laboratory. Analytical data, and electronic deliverables were sent to the designated entity. For BP the data were sent to

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Environmental Standards, Inc. for uploading into the EQuIS[1] database or to CTEH, LLC (Center for Toxicology and Environmental Health). Data for other entities was sent to the appropriate agency (Federal or State) for uploading in to their in-house databases.

- The data originator was charged with performing a Level 2 data validation, and the data was subsequently published to Scribe (Table B-1).

Table B-1. Entities that published data to Scribe.

PROJECT NAME	ORGANIZATION
R06 Deep H2O Horizon Reporting	General Dynamics
DW_Operations	USEPA/ERT
DW_Reporting	USEPA/ERT
CTEH_DW_Sampling_Analytical	CTEH
BP_DW_Sampling_Analytical	Weston Solutions
LDEQ_DW_Sampling_Analytical	Weston Solutions
EnvStds_DW_Sampling_Analytical	Environmental Standards
ADEM_DW_Sampling_Analytical_Monitoring	Alabama DEM
CTEH_DW_Monitoring	CTEH
TS_DW_Monitoring	CTEH
MSDEQ_DW_Sampling_Analytical	MDEQ
FLDEP_DW_Sampling_Analytical	Florida DEP
NPS_DW_Sampling_Analytical	ERT
ALECI_DW_Sampling_Analytical	EnviroChem
Envstd_Sampling_Analytical	Environmental Standards
NOAADW	Weston Solutions
USGSDW	Weston Solutions

The Unified Area Command (UAC) located in Robert, Louisiana, and later in New Orleans, was advisory to the ICPs. Sampling plans were developed by various partners within the UAC and ICPs to address a diverse spectrum of objectives. Continuous improvement in procedures occurred as sampling plans, data management procedures, and QA plans were cross-referenced and standardized as much as possible as the response progressed.

Soon after the NIC directive of 3 August, the UAC began sampling of water and sediments beyond the shoreline. The Implementation Plan (13 September) and Operational Annex (1 October) assessed the existing and completed subsurface sampling and developed a framework to address identified sampling gaps. Data flow from existing

[1] A commercial data management system

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programs tended to follow the existing structures. New data flow followed GC-IMT procedures. All analytical data collected under this plan is or will be published to Scribe.

B.6 Long-Term Data Availability and Access

Long term data management, archive, and public dissemination of all analytical and oceanographic data collected in association with this response will be provided by the National Oceanographic Data Center (NODC) and the National Geophysical Data Center (NGDC).

Oceanographic data records are currently publicly available at the NODC DWH web site[1], as well as the NODC THREDDS Server[2] and the NCDDC EcoWatch THREDDS Server[3]. Geospatially-enabled direct data access is also available on the DWH Atlas[4]. As the data cleanup is completed within the Scribe database, the analytical chemistry data will also become publicly available.

[1] http://www.nodc.noaa.gov/General/DeepwaterHorizon/support.html
[2] http://data.nodc.noaa.gov/thredds/catalog/DeepwaterHorizon/catalog.html
[3] http://ecowatch.ncddc.noaa.gov/thredds/catalog_dwh.html
[4] http://www.ncddc.noaa.gov/website/google_maps/DWHAtlas/mapsAtlas.htm

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C.1 Issues Encountered with Response Dataset in Scribe

Through the course of the response, approximately 17 entities published data to Scribe. Many of these entities also used a number of different labs for sample analysis. Laboratory standard operating procedures, analysis methods, and data formats varied, particularly early in the response. In addition, different naming conventions and relational data structures utilized by the different data originators resulted in numerous issues in the actual transfer of data to Scribe. For example, although sample latitude, longitude, depth) existed, it was often unsuccessfully transferred resulting in missing metadata within Scribe.

A significant issue that was not resolved at the time of this report had to do with non-unique sample names for different analyses conducted on the same sample. For example, in many cases, analyses for VOCs were run at a different lab than analyses for PAHs. Because the sample names were different (and not in one systematic way) the queries used to access the data in Scribe were not always able to match up all the analyses required for comparison to the aquatic life benchmark for PAHs. This also applied to sediment samples in which different analyses were run on the different cores originating from one cast (e.g. total organic carbon from one core barrel, PAHs from another). In this case, these were somewhat unique samples, as they originated from different cores within one cast, however, the benchmark calculation required them to be normalized together. Although the data managers have attempted to address this through the use of "linked" sample numbers, this has not been successfully resolved within a Scribe query to date. Therefore, as noted in the report body, multiple samples included in this report excluded VOC analyses in the benchmark calculation and/or used a default value for TOC even though it was measured.

Inconsistent naming conventions for the various analytes in data published to Scribe resulted in numerous difficulties with utilizing the entire available database. These inconsistent naming conventions resulted both in potential missing analytes (i.e. analyte name used by data originator was not included in search query), or double counting of analytes (i.e. data originator revised the analyte name but the data was inadvertently published twice). This posed a significant challenge to establishing confidence in the PAH aquatic toxicity ratios in particular, as they required the addition of results from numerous analytes. Many of these inconsistencies were ultimately resolved, but preliminary maps and analyses had errors and were continually revised as the underlying dataset in Scribe was revised. This issue was ameliorated in more recent sample analyses when input was standardized, including analyte naming conventions, concentration units, and inclusion of metadata such as location, depth, matrix, etc.

The datasets pulled from Scribe by the OSAT were also inconsistent — data available one day was not retrieved consistently on subsequent queries. Note, the raw data itself was unchanged, but the number of samples retrieved by the query was not consistent. Explanations of this inconsistency include the instability in the underlying data structure as data originators refreshed their datasets to Scribe, changes in query methods used by

rotating data support personnel, or QA/QC activities of which the OSAT was unaware. OSAT continued to work this issue through the final data retrievals.

On a few occasions, Scribe was unable to handle the volume of data being uploaded (these occurred after data originators performed substantial revisions and re-uploaded large datasets). The solution was to limit the upload to 75,000 records at a time – however, this meant that refreshing the data took almost 12 hours to complete.

In addressing all of the issues encountered with utilizing the Scribe database, corrections were cumbersome and time consuming, and required multiple republishing of data from the various sources. It took an inordinate number of attempts and a great deal of time to get consistent, reliable downloads of datasets from Scribe after all of the data had been published. Only very technically skilled individuals could accomplish this operation, thus limiting the utility of the database.

C.2 Excluding Data Inappropriate for OSAT Review

During the response, many sampling programs were designed for diverse objectives. Although suitable for their intended purpose, not all of the data input to Scribe for the response were appropriate for use in OSAT reviews of the three defined areas. For example, Rapid Assessment Team (RAT), Forensic Rapid Assessment Team (FRAT), Coast Guard Special Monitoring of Applied Response Technologies (SMART), anomalous phenomena, waste samples, and others were not designed for evaluation of the environmental conditions. For example, RAT and FRAT data were actual samples of oil and not of water or of tarballs. Well over 40,000 samples were analyzed for the response; of those samples, approximately 17,000 (less than 50%) were appropriate for OSAT review

As data from many of these programs was also published, it was necessary to exclude these samples from consideration. Unfortunately, as they did not always have a unique identifier, this was more difficult in practice than anticipated. The inclusion of these samples overestimated exceedances of toxicity benchmarks, as they were in many cases, actual oil samples.

C.3 Alkylated PAHs

The EPA aquatic toxicity ratios (Appendix A.3) are meant to be calculated using the 41 oil-related organic compounds (i.e., including benzene, toluene, ethylbenzene and xylene or BTEX for short, priority pollutant polycyclic aromatic hydrocarbons (PAHs), and the alkylated PAH compounds). However, many samples (particularly in the nearshore sampling zone) were analyzed only for the 16 priority PAHs that are the norm for the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA, or Superfund) style evaluations. The EPA determined that when evaluating a sample in which the alkylated PAHs were not measured, it is necessary to compensate for their contribution by using “alkylation multipliers”: these multipliers are meant to account for the unmeasured alkyl compounds (see Appendix A.3 for more detail on the development of these multipliers).

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EPA's 16 priority PAHs should NOT be the only targeted oil related organic compounds to be measured and monitored during an oil spill. Crude oil generally consists of only 1-2% parent PAH compounds and alkylated homologs: of this fraction, crude oil contains mostly the alkylated PAHs, hence concentrations of these compounds needs to be assessed. Although multipliers have been developed to account for their contributions based on measured concentrations of the parent compounds (Appendix A.3), the parent PAH concentrations in crude oil may be close to the detection limits and hence reported as 0, potentially resulting in the multipliers being ineffective. OSAT recognized this issue soon after they began reviewing the dataset and made a recommendation that all future samples be analyzed for the complete set of analytes (20 September, Appendix F). However, a substantial number of earlier samples in the nearshore zone (and a much smaller subset in the other zones) included only the 16 priority PAHs.

As it was necessary to use the multipliers for many nearshore samples collected pre-20 September, OSAT attempted to assess the efficacy of drawing conclusions using toxicity ratios calculated from samples in which only the 16 priority pollutants were measured. A statistical comparison was performed. For all samples with the full suite of 41 analytes, toxicity ratios were calculated twice: once using all 41 analytes and a second time using only the 16 priority PAHs (with the multipliers). Toxicity ratios calculated using priority PAHs alone were positively correlated (though statistically not significant, P-value >0.05) with those from the 41 analytes using Pearson correlation coefficient. High variability in toxicity ratios precluded estimating an accurate and precise relationship between the two. This high variability likely resulted from the broad range in concentrations and weathering states.

Although this analysis indicated some limitations in the applicability of using these multipliers, the OSAT decided it was more conservative to include these data (with appropriate caveats) for assessing potential exceedances of the aquatic life benchmark. Therefore, we include in this report toxicity ratios calculated using both the 16 priority PAHs alone and using all 41 analytes. Fortunately, the most recent samples (post the 20 September OSAT recommendation) were analyzed for the full suite of analytes[1] – these samples are the most essential for determining post-impact environmental conditions.

C.4 Total organic carbon

Total organic carbon (TOC) is necessary to calculate the aquatic life ratio for sediments (Appendix A.3). Because organic chemicals tend to adhere to organic matter in sediment, the higher the TOC is in sediments, the lower will be the hazard to organisms from toxic organic compounds.

TOC analysis was not always performed on sediment samples early in the response. In addition, in multi core sampling, the TOC was analyzed from a separate barrel from the PAHs and the samples were not able to be linked within the Shoreiqueries (Appendix C.1). In cases where TOC was not available, OSAT used an assumed TOC of 1% in the calculations. Using this TOC value provided a modestly conservative estimate of the risk hazard (the mean TOC detected in samples was 1.22%). However, as TOC generally

[1] With the exception of EPA Region 6 samples which are currently being reanalyzed.

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decreases in the deep water, this may not be a conservative estimate for all deep-water sample sites. An independent calculation of the aquatic life ratio in offshore and deep-water samples which utilized the measured TOC was performed outside of the Scribe query. Results of this analysis indicated one additional exceedance in deep-water sediments within 3 km of the wellhead.

C.5 Volatile organic compounds

Volatile organic compounds (VOCs) such as BTEX, are required for calculating the aquatic toxicity ratios. Although the analysis for these compounds was standard in samples from the deep-water and offshore zones, some nearshore samples did not include these compounds in their analysis. In addition to this, in many recent water and sediment samples from the offshore and deep-water sampling zones, non-unique or non-linked sample names for different analyses precluded matching up VOC analyses with PAH analyses (Appendix C.1/C.7).

However, in samples that were analyzed for BTEX, these compounds were never detected in MC252 oil that reached the shore, nor were BTEX compounds detected in nearshore sediments. Analyses of oil samples from the source toward the shore showed that VOCs were either dissolved or evaporated from the MC252 oil near the source, and oil that approached the nearshore environment no longer had BTEX compounds present (Gong et al., 2010, Brown et al., 2010).

C.6 Method detection limits

Different data sets had different method detection limits (MDLs), for specific analytes, which complicated the comparison of data and calculations of aquatic life benchmark indices across the response. In some cases, such as at CERCLA sites, the EPA recommends using half (0.5) the MDL in calculating environmental benchmarks. Some analyses from the response had such high MDLs, however, that this adjustment resulted in exceedances where no analytes were actually detected. The EPA example method of calculating benchmarks for this incident used a zero value for results below the method detection limits. Therefore, only analytes that were "detected" at or above the MDLs were used for calculation purposes. This process is in conformance with the example calculation method provided by the EPA on their website[1]. Data with concentrations below the MDL were labeled "U" for non-detect in the qualification column in Scribe.

C.7 Multi Core Sediment Samples from Multiple Barrels

During some sediment sampling activities both offshore and deep water, multi-core devices were used that took several sediment core samples with a single device in one cast. In some cases, samples for volatile organic compounds (VOCs) were taken from one core in the array, and samples for semi-volatile organic compounds (SVOC) were taken from another. Because the samples for VOC analyses were from different core barrels, they were given names slightly different from the samples for SVOC analysis. Calculating aquatic life

[1] http://www.epa.gov/bpspill/water/explanation-of-pah-benchmark-calculations-20100622.pdf

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toxicity ratios combines both analyses. Unfortunately, the naming conventions utilized precluded matching up the samples within the Scribe queries (Appendix C.1).

C.8 Exceedances Resulting From LSU samples

As noted in the section on QC, QA, and data validation, a number of analyses from LSU could not be validated because some documentation lacked chain of custody or laboratory standard operating procedures for preparing and analyzing samples. These samples also displayed some of the highest aquatic toxicity ratios. From audit interviews, it appears that these samples were screened after extraction and that only the samples with the greatest indication of contamination were analyzed. Additionally, some samples of oily water that included free-phase petroleum were included in the "water" analyses. Nevertheless, these were key samples in the early stages of deep water monitoring and were included in the OSAT review.

C.9 J data

Data labeled J in the qualification column were used in benchmark calculations. A J value indicated that an analyte was positively identified, but the associated concentration was estimated because the confidence in the quantitation of that analysis was below the laboratory's quality performance measure. Although in many environmental reviews J data is excluded from regulatory consideration, these data were kept in the OSAT review to err on the conservative (high) side of considering analyte concentrations..

C.10 Tentatively identified compounds

The TICs (labeled **N** in the qualification column) were not used in benchmark calculations.

C.11 DEEP

The Dispersant Environmental Effects Project (DEEP) monitored dispersant effectiveness during the response. This study involved sampling water beneath observed surface oil (at 1 m and 10 m depth) both before and after dispersant application (as well as some additional background sampling in areas without visible sampling was conducted across all three zones, in the months of May, June and July 2010. Although this study was not intended for assessing distribution of oil or dispersant products in the subsurface environment, as it directly targeted known impacted areas, it is included in the dataset reviewed by the OSAT.

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Appendix D: Characterization of Source in Exceedance Samples

This section describes the data evaluation process used by OSAT to determine the source of analytes present in water and sediment samples that exceeded the chronic aquatic life benchmark. In general, the focus was on determining whether exceedance samples were "consistent" or "not consistent" with MC252 oil, with some samples remaining of indeterminate origin when not enough data was available. Samples whose source was indeterminant were considered as consistent with MC252 in all plots and tabulations of exceedances within this report. Depending on the analytes available for a sample, different methods were used for source determination. The following describes source determination for BTEX-only samples, samples containing only priority pollutant PAHs, and full-alkyl PAH samples.

D.1 BTEX-Only Samples

If BTEX analytes were the only compounds detected for a sample[1] with an aquatic life exceedance, then the BTEX compounds present were considered to be from a petrogenic (MC252) source. This was a conservative approach that assumed the presence of BTEX in water samples was indicative of a water soluble fraction or water accommodated fraction (WAF) from petroleum. Since a source determination cannot be based on BTEX data alone, the source was considered to be MC252. Nearly all samples in this category were from the deep water areas, and likely associated with the deepwater fluorescence (~1100-1300 m) documented by the oceanographic surveys.

D.2 Types of PAH Samples

Two types of PAH sample data were available for the water and sediment samples, priority pollutant (PP) PAH and full alkyl PAH. The evaluation process to determine the presence of MC252-based hydrocarbons for samples with aquatic life exceedances differed based on whether PP PAH or alkyl PAH data were available. A description of the procedure for each of the two data types is described below.

D.3 Priority Pollutant PAH Samples

The first step was to evaluate the distribution of PAHs in the sample using a bar graph of the analyte concentrations. This was done to determine if: a) a possible petrogenic (petroleum related) signature was present, or b) a possible pyrogenic (combustion related) signature was present. For water samples, the evaluation included a possible water accommodated fraction distribution.

A petrogenic PAH distribution was interpreted if the PAH analytes present were consistent with fresh or weathered petroleum. This included the presence of naphthalene, fluorene, and phenanthrene, with lower levels of chrysene. A possible petrogenic WAF was interpreted if naphthalene, fluorene and phenanthrene were present, with a general pattern of naphthalene higher in abundance than fluorene which is higher than phenanthrene. A PP PAH petrogenic

[1] Detectable concentrations of BTEX were found almost exclusively in water samples and only rarely in any sediments.

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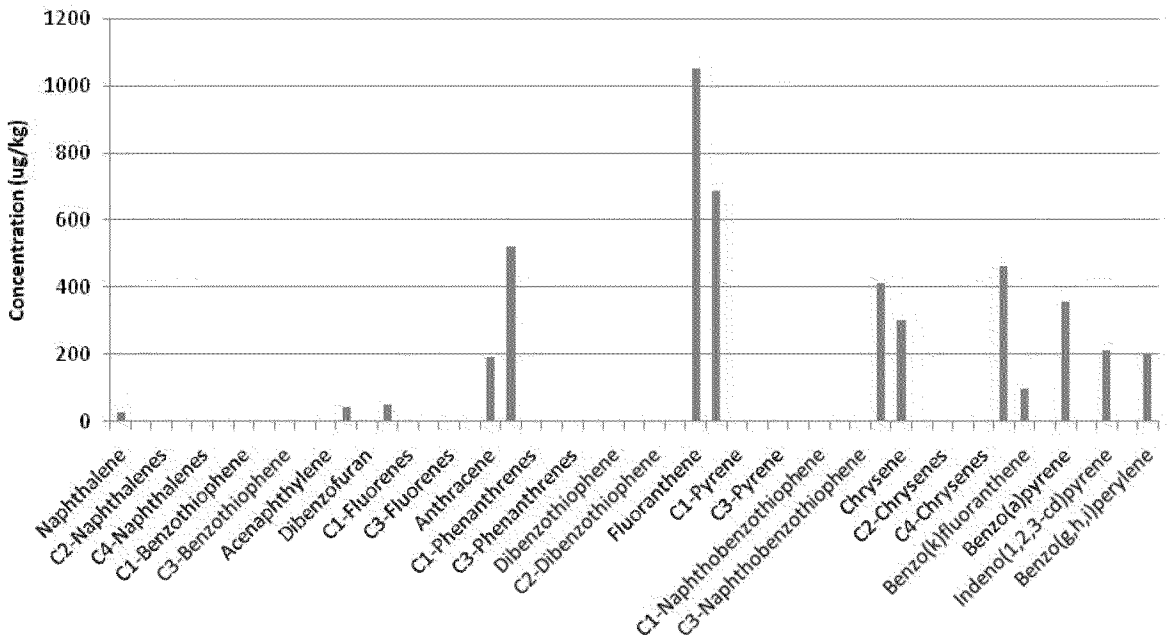
distribution was categorized as "consistent with MC252 oil", since determining the source is not definitive based on the PP PAH data alone. If only one or two petrogenic PAH analytes were detected (e.g., naphthalene alone, which is a common lab contaminant), then the sample was categorized as "indeterminate" source, as not enough information is present to make a source determination.

A pyrogenic PAH distribution was interpreted if the common pyrogenic PAHs were the primary components in the PP PAH distribution. The PP PAHs characteristic of a pyrogenic source include: fluoranthene, pyrene, benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benz(e)pyrene, benz(a)pyrene, indeno(1,2,3-c,d)pyrene, dibenz(a,h)anthracene and benz(g,h,i,)perylene. As is common with pyrogenic PAH distributions, fluoranthene and pyrene were often the most abundant analytes in the overall distribution. Samples identified as pyrogenic PAH were classified as being from "not consistent with MC252 oil". An example histogram of PAHs from a pyrogenic sediment sample is shown in Fig. D-1.

Fig. D-1. Histogram of PAHs from a pyrogenic sediment sample. The sample ID is T007-1331-100807-SD-1 collected on 7 August.



In some cases additional hydrocarbon data, such as total petroleum hydrocarbon (TPH), gas range organics (GRO), diesel range organics (DRO), and oil range organics (ORO), were available in addition to the PP PAH. If available, the other hydrocarbon data was evaluated and used to confirm the presence of crude oil in the samples. For example, in one case the absence of crude ORO, but the presence of DRO hydrocarbons was used to categorize a sample with a petrogenic PP PAH designation as "not MC252 oil" since diesel was the primary hydrocarbon constituent.

D.4 Full-Alkyl PAH Samples

The initial evaluation of the alkyl PAH data followed a similar procedure, with the generation of PAH distribution plots for all of the target analytes. The samples were evaluated using the same process to interpret whether PAH distribution were petrogenic or pyrogenic. A pyrogenic PAH signature was determined using the same analyte and distribution criteria as described above for the PP PAH. Petrogenic PAH distributions were identified by the presence of a suite of alkyl PAHs. The characteristic petrogenic alkyl PAH distribution generally included alkyl naphthalenes, phenanthrenes and chrysenes, where the C-2 or C-3 alkyl groups were the most abundant in the distribution. Samples with aquatic life exceedances that had a petrogenic alkyl PAH distribution were categorized as "MC252 oil" if there was no other data available for review.

However, most samples with alkyl PAH data had additional hydrocarbon data including alkane and biomarker (steranes and triterpanes) concentrations, gas chromatography/mass spectrometry (GC/MS) biomarker extracted ion chromatogram profiles (extracted ion current profiles [EICPs]) and gas chromatography/flame ionization detec tion (GC/FID) chromatograms. In all cases for which additional data were available, the presence of MC252 oil was confirmed by comparisons of the biomarker and alkane distributions in the samples to those of the Q4000 (MC252) control oils analyzed along with each batch samples. A histogram of the PAHs in the Q4000 control oil is shown in Fig. D-2. An example histogram is also shown for PAHs consistent with MC252 oil found in a sediment sample near the wellhead (Fig. D-3). If the biomarker and alkane comparisons were not consistent with the MC252 oil, then the sample exceedance was categorized as "not MC252 oil".

Fig. D-2. Histogram of PAHs in the Q4000 MC252 control oil.

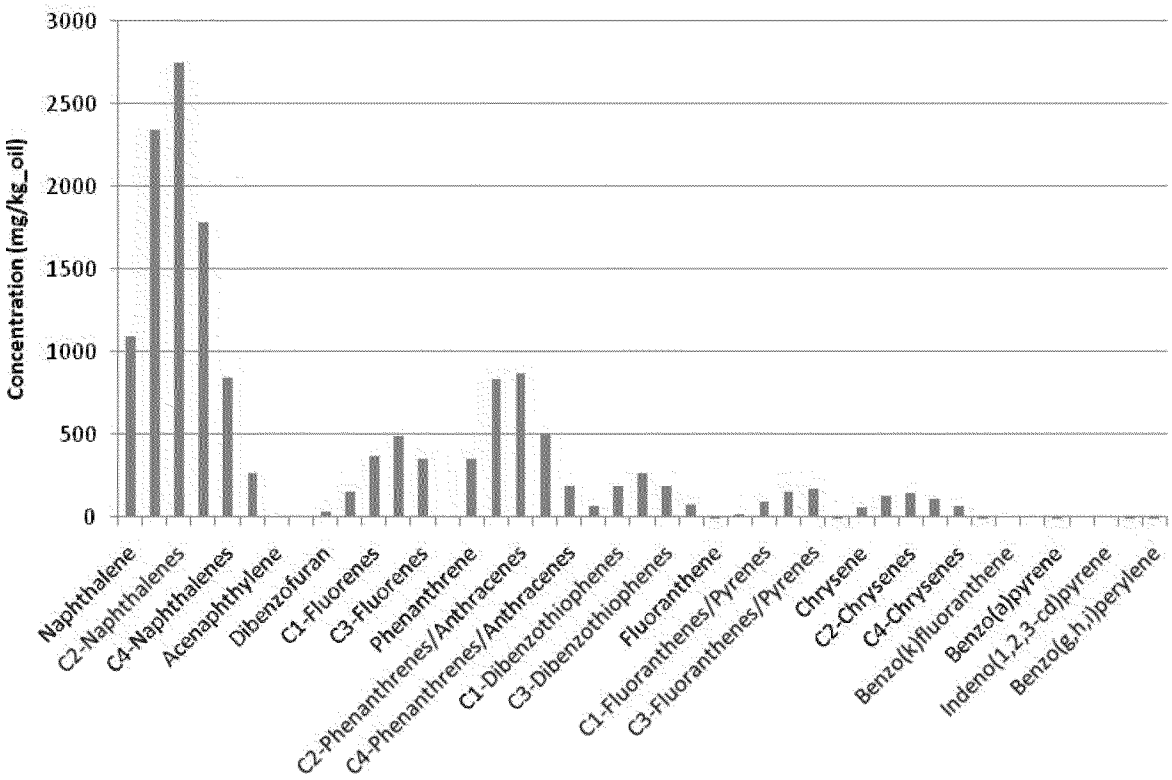
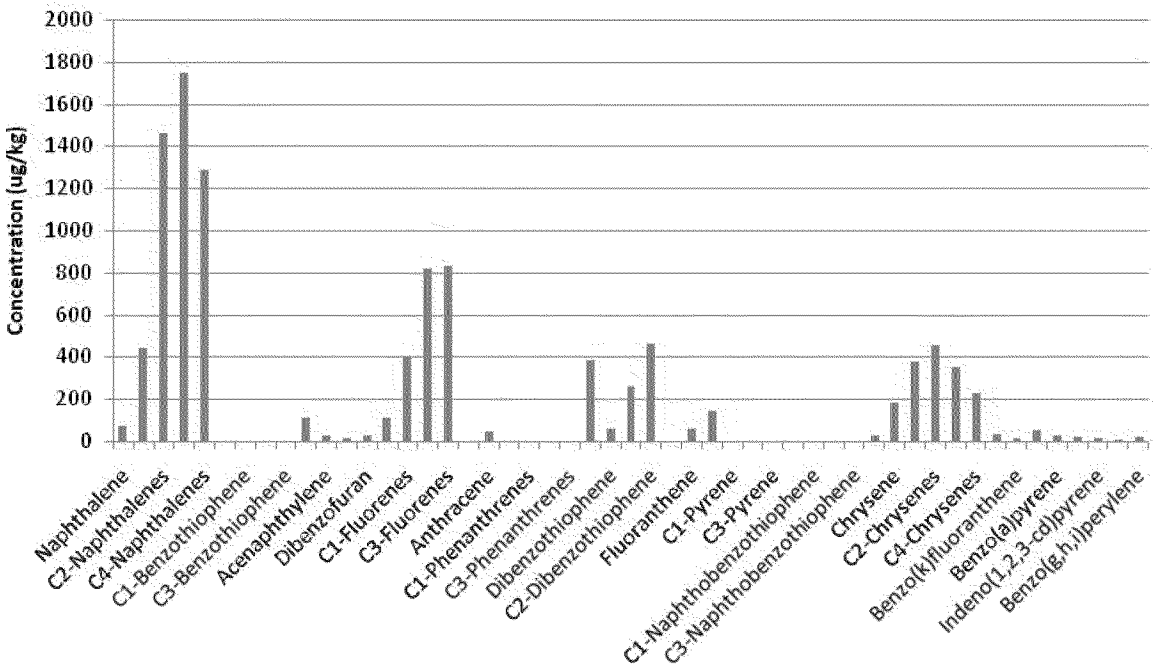


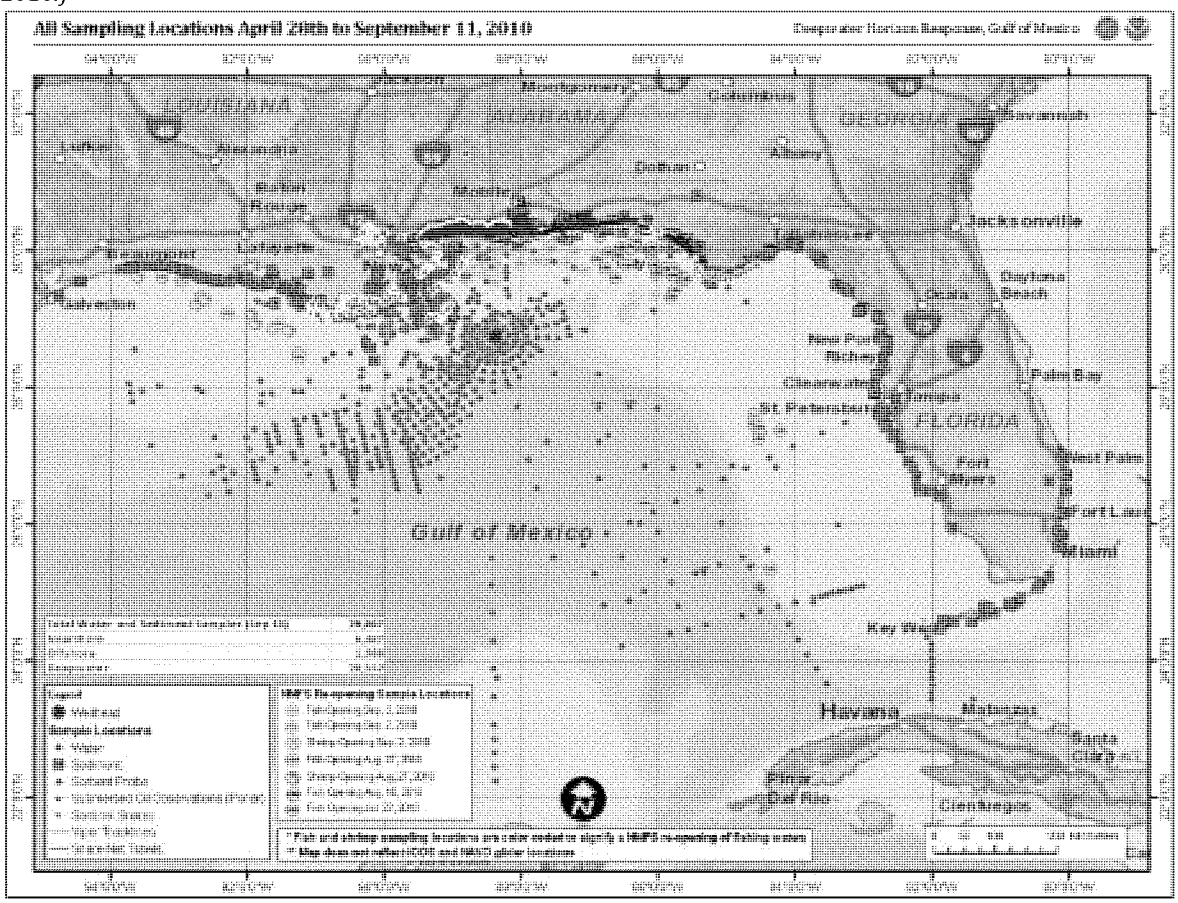
Fig. D-3. Histogram of PAHs in a sediment sample that was consistent with MC252 oil within 3 km of the wellhead. The sample ID was SE-20101017-GY-D038SW-HC-053_BDO collected on 17 October by the R/V Gyre.



Appendix E: Sampling Programs Addressed in the OSAT Summary Report

An overwhelming number of scientific observations and samples have been collected since the beginning of the response. There were also simultaneous sampling efforts being conducted as part of the NRDA process. Prior to the systematic sampling plan laid out in the Implementation Plan (13 September) and refined in the Operational Annex (1 October), the majority of these samples were collected under specific programs designed and managed independently by the Houma and Mobile Incident Command Posts (ICPs) for a diverse set of objectives (see Figure E.1 for locations of samples taken between 28 April and 11 September). Building on previous and existing sampling programs, the Implementation Plan and the Operational Annex proposed a statistically based adaptive approach for enhanced sampling.

Figure E.1. Sample locations from 28 April 2010 to 11 September 11 2010. (*From* Implementation Plan, 13 September 2010.)



The following sections describe some of these independent sampling programs undertaken by the Houma and Mobile ICPs (Sections E.1/E.2), and provide additional details about the sampling rationale developed in the Implementation Plan and the Operational Annex (Section E.4). This is not meant to be a comprehensive list, but details programs that created data which were utilized by the OSAT in preparing this report.

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E.1 Mobile sampling programs in existence before the Implementation Plan

Nearshore water and sediment sampling program

The purpose of this sampling and analysis plan was to guide nearshore water sampling within approximately 3 nautical miles (nmi) from the coastline through Mississippi and Alabama state waters and within approximately 9 nmi from the coastline through the represented portion of Florida's state waters to evaluate water quality. Sampling sites were located near ecologically sensitive areas with a spatial separation of at least 5 miles to achieve even representation across the impacted areas. Samples were taken by CTEH at regular intervals, ranging from every other day early in the Response to monthly in October. Sampling areas were expanded or reduced, as needed, based on the results of consecutive sampling events or the identification of other sources. Water samples were analyzed for VOCs (EPA 8260), SVOCs (EPA 8279), DRO/ORO, propylene glycol and 2-butoxy ethanol (EPA 8015B), GRO (EPA 8015), and Target Analyte List Metals plus mercury

Sediment samples were collected from areas suggested by specific state agencies and were collected at the same frequency as the above water samples. Sediment samples were analyzed for VOC, SVOC, GRO/DRO/ORO, Metals (TAL), plus mercury, propylene glycol, and 2-butoxy ethanol.

Subsurface Oil Assessment Project

The Mobile area operations conducted an extensive program for sub-surface oil assessment to identify the presence of submerged and sunken oil in sub-tidal and nearshore areas. Samples were taken from Vessels of Opportunity by various contract personnel from the ICP. This program included five separate projects from Mississippi to NW Florida including: the Mississippi Sound Subsurface Assessment Project, the Coastal Subsurface Assessment Project, the Snare Sentinel Monitoring Project, the Vessels with Intrinsic Petroleum Ensnaring and Recovery Systems (VIPER) project, and the Snorkel SCAT Project (Mobile Oil Survey Summary 11-14-10).

USEPA Region 4

The EPA conducted a survey program to collect water and sediment samples from locations along the Mississippi, Alabama, and NW Florida coastlines. Samples were taken by EPA contractors. Samples were collected from National Coastline Condition Assessment (NCCA) locations and additional sites where oil was observed. A wide range of analyses were conducted, including oil chemistry analyses, dispersant tests and toxicity tests. Pre-impact data is available and monitoring is continuing. The NCCA program is continuous and was in existence before the DWH incident.

E.2 Houma sampling programs in existence before the Implementation Plan

Fate of Oil Research Team

The Fate of Oil Research Team (FORT) program was developed to collect water column and sediment samples (top 2 cm) in coastal offshore (<200 ft) water and nearshore areas along the Louisiana and Texas coast. Samples were collected for chemical analysis and toxicity

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studies. The M/V *International Peace* (which was previously used for dispersant studies) was used for the deeper areas and Vessels of Opportunity were used for the shallower water. Locations for sampling were identified using information from FRAT, RAT[1] and Snare data and areas where subsurface oil had been reported. Analyses from the FORT program were used in the OSAT dataset.

Snare Sentinel

The key objectives of the Snare Sentinel program was to monitor for the presence of sub-surface oil in nearshore areas that could threaten unoiled, sensitive shoreline and shallow subtidal habitats, and to monitor for the presence of subsurface oil in proximity to previously oiled shorelines caused by remobilization of sediment entrained oil. It was a qualitative presence or absence program designed to detect any oil that moved beneath the surface. Samples were taken primarily from vessels of opportunity or skiffs by contractors from the ICP.

Other qualitative submerged oil sampling programs

Houma ICP had three other qualitative programs for detecting submerged oil or oil on the seabed nearshore. They were designed to drag nets or pom-poms along the seabed and in the subsurface to detect submerged oil or oil at the seabed. These programs ran from June through early August.

USEPA Region 6

The EPA conducted a survey program to collect water and sediment samples from locations along the Louisiana coast. Samples were taken by EPA contractors. As in Region 4, samples were collected from National Coastline Condition Assessment (NCCA) locations and additional sites where oil was observed. A wide range of analyses were conducted, including oil chemistry analyses, dispersant tests and toxicity tests. Pre-impact data is available and monitoring is continuing. The NCCA program is continuous and was in existence before the DWH incident.

E.3 Subsurface Monitoring Unit

The Subsurface Monitoring Unit (SMU) originally operated out of Houma, LA before moving to the UAC in New Orleans, LA in September. A wide range of studies were coordinated by the SMU. These included early surveys conducted from the M/V *Brooks McCall* and M/V *Ocean Veritas*. Studies focused on the water column and a wide range of sampling and data collection techniques were used. The SMU also directed the comprehensive water column and sediment sampling laid out in the Implementation Plan and Operational Annex.

[1] Forensic Rapid Assessment Team, Rapid Assessment Team, and Hotshot teams took samples of surface oil and oil that washed on shore for characterization.

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E.4 Implementation Plan/Operational Annex Sampling

The sampling program detailed in the Implementation Plan (13 September) and refined in the Operational Annex (1 October) was designed to be a hybrid approach that was robust and science driven and that used an adaptive program to fill in gaps between the existing data points to:

1. Monitor and assess the distribution, concentration, and degradation of the portion of the oil that remains in the water column and/or bottom sediments.
2. Evaluate the distribution of indicators of dispersant or breakdown products of dispersants used in oil-spill response activities.
3. Identify any additional response requirements that may be necessary to address remaining subsurface oil.

In the context of the previous sampling, the plan proposed a statistically-based adaptive approach for enhanced sampling in three spatial domains: (a) the *nearshore* from the marshes and beaches (including bays and back-barrier environments) to 3 nmi offshore; (b) the *offshore*, from 3 nmi to the shelfbreak (defined as the 200 m water depth contour); and (c) the *deep water*, from 200 m to about 2,000 m water depth (the well is in 1500 m water depth). The spatial extent of the sampling of shallow waters was guided by previous observations of the extent of oil at the surface (from ships, aircraft, satellites and in situ sampling and observation) and by knowledge of the nearshore physical oceanography (i.e., movement of water and sediments). Sampling in offshore and deep waters was guided by: (a) monitoring results obtained to date, (b) trajectory models for the deep-water layer where hydrocarbons have previously been observed, and (c) a set of hypotheses for likely locations of remaining oil. The effort emphasized enhanced sediment sampling on the continental shelf and in deep water. The location of sampling sites was science driven and included information and input from sampling conducted on academic and non-Response cruises.

Statistical power analyses were conducted to determine sample sizes needed for the evaluation of pre-impact to post-impact periods in the nearshore, where pre-impact data were available. Targeted sampling in specific locations and areas of interest and consideration of both quantitative and qualitative sampling were also incorporated into the design based on theories regarding where oil may have encountered sediment or conditions that may have caused it to sink to the seafloor, such as surface oil encountering sediment laden water in the Mississippi River convergence zone.

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Figure E-2. Sediment sample locations completed as part of the Implentation Plan/Operational Annex sampling.

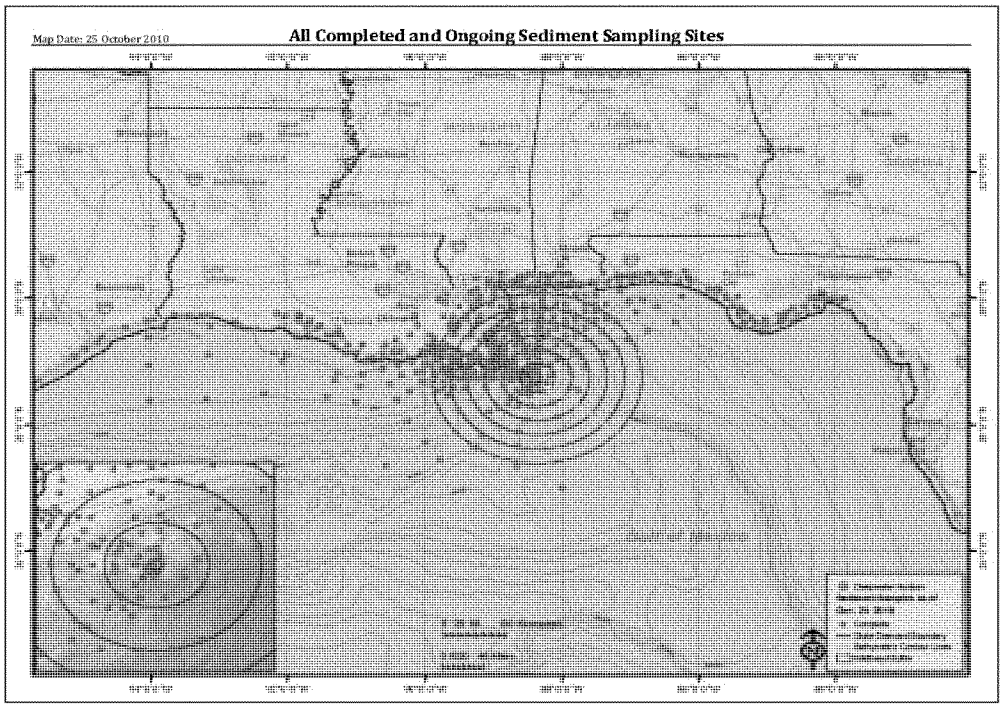
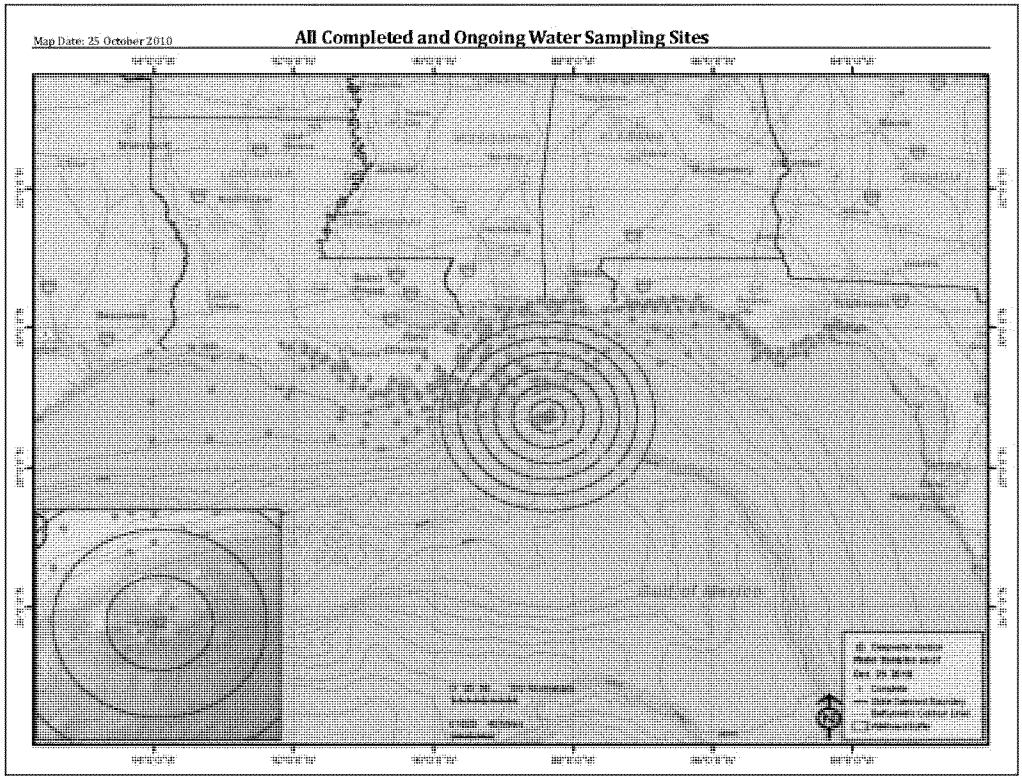


Figure E-3. Water sample locations completed as part of the Implentation Plan/Operational Annex sampling.



Appendix F: OSAT Recommendations, 30 August–01 November 2010

The OSAT analyzed data from the sub-surface and sub-sea monitoring effort and provided the analysis to the Federal On-Scene Coordinator (FOSC). The OSAT also made recommendations on sampling gaps based on data collected and planned execution of the sub-surface monitoring effort. Between 30 August and 01 November 2010, OSAT submitted seven significant recommendations and several minor recommendations to the FOSC via the SSC and EU. This appendix contains the OSAT recommendations and relevant background information. The following table summarizes key OSAT recommendations and outcomes.

Table F.1. Summary of Key OSAT Recommendations and Outcomes

Key OSAT Recommendations (30AUG - 01NOV)	Date of OSAT Recommendation	Outcomes to OSAT Recommendations
Recommendation 1: Discontinue water column sampling in the far field grid (WSW of wellhead).	**20-Sep-2010**	**Outcome**: Recommendation accepted. NRDA sampling may continue but water column effort WSW of wellhead now complete.
Recommendation 2: Conduct more water column sampling in the near field grid (30km circle around wellhead).	**20-Sep-2010**	**Outcome:** Recommendation accepted. Additional sampling locations were established and OSAT sent 15 coordinates to R/V PISCES for sampling which were completed.
Recommendation 3: Future samples should be more thoroughly analyzed. Future sample analysis should include all 43 analytes for PAHs, metals and other organic compounds required for comparison to EPA PAH aquatic life benchmarks in water and sediment samples. In the early stages of the DWH incident response, PAH concentrations were compared to acute exposure benchmarks for aquatic life. During the course of the DWH incident response chronic exposure benchmarks were added due to the longevity of the release.	**20-Sep-2010**	**Outcome**: Recommendation accepted. Sample analysis now includes all 43 analytes for PAH, metals and other organic compoundes. Post-03AUG samples analyzed for 16 analytes are being reanalyzed for 43 analytes.
Recommendation 4: A thorough Scribe data review should be conducted by technical specialists assigned to the Environmental Unit. Scribe data pulls should be continually managed by technical specialists.	**4-Oct-2010**	**Outcome:** Recommendation accepted. A data management branch for Scribe data was established within the Environmental Unit to conduct major QA/QC review of the Scribe metadata to ensure that an accurate and defensible record of sampling and monitoring activities is maintained.
Recommendation 5: OSAT should remain at current staffing levels in anticipation for influx of data. A GIS analyst with Scribe expertise has been assigned by EPA but want to relay to command how vital their function is for the OSAT to report.	**4-Oct-2010**	**Outcome:** Recommendation accepted. A GIS analyst with Scribe expertise has been assigned by EPA and is providing products for OSAT Reports.

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Key OSAT Recommendations (30AUG - 01NOV)	Date of OSAT Recommendation	Outcomes to OSAT Recommendations
Recommendation 6: OSAT sees little need in continuing the Snare Sentinel Program as part of the Subsurface Monitoring effort for the response. OSAT recommends phasing out the program beginning with areas that have shown no oiling of snares to date and are not adjacent to oiled shorelines as determined by SCAT.	**18-Oct-2010**	**Outcome:** Recommendation accepted. GCIMT has initiated the phasing out of this monitoring effort.
Recommendation 7: The sediment sampling strategy laid out in the Implementation Plan calls for an adaptive approach based on observations of oil in sediment samples. Based on recent qualitative observations of oil associated with sediment cores from sites offshore of the Mississippi Delta, the OSAT recommends further targeted sampling in this region to determine the extent of the potentially impacted area. Ideally, protocols would be identical to those used for previous sediment sampling; however, if core barrels are limited then sediment chemistry is the priority.	**19-Oct-2010**	**Outcome:** Recommendation accepted. Further targeted sampling in this region was conducted to determine the extent of the potentially impacted area offshore of the Mississippi Delta. Twelve new stations were added.

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F.1 20 September Memo #1

OPERATIONAL SCIENCE ADVISORY TEAM MEMORANDUM #1

TO: RADM PAUL ZUKUNFT, FEDERAL ON-SCENE COORDINATOR (FOSC)

THROUGH: EDWIN LEVINE, SCIENTIFIC SUPPORT COORDINATOR (SSC)

FROM: OPERATIONAL SCIENCE ADVISORY TEAM (OSAT)

SUBJECT: RECOMMENDATIONS AND FINDINGS

DATE: SUBMITTED 20SEP2010, UPDATED 28SEP2010

CC: CDR DANIEL NORTON, ASSISTANT ENVIRONMENTAL LEADER, USCG

RECOMMENDATIONS AND FINDINGS

Recommendation 1: Deepwater Sampling Zone, Far Field grids.

OSAT has reviewed and evaluated data collected in association with the Deepwater Horizon response Subsurface Monitoring Activities in the Deepwater Zone, including reports issued by the Joint Advisory Group and recent peer reviewed literature. A summary and discussion of this data review has been drafted. Based on this review, the OSAT found that:

Findings:

1. In far-field subsurface samples, PAH concentrations have never exceeded the acute or chronic aquatic life benchmarks in the 81 samples collected. EPA's benchmarks for PAHs are assessed through a mixture approach, whereby unique potency divisors are specified for 41 different oil-related organic compounds.

2. In far-field subsurface samples, dispersant indicator products (DPNB, Propylene Glycol, 2-Butoxy Ethanol) have never exceeded EPA screening levels .[1]

3. Minimum DO levels in the deep plume were measured on 27 May (4.23 mg L-1; 18 km from wellhead) and 28 July (3.66 mg L-1; 40 km from wellhead). Recent

[1] The following represent screening levels (based on dissolved seawater concentrations) that may be used to communicate and explain the relevance of measured concentrations of individual dispersant chemical components from Gulf of Mexico waters and that concentrations above these levels are levels of concern. These compounds represent the major constituents of Corexit, those with known toxicology data and those with newly established analytical methods. The screening levels are based on available biological effects data and are designed to conservatively protect aquatic life. Each of these compounds is subject to wide commercial use in other products beyond Corexit 9500. If a screening level is exceeded, the potential for other source(s) of the chemical will be evaluated.

Compound	Detection Limits	EPA Aquatic Life Screening Level
Propylene Glycol	500 ug/L	500,000 ug/L
2-Butoxyethanol	125 ug/L	165 ug/L
Di(Propylene Glycol) - Butyl ether	1 ug/L	100 mg/L acute 1 mg/L chronic
Dioctylsulfosuccinate	20 ug/L	360 ug/L acute 40 ug/L chronic

measurements in the far field have detected DO sags (typically < 1mg L-1) as far as 450 km from the wellhead (to the SW) Minimum DO values measured since mid-August are above 4 mg L-1.

4. The signature of the plume in both fluorescence and DO is becoming attenuated. The subsurface plume has been delineated WSW of the wellhead based on regions where DO readings have sagged and fluorescence peaked at the same time.

5. National Marine Fisheries Service (NMFS) recommends precautionary closures once oil or chemical contaminants are visually observed on the surface. NMFS has maintained a closure in the far field sampling area; oil was last sited mid-July. NMFS has begun to reopen fisheries.

Conclusions:

i. Oil and dispersant break down product concentrations do not exceed screening levels or benchmarks and are decreasing;

ii. Dissolved oxygen levels are above hypoxic thresholds and are not expected to reach hypoxia; and

iii. The movement and extent of the subsurface plume of dispersed oil has been characterized and delineated and is consistent with predictions based on measured and predicted deep ocean currents.

Recommendation: Sufficient water column data has been collected in the far field sampling grids located to the SW of the wellhead (from approx. 100-400 km).

<u>Recommendation 2: Deepwater Sampling Zone, Near Field.</u>

Findings:

In the near field area, the area within 30 km of the Deepwater Horizon wellhead, no water samples have exceeded the acute aquatic life benchmark for PAHs since 22 June. However, samples continued to exceed the chronic aquatic life benchmark through the most recent samples available in Scribe (as of 19 Sep; data collected 22 July). Thus, the chronic benchmark was still being exceeded on July 22nd and more recent data is required to determine whether this is still the case.

Conclusion:

A systematic sampling of this area, coincident temporally and spatially with the upcoming sediment work would provide a comprehensive dataset for comparison with decision indicators. With the permanent sealing of the well, this systematic near field sampling would also provide a post event assessment: a step necessary for meeting response transition points.

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Recommendation: OSAT recommends that, concurrent with the Deep water sediment sampling plan, a small number of stations (~25) within 30 km of wellhead should be targeted for water sampling. These stations will be chosen based on co-location with deep water sediment sampling and reoccupation of stations previously observed to have significant exceedances of water quality benchmarks.

Recommendation 3:

In the early stages of the Deepwater Horizon incident response, PAH concentrations were compared to acute exposure benchmarks for aquatic life. During the course of the Deepwater Horizon incident response chronic exposure benchmarks were added due to the longevity of the release.

Finding:

Forty-three analytes are required for comparison to EPA aquatic life and human health benchmarks, which are considered by the OSAT in their recommendation process.

Conclusion: Data being collected for analysis pursuant to the response to the Deepwater Horizon response operation needs to meet current standards for analysis.

Recommendation: Future sample analysis should include all 43 analytes for PAHs, metals and other organic compounds required for comparison to EPA PAH aquatic life benchmarks in water and sediment samples.

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F.2 27 September Memo #2

OPERATIONAL SCIENCE ADVISORY TEAM MEMORANDUM #2

TO: EDWIN LEVINE, SCIENTIFIC SUPPORT COORDINATOR (SSC)

FROM: OPERATIONAL SCIENCE ADVISORY TEAM (OSAT)

SUBJECT: RECOMMENDATIONS AND FINDINGS

DATE: 9/27/2010

CC: CDR DANIEL NORTON, ASSISTANT ENVIRONMENTAL LEADER, USCG

RECOMMENDATIONS AND FINDINGS

OSAT continues to engage in data collection and analysis, conducted in accordance with the Subsurface Monitoring Implementation Plan (13 Sept 2010). During the reporting period (beginning 20 Sept 2010), OSAT focused its efforts on the nearshore zone, defined in the plan as the "coast to 3nm." The use of the term "coast" to delineate the landward extent of the zone is not optimal, and an OSAT request for clarification pends with NOAA.

OSAT has received new products created by the Subsurface Monitoring Unit (SMU) that improve visibility of vessels underway and those that are preparing to get under way. These products are helpful and address an existing need. Further improvements would assist the OSAT by providing context for deliberations on decision indicators.

Recommendation 1: Continued Improvements with respect to Communications, Awareness.

Findings: Near real time information regarding vessel locations and activities provides an important context for OSAT deliberations and workflow, and changes to vessel activities may bear on OSAT's work to identify sampling gaps and to do so in a timely manner. OSAT supports improved transparency and data availability within the Environmental Unit, particularly between OSAT and SMU.

Conclusions: In addition to the "just in time" approach that has been applied to cruise planning, lapses in awareness /coordination regarding the sampling strategies of response vessels have resulted in confusion and diminished the efficiency of work products that are rendered moot when work is already in the pipeline or when gaps thought to be addressed, are found to persist.

Recommendation: OSAT requests an advanced (24 hr) briefing or synopsis of cruise and study plans for vessels about to embark on response missions. OSAT also recommends incorporation of a weekly brief on the mission plan of outbound vessels into the 1700 EU meeting.

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Recommendation 2: Further water column sampling is required on the shelf.

Findings:

1. A large gap in water sampling remains along and between the corridor regions on the shelf, where only sediment sampling is currently planned.

2. OSAT is aware of at least one mission performing water column sampling in a distinct region of the shelf (i.e., M/V International Peace).

Conclusions: OSAT's decision indicators for the offshore (shelf) include EPA's benchmarks for poly aromatic hydrocarbons (PAHs) and dispersants in the water column. There is currently insufficient data on the shelf to assess whether or not these benchmarks have been met.

Recommendation: OSAT recommends that water sampling profiles for chemical measurements be collected in and between the corridors that are currently planned for sediment sampling. Where sediment sampling is densely spaced, water samples may be collected at a subset of locations.

Recommendation 3: Confirm that NMFS is informed of May/June Benzene exceedance.

Findings:

1. OSAT found that Benzene levels in approximately 20 deepwater samples collected in May and June slightly exceeded a conservative cancer risk guideline EPA recommended for setting state water quality criteria. No samples collected since early June exceeded the guideline for Benzene.

2. OSAT considered this information and does not recommend changes or amendments to its 20 September recommendation regarding farfield monitoring.

Conclusion: The Subsurface Monitoring Implementation Plan indicates that the SSC should communicate information pertaining to seafood safety to NMFS. EPA pulled data to specifically address the identified exceedance and reviewed language drafted by OSAT's NOAA representative, for conveyance to NMFS. OSAT recommends that a specific NMFS liaison be designated to improve communications from OSAT to that agency.

Recommendation: OSAT recommends that the information be communicated to NMFS for confirmation that NMFS already addressed the matter, or else has the OSAT data for further analysis if necessary.

Recommendation 4: Recover data from missing response cruises identified by EPA analysis.

Finding: EPA comparison of data available in the Scribe database and monitoring cruises on file in the Subsurface Monitoring Unit files (attached) reveals cruises for which data has not been loaded into Scribe.

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Conclusion: Complete datasets are necessary for OSAT to properly consider results and make well-founded recommendations.

Recommendation: Steps should be taken to recover unreported or unavailable data, or to document and address the cause of the loss of data or visibility.

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F.3 29 September Memo #3

OPERATIONAL SCIENCE ADVISORY TEAM MEMORANDUM #3

TO: RADM PAUL ZUKUNFT, FEDERAL ON-SCENE COORDINATOR (FOSC)

THROUGH: CHARLIE HENRY, SCIENTIFIC SUPPORT COORDINATOR (SSC)

FROM: OPERATIONAL SCIENCE ADVISORY TEAM (OSAT)

SUBJECT: SUMMARY OF RECOMMENDATIONS

DATE: 29SEP2010

CC: CDR DANIEL NORTON, ASSISTANT ENVIRONMENTAL LEADER, USCG

SUMMARY OF 20SEP RECOMMENDATIONS

Purpose: To summarize recommendations and findings outlined in OSAT's 20SEP memo to the FOSC.

Recommendation 1: Discontinue water column sampling in the far field grid (WSW of wellhead).

Rationale:

1. Oil and dispersant component concentrations do not exceed screening levels or benchmarks and are decreasing.
2. Dissolved oxygen levels are above hypoxic thresholds and are not expected to reach hypoxia.
3. The movement and extent of the subsurface dispersed oil plume has been characterized, is delineated and is consistent with predictions based on measured and predicted deep ocean currents.
4. Sufficient water column data has been collected in the far field sampling grids located to the SW of the wellhead (from approx. 100-400 km).

Recommendation 2: Conduct more water column sampling in the near field grid (30km circle around wellhead).

Rationale:

1. Based on data reviewed as of 19SEP, the chronic benchmark for PAH was being exceeded on 22JUL. Further analysis is required to determine whether this is still the case.
2. Concurrent with the deepwater sediment sampling plan, a small number of stations (~25) within 30 km of wellhead, collocated with deep water sediment sampling, should be targeted for water sampling. Stations previously observed to have significant exceedances of water quality benchmarks should be chosen.

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3. A systematic sampling of this area would provide a comprehensive dataset for comparison with decision indicators. With the permanent sealing of the well, this systematic near field sampling would also provide a post event assessment: a step necessary for meeting response transition points.

<u>Recommendation 3</u>: Future samples should be more thoroughly analyzed.
Rationale:

1. Future sample analysis should include all 43 analytes for PAHs, metals and other organic compounds required for comparison to EPA PAH aquatic life benchmarks in water and sediment samples.
2. In the early stages of the DWH incident response, PAH concentrations were compared to acute exposure benchmarks for aquatic life. During the course of the DWH incident response chronic exposure benchmarks were added due to the longevity of the release.

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F.4 30 September Memo #4

OPERATIONAL SCIENCE ADVISORY TEAM MEMORANDUM #4

TO:	RADM PAUL ZUKUNFT, FEDERAL ON-SCENE COORDINATOR (FOSC)
THROUGH:	CHARLIE HENRY, SCIENTIFIC SUPPORT COORDINATOR (SSC)
FROM:	OPERATIONAL SCIENCE ADVISORY TEAM (OSAT)
SUBJECT:	RESPONSES TO QUESTIONS FROM 20SEP
DATE:	30SEP2010
CC:	CDR DANIEL NORTON, ASSISTANT ENVIRONMENTAL LEADER, USCG

RESPONSES TO QUESTIONS FROM THE 20SEP Recommendations

Purpose: To respond to questions from FOSC review of OSAT's 20SEP memo.

Question 1: What are the benchmarks used to test the far field (area WSW of the wellhead) subsurface samples? How many samples are needed?

Response:

1) For a view of the far field area, see attachment (1).
2) The benchmarks used for the far field area are EPA's screening levels for poly aromatic hydrocarbons (PAH). These are assessed through a mixture approach, whereby unique potency divisors are specified for 41 different oil-related organic compounds. See attachment (2) for a discussion of how the PAH screening levels were developed.
3) A benchmark is a conservative value below which adverse effects are unlikely to be observed. Benchmarks are similar to screening levels, but this response has deemed them separate; screening levels for established values and benchmarks for event-specific needs (i.e., dispersant constituents). A full explanation of benchmarks is available in attachment (3).
4) The benchmarks used are available at http://www.epa.gov/bpspill/water-benchmarks.html
5) In far field subsurface samples, PAH concentrations have never exceeded the acute or chronic aquatic life benchmarks in the 81 samples collected.

Question 2: Define EPA screening levels for dispersant indicators.

Response:

1) A screening level is an exposure level below which it is unlikely to cause adverse impacts.
2) There are four dispersant indicators screened for, propylene glycol, 2-Butoxyethanol, Di(Propylene Glycol)-Butyl ether and Dioctylsulfosuccinate. These

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indicators can also be found in other products and their presence is only an indicator that dispersant may have been in an area.

3) There are detection limits and EPA aquatic life screening levels set for each of the indicators.
4) Attachment (2) goes into detail about how a screening level is set.
5) Attachment (4) gives detail regarding dispersant indicators.

Question 3: A NMFS closure remains in effect in the far field sampling area, but there is no visible oil. Provide finding of time elapsed since we've had visible MC252 oil.

Response:

1) There has been no observable oil in the far field since at least 04AUG.
    2) Since 22JUL, NOAA has reopened about 52,000 square miles of oil-impacted federal waters in accordance with the re-opening protocol agreed to by NOAA, the U.S. Food and Drug Administration, and Gulf states.
3) Based on data reviewed as of 19SEP, the chronic benchmark for PAH was being exceeded on 22JUL. We do not have sample data for dates later than that; the R/V PISCES is now collecting the samples the OSAT needs. Further analysis is required to determine whether the chronic benchmarks are still being exceeded.

Question 4: Request a conclusion as it pertains to fishery closures, ie. aggressive seafood sampling procedures to facilitate reopening.

Response:

1) Since 22JUL, NOAA has reopened about 52,000 square miles of oil-impacted federal waters in accordance with the re-opening protocol http://sero.nmfs.noaa.gov/sf/deepwater_horizon/attachment1(3).pdf agreed to by NOAA, the U.S. Food and Drug Administration, and Gulf states.
2) Prior to re-opening an area, the protocol requires NOAA to demonstrate the area is oil free, the area has little risk of being re-exposed to oil, and seafood tissue samples collected from within the area have passed both sensory and chemical analysis for hydrocarbons. This protocol involves sensory testing for polycyclic aromatic hydrocarbon (PAH) components of the oil and dispersant along with chemical based testing for PAH as a confirmatory measure.
3) After areas are re-opened NOAA Fisheries Service will maintain a seafood safety monitoring program continuing the collection and testing of seafood to ensure that Gulf seafood remains safe for consumers.

Question 4: Water samples continued to exceed the chronic aquatic life benchmark through the most recent samples available in Scribe (as of 19SEP, data collected 22JUL). Are we still at the chronic level or was 22JUL and end point?

Response:

1) Question 3 response is also related to this question.

2) OSAT outlined a request in Memo #5 to R/V PISCES for more water column sampling near the wellhead. OSAT worked with the Subsurface Monitoring Unit (SMU) to direct the PISCES to the exact coordinates where the chronic levels were found in 22JUL. PISCES is currently collecting those samples and once they are processed in a lab and uploaded into EPA's Scribe database, they will be available for review to determine if more recent samples exceed chronic levels.

Question 5: Standards for analysis of materials for PAH concentrations for comparison with aquatic life and human health have changed during the response. Who changed them and why?

Response:

1) EPA Region 6, Region 4 & Headquarters, in conjunction with Health and Human Services (HHS) and State Health Departments developed benchmarks in a collaborative way to assess potential human health risks from exposure to oil-contaminated water.
2) The higher number of analytes is considered a standard and is more comprehensive than the lower number of analytes.

Attachments:

1) Graphic of near field and far field.
2) Explanation of PAH Screening levels
3) Explanation of Benchmarks
4) Explanation of Dispersant Screening levels

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F.5 29 September Memo #5

OPERATIONAL SCIENCE ADVISORY TEAM MEMORANDUM #5

TO:	NOAA SHIP PISCES
THROUGH:	NOAA SUBSURFACE MONITORING UNIT (SMU)
	CHARLIE HENRY, SCIENTIFIC SUPPORT COORDINATOR (SSC)
FROM:	OPERATIONAL SCIENCE ADVISORY TEAM (OSAT)
SUBJECT:	REQUEST FOR WATER SAMPLING NEAR WELLHEAD
DATE:	29SEP2010
CC:	CDR DANIEL NORTON, ASSISTANT ENVIRONMENTAL LEADER, USCG

Purpose: To request new water sampling near the wellhead.

Recommendation 1: OSAT requests the R/V PISCES take 15 water samples near the wellhead during her current cruise.

Rationale:

1) No water samples have been taken in this area since 22JUL.
2) Based on data reviewed as of 19SEP, the chronic benchmark for PAH was being exceeded on 22JUL. Further analysis is required to determine whether there are still any exceedances.
3) 15 stations within 30 km of wellhead were chosen because these stations previously observed to have significant exceedances of water quality benchmarks.
4) A systematic sampling of this area would provide a comprehensive dataset for comparison with decision indicators. With the permanent sealing of the well, this systematic near field sampling would also provide a post event assessment: a step necessary for meeting response transition points.
5) Sufficient water column data has been collected in the far field sampling grids located to the SW of the wellhead (from approx. 100-400 km) and we are not asking for sampling of those areas.

Sampling plan:

Sampling should include bottom and interval water column, as well as at least one (1) and possibly more samples within the 27.70 sigma-θ layer (if present), as the Chief Scientist sees fit. The 27.70 sigma-θ layer is typically found between 1100-1300 meters, it should be calculated from downcast CTD data and targeted for sampling on the upcast. Samples should also be taken where fluorometer peaks and/or DO sags are detected following standard procedure (i.e. one bottle below, middle, and above peak/sag).

Attachments:

1) List of coordinates,
2) Added deepwater near field sampling points,
3) ERMA display of near field and far field areas.

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F.6 4 October Memo #6

OPERATIONAL SCIENCE ADVISORY TEAM MEMORANDUM #6

TO:	RADM PAUL ZUKUNFT, FEDERAL ON-SCENE COORDINATOR (FOSC)
THROUGH:	CHARLIE HENRY, SCIENTIFIC SUPPORT COORDINATOR (SSC)
FROM:	OPERATIONAL SCIENCE ADVISORY TEAM (OSAT)
SUBJECT:	SUMMARY OF RECOMMENDATIONS
DATE:	04 OCT 2010
CC:	CDR DANIEL NORTON, ASSISTANT ENVIRONMENTAL LEADER, USCG

SUMMARY OF RECOMMENDATIONS

Purpose:

Summarize completed, in progress and outstanding recommendations.
Provide two new recommendations this week.

Recommendation 1 (COMPLETED): Discontinue water column sampling in the far field grid (WSW of wellhead). **UPDATE:** NRDA sampling may continue but water column effort WSW of wellhead now complete.

Recommendation 2 (COMPLETED): Conduct more water quality sampling in the near field grid (30km circle around wellhead). **UPDATE:** OSAT sent 15 coordinates to R/V PISCES for sampling which were completed.

Recommendation 3 (COMPLETED): Sample analysis

Findings: Future sample analysis should include all 43 analytes for PAH, metals, and other organic compounds required for comparison to EPA PAH aquatic life benchmarks in water and sediment samples.

Recommendation: Future samples should be more thoroughly analyzed. **UPDATE:** This is now being completed onshore.

Recommendation 4 (NEW): Analytical Chemistry Data Management

Findings:

- There are significant errors and omissions in the metadata (such as information on sample location and depth) associated with sediment and water samples collected for PAH (and other?) analysis.
- There are no known errors in the analytical chemistry data (laboratory data) on PAH concentrations.

Recommendation:

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A major QA/QC review of the Scribe metadata is needed to ensure that an accurate and defensible record of sampling and monitoring activities is maintained. A data management branch for Scribe data is needed within the Environmental Unit.

- Due to the importance of data management, Memo #8, forthcoming, is dedicated fully to this recommendation.

<u>Recommendation 5 (NEW, IN PROGRESS ALREADY):</u> Staffing levels and GIS analyst

Findings:

- There is a lag time between when a sample is collected to the time the data are ready for analysis. This lag time can be up to two months. OSAT anticipates a flood of data as the labs catch up with sample processing. In anticipation of this flood of data, a GIS analyst was requested from EPA under the PRFA to produce maps for regular reporting.
- It should display exceedances and other (non-detects + below benchmark).
- Separate maps may be needed for sediments and water.
- Depending on the resolution of the data, separate maps may be needed for different geographic regions.

Recommendation: OSAT should remain at current staffing levels in anticipation for influx of data. A GIS analyst with Scribe expertise has been assigned by EPA but want to relay to command how vital their function is for the OSAT to report.

Attachment: Display of near field and far field areas.

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F.7 4 October Memo #7

OPERATIONAL SCIENCE ADVISORY TEAM MEMORANDUM #7

TO:	RADM PAUL ZUKUNFT, FEDERAL ON-SCENE COORDINATOR (FOSC)
THROUGH:	CHARLIE HENRY, SCIENTIFIC SUPPORT COORDINATOR (SSC)
FROM:	OPERATIONAL SCIENCE ADVISORY TEAM (OSAT)
SUBJECT:	DATA MANAGEMENT RECOMMENDATIONS
DATE:	04 OCT 2010
CC:	CDR DANIEL NORTON, ASSISTANT ENVIRONMENTAL LEADER, USCG

ANALYTICAL CHEMISTRY DATA MANAGEMENT

Purpose: To recommend a review and ongoing maintenance of Scribe data by technical specialists.

Recommendation 4 (NEW): A thorough Scribe data review should be conducted by technical specialists assigned to the Environmental Unit. Scribe data pulls should be continually managed by technical specialists.

Findings:

- Critical field and analytical data from surface and subsurface monitoring activities are being managed using the Scribe database.
- There are significant errors and omissions in the metadata (such as information on sample location and depth) associated with sediment and water samples collected for PAH (and other) analysis.
- Data pulled from Scribe by the OSAT are inconsistent - data available one day is not retrieved on subsequent pulls. The reason for this inconsistency is unknown. Potential explanations include: instability in the underlying data structure, changes in query methods used by rotating data support personnel, or QA/QC activities of which the OSAT is unaware.
- A large influx of new data into Scribe from current and planned sampling activities is anticipated within the next several weeks.
- The OSAT has been unable to confirm that a systemic QA/QC process is in place for data in Scribe.
- The OSAT has been unable to locate a central point of overall responsibility for maintaining the Scribe database for this response.

Recommendation:

The Environmental Unit should conduct a major QA/QC review of the Scribe metadata to ensure that an accurate and defensible record of sampling and monitoring activities is maintained. The existing pool of Scribe technical specialists should be increased in order to conduct this review. These technical specialists should conduct a major QA/QC review of

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the Scribe data, metadata and data architecture and work with the OSAT and other parties requiring access to Scribe to develop robust and consistent queries. These activities are required for the OSAT to have confidence in its analysis of analytical data and to ensure that an accurate and defensible record of sampling and monitoring activities conducted by this response is maintained.

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F.8 4 October Memo #8

OPERATIONAL SCIENCE ADVISORY TEAM MEMORANDUM #8

TO:	RADM PAUL ZUKUNFT, FEDERAL ON-SCENE COORDINATOR (FOSC)
THROUGH:	CHARLIE HENRY, SCIENTIFIC SUPPORT COORDINATOR (SSC)
FROM:	OPERATIONAL SCIENCE ADVISORY TEAM (OSAT)
SUBJECT:	RECOMMENDATION FOR A DATA MANAGEMENT BRANCH
DATE:	04 OCT 2010
CC:	CDR DANIEL NORTON, ASSISTANT ENVIRONMENTAL LEADER, USCG

DATA MANAGEMENT

Purpose: To recommend a review of Scribe data and create a Scribe data management unit.

Recommendation 4 (NEW): Analytical Chemistry Data Management

Findings:

- Critical field and analytical data from surface and subsurface monitoring activities are being managed using the Scribe database.
- There are significant errors and omissions in the metadata (such as information on sample location and depth) associated with sediment and water samples collected for PAH (and other) analysis.
- Data pulled from Scribe by the OSAT are inconsistent - data available one day is not retrieved on subsequent pulls. The reason for this inconsistency is unknown. Potential explanations include: instability in the underlying data structure, changes in query methods used by rotating data support personnel, or QA/QC activities of which the OSAT is unaware.
- The overwhelming majority of the chemical analyses conducted thus far have detected no measurable concentration of PAH's. A similarly large percentage of the samples with detectable quantities of PAH's have been below the benchmark levels for these chemicals.
- A large influx of new data into Scribe from current and planned sampling activities is anticipated within the next several weeks.
- The OSAT has been unable to confirm that a systemic QA/QC process is in place for data in Scribe.
- The OSAT has been unable to locate a central point of overall responsibility for maintaining the Scribe database for this response.
- Due to the small number of samples exceeding benchmark levels, the OSAT is able to perform its mission without concern for the overall quality of the metadata.

Recommendation:

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The Environmental Unit should conduct a major QA/QC review of the Scribe metadata to ensure that an accurate and defensible record of sampling and monitoring activities is maintained. A Scribe data management unit should be created within the ICS. This unit should be charged with conducting a major QA/QC review of the Scribe data, metadata and data architecture and working with the OSAT and other parties requiring access to Scribe to develop robust and consistent query's. These activities are required for the OSAT to have confidence in its analysis of analytical data and to ensure that an accurate and defensible record of sampling and monitoring activities conducted by this response is maintained.

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F.9 11 October Memo #9

OPERATIONAL SCIENCE ADVISORY TEAM MEMORANDUM #9

TO:	STEVE LEHMANN, SCIENTIFIC SUPPORT COORDINATOR (SSC)
THROUGH:	CDR DANIEL NORTON, ASSISTANT ENVIRONMENTAL UNIT LEADER, USCG
FROM:	OPERATIONAL SCIENCE ADVISORY TEAM (OSAT)
SUBJECT:	SUMMARY OF OSAT RECOMMENDATIONS AND ACTIVITIES
DATE:	11 OCT 2010
CC:	JOHN BENNINGTON, ENVIRONMENTAL LEADER

SUMMARY OF RECOMMENDATIONS

Purpose: Summarize completed, in progress and outstanding recommendations.

Recommendation 1 (COMPLETED): Water column sampling effort in far field (greater than 30km WSW) of wellhead now complete.
Recommendation 2 (COMPLETED): Conduct more water quality sampling in the near field grid (30km circle around wellhead). **UPDATE:** All deep water column sampling completed.
Recommendation 3 (COMPLETED): All sample analysis include the 43 analytes for PAH, metals, and other organic compounds required for comparison to EPA PAH aquatic life benchmarks in water and sediment samples.
Recommendation 4 (IN-PROGRESS): A major QA/QC review of the Scribe metadata is needed to ensure that an accurate and defensible record of sampling and monitoring activities is maintained. As a result, a data management branch for Scribe data was established within the Environmental Unit..
Recommendation 5 (COMPLETED): A GIS analyst with Scribe expertise has been assigned by EPA and is providing products for Status Reports.

SUMMARY OF OSAT ACTIVITIES

Purpose: Summarize OSAT activities for the past week.

In anticipation of the data influx that will occur once Activities 1 and 2 (from the Operational Annex) are completed, OSAT has been working to standardize data output from Scribe and resolve problems from inconsistent data entry. Other issues include changes caused by the recent geo-labeling update, where all unique sample IDs were linked to a particular zone (nearshore, offshore, or deepwater), and the continuing validation of data in Scribe. Currently, only approximately 59% of Scribe data has undergone QA/QC which may result in fluctuations between current and future results. OSAT continues to work with our EPA Scribe contractors and the BP Exponent contractors, as well as

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Environmental Standards, to produce the highest quality data analysis possible. In addition, OSAT has been working with our dedicated GIS person to develop map products to include in future Status Reports to the SSC and for eventual upload to ERMA.

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F.10 18 October Memo #10

OPERATIONAL SCIENCE ADVISORY TEAM MEMORANDUM #10

TO:	RADM PAUL ZUKUNFT, FEDERAL ON-SCENE COORDINATOR (FOSC)
THROUGH:	STEVE LEHMANN, SCIENTIFIC SUPPORT COORDINATOR (SSC)
FROM:	OPERATIONAL SCIENCE ADVISORY TEAM (OSAT)
SUBJECT:	RECOMMENDATION FOR SNARE SENTINAL PROGRAM
DATE:	18 OCT 2010
CC:	CDR ANDREW TUCCI , ASSISTANT ENVIRONMENTAL LEADER, USCG

REVIEW OF LOUISIANA SNARE SENTINEL PROGRAM

Findings:

- Observational Snare Sentinel data was analyzed for the period 28 July to 2 October. Oil observations were infrequent, with <1% of Snare Sentinel retrievals showing any degree of oiling (40 of 6911 retrievals).
- Oil was detected at 7.1% of Snare Sentinel sites (32 of 449). Five of the total number of sites were oiled more than once. Of these five sites, the highest percentage of oiling was 19% (3 detects in 16 retrievals at one site).
- Oil detections have decreased significantly.
  - Last surface detection was 31 July in Barataria Bay (78 days ago).
  - Last sub-surface detection was 20 September in Barataria Bay (28 days ago).
- All observations of sub-surface oiling have been located off previously oiled shorelines – weathering and biodegradation patterns from these observations indicate that this oil originated from surface oil that was mixed with sediments, became heavier than water, sank and was remobilized.
- Any future detection of subsurface MC252 oil by Snare Sentinels would most likely be due to remobilization of oil already present in the nearshore environment – i.e. in known regions of heavily oiled shorelines.

Recommendation:

Based on these findings, OSAT sees little need in continuing the Snare Sentinel Program as part of the Subsurface Monitoring effort for the response. OSAT recommends phasing out the program beginning with areas that have shown no oiling of snares to date and are not adjacent to oiled shorelines as determined by SCAT.

ED_001324_00007095-00122

F.11 19 October Memo #11

OPERATIONAL SCIENCE ADVISORY TEAM MEMORANDUM #11

TO:	RADM PAUL ZUKUNFT, FEDERAL ON-SCENE COORDINATOR (FOSC)
THROUGH:	STEVE LEHMANN, SCIENTIFIC SUPPORT COORDINATOR (SSC)
FROM:	OPERATIONAL SCIENCE ADVISORY TEAM (OSAT)
SUBJECT:	RECOMMENDATION FOR ADDITIONAL OFFSHORE SEDIMENT SAMPLING
DATE:	19 OCT 2010
CC:	CDR ANDREW TUCCI, ASSISTANT ENVIRONMENTAL LEADER, USCG

ADDITIONAL SEDIMENT SAMPLING IN MS RIVER CONVERGENCE ZONE

Findings:

1. Sensory inspection of 5 sediment cores obtained by the R/V Ocean Veritas between 5-10 October has indicated potential presence of oil (visible sheen associated with sediment or overlying water).

2. The five sample sites are located approximately 15-30 km offshore of the Mississippi Delta. This region was identified in the Implementation Plan as having a higher likelihood of transport of surface oil to the bottom, due to interaction of surface oil with the sediment laden Mississippi River plume in regions of freshwater convergences.

3. There are a number of known seeps and other potential sources of oil located in the vicinity of these sample sites (within 10-20 km). Further sampling and forensic chemistry will determine the source of the observed oil.

Recommendation:

The sediment sampling strategy laid out in the Implementation Plan calls for an adaptive approach based on observations of oil in sediment samples. Based on recent qualitative observations of oil associated with sediment cores from sites offshore of the Mississippi Delta, the OSAT recommends further targeted sampling in this region to determine the extent of the potentially impacted area. Ideally, protocols would be identical to those used for previous sediment sampling; however, if core barrels are limited then sediment chemistry is the priority.

Attachments: Existing and proposed sampling locations

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F.12 26 October Memo #12

OPERATIONAL SCIENCE ADVISORY TEAM MEMORANDUM #12

TO:	RADM PAUL ZUKUNFT, FEDERAL ON-SCENE COORDINATOR (FOSC)
THROUGH:	CHARLIE HENRY, SCIENTIFIC SUPPORT COORDINATOR (SSC)
FROM:	OPERATIONAL SCIENCE ADVISORY TEAM (OSAT)
SUBJECT:	RECOMMENDATION FOR A DATA MANAGEMENT GROUP
DATE:	26 OCT 2010
CC:	CDR ANDREW TUCCI , ASSISTANT ENVIRONMENTAL LEADER, USCG

DATA MANAGEMENT GROUP TO RESOLVE DATA ISSUES

Findings:

- Inconsistencies with data uploaded to Scribe by data originators continue to hamper OSAT's ability to provide data analysis products. Roughly 20% of the data entries within Scribe have at least one major problem and 25% of the data has not gone through QA/QC review.
- Until the identified issues have been resolved, OSAT and other end users of Scribe cannot analyze response data with a satisfactory level of confidence in the results.
- The major issues discovered to date are in two groups: 1) Location (absent or improperly reported latitude and longitude and missing depth values) and 2) Duplicates (analytes not being reported in a standard way causing duplicate analytes, and upload issues that cause multiple entries for one sample).
- The vast majority of issues with data in Scribe are outside of the purview of OSAT's Scribe data manager to remedy, and consequently can only be resolved by engaging with data originators and intermediate databases.
- OSAT's Scribe data manager (an EPA START contractor) has compiled a list of these issues (attached).

Recommendation:

The magnitude and urgency of this problem requires establishing a Data Management Group to 1) resolve data problems found by OSAT, 2) enforce the use of the Data Dictionary by all providers of response data, and 3) proactively search for problems and provide solutions. Given the pace of ongoing response activities, it is necessary that this recommendation be implemented **within two weeks** of today's date to avoid negatively impacting the response. The resolution of these issues needs to be a priority for all parties (both government and BP), especially those who are data originators and those who have contracted data originators to provide data to Scribe for the response. OSAT further recommends that this group report to the Deputy Planning Section Chief –Environmental and the Environmental Unit Leader. The

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membership of this Data Management Group should be determined by the Environmental Unit (EU) with some OSAT input and may be expanded as needed to quickly and efficiently solve the listed problems. At a minimum it needs to include OSAT's data manager, an EPA START contractor, and BP personnel and contractors from the data management/sample tracking section and the data quality assurance section of the EU. Also, a NOAA data manager and an EPA representative are requested to be members.

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Appendix G: List of Acronyms

ADCP	Acoustic Doppler Current Profilers
AOR	Area of Responsibility
AUV	Autonomous Underwater Vehicle
BOEMRE	Bureau of Ocean Energy Management, Regulation, and Enforcement
BTEX	Benzene, Toluene, Ethylbenzene, and Xylene
CEQ	Council on Environmental Quality
CO-OPS	Center for Operational Oceanographic Products and Services
COP	Common Operating Picture
CTD	Conductivity, Temperature and Depth
CTEH	Center for Toxicology and Environmental Health, LLC
DIF	Data Integrated Format
DO	Dissolved Oxygen
DOI	Department of Interior
DOSS	Dioctylsulfosuccinate Sodium Salt (a dispersant-related chemical)
DPnB	Di(Propylene Glycol) Butyl Ether (a dispersant-related chemical)
DRO	Diesel Range Organic
DWH	Deepwater Horizon
EPA	Environmental Protection Agency
ERMA	Environmental Response Management Application
FDA	Food and Drug Administration
FORT	Fate of Oil Research Team
FOSC	Federal On-Scene Coordinator
FWS	Fish and Wildlife Service
GC/MS	Gas Chromatography/Mass Spectrometry
GCOOS	Gulf of Mexico Coastal Ocean Observing System
GIS	Geographic Information System
GOM	Gulf of Mexico
GRO	Gasoline Range Organic
IOOS	Integrated Ocean Observing System
JAG	Joint Analysis Group

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MC252	Mississippi Canyon, Block 252
MMS	Minerals Management Service
NCCA	National Coastal Conditions Assessment
NCDDC	National Coastal Data Development Center
NCP	National Contingency Plan (40 CFR 300)
NDBC	National Data Buoy Center
NIC	National Incident Command
NMFS	National Marine Fisheries Service (NOAA)
NOAA	National Oceanic and Atmospheric Administration
NODC	National Ocean Data Center
NPS	National Park Service
NRDA	Natural Resource Damage Assessment
NSF	National Science Foundation
NSF	National Strike Force (USCG)
OGC	Open Geospatial Consortium
OPA90	Oil Pollution Act of 1990
ORO	Oil Range Organic
ORR	Office of Response and Restoration (NOAA)
OSAT	Operational Science Advisory Team
OSTP	Office of Science and Technology Policy
PAH	Polycyclic Aromatic Hydrocarbon
QA/QC	Quality Assurance/Quality Control
QAPP	Quality Assurance Project Plan
SDF	Standard Data Format
SMB	Sub-surface Monitoring Branch
SMU	Sub-surface Monitoring Unit
SOS	Sensor Observation Service
SSC	Scientific Support Coordinator
SVOC	Semi Volatile Organic Compounds
UAC	Unified Area Command
UNOLS	University-National Oceanographic Laboratory System
USCG	United States Coast Guard

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USGS	United States Geological Survey
USPHS	United States Public Health Service
VIPERS	Vessels with Petroleum Ensnaring and Recovery Systems
VOC	Volatile Organic Compounds
VSP	Visual Sample Plan

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Appendix H: OSAT Membership

The Operational Science Advisory Team (OSAT) is a small group of agency representatives, or members, located at Deepwater Horizon Unified Area Command, within the Environmental Unit. The Unified Area Command operates under the direction of the Coast Guard's Federal On-Scene Coordinator. The Environmental Unit is under the operational control of the National Oceanic and Atmospheric Administration. OSAT acts as an advisory board, providing a cross-agency perspective based on near real-time analysis of data from the sub-surface and sub-sea monitoring effort to inform operational decision making.

Composition: Six scientific specialties were identified to coordinate daily analysis of data, trends and other considerations, based on the framework established by the Subsurface Monitoring Implementation Plan. Specialties were been further matched to agencies to provide the expertise needed to quickly and efficiently process the data.

Oceanography – NOAA (Lead)

General Environmental Science – US Coast Guard

Coastal Science and oil/contaminant chemistry – BP

Sedimentary Geology – US Geologic Survey

Microbiology – BOEMRE

Chemistry/toxicology – EPA

OSAT is also supported by staff from the U.S. Coast Guard (administrative support and coordination) and a data management specialist working under the auspices of NOAA. Below is the list of individual members of the OSAT.

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Agency	OSAT Member	Email
NOAA	Amy MacFadyen	amy.macfadyen@noaa.gov
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	Lyle Bruce	Lyle.bruce@bp.com

The members of the OSAT would also like express our gratitude to the many other individuals who supported us from early participation in organizing efforts and throughout the development and review process by providing discussion, data management support and the production of plots, graphs and maps. This report would not have been possible without your support.

ED_001324_00007095-00130

To: Conmy, Robyn[Conmy.Robyn@epa.gov]; 'fingasmerv@shaw.ca'[fingasmerv@shaw.ca]; 'tchazen@utk.edu'[tchazen@utk.edu]; 'Robert Jones - NOAA Federal'[robert.jones@noaa.gov]; 'Samantha Joye'[mandyjoye@gmail.com]; 'ken.lee@csiro.au'[ken.lee@csiro.au]; 'mbleigh@alaska.edu'[mbleigh@alaska.edu]; 'karl.linden@colorado.edu'[karl.linden@colorado.edu]; 'kmmcfarlin@alaska.edu'[kmmcfarlin@alaska.edu]; msmiles@lsu.edu[msmiles@lsu.edu]; 'thomas.s.coolbaugh@exxonmobil.com'[thomas.s.coolbaugh@exxonmobil.com]; 'Mathijs.Smit@shell.com'[Mathijs.Smit@shell.com]; Sprenger, Mark[Sprenger.Mark@epa.gov]
Cc: 'Lindsey R Howard'[lrw23@wildcats.unh.edu]; nancy.kinner@unh.edu[nancy.kinner@unh.edu]; Kinner, Peter[Peter.Kinner@unh.edu]; Wilson, Gregory[Wilson.Gregory@epa.gov]; Principe, Vanessa[Principe.Vanessa@epa.gov]; 'tracee.nguyen@csiro.au'[tracee.nguyen@csiro.au]; 'Ian Gaudreau'[ipu3@unh.edu]; 'doug.helton@noaa.gov'[doug.helton@noaa.gov]; 'Gary Shigenaka - NOAA Federal'[gary.shigenaka@noaa.gov]
From: Mandsager, Kathy
Sent: Tue 3/31/2015 4:24:12 PM
Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET
Biodegradation of dispersed Macondo oil in seawater at low temperature and different oil droplet sizes - 1-s2.0-S0025326X15000855-main.pdf

Attached is the paper in pdf format.

From: Mandsager, Kathy
Sent: Tuesday, March 31, 2015 11:14 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET

Additional paper for review. http://ac.els-cdn.com/S0025326X15000855/1-s2.0-S0025326X15000855-main.pdf?_tid=a972873c-d7b7-11e4-9464-00000aab0f27&acdnat=1427814635_2731a355cf42e8b64679699bc221613

From: Mandsager, Kathy
Sent: Monday, March 30, 2015 4:43 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'; Mandsager, Kathy
Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET

This is a reminder of our WebEx meeting tomorrow.

From: Mandsager, Kathy
Sent: Friday, March 20, 2015 6:19 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION - 3/31 at 11 ET

Mark your calendar! This is to confirm our next meeting for **Tuesday, March 31** beginning at 11:00 am ET.

Her are the WebEx directions to join this meeting. Thank you!

Degradation & Fate Group
Tuesday, March 31, 2015
11:00 am | Eastern Daylight Time (New York, GMT-04:00) | 2 hrs

Join WebEx meeting

Meeting number: 734 916 289

Join by phone
1-855-244-8681 Call-in toll-free number (US/Canada)
1-650-479-3207 Call-in toll number (US/Canada)
Access code: 734 916 289
Global call-in numbers | Toll-free calling restrictions

Can't join the meeting? Contact support.

From: Mandsager, Kathy
Sent: Thursday, March 19, 2015 8:31 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'; 'doug.helton@noaa.gov'; 'Gary Shigenaka - NOAA Federal'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION

I greatly appreciate your patience with this process. Please **cancel** the tentative hold on your calendar for tomorrow 3/20. Since we would greatly appreciate a majority of this group to participate in this 2-hour WebEx meeting, I am making another attempt at scheduling this call. Please use this doodle poll (all times noted are ET). Thank you to each of you!

Doodle poll here>> http://doodle.com/2w3gyb3gv4sv7iek

From: Mandsager, Kathy
Sent: Monday, March 16, 2015 4:39 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION

In the spirit of trying to keep everyone informed – there will NOT be a meeting tomorrow at 2

(see request below). I was unable to gain a quorum. Please continue to hold this Friday 3/20 as a potential meeting time. And if you have not yet responded with your availability, please let me know if you could possibly make in on **3/20 beginning at 1pm ET.**

Thank you.

From: Mandsager, Kathy
Sent: Monday, March 16, 2015 9:38 AM
To: 'Ian P Gaudreau'; 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: 'Lindsey R Howard'; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; 'tracee.nguyen@csiro.au'; 'Ian Gaudreau'
Subject: RE: Degradation & Fate update - NEXT DISCUSSION
Importance: High

While these papers are fresh on your mind, I am offering some dates as soon as possible for our next meeting. Kindly let me know if you can make yourself available for about a 2-hour timeslot at any of the following times:

Tuesday (tomorrow) 3/17 beginning at 2:00 pm ET

Friday 3/20 beginning at 12noon ET

Friday 3/20 beginning at 1:00 pm ET

Friday 3/20 beginning at 2:00 pm ET

Friday 3/20 beginning at 3:00 pm ET

Thursday 3/26 at 10:00 am ET

Monday 3/30 beginning at 2:20 pm ET

A speedy reply will be greatly appreciated. Thank you!

From: Ian P Gaudreau [mailto:ipu3@wildcats.unh.edu]
Sent: Friday, March 13, 2015 10:16 AM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'msmiles@lsu.edu'; 'thomas.s.coolbaugh@exxonmobil.com'; 'Mathijs.Smit@shell.com'; 'Sprenger, Mark <Sprenger.Mark@epa.gov> (Sprenger.Mark@epa.gov)'
Cc: Lindsey R Howard; Mandsager, Kathy; Kinner, Nancy; Kinner, Peter; 'wilson.gregory@epa.gov'; 'principe.vanessa@epa.gov'; tracee.nguyen@csiro.au; Ian Gaudreau
Subject: Degradation & Fate update - Official Papers

Hi folks,

Thank you for hanging in there on the conference call Wednesday. We really appreciate all of the hard work you have been doing. The 13 official papers you decided you would review and use as the basis for your comments (e.g. knowns, uncertainties) on the effect of chemical dispersants on oil biodegradation are:

- Venosa & Holder 2007
- Prince et al. 2013
- Prince & Butler 2014
- Mcnaughton et al. 2003
- McFarlin et al. 2014
- Mason et al. 2012
- Lindstrom & Braddock 2002
- Kleindienst et al. 2015
- Chakroborty et al. 2012
- Campo et al. 2013
- Bruheim et al. 1997
- Bruheum et al. 1999
- Baelum et al. 2012

These papers are all located in a folder on BOX entitled "Papers selected by panel - Effect of Chemical Dispersants on Oil Biodegradation". This link can be found here: https://unh.app.box.com/files/0/f/3252405678/Papers_selected_by_panel_-_Effect_of_Chemical_Dispersants_on_Oil_Biodegradation

Kathy will be contacting you to schedule what will hopefully be our final meeting of the Degradation & Fate group.

In preparation for the meeting, please re-read the paper and if you have time, send Kathy the conclusions that you derive from these references regarding what is known and uncertain about the effect of chemical dispersants on the biodegradation of oil, particularly as it applies to the Arctic.

Also, we need your feedback on a paper by Harris et al. 2002 (paper is attached in this email). Would you like us to add this paper to the "Papers selected by panel - Effect of Chemical Dispersants on Oil Biodegradation" folder on BOX? Here is the citation:

Harris, B.C. et al. 2002. Nutrient effects on the biodegradation rates of chemically-dispersed crude oil. *In* Twenty-Fifth Arctic and Marine Oilspill Program (AMOP) Technical Seminar, Nineteenth Technical Seminar on Chemical Spills (TSOCS) and Fourth Biotechnology Solutions for Spills (BIOSS): June 11 to 13, 2002, Westin Calgary Hotel, Calgary, Alberta, Canada: Proceedings. Ottawa, Ont.: Environment Canada. pp. 877-893.

Thanks again for your time and energy. I look forward to talking with you again.

Nancy E. Kinner, Ph.D.

University Professor

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Biodegradation of dispersed Macondo oil in seawater at low temperature and different oil droplet sizes

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article info

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Dispersions
Seawater
Low temperature
Droplet size

abstract

During the Deepwater Horizon (DWH) accident in 2010 a dispersant (Corexit 9500) was applied at the wellhead to disperse the Macondo oil and reduce the formation of surface slicks. A subsurface plume of small oil droplets was generated near the leaking well at 900–1300 m depth. A novel laboratory system was established to investigate biodegradation of small droplet oil dispersions (10 lm or 30 lm droplet sizes) of the Macondo oil premixed with Corexit 9500, using coastal Norwegian seawater at a temperature similar to the DWH plume (4–5 ffC). Biotransformation of volatile and semivolatile hydrocarbons and oil compound groups was generally faster in the 10 lm than in the 30 lm dispersions, showing the importance of oil droplet size for biodegradation. These data therefore indicated that dispersant treatment to reduce the oil droplet size may increase the biodegradation rates of oil compounds in the deepwater oil droplets.

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1. Introduction

During the Deepwater Horizon (DWH) incident some of the oil was dispersed as small droplets in the deep ocean at depths of 900–1300 m near the leaking well, and at water temperatures of 4–6 ffC (Camilli et al., 2010; Reddy et al., 2012). Simulations of the plume trajectories predicted that oil droplets with diameters between 10 and 50 lm would form in a distinct subsurface layer and be transported horizontally, while droplets larger than 90 lm rapidly rose to the surface (North et al., 2011). Samples from the deepwater plume showed enrichment of water-soluble components (Camilli et al., 2010). Measurements of PAH in water samples during the release showed rapid decrease with distance from the release point. The responders began injecting the chemical dispersant Corexit 9500A at the wellhead in early May 2010 to reduce the amount of oil that formed surface slicks and reached the Gulf of Mexico (GoM) coastlines (Atlas and Hazen, 2011; Kujawinski et al., 2011). In addition, surface operations included mechanical recovery techniques and application of the chemical dispersants Corexit 9527 and 9500A (Coastal Response Research Center, 2010).

It was early observed that the deepwater plume stimulated bacteria associated with hydrocarbon biodegradation in cold seawater (Oceanospirillaceae, Pseudomonas, Cycloclasticus, Colwellia, Pseudoalteromonas, Methylophaga) (Valentine et al., 2010; Hazen et al., 2010; Redmond and Valentine, 2012; Dubinsky et al., 2013), and data from the field and from laboratory experiments indicated short biodegradation half-lives for nC_{13}–nC_{26} alkanes, ranging from 1 to 8 days (Hazen et al., 2010). A sequential and pulsed propagation of bacteria degrading specific hydrocarbons was suggested, including n-alkanes, aromatics and C_1–C_3 gas components (Valentine et al., 2012; Dubinsky et al., 2013). Results from stable isotope analyses reported that close to 70% of the oxygen consumption in the deepwater plume was related to bacterial respiration of propane and ethane (Valentine et al., 2010), while methane oxidation was delayed (Valentine et al., 2010; Kessler et al., 2011).

Biodegradation rates of components in dispersed oil are normally expected to increase when oil droplet size is reduced, for instance by treatment of the oil with chemical dispersants. Smaller droplets will generate larger oil–water interphase areas for the oil-degrading bacteria to attach to, and dissolution of water-soluble compounds will increase. Volatile oil compounds will rapidly dissolve during subsurface spills and thereby become subject to biodegradation. In contrast volatiles will largely evaporate after surface spills. Several laboratory studies have shown that dispersant treatment of dispersible oils increase oil compound biodegradation (Siron et al., 1995; Venosa and Holder, 2007; Prince et al., 2013; McFarlin et al., 2014; Brakstad et al., 2014).

To our knowledge, no laboratory studies have attempted to relate the biodegradation of oil compounds to measured droplet sizes in the dispersions. We therefore established a novel

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experimental system for comparing oil dispersions of different small droplet sizes in natural seawater. This system was based on combining an oil dispersion generator (Nordtug et al., 2011) and a slowly rotating carousel system to generate small droplet dispersions and avoid droplets rising to the surface. The primary aim of our study was therefore to quantify biodegradation of hydrocarbons in dispersions with defined oil droplet sizes relevant for chemically dispersed oil, and at low seawater temperatures, corresponding to deepwater discharges. Biodegradation of chemically dispersed Macondo oil at two median oil droplet sizes (10 and 30 lm) in natural seawater at 4–5 ffC was investigated with this system. Norwegian coastal seawater was used in the study, but experimental conditions (oil, oil droplet size distributions and seawater temperature) were relevant to the DWH deepwater plume.

2. Materials and methods

2.1. Oil, dispersant and seawater used in experiments

Unweathered Macondo (MASS) oil was collected from a riser pipe connected to the well on 22 May 2010. COREXIT 9500A (Nalco, Sugar Land, TX) dispersant was used at a dispersant-to-oil ratio (DOR) of 1:100. Seawater was collected from a depth of 80 m (below thermocline) in a Norwegian fjord (Trondheimsfjord; 63ff26°N, 10ff23°E), outside the harbor area of Trondheim, the water is supplied via a pipeline system to our laboratories. The water source is considered to be non-polluted and not influenced by seasonal variations. The salinity of the seawater was 34‰, with a water temperature of 6–8 ffC and dissolved oxygen (DO) of approximately 8 mg/L when reaching our laboratory (Brakstad et al., 2004).

2.2. Oil dispersion generator and carousel system

An oil droplet generator was used for preparing oil dispersions with defined droplet size distributions, as previously described (Nordtug et al., 2011). The system consists of 3 chambers with an inner diameter of 8 mm connected by nozzles with a diameter of 0.5 mm. Oil dispersions with defined oil droplet size distributions and concentrations are generated by injecting oil via a capillary into a flow of seawater which moves through the chambers (Nordtug et al., 2011). Oil droplet concentrations and size distribution were determined by Coulter Counter analyses (see below). Flasks (2 L; Schott) were baked (450 ffC; 3 h) and soaked overnight (4% Deconex 11 Universal, Borer, Switzerland), washed with detergent (Neodisher N, Dr. Weigert, Germany), and thoroughly rinsed and autoclaved (121 ffC, 20 min) before use.

The flasks were completely filled with oil dispersions (no headspace or air bubbles) and mounted on a carousel system. During mounting of flasks and biodegradation experiments the wheels were slowly rotated around the carousel axis (0.75 r.p.m.) by a gear motor (SEW Eurodrive, Moss, Norway). In the main biodegradation experiment described here, four carousels with double sets of wheels, holding up to 16 flasks each, were used.

2.3. Biodegradation experiment

The MASS oil was heated at 50 ffC for 30 min, mixed by shaking for 3 min, and cooled to room temperature, after which it was mixed with the dispersant Corexit 9500A (DOR 1:100) at room temperature. This dispersant was shown to effectively disperse the Macondo oil (Daling et al., 2014). Pre-adaption of the seawater prior to the biodegradation experiments was conducted by diluting a stock dispersion (200 mg/L oil; oil droplet size 10 lm) to 0.2 mg/L Macondo oil (measured by Coulter Counter) in natural unfiltered seawater (pre-incubated over night at 5 ffC) in 2-L flasks completely filled (no air bubbles) with the dispersions, with incubation in the carousel system at 5 ffC for 5 days (rotation 0.75 r.p.m.). The experiment was then started by generating dispersions in the pre-adapted seawater with median oil droplet sizes of 10 lm or 30 lm and nominal oil concentration of 2 mg/L from a stock oil dispersion (200 mg/L oil) in natural unfiltered seawater. Dispersions of 10 lm or 30 lm median oil droplet sizes and 2 mg/L nominal concentrations were also prepared in sterile-filtered (0.2 lm) seawater (incubated at 4–5 ffC overnight before use) supplemented with $HgCl_2$ (100 mg/L), to minimize bacterial growth. Immediately after generation of dispersions flask were mounted on the carousel at slow rotation (0.75 r.p.m.). Flasks with experimental blank samples (unfiltered seawater without oil) were also included in the experiment for background analyses. Experiments were conducted at 5 ffC for up to 64 days. Flasks (triplicate samples; 2.3 L sample) of dispersions in natural seawater were sacrificed for analyses after 15 min on the carousel (0-day samples) and after 2, 4, 8, 16, 32, or 64 days of incubation on the carousels. One sterilized control flask and one experimental blank flask were also sacrificed for analyses at the each sampling point.

2.4. Analyses

2.4.1. Oil droplet analyses

Oil droplet concentrations and size distributions were determined by Coulter Counter measure (Beckman Multisizer 4; Beckman Coulter Inc., Brea, CA, USA) fitted with either 100 lm or 280 lm apertures, for measurement of droplets within a diameter range of 2–60 lm or 5.6–100 lm, respectively. Filtered (0.22 lm) seawater was used as electrolyte. All median droplet sizes reported here are expressed as median droplets diameter on droplet volume if not otherwise mentioned.

2.4.2. Chemical analyses

Samples of dispersions and seawater were solvent–solvent extracted with dichloromethane (DCM) for measurements of semivolatile organic compounds (SVOC) by gas chromatographic methods. The flask glass walls were also rinsed with DCM after removal of dispersions to extract material attached to the glass walls. All extracts were pooled before analyses. A gas chromatograph coupled to a flame ionization detector (GC-FID; Agilent 6890 N with 30 mDB1 column; Agilent Technologies) was used for quantification of extracted C_{10}–C_{36} saturates separated according to boiling points, termed as total petroleum hydrocarbons extractable organic carbon (TEOC), while 96 individual targeted compounds (C_{10}–C_{36} n-alkanes, decalines, phenols, 2- to 5-ring polyaromatic hydrocarbons (PAH) and 17a(H),21b(H)-Hopane (30ab Hopane) were analyzed in a gas chromatograph coupled to a mass spectrometer (GC–MS; Agilent 6890 plus GC coupled with an Agilent 5973 MSD detector, operated in Selected Ion Monitoring [SIM] modus; Agilent Technologies), as recently described (Brakstad et al., 2014). Dispersions were acidified (pH < 2) for analyses of 87 targeted volatile organic carbon (VOC) compounds in a Purge & Trap unit (Teledyne Tekmar Atomx; Mason OH, USA.) coupled to a GC–MS (Agilent 6890 N GC and an Agilent 5975B MSD detector; Agilent Technologies)(P&T GC–MS). The VOC and SVOC target compounds included in GC–MS analyses are shown in Table S1. The response values for individual target analytes were determined, with a signal-to-noise ratio of 10 as the lower detection limit, and a lower limit of detection (LOD) of 0.01 lg/L was defined for individual oil compounds.

2.4.3. Temperature, dissolved oxygen, nutrients, and biomass

Air and seawater temperatures were logged through the experimental period. Dissolved oxygen was measured by a

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dissolved oxygen meter (YSI, Inc., Yellow Springs, OH). Nutrient analyses included NO_3–N + NO_2–N (ISO 13395), NH_4–N (ISO 11732), o-PO_4–P (ISO 15681-2), and total Phosphorous (ISO 15681-2), analyzed by Eurofins Environment Testing Norway, Bergen, Norway. Microbial biomass concentrations were determined by epifluorescence microcopy analyses of samples stained by the nucleic acid stain 4⁰,6-diamidino-2-phenylindol (Porter and Feig, 1980).

2.5. Calculations and statistics

Non-linear regression analyses were performed by a 1st order rate approach with determination of lag-phases included using the option "plateau followed by one-phase decay" in GraphPad Prism vs. 6.0 (GraphPad Software Inc., La Jolla, CA, USA). Rate coefficients (k1) were determined for the decay-period, the plateau period defined the lag-phase, and half-lives were determined from rate coefficients and plateau periods (t1/2 = plateau period + 0.693/k1). Rate data were determined for both single oil components and for 22 oil compound groups described in the Oil-Spill Contingency and Response model OSCAR (Reed et al., 2000). The group composition is described in Table S1.

Column statistics were compared by Wilcoxon matched paired test in GraphPad Prism vs. 6.

3. Results and discussion

3.1. Generation and maintenance of oil dispersions over time

Dispersions of Macondo oil mixed with Corexit 9500A (DOR of 1:100) were generated by different seawater flow-rates through the oil droplet generator system, from 50 to 150 ml/min. This resulted in oil dispersions with median droplet size distributions of approximately 10 l m to 30 l m in diameter (Fig. 1). The oil pump of the dispersion generator was set to generate fixed nominal oil concentrations during the dispersion generation (0.2–200 mg oil per L of water), and good agreement between nominal oil concentrations and measured oil droplet concentrations was recorded (data not shown). Recent studies have shown that nearly the same oil droplet size distribution is generated in this system both with and without premixing of oil with chemical dispersant (Nordtug et al., 2011).

The carousel system was developed for maintaining the droplet size distribution in the oil dispersions while incubating the dispersions over time. All experiments in the carousels were conducted in air-tight flasks without headspace to avoid evaporative depletion. The system was able to handle dispersions of fresh Macondo oil with small droplets, in the diameter size range of 10–30 l m, with rising velocities of 1.6–14.4 cm/h, according to Stoke's law. We believe the ability of keeping the droplets in suspension is due to the combined effect of the turbulence created in the flasks and the fact that the oil droplet rising velocities will make them constantly change direction in relation to any fixed point at the flasks inner surface. Larger than 30 l m oil droplets resulted in inhomogeneous dispersions during transfer from dispersion generator to flasks in carousel system because of elevated rising velocities. In initial experiments with 10 l m median volumetric droplet dispersions in a sterilized system (filtered seawater poisoned with $HgCl_2$), the droplet sizes were maintained for at least 28 days in the carousel system, while measured droplet concentration decreased (see Supplementary Information, Fig. S1).

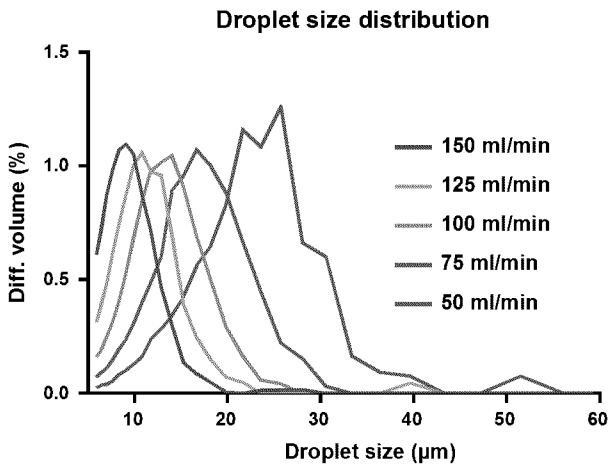

Fig. 1. Average oil droplet size distributions generated in the oil dispersion generator at different seawater flow-rates after premixing of the oil with Corexit 9500A.

3.2. Biodegradation experiment with two dispersions of different oil droplet size distributions

An oil dispersion biodegradation experiment was performed at 5 fC (64 days) with nominal oil concentrations of 2 mg/L generated in the dispersion generator at median oil droplet sizes on volume of 10 l m and 30 l m in diameter, and with natural seawater pre-adapted to the oil and temperature (see Section 2). The low oil concentration used in this experiment is consistent with recent recommendations to avoid too high oil loading during biodegradation studies of dispersed oil (Lee et al., 2013). Seawater pre-adaption was included since many offshore oil production areas may be exposed to hydrocarbons through natural seepage or produced water releases. For instance, a recent hydrocarbon seepage flux of 2.2–18 ┐ 10^7 tons per year has been estimated for the GoM region, corresponding to 14–120% of the DWH discharge rate (Smith et al., 2014). Analyses of macronutrients (NO_3 + N O_2–N, NH_4–N and o-PO_4–P) and dissolved oxygen showed no apparent limitations in oxygen or inorganic nutrient levels during the biodegradation experiment (Supplementary Information, Figs. S2 and S3).

3.2.1. Oil droplet sizes and concentrations

The oil droplet size distributions and concentrations were followed in the dispersions during the biodegradation experiment (Fig. 2). Results from Coulter Counter analyses showed that the initial median oil droplet size distributions in the 10 l m and 30 l m dispersions were 11.0 ± 0.04 l m and 29.4 ± 3.5 l m, and that the 10 l m and 30 l m dispersions represented oil surfaces of 10.9 ± 0.2 ┐ 10^5 and 5.4 ± 0.9 ┐ 10^5 l m^2/ml, respectively. The dispersions with the smaller droplets therefore contained approximately twice the surface area of the dispersions with the larger droplet. Assuming a microbial size of 1 l m in diameter and the maximum microbial density of 1 cell/l m^2 of oil droplet surface area (Horowitz et al., 1975; Vilcaez et al., 2013) the initial oil droplet concentrations represented sufficient surface areas for colonization of up 10 ┐ 10^5 cells/ml in the 10 l m dispersions and 5 ┐ 10^5 cells/ml in the 30 l m dispersions. Initial cell concentrations determined by epifluorescence microscopy were 8 ┐ 10^5 and 12 ┐ 10^5 cells/ml in the 10 l m and 30 l m dispersions, respectively. The oil droplets therefore represented surface areas for attachment of the entire bacterial population in the 10 l m dispersions and approximately 40% of the population in the 30 l m dispersions at the start of the experiment.

The oil droplet size distributions during the biodegradation period (Fig. 2A) were maintained in the 10 l m dispersions (average median droplet size of 3 replicates ranging from 10.7 to 13.4 l m in the natural unfiltered seawater and 8.5–11.1 l m in sterile dispersions). However, the 30 l m dispersions in natural

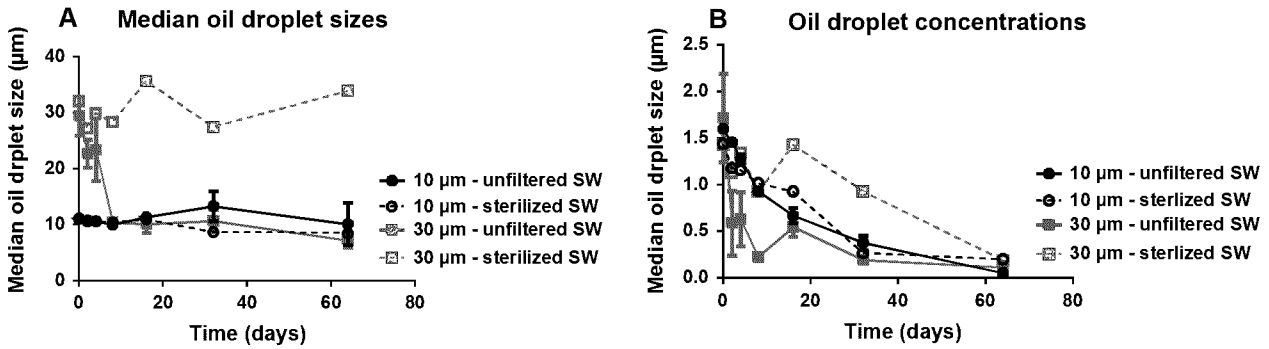

Fig. 2. Median oil droplet sizes (A) and concentrations (B) in oil dispersions generated during a biodegradation period of 64 days at 5 fC in natural unfiltered seawater (unfiltered SW), or in filtered (0.1 l m) and sterilized ($HgCl_2$) seawater (sterilized SW). The dispersions were generated at nominal particle sizes of 10 and 30 l m diameter. The dispersions in unfiltered seawater were analyzed in triplicate (error bars show standard deviation), while sterile samples were analyzed as single samples.

seawater experienced a rapid decline in droplet size between days 0 and 8, from 29.4 to 10.4 l m in average diameter (3 replicates), with a subsequent particle size between 10.2 and 13.4 l m median in the period from day 8 to 64. In contrast, the median oil droplet diameter was maintained in the sterile 30 l m dispersions, ranging from 27.2 to 34.9 l m during the experimental period (Fig. 2A). When oil droplet concentrations were determined by Coulter Counter analyses the concentrations declined more rapidly in natural unfiltered seawater than in the sterilized dispersions. In the 10 l m dispersions the concentrations were decreased from days 0 to 8 by 41.9% (from 1.60 to 0.93 mg/L average values) in natural unfiltered seawater and by 29.2% (from 1.44 to 1.02 mg/L) in the sterilized dispersions, while the reductions in the 30 l m dispersions were 86.9% (from 1.72 mg/L to 0.22 mg/L) and 35.0% (from 1.43 mg/L to 0.93 mg/L) in unfiltered and sterilized seawater, respectively (Fig. 2B). In the sterilized systems with 30 l m oil droplets it was evident that the larger droplets were removed faster than the smaller droplets (Supplementary Information, Fig. S4D). Reduced concentrations of droplets may therefore be caused by removal of the larger droplets with the fastest rising velocities or by droplet coalescence, generating droplets of sizes outside the measuring range of the Coulter Counter instrument (5.6–100 l m), as shown in oil droplet coalescence models (Sterling et al., 2004). The 10 l m dispersions also included oil droplets smaller than the lower instrumental measuring size of 5.6 l m (Supplementary Information, Figs. S4A and S4B). In addition, oil droplets could be removed from the system by attachment to the glass walls of the flasks, as measured by chemical analyses (see below). The higher reduction in oil droplet concentrations in unfiltered than sterilized dispersions may be caused by integration of droplets in "clusters" or "flocs" in natural seawater, which were visually observed in the experiment. These aggregates have been observed in several biodegradation studies of dispersed oil (e.g. Swannell et al., 1997; Harris et al., 2002; MacNaughton et al., 2003; Bælum et al., 2012).

3.2.2. Biodegradation of targeted oil compounds

Concentrations of 183 targeted semivolatile or volatile oil compound measured by GC–MS analyses showed that 81.4% of these were detected in the dispersions above the LOD (0.01 l g/L) at the start of the experiment. Compounds not detected above LOD included C_0–C_4 phenols and benzothiophenes (Supplementary Information, Fig. S5), while additional 13 compounds (7.1%) with concentrations close to LOD (60.1 l g/L) were excluded for biodegradation determination (Supplementary Information, Fig. S5).

Depletion of semivolatile nC_{10}–nC_{36} alkanes and non-alkylated and alkyl-substituted decalines, naphthalenes, fluorenes, phenanthrenes, dibenzothiophenes, fluoranthenes and chrysenes were examined in the dispersions after normalization against the persistent biomarker 17a(H),21b(H)-Hopane (30ab Hopane), commonly used in biodegradation studies (Prince et al., 1994; Page et al., 1995; Wang et al., 1998). The depletions were determined as percentages of normalized data at the start of the experiment (day 0) for each target oil compound (Fig. 3). In average, the oil compound groups showed low to negligible depletion in sterilized dispersions during the first 32 days (average <1%), although decaline concentrations were reduced by more than 20%. After 64 days the oil compounds were further depleted in the sterilized dispersions (average reduction of 19%). However, compounds in natural unfiltered seawater were mostly reduced to near completion before they were depleted in the sterilized dispersions. The oil compound depletion in natural unfiltered seawater was therefore mainly the result of biotransformation.

Low-range (nC_{10}–nC_{15}) and medium-range (nC_{16}–nC_{21}) molecular weight (MW) n-alkanes were rapidly biotransformed after 4 days in the 10 l m dispersions (76.3 ± 2.7 and 74.5 ± 1.9% depletion, respectively), while biotransformation of these n-alkanes started later in the 30 l m dispersions and did not become evident until 8 days (84.2 ± 4.6 and 82.2 ± 3.4% degradation, respectively) in the 30 l m dispersions (Fig. 3A). Faster biotransformation of the low-range and medium-range MW n-alkanes was therefore measured in the 10 l m than the 30 l m dispersions. Also decaline biotransformation started faster in the smaller droplet dispersions, between days 8 and 16 in 10 l m dispersions (60.6 ± 3.6% degradation), and between days 16 and 32 (88.7 ± 9.5% degradation at 32 days) in the 30 l m dispersions (Fig. 3A). However, high-range MW n-alkanes (nC_{22}–nC_{36}) shower slower and more similar biotransformation in the two dispersions (73.0 ± 4.8 and 63.5 ± 0.5% degradation in the 10 l m and 30 l m dispersions after 16 days)(Fig. 3A).

Low-range and medium-range MW PAH-compounds (2- to 3-ring PAH) were biotransformed between day 8 and 16 in both dispersions, with 99.7 ± 0.2 and 88.9 ± 9.0% degradation after 16 days in the 10 and 30 l m dispersions, respectively (Fig. 3B). Also for these PAH compounds faster biodegradation in the 10 l m than the 30 l m oil droplet dispersions was indicated. However, larger PAH (4- to 5-ring) were only biotransformed between 32 and 64 days, with 95.7 ± 5.2 and 88.7 ± 2.1% decay in the 10 and 30 l m dispersions after 64 days.

VOC compounds determined by P&T GC–MS analyses could not be normalized against a biomarker, since analyses of these did not include any recalcitrant biomarker. Determination of % concentrations relative to 0-day concentrations for the alkane and aromatic VOC compound groups showed biotransformation mainly between 8 and 16 days, resulting in 99.6 ± 0.05 and 96.5 ± 5.0% depletion of

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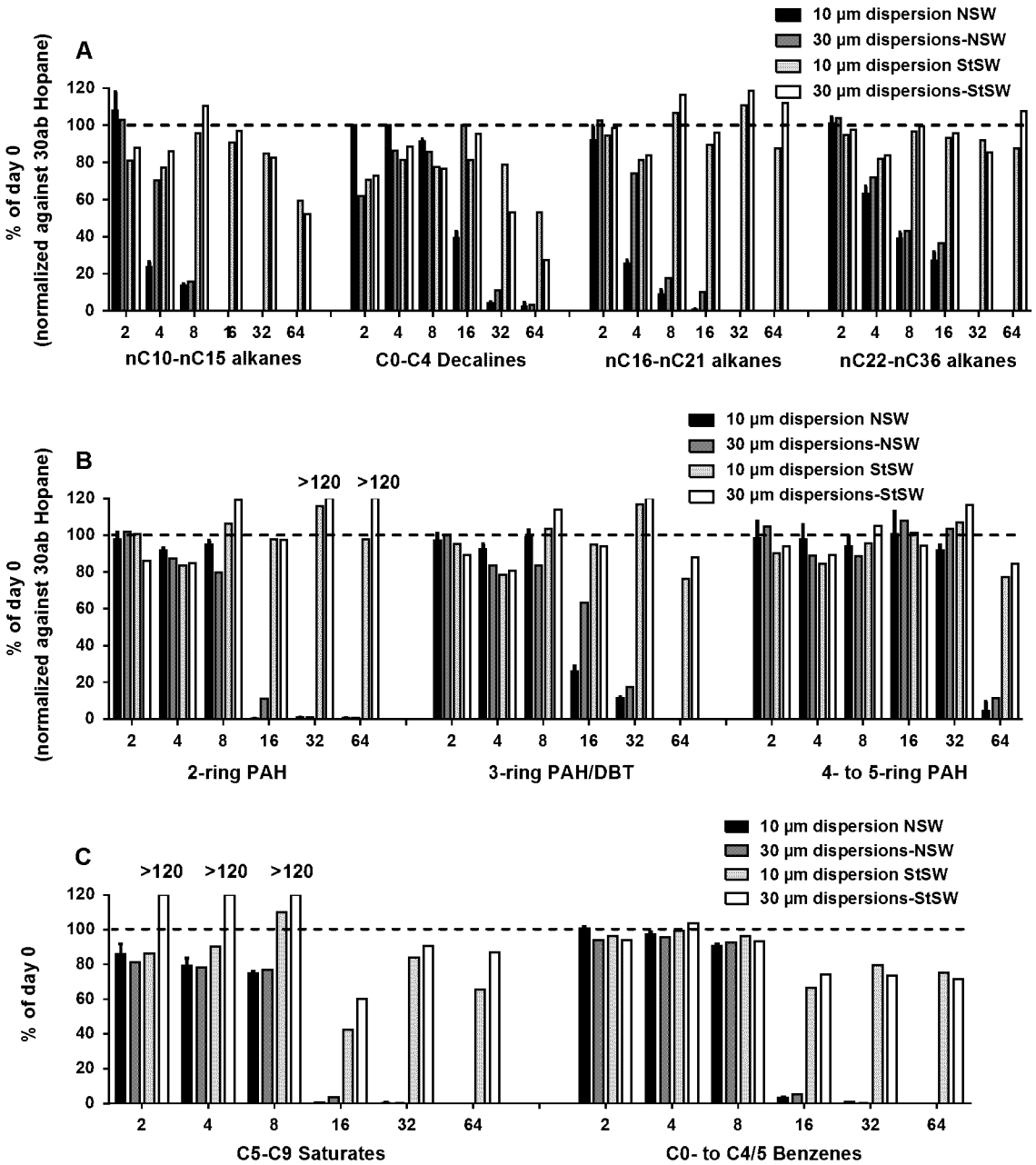

Fig. 3. Percentage concentrations compared to day 0 of semivolatile n-alkanes and C_0–C_4 alkylated decalines (A), semivolatile 2–5 ring PAH (B), and volatile alkanes and aromatic hydrocarbons (C) in 10 l m and 30 l m dispersions in natural seawater (NSW) and sterilized seawater (StSW) during an incubation period of 64 days, with samples collected after 2, 4, 8, 16, 32 and 64 days. The percentages of semivolatile compounds were determined from GC–MS analyses (triplicate) normalized against the biomarker 30ab Hopane with standard deviation error bars of 3 replicates. Volatile compounds were determined without normalization. The broken line shows the 0-day 100% for each target compound.

C_5–C_9 alkanes in 10 l m and 30 l m dispersions, respectively, while corresponding data for C_0- to C_4/C_5 benzenes were 96.8 ± 0.5 and 94.4 ± 2.3% depletion (Fig. 3C). Although sterilized dispersions also showed decreased concentrations of volatiles after 16 days (39.7–57.7% and 25.9–33.5% depletion of saturates and benzenes, respectively) most of the depletion in natural unfiltered seawater may therefore be accounted for by biodegradation.

Non-linear regression analyses of single oil compounds (normalized against 30ab Hopane) were performed by 1st order rate kinetics not including the determined lag-phases, and rate coefficients and half-lives for targeted oil compounds were determined in the dispersions. Low oil concentrations with no significant increase in microbial concentrations (epifluorescence microscopy) during the biodegradation period (average cell numbers differed with a factor of 1.02 between days 0 and 64) argued for 1st order kinetics to determine biotransformation coefficients and half-lives (Battersby, 1990). Non-responsive periods (lag-phases) were observed for most of the targeted compounds (Fig. 3). During the DWH spill it was suggested that deepwater with bacteria re-entered the spill site and caused accelerated hydrocarbon biodegradation (Valentine et al., 2012), resulting in a situation in which hydrocarbon-stimulated bacterial populations eventually could degrade oil compounds without a preceding lag-phase.

First-order rate calculations of targeted semivolatile n-alkanes and 2- to 4-ring PAH normalized against 30ab Hopane were performed (Fig. 4, Table S2). Half-lives calculated from the rate

coefficients varied from 0.6–5.6 days (median 1.1 days) and 1.1–11.1 days (median 2.5 days) for nC_{10}–nC_{30} alkanes in 10 l m and 30 l m dispersions, respectively, while corresponding data for 2- to 4-ring PAH were 0.9–31.9 (median 5.3 days) and 0.9–54.9 days (median 10.2 days). The half-lives for most of the targeted compounds (44 of 48 compounds) were lower in the 10 l m than the 30 l m dispersions, and the differences between the dispersions were significant ($P < 0.05$; Wilcoxon non-parametric t-test). The n-alkane half-lives were increased with longer carbon-chains in both dispersions, primarily in the high-boiling-point range (P nC_{21}). PAH half-lives increased in both dispersions with increasing numbers of aromatic rings and with degree of alkyl-substitution, except for the chrysenes (Fig. 4). Only small differences in half-lives were determined for chrysenes, which appeared in low concentrations in the dispersions (sum of C_0–C_4 chrysenes of 0.59 and 0.79 l g/L in 10 l m and 30 l m dispersions, respectively; supplementary information Fig. S5). Small oil droplets therefore had a positive impact on biotransformation of both n-alkanes and PAH. In a recent study of Macondo oil biodegradation under a near-surface temperature relevant for the GoM, comparing physically and chemically dispersed oil, we observed higher biodegradation differences related to droplet size for n-alkanes (nC_{10}–nC_{30}) than 2- to 4-ring PAH (Brakstad et al., 2014). However, in the near-surface oil biodegradation study both temperature (30–32 fC), experimental system and oil droplet size distributions differed from the current experiment. The different temperatures will also stimulate different taxa of oil-degrading bacteria, as shown from field analyses of the DWH incident, in which none of the predominant bacteria in the deepwater plume (Oceanospirillales, Colwellia and Cycloclasticus) were abundant in surface slicks (Redmond and Valentine, 2012). Detailed results from characterization of cell counts and bacterial communities from the current study will be presented in a separate manuscript (Brakstad et al., in preparation). And importantly, the oil characteristics will be different at the two temperatures. For instance some of the larger n-alkanes may generate wax crystals at low temperature, thereby becoming less susceptible to biodegradation.

First-order rates are often calculated without accounting for the lag-phases. In these studies the lag-periods become a part of the half-live determination. In Table S2 (Supplementary Information) the half-lives are also determined as the sum of the lag-periods and the half-lives determined from the rate coefficients. By these calculations the n-alkane half-lives varied from 2–17.6 days in the 10 l m dispersions and 3.1–20.8 days in the 30 l m dispersions. PAH half-lives varied correspondently from 8.8–49.9 and 8.7–79.9 days in the two dispersions. The same trends for n-alkanes and PAH were observed with these half-life calculations as described above, with generally shorter half-lives of the same compounds in the 10 l m than in the 30 l m dispersions. The sums of half-lives and lag-phases also differed significantly between the 10 l m and the 30 l m dispersions ($P < 0.05$; Wilcoxon non-parametric t-test).

Our n-alkane and PAH biodegradation data, using coastal Norwegian seawater from 80 m depth, mainly coincided with results from studies with chemically dispersed oils incubated at low temperature (L 1 to +8 fC), in which n-alkane depletion corresponded to half-lives of 2 to approximately 10 days, while 2- to 4-ring half-lives ranged between 2 and 37 days (Siron et al., 1995; Venosa and Holder, 2007; Prince et al., 2013; McFarlin et al., 2014). Also results from the DWH deepwater plume samples and laboratory studies with microbial consortia or seawater from GoM indicated average n-alkane (nC_{13}-nC_{26}) half-lives of 1.2–6.1 days (Hazen et al., 2010), showing good agreement with our results. In a recent study with small droplet (10–30 l m) dispersions of Macondo oil in GoM deepwater, biotransformation of 2–3 ring PAH appeared mainly between 9 and 39 days of incubation, while 4–6 ring PAH

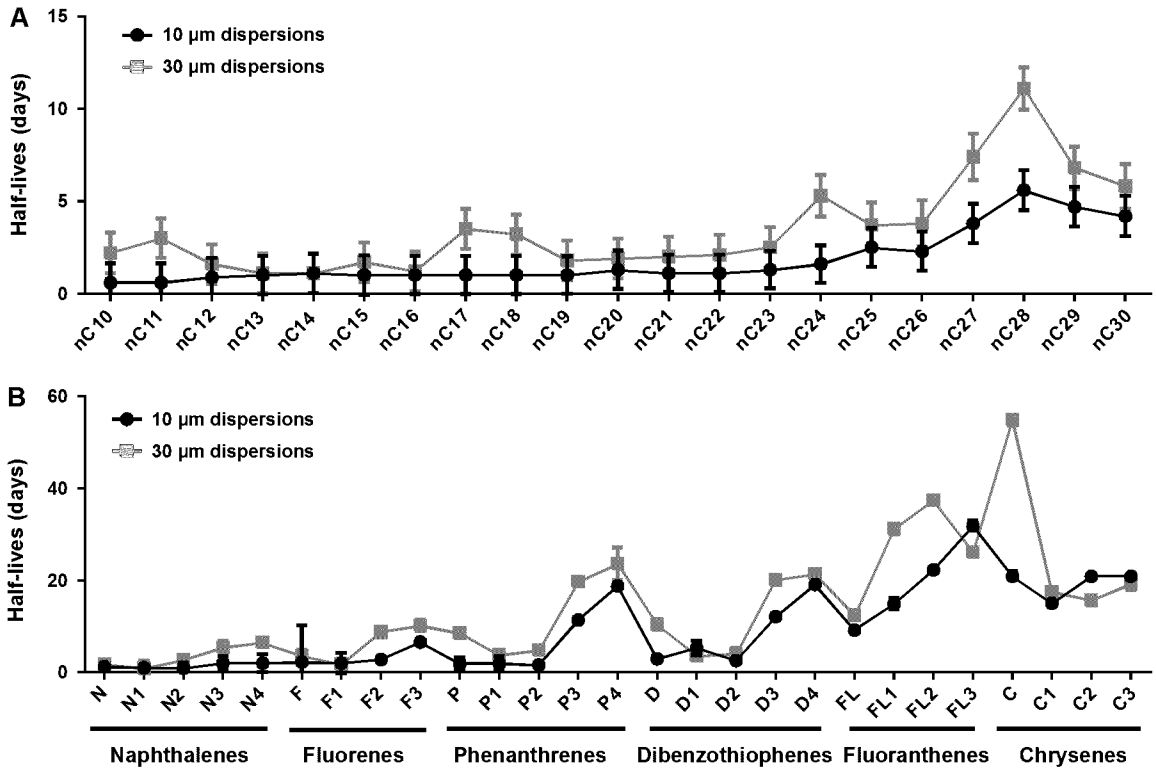

Fig. 4. Half-lives of nC_{10}–nC_{30} alkanes (A) and naphthalenes, fluorenes, phenanthrenes, dibenzothiophenes, fluoranthenes and chrysenes (B) in 10 l m and 30 l m dispersions with standard deviation errors bars of 3 replicates. Half-lives were determined from rate coefficients without lag-periods included. The numbering of the aromatic groups describes the degree of alkyl-substitution.

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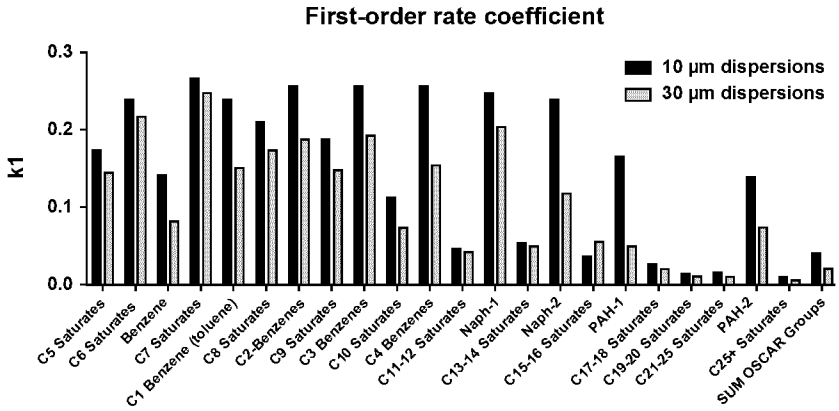

Fig. 5. First-order rate coefficients for oil compound groups of the Macondo oil in 10 and 30 l m dispersions.

transformation had started after 39 days of incubation (Wang et al., 2014), consistent with PAH results from our study.

Oil compound glass wall attachment is well known, and comparison of oil concentrations (TEOC analyses) in water and on glass walls rinsed with solvent (days 0, 8 and 32) showed a gradual increase of wall-attached material, from <3% of total TEOC (day 0), to up to 45% (30 l m dispersions at day 32). Targeted analyses of compounds normalized against 30ab Hopane showed relative preference of 2-ring and non-alkylated 3-ring PAH for the water-phase, while larger alkylated 3-ring and 4- to 5-ring PAH, decalines and n-alkenes gradually distributed between the water- and glass-phase (results not shown). These data emphasize that degradation of dispersed oil in laboratory systems is a result of processes in both water and glass wall surfaces.

3.2.3. Biodegradation of oil compound groups

The well-known targeted compounds described above only cover a fraction of the oil. Comparison of TEOC concentrations (GC-FID analyses) with the targeted SVOC and VOC saturate and aromatic oil compound (GC–MS analyses) showed that the sum of the targeted compounds (n-alkenes, 2–5 ring PAH, volatile C_5–C_9 saturates and C_0–C_5 benzenes) constituted 33.5% and 40.4% of the sum of TEOC and VOC concentration at the start of the experiment in the 10 l m and 30 l m dispersions, respectively (Supplementary Information, Fig. S6). The TEOC concentrations determined by GC-FID analyses represented 72% and 81% of the oil droplet concentrations measured by the Coulter Counter analyses for the two dispersions, and these results corresponded well with the assumption from true boiling point curves, assuming that C_5–C_{36} saturates (boiling points of 27 to approximately 500 fC) represent 70–80% of typical crude oil (Pasquini and Bueno, 2007).

Biotransformation rate coefficients and half-lives of 22 volatile and semivolatile oil compound groups, representing the boiling point range described above (Table S1) were calculated, including C_5–C_{36} saturates and 1- to 5-ring aromatic compounds (Reed et al., 2000). The results of Fig. 5 and Table S3 showed rate coefficients of 0.0096–0.2665 (median 0.1692) in the 10 l m dispersions and 0.0054–0.2475 (median 0.0995) in the 30 l m dispersions. These rates resulted in half-lives of 2.8 to 72.2 days (median 4.1 days) and 2.8 to 128.3 days (median 7.2 days) in the 10 and

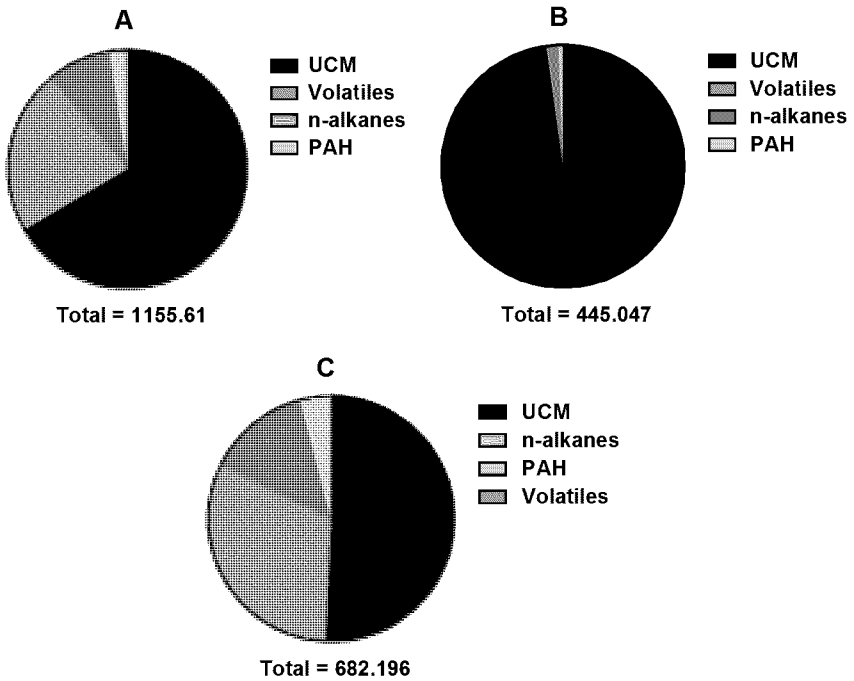

Fig. 6. Distributions of targeted oil compounds and UCM at the start of the biodegradation (A) and after 32 days of incubation in unfiltered (B) and sterilized (C) dispersions. The total concentrations of TEOC (l g/L) are shown below each pie chart.

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30 l m dispersions, respectively. The rate coefficients were higher in the 10 l m than the 30 l m dispersions for all oil compounds, except for C_{15}–C_{16} saturates. Comparison of the rate coefficients in the two dispersions showed significant differences ($P < 0.05$; Wilcoxon non-parametric t-test). The rate coefficients were higher in favor of the 10 l m dispersions by factors of 1.21 and 1.67 (median values) for the saturate and aromatic compound groups, respectively. The rate coefficients were generally higher for the aromatic (average of 0.175039) than the saturate (0.099304) oil compound groups when data from both dispersions were compiled. This is probably associated with the water-solubility of the aromatic hydrocarbons, as well as the presence of poorly biodegradable branched and cyclic alkanes in the semivolatile saturate groups.

In previous studies we have compared dissolution and biodegradation of oil compounds from thin oil films (10–20 l m thickness) immobilized on hydrophobic adsorbents, showing that fast dissolution of naphthalenes and non-alkylated 3-ring PAH resulted in biodegradation mainly of dissolved compounds, while negligible dissolution of n-alkanes (nC_{12}–nC_{36}) resulted in biodegradation of these compounds exclusively on the oil–water interphases. For the larger PAH-compounds dissolution decreased with increasing numbers of aromatic rings and alkyl substitution, with biodegradation of these both as dissolved and as oil-associated compounds (Brakstad et al., 2004; Brakstad and Bonaunet, 2006). Oil compound biodegradation is therefore a complex process, involving bacterial communities in the water-phases as well as bacteria attaching the oil surfaces.

The rate for the sum of the 22 oil compound groups in this study was also faster in the 10 l m ($k1 = 0.03984$) than the 30 l m ($k1 = 0.02004$) dispersions, resulting in half-lives of 17.4 and 34.6 days in the two dispersions (Fig. 5; Table S3). Since the sum of these groups may represent most of the oil, these numbers may be used as biotransformation rates for the oil. The oil droplet size was therefore important both for the biotransformation rates of the oil compound groups and the sum of these groups used in this study and presenting a boiling point range of 27–>500 fC.

The saturates representing the C_{10} to C_{25+} oil compound groups are mostly unidentifiable oil compounds, often termed as the unresolved complex mixture (UCM) (Gough and Rowland, 1990; Killops and Al-Juboori, 1990), and accounts for 60–70% of the groups. Comparison of the sum of TEOC and VOC concentrations with targeted compound groups of n-alkanes, PAH and volatile hydrocarbons showed that the UCM became the predominant oil fraction during the biodegradation period (Fig. 6; Fig. S6, Supplementary Information). Several studies have shown that the UCM fraction includes compounds that are not readily susceptible to biodegradation (Reddy et al., 2002; Booth et al., 2007). The UCM was predominant in each of the saturate groups from C_{11}–C_{12} to C_{25+} (Table S1). The degradation of these compounds decreased by increasing boiling point ranges in both 10 l m and 30 l m dispersions (Table S3), and the rate calculations showed that their degradation was mainly faster in the 10 l m than the 30 l m dispersions (Fig. 5; Table S3).

The result from the current study show that reduced oil droplet sizes resulted in increased biodegradation rates. The use of chemical dispersants will generate small droplet oil dispersions, and the result from this and other studies (e.g. Siron et al., 1995; Venosa and Holder, 2007.; Prince et al., 2013; McFarlin et al., 2014; Brakstad et al., 2014) demonstrate that the use of dispersants to generate small droplets will promote biodegradation in seawater at different environmental conditions. Dispersants like Corexit 9500 are considered to have moderate to low acute toxicity (e.g. Mitchell and Holdway, 2000; Judson et al., 2010), and surfactants like alkyl sulfosuccinates may be biodegradable (Garcia et al., 2009; Bælum et al., 2012). The positive influence of chemical dispersants on the biodegradation of potentially toxic oil compounds may therefore compensate for possible negative effects of dispersant components in the marine environment.

Operationally, dispersants have mainly been used for surface oil spills to remove the slick from the seawater surface by generation of small droplet dispersions in the water column. We have previously shown that oil compounds in surface oil emulsions are poorly biodegradable compared to chemically dispersed oil (Brakstad et al., 2014). The dispersant application during the DWH incident aimed at reducing surface slick generation and may also have contributed to the generation of the small oil droplets in the Macondo deepwater plume. If deepwater injection of dispersant during an oil spill is able to reduce oil droplet size and avoid oil surfacing, this may enhance oil compound biodegradation and thereby additionally reduce the impacts of the spill. However, it is important to emphasize the lack of knowledge related to the many unidentifiable oil compounds, both with respect to biodegradability and toxic effects.

Although the water used in the current study was Norwegian coastal seawater, the results may be related to the Macondo deepwater plume. Both oil, oil droplet size distribution (North et al., 2011) and seawater temperature was similar in the current experiment and the DWH deepwater plume. Our experiments were performed at atmospheric pressure, but laboratory experimental studies have shown that bacteria associated with deepwater plume biodegradation of the Macondo oil also biodegraded the oil at atmospheric pressure (Hazen et al., 2010; Bælum et al., 2012).

4. Conclusions

The study presented here compares, for the first time to our knowledge, biodegradation rates in oil dispersions with pre-defined droplet sizes, and under temperatures relevant for a deepwater discharge. The results of this study showed that biodegradation of alkane and aromatic hydrocarbons in a dispersible oil (Macondo) was faster in 10 l m than 30 l m dispersions for targeted oil compounds and oil compound groups, as well as the sum of the groups representing most of the oil. These data indicate that dispersant treatment of deepwater oil spills will be beneficial for the oil compound biodegradation, if oil droplet sizes are reduced. Our data showed good agreement with results from other biodegradation studies performed with chemically dispersed oil at low seawater temperatures. Although the current study was performed in seawater from a Norwegian fjord and under atmospheric pressure, these data may also have relevance for the DWH deepwater oil plume. We used the same oil, similar temperatures (4–5 fC), and oil droplet size distributions were comparable to the DWH deepwater plume. Published oil compound degradation data with GoM seawater or enrichment cultures also showed agreement with our results. Comparison of fate model predictions, laboratory results and field data may further substantiate the fate of oil dispersions in deepwater spills and improve the information concerning the impacted deepwater area of a spill. This information is important to include in risk assessment analyses.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2015.02.006.

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Cc: Principe, Vanessa[Principe.Vanessa@epa.gov]; Wilson, Gregory[Wilson.Gregory@epa.gov]
From: Mandsager, Kathy
Sent: Fri 2/27/2015 8:45:25 PM
Subject: FW: Dispersant Science in Arctic Waters - Degradation and Fate
Biodegradation Database.xlsx
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From: Mandsager, Kathy
Sent: Friday, February 27, 2015 3:45 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'thomas.s.coolbaugh@exxonmobil.com'
Cc: Kinner, Peter; Kinner, Nancy; Mandsager, Kathy
Subject: Dispersant Science in Arctic Waters - Degradation and Fate
Importance: High

Dear Degradation & Fate group members:

Our next meeting to discuss the outstanding items on this document, particularly with information from the older published papers (LUMCON) that address biodegradation, will be held **Wednesday, March 11 beginning at 1:30 pm ET**. Please mark your calendar and plan to participate.

Attached is the biodegradation spreadsheet for this discussion.

This meeting will be via WebEx and the instructions are noted below.

Degradation & Fate Group
Wednesday, March 11, 2015
1:30 pm | Eastern Daylight Time (New York, GMT-04:00) | 3 hrs

Join WebEx meeting

Meeting number: 312 666 165

Join by phone
1-855-244-8681 Call-in toll-free number (US/Canada)
1-650-479-3207 Call-in toll number (US/Canada)
Access code: 312 666 165
Global call-in numbers | Toll-free calling restrictions

Can't join the meeting? Contact support.

Kathy Mandsager

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Cc: Kinner, Peter[Peter.Kinner@unh.edu]; nancy.kinner@unh.edu[nancy.kinner@unh.edu]; Mandsager, Kathy[kathy.mandsager@unh.edu]; Principe, Vanessa[Principe.Vanessa@epa.gov]; Wilson, Gregory[Wilson.Gregory@epa.gov]
From: Mandsager, Kathy
Sent: Fri 2/27/2015 8:41:36 PM
Subject: FW: Dispersant Science in Arctic Waters - Organizing Committee
2015.02.27_Efficacy and Effectiveness_reformated.pdf
Efficacy_Effectiveness_COMMENT sheet.xlsx
2015.02.27_Degradation and Fate_reformated.pdf
2015.02.27_Toxicity and Sublethal Impacts_reformated.pdf
2015.02.27_Public Health and Food Security_reformated.pdf
2015.02.27_Physical Transport and Chemical Behavior Final_reformated.pdf
Biodegradation Database.xlsx

Dear Organizing Committee members and Observers,

Below is a sample email and documents that were sent to each of the 5 groups. Copies are all attached here for your perusal. Although you are unable to make direct comments and edits to these documents, we would appreciate if you could let us know if you feel anything has been missed or overlooked in any of these topic areas.

Also, for your information, Group 3 on Degradation and Fate is scheduled for yet another discussion via WebEx. An additional spreadsheet was pulled together from the older published papers (LUMCON) that address biodegradation which will further this discussion. Obviously, this document is still in discussion.

From: Mandsager, Kathy
Sent: Friday, February 27, 2015 1:13 PM
To: 'catherine.berg@noaa.gov'; 'Conmy, Robyn'; 'ben.fieldhouse@ec.gc.ca'; 'fingasmerv@shaw.ca'; 'ken.lee@csiro.au'; 'tim.j.nedwed@exxonmobil.com'; 'Chris Reddy'; 'ken@slross.com'; 'Timothy Steffek (timothy.steffek@bsee.gov)'
Cc: Kinner, Nancy; Kinner, Peter; Mandsager, Kathy
Subject: Dispersant Science in Arctic waters - Efficacy and Effectiveness
Importance: High

Dear Efficacy & Effectiveness group members:

Please find attached the latest draft document for your review. We would ask that you review this very carefully and add any comments you may have to the spreadsheet that is also attached. Please especially note any items with yellow highlights, red lettering or with your name noted specifically.

And finally, this is your opportunity (as you review this whole document) to let us know if any topic has been overlooked in our group discussions thus far.

Please return your comments to me (kathy.mandsager@unh.edu) by **Thursday, March 12 COB.**

Kathy Mandsager

Program Coordinator

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Turbulent Shearing of Crude Oil Mixed with Dispersants Generates Long Microthreads and Microdroplets

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Using cinematic digital holography, we demonstrate that turbulent breakup of crude oil mixed with dispersants into microdroplets starts with the formation of very long and quite stable, single or multiple microthreads that trail behind 300–1400 m droplets. These threads extend from Reynolds number dependent regions with high surfactant concentration, which, along with associated viscosities and stretching by turbulence stabilizes the threads. The subsequent breakup, producing 2:8 m droplets, is due to an increasing surface area and diffusion of dispersants into the continuous phase.

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The addition of dispersants, oil and water soluble surfactants, lowers the interfacial tension between oil and water thereby accelerating the breakup of oil spills into droplets by the action of oceanic turbulence [1]. These droplets are dispersed by the fluid and are subsequently biodegraded. The small droplet sizes resulting from the addition of dispersants significantly increase the interfacial area available to the marine organisms, thereby accelerating the rate of biodegradation as long as they are not toxic to these organisms [2,3]. The current experimental study examines the effect of dispersants on the breakup of oil droplets and film, in an attempt to explain the formation of micron size droplets and a bimodal distribution of droplet sizes observed in experiments that simulate oil spill breakup [4].

The breakup of a cylinder of immiscible fluid due to the so-called capillary instability was first shown by Rayleigh [5]. Its dependence on flow conditions and fluid properties was subsequently characterized by Hinze [6]. In a laminar flow, droplet breakup requires that the capillary number, $_c GD=\neg$, exceeds a critical value, which depends on the viscosity ratio, $_d= _c$, and flow conditions [7]. Here is dynamic viscosity, G is strain rate, D is droplet diameter (measured along the major axis in the current paper), $\neg$ is interfacial tension and the subscripts c and d refer to disperse and continuous phases. In turbulent flows droplet breakup typically occurs when the Weber number (We), $\llcorner u\ ^2D=\neg$, exceeds a critical value, whose magnitude is scattered over a wide range [8]. Here $\llcorner$ is density and u is characteristic velocity. The effect of disperse phase viscosity is quantified by the Ohnesorge number (Oh), $_d=\eth\llcorner_d D\neg \text{Þ}^{0:5}$, whose increase raises the critical Weber number [9].

High disperse or continuous phase viscosity along with the surfactants may delay the pinch-off, resulting in formation of extended segments, threads, that remain attached to the droplet. Doshi et al. [10] injected a droplet into a quiescent medium with higher viscosity, and demonstrated formation of short lived, 8 m radius, threads connecting the droplet to the needle. The same phenomenon, but involving a high droplet viscosity, was reported by Zhang and Basaran [11]. Short lived threads attached to droplets were also seen by Vukasinovic et al. [12] during ultrasonic atomization of a high viscosity droplet. Numerical simulations by Jin et al. [13] showed that increasing surfactant concentration should cause formation of threads attaching droplet to needles. Another mode of thread formation from a droplet, typically referred to as tip streaming, occurs when a section of the immiscible fluid is extended into a conical shape due to: (i) accumulation of surfactants [14], (ii) shearing by coflowing liquid [15], and (iii) electrohydrodynamic forcing [16]. Microdroplets are formed when the tip of the cone extends and then breaks up. However, to the best of our knowledge, no study has reported the formation of long, stable, and (sometimes) multiple microthreads trailing behind droplets advected in a turbulent flow.

In this experiment we use Alaskan North Slope (ANS) crude oil premixed with the dispersant COREXIT 9527, from Nalco Energy Services, at dispersant to oil volumetric ratio (DOR) of 1:20 to 1:15. The continuous medium is filtered tap water at 20 C, with a kinematic viscosity ( ) of 10^{-6} m^2=s. Measured values of specific gravity, viscosity, and surface tension of the mixtures with water are available in Table I. The surface tension for lower dispersant concentrations, DOR of up to 1:20, is measured from the height difference required to transform a flat surface of the mixture, at the end of a capillary tube immersed in water, into a hemisphere. For higher DOR, the surface tension is estimated from the oblateness and quiescent rise rate of the droplet (U_q), following Hu et al. [17].

In order to decouple the effect of droplet injection and breakup, we use a 0.15 mm (inner diameter) hypodermic needle to inject a mixture with DOR of 1:15, the concentration required for repeatable thread formation, into a nearly quiescent glass tube which lies within the turbulent facility. The mixture injection rate through the needle is adjusted so that the droplets are formed via a dripping mode [11]. Subsequently, the droplets rise up into a nearly homogeneous, stationary, and isotropic turbulent flow gen-

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TABLE I. Measured properties of the present ANS crude oil when it is mixed with the dispersant COREXIT 9527.

	Specific gravity (ρ_c ¼ 1000 kg=m^3)	Kinematic viscosity (mm^2=s)	Surface tension (mN=m)	Ca (u^0 ¼ 4:63 cm=s)
Crude oil	0.847	7:1 • 0:08	16.7	0.007
DOR 1:20	0.851	8.99	0.69	0.19
DOR 1:18:2	0.852	9.25	0.63	0.21
DOR 1:15:2	0.853	9.60	0.16	0.83

erated by four symmetrically spinning grids located at corners of a rectangular water tank that generates a high turbulence level with a very low mean velocity [18,19]. Consequently, droplets remain in the sample volume for an extended period. The turbulent characteristics in the facility, near the central region, have been carefully measured using 2D PIV at several planes and two perpendicular orientations, at all relevant experimental conditions. For the turbulent condition at which the bulk of the data have been obtained, the mean velocity is ¼ 12% of the root mean square of fluid velocity fluctuations (u^0 ¼ 4:63 cm=s), the turbulence kinetic energy dissipation rate (") is 19 cm^2=s^3, the Kolmogorov length scale [η ¼ $(\nu^3/\varepsilon)^{0.25}$] is 151 m and the integral length scale (L ¼ u^{03}=") is 5.2 cm [19]. The droplet motion is observed in a 1:7 × 1:7 × 7 cm^3 sample volume, located at the center of the tank, using high-speed inline digital holography. The optical setup consists of a collimated laser beam illuminating the sample volume. The interference pattern between the light scattered from droplets and the remaining part of the illuminating beam is recorded by a high-speed 1k × 1k camera at 500 frames=s. The reconstruction of the recorded holograms, plane by plane in the beam axis direction [19,20], brings all the objects, located within the "depth of focus," to come into focus. The observations are performed at different distances from the exit of the tube. At this turbulent level, breakup of 0.3–1.4 mm droplets (We, $\rho_d u^{02} D/\sigma$ ¼ 3:5–16 and Oh ¼ 1:28–0:58) due to capillary instability [21], which is demonstrated in Fig. 1, occurs infrequently.

Shortly after entering the turbulent domain, droplets larger than ¼ 0:9 mm have a series of short threads extending from ¼ 110° to 135° with respect to their leading edge [Fig. 2(a)], decreasing with increasing Weber number (droplet oblateness). Smaller droplets, about 0.6 mm and smaller, typically have a single thread extending from their tail [Fig. 2(c)]. After a brief period (0.02–0.15 s) one or more of these threads extends into a substantial length (l), Fig. 2(b), reaching l=D ~ 14. The length scale of the threads becomes comparable to the turbulent integral length scale, while the thickness (d) rapidly becomes smaller than the present spatial resolution of our holographic system (17 m). Occasionally, a segment of the long thread pinches off, and the section that remains attached does not retract completely, due to the low surface tension and high viscosity, and eventually starts stretching again along with the formation of additional threads. The pinched section is subsequently advected away by the turbulent flow. In some cases simultaneous multiple threads are pulled from the same droplet [Figs. 2(d) and 2(e)]. Slip of the buoyant oil droplets relative to the

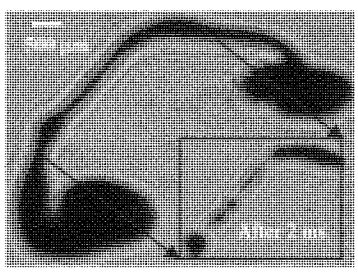

FIG. 1 (color online). Reconstructed holographic images showing turbulent stretching of a crude oil droplet for DOR of 1:20, with inset showing the capillary breakup of a section 2 ms later.

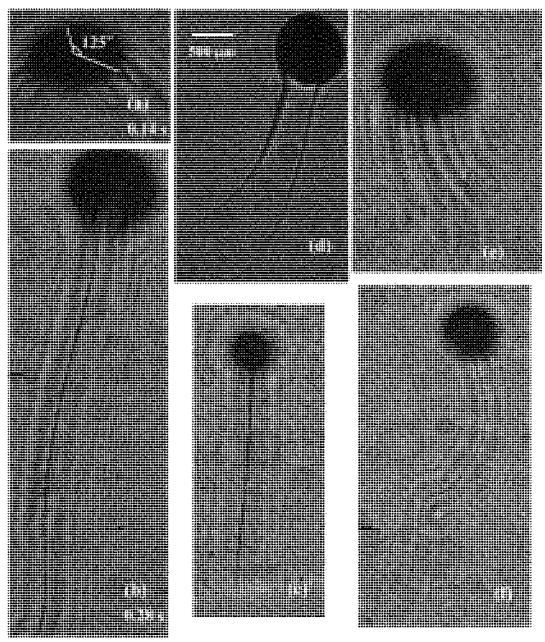

FIG. 2. Reconstructed holograms of threads extending from crude oil droplets for DOR of 1:15 shown at the same magnification. (a) Threads extending at ¼ 125° from the leading edge, 0.14 s after the droplet enters the turbulent flow, D ¼ 1:17 mm. (b) The same droplet 0.14 s later, after stretching of the thread by the turbulent flow. (c) A single thread extending at 180° from a 0.46 mm droplet. (d) and (e) Multiple threads stretched from 0.85 and 1.07 mm droplets. (f) A twisted thread extending from a 0.6 mm droplet. Black bar indicates the boundary between two image sections of adjacent domains, which are reconstructed at different depth to keep the thread in focus.

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local flow [18] stretches the thin threads initially. As their length becomes comparable to L, turbulence induced differences in the local velocity along the length of the thread deforms it [Fig. 2(f)] and extends it further. Consequently, the measured rate of stretching (1–7 cm=s), is of the same order as u^0.

To understand the process of thread formation and separation, we have also injected the mixture into quiescent water and observed their behavior using inline digital holographic microscopy [20], at a resolution of 1.4 and 1:7 m. Holograms are recorded at 15 frames=s using a 2k | 2k digital camera and at 60–250 frames=s by a 1k | 1k camera. A single thread extending behind a 0.43 mm droplet is evident in Fig. 3(a) for DOR of 1:15. Reducing the DOR to 1:18 to stop the thread formation and increasing the droplet size to ┘ 2:1 mm generates the flow pattern shown in Fig. 3(b), as the surfactant is entrained by the flow around the droplet. Clearly, flow separation occurs at 124┐, and near the separation point, the surface of the droplet is sheared towards the separation point from both directions, by the attached flow upstream and the reverse flow within the separated region downstream. Such a flow concentrates the surfactant near the separation region and reduces the local surface tension in a process similar to tip streaming in extensional flows [7], making them susceptible to thread formation. As the droplet Reynolds number ($U_q D$=┘) decreases below 10, i.e., for droplets smaller than 0.6 mm, the separated region disappears. Consequently, for such small

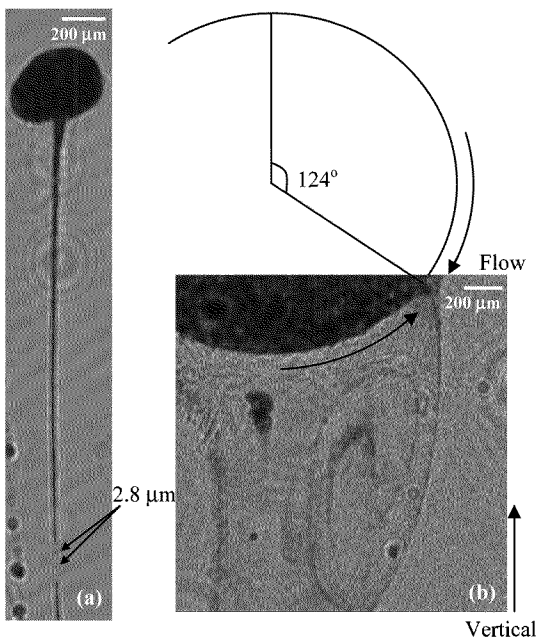

FIG. 3. Crude oil droplets mixed with dispersant rising in a quiescent domain. (a) A 0.43 mm droplet with DOR of 1:15. The thread breaks up to form two 2:8 m droplets, which are indicated by arrows. (b) Part of a 2.1 mm droplet with DOR of 1:18 and without oil shedding, showing that dispersant emerges from the droplet at the point of boundary layer separation, and is entrained into its wake.

droplets the surfactants is sheared towards the tail of the droplet (180┐), resulting in formation of a single thread [Fig. 2(c)]. Differences between the threshold of Reynolds number for separation in comparison to that of solid spheres, observed by Taneda [22], is most likely a result of droplet deformation. It seems that thread formation occurs due to localized increase in concentration of surfactant, but the location of high concentration varies with Reynolds number and Weber number, the latter through shape deformation. This explanation can be extended to the turbulent flow, as shown subsequently.

The capillary number based on the droplet size (D┘ 1 mm), strain rate associated with the turbulent flow, G┘ ð"=┘Þ$^{0:5}$ [23], and bulk mixture properties is ┘ 0:27, i.e., sufficient to deform the droplet and cause breakup, even with the presently high viscosity ratio [7]. However, exposure to such shear is intermittent and associated breakup is rare, because of the unsteady nature of turbulence and migration of the droplet relative to the local flow. On the other hand, the capillary number is higher in regions where flow separation causes accumulation of surfactants, both due to the low surface tension and high local shear, causing the formation of threads seen in Fig. 2. The effect of high droplet viscosity, low surface tension, and turbulence shearing in stabilizing the threads [7,24] can also be quantified using a modified capillary number, Ca ¼ ð $_c$ $_d$Þ$^{0:5}$u^0=┐ (see values in Table I), which has been used successfully by Gañán-Calvo et al. [25,26] to determine the stability of thin threads in coflowing liquids. Interestingly, we find that when Ca ¼ 0:007–0:21, i.e., lower than or close to the threshold level for coflowing liquids [25,26], the thin threads do not develop. However, for Ca ¼ 0:83, i.e., exceeding the threshold value, threads are observed extensively and repeatedly.

As the threads stretch, the increasing surface area decreases the surfactant concentration at the interface. Furthermore, diffusion of surfactants into the water becomes significant when the diameter of the thread becomes comparable to the molecular diffusion length scale ðD$_m$l=u^{0}Þ$^{0:5}$, where D_m is the molecular diffusion coefficient. Using D_m ┘ 10$^{\llcorner 9}$ m^2=s and l ┘ 7 mm, the diffusion length scale is ┘ 12 m, i.e., comparable to a typical thread diameter. Hence one should expect significant diffusion away from the droplet and a reduction in surfactant concentration. Consequently, the surface tension rises, making the thread prone to capillary instability. In the quiescent case, breakup of a thread section produces droplets as small as 2:8 m diameter [Fig. 3(a)]. To determine whether such instabilities occur in the turbulent flow, we examine available holograms obtained at high turbulence levels (" ¼ 256 cm^2=s^3, u^0 ¼ 9:4 cm=s, Ca ¼ 0:38), where the wavy surface of threads [Fig. 4(a)] provides clear evidence of capillary instabilities. Another possible mode of thread breakup involves thread contraction. Conditions for contraction and related breakup depends on the local Ohnesorge number (Oh_d), $_d$=ð$^{\llcorner}$$_d$d┐Þ$^{0:5}$, based on the bulk mixture properties and thread diameter

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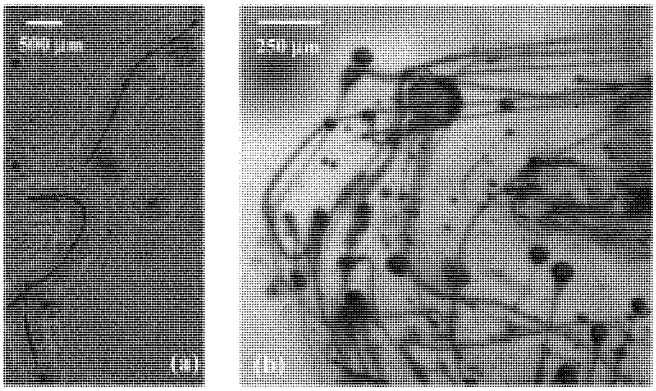

FIG. 4. (a) Reconstructed hologram showing capillary waves on the surface of detached oil threads with initial DOR of 1:20 at a high turbulence level (" ¼ 256 cm^2=s^3, u^0 ¼ 9:4 cm=s). (b) Image of droplets with long threads trailing behind them, which are produced when a water jet impinges on a surface layer of oil mixed with dispersant (DOR ¼ 1:15).

(d ┘ $10^{└5}$ m). Its value, ┘ 7, is a very large number [27]. Hence, significant contraction of the threads and its associated instabilities do not occur in our case. However, we do see the threads contracting partially after a breakup, suggesting that locally the Oh_d decreases to a sufficiently low level.

To confirm that the thread formation and subsequent breakup indeed plays a role in breakup of oil surface layer, we establish a ┘ 2–3 mm layer of oil with DOR of 1:15, on the surface of quiescent water. The interface is impinged with a ┘ 3–4 mm diameter water jet at a velocity of 0:4–0:7 m=s, i.e., comparable to typical breaking wave velocity [28]. The resulting process is observed by high-speed shadowgraphy, at a spatial resolution of 5:6 m. As shown in Fig. 4(b), jet impingement and associated downward fluid motion entrains oil from the surface, resulting in formation of numerous droplets with threads extending from them. Subsequent residual flows keeps on stretching these threads and generating new ones long after the initial impingement. Experiments by Li et al. [4] on the breakup of oil film treated with dispersants have shown that wave breaking produces a bimodal distribution of droplet sizes with peaks at 1 and 150 m. We believe that the presently observed thread formation, which seems to depend on Ca , explains the 1 m peak, while the larger droplets are a result of capillary instability among other phenomena.

In conclusion, shearing of a crude oil droplet mixed with dispersants, and resulting formation of localized regions with low surface tension produces very long, micron sized oil threads that trail behind the droplets. The shearing may be caused by interaction with turbulence, including that caused by the breaking waves, or simple buoyant rise. The point of thread extension from the surface depends on the droplet Reynolds number. Associated Ca exceeds the threshold level needed for maintaining thread stability owing to high turbulence, low surface tension, and viscosities involved. Eventual breakup of these threads to micron sized droplets occurs due to presumed reduction of surfactant concentration, as the surface area increases and the water soluble dispersant diffuses into the surrounding fluid.

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Oil Dispersants and Human Health Effects

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Introduction & Background

The explosion and subsequent blowout of the Deepwater Horizon (DWH) offshore drilling rig on April 20, 2010, led to the largest accidental offshore oil spill since the advent of the petroleum industry, dwarfed only by the deliberate release of crude oil by Iraqi forces during the Persian Gulf War. Over the time until the well was capped on July 15, approximately 200 million gallons of oil spilled into the Gulf of Mexico from the ocean floor beneath the well site located approximately 50 miles off the coast of Louisiana. For perspective, this amount is nearly 20 times the amount of oil discharged during the Exxon Valdez incident in Alaska. As a result, massive mitigation efforts took place during and after the flow of oil which entailed mechanical recovery, controlled burning, and chemical dispersion. As a result unprecedented application of oil dispersant agents was employed by BP during this time until their use was curtailed by the EPA on May 26, 2010. Overall, about 17 - 20% of the crude oil was mechanically recovered and 6 – 8% burned. For the oil remaining in the environment, about 40% (of original input) was evaporated, dissolved, or dispersed into small droplets by natural processes. Initially, it was estimated that only 16.5 million gallons of oil (<10% of total spill) were dispersed into the environment by chemical means, however this approximation was revised upward by 2-fold. The unaccounted for percentages of original oil presumably remain on the surface or washed on shore[1].

Oil dispersants are chemical mixtures of surface active agents and solvents designed to combine with large floating masses of oi l and facilitate the dispersion of the oil into small microscopic droplets that then disperse throughout the water column. The micro -sized oil droplets can then be carried and diluted into the open ocean rather than wash ashore or adhere to wildlife and marine equipment. While it is assumed that dispersed oil is more readily degraded by microbial or physical processes, it can also increase the bioavailability of oil constituents and alter routes and extent of exposure to various toxic chemicals contained in the oil.

Potentially hazardous constituents of concern in dispersants

Currently there are 12 oil dispersant products approved for use by the US EPA and the chemical composition of most remains proprietary information. Even when the specific chemical ingredients are made available, the precise proportion of each entity contained in the product mixture is either not declared, or else specified only over a rather broad range. Table 1 lists these products along with their ingredients, if available. It is estimated that over 1.8 million gallons of

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COREXIT 9500 and COREXIT 9527 were applied during these efforts, including the novel deepwater use of about 800,000 gallons injected below the ocean surface in an attempt to intercept the gushing oil plume locat ed near the source. Early on in the mitigation effort COREXIT 9527 was used, however, due to limited on -hand availability, COREXIT 9500 was substituted as the primary product employed. It remains unclear as to how much of each particular product was used . As the crisis unfolded public perception and opinion became concerned with the additional threat to the environment and human health posed by application of hazardous chemicals in dispersants, as well as the toxic components within the oil itself. The initial withholding of information concerning the chemical composition of the dispersant contributed greatly to public concern. Identification of specific ingredients allows some estimation of their potential toxicity, however, it must be emphasized that human exposures to oil dispersants represent exposures to complex mixtures of the specific ingredients, as well as, in combination with oil components. Much less information is available regarding how such combinations of chemical agents might interact.

Broadly, the potentially hazardous effects represented by the oil dispersants can be divided into two classes. The first are direct toxic effects of the agents contained in the oil dispersant product. The second is the potential to modify the environmental deposition and/or bioavailability of toxic principals within the oil itself. This will be discussed in more detail below. It is difficult to comment on the specific ingredients contained in the products whose formulation remains a trade secret. We can , however, provide some insight regarding those whose formulation is known and, in fact, represent those used almost exclusively in the DWH incident (COREXIT 9500 and 9527). Therefore, we will restrict our discussion primarily to the following specific ch emicals: Dioctyl sodium sulfosuccinate, 2 -butoxyethanol, 1 -(2-butoxy-1-methylethoxy)-2-propanol, and ethoxylated alcohols. Some of the products also contain various petroleum-derived products. For example, COREXIT 9500 contains a large amount (10 – 30%) of hydrotreated light petroleum distillate (CAS #64742 -47-8), which contains primarily $C_9 – C_{12}$ saturated paraffinic hydrocarbons with less than 1% aromatic hydrocarbon content. Other distillate fractions employed as solvents may contain varying amounts of additional aliphatic and aromatic compounds depending on the distillation process. Numerous toxic effects, including malignancy, on a variety of target organs including central nervous system, lung, skin, liver, and bone marrow have been established for several of these components and thus they can contribute to the overall burden of toxic chemicals released during the DWH incident. Since, however, these chemicals are also present as components of the crude oil itself, we will limit our further discussion of toxicologic profiles to those agents specific to the dispersants. Sorbitan octanoate and its polyoxyethylene derivatives, used extensively as food and cosmetic additives, appear to be relatively non-toxic, aside from occasional reports of hypersensitivity and will not be discussed further. They may, however, like other surface active agents, contribute to the ability of dispersants to modulate exposure to oil components. We present here primarily toxicity data pertinent to single agents, however, i t is important to remember that dispersants are complex mixtures and, thus, the potential for interactions between individual components is high, yet

difficult to predict.

Dioctyl sodium sulfosuccinate (DSS) (IUPAC: Sodium 1,4-bis(2-ethylhexoxy)-1,4-dioxobutane-2-sulfonate) (CAS #577-11-7) is an anionic surfactant and a common ingredient in several household products. It is best known as the active ingredient contained in many over-the-counter stool softeners and laxatives (ex. Colace ™, Ducosoft™, Ducolax™, Ex-lax™ stool softener among others). As such it is usually taken orally but can also be given by rectal enema. The recommended daily dose is between 50 and 200 mg (0.7 – 2.9 mg/kg b.w.) with up to 500 mg/day sometimes used. It also has been used as a pesticide on grapes, oranges, feed corn, almonds, nectarines [2]. Systemic absorption after oral administration has been documented in humans[3], but its extent has not been well studied. Absorption in the rat appears extensive with subsequent metabolism and combined urinary (60%) and biliary (40%) excretion. Some concern has been given regarding the potential to produce 2-ethyl-hexanol as a metabolite but so far this pathway appears to be nominal[3b, 4].

Most of the untoward effects seem to be mechanism related and usually manifest as gastrointestinal symptoms including bloating, diarrhea, cramping, GI upset/pain. Prolonged use in the face of such symptoms can conceivably produce dehydration and electrolyte imbalances. The acute LD_{50} in mice ranges from 1.5 g/kg[5] to 4.8 g/kg [6]. The LD_{50} in guinea pig was only 0.65 g/kg and horses appeared similarly susceptible to the adverse effects of the drug [7]. Cause of death was hypovolemic shock and circulatory collapse attendant with loss of fluid into the intestinal lumens, thus is essentially related to the mechanism of its therapeutic action. Several prolonged exposure studies similarly noted GI changes, however, consistently failed to show any changes in other systemic organ systems [8]. No evidence appears that DSS is carcinogenic. Chronic feeding of DSS (1% of diet) failed to show any promotional activity of tumors induced in response to 1,2-dimethylhydrazine (20 mg/kg/week, s.c) and, in fact, reduced the number of tumors seen at lower doses of initiator (10 mg/kg/week, s.c)[9]. In a three generation feeding study in rats 0.5 and 1% DSS in the diet caused a reduction in body weight, however, reproductive performance remained normal throughout the study and no treatment-related macroscopic changes were observed [10]. In a retrospective study where 6,937 women were prescribed drugs during the first trimester of pregnancy, 473 received DSS with only a single birth of a child with an unspecified congenital disorder[11]. Allergic hypersensitivity reactions have been reported[12] but the incidence of anaphylaxis appears low. As reported by eHealthMe.com, a website that tracks post-marketing adverse event reporting to the FDA, only one case of anaphylaxis was reported out of the 411 people who reported side effects to Colace ™[13]. Prescribing information for products containing DSS warn against concomitant use of mineral oil since therapeutic doses of DSS may enhance systemic absorption of mineral oil. This effect serves as a harbinger of the possible toxic interactions between oil dispersants and oil components.

2-Butoxyethanol (2-BE): 2-BE (CAS #111-76-2) (ethylene glycol monobutyl ether, monobutyl glycol ether, Butyl CelluSolve ™, Dowanol™ EB) is a high-production volume solvent in the chemical class of glycol ethers. The structural formula of 2-BE is $CH_3CH_3CH_2$-O-CH_2CH_2-OH.

It is a member of a larger class of ethylene gycol ethers that include 2 -methoxyethanol and 2 -ethoxyethanol, as well as higher series of ethoxylated fatty alcohols. 2 -BE is widely used in the manufacture of various enamels, lacquers, paints and other surface coatings. In addition, it is also commonly found in a variety of household cleaners and products. Because of its relatively high vapor pressure it can exist in the atmosphere as a vapor. 2 -BE is also easily miscible in water and most organic solvents. Because of its aqueous miscibility, the propensity to produce a vapor phase is reduced upon addition to water. As such, the primary routes of exposure thought to be of concern are respiratory and d ermal, although accidental/intentional ingestion of some 2 -BE containing products have been documented. 2-BE can be readily absorbed via all three major routes of exposure. In fact, percutaneous absorption through the skin is thought to be a significant route of exposure for vaporous 2 -BE within the atmosphere[14]. In addition, it appears that 2 -BE is much more efficiently absorbed from an aqueous solution applied to the skin compared to an equivalent dose applied as a neat solution [15]. While most of the dispersant products are recommended for use as undiluted solutions for aerial application, others like COREXIT EC7664A, a surface washing agent, are applied as a 1 - 3% diluted solution [16]. Boat spraying of COREXIT 9500 and 9527 products requires specialize d low -pressure low -volume pumps, which if unavailable, may necessitate use of diluted product down to 5 – 10%.[17]

The metabolism of 2 -BE proceeds mostly through typical alcohol and aldehyde dehydrogenase pathways with formation of 2-butoxyacetaldehyde and 2-butoxyacetic acid (2-BAA), the principal metabolite [18]. This raises the possibility of competitive inhibition of metabolism by other primary alcohols like ethanol and altered kinetics during consumption of alcohol. Administration of ethanol to rats sig nificantly increased blood levels of various ethylene glycol ethers after their inhalation [19]. At higher concentrations this pathway is likely saturated and alternate pathways of O-dealkylation and glucuronidation become more quantitatively important[18b]. An amino acid conjugate, n -butoxyacetylglutamine, has been identified in humans but not experimental animals[20].

The principal health effect of 2 -BE observed in humans is central nervous system toxicity with additional kidney and liver injury at high doses. 2 -BE can produce an acute CNS syndrome typical of exposure to other organic solvents consisting of dizziness, nausea, vomiting, loss of coordination, ataxia, confusion, depression, loss of consciousness. Severity is related to the dose. 2 -BE is also an irritant to mucosal surfaces and skin, therefore ocular, oro -pharyngeal, nasal, respiratory, and dermal symptoms are also observed. 2 -BE does not appear to be a skin sensitizer in humans[21].

Acute LC_{50}s or LD_{50}s have been established in several sp ecies and are summarized in Table 2 [22]. Much of the concern regarding 2 -BE stems from its established ability to produce profound intravascular hemolytic anemia in experimental animals. This effect is characterized by a decreased number of circulating red blood cells (RBCs) and elevations in free hemogoblin. Free hemoglobin is believed to be responsible for the observed tissue damage especially in the kidneys and liver. Inhalation of 2 -BE by female rats (62 ppm, 299 mg/m ^{3}) for 4 hrs increased

osmotic fragility of erythrocytes [23]. This effect has been documented repeatedly in multiple species including dog, rabbit, and with both acute and longer term -exposure[23-24]. Older rats appear more sensitive than younger animals [25], as well as female compared to m ale rats[26]. These observations likely reflect the greater accumulation of the metabolite 2 -BAA in both sensitive groups (see below) [25, 27]. In vitro studies using isolated erythrocytes have provided important insights into 2-BE induced hemolysis. The 2-BAA metabolite of 2-BE appears to be the primary offending species for these effects since in vitro incubation of isolated red blood cells with BAA produced hemolysis at between 20 – 40-fold lower concentrations than the parent compound [24b, 28]. Importantly , the same studies observed marked species differences in sensitivity to the hemolytic effects. Human RBCs were markedly more resistant to these effects than rats [24b, 28] requiring nearly 10 times more BAA to produce hemolysis. Other sensitive species inc lude mice, hamsters, rabbits, and baboons, while resistant species include pigs, dogs, cats, and guinea pigs[29]. These species differences in part reflect intrinsic differences in the red blood cells themselves, presumably at the level of membrane composition. 2-BE-induced frank hemolysis is rarely reported in humans, even during severe poisonings following suicide attempts (ingestion of 25 – 60 gm) [30]. Occupationally relevant exposures (100 ppm) could produce headache and vomiting but no signs of hemolysis, although higher exposures have been shown to alter osmotic fragility when tested in vitro [23, 31]. During a controlled human exposure study, vomiting and headache were observed after breathing 100 ppm (483 mg/mm ^{3}) for 8 hrs. No clinical signs of hemolysis were observed at any level although exposure to 195 ppm (942 mg/m ^{3}) did increase osmotic fragility of RBCs when assessed in vitro [23]. After in vitro incubation of human RBCs with 2 mM BAA, a concentration which causes complete lysis rat RBCs, Udden obse rved no changes in morphology or deformability even in cells derived from patients with hereditary spherocytosis, a disorder characterized by red cells with high osmotic fragility, and sickle cell disease[32].

Reproductive toxicity (both male and female) has been observed with the related glycol ethers, 2-methoxyethanol and 2-ethoxyethanol, however, 2-BE appears relatively devoid of reproductive and developmental effects. No testicular effects were observed in rodents exposed to 2 -BE by inhalation of 800 ppm for 3 hrs [33] or oral administration of up to 2000 mg/kg/day, 5 days/week, for 5 weeks [34]. Developmental studies exposing pregnant dams to 2 -BE by a variety of routes failed to show any fetotoxic or teratogenic effects except at doses that produced signific ant maternal toxicity [35]. While in vitro tests for mutagenic and genotoxic effects have yielded equivocal results [36] in vivo tests have been largely negative. 2 -BE was negative in the bone marrow micronucleus test after i.p. administration in rats and mice [36c, 37]. Using [^{32}P]-post-labelling assay, no DNA adducts were observed in multiple organs of orally dosed -rats[38]. Keith et al. showed no effects on DNA methylation in multiple organs and tumor formation in FVB/N transgenic mice[38]. 2-BE has been associated with formation of hemiangiosarcomas in liver and other organs of mice [39], however, these tumors are now thought to arise secondarily through the heme-dependent generation of reactive oxygen species and hypoxia-dependent proliferative signaling in endot helial cells, which arise during the hemolytic destruction of RBCs [40]. Since

these tumors appear only in the context of profound hemolytic effects, they are thought not to be of significance in human exposures.

1-(2-butoxy-1-methylethoxy)-2-propanol (CAS # 29911-28-2), more commonly referred to as dipropylene glycol n-butyl ether (DPnB), is a component of both COREXIT 9500 and 9527. Synonyms for this compound include 2-(butoxypropoxy)-2-propanol, dipropylene glycol monobutyl ether, ARCOSOLV™ DPNB, or DOWANOL™ DPNB. DPnB is also a glycol ether, except it is classified as a P-series glycol ether (synthesized from propylene oxide) unlike 2-BE discussed above whose synthesis is based on ethylene oxide (E-series) as the starting material. In general, the P-series glycol ethers are frequently considered safer alternative to E-series compounds as they lack the hemolytic toxicity and appear to have less potential to disrupt reproductive function and fetal development [41]. Little of the descriptive toxicology, however, appears in the peer-reviewed literature but instead relies upon industry-sponsored unpublished studies. DPnB (Table 2), as well as other propylene glycol ethers, have very low acute toxicity with LD_{50}s greater than 1,800 mg/kg in oral studies, 2,000 mg/kg for dermal exposures, and >250 ppm for inhalation exposures [42]. In many cases, the actual LD_{50} were not obtained within the dose range employed. When signs of toxicity were observed, they usually included generalized CNS and respiratory depression common with exposure to other solvents. DPnB was classified as "slight irritating" to skin and eyes [43], but no evidence of sensitization was observed[44]. An intriguing peer-reviewed study, however, has recently appeared that begs further consideration. Choi et al. [45] conducted a case-control study correlating household levels of different classes of indoor air volatile organic compounds (VOCs) with allergic disease and IgE sensitization. Of the 8 different classes of VOCs including aromatic hydrocarbons, aldehydes, organic acids, and others, only the propylene glycol and glycol ethers were associated with increased risk of multiple allergic symptoms and atopy. Therefore, the association of glycol ethers to asthma and other allergic diseases deserves further attention although the actual offending chemical(s) have not been identified.

Longer term exposure studies also revealed relatively benign effects. Two-week inhalation studies in rats exposed to DPnB demonstrated a threshold for toxicity somewhere between 320 mg/m^3 (40 ppm) and 810 mg/m^3 (104 ppm)[46]. Toxicity manifested primarily as histopathological lesions in the liver and nasal mucosa. In this regard, DPnB appears slightly more toxic that other propylene glycol ethers such as propylene glycol n-butyl ether[47]. [47c]Dermal exposure to rats for up to 13 weeks produced some localized skin irritation at all doses but little in the way of systemic toxicity except small decreases in body weight and elevated neutrophil counts with a NOAEL of 0.1 ml/kg-day (91 mg/kg-day) and LOAEL of 0.3 ml/kg-day (273 mg/kg-day)[48]. Prolonged oral exposure (13 weeks) produced slight elevations in liver and kidney weights without histopathology and mild changes in clinical chemistries only at the highest dose tested (1,000 mg/kg/day)[49]. Importantly, these studies, as well as one specifically designed to test hematological effects [50], demonstrated that propylene glycol ethers do not share the hemolytic effects manifest by their E-series relative, 2-BE, as discussed above. Functional

reproductive studies with DPnB have not been carried out but no changes in the reproductive organs were observed at necropsy in any of the repeated dosing studies listed above and reproductive endpoints after exposure to the structurally -related propylene glycol monomethyl ether[51] were negative. Developmental studies during dermal exposure of rats established a LOAEL of > 910 mg/kg/day (the highest doses applied in each study), respectively, for maternal toxicity, embryo-/fetal toxicity, or developmental aberrations[52]. In most studies DPnB is not mutagenic by in vitro or in vivo assays [53], however, three tests all from a single laboratory showed chromosomal aberrations in CHO cells [54]. National Toxicology Program testing for carcinogenic effect of the related agent, propylene glycol monobutyl ether, observed an increase in hepatic tumors in male and female mice, but not rats exposed to 1,200 ppm by whole -body inhalation (the highest dose tested) for 2 years [55]. Male mice showed exposure-related increases in non-neoplastic lesion in the kidney with equivocal increases in renal neoplasia. DPnB has not been similarly tested for tumorigenic potential in long-term studies. Therefore, with the paucity of in vivo data, as well as, equivocal in vitro results, formation of hepatic lesions with preneoplastic potential, and tumorigenic effects of structurally -related compounds, labeling DPnB as non-carcinogenic should be taken with some caution.

One likely explanation for the dramatic differences between the structurally similar 2-BE and PGBE relates to differences in metabolism. It is believed that the major offending species for hematologic and reproductive toxicity seen with the E-series glycol ethers is the corresponding acid produced during in vivo metabolism by alcohol and aldehyde dehydrogenases (2-butoxy acetic acid in the case of 2-BE). The major species (>95%) contained in commercial preparations of PGBE, however, is the alpha isomer which represents a secondary alcohol, thus is not a substrate for alcohol dehydrogenase and incapable of forming an alkoxypropionic acid. Instead, metabolism of PGBE proceeds largely by typical mixed function oxidase-dependent O-dealkylation yielding *t*-butanol and propylene glycol. Propylene glycol is readily converted to lactate and pyruvate for consumption in the Krebs cycle. *t*-butanol, as well as some of the parent compound, is excreted as a glucuronide conjugate[56].

Ethoxylated alcohols deserve a brief mention here in that they are chemically related to simpler glycol ethers. They are usually composed of a long chain fatty alcohol ($C_8 - C_{15}$) linked to a polyethelyene glycol chain also of varying length (1-20). Modulation of the length of the carbon chains as well as the number of ethoxy units can be used to determine specific properties of these non-ionic surfactants. One of the approved oil dispersant products, DISPERSIT SPC 1000 contains ethoxylated alcohol specified as a mixture of $C_{12} - C_{14}$ fatty alcohol without noting the relative degree of ethoxylation. While these chemicals have undergone considerable scrutiny in terms of their potential environmental toxicity, there is very little information regarding their effects on humans or other mammals. Various MSDS sheets for these compounds note them to be significant irritants upon ocular or dermal exposure, but no long term systemic toxicities are reported at typical usage exposures. One possible issue to consider is the fact that atmospheric (and perhaps microbial) oxidation of these chemicals can give rise to reactive aldehydes with

potential to produce contact sensitization and subsequent allergic reactions upon re-exposure[57].

Secondary effects by altering oil component exposure

Crude oil represents a complex mixture containing a vast array of aliphatic and aromatic hydrocarbons, heavy metals, and other substances. The total petroleum hydrocarbon (TPH) fraction represents the greatest concern to human health. Depending on the carbon cha in length or number of aromatic rings each compound has its unique profile of volatility, solubility, and physical-chemical properties that ultimately determine its toxicokinetics and toxicodynamics. The low-molecular weight BTEX fraction (benzene, toluen e, ethylbenzene, xylene) is of concern because they can diffuse into aqueous media as well as readily volatalize from a surface film of oil. The carcinogenic effects of benzene are well-known as a leading cause in acute myelogenous leukemia [58]. Larger mol ecular weight species (naphthalene, benzopyrene) may remain more associated with the crude oil mass, but still possess toxic potential. An actual description of the specific adverse health effects of TPH is beyond the scope of this discussion, but the interested reader is referred to ATSDR profile for Total Petroleum Hydrocarbons[59].

Perhaps, the biggest question regarding the action of oil dispersants is how they might modulate the fate and transport of various oil constituents within the environment. B y their nature they are designed to break up the oil mass into tiny micro-sized droplets that remain suspended within the water column rather than form a "slick" on the water surface. Wave tank experiments indicate the size of chemically -dispersed oil dro plets to be in the 10 – 50 μm range with some even smaller, although the size of oil droplets formed over time after application of dispersants in the natural setting of an accidental spill is not well studied [60]. This might reduce the evaporation of BTEX components, for example, reducing atmospheric concentrations and thus inhalational exposure. The concentration of these species, as well as heavier compounds, however, are now also increased within the water, and may promote exposure via dermal contact (swimming, water-on-skin exposure during clean -up operations, aerosol generation during wave action), as well as increasing the possibility that such chemicals might sequester in various marine biota because of their potential to bioaccumulate. Such physic al dispersion of the oil mass into an emulsion of microscopically-sized particles dramatically increases the surface area of the overall oil-water interface where diffusion and absorptive processes proceed. The absence of a distinct odor of volatile oil c omponents, as well as visual evidence of an oil slick could also impart a false sense of security when it comes to use of personal protection equipment such as respirators/filters, gloves, and other body coverings.

Of note is the hypothesis that the presen ce of oil dispersants can also directly affect how various chemicals enter the body. Again the increase in surface area whereby oil -derived chemicals might contact the skin and lung lining might facilitate absorption by the dermal and inhalation routes. W e mentioned above that water mixtures of glycol ethers showed enhanced dermal absorption above that seen when glycol ethers are exposed neatly to the skin [15], however, the dermal absorption of dispersed TPH components has not been compared to those in undis persed

oil. DSS, under the trade name Aerosol -OT, has received recent attention as a means to enhance oral absorption of various pharmaceuticals and is the subject of patents for improved drug - delivery systems [61]. DSS enhanced the efficacy of tetracycline on various microorganisms, including some normally resistant to the drug, by enhancing intracellular permeation of the drug[62]. Aerosol OT/1-butanol emulsions were also found to markedly enhance penetration of the antibiotic, clindamycin phosphate, through human epidermis when compared to a 70% isopropanol vehicle[63]. The ability of Aerosol OT to similarly enhance diffusion of 5 -fluorouracil through skin was accompanied by modifications in the lipid structure and degree of hydration of the stratum corneum layer of the skin [64]. Thus, it is entirely possible that DSS and various other surface active agents in dispersant products can enhance absorption of specific TPH components and thus potentiate any adverse effects resulting from such exposures. Because of its volatility, most benzene applied to skin is expected to evaporate before substantial systemic absorption. If, however, benzene is sequestered into an emulsified aqueous suspension by the action of dispersants its potential for evaporation and, theref ore, dermal absorption might be modified. While direct administration of DSS (1%) into the lungs of dogs produced some pulmonary edema[65], DSS aerosols (5%) accelerated lung clearance of the tracer, ^{99}Tc-diethylenetriamine pentaacetate (^{99}Tc-DPTA) without affecting gas exchange or lung mechanics[66]. In fact, DSS has been considered as a means to enhance delivery of pharmaceutical agents via enhancing alveolar absorption[67]. In rabbits, DSS successfully enhanced the absorption and biological action of insulin delivered by aerosol inhalation[68].

Probable exposure scenarios and possible at-risk groups

It is expected that those individuals directly involved with the clean -up operations and direct handling/application of the dispersants would have received t he highest exposure and, therefore, are the most at risk for adverse effects. In these cases their most likely route of exposure was via inhalation or dermal absorption. The National Institute for Occupational Safety and Health conducted a limited series of health hazard evaluations for several groups of responders employed during the spill clean -up[69]. The most commonly reported symptoms were headache, upper respiratory symptoms, and symptoms related to heat stress. While workers who reported exposures to oil and dispersants reported higher prevalence of all types of symptoms, no assignment of specific causative agents could be made. Health symptom surveys taken aboard two vessels actively engaged in releasing dispersants were similar in scope and magni tude to those obtained from other workers who had not worked on boats and had no exposures to oil or dispersants. Personal breathing zone and area air sampling was used to evaluate exposure to a variety of chemicals, including propylene glycol, a COREXIT component, and measured levels that that were consistently below the acceptable occupational exposure limit (OEL). Moreover, NIOSH investigators consistently noted that workers generally complied with wearing the necessary personal protection equipment required for the task at hand.

Local populations residing on the shores are likely at minimal risk for toxicity based on dilution of the chemicals in surrounding water and air. Aerial application of dispersants occurred only at

distances greater than 3 mi les from shore and serves to severely limit respiratory and dermal exposure on the shoreline. Some concern has been raised about potential contamination of seafood with chemical dispersants. The environmental half-life of these compounds is short and there is little evidence that any of the chemicals discussed above appreciably bioconcentrate or accumulate within the food chain. Trace levels of DSS measured in seafood samples tested after the opening of previously closed waters were considered insignific ant to human health by the FDA.

As always the primary at -risk groups based on the limited knowledge we have are the very young, the elderly, and those with preexisting conditions especially chronic lung disease. The clean-up worker investigation conducte d by NIOH noted a disproportionate number of smokers among the clean-up workers[69]. Pregnant and nursing women should also be advised to minimize potential exposure simply as a matter of common sense. It is possible that the capacity for metabolism can al so determine sensitivity. For example, individuals who possess high levels alcohol dehydrogenase activity (those of Asian or Amerindian descent, for example) might actually be sensitive to some of the effects of 2 -BE compared to others with less functiona l capacity to generate the more toxic metabolite, 2-BAA. Similarly, genotypic/phenotypic variability in the cytochrome P450(s) responsible for O -dealkylation of propylene glycol ethers could contribute to alterations in the physiological disposition of P -series glycol ethers. No specific studies, however, have addressed these issues.

Perceived Safe Levels

No environmental or occupational regulatory/occupational standards for air or water exist for DSS. Because of its use as an OTC medicine and possible application of as food additive, the Acceptable Daily Intake as set by the WHO Joint Expert Committee on Food Additives is 6 mg/person/day and that set by the U.S. FDA is 30 mg/person/day. As a stool softener the recommended adult doses are in the range of 100 – 500 mg/day and appear to be well tolerated over extended periods of time. Because of their high vapor pressures, regulatory guidelines have been set for the glycol ethers. The following air standards for 2 -BE have been set: TLV TWA (Threshold Limit Value, AIGIH) = 25 ppm, PEL (Permissible Exposure Limit, OSHA) = 50 ppm, and IDLH (Immediately Dangerous to Life or Health, NIOSH) = 700 ppm. No limits have been set specifically for DPnB, but those corresponding to dipropylene glycol monomethyl ether are TLV =100 ppm and STEL (short-term exposure limit, ACGIH) = 150 ppm.

Potential relevant biomarkers and future studies

It will be a challenge to ascertain whether the application of oil dispersants into the Gulf of Mexico will have any perceptible effects on human health. The NIH -sponsored Gulf Long term Follow-up (GuLF) Study, led by NIEHS, is set to begin to study clean-up workers and volunteers to understand the scope and diversity of adverse health effects amongst those individuals most highly exposed to the toxic agents in question. One of its major challenges, however, will be to

accurately characterize and quantify exposure to specific oil and dispersant chemicals alone, as well as in mixtures. Clearly, one of the prime issues will be to determine specific populations who were exposed to these agents and quantify the extent of their exposure in terms of time and amount. Detailed clean-up worker histories might allow grouping of workers based on their proximity in time and space to actual application of dispersants and comparing their ultimate health outcomes to oil clean-up workers with similar tasks in regions where dispersants were not applied. Clearly, a more accurate way to document exposure (an internal dose) would be to measure parent compounds or their metabolites in biological samples (blood, urine, other). However, the pathways of metabolism of DSS are not well described. Measurement of urinary 2-BAA has proven useful in monitoring employees potentially exposed to 2-BE in other settings[70]. It is important to remember, however, that these approaches are most useful only in the early stages following exposure since the compounds are presumably cleared fairly rapidly in the absence of a continuous exposure source. Moreover, there clearly are other sources of exposure for these agents such as laxative use and various household cleaning products containing glycol ethers. Therefore, for local residents not directly involved in clean-up activities, the background levels of exposure to many of these agents from other sources may approximate, or even exceed, those specifically from dispersant use. While biomarkers of effect would be useful, there are relatively few, if any, specific for these compounds. Measurement of RBC osmotic fragility could be used to monitor the hemolytic signature effect of E-series glycol ethers, but recall that humans are amongst the least sensitive species for this effect. Various measures of DNA damage and adduct formation in peripheral blood cells has provided some utility in measuring potential genotoxic effects after other oil spills.

The most fruitful future studies might be in regard to studying the interactions between oil dispersants and specific TPH components within the oil itself. Some chemicals in TPH might be more persistent than the dispersant chemicals so measurement of body burden with and without dispersant exposure might prove informative. Animal and in vitro studies that address availability and toxicity of TPH components in the presence and absence of dispersants should be carried out. For example, does simultaneous inclusion of dispersants in TPH component feeding studies alter the genotoxic and tumorgenic effects? Direct in vitro studies can easily be performed to determine if DSS or other oil dispersant components can increase permeation of oil components across skin. Human skin models employing cadaver-derived or tissue-engineered skin are routinely used to assess xenobiotic transport across this barrier in specifically-designed diffusion barrier chambers.

As pointed out earlier, the dispersant products themselves represent complex mixtures whose toxicity may not be adequately predicted by knowledge of the single ingredients alone. Few studies have directly tested the dispersant products for toxicity. Recently, the irritant and sensitizing properties of COREXIT 9500A and DSS were compared in a dermal application model in rats[71]. COREXIT was found to be about 10-fold more potent than would be expected based in its content of DSS alone. Acute 5 hr exposure of rats (27 mg/m^3) of COREXIT aerosols

was found to induce a small change in lung compliance without inflammation [72] and changes in peripheral vascular reactivity [73]. These effects, however, were transient and extrapolation of the exposures conditions to those encountered in the real world is problematic.

Summary

The massive deployment o f oil dispersants in the Gulf of Mexico in response to the DWH oil spill has raised concerns regarding their potential adverse effects to the environment and human health. The specific ingredients contained in many oil dispersant products remain proprieta ry information, however, those contained in COREXIT 9500 and COREXIT 9527, the products used almost exclusively in the Gulf, were available for review. Exposure of the general populace of Gulf shore to the major ingredients dioctyl sodium sulfosuccinate, 2-butoxyethanol, propylene glycol butyl ether, and other ethoxylated alcohols should be considerably below the range expected to produce adverse effects based on a review of their toxicological profiles. Of note, however, is the severe paucity of both hum an and laboratory data regarding the potential effects of chemical mixtures as represented by oil dispersant products. Those individuals involved in clean-up operations that directly handled oil dispersants or worked in the immediate area of application p robably encountered greater amounts of dispersants and might a greater risk of adverse effects, but, in general these should be mild and self-limiting. Importantly, for several of the major toxicities described in experimental animals, humans appear to comparatively resistant. Perhaps a greater question pertains to the ability of dispersants to alter the toxicological properties of the chemicals contained in the oil itself. By their nature they are designed to alter the fate and transport of crude petrol eum and its constituents and, therefore, can change the route and extent of human exposures. The physico -chemical properties of petroleum hydrocarbons contained in micro- -sized oil droplets desperately needs to evaluated and compared to petroleum hydroca rbons alone, in simple aqueous solution, and in air. Moreover, some the oil dispersant products themselves have potential to directly modify biological barriers and, thus, alter permeation of oil-derived chemicals at various routes of exposure.

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Table 1. Approved Oil Dispersant Products and Their Ingredients

Product	Ingredients
BIODISPERS (Petrotech America)	Proprietary
JD-109 (GlobeMark Resources Ltd.)	Proprietary
JD-2000 (GlobeMark Resources Ltd.)	Proprietary
NOKOMIS 3-AA (Mar-Len Supply, Inc.)	Proprietary
NOKOMIS 3-F4 (Mar-Len Supply, Inc.)	Proprietary
COREXIT 9500 (Nalco Energy Services)	Sorbitan, mono-(9Z)-9-octadecenoate Sorbitan, mono-(9Z)-9-octadecenoate, poly(oxy-1,2- ethanediyl) *derivs*. Sorbitan, tri-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) *derivs.* **Butanedioic acid, sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (Dioctyl sodium sulfosuccinate)** (10 – 30%) **1-(2-butoxy-1-methylethoxy)-2-propanol** (1 – 5%) Distillates (petroleum), hydrotreated light (10 – 30%)
COREXIT 9527 (Nalco Energy Services)	Sorbitan, mono-(9Z)-9-octadecenoate Sorbitan, mono-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) *derivs.* Sorbitan, tri-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) *derivs.* **Butanedioic acid, sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (Dioctyl sodium sulfos uccinate)** (10 – 30%) **1-(2-butoxy-1-methylethoxy)-2-propanol** (1 – 5%) Distillates (petroleum), hydrotreated light **2-Butoxy-ethanol** (30 – 60%)
MARE CLEAN 200 (Taiho Industries Co. Ltd.)	Poly(oxy - 1,2 - ethanediyl), α- hydro - ω - hydroxy - , ether with 1,2,3 - propanetriol (9Z) - 9 - octadecenoate Poly(oxy - 1,2 - ethanediyl), α- (9Z)- 1 - oxo - 9 - octadecen - 1 - yl - ω-hydroxy- Poly(oxy - 1,2 - ethanediyl), .α- (9Z) - 1 - oxo - 9 - octadecen - 1 - yl -ω-(9Z) - 1 - oxo - 9 - octadecen - 1 - yl oxy - (Polyethylene Glycol Dioleate) Sorbitan, tri-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) *derivs*. (Polysorbate 85)

□ð

	Alkanes, C14-30

Table 1. Approved Oil Dispersant Products and Their Ingredients (con't)

DISPERSIT SPC 1000 (U.S. Polychemical Corp.)	Poly(oxy - 1,2 - ethanediyl), α- (9Z)- 1 - oxo - 9 - octadecen - 1 - yl - ω- hydroxyl Ethoxylated Amines, tallow alkyl N,N-bis(hydroxyethyl)- Amides, coco **Ethoxylated Alcohols, C_{12-14}-secondary,** 1(or 2) - (2-methoxymethylethoxy)- propanol
SAF-RON Gold (Sustainable Environmental Technologies, Inc.)	Proprietary
NEOS AB3000 (Neos Company, Ltd.)	Proprietary
SEA BRAT 4 (Alabaster Corp.)	Proprietary

□ð

Table 2. Acute Toxicity of 2-Butoxyethanol (2-BE) and Dipropylene Glycol n-*t*-Butyl Ether (DPnB) in Various Species and Routes of Exposure

Route of Administration	Species Tested	LC_{50} or LD_{50}
Inhalation		
2-BE	Male rats (4 hr)	486 ppm (2347 mg/m^3)
	Female rats (4 hr)	450 ppm (2174 mg/m^3)
	Mice (7 hrs)	700 ppm (3381 mg/m^3)
	Guinea Pigs (1 hr)	650ppm (3140 mg/m^3)
DPnB	Rats (4 hr)	> 42 ppm (> 328 mg/m^3) (no deaths)
	Rats (4 hr)	> 262 ppm (> 2,040 mg/m^3) (no deaths)
Oral		
2-BE	Rats	2500 mg/kg b.w.
	Mice	1400mg/kg b.w.
	Guinea Pigs	1200 mg/kg b.w.
	Rabbits	320 mg/kg b.w.
DPnB	Rats	4000 mg/kg
	Rats	1850 mg/kg
	Mice	2160 mg/kg
Dermal		
2-BE	Rabbits	404-502 mg/kg b.w.
	Guinea Pigs	2000 mg/kg b.w.
DPnB	Rat	> 2000 mg/kg (no deaths)

(length of exposure)

To: sarah.allan@noaa.gov[sarah.allan@noaa.gov]; Barron, Mace[Barron.Mace@epa.gov]; abejarano@researchplanning.com[abejarano@researchplanning.com]; DFrenchMcCay@asascience.com[DFrenchMcCay@asascience.com]; michel.gielazyn@noaa.gov[michel.gielazyn@noaa.gov]; peter.hodson@queensu.ca[peter.hodson@queensu.ca]; sharon.hook@csiro.au[sharon.hook@csiro.au]; rrhopcroft@alaska.edu[rrhopcroft@alaska.edu]; john.incardona@noaa.gov[john.incardona@noaa.gov]; Angela_Matz@fws.gov[Angela_Matz@fws.gov]; Teri.Rowles@noaa.gov[Teri.Rowles@noaa.gov]; Mathijs.Smit@shell.com[Mathijs.Smit@shell.com]; Sprenger, Mark[Sprenger.Mark@epa.gov]; rstjeerdema@ucdavis.edu[rstjeerdema@ucdavis.edu]; dana@mote.org[dana@mote.org]; john.wise@louisville.edu[john.wise@louisville.edu]; mhziccardi@ucdavis.edu[mhziccardi@ucdavis.edu]
Cc: nancy.kinner@unh.edu[nancy.kinner@unh.edu]; Gary Shigenaka - NOAA Federal[gary.shigenaka@noaa.gov]; doug.helton@noaa.gov[doug.helton@noaa.gov]; Principe, Vanessa[Principe.Vanessa@epa.gov]; Wilson, Gregory[Wilson.Gregory@epa.gov]; Mandsager, Kathy[kathy.mandsager@unh.edu]
From: Mandsager, Kathy
Sent: Fri 1/13/2017 2:14:21 PM
Subject: State-of-Science: Eco-toxicity and Sublethal Impacts final document review
2017.01.09_EcoToxicity and Sublethal Impacts.docx

Dear Scientific Panel,

We would very much appreciate your review of this final version of the *Eco-toxicity and Sublethal Impacts* document. This document incorporates the edits and suggestions made via email and final discussion.

If you could reply by email with your approval of this document by **Friday, January 27** (2 weeks for this review) that would be greatly appreciated. I will need each of you that participated in this discussion to reply in the affirmative that you approve this document. [The next step is for this document to be submitted publicly for input.]

Please also confirm that your name and affiliation are listed correctly at the end of this document.

You deserve kudos for this great accomplishment! Thank you very much!

Kathy Mandsager

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Cc: doug.helton@noaa.gov[doug.helton@noaa.gov]; Gary Shigenaka - NOAA Federal[gary.shigenaka@noaa.gov]; Principe, Vanessa[Principe.Vanessa@epa.gov]; Wilson, Gregory[Wilson.Gregory@epa.gov]; nancy.kinner@unh.edu[nancy.kinner@unh.edu]; 'Abigail McEachern'[acm2005@wildcats.unh.edu]
From: Mandsager, Kathy
Sent: Thur 1/12/2017 8:10:19 PM
Subject: Public Health panel
fabs tox addition for dispersant doc_RWD.docx

Please see attached document provided by Jim Fabisiak and Robert Dickey for the panel review tomorrow (1/13 at 2pm ET). This is their suggested rewrite for the Potential Toxicological Impacts section.

Kathy Mandsager
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Cc: doug.helton@noaa.gov[doug.helton@noaa.gov]; Gary Shigenaka - NOAA Federal[gary.shigenaka@noaa.gov]; Principe, Vanessa[Principe.Vanessa@epa.gov]; Wilson, Gregory[Wilson.Gregory@epa.gov]; nancy.kinner@unh.edu[nancy.kinner@unh.edu]; Abigail McEachern[acm2005@wildcats.unh.edu]
From: Mandsager, Kathy
Sent: Mon 1/9/2017 8:18:09 PM
Subject: State-of-Science: Public Health/Food Safety WebEx
2017.01.06_Public Health and Food Security.docx

This is a reminder of our call scheduled for this **Friday 1/13 beginning at 2 pm ET**. Attached is the latest version of the document (since call on 1/6). Below are the login instructions.

State-of-Science: Public Health and Food Safety panel
Friday, January 13, 2017
2:00 pm | Eastern Standard Time (New York, GMT-05:00) | 2 hrs

Meeting number (access code): 739 967 405

When it's time, join the meeting.

Join by phone
1-855-244-8681 Call-in toll-free number (US/Canada)
1-650-479-3207 Call-in toll number (US/Canada)
Global call-in numbers | Toll-free calling restrictions

Can't join the meeting?

Kathy Mandsager

Coastal Response Research Center

Center for Spills and Environmental Hazards

220 Gregg Hall, 35 Colovos Rd

University of New Hampshire

Durham, NH 03824

603.862.1545

From: Mandsager, Kathy
Location: via WebEx (login directiosn below)
Importance: Normal
Subject: State-of-Science: EcoTox panel meeting
Start Date/Time: Tue 12/20/2016 7:30:00 PM
End Date/Time: Tue 12/20/2016 9:30:00 PM
2016.11.17_EcoToxicity and Sublethal Impacts.docx
2016.12.16_EcoToxicity and Sublethal Impacts.docx

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MEETING REMINDER! Attached is the latest version of the document.

This is to confirm our next meeting for **TU December 20 from 230 – 430 ET**.
Attached is the latest version of our document.
Below are the directions to login to WebEx:

Ecotox

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From: Mandsager, Kathy
Sent: Thursday, December 01, 2016 12:49 PM
Subject: RE: ECOTOX panel for state-of-science for dispersant use in Arctic waters

Dear Ecotox panel members:

It appears that perhaps one more meeting will finish up this discussion and document. Please take a moment and submit your availability on this doodle poll by Wednesday 12/7. It has been time-zone enabled for your convenience. I will do my best to find a time that suites the majority of you as, like you, we are eager to complete this task.

Click here for the doodle poll>> http://doodle.com/poll/nir5p37i2f68u99t

Thank you!

From: Mandsager, Kathy
Location: via WebEx (login instructions below)
Importance: Normal
Subject: State-of-Science for Dispersant Use in Arctic Waters DEGRDATION & FATE group
Start Date/Time: Wed 12/14/2016 7:00:00 PM
End Date/Time: Wed 12/14/2016 9:00:00 PM
2016.11.28_Degradation and Fate.docx

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,,,,,,,
This is a reminder that we have TWO meetings scheduled this week: tomorrow/Wednesday 12/14 AND Thursday 12/15; both begin at 2:00 pm ET. The WebEx login instructions are below and attached is our latest version of the document for your preparation in the discussion.

Degradation & Fate group

Wednesday, December 14, 2016

2:00 pm | Eastern Standard Time (New York, GMT-05:00) | 2 hrs

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To: chris.barker@noaa.gov[chris.barker@noaa.gov]; CJ.Beegle-Krause@sintef.no[CJ.Beegle-Krause@sintef.no]; Conmy, Robyn[Conmy.Robyn@epa.gov]; Thomas.s.coolbaugh@exxonmobil.com[Thomas.s.coolbaugh@exxonmobil.com]; fingasmerv@shaw.ca[fingasmerv@shaw.ca]; ali.khelifa@ec.gc.ca[ali.khelifa@ec.gc.ca]; jrpayne@sbcglobal.net[jrpayne@sbcglobal.net]; wspegau@pwssc.org[wspegau@pwssc.org]; creddy@whoi.edu[creddy@whoi.edu]; doug.helton@noaa.gov[doug.helton@noaa.gov]; Gary Shigenaka - NOAA Federal[gary.shigenaka@noaa.gov]; Principe, Vanessa[Principe.Vanessa@epa.gov]; Wilson, Gregory[Wilson.Gregory@epa.gov]; Abigail McEachern[acm2005@wildcats.unh.edu]; nancy.kinner@unh.edu[nancy.kinner@unh.edu]
From: Mandsager, Kathy
Sent: Fri 9/30/2016 1:09:54 PM
Subject: State-of-Science Transport & Behavior Group
t.Behavior of Oil Spills in Ice and Implications for Arctic Spill Response.2011_OTC22126LR.pdf
2016.09.29_Physical Transport and Chemical Behavior.docx

Transport & Behavior Group,

Our next meeting in next week: Wednesday, October 5 beginning at 3 pm ET (next week).

Please find attached the latest version of our paper, after yesterday's meeting.

Per your request, I have attached: Dickins 2011 "Behavior of Oil Spills in Ice and Implications for Arctic Spill Response".

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Environ Sci Pollut Res
DOI 10.1007/s11356-013-2053-7

A protocol for assessing the effectiveness of oil spill dispersants in stimulating the biodegradation of oil

Roger C. Prince & Josh D. Butler

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The Author(s) 2013. This article is published with open access at Springerlink.com

Abstract Dispersants are important tools in oil spill response. Taking advantage of the energy in even small waves, they disperse floating oil slicks into tiny droplets (<70 μm) that entrain in the water column and drift apart so that they do not re-agglomerate to re-form a floating slick. The dramatically increased surface area allows microbial access to much more of the oil, and diffusion and dilution lead to oil concentrations where natural background levels of biologically available oxygen, nitrogen, and phosphorus are sufficient for microbial growth and oil consumption. Dispersants are only used on substantial spills in relatively deep water (usually >10 m), conditions that are impossible to replicate in the laboratory. To date, laboratory experiments aimed at following the biodegradation of dispersed oil usually show only minimal stimulation of the rate of biodegradation, but principally because the oil in these experiments disperses fairly effectively without dispersant. What is needed is a test protocol that allows comparison between an untreated slick that remains on the water surface during the entire biodegradation study and dispersant-treated oil that remains in the water column as small dispersed oil droplets. We show here that when this is accomplished, the rate of biodegradation is dramatically stimulated by an effective dispersant, Corexit 9500®. Further development of this approach might result in a useful tool for comparing the full benefits of different dispersants.

Keywords Oil spill dispersants · Crude oil · Biodegradation · Oil spill response

Responsible editor: Robert Duran

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Introduction

Dispersants are important options in the "tool box" for responding to marine oil spills. Developed after initial attempts to disperse oil with simple industrial cleaners (National Research Council 1989), current products are carefully blended mixtures of usually food-grade surfactants and solvents (National Research Council 2005) that can disperse a range of crude oils and refined products (Lessard and DeMarco 2000) over a wide range of conditions ranging from arctic (Belore et al. 2009) to tropical (Zahed et al. 2011).

Several laboratory protocols have been developed for testing the relative efficacy of different dispersants: the air current test (Mackay et al. 1978), the Warren Springs rolling flask test (Martinelli 1984), the swirling flask test (Clayton et al. 1993), the Exxon dispersant effectiveness test (Becker et al. 1993), and the baffled flask test (Venosa et al. 2002) among others. Comparisons of these tests with field and tank data have been reported by Clark et al. (2005), and the baffled flask test was used to compare eight potential dispersants during the response to the Deepwater Horizon spill (Venosa and Holder 2012).

Useful as they are, these are very small volume tests, and they are not amenable to studying long-term processes (days to weeks) such as biodegradation. In practice, dispersants are only used on significant slicks, and the tests use relatively high concentrations of oil to mimic this. In the field, the dispersed oil droplets (typically <70 μm) diffuse apart, dropping to concentrations below 1 ppm in hours to days (Delvigne 1993; Li et al. 2009; Lee et al. 2013), and they do not re-coalesce. Until now, laboratory tests have used volumes that keep the oil concentration in the many hundreds to many thousands of parts per million, and re-coalescence occurs in hours to days.

The ultimate purpose of oil spill dispersants is to dilute spilled oil so that indigenous microbes in the sea can consume the oil. Hydrocarbons are excellent sources of carbon and energy for those organisms, prokaryotic (Prince et al. 2010)

Published online: 13 August 2013

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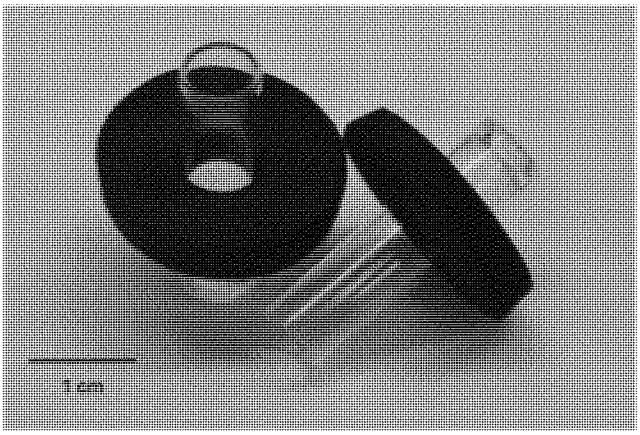

Fig. 1 Glass "booms" to maintain small volumes of oil as a floating slick. The thin glass tubing is held upright by the closed cell ethylene vinyl acetate foam

and eukaryotic (Prince 2010), able to initiate biodegradation, but they lack biologically available nitrogen, phosphorus, and other essential elements for microbial growth. Fortunately, seawater usually provides enough of these nutrients if the hydrocarbons are in the few parts per million concentration or less, and biodegradation of dilute oil has a "half-life" of days to weeks (Hazen et al. 2010; Baelum et al. 2012; Prince et al. 2013).

Quantitatively demonstrating that oil dispersants do indeed stimulate oil biodegradation under controlled conditions has been a major challenge. Current protocols for testing the efficacy of dispersants generate dispersions that are far too concentrated for the available nitrogen, phosphorus, etc. in seawater to allow prompt biodegradation, and experiments are rarely carried out long enough for biodegradation to occur even if the nutrient problem is overcome by adding fertilizer (although see MacNaughton et al. 2003). Conversely, oil at the few parts per million level, where biodegradation is rapid, is dispersed fairly well by laboratory stirring even without adding dispersants, and dispersants have only a minimal stimulatory effect on biodegradation (Prince et al. 2013). Here, we describe experiments where the total oil available in the system is dilute enough that there are potentially enough nutrients in the seawater for rapid biodegradation, but where natural dispersion is slowed by enclosure of the oil in a floating boom unless dispersant is added. These concentrations approximate those found after the successful application of dispersants at sea (Lee et al. 2013), and are below levels where any measurable toxicity occurs, even in 48 (Americamysis bahia) o r 96 h (Menidia beryllina) tests (Hemmer et al. 2011). Under these conditions, Corexit 9500, the dispersant used most widely following the Deepwater Horizon spill, dramatically stimulates the rate of oil biodegradation.

Methods

Seawater was collected from the New Jersey shore in August and September, 2012 (summer conditions; salinity=28 ppt, temperature=21 °C). Nitrate and phosphate levels were below detection limits with simple laboratory colorimetric tests, but are likely to have been near 7 and 0.5 μM, respectively (Louanchi and Najjar 2001). The experiments used 4 l of this seawater in 5 l carboys, maintained at 21 °C in a growth chamber with a diurnal light cycle (16 h on, 8 h off). The oil was Alaska North Slope crude (ExxonMobil 2013), weathered by evaporation at laboratory room temperature in a hood

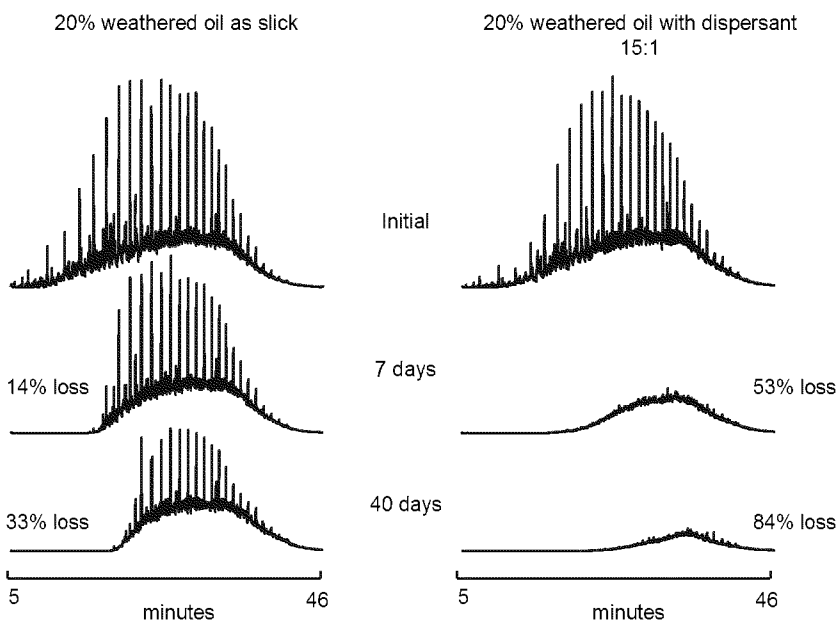

Fig. 2 Total ion chromatograms of the oils (without and with dispersant added at a dispersant to oil ratio of 1:15) extracted after 15 min (initial), 7 and 14 days. The "booms" were removed from the experimental vessels, separated from their flotation collars, and extracted with methylene chloride, as was the water phase. No detectable hydrocarbons were found in the water phase of the oil without dispersant, and none on the glass boom in the samples with dispersant

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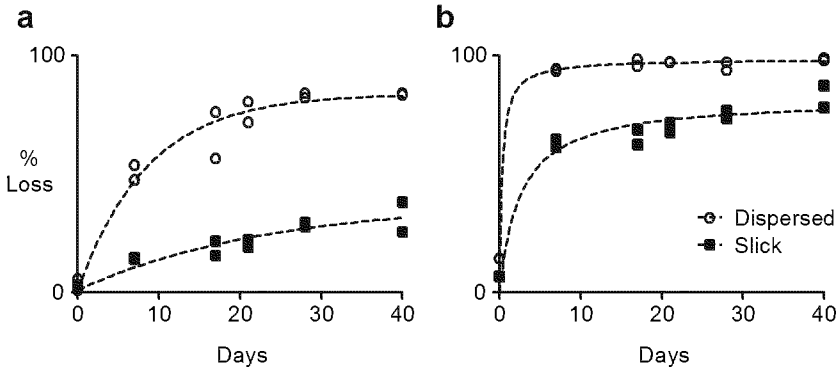

Fig. 3 Loss of a total detectable hydrocarbons and b the sum of the USEPA priority pollutants (Keith and Telliard 1979) from samples with and without dispersant

until it had lost 20 % of its weight; this is a reasonable simulacrum of oil that has weathered between a spill and the application of dispersant from the air (National Research Council 1989, 2005). Oil (10 µl) was added into small floating booms (Fig. 1), some receiving oil alone, others receiving oil that had been premixed with Corexit 9500 at a dispersant to oil ratio of 1:15. The glass booms enclosed a surface area of 28 mm^2, so the 10 µl resulted in a slick with a nominal thickness of 350 µm, although of course there was a meniscus around the edge. The vessels were stirred with a magnetic stirrer to generate a 2-cm vortex, and within minutes, the oil with dispersant had dispersed into the water (to a nominal concentration of 2.5 ppm), while the oil without dispersant remained in the boom. Carboys were incubated up to 40 days, with duplicate vessels with and without dispersant sacrificed at various times throughout the study.

Upon sacrifice, the booms were carefully removed and the carboys extracted three times with methylene chloride. The glass booms were removed from their foam flotation collars and washed twice with methylene chloride. The methylene chloride extracts were collected with a pipette, evaporated carefully to a few milliliters, dried over anhydrous sodium sulfate, and then evaporated to approximately 1 ml, with care to prevent concentration to dryness. In the absence of dispersant, there were no detectable hydrocarbons in the bulk phase, while with dispersant, there was no oil remaining on the glass booms.

Oils were analyzed by gas chromatography coupled with mass spectrometry (Douglas et al. 1992). Hydrocarbon biodegradation was followed with respect to 17α(H),21β(H)-hopane as a conserved internal marker within the oil (Prince et al. 1994).

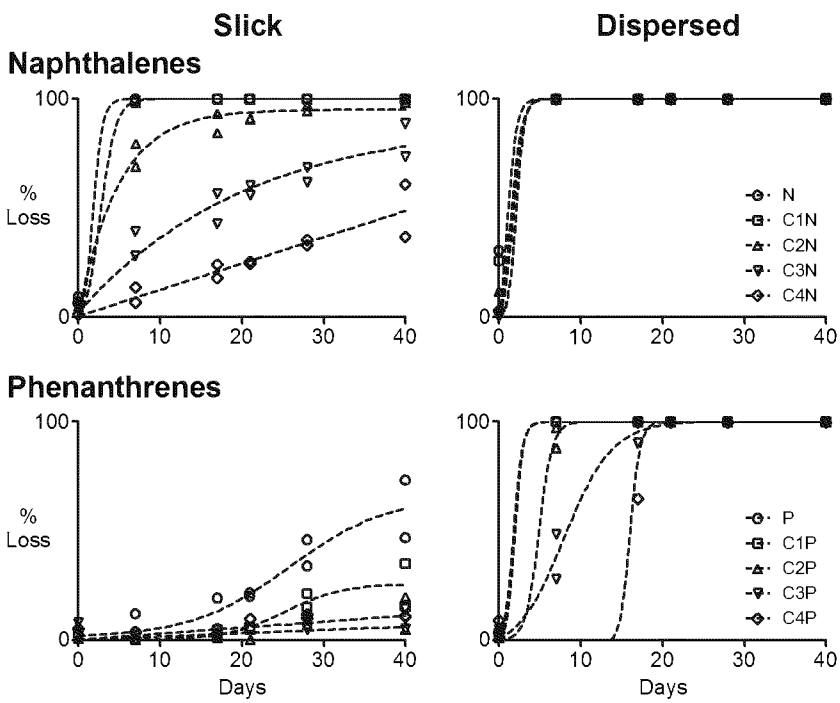

Fig. 4 Loss of naphthalene, phenanthrene, and their alkylated congeners from samples with and without dispersant. N indicates naphthalene, C1N indicates the sum of the methylnaphthalenes, C2N indicates the sum of the dimethyl- and ethyl-naphthalenes, C3N the sum of the trimethyl-, methylethyl-, propyl-, and isopropyl-naphthalenes, etc., while P indicates phenanthrene, etc. We detected no significant preferences for the biodegradation of any isomers over others

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Results

Figure 2 shows representative chromatograms of oils extracted at the initiation of the experiment (15 min after the assembly of the experiments) and after 7 and 40 days of incubation. The sharp peaks are the n- and branched alkanes, while the broad features are the "unresolved complex mixture" in which reside the polycyclic aromatic hydrocarbons. It is clear that biodegradation and evaporation are occurring in samples with and without dispersant, but it is obvious that biodegradation is far more extensive in the dispersed oil than in the slick. Within a week, the dispersed oil had lost approximately half of its detectable hydrocarbons, while the slick had lost only 14 %, and by 40 days, the slick had still not degraded as much as the dispersed oil had in 7 days. Meanwhile, primary biodegradation of the hydrocarbons had reached 84 % in the dispersed oil. Figure 3 shows this graphically, with panel a showing loss of total detectable hydrocarbons, and panel b the loss of the polycyclic aromatic hydrocarbons on the USEPA priority pollutant list (Keith and Telliard 1979). Naphthalene is the most abundant of these molecules in this oil, and the loss of this compound can be attributed to both evaporation and biodegradation (Fig. 4), but its alkyl congeners, and the phenanthrenes, are not significantly volatile, and their disappearance (Fig. 4) can be attributed to biodegradation. Clearly, dispersed oil degraded much more rapidly and extensively than undispersed oil.

Discussion

As far as we are aware, this is the first laboratory demonstration of a substantial and dramatic stimulation of the rate of biodegradation of crude oil by the addition of an oil dispersant. At first glance, this seems to contradict a substantial body of work that found only minimal stimulation at best (Van Hamme and Ward 1999; Lindstrom and Braddock 2002; Venosa and Holder 2007; Prince et al. 2013). We believe that this can be entirely attributed to the fact that in those experiments the relatively low concentrations of oil dispersed quite naturally, almost as well as with dispersants, albeit with slightly larger droplet size (Shaw and Reidy 1979). Since biodegradation is likely dependent on the surface area available for microbial colonization, there is only a small increase of accessibility in decreasing droplet size once small droplets have formed. In contrast, going from a floating slick to 70 μm droplets increases surface area by at least 20-fold, and the stimulation of biodegradation seen here is consistent with this increase.

Several things are noteworthy. The first is that the biodegradation of dispersed oil in these experiments is only marginally faster than it was in water collected at the same site under winter conditions, an apparent "half-life" of about 1 week at 21 °C (Fig. 2) compared with 11 days at 8 °C (Prince et al. 2013), which compares with 28 days (at 100 ppm oil) at 27.5 °C off the Penang, Malaysia shore (Zahed et al. 2011). The second is that these experiments used fresh seawater, not an acclimated inoculum (Venosa and Holder 2007; Campo et al. 2013), thus allowing the indigenous microbes to respond to the oil with the indigenous levels of biological nitrogen, phosphorus, and iron, etc. Next is the observation that the water under the floating slick in the undispersed experiments contained no detectable hydrocarbons (detection limit of the order of a few parts per billion for individual compounds), indicating that any hydrocarbons that dissolved out of the floating slick were promptly degraded by the indigenous microbes.

Our experiments offer the potential for developing a protocol for assessing the biodegradability of dispersed oil that might reveal differences between the biodegradability of dispersions generated by different dispersants. The tests described in the Introduction already offer a way of comparing the dispersion effectiveness of different commercial and experimental products. Building on our protocol, especially to assess and ensure its precision, might allow the development of tests for assessing whether particular formulations stimulate or transiently inhibit biodegradation (Varadaraj et al. 1995). As the protocol is developed, it will be important to ensure that the stirring is well controlled, since it is well known that mixing energy is a primary determinant of the differences seen in the current tests (Venosa et al. 2002; Clark et al. 2005). We use fresh seawater as inoculum because we do not have a clear idea of how to preserve an inoculum for repeated use. This may introduce variability, but attempts to maintain or preserve cultures also have their difficulties, most notably prolonged lag phases before biodegradation begins (Venosa and Holder 2007). More work is needed to determine the better option.

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Degradation of Dispersants and Dispersed Oil

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Introduction

Chemical oil dispersants are proprietary mixtures of surfactants and solvents which are directly applied to a spill in order to reduce the natural attractive forces of the oil. When oil treated with dispersants is exposed to mixing energy, typically from wind and wave action, it is broken up in to small droplets which may then become entrained in the water column (Li et al., 2009a; Li et al., 2009b ; Li, 2008; Lunel, 1995). Many of these droplets are small enough to be neutrally buoyant, and therefore, advection and diffusion forces dilute the plume and transport the droplets far from the site of the original spill. As compared to a surface oil slick or larger and more buoyant physically dispersed oil droplets, these chemically dispersed droplets are much easier for oil-degrading bacteria to colonize and break down (Venosa and Holder, 2007; Venosa and Zhu, 2003). In addition, small droplets enhance dissolution of soluble and semi-volatile compounds into surrounding waters, wherein biodegradation is carried out by aqueous phase microbes. Under these conditions, oil concentration are effectively reduced below toxicity threshold limits, and biodegradation becomes the most important process in reducing the total mass of petroleum hydrocarbons in the environment. By enabling rapid dispersion and biodegradation of surface oil slicks at sea, the use of chemical oil dispersants can be effective in preventing heavy oiling of sensitive coastal environments such as beaches and wetlands, and consequently mitigates risk associated with marine and terrestrial wildlife coming into direct contact with a slick.

Biodegradation of Oil

Any discussion on the biodegradation of chemically-dispersed oil must consider the degradation of the oil itself. A variety of microorganisms in both terrestrial and marine environments have the capacity to utilize petroleum hydrocarbons as the sole source of carbon and energy (Head et al., 2006 ; Leahy and Colwell, 1990; Atlas, 1981 , 1984 ; ZoBell, 1973). Recently a 181 genera of bacteria, 163 genera of filamentous fungi and yeast, and 22 genera of algae have been identified to have the ability to degrade hydrocarbons by metabolizing them in order to grow (Prince, 2010a,b). These findings are not surprising considering the fact that marine microorganisms have long been exposed to significant quantities of petroleum hydrocarbons from natural seepages.

From 1990 to 1999, approximately 600,000 tons of petroleum were released into the world's oceans per year from natural seepages (NRC, 2003; Stout and Wang, 2008) . Biodegradation by indigenous microbial communities is the major process responsible for the weathering and eventual removal of oil from natural seeps that enters the marine

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environment (Atlas, 1995; Atlas and Bartha, 1992; Leahy and Colwell, 1990). Within the marine environment, bacteria are the predominant hydrocarbon degraders (Head et al., 2006; Venosa and Zhu, 2003). Studies from tropical to cold Antarctic and Arctic environments have verified their ubiquitous distribution and their ability to multiply rapidly upon the introduction of oil (Atlas, 1995).

Biodegradation rates have been shown to be the highest for saturates, followed by light aromatics, with high-molecular-weight aromatics and polar compounds exhibiting extremely low biodegradation rates (Prince, 2010c). Co-metabolism plays an important role in oil biodegradation and may require microbial consortia or syntrophic interspecies cooperation (McInerney et al., 2008). Many complex branched, cyclic, and aromatic hydrocarbons, which otherwise would not be biodegraded individually, can be oxidized through co-metabolism in an oil mixture due to the abundance of other substrates that can be metabolized easily within the oil (Atlas, 1981).

It is important to note that microorganisms produce extracellular biosurfactants to promote the formation of oil-in-water emulsions that aid in the uptake and subsequent degradation of hydrocarbons (Desai and Banat, 1997). The hydrophilic and hydrophobic components within the biosurfactants emulsify hydrophobic hydrocarbons, and allow for transport into the hydrophilic intracellular space for biodegradation (Southam et al., 2001). In addition, the fatty acid moieties of biosurfactants promote the growth of microorganisms on the surface of oil droplets (Rosenberg et al., 1979). Nikolopoulou and Kalogerakis (2008) reported that the use of rhamnolipid biosurfactants increased removal of weathered petroleum hydrocarbons (96% removal of C19–C34 n-alkanes within a period of 18 days) and reduced the lag phase prior to the onset of biodegradation. Saeki et al. (2009) showed that addition of biosurfactant JE1058BS to seawater stimulated the degradation of weathered Alaska North Slope 521 crude oil by stimulating the activity of the indigenous marine bacteria and facilitating the removal of oil from the surface of contaminated marine sediments.

In terms of the influence of environmental factors controlling natural oil biodegradation rates, field studies have shown that active microorganisms living in low-temperature environments are dominated by two groups: psychrophilic and psychrotolerant, which are sometimes called psychrotrophic (Atlas, 1984). As defined by Morita (1975), psychrophiles experience optimum growth at less than 15°C, with a maximum growth temperature below 20°C and a minimum growth temperature at or below 0°C. Despite living at these low temperatures, psychrophiles often have metabolic rates comparable to those displayed by the mesophiles adapted to more moderate temperatures. For example, Delille et al. (2009) reported that a temperature of 4°C in the Antarctic had little effect on biodegradation efficiency and that the nutrients, nitrogen and phosphorus, were the limiting factors. Results obtained by Siron et al. (1995) indicated that the temperature threshold for observing significant oil biodegradation was around 0°C. Decreases in solubility associated with low temperatures were considered to be a causal factor for the cases of observed recalcitrance of hydrophobic compounds in cold-water. However, recent reports have indicated that some bacteria may have adapted to the low solubility of hydrophobic environmental chemicals (Deppe et al., 2005; Wick et al., 2002). Indeed there is now evidence that hydrocarbon-degrading microbes may have novel uptake

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mechanisms that enable them to degrade hydrocarbons at rates that exceed their r ates of dissolution in the aqueous phase (Leahy and Colwell, 1990; Thomas et al., 1986).

Throughout the world, the salinity of seawater averages about 35 ‰ (parts per thousand). Salinity variations, albeit small, are mainly caused by such factors as melting of ice, inflow of river water, evap oration, rain, snowfall, wind, wave action, and ocean currents that cause horizontal and vertical mixing of the saltwater (Lagerloef et al., 1995) . Most marine species have an optimum salinity range of 25 –35‰ (ZoBell, 1973) and speci es living in the transition environments are well adapted to fluctuations in salinity. Microorganisms requiring salt for growth are referred to as halophiles. Whereas halophilic hydrocarbon -metabolizing bacteria perform well in this salinity range, there have been reports of the isolation of bacteria capable of degrading hydrocarbons above a salinity of 35 ‰. Bertrand et al. (1990) reported the isolation from a sal t marsh of an extremely halophilic archaea bacterium capable of degrading hydrocarbons in 204 ‰ NaCl, but not below 105 ‰. Diaz (2008) reported the isolation of a bacterial consortium, which mainly included members of the genera *Marinobacter*, *Erwinia* and *Bacillus*, from a crude oil sample from the Cormorant field in the North Sea. This consortium was able to metabolize petroleum hydrocarbons in a salinity range from 0 to 220 ‰ NaCl. Total oil degradation ranged from 48% to 75%, with the greater degradation occurring at the lower salinities.

At the sea surface, wind and wave action maintain a constant supply of o xygen, thus aerobic catabolism of hydrocarbons is usually the preferred biochemical pathway (Leahy and Colwell, 1990) . Oxygen may become limiting in subsurface sediments and anoxic zones of the water column. Oxygen limitation is also a concern for most fi ne-grained marine shorelines, freshwater wetlands, mudflats and salt marshes (Venosa et al., 2002a; Venosa and Zhu, 2003). It is commonly believed that biodegradation rates under anaerobic conditions are almost negligible, while aerobic biodegradation of hydrocarbons occurs rapidly. However, the importance of anaerobic biodegradation should not be underestimated as it has been shown to be a major process under certain conditions. In anoxic marine sediments, reductions of sulphate, Mn(IV) and Fe(III) are the primary terminal electron-accepting processes (Canfield et al., 2005; Finke et al., 2007). Hydrocarbon degradation coupled with sulphate reduction prevails in marine anoxic sediments (Lovley et al., 1997).

With recent advances in analytical methods such as genomics, we are now able to determine the potential of whole microbial communities for oil biodegradation at low temperatures. New evidence as a result of advances in the field of environmental genomics suggests that crude oils are degraded by indigenous organisms in cold water environments at a higher rate than previously reported. This is not surprising since natural oil seeps occur in the world's o ceans at great depths and low temperatures – microbes have become well adapted to their surrounding environment. S tudies have conclusively shown that elevated concentrations of hydrocarbons in the environment increase the number of catabolic -gene copies a mong the microbial community (Heiss-Blanquet et al., 2005; Stapleton and Sayler, 2000; Whyte et al., 2002).

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Biodegradation of Chemically Dispersed Oil

The effect of chemical dispersion on the biodegradation rate of petroleum hydrocarbons has been studied for several decades, and it is generally agreed that chemically dispersed oil is biodegradable. However, the observed effects of chemical dispersants on the rate of oil biodegradation have varied significantly among studies (National Research Council, 2005). Whereas some studies observed stimulation of biodegradation rates by the use of chemical dispersants (Swannell and Daniel, 1999; Traxler and Bhattacharya, 1978), chemical dispersion inhibited the biodegradation rate or had no effect in other studies (Foght and Westlake, 1982; Lindstrom and Braddock, 2002). The effect of chemical dispersion on the rate of oil biodegradation has been further complicated by substrate-dispersant interactions associated with differences in the experimental test conditions, which caused the biodegradation of individual hydrocarbons to be stimulated by some dispersants and inhibited by others (Foght et al., 1987; Van Hamme and Ward, 1999). As a result, it is difficult to predict the effect of dispersants on the biodegradation of specific hydrocarbons based on chemical class (e.g., aliphatic vs. aromatic) (Foght et al., 1987; Lindstrom and Braddock, 2002). Similarly, the effects of specific dispersants on biodegradation cannot be predicted based on the chemical characteristics of the surfactants or the hydrophile-lipophile balance (HLB) of the mixture (Van Hamme and Ward, 1999; Varadaraj et al., 1995).

Attempts have been made to predict the rate of oil biodegradation in the environment based on the results of laboratory studies using scalable, quantitative biodegradation kinetics models that treat oil as droplets suspended in water rather than as homogenous solutions of hydrocarbons in water and consider the growth of the organisms responsible for the biodegradation of oil (National Research Council, 2005). To date only two studies have made an attempt to estimate biodegradation kinetic parameters (Venosa and Holder, 2007; Zahed et al., 2011), by measurement of first-order (in oil concentration or the concentrations of specific oil components) rate coefficients to enable comparison among treatments. However as only one independent rate coefficient was estimated for each treatment, treatment effects could not be rigorously evaluated.

Conducting representative biodegradation studies on dispersed oil in microcosm-scale test systems has at least two important challenges that researchers need to consider as they develop test protocols (Lee et al., 2011). One challenge is to conduct tests at the low dispersed oil concentrations representative of field conditions. Many previous biodegradation studies were conducted at unrealistically high concentrations of dispersed oil in closed microcosms. Prior research either failed to recognize the rapid dilution that occurs at sea or employed methods that were not sufficiently sensitive to study low concentrations. Studying dispersed oil biodegradation at concentrations several orders of magnitude above expected at-sea concentrations in closed systems could limit biodegradation rates and total degradation by exhausting the available nutrients. Some researchers attempted to address this by adding nutrients to the system, but this can lead to unrepresentative modification of the microbial community.

The second challenge with studying dispersed oil biodegradation in a closed system is the difficulty of maintaining a stable dispersion in the laboratory. Dispersed oil in the water

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column exists as small droplets that neither surface nor sink, and the droplets beco me so scattered that they cannot coalesce. It is challenging to maintain stable dispersions of oil in closed systems during the often multi-week test periods required to conduct biodegradation studies. To simulate the dispersion of oil at sea, biodegradati on studies require formation of a stable dispersion containing droplets no greater than 70 - 100 microns and enough mixing energy to keep droplets from resurfacing during an experiment.

A recent Joint Industry Program (McFarlin et al., 2011) on the biodegradation of oil under Arctic conditions has assessed biodegradation of chemically and physically dispersed Alaska North Slope oil as indicated by both primary degradation and hydrocarbon mineralization. The study was conducted under low ambient temperature conditions (-1oC – 2oC) and relatively low oil concentrations (10 -12 ppm crude oil in seawater) and under low level nutrients (0.5 – 1% of the OECD recommended volume of Bushnell Haas Broth). The results of the study demonstrated that the use of dispersant increased the primary biodegradation of fresh oil from 37% to 56% and the mineralization from 12% to 27%. Further increment w as due to the addition of nutrients by 10 % for both primary degradation and mineralization.

Biodegradation of Dispersants

Most studies on surfactant biodegradation focus on surfactants that are used in high - volume consumer products, such as laundry deterg ents (e.g., linear alkyl sulfonates), or other cleaning agents which have known environmental health and safety concerns (e.g., alkylphenol ethoxylates). In general, the results of these have shown that most surfactant formulations are fairly readily biode graded under aerobic (oxygen present) conditions by marine bacteria (Lee et al., 1985; Liu, 1983; Una and Garcia, 1983). The rate of biodegradation under anaerobic (absence of oxygen) conditions tends to be much lower (Berna et al., 2007; Ying, 2006). [redacted] Through the use of anaerobic digesters and analyses of bacterial biogas production, Garcia et al. (2009) conducted a series of batch degradation experiments which revealed that the anaerobic biodegradability of branched alkyl sulphosuccinates such as those used in Corexit were much lower (≤50% mineralization after 50 days) than that of lin ear alkyl sulphosuccinates (≥ 80% mineralization after 50 days). It is clear that different types of surfactants, and even individual surfactants of the same class, can biodegrade at very different rates depending on the structural complexity of chemical branching.

A report by the Fraunhofer Institute (2003) discusses in detail the anaerobic biodegradation of detergent surfactants such as those used in household cleaners. Unfortunately, little attention is paid to the surfactants commonly found in chemical oil dispersants. Generally speaking, the report states that non -ionic surfactants are readily biodegradable under both aerobic and anaerobic con ditions. [redacted]

The biodegradation of surfactants used oil spill treatment agents h as been studied fo r years (Baumann et al., 1999; García et al., 2009; Lindstrom and Br addock, 2002; Liu, 1983; Odokuma and Okpokwasili, 1992; Una and Garcia, 1983). Surfactant biodegradation studies usually distinguish between primary biodegradation, which was calculated from the overall mass balance of surfactant in the reactors (García et al., 2009; Una and Garcia, 1983), and ultimate biodegradation, which also considers the removal of the intermediate products, usually based on oxygen consumption (Odokuma and Okpokwasili, 1992) or carbon dioxide production (García et al., 2009; Lindstrom and Braddock, 2002) relative to the amount expected based on the compound structure. [redacted] (Una and Garcia, 1983). Primary biodegradation of Span 80, the unethoxylated non-ionic surfactant used in both Corexit products, was less than 20% in the same study, but the authors suggested that the poor biodegradation may have been caused by subst rate inhibition due to the extremely [redacted] Ultimate biodegradation of Tween 80 was about 50% in another study (Baumann et al., 1999) , and [redacted] (García et al., 2009), but the observed oxygen consumption or carbon dioxide production were much lower than expected for Corexit 9527 (Odokuma and Okpokwasili, 1992) and Corexit 9500 (Foght et al., 1987), respectively, suggesting that biodegradation was incomplete within the testing period. Some studies have suggested that partial biodegradation of Tween 80 involves metabolism of the oleic acid portion of the molecule, leaving the polyethoxy groups untouched or only partially metabolized (Baumann et al., 1999; Kim and Weber Jr., 2003). Note, however, that enzymatic oxidation and subsequent metabolism of polyethoxylate groups has been described (Nguyen and Sigoillot, 1997; Owen et al., 1997).

[redacted] Unfortunately, until the recent Deepwater Horizon oil spill in the Gulf of Mexico, little information is available on the fate of surfactants in the presence of natural microbial seawater communities at concentrations expected during actual spill response operations. .

Toxicity and Bioaccumulation of Dispersant Surfactants

The toxicity of dispersants may influen ce trophic level dynamics including microbial processes responsible for oil degradation (Lee et al,, 1985). The premise of dispersant use is based on the reduction of oil to concentrations be low toxicity threshold limits. Based on the results of toxicity tests for EPA-approved dispersants such as Corexit 9527 and 9500 listed on the National Contingency Plan (NCP) Product Schedule and the

recommended dispersant-to-oil (DOR) application rates, major environment impacts were not expected at the concentrations to be encountered during their operational use for the treatment of oil spills. A report from the Centers for Disease Control and Prevention (2010) also concluded that "because of the strict guidelines that must be followed to utilize dispersants, it is unlikely that the general public will be exposed (directly) to (the) product." The report further states that "ingredients are not considered to cause chemical sensitization; the dispersants contain proven, biodegradable and low toxicity surfactants."

Despite the development of a regulatory approval mechanism for the support of their operational use, a considerable amount of ongoing research has been funded to evaluate the toxicity of chemical oil dispersants in the marine environment . Public concerns remain high in regards to this topic as the data collected to date has been highly variable due to factors such as differential sensitivity between species, the particular dispersant formulation used, and experimental conditions used (George-Ares and Clark, 2000; Lyons et al., 2011).

Ramachandran et al. (2004) reported that chemically-dispersing different crude oils increased the exposure of fish to the constituents of oil by 10 to 1000-fold in comparison to undispersed oil. The enhanced exposure was demonstrated by an increased activity of liver enzymes that oxygenate compounds accumulated from water containing chemically-dispersed oil droplets. The implication of this work is that the toxicity of oil to fish increased following chemical dispersion in proportion to the extent to which the oil was dispersed. It was noted that the amount of a solution of chemically-dispersed oil that caused toxicity was 100 times less than the amount of undispersed oil required to cause the same effect. However, the measured concentrations of hydrocarbons that were toxic was virtually the same between solutions of dispersed and undispersed oil. Thus, the effect of chemical dispersion was to transfer more compounds from oil to water, and not to make these compounds more toxic.

A study conducted by Fuller et al. (2004) using two fish species, *Cyprinodon variegatus* and *Menidia beryllina*, one shrimp species, *Americamysis bahia* (formerly *Mysidopsis bahia*), and the luminescent bacteria *Vibrio fisherithese* indicated that the toxicity of chemically dispersed oil preparations was equal or less toxic than that of the oil alone. A separate study by Hemmer et al. (2010) looking at toxicity of Louisiana Sweet Crude (LSC), chemical oil dispersants, and chemically dispersed LSC on *M. beryllina*, and *A. bahia* reported that the toxicity of the dispersant alone was lower than that of LSC or dispersed LSC which both showed moderate to high toxicity. Similarly, Milinkovitch et al. (2011) found that while containment and recovery of spilled oil is optimal, there is no significant difference between the toxicity of naturally and chemically dispersed oil when looking at a series of biomarker responses in the gills of golden grey mullet (*Liza aurata*). Further work by Judson et al. (2010) has investigated the potential for chemical oil dispersants to interfere with hormone and other bio-chemical processes in marine organisms. The results of this study concluded that while some dispersants did show low potential for endocrine disruption, most (including Corexit) did not show any significant effect.

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Brief pulses of chemically-dispersed oil can be just as toxic to fish embryos as prolonged exposures. McIntosh et al. (2010) recently reported that exposures to chemically-dispersed crude oil as brief as one hour prevented the fertilization of Atlantic herring embryos. Similarly, one -hour exposures immediately following egg fertilization were sufficient to cause deformities, interfere with development, and kill herri ng embryos at oil concentrations typical of those measured near actual marine oil spills. Thus, contrary to expectations, the primary concern about oil dispersion may not be the toxicity of chemical dispersants, nor the enhanced toxicity of oil. Rather, it is the greatly increased exposure of highly sensitive embryos to the toxic components of oil. As the result, there is the possibility that even brief exposures of fish embryos to dispersed oil can cause embryo toxicity at oil concentrations typical of actual spills. The results of these laboratory studies have highlighted the need for future research to determine effects under the environmental conditions encountered under operational response operations.

In light of the toxicity research study results obtained for chemical oil dispersants, it is suggested that factors such as toxicity of dispersed oil (rather than dispersant itself), dilution and degradation in the environment, species/resources requiring priority protection, potential adverse effects of all response options, and the potential for recovery of sensitive habitats and populations should weigh more heavily into the decision making process than dispersant toxicity alone George-Ares and Clark (2000). Supporting this view, a recent review of the use of chemical dispersants in Europe found that ongoing improvement in dispersant formulation has now reached a point where the toxicity of the dispersant itself is much less important than the toxicity of the oil it is dispersing (Chapman et al., 2007).

While the focus of studies on chemically dispersed o il has been on the induction of acute and/or chronic toxic effects for risk assessments associated with dispersant use, some consideration has also been given to the bioaccumulation of surfactants. In a study on the uptake of two linear alkylbenzene sulfo nates by a freshwater oligocheate (*Lumbriculus variegatus*) and a larval insect (*Chronomus riparius*) using radioactive tracers Mäenpää and Kukkonen (2006) reported that the surfactant residue in the body of the test organisms was more highly dependent on the organic content of the test sediment than on the initial exposure concentration . It was concluded that the high organic content of the test sediment reduced body residue concentrations due to the adsorption of the surfactant to the organic material cont ained in the sediment. This result is consistent with earlier research that concluded surfactants of all classes are readily taken up across the gills but that environmental variables could reduce the concentration of surfactants associated with the test species (EOSCA, 2000).

Following a comprehensive review on the bioaccumulation potential of surfactants , the European Oilfield Specialty Chemicals Association (EOSCA) concluded that although surfactants and their metabolites can be found in aquatic organisms following exposure, there is no evidence to support biomagnification of surfactants through the food chain (EOSCA, 2000). There is also evidence that non-ionic and anionic surfactants (such as those found in most oil dispersants) are biotransformed and eliminated via the gall bladder (Tolls et al., 1994) As a result of surfactant metabolism , Comber et al. (2003)

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suggested that linear alkylbenzene sulfo nates, alcohol ethoxylates, and other structurally similar surfactants are unlikely to bioaccumulate to any significant degree.

Dispersant Use During the DWH Spill Response

The decision by the EPA, NOAA and BP to use chemical dispersants at the Deepwater Horizon spill site did not come lightly. The United States Clean Water Act specifically addresses the use of dispersants in response to oil spills in Section 311(d)(2)(G) which requires that the federal National Contingency Plan for oil spill response contain a schedule identifying:

> (i) dispersants, other chemicals, and other spill mitigating devices and substances, if any, that may be used in carrying out the Plan,
>
> (ii) the waters in which such dispersants, other chemicals, and other spill mitigating devices and substances may be used, and
>
> (iii) the quantities of such dispersant, other chemicals, or other spill mitigating device or substance which can be used safely in such waters, which schedule shall provide in the case of any dispersant, chemical, spill mitigating device or substance, or waters not specifically identified in such schedule that the President, or his delegate, may, on a case -by-case basis, identify the dispersants, other chemicals, and other spill mitigating devices and substances which ma y be used, the waters in which they may be used, and the quantities which can be used safely in such waters.

Although Corexit 9527 and 9500 were both pre-approved by the EPA for use in the event of an oil spill, until the incident in the Gulf, little consideration was given to the suitability of these products for subsurface application. Subsurface injection of dispersant was considered as a means to reduce VOC levels and the volume of dispersant to be used (as application at the well head would improve con tact between dispersant and the oil). Thus, regulatory approval subsurface application was withheld until its efficacy and potential effect on the environment could be assessed (EPA Press Conference Call, 2010).

Following a Net Benefit Environmental Analysis (NEBA) process, a decision was made to apply dispersions an operational countermeasure during the Deepwater Horizon response operations. In Total, 43,884 barrels of Corexit brand chemical oil dispersant was applied (1); 25,505 barrels of Corexit 9527 and Corexit 9500A at the surface (by spraying from vessels at sea and aircraft) and 18,379 barrels of Corexit 9500 via subsurface injection (Federal Interagency Solutions Group, 2010).

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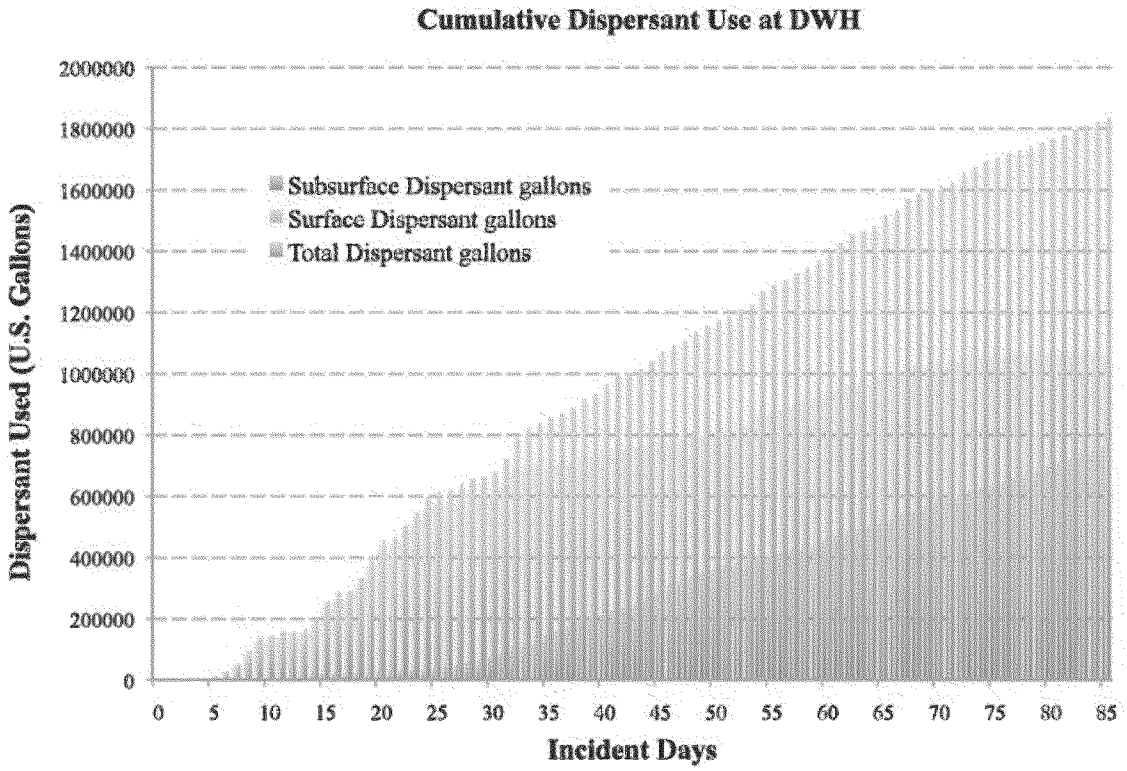

Figure 1. Cumulative surface and subsurface dispersant use by day of spill response (Federal Interagency Solutions Group, 2010).

Dispersant Transport and Fate following the DWH Spill

Corexit 9500 and 9527 were the main dispersants used in response to the BP *-Deepwater Horizon* oil spill. Detailed formulation and ingredient information on COREXIT dispersant products were released to the US EPA for its Gulf monitoring and environmental risk assessment program (Nalco, 2011). The surfactants in these products are similar, including several non-ionic compounds-sorbitan monooleate (Span 80), polyethoxylated sorbitan monooleate (Tween 80), and polyethoxylated sorbitan trioleate (Tween 85)—and the anionic surfactant diethylhexyl sulfosuccinate (DOSS) (Nalco, 2011), but the relative proportions of these compounds may d iffer somewhat between products (Kujawinski et al., 2011) . The biodegradability of chemic al constituents was a criterion in the selection of their formulation by the manufacturer to minimize potential for risks to the environment or public health associated with its use.

As the dispersants could be br oken down rapidly in seawater; the individu al chemical constituents 2-butoxyethanol, dipropylene glycol n-butyl ether (DPnB), propylene glycol, and dioctyl sodium sulfosuccinate (DOSS) were selected for analysis of water samples to determine the expanse of the Deepwater Horizon dispersed oil and Corexit (Operational Science Advisory Team , 2010). These compounds represent major constituents of Corexit, including those with known toxicology data, a those with newly established analytical methods.

Benchmark levels for the individual compounds are established to explain the relevance of measured concentrations (concentrations above benchmark levels are levels of concern.") The benchmarks were based on available biologi data were set at a conservative level to be protective. Aquatic also recognized that the target compounds were also used in other commercial products besides Corexit. Thus, the other sources of the individual chemicals were to be consi the benchmark values were exceeded.

Except for offshore water column samples (79% positive), the dispersant indicators were observed in a small fraction (< 10%) of the samples that were tested. DPnB was one of the most commonly observed dispersant indicator compounds (57 of the 60 positive water samples), but its concentration never exceeded 3 µg/L (Table 1).

Table 1. Samples from the Gulf of Mexico analyzed for the presence of Corexit dispersant indicators; data from the Operational Science Advisory Team (2010).

Zone	Profile	Undetected	Below Benchmark
Nearshore	water column	4790	60
	Sediment	406	6
Offshore	water column	251	199
	Sediment	242	1
Deep Water	water column	3761	353
	Sediment	119	1

* no exceedence of EPA's dispersant benchmarks were observed.

Propylene glycol was the only dispersant indicator detected in the nearshore sediments. Concentrations of DPnB decreased over time (Figure 2), with all values less than 5 µg/L by July 30. The DPnB concentration was highest at the surface and subsurface between 1000 and 1400 m (Figure 3). Deep water concentrations ranged from 0.0170 to 113.4 ìg/L with a mean of 4.3 ìg/L (Operational Science Advisory Team, 2010).

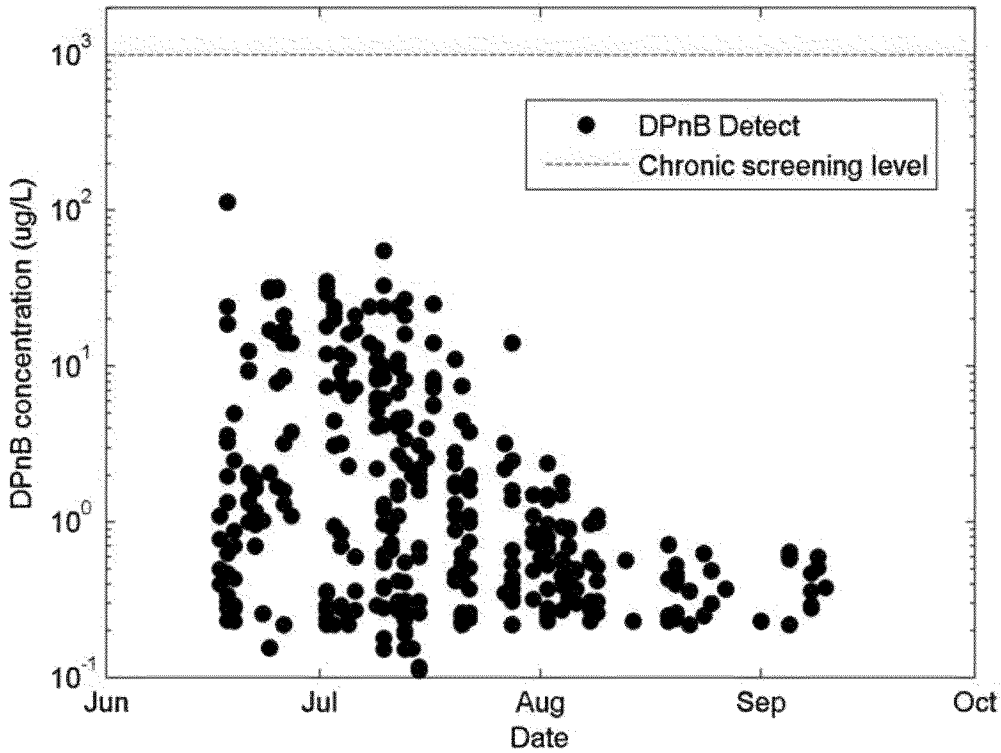

Figure 2. Offshore DPnB concentration over time; the United States Environmental Protection Agency benchmark (chronic screening level) is 1 mg/L (Operational Science Advisory Team, 2010).

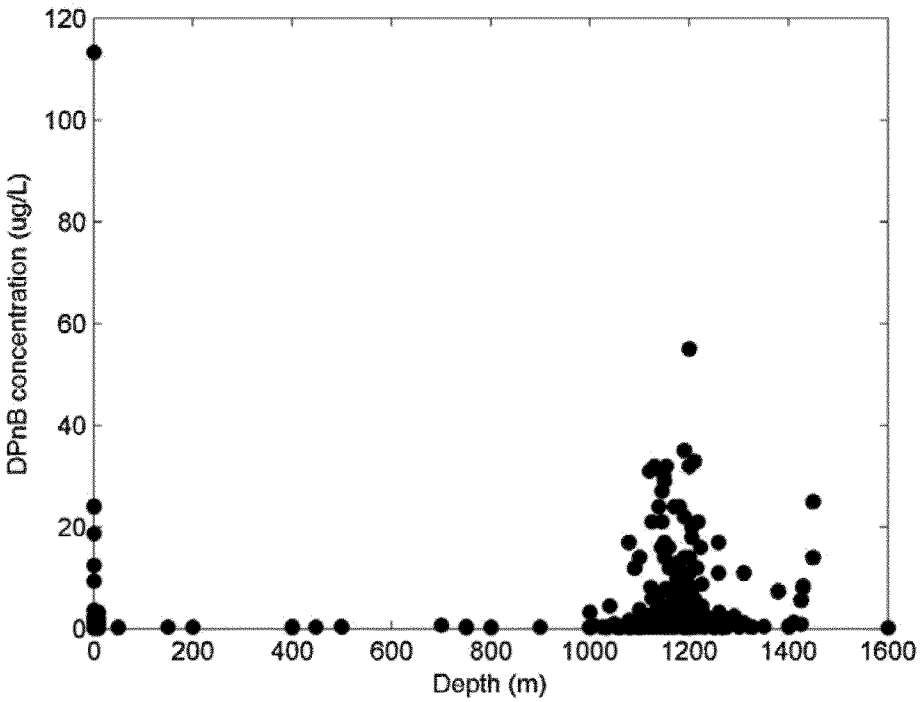

Figure 3. Concentration of DPnB with depth in water samples collected in the deep water zone of the Gulf of Mexico, defined as water depths of greater than 200 m (Operational Science Advisory Team, 2010).

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Using advanced liquid chromatography Fournier transform ion cyclotron resonance mass spectrometry, DOSS was detected both during dispersant application, and up to 300 km from the wellhead 64 days after deepwater dispersant injection had ceased (Kujawinski et al., 2011). It was reported that the majority of the DOSS associated with the subsurface injection of Corexit 9500 moved to, and remained in the bottom water layer between 1000 and 1200 m depth rather than rising to the surface (Figure 4). The possibility could not be dismissed that some dissolution with subsequent vertical transport, as well as partitioning with gas or hydrate, might have occurred. The near 1:1 correlation between DOSS and methane (which was found to act as a conservative marker in this situation) and the consistency between their release rates, indicated that DOSS was not biodegraded or otherwise lost near the well head during conditions of active flow, and thus was transported to the 1000 -1200 m layer (Figure 5). The measured concentrations, ranging from 0.4 to 12 µg/L, were remarkably similar to the expected estimated concentrations. Based on the data, Kujawinski et al. (2011) concluded that although biodegradation might have occurred, the most significant factor that caused a decrease in DOSS concentration at depth was dilution. They calculated that biodegradation rates would have had to be approximately ten times the dilution rate in order for it to have been observed (Kujawinski et al., 2011).

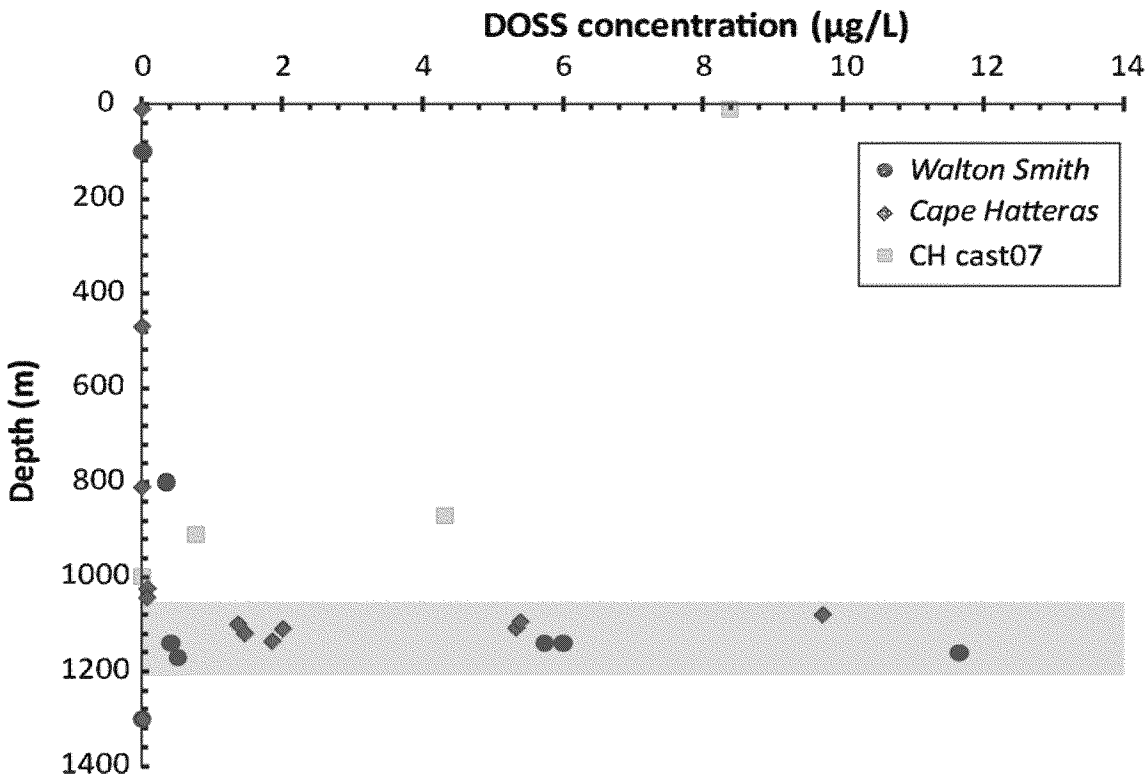

Figure 4. Depth profile of DOSS concentrations falling mainly in the high chromophoric dissolved organic matter zone which included high levels of methane (yellow area), during May and June,

2010, from samples collected by the research vessels *Cape Hatteras* (CH) and *Walton Smith*, with anomalous values from cast07 (Kujawinski et al., 2011).

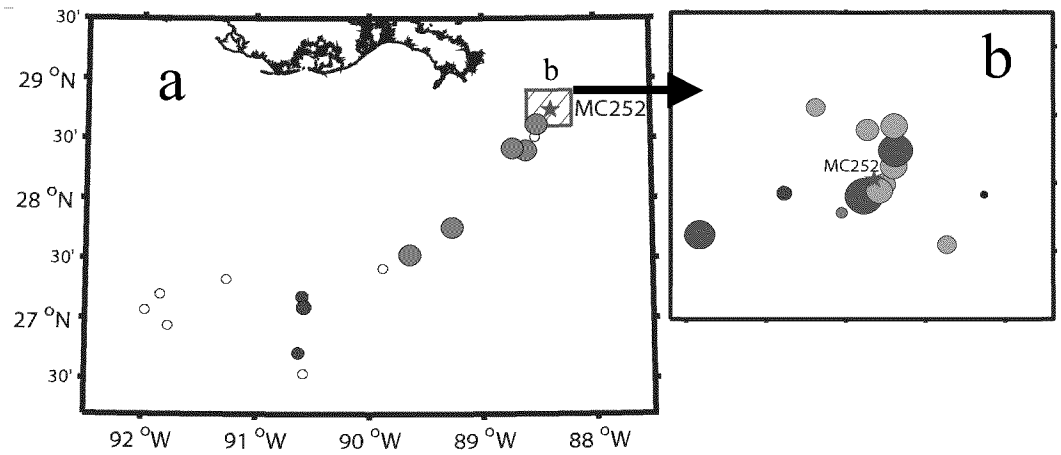

Figure 5. Bird's-eye view of DOSS concentrations at the plume depth of 1000-1200 m in September (a) and May/June (b) with circle size and colour indicative of concentration: white is below detection; blue < 0.01 µg/L; cyan 0.011-0.1 µg/L; green 0.11-1.0 µg/L; yellow 1.0-9.0 µg/L; red > 9.1 µg/L; black indicates the samples were not taken in the plume layer; the star indicates the Deepwater Horizon oil spill site (Kujawinski et al., 2011).

Oil Degradation following the DWH Spill

The recent *DWH* spill has highlighted the importance of natural oil degradation in the recovery of marine ecosystems impacted by crude oil spills. Hazen et al. (2010) reported that the disappearance of residual oil in the Gulf of Mexico from the *DWH* spill was associated microbial degradation processes based on the results of metagenomics. Temperature did not appear to be a major limiting factor as significant rates of oil degradation were observed within the subsea plume of dispersed oil at a depth of 1300 m and temperature below 4 °C. Unlike oil spills occurring at the sea surface, during the *DWH* spill, petroleum hydrocarbons experienced a prolonged, buoyancy -driven ascent through the 1500 m water column (Hazen et al., 2010) . Consequently a unique set of processes affected the released hydrocarbons during their trajectory in the deep sea. Some oil and gas never reached the sea surface, but instead formed hydrocarbon -rich plumes within the cold waters present at about 1100 m depth, supporting an active deep -sea microbial community (Hazen et al., 2010; Valentine et al., 2010) . A combination of integrated chemical, physical and biological processes regulated the transport and fate of hydrocarbons in the deep marine environment. Considering the natural levels of variability and the availability of data at this point of time, it is difficult to disentangle the role of natural processes from the effects of countermeasures such as the use of dispersants.

A considerable amount of research has been focused on the resultant plume of dispersed oil and gas released from the well blow -out that extended southwest from the wellhead between about 1000 and 1200 m below the surface (Camilli et al., 2010; Diercks et al., 2010; Hazen et al., 2010; Kessler et al., 2011; Valentine et al., 2010) . This plume was identified based on fluorescence (Camilli et al., 2010; Diercks et al., 2010; Hazen et al.,

2010), light scattering (Diercks et al., 2010), or the concentrations of specific hydrocarbons (Camilli et al., 2010; Diercks et al., 2010; Kessler et al., 2011; Valentine et al., 2010) and was detectable up to 35 km from the MC252 wellhead (Camilli et al., 2010). The average temperature in the plume was about 5 °C (Camilli et al., 2010; Hazen et al., 2010). Most of these studies also observed a local dissolved oxygen (DO) minimum in the vicinity of the hydrocarbon plume. Camilli and colleagues attributed this minimum to hydrocarbon interference with the *in-situ* DO probes that were used because Winkler titration data did not show oxygen depletion within the plume (Camilli et al., 2010). Other studies, however, showed good agreement between data from the *in-situ* DO probe and Winkler titrations (Kessler et al., 2011; Valentine et al., 2010), suggesting that the rate of aerobic microbial metabolism within the plume was higher than in the surrounding water. Evidence supporting biodegradation of gaseous alkanes (e.g., methane, ethane, propane) (Kessler et al., 2011; Valentine et al., 2010) and higher molecular weight normal alkanes (Kessler et al., 2011) was obtained based on compositional changes that reflected preferential utilization of specific compounds and (for ethane and propane) changes in °C (Valentine et al., 2010). One study estimated that about 70% of the oxygen depletion that was observed within the plume was due to microbial metabolism of ethane and propane (Valentine et al., 2010). Microbial degradation of other hydrocarbons, including butane and longer chain alkanes, was responsible for the additional oxygen depletion. Hazen and colleagues estimated half-lives between about 1.2 and 6.1 days for higher molecular weight normal alkanes based on *in-situ* and microcosm data (Comber et al., 2003). Because their biodegradation rate model did not include biomass concentration, however, and the *in-situ* half-lives did not consider dilution as a factor contributing to the observed changes in compound concentration, the similarity among the observed half-lives should not be over interpreted.

Flocs from samples collected within the plume between May 25 to June 2 were rich in microbes, oil, and oil degradation products, and bacterial counts were elevated within the plume (Hazen et al., 2010). The abundance of genes involved in hydrocarbon degradation were significantly enhanced ($p < 0.05$ or 0.01) in plume samples, and there was a positive correlation with the concentration of low molecular weight components in the oil, suggesting that the composition of the bacterial community changed in response to the presence of oil (Hazen et al., 2010). Cloning and sequencing of 16S rRNA genes showed that the relative abundance of 16 taxa of ã-Proteobacteria, including representatives of known psychrophilic and psychrotolerant hydrocarbon degraders, were higher inside the plume. The most abundant species in samples from within the plume (comprising about 90% of sequences) belonged to a single operational taxonomic unit that was closely related to *Oceanospirillales* (Hazen et al., 2010). Note that observations of samples collected in the same area by another research group about two weeks later, while oil was still being released from the wellhead, did not confirm high levels of *Oceanospirillaceae*, but the samples were dominated by other putative hydrocarbon degraders, especially relatives of *Colwellia* and *Cycloclasticus*, which were thought to be growing on propane, ethane, and butane (Valentine et al., 2010).

Recently, Lu et al. (2011) showed that the microbial community functional composition and structure were dramatically altered in the deep-sea from the Deepwater Horizon spill.

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A variety of metabolic genes involved in aerobic and anaerobic hydrocarbon degradation were highly enriched in the plume than outside the plume. Various other microbial functional genes that are associated w ith carbon, nitrogen, phosphorous, sulfur, and iron cycling, metal resistance, and bacteri ophage replication were also enriched in the plume. The authors suggest that the indigenous marine microbial communities could have a significant role in biodegradation of oil spills in deepwater.

In summary, the size and composition of the Gulf of Mexico microbial community was altered as microbes responded to the presence of oil. Bacterial cell densities were significantly higher in the plume, 10^{5} cells mL^{-1}, as com pared to numbers outside the plume, which was 10^{3} cells mL^{-1} (Atlas and Hazen, 2011). As the community responded, hydrocarbon degraders dominated, resulting in reduced community diversity. DNA surveys for bacterial 16S rRNA genes from samples collected in June revealed dominance of *Cycloclasticus* and *Colwellia*, likely degrading propane and ethane preferentially (Kessler et al., 2011; Valentine et al., 2010). Sixteen taxa of the γ-proteobacteria dominated by the order *Oceanospirillales* occurred in high numbers and dominated the co mmunity in plume samples collected in the same time frame (Hazen et al., 2010). Among these were *Oliespira antarctica, Thalassolituus oleivorans,* and *Oliphilus messinensis*, bacteria known to degrade hydrocarbons and tolerate low temperatures that occur in the deep sea. Sam ples collected later (September) indicated a shift away from these hydrocarbon degraders to methanotrophs, including *Methylococcaceae*, *Methylophaga*, and *Methylophilaceae*. The enhanced abundance of methanotrophs and bacteria containing the particulate methane monooxygenase gene (pmoA) indicated that methane was consumed later in the spill sequence by a different bacterial assemblage (Kessler et al., 2010).

Propane and ethane were degraded relatively rapidly and likely before alkanes >5 carbons in length (Va lentine et al., 2010). The occurrence of natural seeps in the area of the spill may have supported the development and persistence of microbial communities capable of degrading hydrocarbons. Dissolved propane and ethane may promote rapid hydrocarbon degradation and low diversity communities that can degrade other hydrocarbons as the nature of remaining hydrocarbons changes. Hazen et al. (2010) estimated biodegradation rates for hydrocarbons in the plume based on observed concentrations of C13 -C26 alkanes fr om samples collected near the MC252 plume and from laboratory degradation studies at 5 °C. Based on these observations, degradation of alkanes was estimated to be 1.2–6.1 days. Rapid rates of biodegradation may be expected for alkanes, the least recalcitra nt fraction among the complex mixture of compounds that makes up Sweet Louisiana Crude oil. Rapid degradation rates reported for Sweet Louisiana Crude in the region of the MC252 oil spill may be related to its relatively light character, containing a large volatile component and a large fraction of alkanes, both more amenable to degradation than heavier crude oil. Edwards et al . (2011) reported that microbes within the surface slick showed higher rates alkaline phosphate activity, indicating enhanced phosphate stress. Microbial respiration and lipase activity rates were also higher with the slick and the degradation of hydrocarbons was fairly rapid and supported the majority of respiration. The authors suggest that the microbial community possessed the potential to respire hydrocarbons at an unprecedented rate, potentially great enough to keep pace with the flux of oil reaching the surface from the Macondo well, and

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the observed differences in microbial respiration and activity between stations within the slick and outside the slick is a testament to the rapid response of the microbes in surface waters of the Gulf of Mexico to oil from the Deepwater Horizon spill.

The composition of the oil fraction from MC252 estimated by Reddy et al. (2011) indicated 74% saturated hydrocarbons, 16% aromatic hydrocarbons and 10% polar hydrocarbons. Gas chromatographic analysis for several monoaromatic compounds indicated benzene, toluene, ethylbenzene and total xylenes (BTEX) concentrations exceeding 50 $\mu g\ L^{-1}$ within the plume in June 2010 (Camilli et al., 2010). Estimates for hydrocarbon degradation in the plume range from 2 –7 $\mu g\ L^{-1} d^{-1}$, which translates to an estimated half-life of about 1 month for petroleum hydrocarbons (Reddy et al., 2011). Methane was estimated to take longer to degrade. Kessler et al. (2011) estimated the oxidative lifetime of methane resulting from the spill to be 120 days.

Lessons Learned from Deepwater Horizon Spill Response and Future Challenges

About 2.1 million gallons of dispersant were used during the *Deepwater Horizon* spill response, and about 8% of the oil that was released is thought to have been chemically dispersed (Lubchenco et al., 2010). Based on current knowledge, it is believed that most of the chemically dispersed oil, including the MC252-derived hydrocarbons in the deep plume, may have been biodegraded within the environment following its release. Indirect evidence consistent with the expected biodegradation included identification of genes known to be involved in hydrocarbon biodegradation, enrichment of 16S rRNA sequences related to known hydrocarbon degraders, and depletion of dissolved oxygen within the deep dispersed oil plume. In light of the large uncertainties associated with measurements of hydrocarbon fate following accidental spills, fate and transport modeling may offer the best means for evaluating the relative impacts and benefits of chemical dispersion in spill response.

Due to the low concentrations of dispersant following its application in the Gulf of Mexico, as the result of physical dispersion and dilution processes, as well as the intrinsic levels of variability within an open-ocean environment, it is impossible to extract concrete evidence to support the hypothesis that the dispersant surfactants biodegraded rapidly in subsurface waters. Additional research is warranted to enhance better and more quantitative understanding of the fate of dispersants and chemically dispersed oil, particularly in subsurface. In terms of potential environmental risk, it is important to note that all of the surfactants used in Corexit 9500 and Corexit 9527 are known to be at least partially biodegradable under appropriate conditions. Indeed, dispersants themselves can enhance the initial rate of petroleum hydrocarbon degradation by being the first substrate utilized by the hydrocarbon degrading bacteria to grow and colonize dispersed oil droplets (Varadaraj et al., 1995).

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Lab tests on the biodegradation of chemically dispersed oil should consider the rapid dilution that occurs at sea

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abstract

Most crude oils spread on open water to an average thickness as low as 0.1 mm. The application of dispersants enhances the transport of oil as small droplets into the water column, and when combined with the turbulence of 1 m waves will quickly entrain oil into the top 1 m of the water column, where it rapidly dilutes to concentrations less than 100 ppm. In less than 24 h, the dispersed oil is expected to mix into the top 10 m of the water column and be diluted to concentrations well below 10 ppm, with dilution continuing as time proceeds. Over the multiple weeks that biodegradation takes place, dispersed oil concentrations are expected to be below 1 ppm. Measurements from spills and wave basin studies support these calculations. Published laboratory studies focused on the quantification of contaminant biodegradation rates have used concentrations orders of magnitude greater than this, as it was necessary to ensure the concentrations of hydrocarbons and other chemicals were higher than the detection limits of chemical analysis. However, current analytical methods can quantify individual alkanes and PAHs (and their alkyl homologues) at ppb and ppm levels. To simulate marine biodegradation of dispersed oil at dilute concentrations commonly encountered in the field, laboratory studies should be conducted at similarly low hydrocarbon concentrations.

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1. Introduction

The primary goal of any oil spill response operation is to minimize harm to people and the environment. Complete containment and removal of oil from the environment is an expectation that is improbable or impossible for large offshore spills due to physical limitations of mechanical recovery systems. In fact, mechanical recovery operations in the offshore only collect a small fraction of oil even under ideal circumstances (ITOPF, 2010). The extensive mechanical recovery operations during the Deepwater Horizon response were estimated to have recovered only 3% of the oil (NOAA, 2010).

Alternative response tools, such as oil spill dispersants, were developed because of the known limitations of mechanical recovery for large offshore spills. Dispersants, applied at the surface or subsea, allow rapid atomization of large volumes of oil, and thereby facilitate the removal of oil from the environment through biodegradation. Dispersants break a surface slick into micron-sized droplets that are transported into the water column and rapidly diluted. The small oil droplets are now a readily accessible food source for oil degrading bacteria. All marine environments contain petroleum-degrading bacteria because of the abundance of natural oil seeps (Prince et al., 2010).

The rapid dilution that occurs in the open oceans allows bacteria to aerobically biodegrade dispersed oil without exhausting natural levels of biologically available nutrients or oxygen. Laboratory studies have shown that oil degrading microbes colonize dispersed oil droplets within a few days (MacNaughton et al., 2003), and the composition of some dispersants even enhances biodegradation by serving as an initial food source for bacterial growth (Lee et al., 1985; Varadaraj et al., 1995). Fig. 1 illustrates the biodegradation process after applying surface and subsea dispersants.

Measurements made in the Gulf of Mexico indicate that biodegradation is rapid in marine environments (Hazen et al., 2010; Valentine et al., 2012), but there is a significant amount of seemingly contradictory evidence on the biodegradation of dispersed oil in the laboratory. We propose that many of the contradictions are likely the result of study designs that are unrepresentative of the field conditions encountered in the Gulf of Mexico and most oil spills at sea, or limitations in lab-based test methods. This paper

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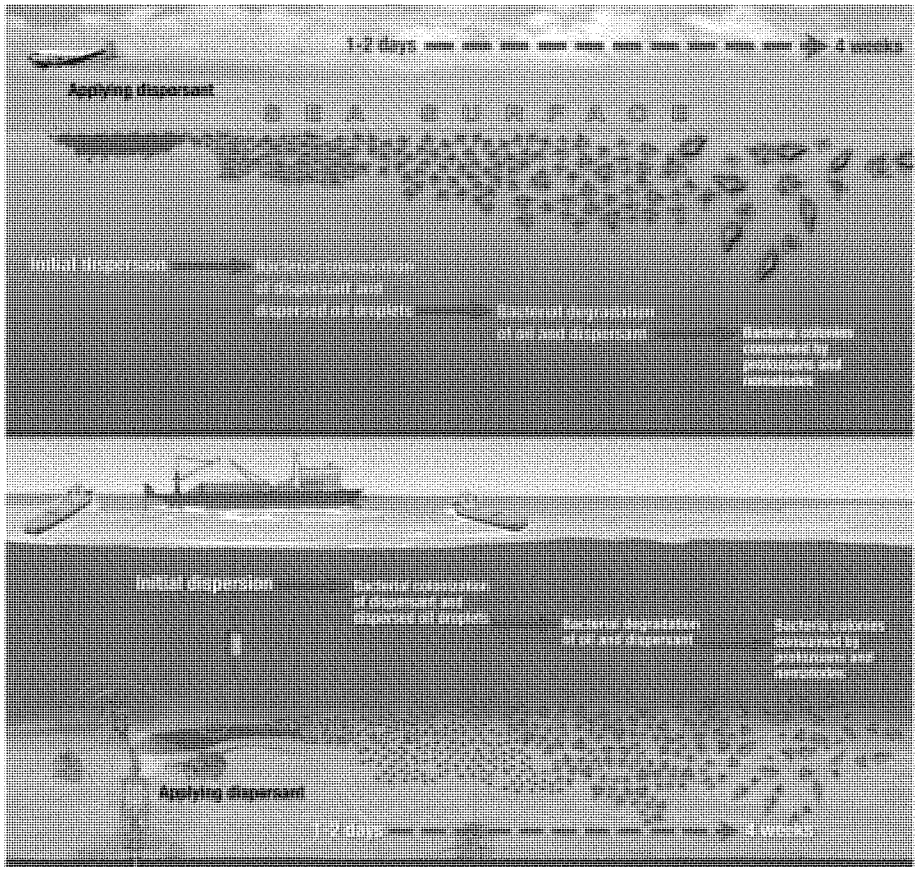

Fig. 1. Diagram illustrating the biodegradation process that begins when dispersants are applied to surface slicks (top) and to a deepwater release (bottom).

describes the important characteristics of plume dilution and dispersion stability that should be accounted for during microcosm studies of dispersed oil fate and effects. We provide evidence from field and wave basin studies to show the speed of dispersed oil dilution and the stability of effective dispersion.

2. Background

Conducting representative biodegradation studies on dispersed oil in a closed microcosm has at least two important challenges that need to be considered. The first challenge is to determine what are the low dispersed oil concentrations representative of field conditions that a researcher wants to investigate, and how to conduct tests at these concentrations. Low-viscosity oil is expected to have an average thickness of 0.1 mm on the sea surface (based on Lehr et al., 1984). Applying dispersants to a slick in 1 m waves causes mixing of dispersed oil into the top 1–1.5 m of the water column (Delvigne and Sweeney, 1988). This results in immediate dilution by a factor of 10,000 producing an average oil concentration of 100 ppm (worst case) or less.

Trudel et al. (2009) confirmed the near immediate dilution in wave tank dispersion tests, finding maximum dispersed oil concentrations between 5 and 147 ppm. Dispersion effectiveness for these tests ranged from 85% to 100%. In another wave tank study under continuous flow conditions, Li et al. (2009) found that the concentration of weathered Alaskan North Slope crude oil dispersed with Corexit 9500 declined from 12 ppm to 2 ppm within 60 min (at a depth of 0.75 m 10 m downstream from the dispersant application). Dispersed oil plumes continue to dilute with time, and are estimated to become very dilute in less than a day (French-McCay and Payne, 2001; French-McCay et al., 2007; Cormack and Nichols, 1977; McAuliffe et al., 1980). Dispersed oil plumes are expected to have average oil concentrations well below 10 ppm before biodegradation becomes significant because microbes require more than one day to colonize dispersed oil droplets (MacNaughton et al., 2003). Further, average concentrations are expected to be well below 1 ppm over the weeks to months that it takes for biodegradation to complete.

Unfortunately published biodegradation studies have been conducted at unrealistically high concentrations of dispersed oil in closed microcosms. Prior research either did not account for the rapid dilution that occurs at sea or employed methods that were not sensitive to low concentrations. Dispersed oil concentrations used in biodegradation studies range from: 1400–4500 ppm (Lindstrom and Braddock, 2002), 266 ppm (Swannell and Daniel, 1999), 250 ppm (Davies et al., 2001), 227 ppm (Yoshida et al., 2006), 100–200 ppm (Zahed et al., 2010), and 830 and 83 ppm (Venosa and Holder, 2007). Studying dispersed oil biodegradation at concentrations orders of magnitude above expected at-sea concentrations in closed systems can limit biodegradation rates and total degradation due to the depletion of nutrients. To counteract this effect, some experiments have been done using high initial concentrations of nutrients in the batch system, but this likely leads to an unrepresentative modification of the microbial community. Prince et al. (2013) found that dispersed crude oil at 2.5 ppm was rapidly and extensively biodegraded without the need for added nutrients or bacteria. Other confounding factors also need to be considered. A recent paper by Hamdan and Fulmer (2011) used Corexit 9500 concentrations between 1000 and 10,000 ppm. These dispersant concentrations are three to five orders of magnitude greater than would be expected even immediately after dispersant application

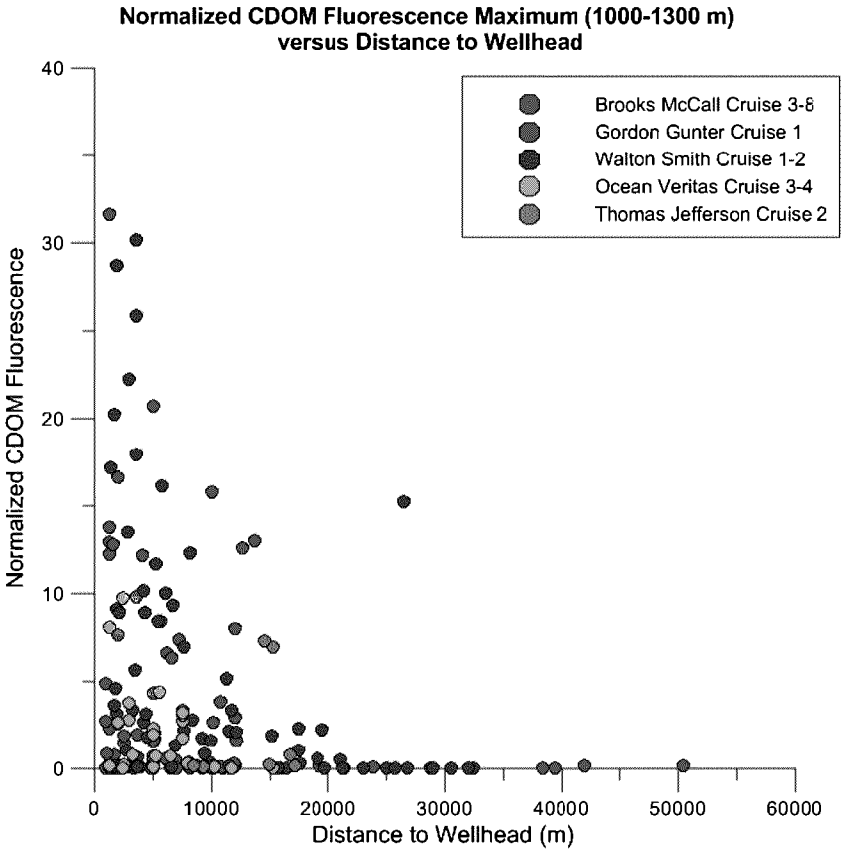

Fig. 2. Mean fluorescence (ppb quinine sulfate dehydrate equivalents, QSDE)) vs. distance from wellhead. Oil concentration (ppm) is derived from the normalized colored dissolved organic matter (CDOM) fluorescence multiplied by a correction factor based on laboratory measurements comparing CDOM fluorescence and MC252 oil with Corexit 9500 at a DOR of 1:25 (calibration factor = 1.2416). Copied from JAG, 2010.

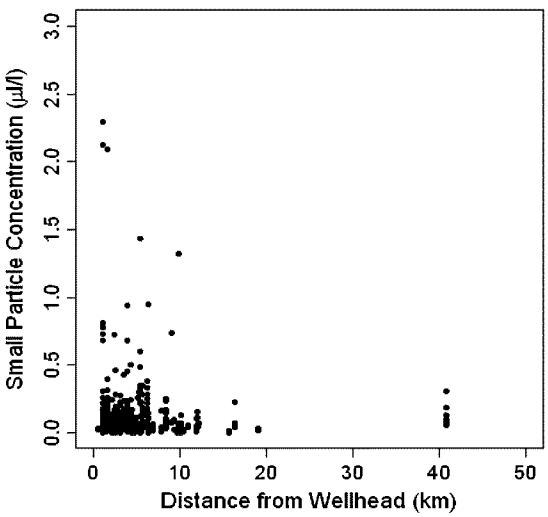

Fig. 3. Small particle concentrations (<70 l m) within the subsurface plume over distance from the wellhead within period of oil release and sub-surface injection of oil dispersants from 8 May to 24 August 2010.

at sea. At these concentrations, it is not surprising that they found that hydrocarbon-degrading bacteria are inhibited by dispersants.

The second challenge with studying dispersed oil biodegradation in a closed system is the difficulty of maintaining a stable dispersion in the laboratory. Dispersed oil in the water column exists as small droplets that neither surface nor sink, and the droplets become so scattered that they cannot coalesce. For biodegradation studies of chemically dispersed oil within the laboratory, mixing is considered essential. If alternative procedures are not used, care must be taken when using centrifugal mixing to minimize the formation of a vortex that concentrates low density oil droplets. It is a challenge to maintain stable oil dispersions in closed systems because natural dilution does not occur, allowing greater droplet-to-droplet contact and coalescence.

Lewis (2004) estimated that the droplet diameter required to keep dispersed oil from resurfacing was <100 l m. The exact diameter depends on the oil density and light oils require a diameter <70 l m to keep droplets from resurfacing in moderate seas. Wave tank tests by Li et al. (2008, 2009) using light-medium oils found dispersed oil droplets with median diameters <50 l m. Lunel (1993, 1994a,b, 1995) reported mean droplet diameters between 15 and 25 l m during field measurements of dispersant-treated oil slicks at sea. Thus, to simulate the dispersion of oil at sea, biodegradation studies require formation of a stable dispersion containing droplets no greater than 70–100 l m and enough mixing energy to keep droplets from resurfacing during an experiment.

3. Field data from the Deepwater Horizon incident

During the Deepwater Horizon spill, oil and gas were discharged from the wellhead at 1544 m below the sea surface for 87 days. A total of 0.78 million gallons of the chemical dispersant Corexit 9500A (Nalco) were applied directly at the wellhead (NOAA, 2010). Turbulent energy from the release of gas and oil from the well resulted in the formation of a subsurface plume of dispersed oil droplets that was observed between 1000 and 1400 m depth;

it began near the release area and trended primarily southwest consistent with water movement along isobaths.

Despite a sustained period of oil release (thought to be 8400 m^3/day) with concurrent additions of dispersant over a prolonged period of time, the field data showed a rapid decrease in the concentration of the plume with distance due to physical dilution processes and the biodegradation of oil (Fig. 2). The highest recorded mean oil concentration measured at 1 km from the source was 10 ppm (R/V Brooks McCall; 30 May 2010), while P 10 km the highest mean oil concentration was 3 ppm, with most concentrations in the ppb range (R/V Gordon Gunter; 2 June 2010) (JAG, 2010).

Samples of water recovered from within the plume during the same R/V Brooks McCall surveys (191 locations) showed that >80% of the subsurface particles were <70 l m (with ffi20 l m median diameter) (Fig. 3). These sizes are in good agreement with previous observations of chemically dispersed oil at sea (Lunel, 1993, 1994a,b, 1995) and in controlled experimental wave tank studies (Li et al., 2008, 2009).

The stimulation of deep-sea indigenous bacteria with the capacity to degrade oil during the Deepwater Horizon spill was clearly demonstrated by Hazen et al. (2010) and Valentine et al. (2012) even though the temperature was 4 fC. Both studies highlight the rapid and prolonged bacterial bloom and subsequent increased biodegradation rates in response to the elevated hydrocarbon presence at low overall oil concentrations.

4. Summary and conclusions

Chemical oil dispersants can rapidly break up surface slicks by diluting the oil into the water column to very low concentrations. This has been clearly demonstrated by wave tank studies and further corroborated by the direct measurements taken during the Deepwater Horizon oil spill. Although recent research cites concentrations almost three orders of magnitude greater, oil concentrations in the low ppm range should be duplicated in the laboratory for oil degradation studies, since it is at these concentrations that the dispersed oil is amenable to biodegradation by microbes, as they will not suffer toxic effects from the dispersant or oil. This situation is a better representation of the environment in field situations in which microbial oil degradation occurs.

It should be noted that up to 90% of indigenous bacteria in marine systems may not survive in culture (Amman et al., 1995); therefore the bioremediation potential of deep-sea microorganisms may not have been accounted for in previous studies. We are now capable of observing changes in the function of whole populations rather than the activity of isolates in culture.

Future laboratory studies that focus on the biodegradation, fate and effects of dispersed oil should attempt to account for the rapid dilution and stability of low concentrations of dispersed oil similar to those found in plumes in the field. When these environmental conditions are used, rapid and significant biodegradation of dispersed oil is expected.

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Short Communication

FATE OF OIL DISPERSANTS IN AQUATIC ENVIRONMENT

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ABSTRACT

Chemical oil dispersants have been used throughout most of the world to combat oil spills. However, their fate in aquatic environment remains uncertain. This study was conducted to investigate the persistence of five oil dispersants in the aquatic environment. The results indicate that petroleum utilizing bacteria possess high potential for the biodegradation of oil dispersants. The order of biodegradability for the five oil dispersants was as follows: Magnus 101 > BP1100X > BP1100 > Linco No. 4 > Corexit 8666. From the results of this study and a review of the literature data, it can be concluded that treatment of oil spills with chemical dispersants is unlikely to cause any build-up of organic contaminants in the aquatic environment.

INTRODUCTION

Accidential spillage of crude oil or petroleum products appears to be an inevitable consequence during the processes of extracting, manufacturing and transportation of petroleum hydrocarbons (Atlas, 1981). Unfortunately, most of these spillages occur in aquatic environments because of the substantial involvement of water transportation for such commodities as well as with the increased oil drilling activities in the continental shelf. Oil spills are, probably one of the major sources of organic contaminants for the aquatic environment. The ecological consequences of each oil spill incident varies, depending on the magnitude of the spill, the chemical composition of the oil and the nature of the contaminated ecosystem.

Various physical and chemical techniques have been developed for cleaning oil spills. The physical approach is intended to confine or remove the spilt oil from the surface water. However, the current state-of-the-art for containment and removal of oil spills from offshore waters has distinct limitations (McCarthy et al., 1978). For instance, containment booms only can be applied to seas with waves not exceeding two feet or currents less

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than two knots. Other available physical options such as burning, sinking, gelling, or the use of sorbents are even less desirable, being either environmentally unacceptable or technically unfeasible. The chemical control of oil spills mainly involves the use of dispersants to break up oil slicks and thus disperse the oil in the water column, which, otherwise, may threaten to pollute shoreline areas (Mulkins-Phillips and Stewart, 1974). Other advantages of using dispersants are the removal of a potential fire hazard, facilitation of evaporation, and enhancement of biodegradation processes (Slade, 1982). Consequently, chemical oil dispersants have been used throughout most of the world to combat oil spills (Albers and Gay, 1982). Much knowledge has been gathered regarding the toxicity of oil dispersants towards birds (Albers and Gay, 1982), fish (Slade, 1982), and algae (Heldal et al., 1978). There has been, in general, a lack of information concerning the persistence of oil dispersant in the aquatic environment. This study was conducted to assess the fate of oil dispersants in the aquatic ecosystem using an oil degrading bacterium.

MATERIALS AND METHODS

Medium

The basal mineral medium used for growing the crude oil degrading bacterium had the following ingredients ($g\ l^{-1}$): K_2HPO_4, 0.66; KH_2PO_4, 0.41; $MgCl_2 \cdot 6H_2O$, 0.10; $FeCl_2 \cdot 4H_2O$, 0.05; $MnCl_2 \cdot 4H_2O$, 0.002; and $(NH_4)_2SO_4$, 0.50. The final pH of the medium was 6.9, and it was sterilized at 121°C for 15 min. The crude oil (Norman Wells) was sterilized separately under the same conditions and was added aseptically to the medium at a concentration of $4\ ml\ l^{-1}$.

Culture

The crude oil-degrading bacterium, CM01, used in this study was originally isolated from a local refinery soil (Liu and Dutka, 1972). The culture was grown in a 2-l Bellco spinner flask containing 1 l of basal mineral medium and 4 ml of crude oil. The flask was incubated on a magnetic stirrer with agitation at room temperature (21°C). Since hydrocarbon-utilizing bacteria can only grow at the oil—water interface (Traxler and Bhattacharya, 1978), the magnetic spindle inside the flask was set at the fast spin setting, (300—400 rpm) to increase the area of such interfaces. With 10% inoculum, an incubation time of 18—20 h was adequate to harvest the cells for use in manometric experiments.

Manometric Studies

The cells were harvested by centrifugation at 10,000 × *g* for 20 min at 4°C, followed by two washes with 0.02 *M* phosphate buffer at pH 7.0. The resulting packed cells were resuspended in cold 0.05 *M* phosphate buffer (pH 7.0) to give a cell concentration of approximately 20 mg dry weight

ml^{-1}. The overall oxygen consumption as determined in a Gilson differential respirometer was taken as a measure of the biodegradation potential ($Q_{O_2} = \mu l\, O_2\, h^{-1}\, mg^{-1}$ cell dry weight) of culture CM01 against oil dispersants. The composition of the assaying preparation, unless otherwise stated, consisted of 1 ml of cell suspension (approx. 20 mg), 1 ml of 0.05 *M* phosphate buffer, 0.8—0.9 ml of distilled water, 100—200 μl substrate, and 0.2 ml of 20% KOH (in the center well of the Warburg flask for CO_2 absorption). Total volume of the reaction mixture was 3.2 ml. The experiments were performed at 20°C with a steady shaking rate of 105 strokes min^{-1}.

Dry weight

Thirty ml of the culture broth were centrifuged at 15,000 × *g* for 20 min, followed by two washes in distilled water. The cell pellet was taken up in 5 ml of distilled water and dried for 20 h at 105°C.

RESULTS AND DISCUSSIONS

The bacterial culture, CM01, was selected for this study from 7 isolates, on the bases of its rapid growth on crude oil. The culture exhibited good potential for the biodegradation of the oil dispersant, BP1100X (Fig. 1). In these experiments, various amounts of BP1100X were added to 250-ml erlenmeyer flasks, containing 100 ml of basal mineral medium, and 1 ml of inoculum. Dry weight determinations were followed at various time intervals (10, 20, and 24 h). The CM01 culture was found to be capable of growing rapidly in different BP1100X concentrations (100—10,000 ppm) as sole carbon and energy source without acclimatization. At BP1100X concentrations below 3 ml l^{-1}, there was a linear relation between cell yield (dry

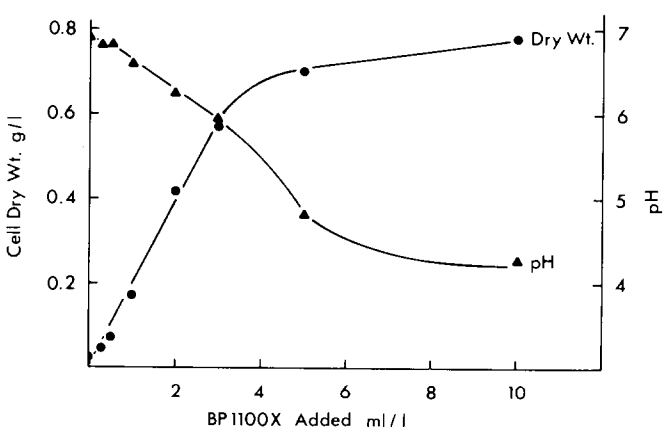

Fig. 1. Effect of BP1100X concentrations on cell yield and final medium pH.

weight) and substrate utilization with a rate of 0.218 ml BP1100X h^{-1} g^{-1} cell dry weight. The cell yield, however, leveled off at higher BP1100X concentrations (4—10 ml l^{-1}), possibily due to the lowering of the medium pH by the culture activity. For instance, an increase of BP1100X concentration in the growth medium from 3 ml l^{-1} to 5 ml l^{-1}, was found to cause a corresponding decrease of the medium pH from 6.0 to 4.8 (Fig. 1).

The ability of the culture CM01 to degrade the oil dispersant BP1100X is further supported by the manometric experiments. In these studies, various amounts of BP1100X (1, 2, 5, 25, 50, 100 and 200 μl) were subjected to oxidation by CM01 in the Warburg flasks. The results presented in Fig. 2 indicate a good correlation between BP1100X concentration and total oxygen consumption. Since BP1100X was the only substrate in the Warburg reaction mixture, the amount of oxygen uptake by CM01 would indicate that BP1100X was utilized by the culture for respiration. Between the BP1100X concentration ranges of 0 to 25 μl $flask^{-1}$ (0—8333 ppm), the amount of oxygen used by the culture CM01 was a direct function of the BP1100X concentrations. It should be noted that with all concentrations of BP1100X used in these experiments, there was no lag period for oxygen uptake, suggesting that the culture CM01 was capable of immediately oxidizing the oil dispersant BP1100X at all concentrations used.

Various oil dispersants have been used for the treatment of oil spills (McCarthy et al., 1978; Albers and Gay, 1982). It would be advisable to determine the order of their persistence in aquatic environment. Thus, high concentrations (33,332 ppm) of five oil dispersants (Magnus 101, BP1100X, BP1100, Linco No. 4, Corexit 8666) and on crude oil (Norman Wells) were

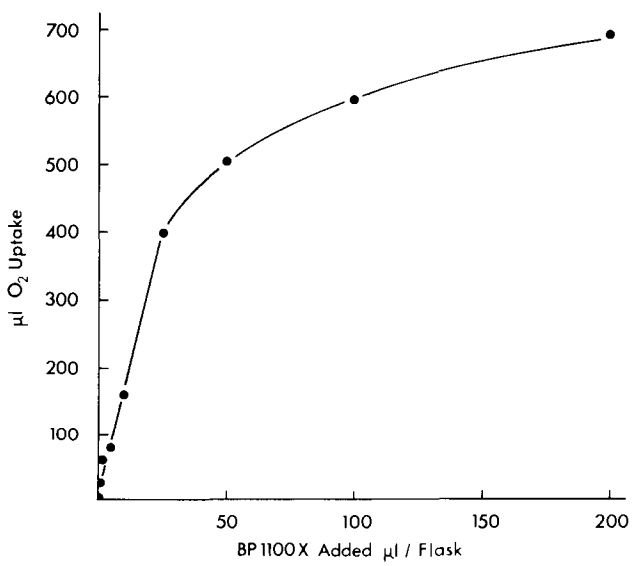

Fig. 2. Total oxygen consumption (60 min) as a function of BP1100X concentrations in the Warburg flasks.

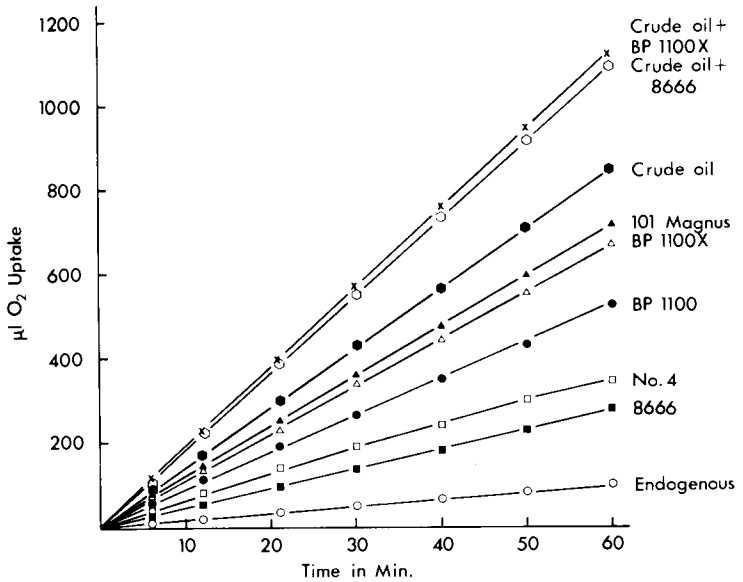

Fig. 3. Oxidation of various oil dispersants by culture CM01.

subjected to culture CM01 oxidation in the Warburg flasks (Fig. 3). Oil dispersant concentrations used in these experiments were about 2.7 times higher than those employed under field situations (Mulkins-Phillips and Stewart, 1974). The immediate response in oxygen uptake (Fig. 3) by the culture CM01, upon addition of the dispersants, suggests that the hydrocarbon utilizing bacteria possess an inherent potential to degrade oil dispersants, i.e., playing an important role in the removal of dispersant from aquatic environment.

The order of biodegradability for the five oil dispersants was determined in terms of Q_{O_2}: Magnus 101 (Q_{O_2} = 34.0), BP1100X (Q_{O_2} = 31.1), BP1100 (Q_{O_2} = 24.6), Linco No. 4 (Q_{O_2} = 16.3) and Corexit 8666 (Q_{O_2} = 13.5). The overall lower Q_{O_2} values (13.5—34.0) for these oil dispersants compared to the crude oil Q_{O_2} (40.0) is probably more desirable in that the dispersants would not be preferentially utilized as carbon sources over the oil by aquatic microorganisms. Petroleum utilizing microorganisms are known to be widely distributed in aquatic environment (Atlas, 1981) and the results in this study demonstrate the susceptibility of oil dispersants to immediate biodegradation by such microorganisms. Thus, it appears that treatment of oil spills with dispersants is not likely to have any significant impact on the build-up of organic contaminants in aquatic environment.

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RESEARCH

Biodegradation of Dispersed Forties Crude and Alaskan North Slope Oils in Microcosms Under Simulated Marine Conditions

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For oil spills in the open sea, operational experience has found that conventional response techniques, such as mechanical recovery, tend to remove only a small fraction of oil during major spills, a recent exception being the Mississippi River spill in Louisiana [Spill Sci. Technol. Bull. 7 (2002) 155]. By contrast, the use of dispersants can enable significant fractions of oil to be removed from the sea surface by dispersing the oil into the water column. It is thought that once dispersed the oil can biodegrade in the water column, although there is little information on the mechanism and rate of biodegradation. Two studies were undertaken on dispersion, microbial colonisation and biodegradation of Forties crude and Alaskan North Slope (ANS) oils under simulated marine conditions. The study using the Forties crude lasted 27 days and was carried out in conditions simulating estuarine and coastal conditions in waters around the UK (15 C and in the presence of nutrients, 1 mg $N\text{-}NO_3/l$), while the ANS study simulated low temperature conditions typical of Prince William Sound (8 C) and took place over 35 days. The results of both studies demonstrated microbial colonisation of oil droplets after 4 days, and the formation of neutrally buoyant clusters consisting of oil, bacteria, protozoa and nematodes. By day 16, the size of the clusters increased and they sank to the bottom of the microcosms, presumably because of a decrease in buoyancy due to oil biodegradation, however biodegradation of n-alkanes was confirmed only in the Forties study. No colonisation or biodegradation of oil was noted in the controls in which biological action was inhibited. Oil degrading bacteria proliferated in all biologically active microcosms. Without dispersant, the onset of colonisation was delayed, although microbial growth rates and population size in ANS were greater than observed with the Forties. This difference reflected the greater droplet number seen with ANS at 8 C than with Forties crude at 15 C. Although these studies differed by more than one variable, complicating comparison, the findings suggest that dispersion (natural or chemical) changes the impact of the oil on the marine environment, potentially having important implications for management of oil spills in relation to the policy of dispersant use in an oil spill event.
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Keywords: Biodegradation, dispersant, dispersed oil, microorganisms

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Introduction

Operational experience following major oil spills has shown that the use of dispersants can enable significant amounts of oil to be removed from the sea surface by dispersing the oil into the water column

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(Green et al., 1982, Lunel et al., 1995). In comparison, conventional response techniques such as mechanical recovery, remove only a small fraction of oil. It is well known that bacteria are able to degrade oil dispersed in the water column (Floodgate, 1984; Bartha & Atlas, 1987). Oil dispersion is thought to stimulate the rate of hydrocarbon degradation by increasing the surface area of the oil available for microbial attack (Singer & Flaherty, 1984; Leahy & Colwell, 1990).

While a substantial amount ofresearch has been conducted into the impact ofchemical dispersants on the fate of dispersed oil, results have often been conflicting. Some workers have proposed that dispersants had little effect on oil biodegradation, while others suggest a positive effect and some note a negative effect (Robichauz & Myrick, 1972; Mulkins-Phillips & Stewart, 1974; Traxler & Bhattacharya, 1979; Foght & Westlake, 1982; Lee et al., 1985; Literathy et al., 1989). It has been suggested that dispersants increase oil biodegradation by increasing the surface area for microbial attack, and encouraging migration of the droplets through the water column making oxygen and nutrients more readily available (Mulyono et al., 1994). However, some dispersants were also found to have a toxic effect on microbial processes retarding the rate of oil decomposition (Mulyono et al., 1994; Varadaraj et al., 1995). Varadaraj et al. (1995) studied this dual capability of dispersants of increasing the surface area of dispersed oil and affecting the growth of hydrocarbon-degraders, and concluded that both of these factors influenced the effectiveness of dispersants at promoting oil decomposition. They found that increasing the sorbitan content of a dispersant stimulated oil biodegradation by acting as a nutrient source for the growth of hydrocarbon-degraders. In these studies little work has been done on the microbial interactions with oil droplets.

Herein are presented the results from two studies conducted between 1994 and 2001, into the effect ofa dispersant (Corexit 9500) on the dispersion, biodegradation and microbial colonisation ofboth Forties crude at 15 C and Alaskan North slope (ANS) oil at 8 C. Both studies were conducted using natural seawater in a Mackay apparatus designed to simulate the marine environment as closely as possible in laboratory conditions. In these studies droplet formation and size were analysed using a chamber slide technique adapted from Delvigne and Sweeney (1988).

Materials and Methods

The marine microcosms used throughout these two studies were based on the apparatus designed by

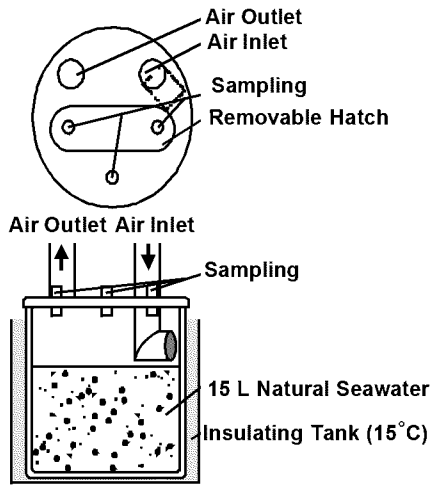

Fig. 1 Schematic ofthe laboratory microcosms.

Mackay and Szeto (1980), adapted and operated as described in Swannell et al. (1997) (Fig. 1). The Forties crude and ANS oils were weathered prior to use. Weathering was performed by distillation of the crude oil at 250 C, a process that removed approximately 25% of oil by weight (Walker et al., 1992). To simulate an oil slick, the weathered oil (10 ml) was added to each microcosm forming a slick approximately 100 Im thick, airflow was then switched on (5.0 ± 0.5 m/s), simulating the wind and wave action encountered at sea and was continued throughout each experiment.

Where required, chemical dispersion was achieved through the addition ofCorexit 9500 (10% w/w ofthe oil). Weathered oil (Forties or ANS) was added to the microcosm and held in a 50 mm diameter PTFE ring. Corexit 9500 was added evenly over the oil. The air flow was switched on and the ring removed, enabling the oil/dispersant mix to spread over the surface of the water.

For the study utilising Forties (study 1), 3 microcosms each containing 15 l seawater were set up as follows:

- Seawater containing oil and Corexit 9500;
- Seawater containing oil, Corexit 9500 and added levels ofnutrients (1 mg N-NO_3/l) simulating conditions found in estuarine and coastal waters in the UK;
- Seawater containing oil, Corexit 9500 and mercuric chloride (300 mg/l, Russell et al., 1992)—a killed control.

In addition, chemical dispersion was compared to natural dispersion (without dispersant) for nutrient amended, unamended and killed controls. Each study was carried out in triplicate at 15 C, conditions typ-

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ical ofthose in UK coastal waters. Throughout these microcosm experiments samples were taken on days 1, 2, 3, 5, 7, 9, 13, 16, 20, 23, and 27.

For the ANS oil (study 2), microcosms were set up as follows:

- Seawater containing ANS (the ffiatural dispersant test-);
- Seawater containing ANS and Corexit 9500;
- Seawater containing ANS, Corexit 9500 and mercuric chloride (300 mg/l)—the killed control.

On day 8 ofthe ANS study, microcosms were amended with 1 mg/l N as sodium nitrate and 0.1 mg/l P as potassium dihydrogen orthophosphate. Day 8 was chosen for nutrient addition as the team estimated that the original seawater nutrient content would become limiting by that time. This reduced the likelihood that the biodegradation ofoil by the indigenous microbial population would be limited by nitrogen or phosphorus over the course ofthe 35 days experiment.

Again, each microcosm study was carried out in triplicate, however the temperature was held at 8 C, conditions typical ofthose in Prince William Sound. Samples were taken on days 0, 1, 4, 7, 10, 14, 17, 21, 24, 28 and 35. For both Forties Crude and ANS experiments sampling consistency throughout was ensured by always using an open pipette from the same place and depth within the microcosm. Visual analysis confirmed that the microcosms remained single phased throughout each study. There was no visible formation of a water-in-oil emulsion.

To determine the impact ofdispersion on droplet formation, microbial biomass and oil degradation, the following analyses were performed common to both studies:

- Droplet formation (looking at both size and number ofdroplets using the Chamber slide method adapted from Delvigne and Sweeney (1988) by Swannell et al. (1997)), droplet size followed a Poisson distribution;
- Microbial biomass (most probable number counts) with the appropriate weathered oil as the C-source, Croft et al. (1995); and
- Oil degradation (GC/MS analysis total hydrocarbons (reported as n-alkane depletion in study 1); GC/MS analysis of the ratio of total hydrocarbons to the biomarker hopane in study 2 (Swannell et al., 1997)).

Analysis ofvariance was used to determine significant differences between microcosm treatments.

Results and Discussion

Study 1 (Forties)

Figure 2 presents semi-log plots ofthe droplet size and number following: (A) the addition of dispersant to the killed control, (B) the addition ofdispersant to natural seawater, and (C) the addition ofdispersant to nutrient amended natural seawater. In the killed control, by day 9 droplet number had reached the maximum detectable amount of4.5 $\cdot 10^6$ per ml and did not fall below this level for the remainder of the study. The majority ofdroplets in this microcosm were 1–2 Im in diameter. In contrast, in the presence of microorganisms the maximum droplet number was reached by day 9, but decreased from day 13 down to $<1 \cdot 10^6$ by the end ofthe study, significantly less than were detected in the killed control ($P < 0:05$). Finally, where nutrients were added, the pattern was similar to that for the dispersant amended seawater except that the peak concentration approximated to $3 \cdot 10^6$ by day 9 before falling off by day 16 (again a significant difference compared to the control ($P < 0:05$)). The decrease in final oil droplet numbers in the two ffive-

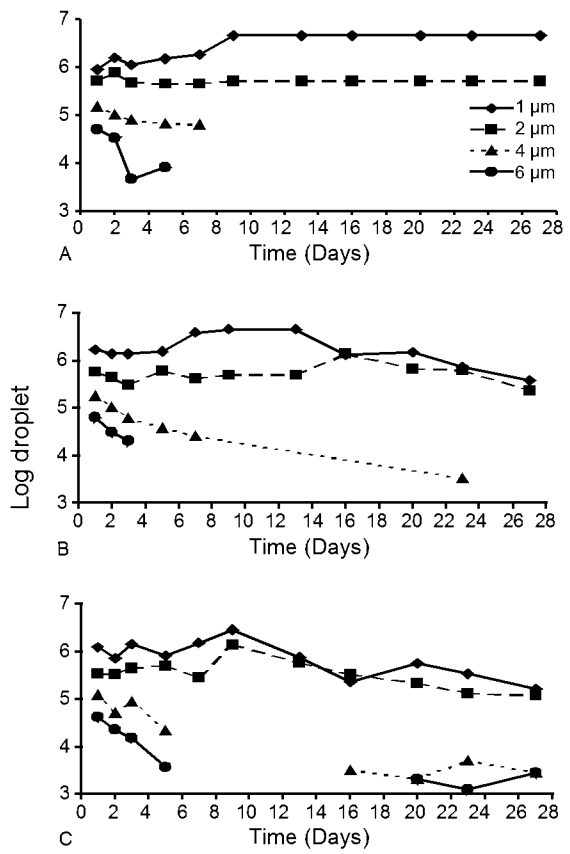

Fig. 2 Forties oil droplet distribution in seawater amended with (A) dispersant and mercuric chloride (the killed control); (B) dispersant; and (C) dispersant and low levels ofnutrient. The legend is presented in A.

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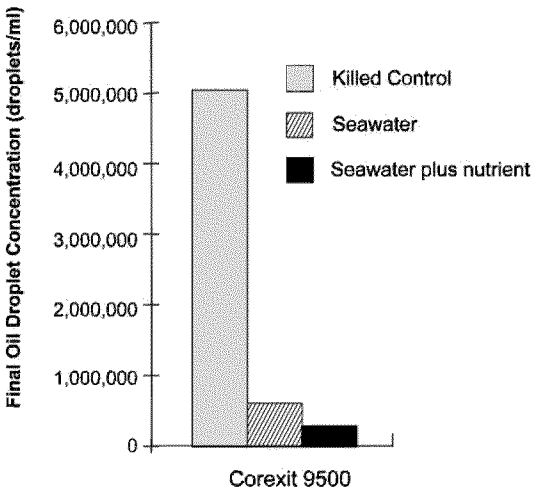

Fig. 3 Reduction in Forties oil droplet concentration in the killed control, seawater and seawater with low levels ofnutrients.

microcosms compared to the killed control is shown in Fig. 3. These results suggest microbial degradation of the dispersed oil droplets in the dispersant amended seawater samples, with said degradation further stimulated by the presence ofa low concentration of additional nutrients.

Analysis ofthe n-alkane depletion in comparison to that in the killed control confirmed this finding, with approximately 90 and 55% of the n-alkanes degraded in the nutrient amended seawater and the non nutrient amended seawater, respectively (Fig. 4(A)). The impact of the dispersant on the microbial population is illustrated in Fig. 4(B) showing the difference in mean maximum microbial growth as measured by MPN/ml/day in the chemically dispersed verses the naturally dispersed Forties crude with and without added nutrients. Max MPN/ml/day were an order of magnitude

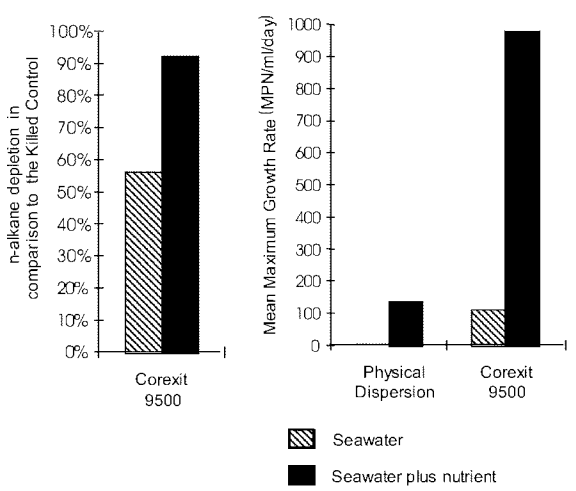

Fig. 4 (A) Depletion ofForties n-alkanes compared to the killed control for seawater with dispersant and seawater, dispersant and low levels of nutrient, and (B) impact of dispersant on microbial growth rate in seawater and seawater with low levels of nutrient.

greater in the chemically dispersed microcosms verses those without dispersant.

Study 2 (ANS)

Results obtained during the ANS slope study at 8 C were more variable than those for the Forties crude at 15 C. Oil droplet size and distribution are presented in Fig. 5(A) and (B) and in Table 1 (the large variation making it impossible to show this result as a figure). This contrasted with the lower variation in the Forties experiment. In the killed controls, oil dispersion was characterised by high numbers ofoil droplets at the start ofthe experiment that depleted with time (30–53% decrease in droplet number, Fig. 5(A)). The oil droplet size distribution showed that large oil droplets present at the start ofthe experiment disappeared over time, consistent with the hypothesis that large oil droplets re-coalesced with the oil slick. Where dispersant was added, the droplet counts pattern was again characterised by high numbers ofoil droplets at the start ofthe experiment that decreased with time (Fig. 5(B)). However the decrease in oil droplet numbers was significantly greater in these microbiologically active microcosms than in the killed controls, with 86–95% depletion in the total number ofoil droplets by the end ofthe experiment ($P < 0:05$). This observation may reflect the biodegradation ofthe oil droplets by indigenous hydrocarbon-degraders over

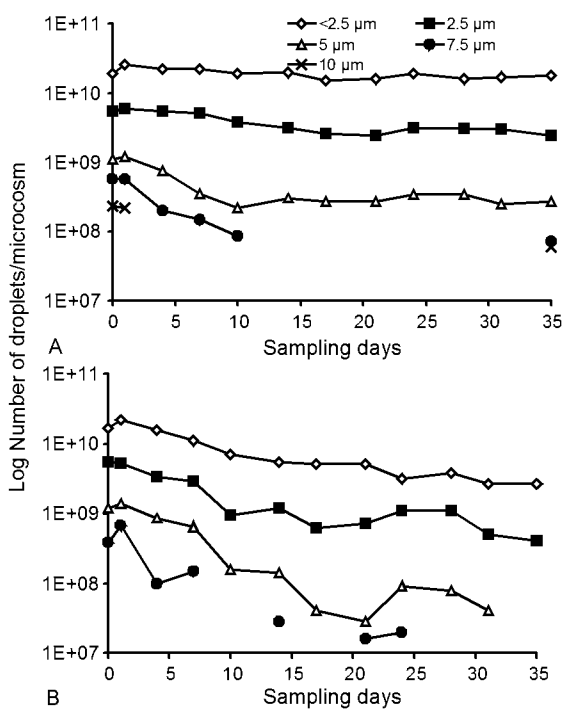

Fig. 5 ANS oil droplet distribution in seawater amended with (A) dispersant and mercuric chloride (the killed control); and (B) dispersant. The legend is presented in A.

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Table 1 Oil droplet distribution in replicate natural dispersion microcosms (ANS study) over time (days)

	7	10	14	17	21	24	28	31	35
Exp 1									
<2.5	7.80	1.25	31	79.3	149	246	125	85.4	41.2
2.5	3.40	0.31		5.45	15.6	39.8	26.3	4.68	3.07
5	1.28					5.16	7.66		
7.5	0.14					1.47			
10									
Exp 2									
<2.5		1.87	5.10	1.20	110	151	278	564	850
2.5		0.47	2.32	0.96	22.7	38.5	43.8	130	74.2
5					3.98	1.75	1.75	12.0	2.39
7.5							0.88	8.58	
10									
Exp 3									
<2.5	1.76	2.76	32.6	57.5	23.9	9.50	4.01	3.88	6.67
2.5			14.0	23.3	12.5	4.75			0.95
5			0.93	3.11	1.04	0.95			
7.5				1.55					
10									

Numbers represent droplets per microcosm ($\cdot 10^7$).

time. The oil droplet size distribution was similar to that observed with the controls, with larger droplet numbers decreasing over time. To a lesser extent than was noted for the killed controls, the number of oil droplets produced still varied substantially between replicates, again reflected in the large standard deviations.

For the natural dispersion tests, the oil slick emulsified to different extents in each replicate, showing a greater degree ofvariability than observed previously with the Forties crude oil at 15 C. This resulted in three different patterns ofoil dispersion (Table 1). In replicate 1, the pattern ofoil dispersion was characterised by the appearance ofa low number ofoil droplets by day 7, increasing to a maximum on day 24 and then decreasing up to the end ofthe experiment. In contrast the oil in replicate 2 emulsified to the extent of forming a "mat" of oil on the surface of the water. Possibly as a result ofthis, the onset ofoil dispersion was delayed until day 10. By day 21 the oil droplet numbers started to increase and, in contrast to that observed in replicate 1, continued to increase until day 35. In the final replicate, the oil slick again emulsified before forming a semi-submerged "ball" of oil and water reducing the surface area of the oil slick. Consequently, this microcosm contained the lowest total number ofoil droplets. In general, a higher number ofoil droplets were noted in the ANS natural dispersion microcosms than in the Forties crude microcosms (Swannell & Daniel, 1999).

Although a consistent pattern ofdroplet distribution was observed for the ANS oil (at least in the killed control and chemical dispersant tests, less so in the natural dispersant test), the number ofoil droplets produced varied substantially between replicates. This finding was at odds with the results from the Forties crude study. This variation in results could be a reflection ofboth the different oil types (ANS is less dispersible and the oil may form a less stable oil-in-water emulsion than Forties in the presence of Corexit 9500), and the temperature (8 C in the ANS verses 15 C for the Forties). The lower temperature may have reduced the stability ofthe dispersed phase and the effectiveness ofthe dispersant.

During the microscopic enumeration ofthe ANS oil droplets, bacterial colonisation ofthe oil droplets was assessed. In microcosms treated with dispersant, by day 4 at least 40 % ofthe oil droplets were colonised by 1 or more bacteria (Fig. 6). The percentage of colonised bacteria increased to approximately 80% by day 17 and stayed at that level for the remainder of the experiments. Thus, in the presence ofdispersant the oil droplets became colonised within days, and bacteria-oil-protozoa and nematode droplet clusters formed of 10 Im to 1–2 mm, a finding also observed in the

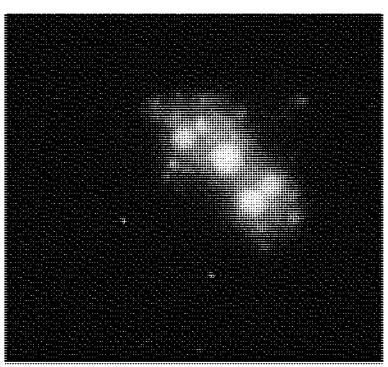

Fig. 6 Cluster ofoil droplet and bacteria under epifluorescence at a magnification of400 ·, the biggest oil droplet diameter is 5 Im.

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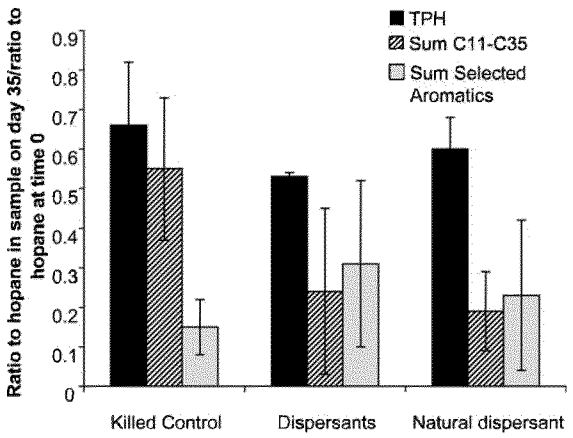

Fig. 7 Ratio ofTPH, the sum ofthe C11 to C35 n-alkanes and the sum of selected aromatics to the biomarker hopane on day 35 of the ANS study to hopane at time 0 (error bars represent standard deviation). Selected aromatics are: (naphthalene, methylnaphthalenes, dimethylnaphthalenes, trimethylnaphthalenes, dibenzothiophene, methyldibenzothiophenes, dimethyldibenzothiophenes, phenanthrene, methylphenanthrenes, and dimethylphenanthrenes).

Forties crude oil study. Similar findings were observed in the natural dispersion microcosms with clusters observed shortly after the onset of oil dispersion (day 10–14). As in the Forties study, the oil droplet colonisation results show that the indigenous microbial populations are interacting closely with the oil and presumably biodegrading it.

To assess the extent ofbiodegradation at the end of the study (day 35) concentrations ofselected biodegradable fractions of oil were compared with the recalcitrant biomarker hopane. Results are presented as the ratio ofthe total volumes ofpetroleum hydrocarbons (TPH), the sum ofthe C11 to C35 n-alkanes, and the sum of selected aromatics, to hopane (Fig. 7). Although the ratio of TPH and n-alkanes to hopane on day 35 was lower in the chemically and naturally dispersed microcosms than in the killed controls, variation between replicates meant this decrease was not significant (P > 0:05). Similarly, there was no significant difference in the ratio of the selected aromatics to hopane between the killed controls and the

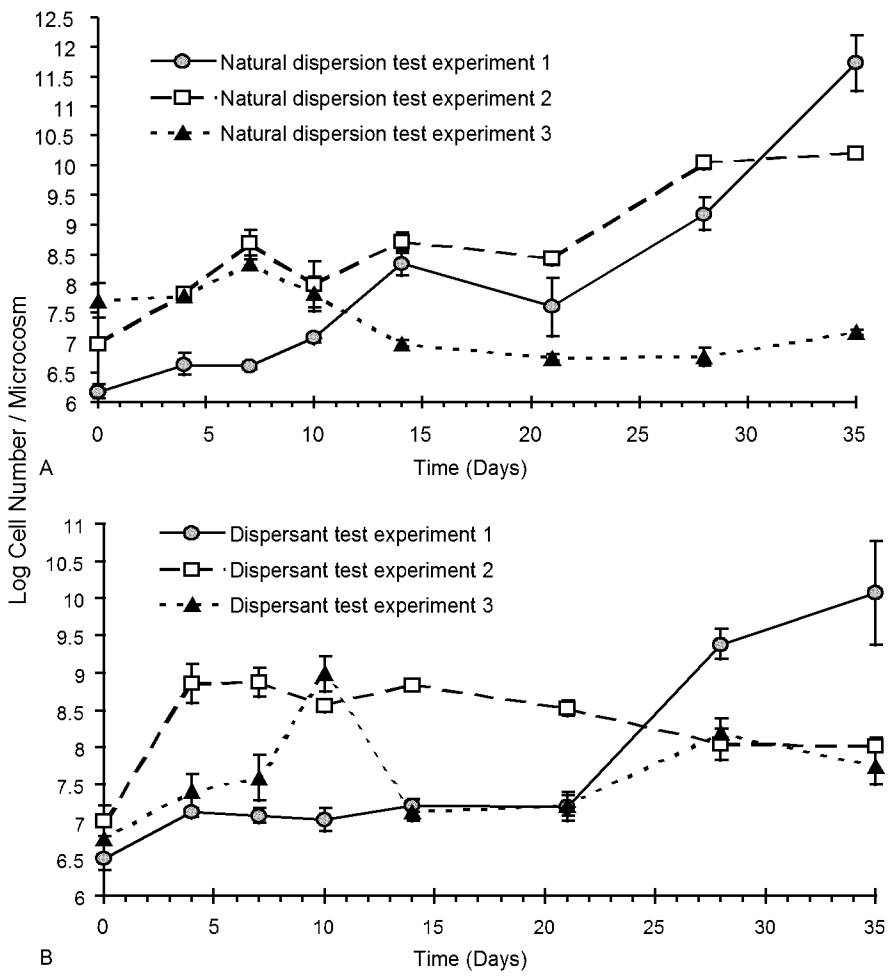

Fig. 8 Hydrocarbon degrading populations in the biologically active ANS study microcosms: (A), natural dispersion experiment; (B), chemical dispersant test (error bars represent the standard deviation).

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dispersion tests (P > 0:05). By comparison, a significant decrease in the ratio of n-alkanes to hopane in chemically dispersed oil compared to killed controls was detected in the Forties crude study (Swannell & Daniel, 1999), indicating biodegradation at 15 C. The loss of hydrocarbons in the killed control was probably the result of evaporation. In the Forties Experiment, the addition of Corexit 9500 resulted in all the oil becoming dispersed and remaining in the water column, thereby reducing additional evaporation. With the ANS, a significant percentage of the oil remained undispersed, potentially increasing the evaporation.

The killed controls from the ANS study did not contain any bacteria as quantified by MPN counts. In both natural and chemical dispersant tests, the MPN counts exhibited a large variation between replicates. For both treatments there was an initial increase in the hydrocarbon-degrading population in all replicates (Fig. 8(A) & (B)). After this initial increase, the hydrocarbon-degrading population ofthe chemical dispersed replicates stabilised over time (Fig. 8(B)) with only replicate 1 exhibiting a significant subsequent increase in MPN counts (an increase ofapproximately 3 orders ofmagnitude by day 35). In replicates 1 and 2 ofthe naturally dispersed microcosms (Fig. 8(A)), however, MPN counts increased over time (by between 3 and 5 orders ofmagnitude by day 35), a pattern that was not repeated in replicate 3. Variation between experiments could be explained by the formation ofclusters. This finding correlated with the lower number ofoil droplets detected in this replicate. Maximum growth rates in the presence ofthe chemical dispersant were approximately one fifth of those observed with Forties crude at 15 C, presumably reflecting slower growth on oil at the lower temperature, an observation made by other researchers (Leahy & Colwell, 1990). However, in the naturally dispersed treatments, microbial growth rates were higher in the ANS samples than had been detected in the Forties study. This result reflected the greater droplet number seen with natural dispersion ofthe ANS at 8 C compared to the Forties at 15 C.

Conclusions

For both Forties crude and ANS oil, the addition of dispersant resulted in increased oil droplet numbers in comparison to natural dispersion. However, the results ofthe killed controls suggested that the oil-in-water emulsion was not as stable with ANS and Corexit 9500 at 8 C as it was with Forties and Corexit 9500 at 15 C. For both oil types, natural processes promoted oil dispersion with microbial colonisation, albeit at lower levels for the Forties crude at 15 C than those observed for the ANS at 8 C. Oil degraders proliferated in all biologically-active microcosms with bacteria colonising the oil droplets forming large bacteria-oil-protozoa-nematode clusters. However, compared to the killed controls, oil biodegradation was confirmed only in the Forties crude study at 15 C.

The results ofthis study suggest that the fundamental microbiological processes that occur after chemical dispersion ofoil in the sea are similar for both Forties and ANS. In both cases, dispersed droplets were colonised within days by bacteria, coalescing to form clusters of droplets and bacteria. This process coincides with an increase in the local numbers ofindigenous hydrocarbon degraders. However, temperature, and possibly oil type, has a large effect on dispersion and microbial growth rate and biodegradation ofoil. It should be remembered that these experiments were conducted in contained microcosms, whereas in open water dispersion also dilutes and disperses the oil and where nutrients may not become limiting.

Given the differences between the experiments, the influence oftemperature on the dispersion ofForties and ANS deserves further investigation, particularly as at higher temperatures the residence time ofdispersed oil in the water column could be significantly shorter. Moreover, these laboratory observations require field evaluation to confirm that they are representative ofthe natural marine ecosystem. Field studies however present a significant challenge to researchers.

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The primary biodegradation of dispersed crude oil in the sea

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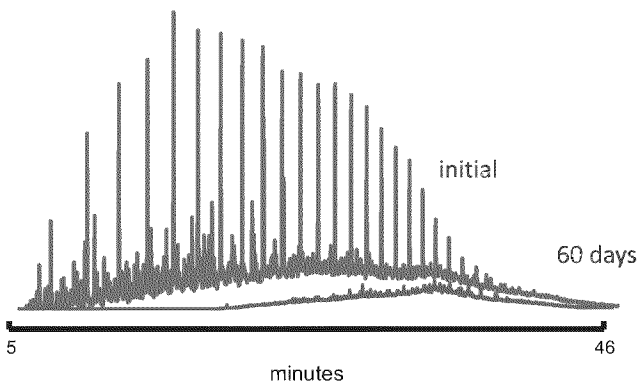
HIGHLIGHTS

- Dispersed crude oil is rapidly and extensively biodegraded in natural seawater.
- This occurs without the need for added nutrients or bacteria.
- This biodegradation extends to all resolvable classes of hydrocarbons except the hopanes.
- The approximate biodegradation half-life is 11–14 d.

GRAPHICAL ABSTRACT



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ABSTRACT

Dispersants are important tools for stimulating the biodegradation of large oil spills. They are essentially a bioremediation tool – aiming to stimulate the natural process of aerobic oil biodegradation by dispersing oil into micron-sized droplets that become so dilute in the water column that the natural levels of biologically available nitrogen, phosphorus and oxygen are sufficient for microbial growth. Many studies demonstrate the efficacy of dispersants in getting oil off the water surface. Here we show that biodegradation of dispersed oil is prompt and extensive when oil is present at the ppm levels expected from a successful application of dispersants – more than 80% of the hydrocarbons of lightly weathered Alaska North Slope crude oil were degraded in 60 d at 8 °C in unamended New Jersey (USA) seawater when the oil was present at 2.5 ppm by volume. The apparent halftime of the biodegradation of the hydrocarbons was 13.8 d in the absence of dispersant, and 11 d in the presence of Corexit 9500 – similar to rates extrapolated from the field in the Deepwater Horizon response.

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1. Introduction

Large amounts of crude oil have been entering the world's oceans for millions of years, and a diverse group of microorganisms has evolved to take advantage of this rich source of reduced carbon (Prince, 2010; Prince et al., 2010). Much of the oil comes from natural seeps, and while some adheres to sediment particles and is degraded on the sea bottom, some rises to the sea surface to form slicks, and some disperses as small droplets in the water column (Farwell et al., 2009). Other oil comes from anthropogenic sources, both as runoff from land and from drilling and shipping accidents (National Research Council, 2003). Oil is an unusual substrate in two main regards. First, only a few molecules such as the smallest saturates (e.g. methane, ethane and propane) and aromatics (e.g. benzene, toluene, ethylbenzene and the xylenes) are significantly soluble – so biodegradation of most oil components must take place at the surface of the oil. Second, while oil provides a rich

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source of carbon and energy, it contains no significant amounts of biologically available nitrogen or phosphorus essential for microbial growth. Together these mean that the biodegradation of significant amounts of oil in aerobic environments is likely to be limited by the available surface area of the oil, and the presence of nutrients. The biodegradation of oil on shorelines has been enhanced by the judicious application of fertilizers to the oil (Bragg et al., 1994; Prince and Bragg, 1997), but adding nutrients to slicks on the open ocean is not usually appropriate in view of the likely competition for uptake by planktonic photoautotrophs. Instead the preferred approach for stimulating the biodegradation of oil slicks and subsea releases is to add chemical dispersants to substantially increase the surface to volume ratio of the oil, and allow the oil to disperse so that the natural background levels of biologically available nitrogen or phosphorus are adequate for microbial growth (National Research Council, 2005). For example, dispersants were used on a large scale following the spill from the *Sea Empress* in South Wales (Lunel et al., 1997), and more recently at the leaking wellhead of the *Deepwater Horizon* blowout in the Gulf of Mexico (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011).

Despite these large-scale uses, and many tests of the effectiveness of dispersants in dispersing crude oil, from laboratory through large tanks, field trials and actual use (e.g. Mukherjee and Wrenn, 2009; Li et al., 2009a,b; Belore et al., 2009; Lewis et al., 2010), few papers have addressed the question of the biodegradation of dispersed oil under environmentally relevant conditions. Oil slicks of light to medium oils spread rapidly until they are only approximately 100 μm or less in thickness (Lehr et al., 2002). Applying dispersant to such a slick in 1-m waves causes nearly immediate mixing of dispersed oil into the top 1–1.5 m of the water column (Delvigne and Sweeney, 1988), resulting in immediate dilution by a factor of about 10000 to give an average oil concentration of ~100 ppm (Belore et al., 2009). The dispersed droplets are so small, typically 10–100 μm in diameter (Li et al., 2011), that they are entrained in the water column as discrete droplets that do not coalesce but diffuse to ever more dilute concentrations. It is technically challenging to do biodegradation experiments at such dilutions, and no published work that we have found (Bruheim et al., 1999; Lindstrom and Braddock, 2002; MacNaughton et al., 2003; Venosa and Holder, 2007; Zahed et al., 2010) comes very close.

We report here the biodegradation of fresh and lightly weathered crude oil (the latter mimicking exposure at sea for 24 h) with and without the dispersant Corexit 9500 (Nalco, 2011), at concentrations approaching those seen following a real spill in natural seawater with no added nutrients or bacteria. This mimics the conditions for natural biodegradation, but at the expense of demonstrating the physical effectiveness of the dispersant – at such concentrations, and in stirred laboratory experiments, Alaska North Slope crude oil disperses quite well with no additions. We show that oil is extensively degraded under such conditions, and that Corexit 9500 exerts no inhibitory effects.

2. Materials and methods

Seawater was collected from the New Jersey shore in April 2010 and January and April 2011 (winter conditions, salinity = ~28 ppt, temperature = ~8 °C). Nitrate and phosphate levels were below detection limits with simple laboratory colorimetric tests, but are likely to have been near 7 and 0.5 μM respectively (Louanchi and Najjar, 2001). The seawater was transferred to a cold room at 8 °C and aerated for 24 h prior to assembly of the experiments in 5 L carboys. Four liters of seawater were placed in each carboy, and were stirred with a large magnetic stirrer to generate a 2 cm vortex. We tried two methods of adding oil to these vessels, with indistinguishable biodegradation results. In the first, Alaska North Slope crude oil was weathered by evaporation at laboratory room temperature in a hood until it had lost 20% of its weight. Corexit 9500 was added at 5% to an aliquot, and 10 μL of the weathered oil or weathered oil plus dispersant was added to each carboy (both in triplicate) with a positive displacement pipette to yield 2.5 ppm oil by volume. As shown below, these experiments revealed no substantial difference in the extensive biodegradation seen after 60 d. In the second pair of experiments, 10 μL of fresh Alaska North Slope crude oil was added to the 4 L batches of seawater without dispersant: the dispersed oil was generated by floating 1 mL of oil on 1 L of seawater in an aspirator vessel. The water was stirred vigorously with a magnetic stir bar to generate a 2 cm vortex, and 67 μL of Corexit 9500 was dropped on the slick with a positive displacement pipette. The oil instantly dispersed throughout the water column, and after 2 min of stirring the stirrer was turned off and the contents allowed to settle for a few minutes. Dispersed oil was drawn off from the bottom of the aspirator bottle, and 10 mL of the dispersed oil/water mixture was added to 4 L of seawater. In this case enough replicate vessels were assembled, in two separate campaigns, that duplicate carboys of oil with and without Corexit could be sacrificed at 0, 4, 6, 11, 24 and 41 d.

At the designated time, oil was extracted from each carboy, three times, with methylene chloride, dried with sodium sulfate, and analyzed by gas chromatography coupled with mass spectrometry (Douglas et al., 1992). Care was taken to prevent complete evaporation of the methylene chloride so as not to lose volatile oil components during extraction. Oil biodegradation was followed with respect to 17α(H),21β(H)-hopane as a conserved internal marker within the oil (Prince et al., 1994). Apparent half-times of the loss of analytes were calculated as before (Prince et al., 2007). Two-dimensional GC followed earlier methods (Wang et al., 2003), but used a flame ionization detector; the first column separated the oil components by boiling point, the second by polarity. This approach separates the oil into eight major classes: 3-ring aromatics, 2-ring aromatic + 1-saturated ring or 5-saturated rings, 2-ring aromatics or 4-saturated rings, 1-ring aromatic + 1-saturated ring or 3-saturated rings, 1-ring aromatic or 2-saturated rings, 1-saturated ring, alkanes, and hopanes.

3. Results

Fig. 1 shows total ion chromatograms of the initial oils and oils extracted after biodegradation with the natural indigenous microbiota and nutrients at 8 °C. The top trace is the original oil before addition to the seawater; the sample with Corexit had this added to the neat oil, although the experiment used oil dispersed by addition of the dispersant to the oil on the water surface, as described above. It is noteworthy that the initial sample with dispersant shows prominent peaks near 28 min that are not seen in the samples extracted from the water phase – these are some of the surfactant molecules (Nalco, 2011) that are not extractable from water with methylene chloride. On the other hand, the features near 14 min can be attributed to the hydrocarbon solvent of the dispersant (Nalco, 2011) – these are extracted from water with our protocol, as shown in the samples labeled Day 0. These samples were extracted within 10 min of the initial assembly of the experimental systems, and it is noteworthy that in this time the samples without dispersant had lost most hydrocarbons smaller than naphthalene. The evaporative loss was much reduced in the dispersed sample, presumably because the hydrocarbons were in the bulk water phase.

The oils were extensively and rapidly degraded, with both the alkanes responsible for the sharp peaks and the unresolved material showing substantial losses. Very similar extensive biodegrada-

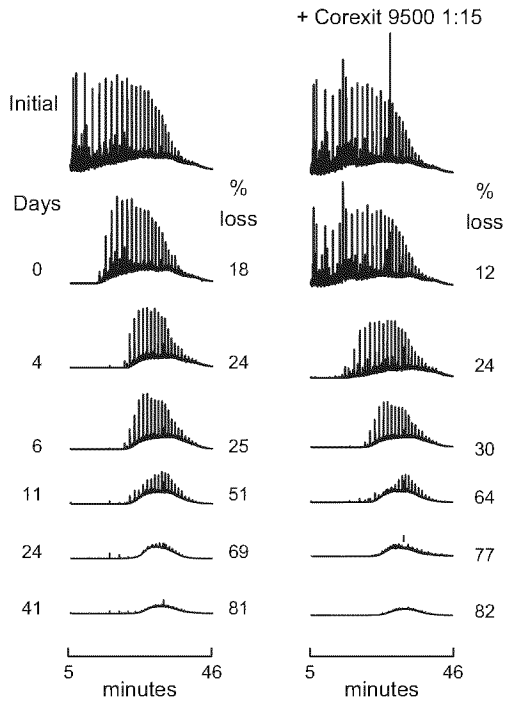

Fig. 1. Total ion chromatograms of fresh crude oil without and with 6.7% Corexit 9500 at the beginning of the incubation, and after the indicated days of stirring in unamended seawater at 8 °C. The 'initial sample' with Corexit is actually a sample of fresh oil plus Corexit, while the experimental samples had the dispersant added to oil floating on water as described in Section 2.

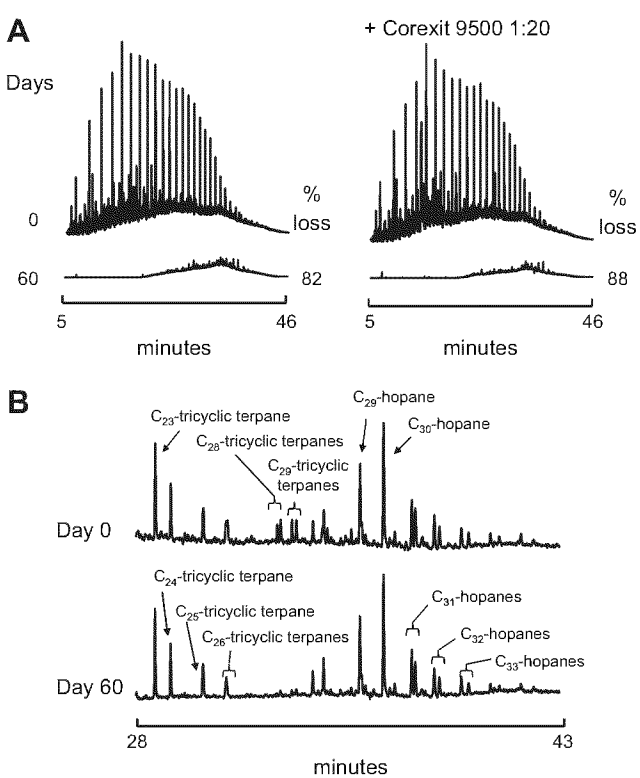

Fig. 2. (A) Total ion chromatograms of artificially weathered crude oil without and with 5% Corexit 9500 at the beginning of the incubation, and after 60 d of stirring in unamended seawater at 8 °C. The Corexit was added to the oil prior to addition to the flask. (B) $m/z = 191$ chromatograms of the initial oil and biodegraded samples with Corexit.

tion was seen when the experiment used 20% weathered crude oil as shown in Fig. 2A. These losses can be quantified using 17α(H),21β(H)-hopane as a conserved internal marker within the oil (Prince et al., 1994). Although hopanes are eventually biodegraded (Prince and Walters, 2007), this process does not appear to have happened in our experiments (Fig. 2B), although there was biodegradation of the C_{28} and C_{29} tricyclic terpanes after 60 d. If 17α(H),21β(H)-hopane had been degraded in our experiments, our estimates of the extent of biodegradation would be underestimates. The biodegradation of representative alkanes and aromatics in the oil are shown in Fig. 3. The essentially complete loss of all the alkylnaphthalenes likely includes some evaporative losses, but the losses of the other aromatics can be principally attributed to biodegradation (Prince and Walters, 2007). Unfortunately GC/MS cannot readily quantify all the individual hydrocarbons of crude oil. The major groups of compounds can, however, be separated using two-dimensional gas chromatography with flame ionization detection, as shown in Fig. 4. Fig. 5 compares the initial oil with samples of weathered oil after 60 d of biodegradation. It is clear that all the resolved components have undergone substantial loss, with the exception of the hopanes. This includes the aromatic compounds with up to at least three rings, the saturated ring compounds with up to at least five rings, multi-ring compounds with both aromatic and saturated rings, and the linear and branched alkanes.

We have enough replicates ($n = 10$) that we can calculate the median apparent half-lives of primary biodegradation of some individual analytes, and these are listed in Table 1. The apparent

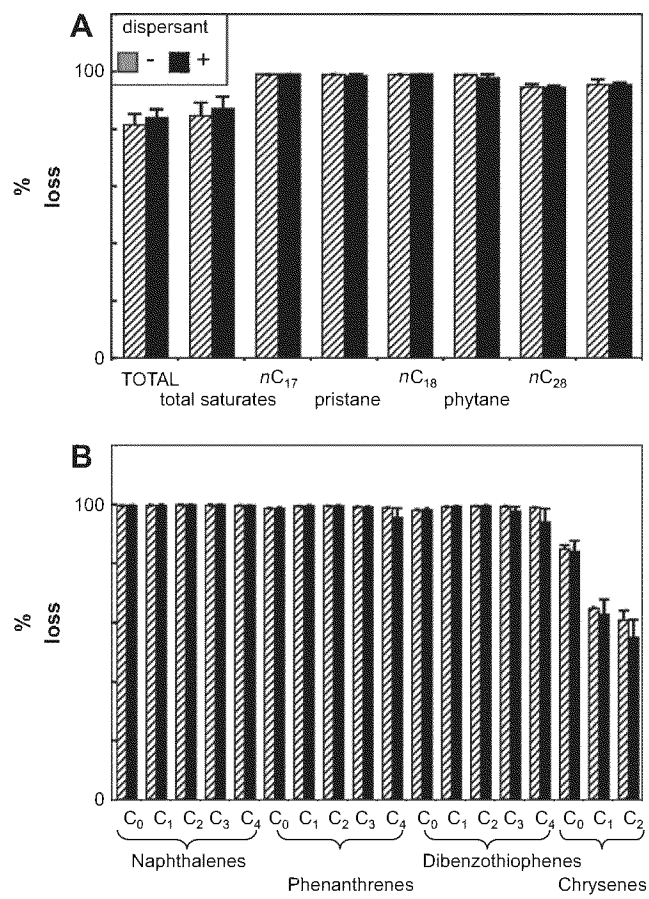

Fig. 3. Loss of (A) total resolved hydrocarbons, total saturates (measured as $m/z = 57$) and representative alkanes, and (B) representative polycyclic aromatic hydrocarbons in the samples of Fig. 2. The C_n nomenclature indicates the number of alkyl carbons on the aromatic nucleus; C_3 for example, includes trimethyl, methylethyl, propyl and isopropyl forms. The error bars are standard errors.

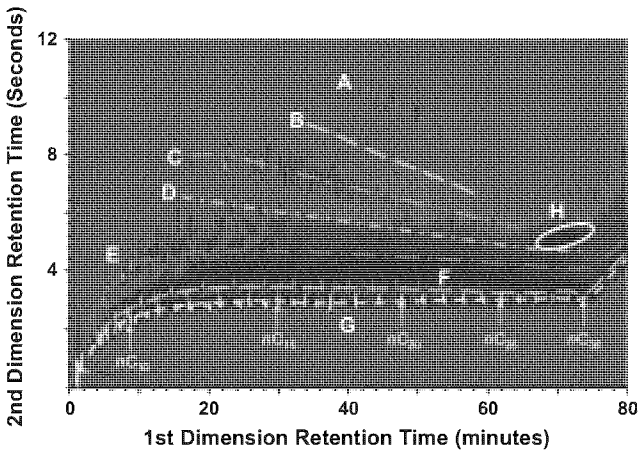

Fig. 4. Two-dimensional GC chromatogram of the initial, partially weathered oil. The first dimension separates by boiling point, the second by polarity. A: 3-ring aromatics series. B: 2- ring aromatic + 1-saturated ring or 5-saturated ring series. C: 2-ring aromatics or 4-saturated ring series. D: 1-ring aromatic + 1-saturated ring or 3-saturated ring series. E: 1-ring aromatic or 2-saturated ring series. F: 1-saturated ring series. G. alkane series – representative n-alkanes are identified. H: hopane series.

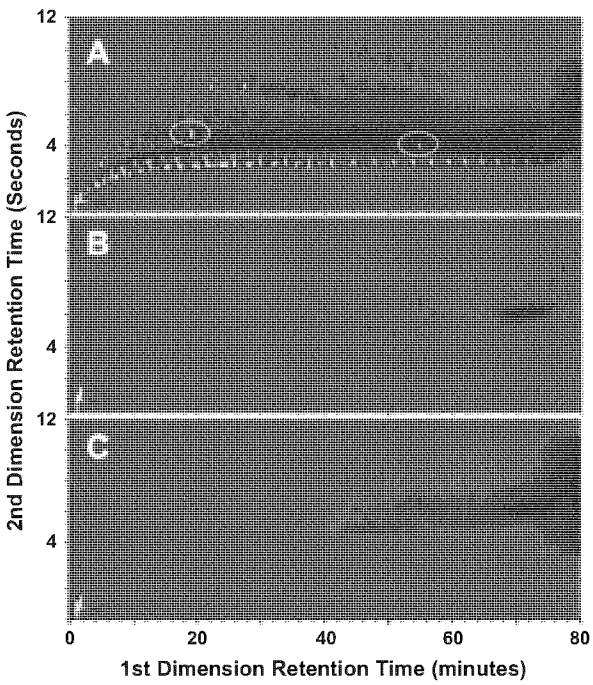

Fig. 5. Two-dimensional GC chromatogram of the initial, partially weathered oil with dispersant (A), and the biodegraded oils with (B) and without (C) dispersant. Components attributable to the dispersant (Nalco, 2011), not present in Fig. 5, are enclosed in ellipses.

half-lives are calculated from the amounts remaining at various times, and they do not include the clear lag-times apparent in Fig. 1. Nevertheless they provide an indication of how rapidly oil can disappear from the environment when it is dispersed at low concentrations. As expected (Douglas et al., 1996), phenanthrenes and dibenzothiophenes were degraded at essentially similar rates, while chrysenes were more resistant to biodegradation, with or without dispersant. It is noteworthy that all the isomers of the alkyl aromatics of Fig. 3 are degraded – 3-methylchrysene is apparently the least degradable methylchrysene, but its degradation was clearly underway at the end of the experiment (Fig. 6). There have been reports that dispersants inhibit the biodegradation of polycyclic aromatic hydrocarbons (Lindstrom and Braddock, 2002) and sulfur containing species such as the dibenzothiophenes (Foght et al., 1983). Neither effect is seen in our experiments.

4. Discussion

Many earlier experiments (e.g. Bruheim et al., 1999; Lindstrom and Braddock, 2002; MacNaughton et al., 2003; Venosa and Holder, 2007; Zahed et al., 2010) used rather higher concentrations of oil and additional nutrients to allow its degradation. A classical medium, used by many, is that of Bushnell and Haas (1941), which provides 25 mM bioavailable nitrogen as a combination of nitrate and ammonium, and 13 mM phosphate. The medium was developed during World War II for studying biodegradation in fuel storage facilities, and it is really quite inappropriate for mimicking marine systems where the levels of nutrients are many orders of magnitude lower. Such high levels might well alter the relative competitive advantage of the microbial populations. Even nearshore Atlantic waters contain only a few micromolar available nitrogen (Levitus et al., 1993; Louanchi and Najjar, 2001; Pelegri et al., 2006; While and Haines, 2010), and less than micromolar phosphate, and most of the world's oceans have only nanomolar concentrations of nutrients (Patey et al., 2010). Clearly these natural levels of nutrients, and the native biota of fresh seawater are adequate for substantial biodegradation of dilute hydrocarbons.

We note that our measurements detect only the hydrocarbons in crude oil, and do not address the potential biodegradability of the asphaltenes and resins (Tissot and Welte, 1984). Furthermore, the disappearance of analytes minimally indicates only the initiation of the biodegradation process – commonly known as primary biodegradation – not their ultimate biological oxidation to water and CO_2. Quite likely much of the carbon is still in the microbial biomass in our experiments – Yerushalmi and Guiot (1998) concluded that the biomass yield of microbes growing on gasoline varied from 0.43 mg/mg to 0.69 mg/mg, and it seems reasonable that these values might be representative of many degradable hydrocarbons. Beolchini et al. (2010) estimated a value of 0.39 mg biomass/mg of hydrocarbons in harbor sediments, which likely contain significant amounts of recalcitrant hydrocarbons since the most readily biodegraded would likely already have been consumed. It has long been recognized that hydrocarbon-degrading organisms incorporate hydrocarbons into cellular lipids (Davis, 1964; Mazzella et al., 2005) and storage products (Klein et al., 2008; Manilla-Pérez et al., 2010; Rontani, 2010). These enter the ocean foodweb, and may eventually give rise to enhanced fisheries (Levy and Lee, 1988).

5. Conclusions

Our experiments demonstrate that even under late winter conditions, in our case a water temperature of 8 °C, the primary biodegradation of oil in seawater can be reasonably rapid, with no additional nutrients, when the concentration of oil is in the few ppm. These are the concentrations likely to occur after a successful application of dispersant (National Research Council, 2005). Recent data from the Gulf of Mexico suggesting the similarly rapid biodegradation of oil dispersed at the sea bottom from the Deepwater Horizon blowout (Hazen et al., 2010), with a lag of several days before biodegradation got underway (Reddy et al., in press), are fully concordant with our findings.

It is important to recognize that the simple comparison of the rate of biodegradation in the presence and absence of dispersant in Table 1 is somewhat misleading in judging the effectiveness of dispersants. As we have shown, the biodegradation of dispersed

Table 1
Initial concentrations and median apparent half-lives of primary biodegradation of some individual analytes. The concentrations assume full solubility in the total volume.

	Initial concentration (ppb)	Median apparent half-life (d)	Median apparent half-life with dispersant (d)
Total detectable hydrocarbons		13.8	11.0
Total saturates ($m/z = 57$)		15.6	11.6
17α(H),21β(H)-hopane	0.48		
Heptadecane	9.24	1.9	1.8
Pristane	4.87	4.7	3.4
Octadecane	5.48	2.5	2.2
Phytane	3.36	5.3	3.8
Triacontane	1.85	13.1	7.8
Naphthalene	1.35	2.3	1.9
C_1-naphthalenes	2.96	2.0	1.7
C_2-naphthalenes	3.73	1.9	1.7
C_3-naphthalenes	2.88	1.9	1.8
C_4-naphthalenes	1.46	2.1	1.9
Fluorene	0.20	3.1	2.6
Phenanthrene	0.56	2.6	2.2
1-Methylphenanthrene	0.36	2.8	2.4
2-Methylphenanthrene	0.27	2.9	2.5
3-Methylphenanthrene	0.22	3.0	2.6
9-Methylphenanthrene	0.41	3.5	2.5
C_2-phenanthrenes	1.48	7.9	9.7
C_3-phenanthrenes	1.05	18.9	22.4
C_4-phenanthrenes	0.63	28.8	35.7
Dibenzothiophene	0.49	2.7	2.4
1-Methyldibenzothiophene	0.17	3.7	4.5
2/3-Methyldibenzothiophenes	0.27	3.0	3.5
4-Methyldibenzothiophene	0.51	2.8	3.5
C_2-dibenzothiophenes	1.43	7.8	7.6
C_3-dihenzothiophenes	1.26	19.5	21.2
C_4-dibenzothiophenes	0.69	33.3	35.9
Benz[a]anthracene	0.03	26.6	17.8
Chrysene	0.10	30.8	36.2
1-Methylchrysene	0.02	14.3	14.3
2-Methylchrysene	0.02	6.3	11.2
3-Methylchrysene	0.11	27.1	36.5
4/6-Methylchrysenes	0.02	11.3	12.4
C_2-chrysenes	0.25	28.1	28.5
Sum 16 EPA priority pollutants	2.24	4.5	4.0

The C_n nomenclature indicates the number of alkyl carbons on the aromatic nucleus; C_3 for example, includes trimethyl, methyl-ethyl, propyl and isopropyl forms. 2- and 3-methyldibenzothiphenes co-elute, as do 4- and 6-methylchrysenes. 5-methylchrysene is below detection. Most of the EPA priority pollutants (Keith and Telliard, 1979) are below detection limits in our experiments; the predominant components are naphthalene and phenanthrene.

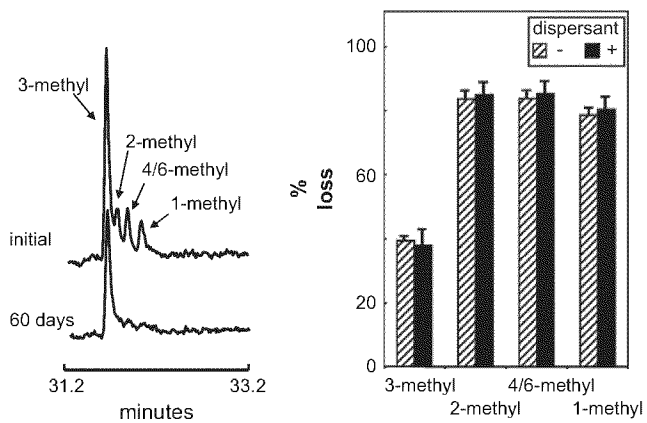

Fig. 6. Loss of methyl chrysenes. The chromatograms are of $m/z = 242$. 4-and 6-methylchrysenes co-elute under our conditions.

oil at the few ppm level is not significantly stimulated by dispersant addition. But this misses the point that dispersants are only used for significant spills – to remove slicks from the water surface, and prevent the formation of water-in-oil mousses and the stranding of oil on shorelines. The rate of biodegradation of heavy concentrations of undispersed oil is much slower than seen here, limited by surface area and nutrient availability, and likely also by oxygen access. The role of dispersants is not to stimulate the biodegradation of dispersed oil, but to allow the dispersion. We show here that the biodegradation of dispersed oil is essentially unaffected and certainly not inhibited by the presence of dispersants, and is rapid and extensive.

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Biodegradation of Non-Ionic Dispersants in Sea-Water

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Summary. In this paper, we describe the aerobic biodegradation of some non-ionic dispersants of the Span, Tween, and Corexit series in sea-water, where they are now more frequently found as a result of their application to the removal of oil spills. First, the extent to which dispersants are biodegraded, as an indication of their suitability for use on a large scale, is discussed. Biodegradation may be carried out by means of monocultures or mixed cultures of marine bacteria of the genera *Aeromonas, Pseudomonas,* and *Flavobacterium.* Analytical techniques based on absorbance measurements were used to follow the process. On the other hand, by determining the kinetics of the biodegradation process a more complete analysis is obtained. The kinetic coefficients controlling the process are deduced and it is shown that for some dispersants the experimental results are in close agreement with the proposed scheme. Where observed values are explained on the basis of ethylene oxides content of the dispersants, estimations of the amount of dispersant present in the sea at a given time can be made, if the amount of the dispersant first used is known.

Introduction

The continous impoverishment in oxygen both in waste and marine waters was soon related to industrial progress and the birth of detergents. The presence of detergents in the sea has been greatly increased due to their use in the treatment of oil spills. In order to carry out this treatment on a large scale with dispersants it is necessary to find out previously how they will affect the medium. Thus it is quite usual to carry out a study on the biodegradation of dispersants, which will allow a prediction of their level in the sea at any time.

Garret (1967) has pointed out the presence in the sea of surfactants and their residual products; the latter result, possibly, from the metabolization of the hydrophobic part of these compounds (Fischer and Gerike 1975).

Non-ionic dispersants have replaced ionic in certain fields, because of advantages such as their compatibility with many substances that react with and inactivate anionic and cationic agents (Cruickshank 1968; Bourrel et al. 1979), although they have not been greatly applied to the treatment of oil spills. Among the non-ionic dispersants an important group is the polyoxyethylene fatty acid esters, which result from the reaction of fatty acids C_{12} to C_{18} with sorbitol and a later addition of ethylene oxides. Due to the wide structural variety shown by this group of substances their study is very complex and although some authors (Fincher and Payne 1962) have related their biodegradation to the content of ethylene oxides, others disagree (Schick 1967).

In order to follow the biodegradation process with a certain facility compounds with oleic acid, thus having double bonds C=C, were first chosen. The theoretical degradation scheme of these compounds was discussed in a previous paper of this department (Bao and Bergueiro 1977). Our study was later extended to other surface active agents of the same or equivalent series.

The micro-organisms used were selected on the basis of reports by many authors who have carried out degradation of dispersants with cultures of *Pseudomonas* and *Flavobacterium* genera (Goodnow and Harrison 1972; Hsu 1965; Atlas and Bartha 1972). It is well known that micro-organisms adapt themselves to the medium, so that by isolating them in polluted areas, satisfactory results can be obtained. Thus for this investigation *Flavobacterium* and *Pseudomonas* together with *Aeromonas* genera were isolated from

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sea-water of the Galicia Estuaries after an oil spill.

As a further step in the work reported in this paper, the simultaneous biodegradation of the dispersants and petroleum in sea-water will be carried out.

Materials and Methods

Micro-organisms. Flavobacterium pictorum, Pseudomonas sp., and *Aeromonas sp.*, isolated from Galicia Estuaries, were used throughout this study. The first two belong to genera which easily degrade dispersants and the third one has been chosen because of its great abundance in the place where the samples were collected.

Substrate. The following non-ionic dispersants were used in sea-water in the following concentrations:
Span 65 = Sorbitan tristearate – 1 g/l.
Span 80 = Sorbitan monooleate – 5 g/l.
Tween 20 = Polyoxyethylen (20) sorbitan monolaurate – 5 g/l.
Tween 80 = Polyoxyethylen (20) sorbitan monooleate – 2 g/l.
Tween 85 = Polyoxyethylen (20) sorbitan trioleate – 1 g/l.
Corexit 7664 = Polyoxyethylen (13.5) glycerol trioleate – 2 g/l.

We have used a range of high concentrations because we think that there may be similar cases to those found in Galicia Estuaries (where the samples of water and micro-organisms have been taken) in which the concentrations of dispersants after an oil spill are higher than average. This can be explained as a result of the slow renewal and movement of water in the absence of naturally generated turbulence.

The dispersants of Span and Tween series, developed by Atlas Powder Co., were supplied by Comercial Química Massó (Barcelona, Spain). The Corexit was supplied by Esso Research and Engineering Co.

Sea-water from Galicia Estuaries was autoclaved (at 121° C for 15 min) before use.

Process Conditions. The pH is maintained between 7.5–7.8, a quite normal value in Galicia Estuaries. The bacteria are cultivated in a relatively poor PYM medium (1 g Peptone, 0.5 g yeast extract, 800 cm^3 sea-water, 200 cm^3 distilled water) keeping the flasks in a rotary shaker at 30° C until high growth occurred (30 h), and the viable cell count had risen to 10^9/ml. This culture was used to inoculate (in a quantity of 0.1 ml) the solutions of dispersants in sea-water; test tubes of 2.5 cm diameter filled up to one third of their capacity (30 ml) were used. Three test tubes were prepared for each measurement of absorbance: one with dispersant and inocule (experimental), other without dispersant and with inocule (blank) and a third one with dispersant and sterile medium (standard). The set of test tubes was incubated at 15° C and 150 rpm for a maximum of 25 days, during which measurements of absorbance are taken in a psychroterm incubator (New Brunswick Sci. Co). The samples were centrifugated at 5,000 rpm for 30 min and their degradation was determined by analytical techniques.

Analytical Methods. The APHA (American Public Health Association) method was used to determine the chemical oxygen demand (COD) whereas the API (American Petroleum Institute) method modified (Núñez 1982) was used to control the biochemical oxygen demand (BOD).

To follow biodegradation two methods were utilized:

Method 1. This method is based on the absorbance shown by the C=C group at 235 nm. The determined concentration corresponds to compounds with double bonds present in the dispersant and in its biodegradation products. In our case it is only valid for dispersants containing oleic acid.

Method 2. This method proposed by Clanet and Viller (1976) is based on the formation of a coloured complex with the salt of a heteropolyacid, such as ammonium cobaltothiocyanate, which is extracted from benzene. Its absorbance is measured at 320 nm (Pye Unicam 1700 Spectrophotometer).

This method is limited because the loss of the dispersant property and not necessarily the full degradation is measured. Our interest is, however, precisely the biodegradation which leads to the loss of that dispersant property, and thus the method is suitable.

Results and Discussion

A first group of dispersants has been chosen on the basis of their content in oleic acid, each one of which contains a different amount of ethylene oxides: Span 80 (0 mol), Tween 80 (20 mol), and Corexit 7664 (13.5 mol).

On the other hand, to select a second group of dispersants, the BOD and COD of a large number of compounds of the Span and Tween series were determined and we chose Span 65, Tween 20, and Tween 85 because they showed the lowest oxygen demand.

The selected micro-organisms were first tested in pure and mixed cultures on Tween 80, the *Aeromonas sp.* being found to be the most effective (Fig. 1). Thus it was used in the experiments with the other dispersants. The *Aeromonas* genus, although not usually studied as a degradator of surface-active agents, was found in large amounts in the place where the samples were collected and thus its high degrading capacity did not surprise us.

The results are summarized in Figs. 1–5. Figures 1–3 were obtained by following the biodegradation process using the analytical Method 1 applicable only to surface active agents that contain oleic acid. Figures 4 and 5 were obtained by applying the analytical Method 2 to all the tested dispersants. It can be assumed that all final degradation values (which are the same for each compounds, irrespective of the analytical method used) indicating that the degradation is in direct relation to the number of ethylene oxides of the molecule.

It must be pointed out that all figures are similar in showing a close resemblance to graphs of bacterial growth, indicating that this and biodegradation take place in a parallel way (Van der Kooij et al. 1982). In each figure a minimum degradation value is observed which arises at different times according to the genus of the bacterium; in some cases biodegradation

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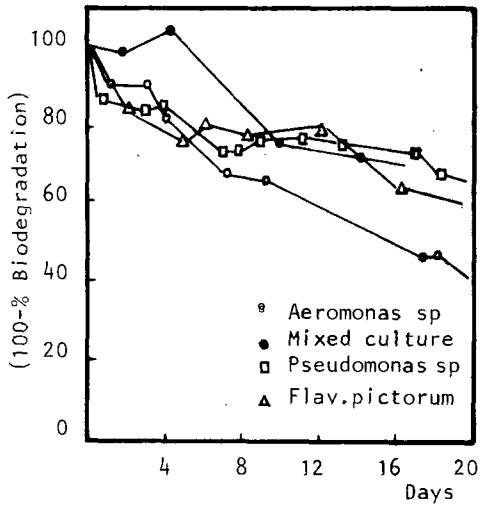


Fig. 1. Degradation of Tween 80 (Method 1) by means of pure cultures of *Aeromonas sp, Pseudomonas sp, Flavobacterium pictorum* and a mixed culture of these micro-organisms

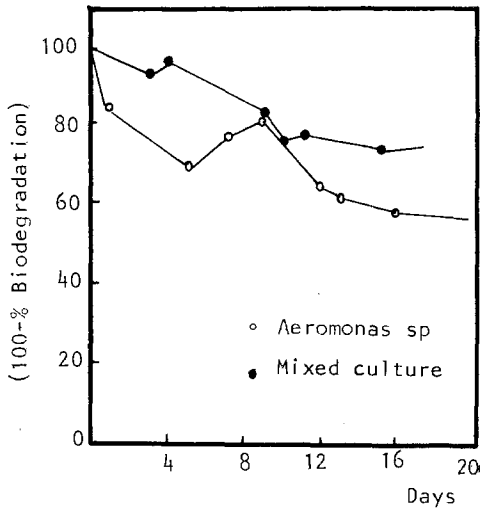


Fig. 2. Degradation kinetics of Corexit 7664 (Method 1) by means of a pure culture of *Aeromonas sp* and a mixed culture of *Aeromonas sp, Pseudomonas sp,* and *Flavobacterium pictorum*

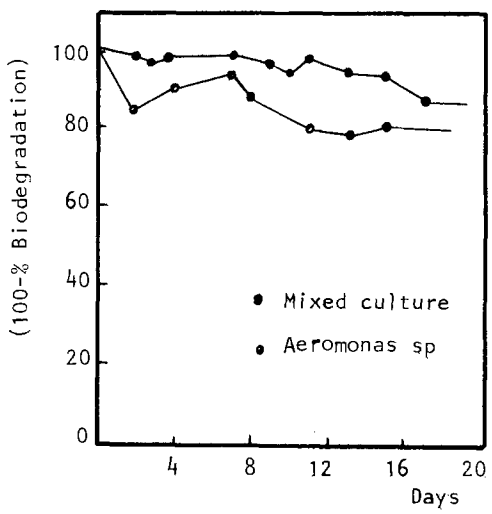


Fig. 3. Degradation kinetics of Span 80 (Method 1) by means of a pure culture of *Aeromonas sp* and a mixed culture of *Aeromonas sp, Pseudomonas sp*, and *Flavobacterium pictorum*

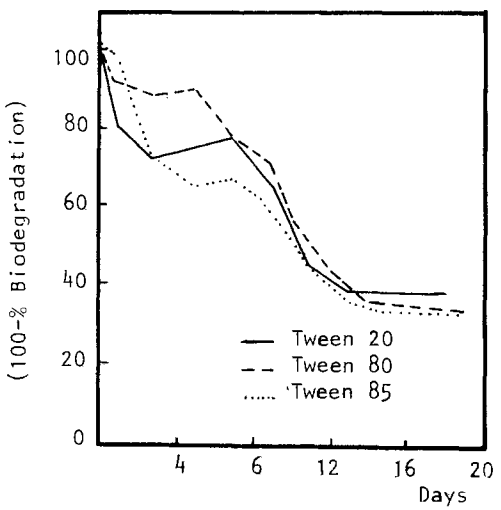


Fig. 4. Degradation kinetics (Method 2) of Tween 20, Tween 80, and Tween 85, by means of a pure culture of *Aeromonas sp*

appears to be reversed, and this can be interpreted as a release from the bacterial membrane of compounds analogous to the one being determined (Socatch 1969). From days 12 to 16 the degradation is almost constant, which indicates that bacterial activity tends to cease.

From Figs. 1–3 it is seen that the mixed culture does not have any advantage over pure cultures for dispersant biodegradation, an observation made previously by other several authors (Soli and Bens 1973). The Span 80 (Fig. 3) shows a very low degradation, which may either be because its concentration is close to the toxic level for bacteria, or because it has an excess in free oleic acid, a factor that has caused bacterial inhibition in similar cases (Rose et al. 1966).

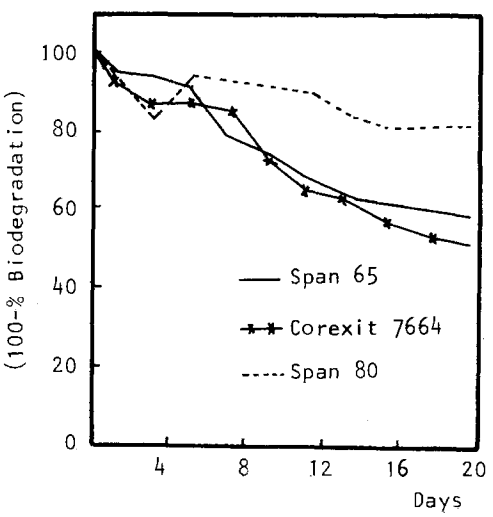
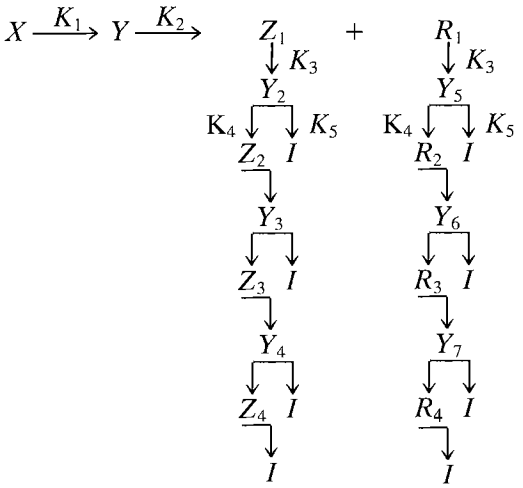


Fig. 5. Degradation kinetics (Method 2) of Span 65, Span 80, and Corexit 7664 by means of a pure culture of *Aeromonas sp.*

The results of degradation by *Aeromonas sp.* of the Span 80, Tween 80 and Corexit 7664, all containing oleic acid, have been compared and with the aim of elucidating the actual process, we set up a kinetic model based on β-oxidation theory (Stumpf 1969) on the following scheme:



where X represents each one of the dispersants containing oleic acid, Y is this acid, and Z_1 and R_1 are the nonanoic and azelaic acids, respectively. The compounds designated by Y are similar to oleic acid, that is, they contain C=C, while Z and R designate compounds with no C=C obtained in the different degradation steps. I is Ac-CoA (acetyl-Coenzyme A) which forms part of the respiratory chain of the bacteria.

Taking into account that reactions $Z \rightarrow Y$, $R \rightarrow Y$ are similar whatever their subindex may be, and considering that the kinetic coefficients for parallel reactions are also very similar, the number of significant coefficients to represent the process is limited to those shown in the scheme.

From the experimental results and using the model equations the kinetic coefficients are calculated. This is carried out by means of a technique (algorithm of the gradient) previously used in our department (Girondo 1975) based on the assumption that the deviation between the experimental value and the corresponding theoretical one is reduced to the minimum. From an assumed value of the coefficients, the algorithm improves the values by iterations according to the least square method in the direction of the maximum gradient. The results were obtained by means of a Fortran V computer program.

The values of the coefficients, summarized in Table 1, show a substantial agreement with the proposed scheme, where it is clear that the coefficients K_4 and K_5 must be equal since they belong to the same reaction. Thus, the values of each one of the kinetic rate coefficients K_3, K_4, and K_5 remain constant for the three tested dispersants, with K_4 almost equal to K_5.

Table 1. Results of the proposed degradative scheme

Dispersant	Span 80	Corexit 7664	Tween 80
Number of moles of E. O.	0	13.5	20
Kinetic coefficients (days^{-1})			
K_1	2.100	1.880	0.570
K_2	0.100	0.670	2.860
K_3	0.012	0.010	0.009
K_4	0.035	0.030	0.030
K_5	0.035	0.030	0.030

The kinetic coefficient K_1, which controls the step:

$$\underset{(X)}{\text{Dispersant}} \xrightarrow{K_1} \underset{(Y)}{\text{oleic acid}}$$

takes values completely different for the three dispersants, these values being higher the smaller the number of ethylene oxides. This can be explained as follows:

1. The enzyme is excited in the presence of polar points (Kieslich 1975).
2. The enyzme may go to the acid group and their interaction can be difficult due to steric hindrance in the presence of ethylene oxides.
3. It could happen that due to the formation of micelles, the concentration of substances containing ethylene oxides decreases.

The rate constant K_2, which controls the step:

$$\underset{(Y)}{\text{Oleic acid}} \xrightarrow{K_2} \underset{(Z_1)}{\text{nonanoic acid}} + \underset{(R_1)}{\text{azelaic acid}}$$

takes different values that are inverted with regard to the previous step and which are increased when the number of moles of ethylene oxides rises.

The most plausible explanation is that the enzyme takes an associated remainder that acts as an enzymatic activator. When the structures of dispersants are compared, it is understood that the activator derives from the ethylene oxide group. The remainder must be released in further steps and this release will explain the fact that the following kinetic coefficients have the same value.

From these results the amount of dispersant present in the sea at any given time can be predicted.

Acknowledgments. We would like to thank the Department of Microbiology of the Faculty of Pharmacy (Santiago de Compostela) for having supplied the micro-organisms and for the use of

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their equipment. We are very grateful indeed to Dr. Juan Luis Barja Pérez, a member of the staff of that department, for his continuous help. We are also grateful to Ldo. E. Patiño for his advice in the preparation of the manuscript in English.

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Received December 29, 1982

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To: Sprenger, Mark[Sprenger.Mark@epa.gov]; Conmy, Robyn[Conmy.Robyn@epa.gov]; Barron, Mace[Barron.Mace@epa.gov]
Cc: Principe, Vanessa[Principe.Vanessa@epa.gov]; Field, Chris[Field.Chris@epa.gov]; Matthiessen, Craig[Matthiessen.Craig@epa.gov]; Greenberg, Marc[Greenberg.Marc@epa.gov]; Runge, Roberta[Runge.Roberta@epa.gov]; Combes, Marcia[Combes.Marcia@epa.gov]
From: Wilson, Gregory
Sent: Wed 1/21/2015 4:32:27 PM
Subject: FW: State-of-Science for dispersant use in Arctic waters OC
2015.01.15_Degradation and Fate Finalpck.docx
2015.01.14_Efficiency and Effectiveness Final pck.docx
2015.01.15_Physical Transport and Chemical Behavior Finalpck.docx
2015.01.15_Public Health and Food Security_pck.docx
2015.01.15_Toxicity and Sublethal Impacts Finalpck.docx

All –

Attached are summary documents from each session of the State-of-Science for dispersant use in Arctic waters workshop that was held in Seattle. You will get the document for your individual session through Nancy/Kathy (CRRC), but we wanted to make sure you had copies of the initial drafts from all the sessions that we received as the organizing committee in case you had questions. While you will be requested feedback from the session(s) you participated in, you may want to contact your fellow EPA participant(s) if you have questions or comments on the other sessions so that they may consider that feedback.

Once again, we would like to thank everyone who participated and attended the sessions. We appreciate all your hard work!

Greg

From: Mandsager, Kathy [mailto:kathy.mandsager@unh.edu]
Sent: Thursday, January 15, 2015 9:49 AM
To: 'doug.helton@noaa.gov'; 'gary.shigenaka@noaa.gov'; 'philip_johnson@ios.doi.gov'; Runge, Roberta; 'lholland-bartels@usgs.gov'; 'stacey.l.crecy@uscg.mil'; 'mark.everett@uscg.mil'; 'kristin.ryan@alaska.gov'; 'mark.swanson@pwsrcac.org'; saupe@circac.org; 'lmajors@alaskacleanseas.org'; nancy.kinner@unh.edu; Kinner, Peter
Cc: Wilson, Gregory; Principe, Vanessa
Subject: RE: State-of-Science for dispersant use in Arctic waters OC

And here are the documents for your review for today's call.

From: Mandsager, Kathy
Sent: Wednesday, January 14, 2015 2:30 PM
To: 'doug.helton@noaa.gov'; 'gary.shigenaka@noaa.gov'; 'philip_johnson@ios.doi.gov'; 'runge.roberta@epa.gov'; 'lholland-bartels@usgs.gov'; 'stacey.l.crecy@uscg.mil'; 'mark.everett@uscg.mil'; 'kristin.ryan@alaska.gov'; 'mark.swanson@pwsrcac.org'; 'saupe@circac.org'; 'lmajors@alaskacleanseas.org'; Kinner, Nancy; Kinner, Peter
Cc: 'Wilson, Gregory'; 'Principe, Vanessa'
Subject: RE: State-of-Science for dispersant use in Arctic waters OC

Mark your calendar for our call tomorrow: Thursday 15 January at 3:00 pm EST.

Dial 866.906.9888 pin: 9606919

Documents for your review forthcoming.

From: Mandsager, Kathy
Sent: Tuesday, January 13, 2015 1:35 PM
To: 'doug.helton@noaa.gov'; 'gary.shigenaka@noaa.gov'; 'philip_johnson@ios.doi.gov'; 'runge.roberta@epa.gov'; 'lholland-bartels@usgs.gov'; 'stacey.l.crecy@uscg.mil'; 'mark.everett@uscg.mil'; 'kristin.ryan@alaska.gov'; 'mark.swanson@pwsrcac.org'; 'saupe@circac.org'; 'lmajors@alaskacleanseas.org'; Kinner, Nancy; Kinner, Peter; Mandsager, Kathy
Cc: 'Wilson, Gregory'; 'Principe, Vanessa'
Subject: State-of-Science for dispersant use in Arctic waters OC

Dear Organizing Committee members:

We wish to thank you for all your efforts in making last week's workshop such a great success!

We will have a draft of the outcomes for your review later this week, but in the meantime, I would like to schedule a conference call as follow-up from our meetings last week and to lay out our next steps. Please kindly let me know your availability for these times for a 1-hour conference call, with the understanding that we may not be able to get all of you together.

Thursday 1/15 at 3:00 pm ET

Friday 1/16 at 11:00 am ET

Friday 1/16 at 1:00 pm ET

Kathy Mandsager

Program Coordinator

Coastal Response Research Center

Center for Spills in the Environment

234 Gregg Hall, Colovos Rd

University of New Hampshire

Durham, NH 03824

603.862.1545

To: 'doug.helton@noaa.gov'[doug.helton@noaa.gov]; 'gary.shigenaka@noaa.gov'[gary.shigenaka@noaa.gov]; 'philip_johnson@ios.doi.gov'[philip_johnson@ios.doi.gov]; Runge, Roberta[Runge.Roberta@epa.gov]; 'lholland-bartels@usgs.gov'[lholland-bartels@usgs.gov]; 'stacey.l.crecy@uscg.mil'[stacey.l.crecy@uscg.mil]; 'mark.everett@uscg.mil'[mark.everett@uscg.mil]; 'kristin.ryan@alaska.gov'[kristin.ryan@alaska.gov]; 'mark.swanson@pwsrcac.org'[mark.swanson@pwsrcac.org]; saupe@circac.org[saupe@circac.org]; 'lmajors@alaskacleanseas.org'[lmajors@alaskacleanseas.org]; nancy.kinner@unh.edu[nancy.kinner@unh.edu]; Kinner, Peter[Peter.Kinner@unh.edu]
Cc: Wilson, Gregory[Wilson.Gregory@epa.gov]; Principe, Vanessa[Principe.Vanessa@epa.gov]
From: Mandsager, Kathy
Sent: Thur 1/15/2015 2:48:46 PM
Subject: RE: State-of-Science for dispersant use in Arctic waters OC
2015.01.15_Degradation and Fate Finalpck.docx
2015.01.14_Efficiency and Effectiveness Final pck.docx
2015.01.15_Physical Transport and Chemical Behavior Finalpck.docx
2015.01.15_Public Health and Food Security_pck.docx
2015.01.15_Toxicity and Sublethal Impacts Finalpck.docx

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Sent: Wednesday, January 14, 2015 2:30 PM
To: 'doug.helton@noaa.gov'; 'gary.shigenaka@noaa.gov'; 'philip_johnson@ios.doi.gov'; 'runge.roberta@epa.gov'; 'lholland-bartels@usgs.gov'; 'stacey.l.crecy@uscg.mil'; 'mark.everett@uscg.mil'; 'kristin.ryan@alaska.gov'; 'mark.swanson@pwsrcac.org'; 'saupe@circac.org'; 'lmajors@alaskacleanseas.org'; Kinner, Nancy; Kinner, Peter
Cc: 'Wilson, Gregory'; 'Principe, Vanessa'
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Cc: 'Wilson, Gregory'; 'Principe, Vanessa'

Subject: State-of-Science for dispersant use in Arctic waters OC

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Kathy Mandsager

Program Coordinator

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Cc: doug.helton@noaa.gov[doug.helton@noaa.gov]; Gary Shigenaka - NOAA Federal[gary.shigenaka@noaa.gov]; Principe, Vanessa[Principe.Vanessa@epa.gov]; Wilson, Gregory[Wilson.Gregory@epa.gov]; Abigail McEachern[acm2005@wildcats.unh.edu]; nancy.kinner@unh.edu[nancy.kinner@unh.edu]
From: Mandsager, Kathy
Sent: Thur 9/1/2016 2:14:53 PM
Subject: RE: Transport & Behavior Panel - NEXT STEPS
Weingartner et al. 2013,Hydrographic variability over the northeastern Chukchi Sea shelf in summer-fall 2008–2010.pdf
xHORNER-DEVINE_annurev-fluid-010313-141408.pdf
xMcPhee & Stanton 1996 Turbulence in the statically unstable oceanic boundary layer under Arctic leads (1).pdf
xMcPhee et al. 2008 Revisiting heat and salt exchange at the ice-ocean interface- Ocean flux and modeling considerations (1).pdf
xNGHIEM_grl51361.pdf
2016 08 16c_state-of-science on Physical Transport and Chemical Behavior (key adjustments).docx

Several important things are included in this email:

1. Per our last meeting, several papers were identified for your review. Once again, I am attaching the papers (including Weingartner et al 2013) so you will be prepared to discuss on our next call. This refers to Section I. A. #2 of our document.

2. I would also like confirm our next meetings. Our goal is to complete this by December! So…

TH 9/29; 2-4 pm ET

WE 10/5; 3-5 pm ET

WE 11/9; 3-5 pm ET

WE 11/16; 3-5 pm ET (if needed)

(Please delete the hold for 12/5; 2-4 ET, mentioned below.)

I will send these all to you via Outlook calendar as well, including the WebEx instructions.

Thank you everyone!

From: Mandsager, Kathy
Sent: Tuesday, August 30, 2016 11:34 AM
Subject: RE: Transport & Behavior Panel - NEXT STEPS

Please submit your availability on each of the doodle polls below. Thanks.

From: Mandsager, Kathy
Sent: Wednesday, August 24, 2016 3:57 PM
Subject: Transport & Behavior Panel - NEXT STEPS

Again, thank you for moving forward on this document and reviewing the public input. I will send our draft as soon as possible. For now, I am attaching a number of papers for your review prior to our next call.

So that brings us to the next step of arranging a few more calls. I would like to confirm a monthly call (we can always cancel if not needed). So please take a moment and fill in each of these doodle polls to secure meeting dates. They are time zone enabled for your convenience and if you could submit these by Monday 8/29 that would be greatly appreciated.

September meeting date: http://doodle.com/poll/fd6aw9waqbf7uxg5

October meeting date: http://doodle.com/poll/agwry9n3m882mvdw

November meeting date: http://doodle.com/poll/tnbsnuydfpgc524k

Please also save **December 5; 2- 4 ET** for a final wrap-up meeting if needed.

Kathy Mandsager

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JOURNAL OF GEOPHYSICAL RESEARCH, VOL. 101, NO. C3, PAGES 6409–6428, MARCH 15, 1996

Turbulence in the statically unstable oceanic boundary layer under Arctic leads

Miles G. McPhee
McPhee Research Company, Naches, Washington

Timothy P. Stanton
Naval Postgraduate School, Monterey, California

Abstract. Measurements of turbulent stress, heat flux, salinity flux, and turbulent kinetic energy (TKE) dissipation were made in the oceanic boundary layer under freezing leads during the 1992 Lead Experiment project in the Arctic Ocean north of Alaska. Results from two instrument systems, one comprising a vertical array of four turbulence-measuring instrument clusters, the other an automated, loose-tethered microstructure profiler, show that forcing by modest surface fluxes (surface friction velocity $u_{*0} \sim 0.7$ cm s^{-1}, surface buoyancy flux $\langle w'b' \rangle_0 \sim -0.7 \times 10^{-7}$ W kg^{-1}) substantially changes the scales and character of boundary layer turbulence relative to forcing by stress alone. Despite continuous freezing at the surface a diurnal cycle of heating and cooling of the mixed layer was seen, with downward oceanic heat flux as high as 70 W m^{-2} observed at middepth in the mixed layer near solar noon. Heat flux was determined both by direct eddy covariance of temperature and vertical velocity at fixed levels and from TKE and thermal dissipation estimates from the profiling instrument, with reasonable agreement. Similarly, there was close correspondence between TKE dissipation estimates obtained from inertial subrange spectral levels at the fixed instruments and from microstructure shear profiles. TKE production was dominated by buoyancy flux through most of the boundary layer. Thermal and saline eddy diffusivities were computed from directly measured fluxes and mixed layer temperature and salinity gradients, with mean values of 0.046 and 0.049 m^2 s^{-1} for temperature and salinity, respectively. Kolmogorov constants for relating thermal and saline dissipations to inertial subrange spectral levels were found to be 0.9 and 1.0, respectively, but with large scatter.

1. Introduction

During the 1992 Lead Experiment (LEADEX) in the perennial sea ice pack north of Alaska, we deployed complementary instrumentation at the edges of active (rapidly freezing) leads. Our goal was to relate direct measurement of turbulent fluxes of momentum, heat, and salt to mean flow properties and to microstructure dissipation and flux estimates. The work was motivated by two factors. First, leads affect the thermohaline structure of the upper Arctic Ocean much more than their relative surface area might suggest, because they provide a direct window to the ocean through an otherwise insulating and highly reflective sea ice/snow cover. In the leads, heat absorbed from solar radiation and salt released by rapid freezing are mixed downward by turbulence. At low temperatures the thermal expansion coefficient for seawater is small and density variations are controlled mostly by salinity variations, hence buoyancy flux at the surface of active leads is nearly always destabilizing [*Morison et al.*, 1992]. How oceanic boundary layer turbulence responds to unstable surface conditions is not very well known. A second, more general motivation for the combined lead turbulence studies is simply that it is quite difficult to make similar measurements in the open ocean. Sea ice is very effective at damping surface waves, and its stable platform provides a remarkably productive natural laboratory which has furnished, for example, unique documentation of Ekman spirals in upper ocean currents [*Hunkins*, 1966] and Reynolds stress [*McPhee and Martinson*, 1994] as well as the first direct measurements of heat and salt flux in the ocean by the eddy covariance technique [*McPhee et al.*, 1987; *McPhee*, 1994]. We know of no previous experiment in which both direct Reynolds fluxes and microstructure measurements were made simultaneously in a statically unstable oceanic boundary layer.

The paucity of turbulence measurements in the open (ice free) ocean mixed layer has hindered understanding of the effects of destabilizing surface buoyancy flux. Convection near the equator has been discussed by *Anis and Moum* [1992]. The most complete observational study in midlatitudes is that reported by *Shay and Gregg* [1986], who investigated dissipation rates measured by dropped shear/microstructure probes during cold air outbreaks over warm water. They found that, disregarding a highly turbulent region near the surface, the ratio of dissipation rate ε to surface buoyancy flux $\langle w'b' \rangle_0$ through most of the mixed layer was consistent with dominance of buoyancy forces over shear in the production of turbulent kinetic energy (TKE). Assuming a linear decrease in buoyancy flux across the mixed layer, their results implied that buoyancy production accounted for roughly 60–80% of the mean TKE dissipation in the two oceanic regimes studied. They deduced that scaling based on the convective turbulent velocity scale, $w_* = (D\langle w'b' \rangle_0)^{1/3}$, was more appropriate

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than that based on the surface friction velocity u_{*0}, i.e., that surface stress was relatively unimportant in determining response of the whole mixed layer to surface conditions. Using the ratio of mixed layer depth D to Obukhov length $L = u_{*0}^3/(\kappa\langle w'b'\rangle_0)$ as a measure of the strength of convection, Shay and Gregg showed that the range of their measurements, $-3 < D/L < -76$, was considerably less convective than most studies of statically unstable atmospheric boundary layers. As a general principle, when the magnitude of the Obukhov length is comparable to or smaller than the other turbulence scales in a flow, buoyancy is an important factor in the TKE budget.

Compared with the atmosphere and even with the "cold outbreak" oceanic measurements, surface buoyancy flux in frozen oceans presents a different set of problems. In a study of turbulent mixing length, *McPhee* [1994] reported an Obukhov length of about $L = -12$ m at LEADEX lead 3, when the mixed layer was around 28 m deep with a light breeze moving the ice pack at about 12 cm s^{-1}. This is less convective than the least convective case presented by *Shay and Gregg* [1986], thus free convection scaling is not necessarily appropriate (see also *Morison et al.* [1992]). Since leads rarely occupy more than a small fraction of the surface during winter, it seems likely that mixing is dominated by storm-driven stress events rather than free convection. Suppose, for example, that a 300-m-wide lead drifts with the surrounding ice pack at 16 cm s^{-1} under moderate stress (u_{*0} = 1 cm s^{-1}) in a mixed layer 40 m thick and that the freezing rate in the lead is sufficient to set up a surface buoyancy flux of -2×10^{-7} W kg^{-1} (all these values are fairly typical of Arctic conditions, except that the lead is relatively wide). It would take about 30 min for the lead to traverse its own width, which is about the same time it would take the signal associated with surface convection to reach the base of the mixed layer, given w_* = 2 cm s^{-1}. Thus by the time a water parcel at the bottom of the mixed layer senses convection from the lead, the lead has passed over, and even if the boundary layer beneath the lead is "freely convecting" (in this example, $D/L \approx -3$), the effect would be rapidly smeared over an area that is large relative to the lead.

From these considerations it seems plausible that the overall importance of lead convection to the structure of turbulence in the under-ice boundary layer lies more in its enhancement of mechanical stirring rather than in the direct "plume entrainment" which often dominates atmospheric boundary layer development over heated terrain. Our analysis therefore is geared toward understanding the interplay of Reynolds stress with heat and salinity flux in determining the turbulence dynamics and energetics, a good example being how destabilizing buoyancy flux enhances eddy diffusion efficiency.

In this paper we focus on two types of upper ocean turbulence measurements from systems deployed at the edges of active leads. One used four turbulence instrument clusters (TICs) spanning 6 m vertically to sample at 1-s intervals the three-dimensional velocity field, along with temperature and conductivity. A second system, the loose-tethered microstructure profiler (LMP), continuously and automatically measured profiles of temperature, conductivity, and small-scale velocity shear. Our basic strategy during LEADEX was to identify an active lead, move our instrumentation to its downstream (upwind) edge as rapidly as possible by helicopter [*LeadEx Group*, 1993], and then measure mixed layer fluxes and microstructure profiles for as long as the ice growth rate remained high. Of four leads occupied within a radius of about 40 km of the main LEADEX ice camp, we obtained simultaneous measurements at two sites, leads 3 and 4. Prior to any of the actual helicopter deployments, the TIC mast was tested by installing it through 1-m-thick ice on the refrozen lead that served as the main camp runway. Fortuitously, a small wind storm materialized as we finished the initial checkout, so the mast was left in place until the storm abated, providing a "control" case in which buoyancy flux was small. The deployments and data discussed in the paper are summarized in Table 1.

Table 1. Boundary Layer Data Summary

Measurements	Levels, m	Times
	Runway Test Deployment	
u_m, v_m, w_m, T, μC	3.0	85.1 to 85.3
	Lead 3	
u_m, v_m, w_m, T, C	3.3, 6.3, 9.3	98.25 to 98.75
u_m, v_m, w_m, T, C	13.8, 16.8, 19.8	98.75 to 98.9
u_m, v_m, w_m, T, μC	4.3	98.25 to 98.75
u_m, v_m, w_m, T, μC	14.8	98.75 to 98.9
LMP, T, C, small-scale shear	profiles to 60 m, 10 per hour	98.0 to 100.0
	Lead 4	
u_m, w_m, T, μC	3.2	103.2 to 103.6
u_m, w_m, T, μC	4.2	103.7 to 104.2
u_m, v_m, w_m, T, C[a]	2.2, 5.2, 8.2[a]	103.2 to 103.6[a]
u_m, v_m, w_m, T, C[a]	3.2, 6.2, 9.2[a]	103.7 to 104.2[a]
LMP	profiles to 60 m, 10 per hour	103.0 to 104.3

Data provide a summary of turbulence measurements discussed in this paper for each deployment site. For a more general description of the experiment, including other measurements made, see *LeadEx Group* [1993]. Time is expressed in decimal days of 1992, where, e.g., 98.75 refers to 1800 UT on April 27, 1992. The velocity components, u_m, v_m, w_m, refer to the individual rotors of each turbulence instrument cluster. LMP is loose-tethered microstructure profiler.

[a]Low flow velocity resulted in stalling of one or more of the current meters in each cluster during these periods.

In section 2 we describe instrumentation and discuss the statistical significance of flux measurements. Time series and statistical analysis are presented and compared in some detail, since there are several novel features to the data set. Results from leads 3 and 4 are presented in sections 3 and 4, respectively, and discussion and summary follow in section 5.

2. Instrumentation, Techniques, and Statistical Significance

2.1. Turbulence Instrument Clusters

Turbulent fluxes were measured directly in the oceanic boundary layer using TICs mounted on a rigid mast which could be lowered as a unit to any level in the mixed layer. Each TIC includes three small, partially ducted current meters oriented along mutually orthogonal axes canted 45° to the horizontal, mounted near fast response thermistors (Sea-Bird Electronics SBE03) and ducted conductivity meters (SBE04). With proper coordinate transformations and engineering calibrations, each cluster thus measures the three velocity components u, v, w, plus temperature T and conductivity C, from which salinity S is derived. Deviatory time series, denoted u', v', w', T', and S' are calculated by subtracting mean values over a suitable averaging period. The basic system, including its capacity to measure heat and momentum flux in the neutrally stratified boundary layer, is described by *McPhee* [1992].

For rapid deployment during LEADEX we developed a mast with four clusters, distributed so that TICs 2, 3, and 4 were 1, 3, and 6 m, respectively, below the topmost TIC 1. The rigid mast was equipped with a vane to maintain alignment into the mean flow. Its orientation and depth were monitored continuously with a fluxgate compass, tiltmeters, and a pressure gauge. For TIC 2 the standard SBE04 conductivity meter was replaced by a microstructure conductivity meter (SBE07, hereafter referred to as μC). We had found from previous experiments that flow through the ducted conductivity cells was restricted at scales comparable to the inertial subrange scales of typical under-ice turbulence, hence that they were not suitable for directly determining $\langle w'S' \rangle$. The exposed electrodes of the SBE07 instrument obviate the ducting problem, and although its voltage drift precluded accurate determination of the absolute salinity, salinity spectra calculated from the μC output demonstrated a well-developed inertial subrange. As illustrated below, we believe the μC-equipped TIC gave accurate measurements of the turbulent salinity flux $\langle w'S' \rangle$ (see also *McPhee* [1994]).

Ensemble average turbulent fluxes, $\langle u'w' \rangle$, $\langle v'w' \rangle$, $\langle w'T' \rangle$, and $\langle w'S' \rangle$, were estimated in the usual way by calculating the zero-lag covariance over a suitable averaging interval (in this case, 1 hour), assuming that turbulent features advected past the TIC site by the mean flow over the specified averaging time are representative of the ensemble at a particular instant (Taylor's hypothesis).

2.2. TIC Examples and Statistical Significance

Four 1-hour long time series from the μC-equipped TIC 2 illustrate the turbulent flux processes observed during LEADEX (Figures 1–4). Scales are the same for all four plots to emphasize the differences. The first segment (Figure 1) is from 4.3 m depth taken before dawn at lead 3, when there was active freezing and a small but persistent upward heat flux in the mixed layer. Note that S' and T' were strongly anticorrelated. The event between minutes 40 and 46, in which a downward ramp in w was accompanied by increasing S' and decreasing T', produced strong instantaneous scalar fluxes of opposite sign. Similar, though less intense events happened every 8–10 min throughout the record. These appear to have been organized convective features, transporting cold, saline fluid produced by freezing at the surface downward in bursts of negative w lasting several minutes. Assuming the plumes were advected past the mast with the mean horizontal velocity of the flow, the downwelling regions were 15–25 m across. Superimposed on the more or less organized plumes were energetic fluctuations indicative of shear-driven turbulence near the surface.

A second time series taken around local noon (incoming shortwave radiation maximum) is shown in Figure 2, after the mast had been lowered so that TIC 2 was about 15 m deep. It shows similar large excursions in w and ramp-like changes in temperature and salinity, again with something like a 10-min timescale. There was somewhat less fine scale variability, both because the large-scale convection more completely dominated shear-driven turbulence at this depth [*McPhee*, 1994] and the natural scale of shear-driven turbulence increased away from the surface. The main differences from earlier, however, were that here T' and S' were positively correlated and there was net downward flux of both salt and heat, the latter arising from solar heating near the surface. Even though there was still freezing at the interface, the convective plumes

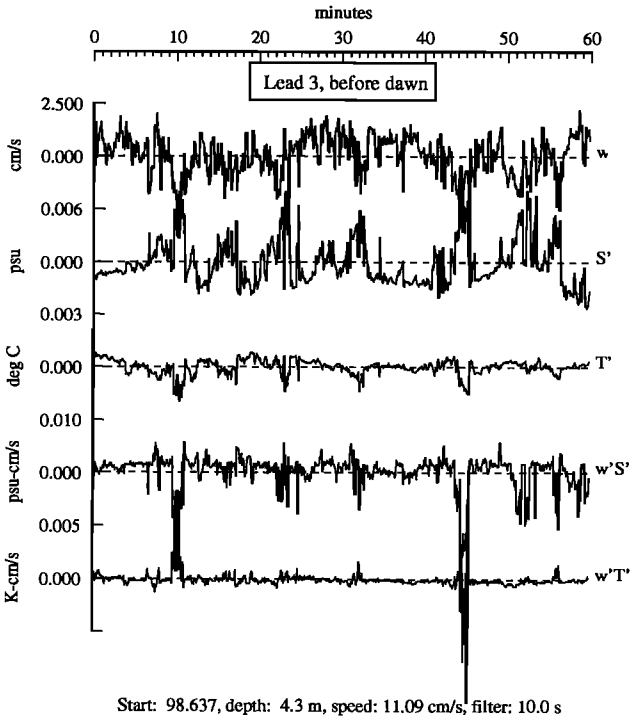

Figure 1. One-hour time series of vertical velocity w and deviatory salinity S' and temperature T' (linearly detrended) for turbulence instrument cluster (TIC) 2 at lead 3 before dawn. Also shown are the instantaneous fluxes, which are in opposite directions. Average values of the fluxes are $\langle w'S' \rangle = -1.54 \times 10^{-5}$ psu m s^{-1} and $\langle w'T' \rangle = 1.44 \times 10^{-6}$ K m s^{-1}.

transported significant heat away from the surface; e.g., for several minutes centered around minute 38, the instantaneous downward heat flux approached 600 W m^{-2}. Averaged over the hour, $-\rho c_p \langle w'T' \rangle$ (where ρ is density, and c_p is the specific heat of seawater) exceeded 70 W m^{-2}. Conductivity of seawater depends on both salinity and temperature, hence there is often concern that a time-response mismatch between measured temperature and conductivity will lead to spurious TS and wS correlations. We will show below that at both leads 3 and 4, salinity flux was always downward, while turbulent heat flux changed sign soon after sunup, following closely the incoming solar flux and mean gradients, thus $\langle w'S' \rangle$ covariances cannot have resulted from spurious effects.

The third time series (Figure 3), is from lead 4, where the freezing rate was comparable to lead 3, but the ice/ocean relative current velocity was much smaller, 4 versus 11 cm s^{-1}. There were still large excursions in vertical velocity, obviously anticorrelated with salinity deviations, but note that the plumes were relatively more frequent, which means that if they were advected with the mean flow, they had much smaller horizontal scales. Also, while w varied as widely as before, there was comparatively less fine scale structure to the turbulence, indicating that in this flow, free convection dominated through almost the entire boundary layer.

A 1-hour time series from the "runway storm" (Figure 4) provides a counterpoint to the lead-edge examples. Shown at the same scales, temperature and salinity deviations appear to be almost flat, although, in fact, the average values of the fluxes are different from zero. Note that excursions in w were about as large as at the lead but were generally of much shorter

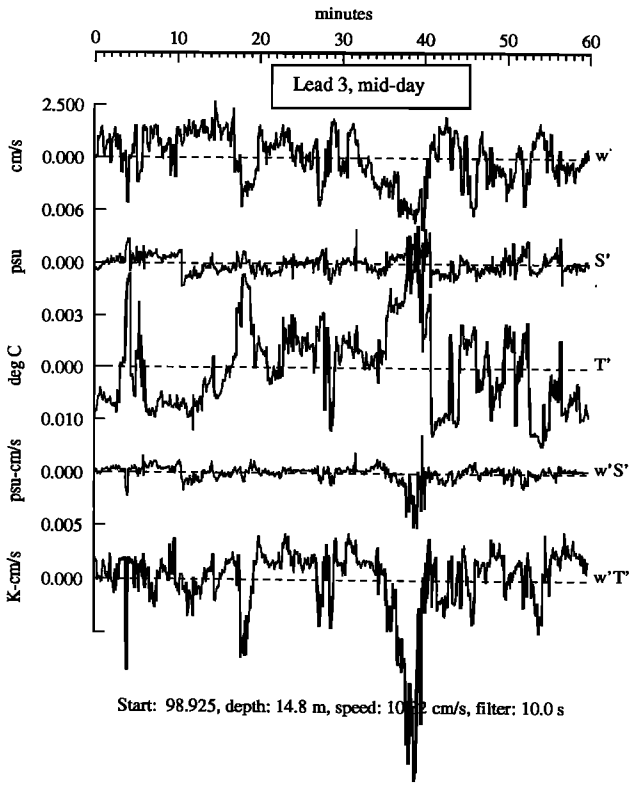

Figure 2. Same as Figure 1, except near solar noon at lead 3. Heat and salinity fluxes are now in the same direction. Average values of the fluxes are $\langle w'S' \rangle = -4.87 \times 10^{-6}$ psu m s^{-1} and $\langle w'T' \rangle = -1.74 \times 10^{-5}$ K m s^{-1}.

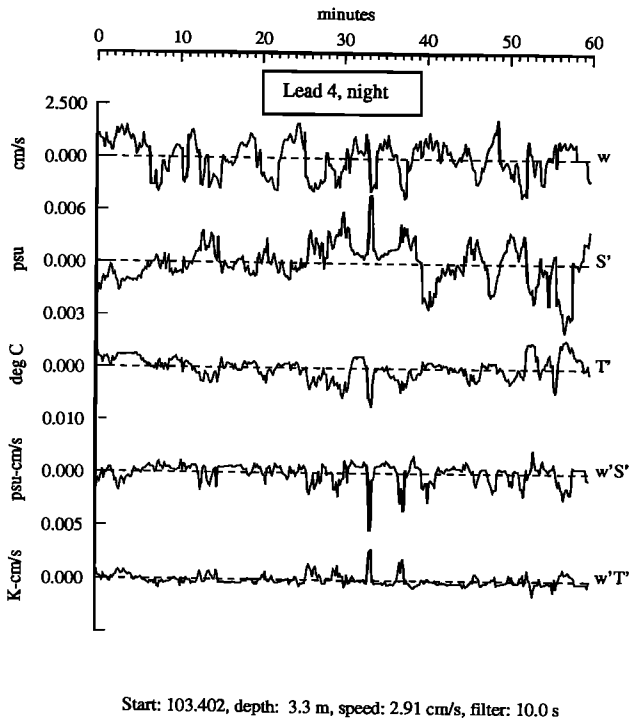

Figure 3. Same as Figure 1, except during the night at lead 4. Average values of the fluxes are $\langle w'S' \rangle = -1.03 \times 10^{-5}$ psu m s^{-1} and $\langle w'T' \rangle = 2.72 \times 10^{-6}$ K m s^{-1}. Note that the mean speed is much smaller than at lead 3.

duration, since shear-driven turbulence dominated. The Reynolds stress during the runway storm was somewhat larger than at lead 3.

Fleury and Lueck [1994] have suggested a straightforward empirical technique for testing whether Reynolds averaged, zero-lagged covariances are significantly different from what might be expected due to random correlations of two time series (e.g., w and S'). They argue that, provided the lag between two series exceeds the autocorrelation scale of each, a random sampling of lagged correlations will approach the probability distribution expected from spurious correlations in two essentially uncorrelated series. The zero-lag correlation is statistically significant to the degree that it lies outside the chance distribution. Their significance test was applied to ten 1-hour data segments from lead 3. For each segment we (1) determined lags beyond which autocorrelations of T' and S' were small; (2) generated histograms of w, T' and w, S' correlations for random distribution of lags exceeding the autocorrelation scales, using circular symmetry; (3) fitted a Gaussian distribution to the histograms summed from all 10 runs; and (4) plotted the probability density function along with the individual zero-lag correlations. Results are shown in Figure 5. The numbered lines representing zero-lag correlations are scaled according to the magnitude of the covariance (e.g., $\langle w'T' \rangle$) relative to the largest value. Note that with the exception of one w, T' correlation (hour 7) when the heat flux was small, it is highly improbable that any of the correlations was due to chance.

Since measured heat and salinity fluxes were small during the runway storm test (Figure 4), we thought a similar analysis

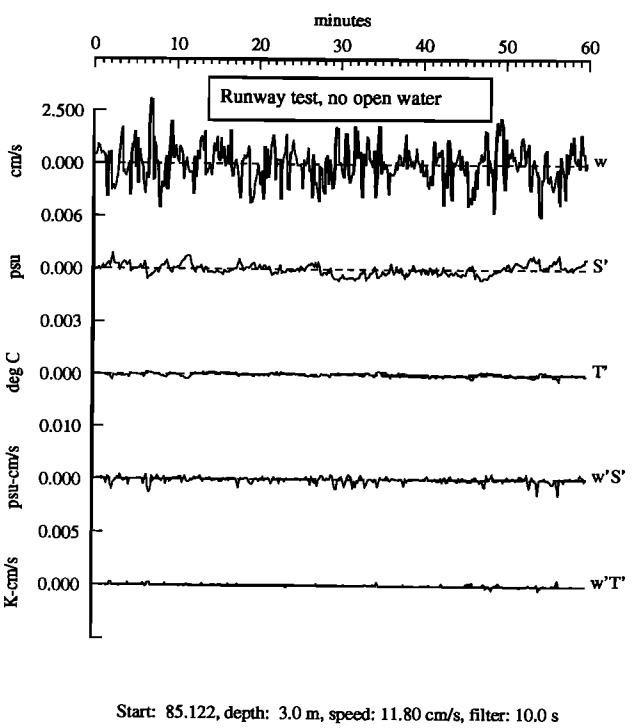

Figure 4. Same as Figure 1, except with TIC mast deployed under the smooth runway ice at the main LEADEX camp during checkout. Mean speed is comparable to lead 3, but heat and salinity fluxes are much smaller. Average values are $\langle w'S' \rangle = -1.63 \times 10^{-6}$ psu m s^{-1} and $\langle w'T' \rangle = 2.81 \times 10^{-7}$ K m s^{-1}.

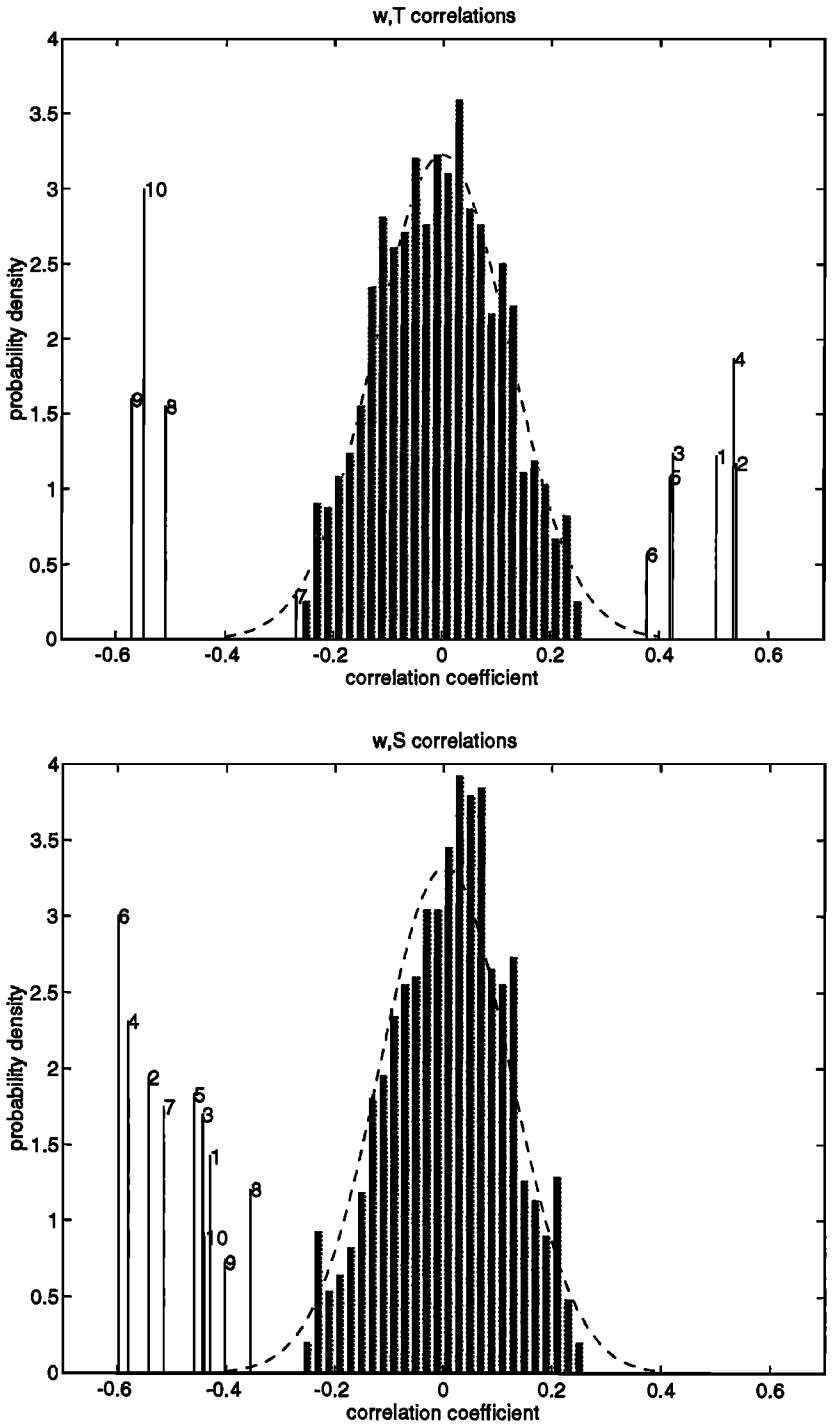

Figure 5. Histograms with fitted Gaussian distributions indicating the probability density functions for random-lagged correlations where the lag exceeds the autocorrelation scale [after *Fleury and Lueck*, 1994] from ten 1-hour-long time series from TIC 2 at lead 3. The numbered solid lines show the zero-lag correlations for each hour (e.g., $\langle w'T' \rangle / (\langle w^2 \rangle^{1/2} \langle T'^2 \rangle^{1/2})$), where the length of the line indicates the magnitude of the flux covariance (e.g., $\langle w'T' \rangle$) relative to the largest hourly value.

might illustrate conditions in which the significance of the vertical flux correlations would be less apparent. We chose a period during a particularly steady part of the storm and analyzed five 1-hour segments in the same way as above. Results, shown in Figure 6, were surprising in that the correlations appear to be nearly as significant relative to chance fluctuations as when the fluxes were an order of magnitude larger at lead 3. Since turbulent energy levels were higher during the runway test, there is little reason to assume that the system was not measuring well into the inertial subrange and hence that despite their small magnitude, the measured fluxes (Figure 7) were relatively accurate. The upward heat flux (order 1 W m^{-2}) may have come either from daytime solar heating through thin ice or from entrainment at the base of the mixed

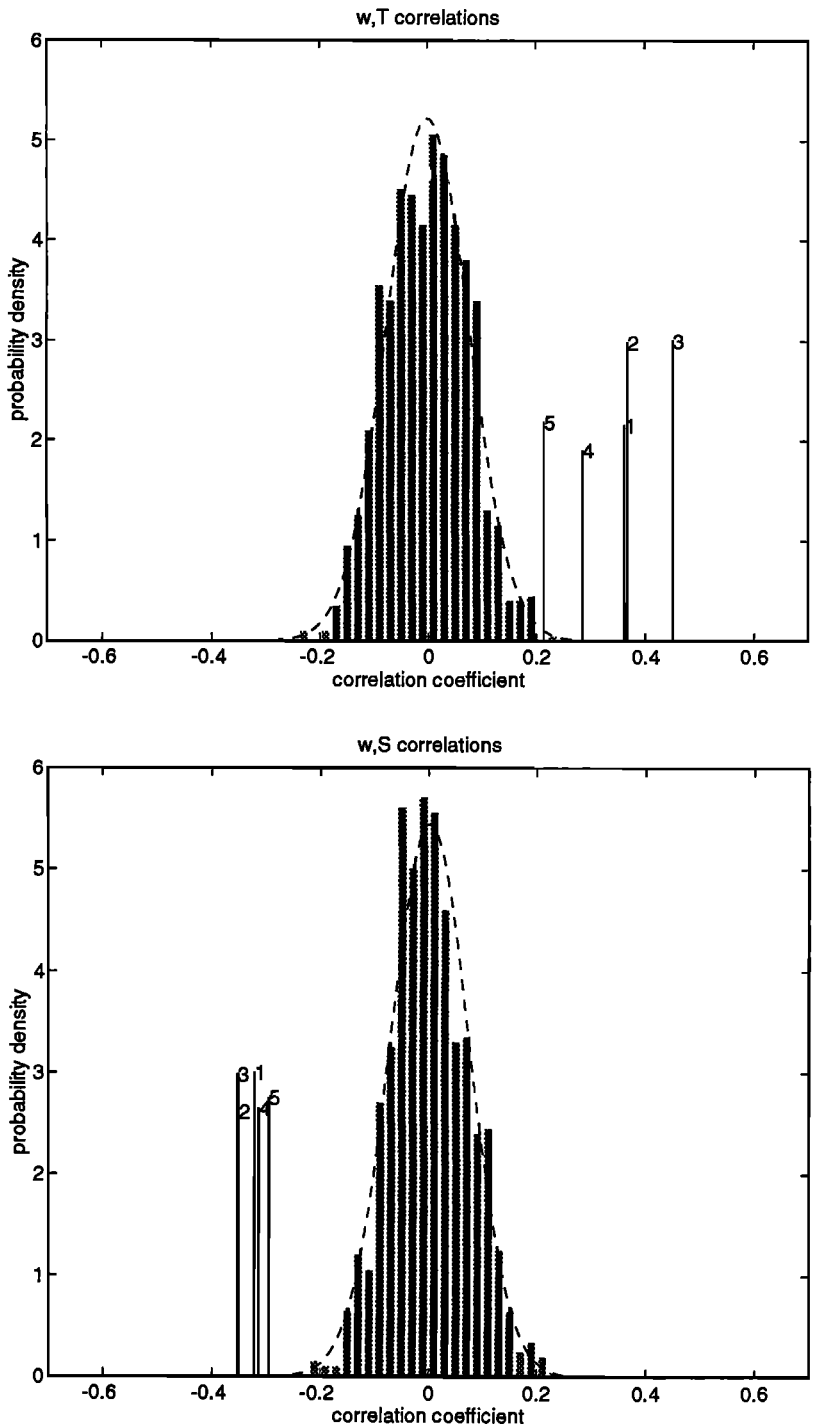

Figure 6. Same as Figure 5, except for five 1-hour time series during the test deployment under 1-m-thick ice near the main camp runway.

layer during the storm. Downward salinity flux probably resulted from freezing of the relatively thin runway ice. The average salinity flux over the 5-hour period corresponds to an ice growth rate of roughly 6 mm d^{-1}, which implies a temperature gradient in the ice of around -10 K m^{-1}. These are reasonable numbers, and while not directly germane to the lead study, the exercise demonstrates that the TIC system is capable of measuring scalar fluxes down to levels we would have until now discounted. Using average values of friction velocity and buoyancy flux from the data in Figure 7, the Obukhov length was around -141 m, hence the boundary layer was indeed near-neutral stability.

2.3. Loose-Tethered Microstructure Profiler

High-resolution profiles of temperature, conductivity, and small-scale velocity gradients were continuously measured at each lead site using a loose-tethered microstructure profile package. A computer-controlled servo winch allowed the instrument package to essentially free-fall from a 1-m start depth to 60 m, spanning the 30-m deep mixed layer. The profiler was

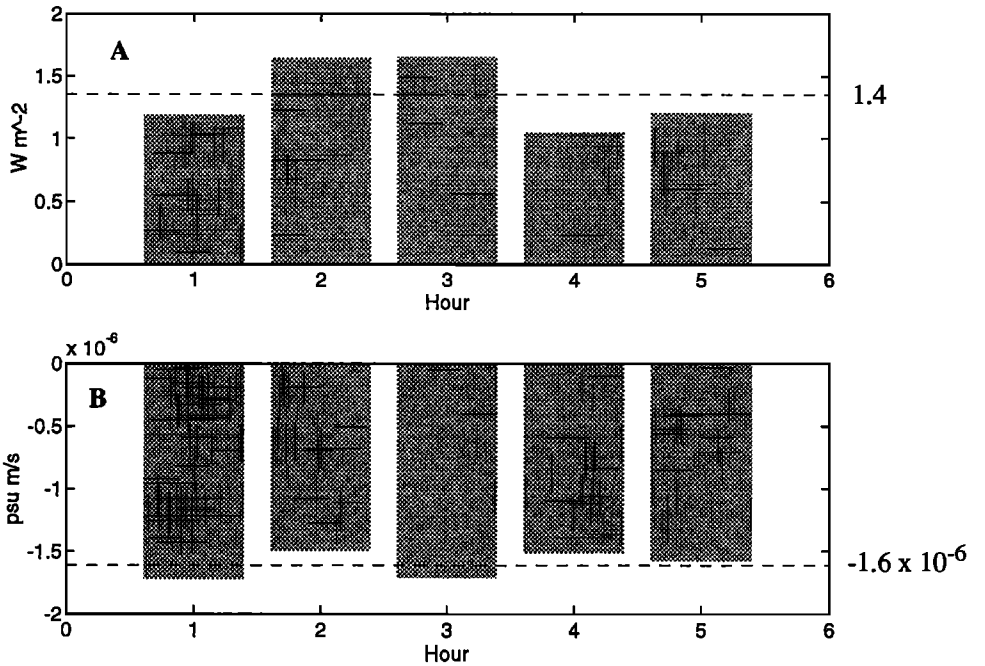

Figure 7. Turbulent (a) heat flux and (b) salinity flux for successive 1-hour averages from cluster 2 (microstructure conductivity meter equipped) during the runway test at depth of 3 m. Mean values are listed at right.

equipped with a fast response FP07 thermistor, a Sea-Bird Electronics SBE07 dual-needle conductivity probe, and an airfoil lift probe which senses small-scale cross-velocity fluctuations as the stable instrument platform descends through the water column (see, for example, *Osborn and Crawford* [1980]). As soon as the programmed maximum depth is reached, the winch retrieves the package to the start depth and automatically repeats the profiling cycle approximately every 6 min.

Spatial responses of the temperature, conductivity, and shear sensors are all good down to scales of order 1 cm. Their high-frequency responses are electronically preemphasized to improve the noise floor of temperature and velocity gradient variance spectra used to infer thermal dissipation rate ε_T (equal to $\chi/2$, where χ is the thermal dissipation rate discussed, e.g., by *Osborn and Cox* [1972]) and molecular kinetic energy dissipation ε. Under assumptions of isotropy the dissipation rates may be estimated from the single-axis gradient measurements of temperature and velocity using

$$\varepsilon_T = 3\nu_T\left\langle\frac{\partial T}{\partial z}\right\rangle^2 \qquad \varepsilon = \frac{15}{2}\nu\left\langle\frac{\partial u}{\partial z}\right\rangle^2 \tag{1}$$

where ν_T is the thermal molecular diffusivity for seawater, ν is molecular viscosity, and the gradient variances were integrated from approximately 0.01- to 1-m scales. A stepped integration of the velocity gradient spectrum was used, following *Shay and Gregg* [1986], to minimize instrument vibration contamination and the effects of the high wavenumber cutoff of the O(60 cpm) half-power probe response. It is estimated that at least 90% of the gradient variance was recovered at the highest dissipation rates measured at the site. The local dissipation rates were used to determine the integration cutoff for response-compensated temperature gradient spectra measured by the FP07 thermistor, minimizing noise contamination at low turbulence levels. A flow-compensated thermistor response function, following *Fleury and Lueck* [1994], provided spectral correction to the thermistor response roll-off in the temperature variance spectral integrations. Fine-scale temperature and salinity structure were resolved to 0.1-m scales with noise levels less than 10^{-4} K and 10^{-4} practical salinity units (psu), respectively, with an absolute temperature accuracy of 1 mK. Calibration of the microconductivity cell is susceptible to drift, so a surface calibration point was measured at the start of each profile using a pumped Sea-Bird temperature-conductivity pair suspended 50 cm from the "parked" profiling package. The low noise performance of the sensors allowed very high resolution vertical gradient measurements of both salinity and temperature to be estimated. Laboratory calibrations were used to determine the very small pressure-dependent term from each thermistor used in the profiler, removing a potential source of apparent mean gradient in the profiles.

Profiling a sensor through the water column allows vertical gradients to be resolved to the limits of sensor noise, short-term stability, pressure-dependent effects and, most importantly, the fine-scale structure in the fluid. To reduce the effects of the turbulence-induced structure in the water column, temporal averages of the rapidly repeated profiles were taken to determine mean vertical structure and vertical gradients. For example, 10-cm resolution time series of successive temperature profiles over a 2.5-hour period are shown in Figure 8, where successive profiles have been offset by 0.0025°C. Significant levels of fine structure over a range of scales can be seen in these 0.1-m vertical resolution profiles, making it difficult to resolve mean gradients from any single profile, while O(1 hour) temporal averaging effectively removed the turbulent, fine-scale contribution to the mean vertical profile. The mean vertical structure within the mixed layer over a 2-day period at lead 3 is illustrated in Figure 9, where the profiles have been

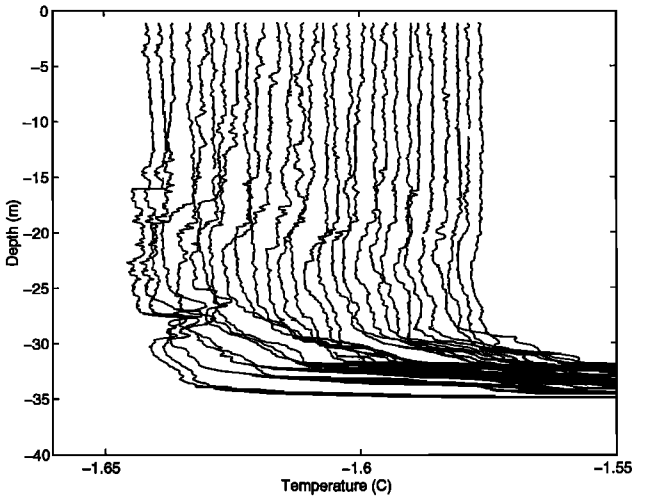

Figure 8. Sequential loose-tethered microstructure profiler (LMP) temperature profiles at lead 3 for a 2.5-hour period. Each profile is offset rightward by the equivalent of 0.0025°C.

averaged over 1-m depth intervals and a 1-hour filter was applied to the profile time series. The z axis span of the plot has been restricted to 40 m to emphasize the diurnal heating cycle within the mixed layer, but this consequently clips off the warm pycnocline structure at greater depths.

3. Lead 3 Results

The third lead deployment most closely matched the ideal of wind-driven ice drift perpendicular to the lead axis so that instruments on the upwind edge of the lead sensed "downstream" relative currents, with a fetch from almost directly across a kilometer-wide expanse of open water and thin ice.

At Lead 3 the LMP was deployed for a period of 57 hours, starting at 2330 UT on day 97 (April 6, 1992) when there was open water right across the lead. An hour later, grease ice had covered most of the lead. No significant areas of open water were observed subsequently, and the ice reached approximately 20 cm thick before the end of the LMP time series. A total of 570 continuous profiles were measured to a depth of 60 m, with a couple of 20-min gaps during ice-rafting events. The TIC mast was operating by 0550 UT on day 98, with measurements recorded until about the end of day 99. However, by early on that day, currents relative to the ice had fallen to levels below that required to maintain all three components of each cluster spinning, hence flux measurements were not reliable.

3.1. Momentum Flux at the Lead Edge

Current vectors at each level on the TIC mast, measured in a reference frame attached to the drifting ice, are shown in Figure 10a for the period when relative currents were strong enough to ensure that all rotors were well above stall speed. The mast was lowered into the middle part of the mixed layer after 98.75. (Unless otherwise noted, time is expressed here in "decimal days of 1992," where, for example, day 98.75 refers to 1800 UT on April 27, 1992.) Vector friction velocity (Figure 10b) is defined by

$$u_* \mathbf{u}_* = \langle u'w' \rangle \mathbf{i} + \langle v'w' \rangle \mathbf{j} \qquad (2)$$

i.e., a vector in the direction of the horizontal Reynolds stress traction, with the square root of its magnitude. This definition of friction velocity is local as distinguished from the friction velocity at the ice/ocean interface. In turbulent flows, stress magnitude varies approximately as the square of flow velocity, thus $\mathbf{u}_*$ and $\mathbf{V}$ are nearly proportional. Friction velocity vectors were computed by calculating the $u'w'$ and $v'w'$ zero-lag covariances over 1-hour averaging periods every 15 min. At depths removed from edge effects (the mast was deployed through a hole in 1.3-m, first-year ice about 3 m from the lead edge), mean and friction velocity are related consistently; e.g., for the 6 hours from 98.5 to 98.75, the complex ratio for cluster 4, $\mathbf{V}_{9.3}/\mathbf{u}_{*9.3}$ is $14.5e^{\iota \times 29.2°}$, where the exponential indicates that stress is 29° clockwise from the mean velocity. After the frame was lowered to midway in the mixed layer for the period 98.75 to 99.0, the average ratio for cluster 4 at about twice the depth was $\mathbf{V}_{19.8}/\mathbf{u}_{*19.8} = 12.8e^{\iota \times 33.8°}$. In other words, the effective drag had increased (drag coefficients for local stress are $c_{9.3} = 4.7 \times 10^{-3}$ and $c_{19.8} = 6.1 \times 10^{-3}$, respectively). In a neutral planetary boundary layer (PBL) (see, e.g., *McPhee and Martinson* [1994, Figure 4]), stress magnitude is found to decrease exponentially with depth, as speed relative to the interface increases. The lack of stress attenuation with depth at lead 3 indicates that buoyancy flux was important in the PBL dynamics.

3.2. Heat Flux

The thermal regime at lead 3 is summarized in Figure 11. Maximum insolation occurred around 2130 UT (Figure 11a), with the diurnal air temperature cycle lagging by a few hours. These were among the coldest temperatures encountered during the entire project, which was, in general, anomalously warm for this region and season. Vertical heat flux in the mixed layer (Figure 11b) was calculated in two ways. From the LMP profiles, temperature variance dissipation rate ε_T was determined from (1). Assuming a local balance between the production of thermal variance and its dissipation

$$-\langle w'T' \rangle \frac{\partial T}{\partial z} = \varepsilon_T \qquad (3)$$

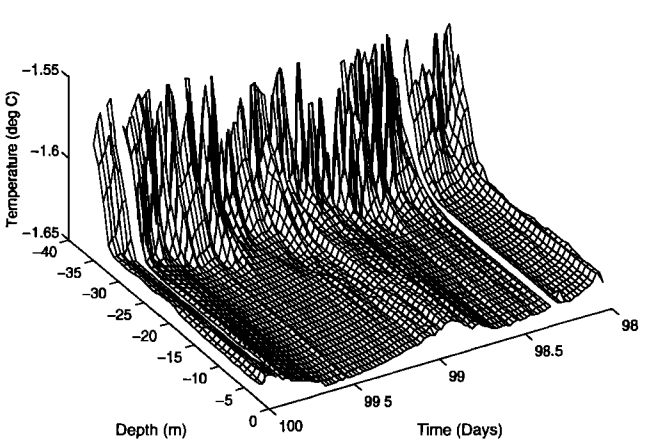

Figure 9. Perspective plot of temperature structure at lead 3 after averaging LMP temperatures in 1-m vertical bins and smoothing with a 1-hour temporal filter. The data are clipped at 40 m to accentuate variation in mixed layer temperature. Note the diurnal signal as midday heating warms the mixed layer slightly.

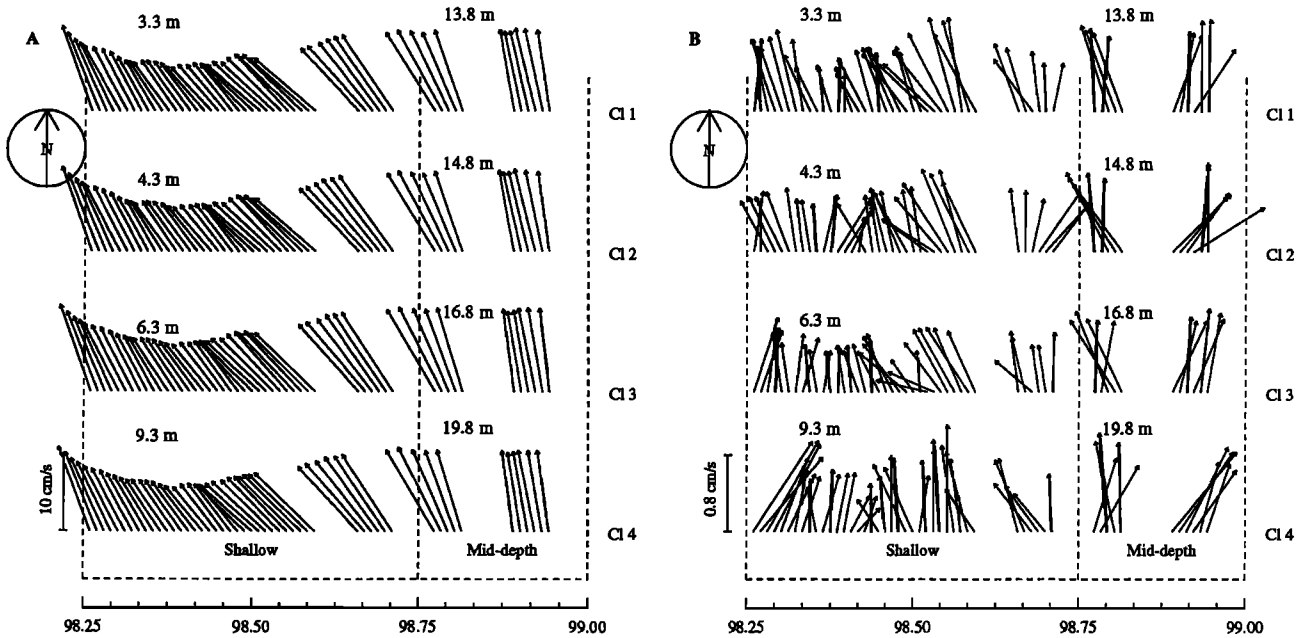

Figure 10. (a) Velocity relative to an observer on the drifting ice at four levels on the TIC mast from 1-hour averages calculated every 15 min. Shortly before 98.75, the mast was lowered from near the surface to about middepth in the mixed layer. (b) Friction velocity $\mathbf{u}_* = \tau/|\tau|^{1/2}$, where $\tau = \langle u'w' \rangle + i\langle v'w' \rangle$ is the local kinematic Reynolds stress.

Heat flux was also calculated directly from point measurement of T' and w at the four levels of the TIC mast, with the ensemble mean estimated by the covariance over time for 1-hour averages, staggered at half-hour intervals.

Prior to time 98.75 the TIC mast was positioned with clusters between 3.3 and 9.3 m depth. It was then lowered to span 13.8 to 19.8 m and later was stationed in the upper pycnocline for a few hours. By the time it was raised again into the mixed layer, currents had fallen off to below stall speed for at least one component of each cluster. LMP measurements were averaged in the depth interval from 7.5 to 12.5 m and are thus representative of conditions at about 10 m. The flux estimates were bin averaged in half-hour (0.02 day) blocks, which were further smoothed with a 2-hour running mean. Our estimates agree reasonably well (Figure 11b) and are highly correlated with the incoming solar radiation. It was not a priori obvious that this would be so, since by the morning (local) of April 7, 5–10 cm of ice had grown on the lead. At midday, 10–20% of the shortwave radiation impinging on the upper surface of the ice was making its way through the still growing ice cover to be distributed downward in the mixed layer by turbulent mixing. At night, heat was extracted from the mixed layer at a rate of about 10–30 W m^{-2}. There are two plausible sources for this upward heat flux. Foremost would be extraction of heat stored in the mixed layer during the previous day's insolation. Since it took only 2 or 3 hours for the ice to traverse the width of our lead, this would have to be a regional rather than purely local phenomenon but is consistent with a relatively large amount of opening observed in the pack around lead 3. Another plausible source is entrainment of heat from below the mixed layer, enhanced by increased mixing associated with destabilizing buoyancy flux (T. Stanton, manuscript in preparation, 1995).

Temperature gradient (Figure 11c) was estimated two different ways with slightly different meanings. First, we obtained a mean value for the mixed layer by calculating the least squares regression coefficient for each 1-hour average of LMP potential temperature profiles between depths of 3 to 20 m. The range was chosen by examining 12-hour mean profiles and noting that the profiles were close to linear above 20 m but showed appreciable curvature for greater depths. A measure of the scatter in each 1-hour average is given by the 95% confidence limits for the regression slope and is indicated by the shaded area in Figure 11c. We also determined the temperature gradient across the 6-m span of the TIC mast by applying small, constant corrections to the four thermometers on the mast so that the gradient vanished when the heat flux was near zero. Using the mixed layer for highly accurate calibration of the mast thermometers has been done for previous projects [*McPhee*, 1992; *McPhee and Martinson*, 1994] with reasonable results. The gradient was again calculated by least squares fitting of the adjusted temperature, with the 95% confidence interval for the regression slope indicated by the error bars (Figure 11c). Despite very small gradient magnitudes the two methods agree reasonably well. During the time late on day 98 when the error bars do not overlap, the TIC mast was positioned deeper in the mixed layer. This was a time of intense solar heating at the surface, hence we would expect a decrease in heat flux with depth, which would also probably correlate with a decrease in $\partial T/\partial z$.

A plot of the vertical structure of LMP heat flux as a function of time is shown in Figure 12. Vertical temperature gradients have been estimated over 8-m depth intervals, and these gradients and ε_T are averaged over 1-hour intervals before heat fluxes were calculated using (3). Unresolved temperature gradients or samples with ε_T below the instrument's noise floor are shown as blank areas in the profiles. Downward heat fluxes of up to 50 W m^{-2} are clearly seen at the start of day 98 and the subsequent two solar heating cycles. Strong fluxes extend

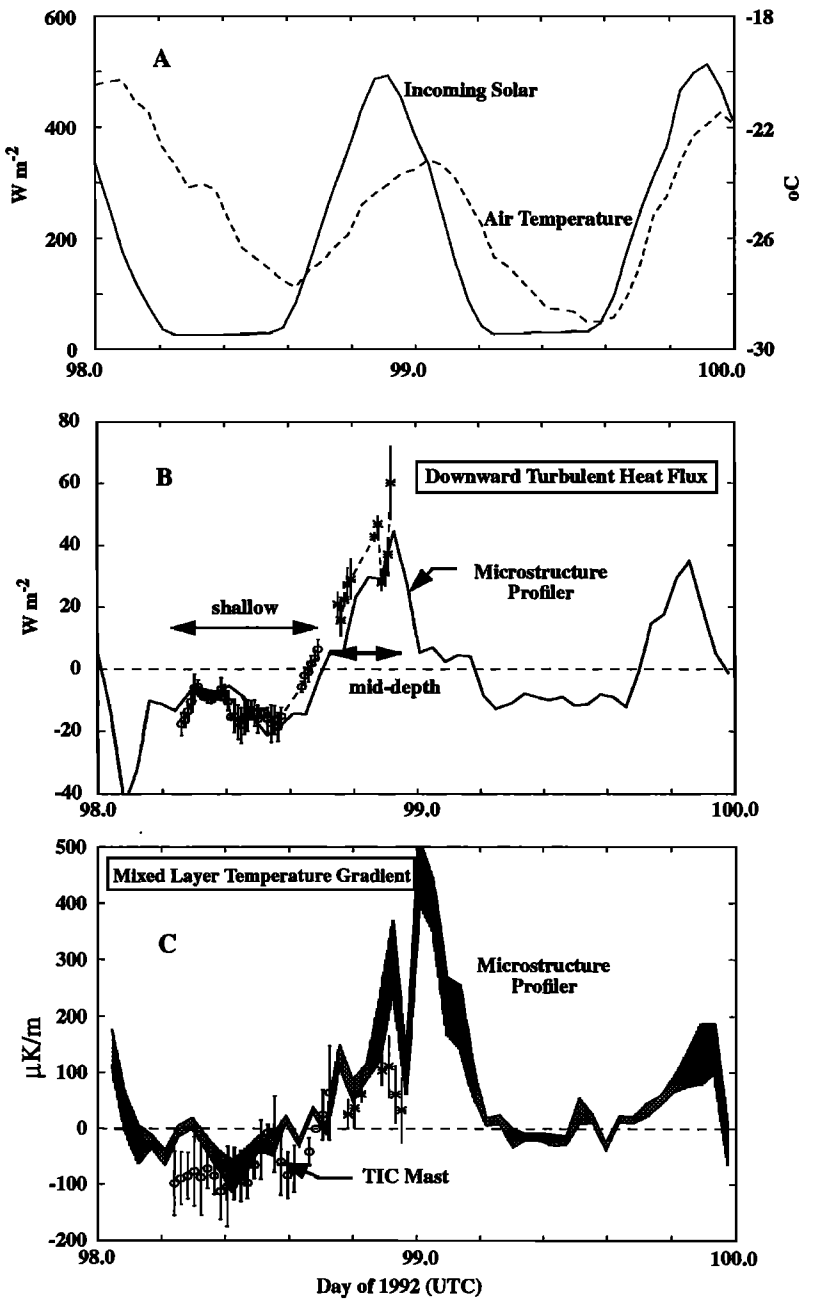

Figure 11. (a) Downward shortwave radiation and air temperature over the ice adjacent to lead 3. (b) Negative turbulent heat flux averaged across the TIC mast (denoted by circles when the mast was shallow, stars when the mast was middepth in the mixed layer; error bars represent 2 standard deviations) and from the LMP data, evaluated at 10 m. (c) Temperature gradient from the TIC mast (symbols as in Figure 11b; error bars represent the 95% confidence interval for the slope), and the 95% confidence interval for the LMP temperature slope in the interval 3–20 m (shaded).

down to over 20 m depth, before the weak salinity stratification and upward temperature gradients near the pycnocline reverse the heat flux sign, and greatly attenuate the flux levels. Upward fluxes of 10–30 W m^{-2} occur between the solar heating events. While weak, episodic heat flux events are observed deeper in the pycnocline (not shown here), the mean upward fluxes between 30 and 40 m are approximately 0.5 W m^{-2}. The significant vertical structure during the warming cycles is indicative of the heterogeneous structure of the mixed layer fine structure even on O(1 hour) timescales.

3.3. Salinity Flux

Salinity flux $\langle w'S' \rangle$ at the edge of lead 3 during the period 98.25 to 98.75 averaged -0.98×10^{-5} psu m s^{-1} at 4.3 m depth as measured with a TIC equipped with a Sea-Bird Electronic SBE07 microconductivity sensor [*McPhee*, 1994]. The standard deviation of the hourly salinity flux estimates was 0.34×10^{-5} psu m s^{-1}.

Since direct salinity and heat flux measurements are uncommon in the ocean, detailed examples from spectral analysis of

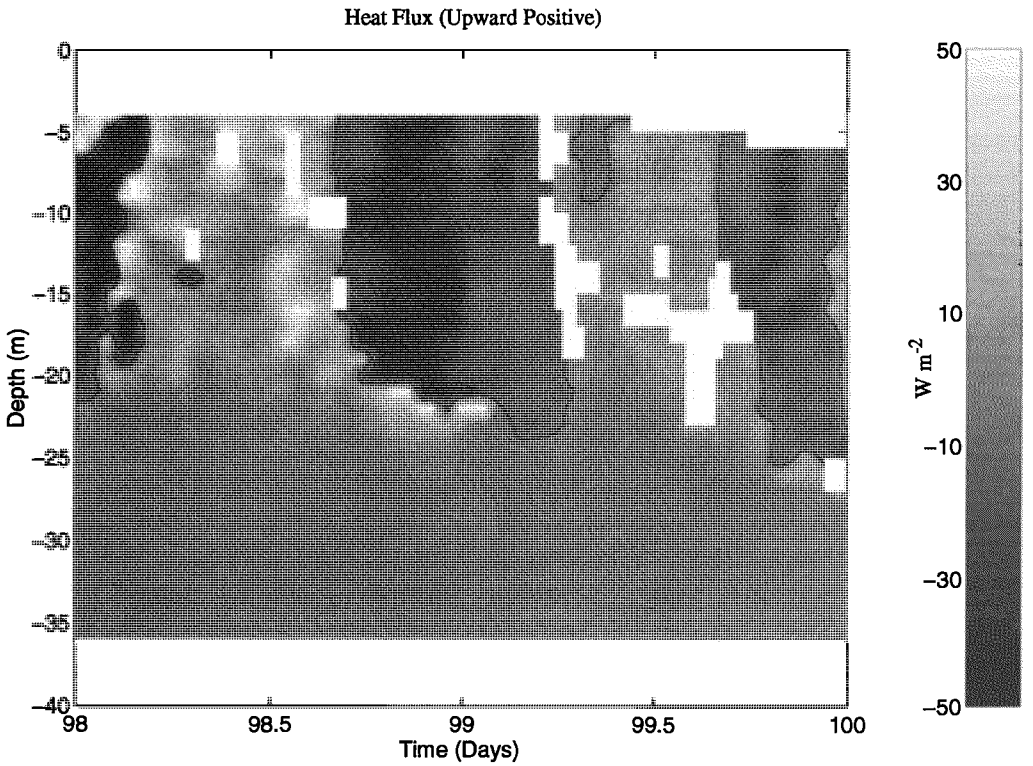

Figure 12. A 2-day heat flux profile time series extending to 36 m depth at lead 3 measured by the LMP. The solid contour is at zero flux level, and blank areas indicate unresolved fluxes.

two 1-hour time series (Figures 1 and 2) are presented here. Following *Bloomfield* [1976], vertical velocity, temperature, and salinity (derived from the microstructure conductivity probe on TIC 2) were linearly detrended and discrete-Fourier transformed. Autospectra of the three time series were obtained by smoothing the periodogram of squared amplitudes of the Fourier components with a Daniell procedure [*Bloomfield*, 1976] comprising three successive passes of a 16-point running mean. Approximate 95% confidence limits for the logarithm of the discrete spectra averages were estimated from the sum of the squared weights of the unit impulse response to the same filter, assuming the spectral estimates to be approximately normally distributed [*Bloomfield*, 1976, chapter 8]. Cross-spectra between vertical velocity and temperature or salinity deviations were estimated similarly by smoothing the complex product of the w Fourier components with the conjugate of the respective T' or S' Fourier components. The complex cross-spectrum is characterized by the squared coherency (i.e., the squared amplitude normalized by the product of the autospectra) and the phase angle, which is the arc tangent of the imaginary part (quadrature spectrum) over the real part (cospectrum). *Bloomfield* [1976] furnishes formulas for estimating confidence levels for the squared coherency under the same assumptions of normality as the autospectra estimates. In general, a wavenumber band will contribute to vertical flux if the cospectrum dominates (phase near zero or pi), whereas bands where the quadrature spectrum dominates (phase near $\pm\pi/2$) will contribute little to the flux.

Spectral energy density for vertical velocity S_{ww}, temperature S_{TT}, and salinity S_{SS}, plus w, T' and w, S' cross-spectra for the time series of Figure 1 are graphed in Figure 13 as functions of the angular wavenumber, $k = 2\pi f/\langle U\rangle$, where f is frequency and $\langle U\rangle$ is the mean current speed. The geometry of the ducted rotor clusters precludes measuring flow disturbances with scales smaller than about 0.25 m, hence a practical Nyquist wavenumber limit on a logarithmic scale is $\log_{10}$ (4π rad m^{-1}) = 1.1. The autospectra are quite similar when normalized by their respective variances and show an extensive region with $-5/3$ slope indicative of the inertial subrange, with drops in spectral densities of about 3 orders of magnitude approaching the high wavenumber resolution of the turbulence clusters. Cross-spectra show significant squared coherency and relatively steady phase up to wavelengths ($2\pi/k$) of about 12 m for temperature and 6 m for salinity. As is obvious from visual inspection of the time series, lower-frequency excursions in temperature are positively correlated with w (phase near zero), while excursions in salinity are negatively correlated (phase near π). Note that turbulent heat flux is relatively small (5.9 W m^{-2}) and salinity flux is relatively large. While there are significant "bumps" in the squared coherency (which is normalized by the product of the autospectral densities) at wavenumbers greater than 1 rad m^{-1} (0 on the log abscissa), they contribute little to the covariance, because (1) the energy levels are much decreased and (2) the phases are often nearer to $|\pi/2|$ (quadrature) than π.

The second example (Figure 14) is from midway through the mixed layer around local noon when downward heat flux was near its maximum. The salinity flux is 1/3 of its previous value, both because of reduced freezing at the surface and the greater sensor depth. Heat flux, on the other hand, is an order of magnitude greater and its phase is reversed. Still, the wavenumber range for which squared coherency falls off for each scalar remains about the same, indicating that the physical mechanisms behind the turbulent flux are similar. Despite its

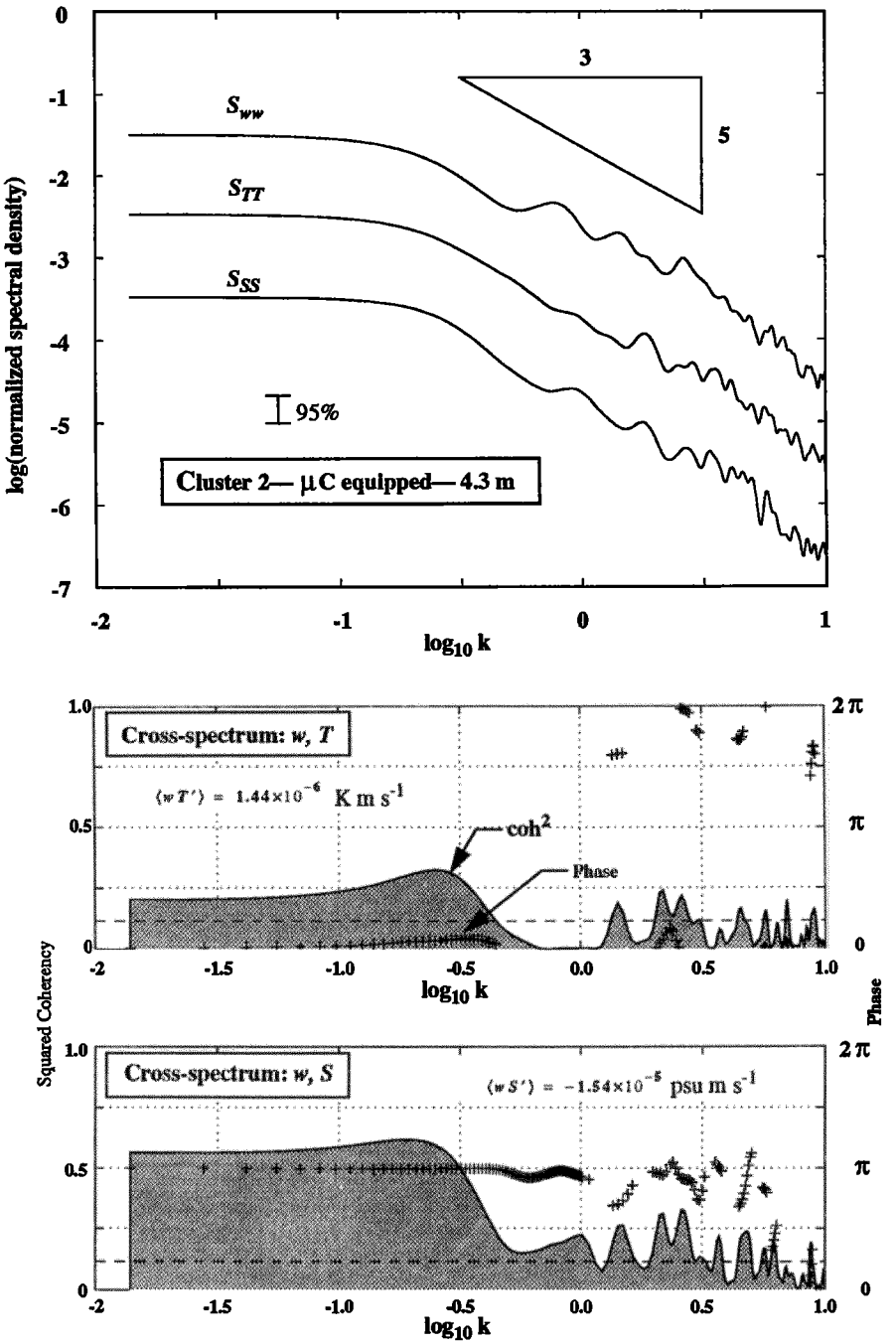

Figure 13. (top) Spectra for w, T', and S', normalized by their respective variances, corresponding to the time series in Figure 1. The T' and S' spectra are offset one and two decades downward, respectively, for clarity. Cross-spectra for the Reynold covariances for (middle) w, T and (bottom) w, S, as squared coherency (shaded curves) and phase (pluses). Units for wavenumber k are radians per meter. See text for details.

large magnitude the impact of heat flux on buoyancy flux is minor compared with salinity flux, because thermal expansion is vanishingly small at temperatures near freezing.

In previous projects, attempts to measure salinity flux using TICs equipped with standard conductivity meters have not been very successful. The meters measure conductivity of water forced through a narrow duct by the ambient flow (since active pumping might disturb flow sensed by the other instruments), and typical under-ice turbulent motions in the "energy-containing" range of the spectrum are often strongly filtered by the ducts. In the LEADEX examples, however, the turbulent scales are relatively large and much of the significant coherency occurs in disturbances with wavelengths in excess of 12 m and timescales of 2 min or longer. Under these conditions the response of the standard conductivity cells might be improved. The concept is explored in Figure 15 from the standard TIC mounted 1 m above TIC 2 for the same time period as Figure 13. This cluster was about 2 m below the ice situated about 3 m in from the lead edge. While its depth was beyond the internal boundary layer that would start at the ice edge, the

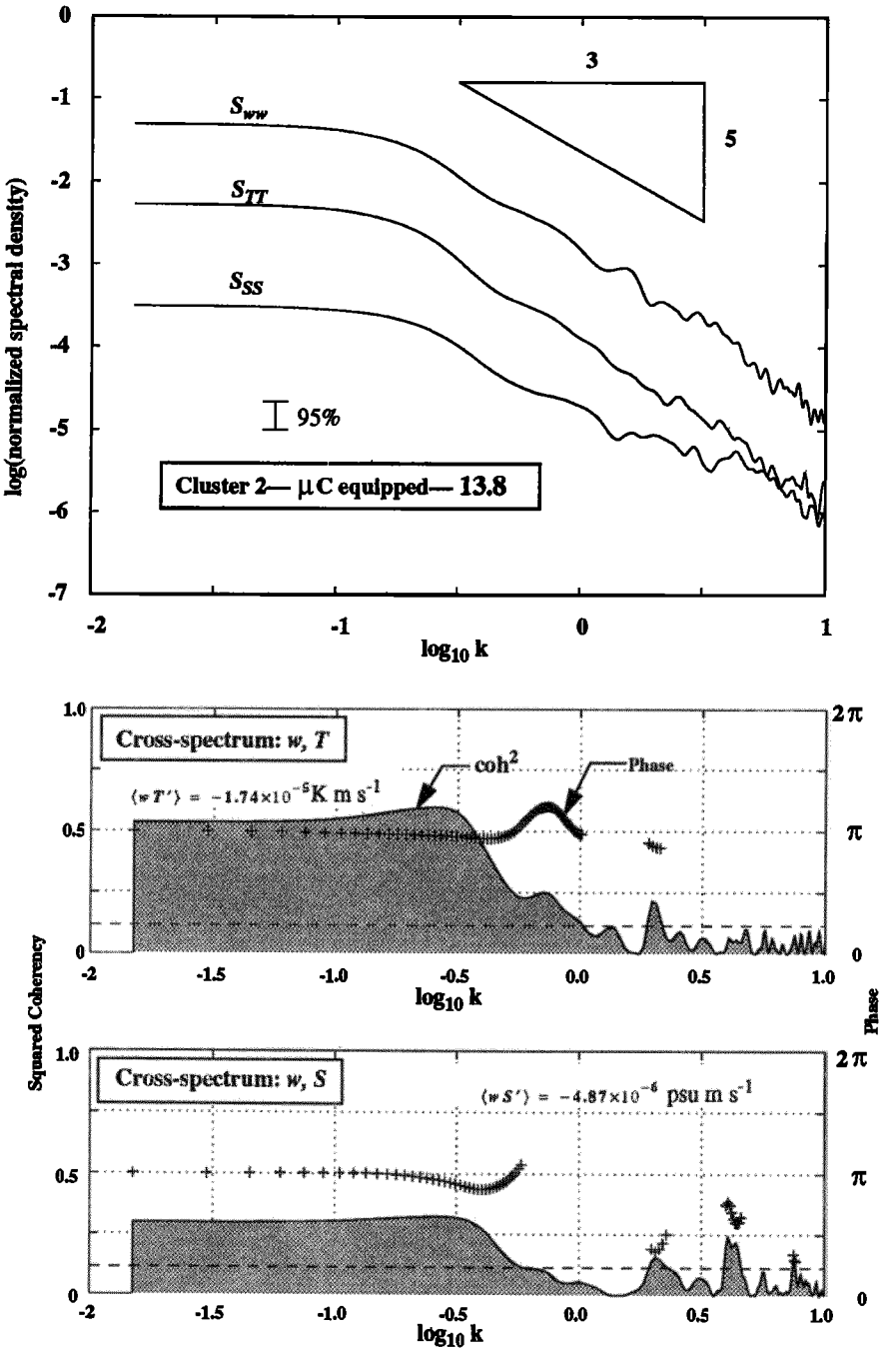

Figure 14. Same as Figure 13, except for the time series of Figure 2, when the mast was at middepth in the mixed layer and heat flux was downward.

local flow regime was probably influenced by horizontal gradients. Nevertheless, there are several interesting features in the spectra which are roughly consistent with a "one-dimensional" interpretation. First, there is a plateau in the w spectrum in the wavenumber range between about 0.5 and 1.6 rad m^{-1}, which also shows up as a marginally significant peak in the T' spectrum and a similar plateau in the S' spectrum. These scales are associated with generation of turbulence by shear in the surface layer, which at this level is often more energetic than the longer convective-scale turbulence [*McPhee*, 1994]. Note, however, that scalar coherencies are dominated by the larger scales and appear to be similar to those found lower in the boundary layer. At higher wavenumbers the S_{SS} spectrum begins to roll off relative to the other normalized spectra, as filtering from the conductivity flow restriction becomes important. Despite secondary peaks in coherency above $k = 1$ rad m^{-1} it appears that most of the covariance occurred at lower wavenumbers, and, in fact, the calculated salinity flux using the standard C meter was about 3/4 of that calculated using the microconductivity TIC 1 m lower. As an experiment, the TIC 2 data were filtered and decimated to give a sampling interval of 20 s, which yields a Nyquist wavenumber, $k_N = \pi/(\langle U \rangle \delta t)$, of 1.4 rad m^{-1}, corresponding approximately to the wavenumber at which the standard cell salinity begins rolling off. Salinity flux

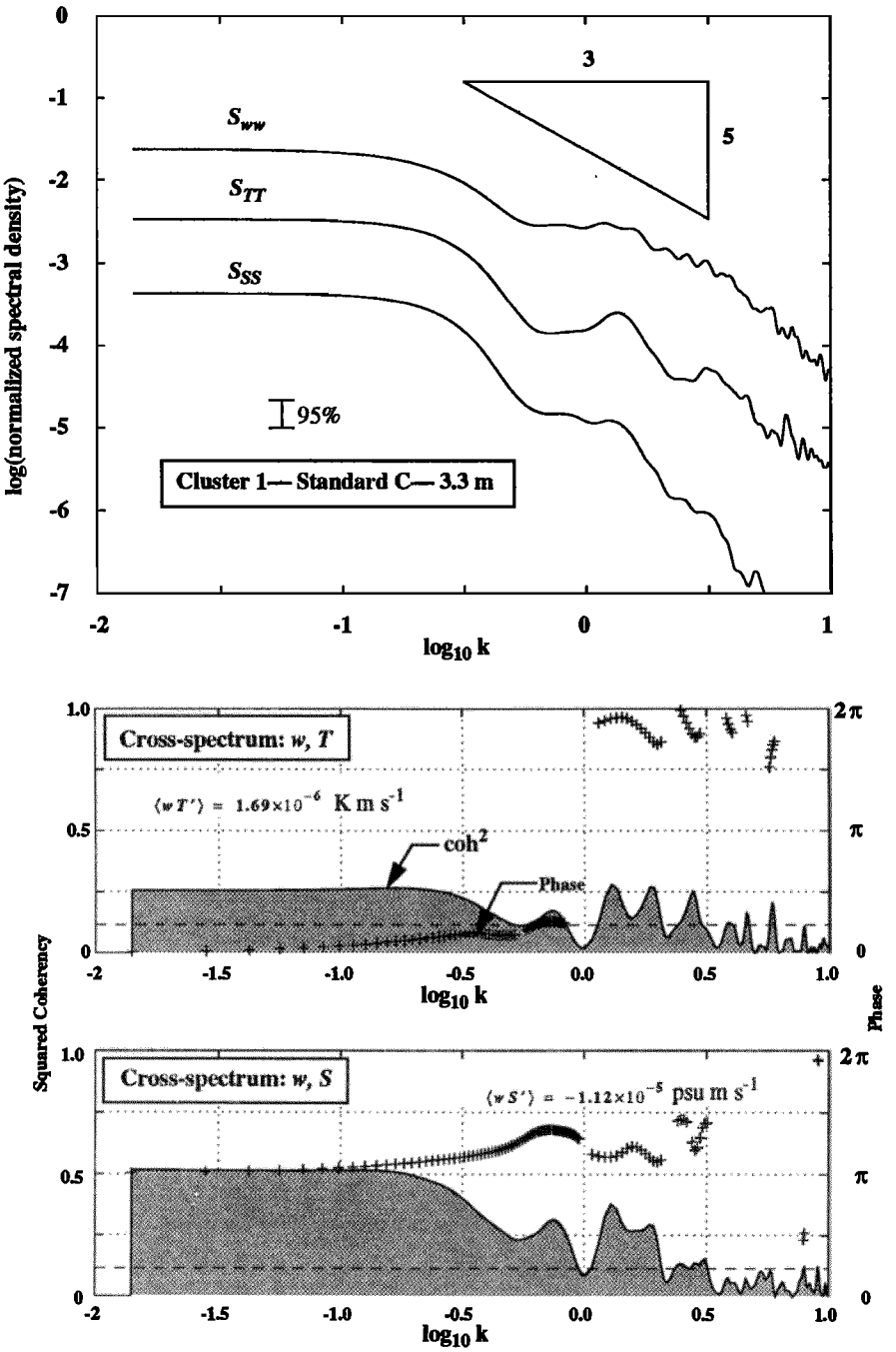

Figure 15. Same as Figure 13, except with TIC 1 mounted 1 m above TIC 2 and standard SBE04 conductivity meter.

calculated from the filtered microconductivity time series is -1.17×10^{-5} psu m s^{-1}, a decrease of 24% from the unfiltered covariance and quite close to the value obtained with the nearest standard cluster. Over the period from 98.25 to 98.75, salinity fluxes calculated using the standard SBE04 conductivity meters in TICs 3 and 4 (6.4 and 9.4 m depth) were smaller than TIC 2 by 26% and 24%, respectively.

3.4. TKE Dissipation

Turbulent kinetic energy dissipation rate ε was calculated from both instrument systems but from quite different points of view. For the profiling system, ε was obtained from the microscale shear according to (1).

Dissipation was also estimated from the power spectrum of vertical velocity measured at discrete depths using inertial subrange arguments and the Kolmogorov relationship (see *McPhee and Martinson* [1994] and *McPhee* [1994] for its application to the TIC system):

$$\varepsilon^{2/3} = \frac{3}{4\alpha_\varepsilon} S_{ww}(k) k^{5/3} \tag{4}$$

where k is the wavenumber, obtained assuming advection of

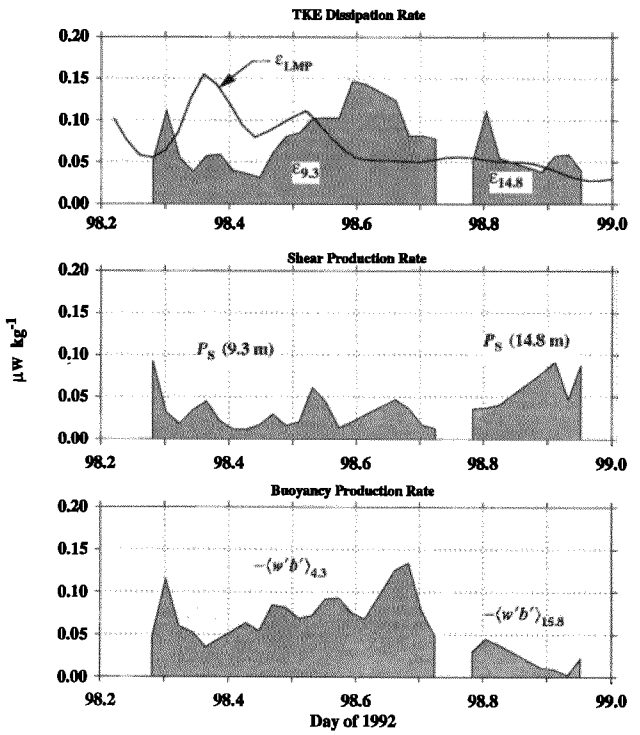

Figure 16. Turbulent kinetic energy (TKE) budget, showing (top) dissipation rate, (middle) shear production rate, and (bottom) buoyancy production rate. Solid curve (top) is from the loose-tethered microstructure profiler; shaded curves are from the turbulence instrument cluster nearest 10 m depth for dissipation (top) and shear production (middle). Buoyancy production (bottom) is from TIC 2, equipped with a microconductivity sensor.

turbulence with the mean flow (Taylor's hypothesis), S_{ww} is the w power spectral density, and α_ε is the Kolmogorov constant, taken here to be 0.51. We compared dissipation estimates made on day 98, when the flow was fairly energetic, as shown in Figure 16. As before, profiler data were averaged over a 10-m depth interval centered at 10 m. During the first part of the period the TIC mast was shallow, so we used data from the lowest cluster, at 9.3 m depth. When the mast was later lowered to the middle part of the mixed layer, we used the topmost cluster, which was at 14.8 m. During the first part of the period the two methods agree well for the average values as follows: 0.75×10^{-7} W kg^{-1} for the profiler versus 0.73×10^{-7} W kg^{-1}, despite the discrepancy around time 98.4. On shorter timescales we believe the differences are due mainly to the natural intermittency of the large convective plumes, as discussed, for example, by *Muench et al.* [1995]. After the mast was lowered (98.75 to 98.95) the average TIC estimate was about 15% larger than the profiler average.

A quantity closely related to ε is the production of turbulent kinetic energy by vertical shearing, approximated by the Reynolds stress squared divided by eddy viscosity:

$$P_S = u_*^3/\lambda \tag{5}$$

where λ is the mixing length. It appears [*McPhee and Smith*, 1976; *McPhee*, 1994] that a consistent estimate of the local value of λ may be obtained from the length scale associated with the peak in $kS_{ww}(k)$, the weighted w spectrum (see also *Busch and Panofsky* [1968]), namely,

$$\lambda = c_\lambda/k_{\max} \tag{6}$$

where $k_{\max}$ is wavenumber at the spectral peak and c_λ is a constant, about 0.85. Combining (5) and (6) has provided a tractable way of estimating shear production in horizontally heterogeneous, rotating flows from previous experiments [*McPhee and Martinson*, 1994; *McPhee*, 1994], despite the fact that the shear production term in the TKE equation is a complicated sum of local mean velocity gradients and Reynolds stress tensor components. Results are shown in Figure 16 (middle). At 9.3 m the average value of P_S is 0.27×10^{-7} W kg^{-1}, which is only 30–40% of the dissipation rate. In a horizontally homogeneous flow the difference would be ascribed to the vertical buoyancy flux according to the simplified TKE equation

$$P_S - \langle w'b'\rangle \approx \varepsilon. \tag{7}$$

However, stationed as we were at the edge of a moving, active lead, we suspect that horizontal divergence terms in the TKE equation were not necessarily negligible. Buoyancy flux time series, based on microconductivity measurements at 4.3 m (when the mast was shallow) and 15.8 m (mast middepth), are shown in Figure 16 (bottom). While the balance is not exact between shear and buoyancy production on the one hand against dissipation on the other, Figure 16 demonstrates approximate equivalence and shows that production is dominated by buoyancy, at least away from the immediate surface layer, except during the later period when freezing had slowed.

4. Lead 4 Results

Boundary layer flow at lead 4 was much less energetic than at lead 3. Within a few hours of first deployment, currents had fallen off to near-background geostrophic levels (<4 cm s^{-1}). LMP casts began at about 103.0, when the lead was covered with a 1-cm skim of ice, and continued for approximately 30 hours. Owing to a computer malfunction, the TIC measurements began later (103.2) and continued for about 24 hours. Both programs were curtailed when ice rafting threatened the instrumentation. In low-energy flows the TIC mechanical current meters are prone to dropout as one or more of the rotors in each triplet falls below threshold. The LMP is not subject to low threshold limits as long as the turbulence levels are above the noise floor of the instrument.

With the TIC system all three velocity components are required to estimate the Reynolds stress tensor. It is possible, however, to make estimates of scalar vertical fluxes with only two rotors turning, provided they are the ones that are canted at an angle to the horizontal (because the third, horizontal component has little effect on the vertical velocity determination). By good fortune, the one cluster for which the canted rotors turned consistently at lead 4 was TIC 2, which was equipped with the microconductivity meter. So although it was not possible to measure turbulent stress directly in the low currents (the horizontal component of TIC 2 was sporadic), we were often able to measure vertical velocity and to form the covariances $\langle w'T'\rangle$ and $\langle w'S'\rangle$. Convective features with transient vertical velocities nearly as large as the mean horizontal current passed the TIC mast frequently (Figure 3), and these were often correlated with large excursions in temperature and salinity, as at lead 3. Using $w'T'$ time series from TIC 2 and dissipation (TKE and thermal) measurements from the LMP,

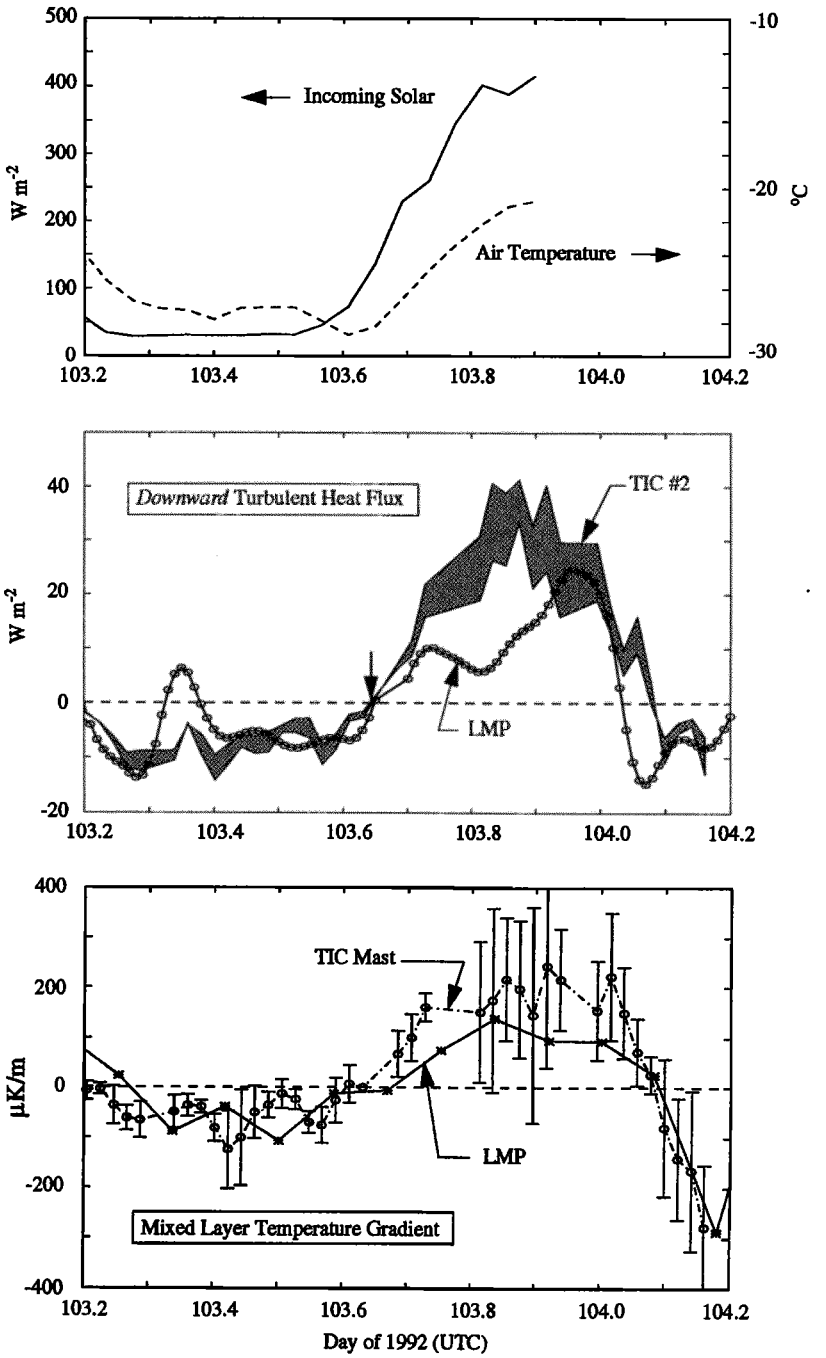

Figure 17. Thermal summary at lead 4, similar to Figure 11, except that the TIC heat flux is from cluster 2 only, which was the only one with consistent w measurements.

we were able to construct a thermal history for 24 hours at lead 4, as illustrated in Figure 17.

The stall speed of each individual TIC rotor is around 1 cm s^{-1}, and the rotors which sense vertical velocity are oriented 45° up and down from the horizontal. As pronounced vertical velocity events passed, the pitch of the mean flow vector would sometimes increase to the point where one of the rotors quit turning until its particular component again exceeded stall speed. This presents a choice in data processing: either (1) include below threshold values in the stalled component as zeros when forming w, which would tend to overestimate scalar covariance by introducing spurious variance in w, or (2) consider only samples for which both components are clearly turning, which would tend to underestimate covariance by selecting against times when the vertical velocity and instantaneous flux are greatest. The calculations were done both ways, with the difference indicated by the shaded envelope for heat flux in Figure 17b. The differing treatment may lead to discrepancies as large as 10–15 W m^{-2}; nevertheless, Figure 17b illustrates a reasonably coherent view of heat flux in the mixed layer similar to that observed at lead 3, with a significant amount of midday solar radiation penetrating the ice/ocean interface and being mixed down. In this case, mixing was dominated almost entirely by "free" convection. At times, there

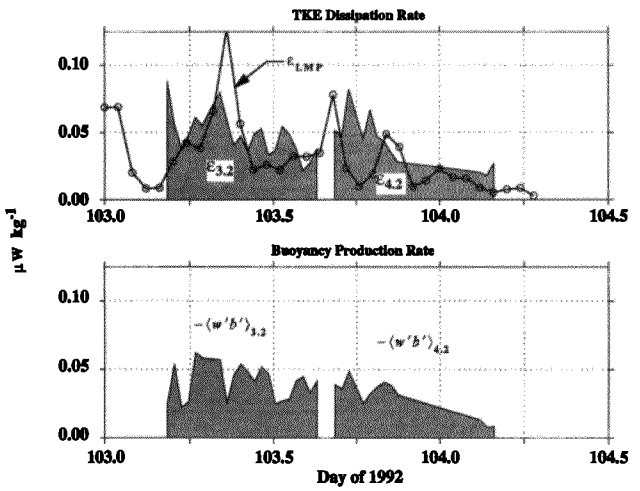

Figure 18. (top) TKE dissipation from the loose-tethered microstructure profiler (solid curve) and from TIC 2 (shaded area) at depths shown. (bottom) Buoyancy production from TIC 2, equipped with a microconductivity sensor. TIC dissipation and buoyancy production were calculated only from 1-hour time series that exhibited well-behaved inertial subranges in w and S' spectra.

were fairly large differences in heat flux estimates from TIC 2 and the LMP, but the LMP was using averages over a 5-m bin centered at 10 m depth; hence one might expect natural variation, especially without strong mechanical mixing.

Temperature gradients were calculated as before by adjusting TIC calibrations to a time when heat flux was negligible (arrow at 103.65 in Figure 17b). Again, there is rough correspondence between the TIC and LMP gradients, despite very small magnitudes.

Calculating TKE dissipation from inertial subrange spectral levels is also tricky when the clusters are near threshold velocity. Even with some confidence in the TIC 2 w measurements, lack of the third component precludes an accurate measure of mean flow speed, which is needed to transform frequency spectra to wavenumber spectra (not to mention uncertainties in Taylor's hypothesis at very low speeds). Measuring as it does near the high end of the spectrum, the LMP shear probe does not suffer the same drawbacks. For the sake of comparison, w power spectra were calculated as a function of frequency as before, then transformed to wavenumber space by substituting the horizontal component of velocity from TIC 3 (which did turn consistently) to get the mean speed. Results of the comparison are shown in Figure 18 (top). During the first part of the period, TIC 2 was at 3.2 m depth, later lowered to 4.2 m. The correspondence between the two ε estimates is not bad; mean values are 4.6×10^{-8} and 2.9×10^{-8} W kg^{-1} for TIC 2 and the LMP, respectively. Again, since the cluster measurement was higher in the boundary layer, one would expect somewhat higher dissipation rates there. Figure 18 (bottom) shows that TKE buoyancy production and dissipation are in approximate balance. Buoyancy flux was calculated from $\langle w'S' \rangle$ using microconductivity at TIC 2, subject to the caveats mentioned above for the heat flux calculations ($\langle w'T' \rangle$ was used in the buoyancy flux calculation but contributed less than 0.5%). The mean value for P_b was 3.7×10^{-8} W kg^{-1}, about halfway between the ε estimates.

Because of the large uncertainties introduced into the TIC calculations as a result of the low mean flow energy, we consider the lead 4 results as indicating qualitative trends only, and they were not used in the quantitative assessment of derived quantities discussed in section 5. A more complete description of microstructure properties at lead 4 is underway (T. Stanton, manuscript in preparation, 1995).

5. Discussion

5.1. Turbulent Diffusivities

From independent flux and scalar gradient measurements at lead 3, we derived eddy diffusivities typical of lead conditions. The scalar eddy diffusivities for heat K_h and salinity K_S are

$$K_h = -\langle w'T' \rangle \Big/ \frac{dT}{dz}$$
$$K_S = -\langle w'S' \rangle \Big/ \frac{dS}{dz} \tag{8}$$

K_h was calculated using thermal gradients from each system, as discussed in section 3.2. Results are summarized as scatter diagrams and linear fits (assuming zero intercept) for lead 3 in Figure 19a. The two lines were obtained from regression of measured $\overline{\langle w'T' \rangle}$ (where the overbar indicates averaging across the mast) against the LMP (mixed layer) thermal gradient and the TIC (6-m span) thermal gradient. On the basis of mean mixed layer thermal gradient from the LMP, the eddy thermal diffusivity was $K_h = 443 \pm 92$ cm^2 s^{-1}, where the errors represent the 95% confidence limit of the regression slope, assuming independent, normally distributed errors in the measurements. Using thermal gradient based on the TIC mast temperatures yielded $K_h = 473 \pm 214$ cm^2 s^{-1}. In the latter estimate the confidence interval is more than twice as large, but mean values are in reasonable agreement.

There was no "zero-flux" calibration point for salinity measurements, and as discussed in section 3.3, only the microconductivity cluster measured consistently into the inertial subrange. Consequently, K_S was determined from the flux measurements of TIC 2 and 1-hour average salinity gradients from the LMP across the mixed layer from 3 to 20 m depth. The data pairs (Figure 19b) are much more widely scattered than the heat flux/thermal gradient pairs, as reflected in a regression slope, which is barely significant at the 95% confidence level, $K_S = 493 \pm 451$ cm^2 s^{-1}. Nevertheless, the average salinity eddy diffusivity is similar to the better sampled thermal diffusivity, in keeping with Reynolds analogy for high Reynolds number flows.

Given measurements of Reynolds stress (momentum flux) and mean velocity, it is possible, in principle, to estimate bulk eddy viscosity for the mixed layer in the same way as eddy diffusivities were estimated above. However, in practice, this is rarely feasible for several reasons. First, a horizontal density gradient, which we expect near leads, will induce vertical geostrophic shear ("thermal wind") unrelated to the momentum flux. Second, stress and mean velocity profiles are easily distorted, especially in direction, by pressure forces associated with nonuniform under-ice topography. Finally, in rotational boundary layers, much of the shear associated with turbulence in the outer part of the layer is manifested as angular shear, with very small gradients in speed. Thus even in the absence of horizontal inhomogeneities, it takes extremely well aligned and accurate current meters to measure the outer-layer shear. An

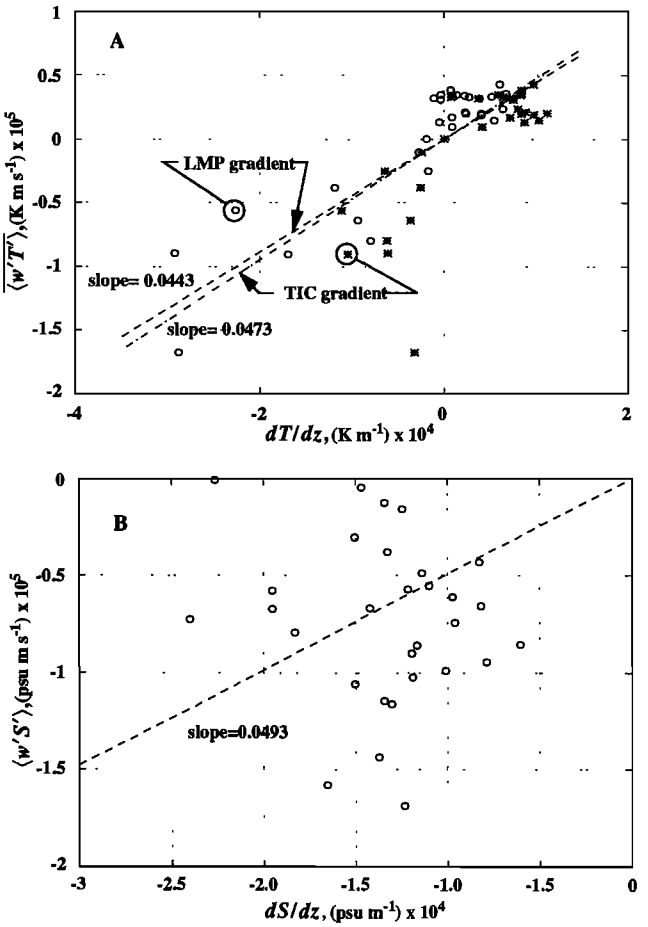

Figure 19. Scatterplots and least squares regression lines (zero intercept) for eddy diffusivity estimates. (a) TIC mast average kinematic heat flux versus LMP thermal gradient and TIC mast gradient after thermometers were adjusted to agree when heat flux was negligible. (b) TIC 2 salinity flux versus LMP salinity gradient.

alternative method for estimating eddy viscosity ($K = u_*\lambda$, where λ is inversely proportional to the wavenumber at the peak in the weighted w spectrum) was used in section 3.4 in the context of estimating TKE dissipation. For comparison, we calculated the product of u_* and λ for each 1-hour sample from the TIC nearest to 10 m depth (9.4 m with the mast shallow, 13.4 m with the mast middepth) and averaged over the same time period as the diffusivity regressions. The result was $K = 551$ cm^2 s^{-1} with a standard deviation of 283 cm^2 s^{-1}. Thus all three eddy diffusion coefficients, despite fairly large sample-to-sample variability, agree in the mean to within about 20%. *McPhee and Martinson* [1994] reported eddy viscosity and thermal diffusivity measured in a boundary layer with negligible surface buoyancy flux during a storm in the western Weddell Sea and suggested that the maximum eddy viscosity (and diffusivity) in the neutrally stable boundary layer could be estimated by the formula $K = 0.02u_{*0}^2/f_{\rm cor}$, where $f_{\rm cor}$ is the Coriolis parameter. During the time at lead 3 when the TIC mast was shallow, the average value of u_* at 3.4 m was 0.61 cm s^{-1}. Using this as an estimate of the interfacial value yields a neutral maximum eddy viscosity of about 53 cm^2 s^{-1}, an order of magnitude less than we found. The present results are thus consistent with the significant increase in mixing length reported by *McPhee* [1994] and illustrate how even modest destabilizing surface buoyancy flux can greatly increase the efficiency of turbulent mixing.

5.2. Kolmogorov Constants

Provided the turbulent flow is approximately horizontally homogeneous, the inertial dissipation technique may be used to estimate scalar fluxes as well as Reynolds stress [*McPhee*, 1994]. A two-step procedure is used. First, an analog of the TKE dissipation/spectral density relation in the inertial subrange (4) is solved for the "scalar variance dissipation" [*Hinze*, 1975]:

$$\varepsilon_S = \frac{\varepsilon^{1/3}}{\alpha_S} S_{\rm SS}(k)k^{5/3} \tag{9}$$

where, e.g., ε_S is the salinity variance dissipation, and α_S is the Kolmogorov constant for salinity. The flux magnitude is then obtained from the steady, horizontally homogeneous variance equation:

$$\left|\langle w'S'\rangle \frac{\partial S}{\partial z}\right| = \frac{\langle w'S'\rangle^2}{u_*\lambda} = \varepsilon_S \tag{10}$$

assuming that eddy viscosity and eddy salt diffusivity are similar.

As far as we know, these are the first measurements of salinity flux in a naturally occurring, high Reynolds number flow, hence there is no other observational base from which to draw the numerical value of α_S. Given the degree of horizontal inhomogeneity implicit in our measurements near the edge of the lead, it is highly questionable whether the assumptions behind (10) hold, i.e., that terms involving horizontal gradients are negligible. However, if scalar temperature and salinity behave similarly in a high Reynolds number flow, we expect the unknown terms to be roughly comparable, thus our objective was to determine whether α_S differed significantly from α_T under these particular flow conditions. If not, there is justification for assuming that the scalar Kolmogorov constants for heat and salt are similar under most conditions, as they are for heat and water vapor in the atmosphere [e.g., *Edson et al.*, 1991]. With this in mind, we derived an average value for α_S from the flux, dissipation, and salinity spectra data (TIC 2) using (9) and (10) and compared it with a similarly derived value of the average thermal Kolmogorov constant α_T at lead 3. In the atmospheric surface layer, α_T is found to be about 0.8, which was consistent with heat flux and temperature spectra in the neutrally stable boundary layer under Ice Station Weddell, 1992 [*McPhee*, 1994].

Results of the calculations are shown for TIC 2, when the mast was shallow, in Figure 20. Fitted 1-hour spectra were used to estimate ε and λ (section 3.4) and weighted spectral densities $kS_{\rm SS}(k)$ and $kS_{\rm TT}(k)$, which were evaluated at the lowest wavenumber for which the spectral slope was $\leq -2/3$. These were combined with the measured fluxes to obtain hourly estimates of $\log_{10}(\alpha_{S,T})$. In the salinity calculation, only samples for which $|\langle w'S'\rangle| > 5 \times 10^{-6}$ psu m s^{-1} were used. Similarly, only samples for which $|\langle w'T'\rangle| \geq 1 \times 10^{-6}$ K m s^{-1} were used in the α_T calculations. The mean values are $\overline{\alpha_S} = 0.98$ and $\overline{\alpha_T} = 0.85$. Standard deviations of $\log_{10}(\alpha_S)$ and $\log_{10}(\alpha_T)$ are each about 0.5, so the difference between the two mean estimates has little significance. Considering that the actual flow was far from the idealization implicit in (10), the correspondence of $\overline{\alpha_T}$ with its commonly accepted atmo-

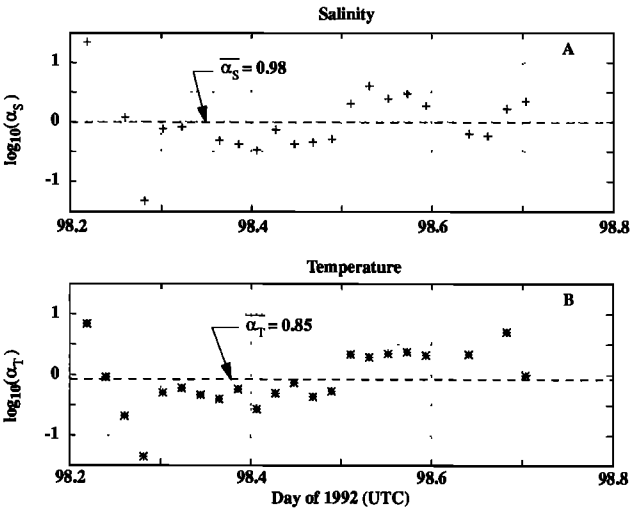

Figure 20. Kolmogorov constants for (a) salinity variance dissipation and (b) temperature variance (thermal) dissipation. Dashed horizons show the mean of the logarithms in each case.

spheric counterpart was surprising. It may have been merely fortuitous but might also indicate that even in relatively inhomogeneous flows, the scalar variance equations (and the w spectrum) are dominated by vertical exchange processes.

Our analysis suggests that credible estimates of salinity flux (as well as heat flux) can be made from spectral levels in the inertial subrange using techniques analogous to the inertial-dissipation method used in the atmosphere, provided the salinity spectrum is adequately resolved and either the mixed layer salinity gradient is measured or the proper length scale in (10) is determined. The Kolmogorov constant for salinity appears to be comparable to that for temperature, of order 1.

5.3. Implications for Modeling

Our results show consistently that the scale of turbulent eddies which are primarily responsible for turbulent transfer under freezing leads is significantly increased by destabilizing surface buoyancy flux. In terms of eddy viscosity (or equivalently, mixing length), the increase at lead 3 was tenfold, despite relatively mild instability ($D/L \approx -2.3$; see section 1). Thus wherever rapid freezing occurs, turbulent mixing will be significantly more efficient. In modeling these effects, however, it is important to consider the increase in length scale. Using the peak in the weighted w spectrum as indicative of the scale of the main energy-containing eddies, typical wavelengths ($2\pi/k_{max}$) were about 70 m at lead 3 (that is, a major convective cell passed about every 10 min, on average). A parameterization of these processes in terms of the increased eddy viscosity or mixing length is only valid on horizontal scales larger than this. A very fine scale numerical lead model, say with horizontal resolution of order 10 m, ought to resolve such eddies and would become, in essence, a large-eddy simulation (LES) model. In this case, a subgrid-scale parameterization of turbulence would be more appropriate than eddy viscosity models based, e.g., on the mixing length suggested by *McPhee* [1994] or Richardson-number-dependent "shape functions" in second-moment closure [*Mellor and Yamada*, 1982]. By the same token, flow statistics in such a model would only be valid averaged over many LES realizations, and the chances of simulating a specific observed event would be very small, due to the chaotic character of the turbulent motions.

5.4. Concluding Remarks

In addition to our investigation of the impact of destabilizing surface buoyancy flux on oceanic boundary layer dynamics, this study provided a rare opportunity to directly compare two turbulence quantities, ε and $\langle w'T' \rangle$, measured in the mixed layer by entirely different methods. In general, we found the results encouraging; mean values of dissipation at lead 3 agreed to within a few percent and both estimates of heat flux showed the diurnal cycle apparent in mixed layer temperature and temperature gradient with roughly similar magnitudes (they are not directly comparable because of different sampling regions within the mixed layer). We also combined data from the two systems for a unique means of demonstrating that eddy salt, heat, and momentum diffusivities are similar, which, as far as we know, has not been done before.

A point worth stressing in conclusion is that relatively mild destabilizing surface buoyancy flux has a large impact on the scales of turbulence in the boundary layer and hence on the efficiency of turbulent exchange. The mean buoyancy flux at lead 3 when TIC 2 was at 4.3 m depth was about -0.73×10^{-7} W kg^{-1}. For temperate water with mixed layer temperature equal to 15°C, this corresponds to a surface heat loss of around 150 W m^{-2}, which is not particularly large; the annual average surface heat loss, for example, in the Atlantic off the east coast of North America exceeds 250 W m^{-2} [*Gill*, 1982, Figure 2.7]. Yet this was enough to increase the eddy diffusivity (viscosity) by an order of magnitude from what we would expect under neutral conditions (Figure 19) and to appreciably alter the character of the turbulent flow (compare Figures 2 and 4). There is currently much active research regarding the role of Langmuir circulations on mixing efficiency in the open ocean, with most of the emphasis on surface wave forcing [*Leibovich and Paolucci*, 1980]. Indeed, numerical modeling by *Li and Garrett* [1995] suggests that wave forcing nearly always dominates over thermal convection in the open ocean. Since surface waves were absent at lead 3 (the surface had frozen by the time flux measurements were underway), these results provide a control which illustrates formation of organized convective features, apparently related to the depth of the mixed layer [*McPhee*, 1994], that significantly increase the effective eddy viscosity. Thus, while we readily acknowledge that Langmuir circulations may increase mixing efficiency under moderate stress conditions in the neutrally or stably stratified open ocean relative to that observed under sea ice, we think the present results show that static instability will play an important, possibly dominant role in open ocean mixing under many commonly encountered situations.

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Cc: Abigail McEachern[acm2005@wildcats.unh.edu]; Terry Hazen[hazenlab.utk@gmail.com]
From: Mandsager, Kathy
Sent: Tue 8/30/2016 3:47:19 PM
Subject: RE: State of Science Degradation & Fate group
White et al. 2014 Long-Term Persistence of Dispersants following the Deepwater.pdf
2016 08 18_LINDEN_Degradation and Fate with public input_MBL.docx

Last minute reminder of call today and since Mary Beth Leigh added a few comments, I am including that version of the document here (which is what we will use on today's call). I am also resending the White et al, 2014 paper for your review, in case you haven't had a chance to look it over recently.

-----Original Appointment-----
From: Mandsager, Kathy
Sent: Tuesday, August 23, 2016 1:22 PM
To: conmy.robyn@epa.gov; fingasmerv@shaw.ca; tchazen@utk.edu; robert.jones@noaa.gov; mandyjoye@gmail.com; mbleigh@alaska.edu; karl.linden@colorado.edu; kmmcfarlin@alaska.edu; msmiles@lsu.edu; thomas.s.coolbaugh@exxonmobil.com; mathijs.smit@shell.com; sprenger.mark@epa.gov; Gary Shigenaka - NOAA Federal; doug.helton@noaa.gov; 'principe.vanessa@epa.gov'; 'wilson.gregory@epa.gov'; mjoye@uga.edu
Cc: Abigail McEachern; Terry Hazen
Subject: State of Science Degradation & Fate group
When: Tuesday, August 30, 2016 2:00 PM-4:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: via WebEx (details below)

This is a reminder of our next WebEx call. Attached is the latest version of our document. Please review carefully, peruse unfamiliar publications, and make verbiage suggestions in your margins to encourage more efficient discussion.
Thank you! << File: 2016.08.18_LINDEN_Degradation and Fate with public input.docx >>

Degradation & Fate group

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Environmental Science & Technology Letters

Letter

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Long-Term Persistence of Dispersants following the Deepwater Horizon Oil Spill

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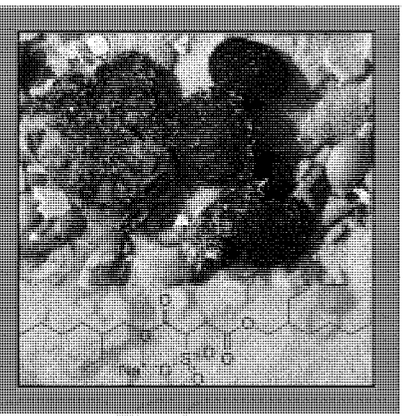
* Supporting Information

During the 2010 Deepwater Horizon (DWH) oil spill, 1.84 M gallons of chemical dispersant was applied to oil released in the subsurface and to oil slicks at the surface. We used liquid chromatography with tandem mass spectrometry to quantify the anionic surfactant DOSS (dioctyl sodium sulfosuccinate) in samples collected from environments known to contain oil persisting from the DWH oil spill. DOSS was found to persist in variable quantities in deep-sea coral communities (6−9000 ng/g) 6 months after the spill and on Gulf of Mexico beaches (1−260 ng/g) 26−45 months after the spill. These results indicate that the applied dispersant, which was thought to undergo rapid degradation in the water column, remains associated with oil in the environment and can persist for ~4 years.



INTRODUCTION

To mitigate the impact of the ~5.0 M barrels (~210 M gallons) of oil released as a result of the Deepwater Horizon (DWH) blowout,[1] dispersants were applied to surface and subsurface waters of the Gulf of Mexico in unprecedented quantities (1.07 and 0.77 M gallons, respectively).[2] The dispersants used (Corexit 9527 and Corexit 9500A, from Nalco Co.) contain the anionic surfactant dioctyl sodium sulfosuccinate (DOSS), which has amphiphilic properties that can lower the interfacial tension between oil and water and hamper the formation of large oil slicks on the surface of the ocean. Little is known about the persistence of DOSS in the marine environment, and studies to date have focused primarily on detection techniques in aqueous samples.[3−6] Examination of Gulf of Mexico subsurface waters following the DWH oil spill indicated that DOSS acts conservatively in the water column and is not removed significantly by biodegradation or sedimentation.[7] In contrast, laboratory incubation experiments have shown that the hydrocarbon components of Corexit are degraded[8] and that DOSS is also rapidly degraded at 25 °C.[9] In addition, DOSS has found to be susceptible to hydrolysis and photodegradation under laboratory-simulated solar conditions that occur in surface waters.[10] The biodegradation of DOSS in the absence of sunlight[10] and at temperatures representative of the subsurface (5 °C),[9] however, has been found to be much slower, reinforcing the finding that DOSS persists in subsurface waters following the DWH spill.[7] Here we examine the long-term persistence of DOSS in deep-sea and coastal sediment environments.

EXPERIMENTAL PROCEDURES

Sampling Information. Samples from deep-sea coral communities were collected in December 2010 from lease blocks Mississippi Canyon (MC) 294 at 28.67°N, 88.48°W, 11 km to the southwest of the site of the Macondo well at 1370 m depth, and MC 388 at 28.38°N, 88.10°W, 22 km east southeast of the Macondo well at 1850 m depth. At the MC294 site, coral colonies showed visible signs of stress and were covered in a brown, flocculent material (floc), which contained weathered oil from a source consistent with the Macondo well.[11] At MC388, corals exhibited no visible signs of stress and were not covered in floc. Coral floc was therefore collected from only the MC294 site, while sediment samples were collected from both MC294 and MC388 (collection details described in ref 11). Additional oiled samples were collected between June 2012 and January 2014 from Florida, Alabama, Mississippi, and Louisiana beaches (see Figure S1 of the Supporting Information). These samples were similar to others that have washed onto Gulf coast beaches since July 2010 and are termed oil-soaked sand patties, tar balls, or surface residual balls. The weathered oil present in these types of samples has been found to be consistent with an origin from the DWH spill.[12−14] After homogenization, each sample was split for the quantification of DOSS (50% of the sample) and oil (50% of the sample).

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Table 1. Dates Collected, and Water, Oil, and DOSS Contents of Coral Flocculent Material and Sediment Samples

sample	date collected	water (%)	oil content (μg/g)[a]	DOSS content (ng/g)[b]	dispersant:oil ratio (%)[c]
flocculent material from MC294					
coral (F6)	12/09/10	NM[e]	9.2	16	0.81
coral (B8)	12/14/10	NM[e]	460	11	0.011
coral (E3)	12/10/10	NM[e]	73	8.3	0.054
coral (A5)	12/14/10	NM[e]	81	6.1	0.036
surface sediments from MC294					
4660 core 1 0−1 cm	12/08/10	71	380	22	0.028
4660 core 1 1−2 cm	12/08/10	83	170	BD[d]	BD[d]
4660 core 2 0−1 cm	12/08/10	83	300	BD[d]	BD[d]
4660 core 2 1−2 cm	12/08/10	80	160	BD[d]	BD[d]
4661 core 1 0−1 cm	12/09/10	74	120	3700	15
4661 core 1 1−2 cm	12/09/10	71	600	BD[d]	BD[d]
4661 core 2 0−1 cm	12/09/10	81	98	BD[d]	BD[d]
4661 core 2 1−2 cm	12/09/10	74	610	BD[d]	BD[d]
4662 core 8 0−1 cm	12/10/10	84	1200	BD[d]	BD[d]
4662 core 8 1−2 cm	12/10/10	93	32	BD[d]	BD[d]
4662 core 11 0−1 cm	12/10/10	81	1400	2400	0.85
4662 core 11 1−2 cm	12/10/10	85	60	BD[d]	BD[d]
4664 core 5 0−2 cm	12/14/10	65	9300	19	0.0010
4664 core 6 0−2 cm	12/14/10	86	120	BD[d]	BD[d]
subsurface sediment from MC294					
4664 core 5 2−5 cm	12/14/10	69	370	BD[d]	BD[d]
4664 core 6 2−5 cm	12/14/10	69	31	BD[d]	BD[d]
4662 core 8 4−5 cm	12/10/10	91	450	BD[d]	BD[d]
4662 core 11 4−5 cm	12/10/10	95	460	BD[d]	BD[d]
sediments from MC388					
4663 core 5 0−1 cm	12/11/10	94	150	9000	29
4663 core 5 1−2 cm	12/11/10	93	12	BD[d]	BD[d]

[a]Oil content measured by gas chromatography coupled to flame ionization detection [GC−FID (see SM)] reported as micrograms per gram of dry sediment for sediment samples and micrograms per filter for flocculent material. [b]DOSS (dioctyl sodium sulfosuccinate) reported as the average of dual or triplicate injections in nanograms per gram of dry sediment for sediment samples and micrograms per filter for flocculent material. [c]Dispersant:oil ratio calculated from DOSS concentrations where DOSS comprises 21% of the dispersant (as described in ref 6). [d]Below detection determined to be <3 times the procedural blank. [e]Not measured.

DOSS Extraction. To examine the persistence of dispersants in all samples, including deep-sea sediment, coral floc, and oil-soaked sand patties, a method was developed to extract DOSS from solid matrices. Initial extraction recovery experiments were conducted with Gulf of Mexico sediments from 10 to 15 cm depths, which were not expected to contain DOSS. These samples (1−2 g wet weight) were spiked with 100 ng of DOSS and examined alongside procedural blanks of combusted (450 °C, 8 h) sand. Sediments were extracted in combusted glass centrifuge tubes with 1−3 mL of a methanol/water mixture at ratios of 3:1, 1:1, and 1:3 by sonication for 30 min at 40 °C. Samples were then centrifuged for 8 min at 800 rpm. The extract was transferred to an autoclaved Eppendorf tube and centrifuged for 8 min at 20000g, to pellet any fine particulate material. The extract was then removed, split between two new Eppendorf tubes, and dried at 30 °C in a speedvac for 1.5−3 h. A re-extraction was performed on the residual sediment for each sample by adding 1 mL of a 1:1 solution of MilliQ water and methanol to the solid phase and continuing the extraction procedure using the same glassware and Eppendorfs. Liquid phases from the re-extraction were split evenly between the two dried first-round extractions and dried for an additional 1.5 h at 30 °C using a speedvac. For each sample, one Eppendorf of the dried extract was analyzed for DOSS content and the other archived. The optimal solvent system for extraction of DOSS was determined to be a 1:1 methanol/water system, which recovered 89−119% of the spiked DOSS (n = 3). Sediment samples (1−3 g wet weight), coral flocculent material (one GF/F filter), and Gulf Coast beach samples (2−5 g wet weight) were therefore extracted with a 1:1 methanol/water mixture. Laboratory blanks (1−2 g of combusted sand) were run with every six samples. All samples were kept dry and frozen at −20 °C until the day of analysis via liquid chromatography with tandem mass spectrometry (details provided in the Supporting Information).

Oil Extraction. Sediment and oil-soaked sand patty samples were air-dried to determine their water content prior to extraction. Sediment (1−4 g) and coral flocculent material (one GF/F filter) were then extracted as described in ref 11 using a microwave-accelerated reaction system at 100 °C and 800 W for 30 min with 20 mL of a dichloromethane/methanol mixture (9:1 DCM:MeOH ratio). The extracts were solvent exchanged into hexane and charged onto a glass column (8 cm × 0.6 cm) packed with fully activated silica gel (100−200 mesh). The column was eluted with 4 mL of 5% DCM in hexane (F1), 1:1 DCM hexane (F2), 1% formic acid in DCM (F3), and 1% formic acid in MeOH (F4). Fraction F1 was analyzed for petroleum hydrocarbons via gas chromatography coupled to flame ionization detection (GC−FID). Samples collected from Gulf Coast beaches were extracted as described in ref 5 using 20 mL of a dichloromethane/methanol mixture (8:2 DCM:MeOH ratio). The total extract obtained was weighed

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Table 2. Dates Collected, Locations, and Water, Oil, and DOSS Contents of Samples Collected from Gulf Coast Beaches

sample	date collected	location	water (%)	oil content (g/g)[a]	DOSS content (ng/g)[b]	dispersant:oil ratio (%)[c]
Perdido Beach, FL (PB)						
PB02−15[d]	06/26/12	30.29°N, 87.48°W	0.20	46	110	0.0012
PB25	07/09/13	30.29°N, 87.48°W	0.14	110	4.1	0.000017
PB26	01/25/14	30.29°N, 87.48°W	1.6	190	260	0.00064
Gulf Shores, AL (GS)						
GS14	06/12/12	30.24°N, 87.74°W	8.9	98	51	0.00025
GS27	07/09/13	30.24°N, 87.74°W	10	160	37	0.00011
GS30	01/25/14	30.24°N, 87.74°W	5.8	92	1.2	0.0000060
Fort Morgan, AL (FM)						
FM02	07/09/13	30.22°N, 88.01°W	5.1	200	45	0.00011
FM03	01/25/14	30.22°N, 88.01°W	1.8	87	18	0.00010
Ship Island, MS (SI)						
SI24	06/27/12	30.21°N, 88.98°W	3.6	0.00	BD[e]	BD[e]
SI30	06/27/12	30.21°N, 88.98°W	0.074	85	32	0.00018
Gulfport, MS (GP)						
GP1,3,5,6[d]	07/18/12	30.36°N, 89.09°W	0.22	200	25	0.000061
GP25	07/10/13	30.36°N, 89.09°W	0.13	150	63	0.00021
GP30	01/26/14	30.36°N, 89.09°W	4.2	160	34	0.00010
Grand Isle, LA (GI)						
GI08	06/14/12	29.23°N, 89.99°W	1.7	91	3.4	0.000018
GI40	07/11/13	29.23°N, 89.99°W	0.18	67	4.0	0.000029
Elmer's Island, LA (EI)						
EI19	06/14/12	29.17°N, 90.08°W	3.2	1000	BD[e]	BD[e]

[a]Oil content determined gravimetrically (see SM), reported as milligrams per gram of dry sand patty. [b]DOSS (dioctyl sodium sulfosuccinate) reported as the average of dual or triplicate injections in nanograms per gram of dry sand patty. [c]Dispersant:oil ratio calculated from DOSS concentrations where DOSS comprises 21% of the dispersant (as described in ref 6). [d]Composite of more than one sand patty collected from the same location. [e]Below detection determined to be <3 times the procedural blank.

to provide the mass of total extractable hydrocarbons, and an aliquot was also analyzed via GC−FID (total resolvable hydrocarbons) as well as gas chromatography coupled to mass spectrometry (GC−MS) operated in selected-ion monitoring mode (SIM). Method extraction and recovery studies were performed by spiking samples with a known quantity of hexatriacontane (recovery standard) and stearyl palmitate (internal standard). Laboratory blanks (1−2 g of combusted sand) were run with every 10 samples and were free of petroleum hydrocarbons. Details of analysis via one-dimensional gas chromatography are provided in the Supporting Information.

RESULTS AND DISCUSSION

All deep-sea sediments examined contained oil [12−9300 μg/g of dry sediment (Table 1)], but DOSS was detected only in select surface sediments (0−1 and 0−2 cm) at concentrations ranging from 22 to 3700 ng/g dry weight (Table 1). The presence of oil and DOSS in the surface sediment was patchy, and cores from the same site taken alongside each other exhibited variable concentrations of both. For example, sediment from the MC294 4664 0−2 cm sample, core 5, contains 9300 μg/g of oil and 19 ng/g of DOSS, whereas core 6 taken at the same time, <1 m away, contains 120 μg/g of oil and nondetectable DOSS (Table 1). In addition, there is no linear relationship between the quantities of oil and DOSS in sediments and calculated ratios of dispersant to oil vary from 0.001 to 29% (Table 1). The variation in the dispersant:oil ratio in sediments may arise from additional contributions of oil from natural seepage, which is commonly found in Gulf of Mexico sediments (e.g., refs 15 and 16), although hopanoid biomarker ratios[11] indicate that natural seep oil is predominantly found at depths greater than 2 cm where DOSS is not detected. As such, the variability of dispersant:oil ratios as well as the absence of DOSS in some surface sediment is most likely due to the heterogeneity of the application of the dispersant (discussed in detail below). The absence of DOSS at sediment depths greater than 2 cm and its presence in the uppermost surface sediments indicate a recent input that has not been mixed into deeper sediment. Floc isolated from corals impacted by the DWH oil spill contained 9.2−460 μg/g of oil and 6.1−16 ng of DOSS (Table 1), with dispersant:oil ratios ranging from 0.011 to 0.81% (Table 1). These ratios fall within the range observed for sediments, suggesting a similar source of dispersant and oil to corals and sediments at this site.

Oil-soaked sand patties were collected from various locations on the beach, including the high-tide line (>4 m from the surf; PB02−15, PB25, PB26, and GI08), the surf zone (<1 m from the surf; GS14, GS27, GS30, FM02, FM03, and GI40), between these two areas, midway up the beach (1−4 m from the surf; SI30), and on rock jetties (GP1,3,5,6, GP25, and GP30). All samples contained various quantities of oil [46−200 mg/g (Table 2)] except for one sample collected from Ship Island, MS [SI24 (Table 2)], which was a mud sample with a color and a texture different from those of the oil-soaked sand patty samples (see Figure S2 of the Supporting Information). The DWH spill was determined to be the origin of the oil in all sand patty samples except EI19, based on their distinctive sandy texture and orange and brown color (see Figure S2 of the Supporting Information and description in ref 13) as well as the hopane and sterane biomarker ratios of the oil residue (see Table S1 of the Supporting Information). In all of these samples, DOSS was found above detection limits (1.2−260 ng/g). DOSS was not detected in EI19, a nonsandy, asphaltlike

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sample that was darker brown in color (see Figure S2 of the Supporting Information). This sample was selected for analysis because it was physically distinct, had different hopane and sterane biomarker ratios (see Table S1 of the Supporting Information), and likely did not originate from the DWH spill. Samples similar to EI19 were examined by Aeppli et al.[12] and discounted from further consideration (as DWH-derived) on the basis of their appearance and biomarker ratios that differed from those of the Macondo well oil.

Calculated ratios of dispersant to oil in oil-soaked sand patties are small, ranging from 0.0000060 to 0.0012%, and are at least 1−2 orders of magnitude lower than the ratios calculated for the deep-sea sediments and coral floc samples. Oil-soaked sand patty samples originate from offshore mats of oil, which formed when weathered oil at the surface moved into shallow waters where oil mixed with sand and then sank. These mats of residual oil persist offshore and are generally meters in width, meters to tens of meters in length, and a few to ten centimeters in thickness.[14] The mats may become buried and unburied with sand, and oil-soaked sand patties are transported to shore when conditions for erosion are favorable.[14] The oil that persists in these samples is severely degraded and is comprised of an unresolved complex mixture (UCM) with an n-alkane range between C_{13} and C_{42}. The DOSS in these offshore mats likely originated from applications of the dispersant to surface oil slicks. We hypothesize that DOSS becomes encapsulated within the weathered oil and sand, rendering it unavailable for exchange with the surrounding water, thus limiting dissolution and/or biological uptake and degradation. This protective mechanism, which would allow DOSS to persist in the environment, is widely observed for labile organic compounds in soils and sediments that are protected from degradation by their association with bulk organic matter and minerals.[17,18]

The precise mechanism for the delivery of DOSS to deep-sea sediments and corals is unknown; however, we propose that an encapsulation step as opposed to a surface sorption mechanism[19] is also required to explain the persistence of DOSS in these samples. Deep-water (800−1200 m) plumes enriched in petroleum hydrocarbons[20−23] and DOSS[7] from the DWH spill and applied dispersant could contribute to the delivery of DOSS and oil to the deep sea. Once in these environments, DOSS would have to become trapped within the coral tissue or buried in sediments to preclude further interaction with water and loss through desorption and/or dissolution. It is unlikely that raining particles from the sea surface could have absorbed DOSS from deep-water plumes, as this mechanism would not entrain DOSS in a matrix that inhibits subsequent dissolution. Further, DOSS was evenly mixed within deep-water plumes,[7] suggesting that its delivery to the seafloor by particle removal would be widespread and even in distribution. Instead, the patchiness of the observed DOSS concentrations implicates the transport of DOSS from the sea surface. In the aftermath of the DWH oil spill, large quantities of the oil sank.[24] This oil was mixed heterogeneously with surface-applied dispersants, and subsequent sinking of this mixture would transport both oil and DOSS to the deep sea. Calculating the expected delivery of DOSS from the sea surface to the deep sea is currently impossible given the patchiness of DWH oil in surface sediments and the range in oil:dispersant ratios expected from surface dispersant applications. Nevertheless, as studies begin to gain a better understanding of the spatial distribution of DWH oil in the deep sea, it may be possible to include estimates of DOSS contributions. Regardless of the mode of delivery, DOSS is detected in surface sediments and on impacted corals ~6 months after the spill, confirming the presence of DWH spill-derived compounds in the deep sea.

Overall, these findings indicate that under certain conditions, DOSS can persist for up to 4 years in the environment. The observed dispersant to oil ratios (0.0000060−0.85%) are much lower than the ratios predicted at the time of the spill (2−10%) for all samples except two deep-sea surface sediments (15 and 29%). These data are consistent with the heterogeneous application of the dispersant at the sea surface as well as the highly variable DOSS concentrations measured in the upper 2 m of the water column immediately following the spill.[25] The heterogeneity in dispersant:oil ratios also represents a range of potential yet currently undetermined toxicological impacts. Aqueous concentrations of DOSS that may be harmful to marine life have been established by the U.S. Environmental Protection Agency;[26] however, the toxicity of DOSS in solid-matrix environments such as sediments or sand patties is not known. Only a few studies concerning the toxicity of both oil and dispersant exist,[27−30] yet understanding the toxicity of oil and dispersant mixtures in sediments and sands is critical as it has the potential to affect sand- and sediment-dwelling fauna. Oil-soaked sand patties continue to wash up along Gulf coast beaches and, unless recovered, may constitute a persistent contamination of oil and dispersant in these environments.

ASSOCIATED CONTENT

* Supporting Information

Additional experimental details about instrumental analysis and locations and photographs of samples collected from Gulf coast beaches. 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.

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Public Health

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Thank you!

Degradation & Fate group

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2:00 pm | Eastern Daylight Time (New York, GMT-04:00) | 2 hrs

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Good afternoon ICCOPR Members:

Happy New Year! I hope everyone had an enjoyable holiday season, and are ready for the year ahead.

1. Please find attached the meeting minutes and presentations from the December 14, 2016 ICCOPR meeting.

2. Our next meeting is scheduled for Wednesday, March 8, 2017. Can I get a volunteer agency to host the meeting? Great opportunity to highlight your offices (and have a shorter commute).

3. The Prince William Sound Regional Citizen's Advisory Committee (PWSRCAC) has requested to meet with ICCOPR during their annual DC visit this spring. Specific dates have not been selected yet, but anticipate near the end of March. I will keep you apprised as PWSRCAC develops their travel plans.

4. Here are a few informational items that may be of interest:
   a. Registration for the International Oil Spill Conference has opened. http://www.iosc2017.org/conference/registration
   b. Chris Reddy, WHOI, shared the following article with ICCOPR "What Happened to Deepwater Horizon Oil? Did dispersants help microbes break down oil"

http://www.whoi.edu/oceanus/feature/what-happened-to-deepwater-horizon-oil

c. The National Academy of Sciences Gulf Research Program awarded their Synthesis Grants for 2016. http://www.national-academies.org/gulf/grants/synthesisgrants/synthesis-grants-2016-awardees/index.htm?utm_source=Gulf+Research+Program+Updates&utm_campaign=fa006e053b-2016_12_15_synthesis_grants&utm_medium=email&utm_term=0_367e0eb01a-fa006e053b-402528737

5. If you are receiving this email, and do not believe you should be included in the ICCOPR membership distribution, please let me know.

Look forward to seeing everyone in March.

Very Respectfully,
Kirsten

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