Formation of Rapidly-Sinking, Oil-Associated Marine Snow

Uta Passow

Marine Science Institute, University of California Santa Barbara, CA 93106, USA,
uta.passow@lifesci.ucsb.edu, phone: 805 893 2363

submission Deep Sea Research special Issue; Guest Editor: S. Joye;
subm. 6th Jan 2014
resubm. 11th June 2014

Short title: Oil associated marine snow

Keywords: microbial marine snow; aggregates, Corexit, Gulf of Mexico, oil distribution, sedimentation, Deepwater Horizon

Abstract

Significant amounts of oil accumulated at the sea surface and in a subsurface plume during the Deepwater Horizon (DwH) spill in the Gulf of Mexico (GoM) in 2010. A substantial fraction of this oil was removed from the marine environment by mechanical recovery or burning, or it reached shorelines, whereas another fraction remained within the marine environment, where it dispersed (chemically or naturally), emulsified or sedimented. After the DwH accident the sedimentation of hydrocarbons to the seafloor via rapidly sinking, oil-associated marine snow has become a focus of attention, and it has been hypothesized that marine snow formation significantly impacted the distribution of the oil from the DwH spill.

Here, roller table experiments are presented that investigated the conditions inducing the formation of oil-associated marine snow, focusing especially on the effects of oil type, photochemical aging of oil, and the presence of phytoplankton or dispersant. Large, mucus-rich marine snow, termed microbial marine snow, formed in treatments incubated with the oil that had accumulated at the sea surface. This bacteria-mediated formation of up to cm-sized marine snow in the absence of particles $> 1\mu\text{m}$, represents a unique formation pathway different from that of the physical coagulation of particles. Microbial marine snow, albeit smaller, also formed in the presence of crude oil that had been aged for ≥ 3 weeks in sunlight, but no particles formed in the presence of unaltered crude. The dispersant Corexit 9500A (Corexit: oil ratio = 1: 100) impeded the formation of microbial marine snow, requiring a re-evaluation of the benefits and detriments of Corexit 9500A as a mediating measure. Phytoplankton aggregates also incorporated fossil carbon, providing an alternate pathway for the formation of oil-associated marine snow. The ubiquitous formation and rapid sedimentation of oil-rich marine snow can explain the high accumulation rate of flocculent material at the seafloor and on corals observed after the DwH spill. These results may raise awareness that oil spill response and assessment need to include sedimentation of hydrocarbons via marine snow as a significant distribution mechanism and may guide future modelling efforts and budget calculations.

1. Introduction

The Macondo blowout associated with the Deepwater Horizon (DwH) drilling platform was the largest marine oil spill in history and the first large spill located in the deep (> 1000 m) ocean (Farrington, 2013). Between the start of the release on 20 April 2010 and the final capping on 15 July, an estimated $780,000 \text{ m}^3$ of crude oil and methane mixture was released from the well head at about 1500 m depth. With a density lower than that of seawater, the hydrocarbon mixture rose toward the surface. Due to dilution and dispersion, some of the rising hydrocarbons split off and formed a subsurface plume, whereas about half of the liquid hydrocarbons reached the sea surface, where a thick layer of oil formed (Atlas and Hazen, 2011; Diercks *et al.*, 2010; Zhou *et al.*, 2013).

Despite the initial buoyancy of the material, a substantial fraction of the hydrocarbons returned to the seafloor, where oil spill contaminated material accumulated in the months after the spill, impacting benthic organisms and leaving a clearly marked footprint of the DwH spill (Montagna *et al.*, 2013; White *et al.*, 2012). The processes which caused the redistribution of oil to the seafloor are currently not well understood; however, the rapid transport of particles from the seasurface to the deep ocean is a common global process that is frequently mediated by marine snow, which are rapidly sinking composite particles $> 0.5\text{mm}$ (Alldredge and Silver, 1988; Armstrong *et al.*, 2009; Asper *et al.*, 1992; Diercks and Asper, 1997; Pilskaln *et al.*, 1998). Additionally, it is known that oil may be transported to depths via oil-mineral aggregates (called OMAs or SPM-oil aggregates; Khelifa *et al.*, 2005; Niu *et al.*, 2011). Tar-like residues from weathered oil may also sink directly.

After the Ixtoc spill of 1979 the formation of pancakes and flakes were described as stages in the weathering cycle of floating petroleum residues (Patton *et al.*, 1981). These pancakes and flakes, which formed on a timescale of months, were products of the aging oil at the sea surface, similar to pelagic tar (Patton *et al.*, 1981). Such aged oil residues may eventually reach the seafloor.

Oil-mineral aggregates are usually $< 50 \mu\text{m}$ (Omotoso *et al.*, 2002; Stoffyn-Egli and Lee, 2002), much smaller than marine snow, but may, depending on clay content and wave action, contribute

appreciably towards moving oil residues to the sea floor (Khelifa *et al.*, 2005; Niu *et al.*, 2011), because of the high excess density of minerals. The wide continental shelf of the Northern GoM receives inorganic particles from continental rivers, run-off and coastal erosion and the mid-depth intermediate nephroid layers may extend far past the shelf edge (Dickson and McCave, 1986). Such subsurface mineral plumes, must have offered ample opportunity for the formation and sinking of OMAs after the DwH spill.

The observations of unusually abundant, uncommonly large (mm to cm – sized), and fast sinking marine snow in the vicinity of the oil layer at the sea surface three to four weeks after the spill, in May 2010 (Passow *et al.*, 2012), suggests that the formation of marine snow also contributed appreciably to the transport of oil contaminated material to the seafloor (Dell' Amore, 2010). In the absence of oil, marine snow is known to form either by zooplankton activity or due to coagulation of particles (Alldredge and Silver, 1988). Coagulation theory describes the formation of aggregates via collision and attachment of individual particles like cells, feces and detritus. Aggregation rates thus depend, among other things, on particle size, concentration and stickiness (Burd and Jackson, 2009; Jackson, 2005; Jackson and Burd, 1998). Because of their transient nature, events forming large quantities of marine snow are difficult to document *in situ*. Moreover, marine snow formation had never been recorded in association with spilled oil; as a result, important details of the marine snow formation event observed after the DwH spill were not documented.

Here, results of six controlled laboratory experiments that explore the formation of marine snow in association with oil are described. The experiments demonstrate specifically how the formation of oil-associated marine snow was influenced by oil type, photochemical aging of oil, the presence of Corexit and the presence of diatoms.

2. Methods

2.1 Experimental set up: The general aim of these laboratory experiments, which were conducted between 2011 and 2013, was to investigate the conditions resulting in the formation of different types of oil-associated marine snow (Table 1). Experiments 1, 2, 5 and 6 were conducted in one

liter, rotating cylindrical Plexiglas tanks, which are the standard to mimic natural marine snow formation (Ploug and Passow, 2007; Shanks and Edmondson, 1989). One liter glass and quartz bottles were used in experiments 3 and 4 to allow aging in natural sunlight prior to the incubation on rolling tables. When rolling tanks are entirely filled and bubble free, they provide a turbulence-free environment (Ploug *et al.*, 2010). Turbulent conditions were achieved by filling the tanks only partially or by using bottles (Jackson, 1994). A turbulent environment was provided in all experiments, except in experiment 1 where both turbulent and non-turbulent treatments were compared, and in experiment 6, because more quiescent conditions promote aggregation of diatoms. Tanks or bottles were rotated at 1.5 – 2.5 rotations per minute; at this speed sinking or floating marine snow never collided with container walls.

Each experiment consisted of 9-10 treatments filled with seawater to which, depending on treatment, oil, dispersant, and/ or phytoplankton was added. All experiments were conducted at 14°C in walk-in environmental chambers with 12: 12 hr. light: dark cycles ($< 30 \mu\text{mol m}^{-2} \text{s}^{-1}$), except one treatment of experiment 1 where the impact of complete darkness was tested and except all treatments with diatoms, which were kept dark to avoid growth. Light conditions had no visible impact on marine snow formation in the absence of diatoms (experiment 1); thus dark treatments were not further pursued. The formation of marine snow was observed over periods ranging from 8 to 66 d, at the end of which any resulting marine snow was analyzed for dry weight, PO^{13}C , and content of transparent exopolymer particles (TEP).

2.2 Seawater: Seawater for experiments 1 to 4 was collected from above a natural seep off Santa Barbara, California (34.22.481N, 119.51.197W) and from above a natural seep in the GoM (GC600: 27°21.311'N, 90°33.196'W) for experiment 5. Seawater from both sites contained no visible oil slicks, but presumably bacterial communities habituated to episodic appearance of low levels of oil were present. Seawater was usually GFF-filtered before use, allowing the presence of the natural bacterial population, but removing particles $> 1 \mu\text{m}$. In experiment 1, microbial control treatments were conducted with $0.2 \mu\text{m}$ pre-filtered or artificial seawater (Kester *et al.*, 1967). Raw (unfiltered) seawater was used in another treatment in experiment 1, but concentrations of natural particles $> 1 \mu\text{m}$ were low. No significant differences in marine snow number, size and characteristics was observed between these different seawater treatments,

indicating that aggregation of particles was not important for the formation of marine snow in experiment 1. Thus the different seawater treatments are all reported as replicates to the GFF-filtered treatments. Diatom cultures were incubated in sterile filtered and UV treated (30 minutes, 50 Watt UV, Emperor Aquatics) seawater media with vitamins and trace metals as in F/2 and 40 μM nitrate, 15 μM phosphate, 100 μM silicic acid (Guillard, 1975).

2.3 Oil types: The effect of oil from different sources and the effect of photochemical aging of crude oil on marine snow formation were investigated (Table 2). Louisiana light oil (from Fisher), termed LA-Oil and Macondo well oil (provided by J. Short: LSU ID # 2010158-12 MC-252 Source oil, 5/20/10 J Short) represented unaltered crude oil and were added at 1 mL per liter of seawater (0.1 %). Oil collected by bucket from the thick layer of oil at the sea surface near the Deepwater Horizon accident site (at 28°42,960'N, 80°23,351'W) on two occasions on 5/12/10, about 3 weeks after the start of the spill will be called *spill-oil*. Spill oil presumably contained seawater, microbes, and potentially dispersant. The oil-water mixture collected at the surface above a natural cold seep in the GoM (GC600: 9/8/12; 27°21.311'N, 90°33.196'W) will be called *seep oil*. Exact concentrations of spill oil and seep oil are unknown. The *spill-oil* was sampled from a thick layer of oil and was thus very concentrated. It was added at 1 mL to 1 liter of seawater. The *seep oil*, which was gathered from a thin oil slick visible at the sea surface (Ziervogel *et al.*, 2014), was much less concentrated and added at a ratio of 1:10 to seawater. All oils were stored in the fridge in the dark until usage. Chemically, unaltered crude oils (e.g. LA-oil and Macondo -oil) differ appreciably from oils collected at the sea surface (e.g. spill oil, seep oil), because dilution, evaporation, dispersion and photo-oxidation effect oil composition (Federal-Interagency-Solution-Group, 2010; Reddy *et al.*, 2011; Ryerson *et al.*, 2012; Spier *et al.*, 2013).

Photo-oxidation experiments (exp. 3 & 4) were conducted by pre-inoculating the different treatments under natural sunlight. Unaltered Macondo oil was mixed with GFF pre-filtered seawater and, depending on treatment, dispersant added. The respective bottles were aged in sun light for 1.5 to 12 weeks. Glass (exposure to PAR only) or quartz bottles (exposure to PAR + UV) were submerged in water in aquaria on a sunny roof top at the University of California Santa Barbara. During aging for 1.5 or 3 weeks (exp. 3) daily water temperature in the warmest

period of the day averaged 29.1°C, but never exceeded 30°C. During the aging experiment 4, (aging for 6 and 12 weeks), which was later in the season than experiment 3, average daily water temperature in the warmest period of the day was lower (24.5°C). And daily integrated light intensity during aging for 6 and 12 weeks was only 57% of that during aging for 1.5 and 3 weeks, with average light values during the period with highest light intensities (12:00 to 16:00 clock) of 608 $\mu\text{Em}^2\text{s}^{-1}$, and 1152 $\mu\text{Em}^2\text{s}^{-1}$, respectively. After aging was complete the bottles were incubated on roller tables and marine snow formation monitored as in the other experiments.

2.4 Dispersant: Treatments with dispersant addition contained Corexit 9500A, Nalco, which was added either at low concentrations (10 μL per liter seawater = dispersant: oil: seawater = 1: 10^2 : 10^5) or at high concentrations (1 mL per liter seawater = dispersant: oil: seawater = 1: 1: 10^3). Experiments 1, 2, 3 and 4 included Corexit treatments, which were always paired with treatments that were identical except that they did not contain Corexit.

2.5 Diatoms: A common formation mechanism of marine snow is the aggregation (physical coagulation) of phytoplankton, especially diatoms (Jackson, 1995, 2005). Diatoms were added to some treatments of experiments 5 and 6 to investigate the formation of oil containing marine snow due to the aggregation of diatoms. In experiment 5 *Thalassiosira weissflogii* cells were added at an end concentration of about 16,000 cells mL^{-1} using a mix of an exponentially growing and a senescent culture (1:1). *Dactyliosolen fragilissimus* in early stationary phase (1-2 days after nitrogen limitation) was used for experiment 6.

2.6 Sampling: Marine snow formation and characteristics was monitored following established procedures (Passow and De La Rocha, 2006; Passow *et al.*, 2014). Operationally, particles > 1 mm will be defined as marine snow in these experiments; marine snow 0.5 to 1 mm can't be handled reliably. Size (longest and shortest axis) and concentration of marine snow was recorded manually every 1-5 days without changing the rotation speed of tanks or bottles. Changing rotation speed would have resulted in marine snow colliding with tank surfaces, which changes their characteristic (Ploug and Grossart, 1999). In treatments where marine snow formed, it appeared within 1-4 days on the roller table and did not change significantly thereafter.

Treatments where no marine snow formed were run for up to 66 days on the rolling table to ensure the absence of particle formation. As the oil-water suspension, rather than the water soluble fraction of oil was used in experiments, representative subsampling or filtration of the non-aggregated material was unachievable in treatments with oil. However, at the end of the experiments marine snow was isolated manually using a cut off pipette (Passow and De La Rocha, 2006), and analyzed.

2.7 Marine snow size and volume: The volume of each marine snow particle was calculated based on ellipsoid shapes assuming that the third dimension equaled the shorter of the two measured axis. Equivalent spherical diameters (ESD) were calculated from volumes for ease of comparisons. Total volume represents the sum of the volumes of all marine snow particles in a tank or bottle and is considered a proxy for the total amount of marine snow that formed.

2.8 Dry weight and PO ¹³C of marine snow: Depending on their size, 1-8 individual marine snow sized particles were placed on preweighed and muffled (450°C for 4 hrs) replicate GFF filters (25 mm). Dry weight was measured by reweighing filters after drying at 60°C (Passow *et al.*, 2014). The ¹³C signature was then measured after acidification using a Finnigan Delta Plus Advantage. PO ¹³C of the different types of oil was also measured. Thus two end member mass balance calculations (Chanton *et al.*, 2012) could be used to estimate the contribution of fossil carbon in oil containing marine snow.

2.9 TEP content of marine snow: TEP or their dissolved precursors, which are rich in acidic polysaccharides, are released by phytoplankton and bacteria (Ortega-Retuerta *et al.*, 2009; Passow, 2002a) and provide the matrix of marine snow (Passow, 2002b). The concentration of TEP in marine snow was determined by mixing 1-8 individually isolated marine snow particles, filtering aliquots of this slurry onto four replicate 0.4 µm PC filters, and analyzing for TEP using the dye Alcian Blue (Passow and Alldredge, 1995). Methodological tests determined that the presence of oil had no impact on staining with Alcian Blue, but TEP could not be measured in treatments containing the dispersant Corexit due to artifacts.

3. Results and Discussion

3.1 Formation of Oil-Containing Marine Snow in the Absence of Particles

Large, cm-sized marine snow formed in all types of seawater (pre-filtered, raw, artificial) when it was inoculated with the spill oil, collected in May 2010 at the surface, near the spill site. This marine snow had an appearance very different from aggregates formed due to coagulation (collision and attachment) of individual particles. It consisted predominately of amorphous mucus, which was extremely sticky. The ^{13}C signature of this marine snow was nearly identical to that of the spill oil (Table 3), supporting the visual impression that the marine snow did not consist of individual particles that aggregated. This mucus-rich marine snow appeared in less than a day in all treatments inoculated with spill oil (exp. 1, 2, 5 and 6), and consolidated into one or two very large particles within four days in all eleven treatments. After four days of incubation, the equivalent spherical diameter (ESD) of this marine snow ranged between 3 to 40 mm (avg. $\pm$ std: 6.4 ± 2.1 mm, $n = 11$) and no systematic change in its appearance was observed in the subsequent 1 to 2 weeks. The marine snow formed in experiment 6, almost 2 years after collection of the spill oil did not differ from that formed during experiments 1 and 2.

The fact that this marine snow formed in the absence of particles $> 1\mu\text{m}$ implies a hitherto unexplored formation mechanism for marine snow. Many microbes release high molecular weight exudates in the presence of oil. These substances physically protect the bacteria, aide in their attachment, or emulsify and solubilize oil products, thus increasing the bioavailability of diverse components of oil (Head *et al.*, 2006; McGenity *et al.*, 2012). The oil floating at the surface after the spill triggered the copious production of such exudates (Gutierrez *et al.*, 2013; Ziervogel *et al.*, 2012). Here we suggest that *in situ* the production of microbial mucus results in patches of “floating biofilm” which transform into marine snow once they sink below the air-sea interface. The ^{13}C signature (Table 3) suggests that the oil provided the substrate for the ubiquitous mucus production. As this marine snow, which will be called *microbial marine snow*, formed even when spill oil was added to artificial seawater, the spill oil must have included the microbial community responsible for its formation.

Bacteria are known to play an important role in the formation, degradation and modification of marine snow (Grossart *et al.*, 2003; Kiørboe, 2003; Simon *et al.*, 2002), but the *in situ* formation of mm to cm sized marine snow by bacteria, in the absence of coagulating particles, has not been described before. However, under laboratory conditions the formation of amorphous particles (usually < 0.5 mm) via bubbling of exudates has been observed (Alber and Valiela, 1994; Bhaskar *et al.*, 2005; Biddanda, 1986; Kepkay and Johnson, 1988; Kepkay, 1994).

The characteristics of the laboratory produced microbial marine snow resembled the large marine snow observed *in situ* in the GoM after the spill, but differed appreciably from other, oil-free types of marine snow (Alldredge and Silver, 1988): Not only was the microbial marine snow larger than more commonly observed marine snow, but the size-normalized dry weight was also substantially (6 to > 50 times) higher than that of other types of marine snow (Table 4; Alldredge *et al.*, 1998; Ploug and Passow, 2007). Moreover, the size-normalized content of TEP was appreciably higher in microbial marine snow compared to diatom aggregates or other natural marine snow (Alldredge *et al.*, 1993; Table 4), confirming the visual impression of a high mucus content.

Whereas *microbial marine snow* (Fig. 1a) formed in all treatments with spill oil (n=11), no particles whatsoever formed in treatments without oil (n=7) or in the presence of unaltered crude oils (LA or Macondo oil; n = 7) even after 41 days, no matter the seawater source or turbulence regime (Fig 2). The combination of the spill oil characteristics and the microbial community in this oil must have been fairly uniquely positioned to promote the formation of this large microbial marine snow. The fact that crude oil did not induce the production of any marine snow by the microbial communities present in the surface water (in raw or GFF pre-filtered seawater treatments), challenges findings that in the presence of Macondo crude a microbial community isolated from 1000 m formed aggregations of bacteria, which grew into small marine snow-sized particles after > 20 days (Baelum *et al.*, 2012). Clearly the specific interactions between bacterial communities, their exudations, and different oil types need further investigations.

Next it was tested if photo-oxidation of crude oil contributed towards altering the oil that surfaced after the spill in such a manner to allow the formation of marine snow. Experiments 3

and 4 examined if the microbial community present in the GFF pre-filtered surface water formed marine snow in the presence of crude oil aged in sunlight. These experiments revealed that prolonged aging, especially in UV light, promoted marine snow formation (Fig 2), although the particles appeared more compact and were smaller compared to the microbial marine snow formed from spill oil (Table 5): No particles formed in treatments ($n = 3$ tanks) with less than three weeks exposure to photosynthetically active radiation (PAR). Some marine snow did form in the tanks aged in PAR for three or six weeks, however, the total amount of marine snow formed after aging in PAR for ≤ 6 weeks was small ($< 10 \text{ mm}^3$). After aging for 12 weeks in PAR ($n = 1$ tank) more and larger ($\text{ESD} = 2\text{-}3 \text{ mm}$) marine snow was observed, reaching a total volume of $> 300 \text{ mm}^3$ (Fig 3).

The presence of UV radiation promoted marine snow formation more rapidly compared to treatments exposed to PAR only, with total marine snow volume after only 3 or 6 weeks an order of magnitude larger ($n = 2$ tanks each; Fig 3). However, the total volume of marine snow that formed after 12 weeks of aging, was smaller in the treatment aged in PAR & UV compared to PAR only ($n = 1$ tank each; Fig 3). This could reflect a real change in marine snow formation dynamics after prolonged exposure to UV, or may be an experimental artifact resulting from differences in light exposure during the aging experiments.

The marine snow formed from photo-chemically aged crude oil differed in size and appearance from the microbial marine snow formed from spill oil (Fig 1, Table 5). Differences in the weathering between both oil types may have played an important role: Besides aging due to sunlight, spill oil underwent extensive compositional changes during its ascent from $\sim 1500 \text{ m}$. Differences in microbial community composition between experiments with spill oil and photooxidised crude oil may have also contributed to the differences in marine snow appearance. However, the formation mechanism appears similar in both cases, and marine snow formed in experiments 3 and 4 also consisted to nearly 100% of fossil carbon (Table 3). The marine snow formed from spill oil and from photo-chemically aged crude oil, both resembled the marine snow observed *in situ* in May 2010, and both may be part of the same phenomenon, suggesting that photochemical aging may have contributed towards the formation of microbial marine snow in the GoM after the DWH spill.

3.2 Formation of Oil-Containing Diatom Aggregates

Phytoplankton have also been implicated in transporting polycyclic aromatic hydrocarbons (PAH) to the seafloor, based on high PAH concentrations in phytoplankton detritus and high correlations between pigments and PAHs in surface sediments (Kowalewska, 1999; Kowalewska and Konat, 1997a; Kowalewska and Konat, 1997b; Lubecki and Kowalewska, 2010). Inclusion of oil in phytoplankton aggregates provides a possible explanation for phytoplankton-associated transport of oil to the seafloor. Oil may sorb to phytoplankton, but alternatively oil droplets may be trapped within the interstices of phytoplankton aggregates, which are extremely porous ($\geq 99\%$ (Alldredge and Gotschalk, 1988)).

Experiments 5 and 6 with *Thalassiosira weissflogii* and *Dactyliosolen fragilissimus* revealed that appreciable amounts of oil were incorporated in diatom aggregates, independent of oil type: Between 16% and 65% of the carbon in diatom aggregates that formed in the presence of oil were derived from oil (Table 3): *D. fragilissimus* aggregates contained 16% and 65% carbon derived from spill oil or Macondo oil, respectively (Table 3). Our data suggests a $\delta^{13}\text{C}$ signature of -30.5 to be appropriate for the collected seep oil (but see (Wang and Chen, 2001), implying a fossil carbon content of about 50% in *T. weissflogii* aggregates (Table 3). Differences in the amount of oil incorporated into diatom aggregates may depend on packaging of cells as well as on the quantity and type of extracellular substances (e.g. hydrophobic or hydrophilic), which differs appreciably depending on species (Ding *et al.*, 2009; Verdugo, 2012).

Diatom aggregates formed in the presence or absence of any type of oil tested, but aggregate appearance and aggregation dynamics were affected by the presence and type of oil. In the absence of oil, small, sub-mm sized, wispy aggregates formed with both species within days, but did not grow larger than ~ 1 mm over time. The addition of seep oil to *T. weissflogii* slightly promoted aggregate formation, as indicated by the earlier onset of aggregation and higher aggregate concentration (Table 6). No significant difference in aggregate size and abundance was observed between treatments of *D. fragilissimus* with or without unaltered Macondo oil (Table 6). However, the addition of spill oil resulted in the rapid formation of very large (ESD = 10 mm) and sturdy aggregates with a total aggregate volume an order of magnitude larger

compared to the corresponding no-oil treatment (Table 6). Size-normalized dry weight (DW) and TEP of these aggregates were in the same range as that of the microbial marine snow (Table 4). Presumably, both, bacteria-mediated mucus production and coagulation of phytoplankton contributed to the formation of these large marine snow aggregates. These composite “diatom-microbial marine snow aggregates” contained a relatively small fraction of fossil carbon (16%).

3.3 The Impact of Corexit on the Formation of Microbial Marine Snow:

The use of chemical dispersants is a common non-mechanical response to an oil spill. The impact of Corexit on marine snow formation in the absence of coagulating particles was thus also assessed. After the DwH oil spill the dispersant Corexit 9500A was used both at the surface and at the source at depth (Federal-Interagency-Solution-Group, 2010). The target Corexit to oil ratio for oil spill mediation is close to 1:15, but Corexit delivery and dispersal during an oil spill is not precise and both higher and lower ratios commonly occur (Dispersants, 2005). The average Corexit concentration in the water after the DwH spill was estimated at 0.5% (1:200), with much higher concentrations near the source (Kujawinski *et al.*, 2011). Experiments investigating the impact of dispersant on the formation of marine snow used Corexit 9500A at either a “high” or “low” Corexit: oil ratio of 1:1 or 1:100, which span the expected range for Corexit in the GoM, although the “high” concentrations would be expected near the source only.

At low concentrations the presence of Corexit 9500A inhibited the formation of marine snow from either the spill oil or the photochemically aged Macondo oil (exp. 1, 2, 3, 4). Microbial marine snow formation in the presence of Corexit was retarded ($n = 1$ tank), reduced ($n = 3$ tanks) or completely inhibited ($n = 2$ tanks) compared to the corresponding treatments without Corexit (Fig 4). On average the total volume of marine snow was 117 mm^3 per liter lower in the presence of Corexit ($n = 6$). Further experiments will be needed to determine how Corexit inhibits the formation of microbial marine snow. Possibly, the presence of Corexit impacted bacteria or their ability to generate exudates. Alternatively, the fact that the oil was already dispersed may have eliminated the stimulus that triggered the release of exudates by bacteria. Or Corexit lead to a shift in composition towards strains less likely to generate mucus.

A different response was observed at high Corexit concentrations (1 mL per 1 Liter seawater), because Corexit self-aggregated. Large aggregates, consisting largely of Corexit formed in all treatments (n = 6 tanks) containing high Corexit concentrations and spill oil. Corexit aggregates also formed in the absence of oil in treatments without turbulence (n = 3 tanks). No particles of any kind formed in the treatment without oil under turbulent conditions even after 8 days (n = 3 tanks), suggesting that the oil contributed to the cohesion and stability of Corexit aggregates. Corexit aggregates formed in the presence of oil were larger and more abundant than those formed in the absence of oil, confirming its role as a stabilizer (Table 7). It is currently unclear if solely physico-chemical interactions between Corexit led to the formation of these aggregates, or if bacterial activity contributed. In contrast to the microbial marine snow (n=11 tanks), Corexit aggregates (n=9 tanks) were always positively buoyant. Thus while they could potentially be ingested by marine organisms, Corexit aggregates would not sediment.

4. Conclusion

Our experimental results provide a mechanistic explanation of the pathways of oil-associated marine snow formation during the DwH oil spill in the GoM. The bacteria-mediated formation of microbial marine snow provides a potentially very effective mechanism for the removal of oil from the ocean surface. The presence of a thick layer of oil after the DwH spill could have fueled the production of a remarkably large amount of microbial marine snow. In experiments one mL oil per liter of seawater produced on average $263 \pm 271 \text{ mm}^3$ of particulate matter (n = 8) with a dry weight of $3.5 \text{ mg} \pm 3.4 \text{ mg}$, which formed within hours to days.

Additionally, the experiments reveal that oil carbon is incorporated into aggregates formed by coagulation of diatoms. Increased phytoplankton concentration (Hu *et al.*, 2011) due to the accident itself, or due to mediating measures like the opening of the floodgates of the Mississippi and diversionary channels, contributed to the formation of exceptionally high concentrations of phytoplankton that potentially aggregated. Possible subsequent sedimentation events would be higher than usual and could transport large amounts of fossil carbon to depths. Moreover,

experimental evidence suggests that the presence of oil increases the stability and cohesion of aggregates, implying faster and more efficient transport to depths.

Both types of marine snow, microbial snow or phytoplankton aggregates, as well as the formation of OMAs, presumably contributed towards transporting oil carbon to the seafloor after the DwH accident. Whereas high sedimentation rates of minerals would be associated with sinking OMAs, the organic matter content would be elevated when sinking marine snow dominates downward flux.

Understanding of the different conditions which led to the formation and sedimentation of oil residues will enable model projections of the spatial and temporal extent of sedimentation pulses after the DwH accident. Modeling efforts that combine data on the spatial distribution of oil, dispersants, minerals and phytoplankton with the mechanistic understanding of the formation mechanisms of oil-containing, sinking particles could hindcast the spatial and temporal extent of sedimentation events after the spill. These estimates can then be compared to measured sedimentation and accumulation rates.

The overall effects of Corexit 9500A on sedimentation of oil-rich marine snow is currently difficult to assess, in part because of the patchy Corexit delivery. Self-aggregation of Corexit may have occurred near the source, but mainly Corexit concentrations in the GoM were too low for self-aggregation to have been significant. However, even very low concentrations of Corexit inhibited marine snow formation in experiments. Likely, the presence of Corexit reduced microbial marine snow formation and sedimentation after the spill. The impact of Corexit on the formation of phytoplankton-oil aggregates remains to be determined. Obviously the inhibitory effect on the formation of sinking particles needs to be considered when evaluating the advantages and disadvantages of mediation by Corexit additions in the future.

The formation of sinking, oil-associated, particles has far reaching consequences for the fate and distribution of oil in marine environments. Marine snow are hotspots of microbial activity (Arnosti *et al.*, 2013; Azam, 1998; Azam and Malfatti, 2007; McGenity *et al.*, 2012; Ziervogel *et al.*, 2012), but also the vehicle for the efficient transport of enclosed material to depths.

The qualitative observation of high *in situ* concentrations of marine snow in May 2010, and the observation of high sedimentation rates in August-September 2010 (Passow unpubl.) suggest that the DwH spill may have directly and indirectly triggered several large sedimentation events. Such sedimentation events would transport hydrocarbons from the surface oil layer or the subsurface plume downward. During transit sticky marine snow scavenges other particles such as organisms, feces, and detritus with great efficiency (Smetacek, 1985), potentially resulting in a marine snow “blizzard”, which could explain the extraordinarily high accumulation rates of material observed at the sediment surface. The mucus-rich character of microbial marine snow is consistent with the material found covering corals (Hsing *et al.*, 2013; White *et al.*, 2012) and the patchy distribution of this material on corals is consistent with sinking marine snow. Future spill response and assessment will have to take the possible formation of rapidly settling, oil-associated marine snow and its deposition on the seafloor into account.

Acknowledgement

This work was made possible with funding from an NSF rapid (OCE-1045330) and by a grant from BP/the Gulf of Mexico Research Initiative to support consortium research entitled "Ecosystem Impacts of Oil and Gas Inputs to the Gulf (ECOGIG)" administered by the University of Mississippi. Thanks go to Vernon Asper and Arne Diercks for oil collected from the surface of the GoM in May 2010, and to Jeff Short (JWS Consulting LLC) who shared 20 mL of his Macondo oil. Nalco provided Corexit EC9500A. Special thanks go to Caitlin Fairfield, Julia Sweet, and Christina Hirdes, without whose help the experiments could not have been conducted. Alice Alldredge's and Avan Antia's comments on earlier versions of the MS are gratefully acknowledged. Discussions with MOSSFA (Marine Oil Snow Sedimentation and Flocculent Accumulation) working group members Kendra Daly, Dave Hollander and Jeff Chanton have contributed. The constructive comments of 2 anonymous reviewers are gratefully acknowledged. This is ECOGIG contribution # 205. The GRIIDC data ID is R1.x132.139.0004.

Figure Legends

Figure 1: A) Examples of microbial marine snow formed in rolling tanks in the presence of oil that accumulated at the sea surface after the accident (exp. 1 & 2). B) Example of microbial marine snow formed after aging of Macondo crude oil in sunlight (exp. 3).

Figure 2: Formation of marine snow in filtered seawater in treatments without oil, with crude oils (LA- or Macondo-oil), with spill oil, or with photo-chemically aged Macondo oil (exp. 1-4). Maximum, minimum and median values of A) the equivalent spherical diameter (ESD), and B) the number concentrations of marine snow are depicted. No Corexit was added in any of these treatments. No particles formed in the absence of oil or in the presence of unaltered crude oil. In the presence of spill oil very large microbial marine snow formed ($n = 7$ tanks), whereas microbial marine snow formed from photo-chemically aged Macondo crude (3-12 weeks; $n = 6$) was more numerous but appreciably smaller. For specifics of oil types see Table 2.

Figure 3: The total volume of microbial marine snow formed as a function of aging time of Macondo oil exposed to Photosynthetically Active Radiation (PAR) only or to PAR + Ultra Violet radiation (UV). The average and standard deviation (error bars) of total volume of marine snow per tank were determined from 4 measurements taken within the first ten days of incubation (experiments 3 & 4). No Corexit was added in any of these treatments.

Figure 4: The total volume of marine snow ($\text{mm}^3 \text{ tank}^{-1}$) that formed in the presence or absence of Corexit (Corexit: oil: seawater = $1:10^2:10^5$). Depicted are observations from a total of 6 individual paired treatments stemming from experiments 2, 3 and 4 (Tr. 2.2; 3.2; 4.1; 4.2; 4.7; 4.8) incubated with either spill oil or photochemically aged Macondo oil. Each of the six treatments is depicted as a time series lasting between 4 and 13 days. The exact response to Corexit addition varied: The presence of Corexit retarded (Tr. 2.2), reduced (Tr. 3.2; 4.2; 4.8) or completely inhibited (Tr. 4.1; 4.7) marine snow formation and overall appreciably fewer particles formed in treatments with Corexit compared to the corresponding treatments without Corexit (avg. 2 vs. 8 tank^{-1} , $n = 6$ tanks each).

Table 1

Experiments 1 to 6 investigated the formation of oil containing marine snow: In experiment 1 both turbulent and non-turbulent treatments were run in parallel. Experiments testing aggregation of diatoms (5 & 6) were run without turbulence; all others (2, 3 & 4) were conducted with turbulence. Different types of oils (see Table 2) were tested in all experiments, except in experiments 3 and 4 where Macondo crude oil was photo-oxidized for varying amounts of time. Treatments with the dispersant Corexit were present in experiments 1, 2, 3, 4. Treatments with diatoms were present in experiments 5 and 6.

Main factors investigated	Exp. #	Oil types used
Microbial marine snow formation in the absence of particles > 1 μm		
Impact of turbulence	1	Spill oil
Impact of oil type	1, 2, 5, 6	Spill oil; LA-oil; Mac.-oil, CS oil
Impact of photo-oxidation of oil for 1.5 and 3 weeks for 6 and 12 weeks	3 4	Mac.-oil
Impact of Corexit Low conc.: (10 μ L Cor.: 1 mL oil) High conc.: (1 mL Cor.: 1 mL oil)	2, 3, 4 1	Spill oil; LA-oil; Mac.-oil
Incorporation of oil into phytoplankton aggregates		
Impact of oil type & diatom species	5, 6	Spill oil; Mac.-oil; CS oil

Table 2

Oil types used in the experiments: DwH = Deepwater Horizon, GoM = Gulf of Mexico. All oils were stored at 4°C in the dark. The impact of spill oil on marine snow formation did not change significantly as a function of storage time within the 2 year period.

Oil name	Description	Source
LA-oil	U.S. EPA-API (Environmental Protection Agency - American Petroleum Institute) Reference Oil, So. Louisiana, Crude Oil, WP 681	Fisher Scientific; Catalog #: NC9063321
Mac.-oil	Macondo oil, the crude oil that spilled in the DwH accident, provided by J. Short; JWS Consulting LLC	Jeff Short, LSU ID # 2010158-12; MC-252 Source oil, 5/20/10
Spill-oil	Collected in the GoM near DwH accident site: 5/12/10; 28°42,960'N, 80°23,351'W	Oil carpet sampled by bucket by V. Asper & A. Diercks
CS-oil	Collected above cold seep GC600: 9/8/12; 27°21.311'N, 90°33.196'W	Visible oil slick sampled by bucket

Table 3

$\delta^{13}\text{C}$ signature of oil, marine snow formed in the absence or presence of oil (average $\pm$ standard deviation of replicate measurements) and calculated fraction of fossil carbon in oil-associated marine snow. The number of replicate measurements is given in brackets. The spill oil used in experiments 5 and 6 was collected on two occasions (#1 or #2) at the same site, on the same day, but the $\delta^{13}\text{C}$ signature differed. Two end member mass balance calculations were used for the estimate of the contribution of fossil carbon to marine snow (Chanton *et al.*, 2012). Only one endmember exists for microbial marine snow, and a contribution of near 100% is deduced from the similarity of the value to that of the oil. [#]Assumption: $\delta^{13}\text{C}$ of Macondo crude oil did not change due to photochemical aging. * (Wang and Chen, 2001) found a $\delta^{13}\text{C}$ signature of GC cold seep oil of -27.0, but this value was higher than that of the CS oil we collected, as indicated by the signal of the microbial marine snow.

Marine snow type	Oil $\delta^{13}\text{C}$	Marine snow in the absence of oil $\delta^{13}\text{C}$	Marine snow in the presence of oil $\delta^{13}\text{C}$	Oil carbon as % of total carbon in marine snow
Microbial marine snow				
Microbial marine snow from spill oil #1, exp. 5	-25.89 (1)	-	-25.90 $\pm$ 1.1 (2)	~100
Microbial marine snow from spill oil #2, exp. 6	-27.59 $\pm$ 0.12 (4)	-	-27.45 $\pm$ 0.49 (6)	~100
Microbial marine snow from photochemically aged Macondo Oil, exp. 3 & 4	-27.43 $\pm$ 0.08 [#]	-	-27.80 $\pm$ 0.1 (3)	~100 [#]
Particles (< 1mm) formed from cold seep oil, exp. 6	-27.00*	-	-30.50 $\pm$ 7.7 (2)	>100*
Diatom aggregates				
<i>D. fragilissimus</i> agg. with spill oil #2, exp. 6	-27.59 $\pm$ 0.12 (4)	-25.89 $\pm$ 0.18 (4)	-26.17 $\pm$ 0.09 (4)	16
<i>D. fragilissimus</i> agg. with Macondo oil, exp. 6	-27.43 $\pm$ 0.08 (2)	-25.89 $\pm$ 0.18 (4)	-26.89 $\pm$ 0.43 (6)	65
<i>D. fragilissimus</i> agg. with Macondo oil & Corexit, exp. 6	-27.43 $\pm$ 0.08 (2)	-25.89 $\pm$ 0.18 (4)	-24.20 $\pm$ 0.15 (2)	-
<i>T. weissflogii</i> agg. with cold seep oil, exp. 6	-30.5	-22.6 $\pm$ 0.8 (2)	-26.60 (1)	51

Table 4

Size normalized comparison of characteristics of microbial marine snow with other, non-oil containing types of marine snow from the literature (Alldredge *et al.*, 1998; Alldredge *et al.*, 1993; Ploug and Passow, 2007). *Skeletonema marinoi* (diatom) aggregates were made in rolling tanks under conditions comparable to ours, but without oil additions (Ploug and Passow, 2007). Oil-free marine snow (11 different types) was collected *in situ* by divers (Alldredge, 1998; Alldredge *et al.*, 1998). For ease of comparison all aggregates were size-normalized to an ESD of 8.3 mm, the average size of microbial marine snow formed under comparable, non-turbulent conditions.

	Oil associated marine snow		Oil-free marine snow	
	<i>Microbial marine snow (from spill oil)</i>	<i>D. fragilissimus aggregates</i>	<i>S. marinoi aggregates</i>	Mixed marine snow
DW mg agg⁻¹	3.97	2.80	0.28	0.07
TEP µg GXeq. agg⁻¹	182	281	42	21

Table 5

Characteristics of *microbial marine snow*: ESD (equivalent spherical diameter), total volume, DW (dry weight) and TEP (Transparent Exopolymer Particles) content. Microbial marine snow from spill oil formed under turbulent conditions was smaller (ESD = 5.3 ± 0.9 mm vs. 8.3 ± 2.8 mm) and contained relatively more TEP (218 ± 180 $\mu\text{gGXeq. tank}^{-1}$ vs. 102 ± 59 $\mu\text{gGXeq. tank}^{-1}$) compared to that formed under quiescent conditions. However, statistically there was no difference (Mann–Whitney U-test; $p \leq 0.1$; $n_1=3$, $n_2=4$) and the data is combined here. Particle concentrations in treatments with photochemically aged Macondo oil were too low to determine DW and TEP. Marine snow formed in the presence of photo-chemically aged oil combines data from treatments aged for ≥ 3 weeks.

	Maximum	Median	Minimum	Average $\pm$ Stand. deviation
Marine snow from spill oil (n = 8 tanks)				
ESD mm	12.5	7.0	4.0	7.2 ± 2.5
Total Volume $\text{mm}^3 \text{ tank}^{-1}$	785	144	47	263 ± 271
DW mg tank^{-1}	11.5	2.1	1.2	3.5 ± 3.4
TEP $\mu\text{g GXeq. tank}^{-1}$	425	115	60	160 ± 136
Marine snow from photo-chemically aged oil (n = 6 tanks)				
ESD mm	5.3	2.5	1.5	2.8 ± 1.4
Total Volume $\text{mm}^3 \text{ tank}^{-1}$	325	140	5	142 ± 130

Table 6

Aggregation dynamics of diatoms in the presence or absence of oil: Experiments were conducted with *Thalassiosira weissflogii* or *Dactyliosolen fragilissimus* and different oil types. Results are expressed as time (in days) until aggregates ≥ 1 mm (longest axis) appeared, size of aggregates ≥ 1 mm (longest axis), number of aggregates (Agg. No.), dry weight (DW as average $\pm$ standard deviation), and total volume of aggregates per tank (Total Vol.). N are the number of replicate tanks. Aggregates formed by *T. weissflogii* were so fragile and wispy that size and numbers represent rough estimates only and aggregates could not be collected for dry weight determination. *Enough material for TEP determination was only available for *D. fragilissimus* plus spill oil: $528 \pm 127 \mu\text{g GXeq L}^{-1}$. NV: no value

Diatom	Oil type	Time till aggregates ≥ 1 mm formed days	Size: long axis mm	Agg. No. # tank ⁻¹	DW mg tank ⁻¹	Total Vol. mm ³ tank ⁻¹	N
<i>T. weissflogii</i>	no oil	13-20	1	~15	NV	8	2
<i>T. weissflogii</i>	CS-oil	8	1	~50	NV	27	2
<i>D. fragilissimus</i>	no oil	2-3	1	25	3.45 ± 1.5	26 ± 0	2
<i>D. fragilissimus</i>	Mac. -oil	3-4	1	20	2.2 ± 0.7	21 ± 0	2
<i>D. fragilissimus</i> *	Spill-oil	1-2	4-5	25-30	5.3 ± 3.0	563 ± 4	2

Table 7

Average $\pm$ standard deviation of ESD (equivalent spherical diameter), total volume, and DW (dry weight) of Corexit aggregates, which formed at Corexit concentrations of 1 mL per liter of seawater: Whereas the degree of turbulence had no significant effect on the formation of Corexit aggregates formed in the presence of oil, Corexit aggregates without oil formed only under quiescent conditions. In contrast of other types of marine snow, Corexit aggregates were buoyant.

	Corexit aggregates with spill oil: Turbulent& non- turbulent	Corexit aggregates without oil: Quiescent
ESD mm	8.5 $\pm$ 2.4	3.1 $\pm$ 0.6
Total Volume mm³ tank⁻¹	652 $\pm$ 644	178
DW mg tank⁻¹	2.3 $\pm$ 1.7	0.3

References

- Alber, M., Valiela, I., 1994. Production of microbial organic aggregates from macrophyte-derived dissolved organic material. *Limnology and Oceanography* 39 (1), 37-50.
- Allredge, A., 1998. The carbon, nitrogen and mass content of marine snow as a function of aggregate size. *Deep-Sea Research Part I Oceanographic Research Papers* 45 (4-5), 529-541.
- Allredge, A.L., Gotschalk, C., 1988. *In situ* settling behavior of marine snow. *Limnology and Oceanography* 33, 339-351.
- Allredge, A.L., Passow, U., Haddock, S.H.D., 1998. The characteristics and transparent exopolymer particle (TEP) content of marine snow formed from thecate dinoflagellates. *Journal of Plankton Research* 20, 393-406.
- Allredge, A.L., Passow, U., Logan, B.E., 1993. The abundance and significance of a class of large, transparent organic particles in the ocean. *Deep-Sea Research I* 40 (6), 1131-1140.
- Allredge, A.L., Silver, M.W., 1988. Characteristics, dynamics, and significance of marine snow. *Progress in Oceanography* 20, 41- 82.
- Armstrong, R.A., Peterson, M.L., Lee, C., Wakeham, S.G., 2009. Settling velocity spectra and the ballast ratio hypothesis. *Deep Sea Research II* 56 (18), 1470-1478.
- Arnosti, C., Ziervogel, K., Yang, T., Teske, A., 2013. Oil-derived marine aggregates - hot spots of polysacchride degradation by specialized bacterial communities. *Deep Sea Research* submitted June 2013.
- Asper, V.L., Deuser, W.G., Knauer, G.A., Lohrenz, S.E., 1992. Rapid coupling of sinking particle fluxes between surface and deep ocean waters. *Nature* 357 (6380), 670-672.
- Atlas, R.M., Hazen, T.C., 2011. Oil biodegradation and bioremediation: A tale of the two worst spills in U.S. history. *Environmental Science & Technology* 45 (16), 6709-6715.
- Azam, F., 1998. Microbial control of oceanic carbon flux: The plot thickens. *Science* 280 (5364), 694-696.
- Azam, F., Malfatti, F., 2007. Microbial structuring of marine exosystems. *Nature* 5, 782-791.
- Baelum, J., Borglin, S., Chakraborty, R., Fortney, J., Lamendella, R., Mason, O., Auer, M., Zemla, M., Bill, M., Conrad, M., Malfatti, S., Tringe, S., Holman, H., Hazen, T., Jansson, J., 2012. Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. *Environmental Microbiology* 14 (9), 2405-2416.
- Bhaskar, P.V., Grossart, H.-P., Bhosle, N.B., Simon, M., 2005. Production of macroaggregates from dissolved exopolymeric substances (EPS) of bacterial and diatom origin. *Fems Microbiology Ecology* 53 (2), 255-264.
- Biddanda, B.A., 1986. Structure and function of marine microbial aggregates. *Oceanologica Acta* 9 (2), 209-211.
- Burd, A.B., Jackson, G.A., 2009. Particle Aggregation. *Annual Review of Marine Science* 1 (1), 65-90.
- Chanton, J.P., Cherrier, J., Wilson, R.M., Sarkodee-Adoo, J., Bosman, S., Mickle, A., Graham, W.M., 2012. Radiocarbon evidence that carbon from the Deepwater Horizon spill entered the planktonic food web of the Gulf of Mexico *Environmental Research Letters* 7 (4), 045303.
- Dell' Amore, C., 2010. "Sea Snot" Explosion Caused by Gulf Oil Spill? *National Geographic*.
- Dickson, R.R., McCave, I.N., 1986. Nepheloid layers on the continental slope west of Porcupine Bank. *Deep Sea Research Part A. Oceanographic Research Papers* 33 (6), 791-818.
- Diercks, A.R., Asper, V.L., 1997. In site settling speeds of marine snow aggregates below the mixed layer: Black Sea and Gulf of Mexico. *Deep-Sea Research Part I: Oceanographic Research Papers* 44 (3), 385-398.

- Diercks, A.R., Highsmith, R.C., Asper, V.L., Joung, D., Zhou, Z., Guo, L., Shiller, A.M., Joye, S.B., Teske, A.P., Guinasso, N., Wade, T.L., Lohrenz, S.E., 2010. Characterization of subsurface polycyclic aromatic hydrocarbons at the Deepwater Horizon site. *Geophysical Research Letters* 37 (20).
- Ding, Y.-X., Hung, C.-C., Santschi, P.H., Verdugo, P., Chin, W.-C., 2009. Spontaneous assembly of exopolymers from phytoplankton. *Terrestrial, Atmospheric and Oceanic Sciences (TAO)* 20 (5), 741-747.
- Dispersants, C.o.U.O.S., 2005. Understanding oil spill dispersants: Efficacy and effects. National Academic Press.
- Farrington, J.W., 2013. Oil Pollution in the Marine Environment I: Inputs, Big Spills, Small Spills, and Dribbles. *Environment Science and Policy for a Sustainable Development*.
- Federal-Interagency-Solution-Group, 2010. Oil Budget Calculator Science and Engineering team: Oil budget calculator technical documentation.
http://www.noaa.gov/stories2010/PDFs/OilBudgetCalc_Full_HQ-Print_111110.pdf.
- Grossart, H., Kiorboe, T., Tang, K., Ploug, H., 2003. Bacterial colonization of particles: Growth and interaction. *Applied and Environmental Microbiology* 69 (6), 3500-3509.
- Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.), *Culture of marine invertebrate animals*. Plenum Press, pp. 108-132.
- Gutierrez, T., Berry, D., Yang, T., Mishamandani, S., McKay, L., Teske, A., Aitken, M., 2013. Role of bacterial exopolymers in the fate of the oil released during the Deepwater Horizon oil spill. *PLoS ONE* 8 (6), e67717.
- Head, I.M., Jones, D.M., Roling, W.F.M., 2006. Marine microorganisms make a meal of oil. *Nat Rev Micro* 4 (3), 173-182.
- Hsing, P.-Y., B. Fu, E.A. Larcom, S.B. Berlet, T.M. Shank, A.F. Govindarajan, A.J. Lukasiewicz, Dixon, P.M., Fisher, C.R., 2013. Evidence of lasting impact of the Deepwater Horizon oil spill on a deep Gulf of Mexico coral community. *Elementa: Science of the Anthropocene*.
- Hu, C.M., Weisberg, R.H., Liu, Y.G., Zheng, L.Y., Daly, K.L., English, D.C., Zhao, J., Vargo, G.A., 2011. Did the northeastern Gulf of Mexico become greener after the Deepwater Horizon oil spill? *Geophysical Research Letters* 38.
- Jackson, G.A., 1994. Particle trajectories on a rotating cylinder: Implications for aggregation incubations. *Deep-Sea Research* 41 (3), 429-437.
- Jackson, G.A., 1995. Comparing observed changes in particle size spectra with those predicted using coagulation theory. *Deep-Sea Research II* 42, 159-184.
- Jackson, G.A., 2005. Coagulation theory and models of oceanic plankton aggregation. In: Droppo, I., Leppard, G., Liss, S., Milligan, T. (Eds.), *Flocculation in natural and engineered environmental systems*. CRC Press, Boca Raton, Florida, pp. 271-292.
- Jackson, G.A., Burd, A.B., 1998. Aggregation in the marine environment. *Environmental Science & Technology* 32, 2805-2814.
- Kepkay, P., Johnson, B., 1988. Microbial response to organic particle generation by surface coagulation in seawater. *Marine Ecology Progress Series* 48, 193-198.
- Kepkay, P.E., 1994. Particle aggregation and the biological reactivity of colloids. *Marine Ecology Progress Series* 109 (2-3), 293-304.
- Kester, D., Duedall, I., Connors, D., Pytkowicz, R., 1967. Preparation of artificial seawater. *Limnology and Oceanography* 12 (1), 176-179.
- Khelifa, A., Stoffyn-Egli, P., Hill, P.S., Lee, K., 2005. Effects of salinity and clay type on oil-mineral aggregation. *Marine Environmental Research* 59 (3), 235-254.

- Kjørboe, T., 2003. Marine snow microbial communities: scaling of abundances with aggregate size. *Aquatic Microbial Ecology* 33 (1), 67-75.
- Kowalewska, G., 1999. Phytoplankton — the main factor responsible for transport of polynuclear aromatic hydrocarbons from water to sediments in the Southern Baltic ecosystem *ICES Journal of Marine Science* 56, 219-222.
- Kowalewska, G., Konat, J., 1997a. Distribution of polynuclear aromatic hydrocarbons (PAHs) in sediments of the southern Baltic Sea. *Oceanologia* 39 (1), 83-104.
- Kowalewska, G., Konat, J., 1997b. The role of phytoplankton in the transport and distribution of polynuclear aromatic hydrocarbons (PAHs) in the southern Baltic environment. *Oceanologia* 39 (3), 267.
- Kujawinski, E.B., Kido Soule, M.C., Valentine, D.L., Boysen, A.K., Longnecker, K., Redmond, M.C., 2011. Fate of dispersants associated with the Deepwater Horizon oil spill. *Environmental Science & Technology* 45 (4), 1298-1306.
- Lubecki, L., Kowalewska, G., 2010. Distribution and fate of polycyclic aromatic hydrocarbons (PAHs) in recent sediments from the Gulf of Gdansk (SE Baltic). *Oceanologia* 52 (4), 669-703.
- McGenity, T., Folwell, B., McKew, B., Sanni, G., 2012. Marine crude-oil biodegradation: a central role for interspecies interactions. *Aquatic Biosystems* 16 (8).
- Montagna, P., Baguley, J., Cooksey, C., Hartwell, I., Hyde LJ, Hyland, J., Kalke, R., Kracher, L., Reuscher, M., Rhodes, A., 2013. Deep-Sea Benthic Footprint of the Deepwater Horizon Blowout. *PLoS ONE* 8 (8), e70540.
- Niu, H., Li, Z., Lee, K., Kepkay, P., Mullin, J.V., 2011. Modelling the transport of oil-mineral aggregates (OMAs) in the marine environment and assessment of their potential risks. *Environmental Model Assessment* 16, 61-75.
- Omotoso, O.E., Munoz, V.A., Mikula, R.J., 2002. Mechanisms of crude oil-mineral interactions. *Spill Science & Technology Bulletin* 8 (1), 45-54.
- Ortega-Retuerta, E., Reche, I., Pulido-Villena, E., Agustí, S., Duarte, C.M., 2009. Uncoupled distributions of transparent exopolymer particles (TEP) and dissolved carbohydrates in the Southern Ocean. *Marine Chemistry* 115 (1-2), 59-65.
- Passow, U., 2002a. Production of transparent exopolymer particles (TEP) by phytoplankton and bacterioplankton. *Marine Ecology Progress Series* 236, 1-12.
- Passow, U., 2002b. Transparent exopolymer particles (TEP) in aquatic environments. *Progress in Oceanography* 55, 287-333.
- Passow, U., Alldredge, A.L., 1995. A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP). *Limnology and Oceanography* 40 (7), 1326-1335.
- Passow, U., De La Rocha, C.L., 2006. Accumulation of mineral ballast on organic aggregates. *Global Biogeochemical Cycles* 20 (GB1013), 7.
- Passow, U., Rocha, C.L.D.L., Fairfield, C., Schmidt, K., 2014. Aggregation as a function of pCO₂ and mineral particles. *Limnology and Oceanography* 59 (2), 532-547.
- Passow, U., Ziervogel, K., Asper, V., Diercks, A., 2012. Marine snow formation in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico. *Environmental Research Letters* 7 (035031), 11.
- Patton, J., Rigler, M., Boehm, P., Fiest, D., 1981. Ixtoc 1 oil spill: Flaking of surface mousse in the Gulf of Mexico. *Nature* 290, 235-238.
- Pilskaln, C.H., Lehmann, C., Paduan, J.B., Silver, M.W., 1998. Spatial and temporal dynamics in marine aggregate abundance, sinking rate, and flux: Monterey Bay, central California. *Deep-Sea Research II* 45, 1-26.

- Ploug, H., Grossart, H.-P., 1999. Bacterial production and respiration in suspended aggregates - a matter of the incubation method. *Aquatic Microbial Ecology* 20, 21-29.
- Ploug, H., Passow, U., 2007. Direct measurements of diffusivity of diatom aggregates containing transparent exopolymer particles. *Limnology and Oceanography* 52, 1-6.
- Ploug, H., Terbrüggen, A., Kaufmann, A., Wolf-Gladrow, D., Passow, U., 2010. A novel method to measure particle sinking velocity in vitro, and its comparison to three other in vitro methods. *Limnology and Oceanography: Methods* 8, 386-393.
- Reddy, C.M., Arey, J.S., Seewald, J.S., Sylva, S.P., Lemkau, K.L., Nelson, R.K., Carmichael, C.A., McIntyre, C.P., Fenwick, J., Ventura, G.T., Van Mooy, B.A.S., Camilli, R., 2011. Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill. *Proceedings of the National Academy of Sciences*.
- Ryerson, T.B., Camilli, R., Kessler, J.D., Kujawinski, E.B., Reddy, C.M., Valentine, D.L., Atlas, E., Blake, D.R., de Gouw, J., Meinardi, S., Parrish, D.D., Peischl, J., Seewald, J.S., Warneke, C., 2012. Chemical data quantify Deepwater Horizon hydrocarbon flow rate and environmental distribution. *Proceedings of the National Academy of Sciences* 109 (50), 20246-20253.
- Shanks, A.L., Edmondson, E.W., 1989. Laboratory-made artificial marine snow: A biological model of the real thing. *Marine Biology* 101 (4), 463-470.
- Simon, M., Grossart, H.P., Schweitzer, B., Ploug, H., 2002. Microbial ecology of organic aggregates in aquatic ecosystems. *Aquatic Microbial Ecology* 28 (2), 175-211.
- Smetacek, V.S., 1985. Role of sinking in diatom life-history cycles: Ecological, evolutionary, and geological significance. *Marine Biology* 84, 239-251.
- Spier, C., Stringfellow, W.T., Hazen, T.C., Conrad, M., 2013. Distribution of hydrocarbons released during the 2010 MC252 oil spill in deep offshore waters. *Environ. Pollut* 173, 224-230.
- Stoffyn-Egli, P., Lee, K., 2002. Formation and characterization of oil-mineral aggregates. *Spill Science & Technology Bulletin* 8 (1), 31-44.
- Verdugo, P., 2012. Marine Microgels. *Annual Review of Marine Science* 4 (1), 375-400.
- Wang, X.-C., Chen, R., 2001. Contribution of "old" carbon from natural marine hydrocarbon seeps to sedimentary and dissolved organic carbon pools in the Gulf of Mexico. *Geophysical Research Letters* 28 (17), 3313-3316.
- White, H.K., Hsing, P.-Y., Cho, W., Shank, T.M., Cordes, E.E., Quattrini, A.M., Nelson, R.K., Camilli, R., Demopoulos, A.W.J., German, C.R., Brooks, J.M., Roberts, H.H., Shedd, W., Reddy, C.M., Fisher, C.R., 2012. Impact of the Deepwater Horizon oil spill on a deep-water coral community in the Gulf of Mexico. *Proceedings of the National Academy of Sciences* 109 (50), 20303-20308.
- Zhou, Z., Guo, L., Shiller, A.M., Lohrenz, S.E., Asper, V.L., Osburn, C.L., 2013. Characterization of oil components from the Deepwater Horizon oil spill in the Gulf of Mexico using fluorescence EEM and PARAFAC techniques. *Marine Chemistry* 148 (0), 10-21.
- Ziervogel, K., D'Souza, N., Sweet, J., Yan, B., Passow, U., 2014. Natural oil slicks fuel surface water microbial activities in the northern Gulf of Mexico. *Frontiers in Aquatic Microbiology* 5, 188.
- Ziervogel, K., McKay, L., Rhodes, B., Osburn, C., Dickson-Brown, J., Arnosti, C., Teske, A., 2012. Microbial activities and dissolved organic matter dynamics in oil-contaminated surface seawater from the Deepwater Horizon oil spill site. *PLoS ONE* 7 (4).

Figure 1

Figure 2

Figure 3

Figure 4