2021 INTERNATIONAL OIL SPILL CONFERENCE

Formation of marine oil snow with surface water from Cook Inlet, AK

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ABSTRACT

The objective of the research is to inform response decision-making and understanding of the potential association of spilled oil with marine snow in Cook Inlet, Alaska. While extensive research has been conducted on minerals aggregating with spilled oil, larger organic aggregates, such as marine snow, have only recently been studied as a transport mechanism. This knowledge gap in understanding the fate of oil was highlighted as part of the Gulf of Mexico Research Initiative (GoMRI) following the Deepwater Horizon (DWH) spill. It was determined that significant percentages of spilled oil reached the seafloor as a result of association with marine snow during both DWH and the Ixtoc 1 blowouts. As development of oil resources continues in Alaska and the Arctic, marine snow is a significant oil exposure pathway that must be considered during oil spill response. In parallel with a corresponding sedimentation study, input from local, federal and industry experts was used to develop laboratory scale oil exposure experiments to evaluate the potential for oil-marine snow aggregate formation in Cook Inlet. Roller-bottle experiments were conducted from May to July 2019 to assess the interactions between a 5 μm sheen of Alaska North Slope crude oil and Cook Inlet surface water. Aggregate formation was documented and sinking flocs were observed and analyzed with fluorescence microscopy to estimate oil content. The total oil volumes estimated in aggregates were between 0.01 to 0.4 μl. Estimates of total oil volume associated with the aggregates ranged from 0.6 to 9.3 % ± 1.4% of the total oil volume (80 μl) that was added to the bottles. The incorporation of spilled oil in surface forming aggregates will contribute to understanding fate and response implications in Cook Inlet and other northern regions at risk of spilled oil entering the benthic food web via association with sinking marine snow.

INTRODUCTION AND BACKGROUND

Marine snow is the phenomenon of particle aggregates sinking throughout the world's oceans. Natural marine snow (NMS)[1] forms in the surface layers of the ocean and consists of biotic and abiotic substances, such as phytoplankton, zooplankton, fecal pellets, and minerals (Alldredge and Silver, 1988). As surface-forming NMS aggregates increase in size and weight, and incorporate suspended sediment, they become negatively buoyant and sink. NMS is used as a food source by pelagic and benthic species or is deposited on the seafloor (Steinberg, 1995; Green and Dagg, 1997; Dilling et al., 2004).

During the 2010 Deepwater Horizon (DWH) oil spill in the Gulf of Mexico (GoM), a portion of the oil settled to the seafloor associated with NMS. The oil spill research community defined the formation, sinking, and fate of these aggregates as Marine Oil Snow (MOS) Sedimentation and Flocculent Accumulation (MOSSFA) and several Gulf of Mexico Research Initiative (GoMRI) consortia explored the impacts and significance of the sedimentation event (CSE, 2013). During DWH, researchers observed mucus-rich MOS aggregates up to 10 cm at the surface of the water near the blowout (Passow et al., 2014). The upper 140 m of the water column also showed a three-fold increase in quantity of marine snow particles, observed by a shadowed image particle profiling and evaluation recorder (SIPPER) system, compared to the four summers following the event (Daly et al., 2016; Daly et al., 2018). From sedimentary oil indicators, it was estimated that 3 to 14% of the total DWH oil released sank to the bottom (Valentine et al., 2014; Chanton et al., 2015).

"Oily-floc" collected in sediment cores showed increased weathering and biodegradation up to 8 km from the well (Stout and Payne, 2016). The deposited material was comprised of oil-

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[1] There are multiple terms used to describe marine snow and oil associated with marine snow. This paper will use the same nomenclature as used in the recent review of the topic by Brakstad et al. (2018).

related compounds, bacterial biomass, surface blooming phytoplankton and zooplankton fecal pellets. The formation of MOS was attributed to a combination of dispersed oil droplets, chemical dispersants, high phytoplankton densities, and the influence of riverine nutrients and clays discharged from the Mississippi River as part of the response (CSE, 2013). The DWH blowout conditions were unique (134 million gallons of crude oil released at a depth of 1525 m over 87 days) and the role of MOS in the overall mass balance of the released oil was unforeseen (U.S. District Court, 2015; Daly et al., 2016).

Documented impacts from DWH MOS to the GoM include oil exposure of benthic species, reduced oxygen conditions in seafloor sediments, and mortality of benthic fauna (Schwing et al., 2015; Brooks et al., 2015; Romero et al., 2015). After DWH, fish that prey on benthic species and those that live close to the sediment (e.g. Red Snapper, Golden Tilefish) exhibited elevated levels of polycyclic aromatic hydrocarbons (PAHs) in their bile (Murawski et al., 2014; Snyder et al., 2015).

Vonk et al. (2015) reviewed 52 large oil spills to investigate past MOSSFA events and found that benthic contamination was documented at two other spills (Santa Barbara (1969) and Ixtoc 1 (1979-1980)), but systematic monitoring of benthic effects during a response was rare in most spill responses (Vonk et al., 2015). During the Ixtoc 1 response, Boehm and Fiest (1982) documented deposition of oil-phytoplankton aggregates, which were also later observed in sediment cores by Schwing et al. (2020) indicating the MOSSFA event. More than 50 researchers met in 2013 and concluded that MOSSFA must be considered as a pathway for the "protracted exposure, uptake and continued metabolism of toxic and carcinogenic petroleum hydrocarbons by ecologically, economically and recreationally important benthic fish" (Kinner et al., 2014). The researchers also concluded that MOSSFA processes should be included in predictive models for the fate of spilled oil, highlighting the need to better understand MOSSFA drivers in regions at risk.

The potential drivers for a significant MOSSFA event may be found throughout Arctic and Subarctic regions due to petroleum shipping and extraction. The drivers for MOSSFA are: (1) oil entering the water column, (2) high content of clay mineral particles, and (3) presence of phytoplankton and/or oil-degrading bacteria (Vonk et al., 2015).

Oil drilling and production in Cook Inlet started in the late 1950s and by 2015 there were 16 active platforms in the region, producing a total of 15,800 barrels per day (bpd) (AOGA, 2015). While the current production platforms are all in state waters, during 2017 14 lease blocks (~120 sq. mi) were sold on the outer continental shelf (OCS) which is governed by BOEM (2018). BOEM estimated that there are 1.01 billion barrels (Bbbl) of undiscovered, but technically recoverable oil reserves in the Cook Inlet OSC region (BOEM, 2017).

In addition to offshore drilling, spills from oil shipping are also a threat in the region. A 2012 vessel traffic study reviewed 500 port calls to Anchorage and summarized that traffic was comprised of Ro-Ro (roll-on/roll-off) cargo vessels (44%), ferries (23%), crude oil tank ships (16%), bulk carriers (7.5%) and other traffic including refined product tank ships, gas carriers, cruise ships and fishing vessels (9.5%) (Cape International, 2012). A workshop held in 2012 considering spill consequence in Cook Inlet as a function of habitat, fish, birds, mammals, commercial and subsistence fishing, and industry examined seven scenarios varying oil type, volume, and time of year (Nuka, 2013). The resulting report concluded that “all areas of Cook Inlet are vulnerable to significant consequences from marine oil spills of any type in all seasons”. In 2014, NOAA (Reich et al., 2014) assessed marine oil spill risk and environmental vulnerability in Alaska and calculated the relative risk per region. It ranked the Cook Inlet region as third highest in the state for environmental vulnerability from a “worst case discharge” due to the severity of potential impacts.

Lower Cook Inlet is known to have areas of high primary productivity exhibiting an annual diatom bloom beginning in late April, peaking in July, and declining to near zero from November to March (Holderied et al., 2018). In addition, Cook Inlet waters have high suspended solids loads (MMS, 1995). Four million tons of sediment are discharged into the inlet annually from six major river basins, with most entering the inlet June through August (USGS, 1999). Suspended sediment concentrations average 200 mg/L (Sharma and Burell, 1970). While Cook Inlet has very strong currents, there are areas where NMS and suspended solids can settle to the bottom.

The objective of this research was to test the hypotheses that surface waters from lower Cook Inlet would form NMS and, if oil were present would form MOS aggregates in roller-bottles to a similar degree as found in studies with water from other regions of high primary productivity (e.g., as observed in the GoM during the DWH spill). We hoped to gain a better understand of MOS as a potential exposure route in Subarctic conditions that could impact critical benthic habitats in lower Cook Inlet and similar highly productive areas in Alaska where oil extraction/production and shipping accidents might occur.

METHODS AND MATERIALS

Oil incubation experiments were conducted in summer 2019 to evaluate the potential for oil sorption and sinking. The studies were conducted at NOAA’s Kasitsna Bay Laboratory using water collected during particle flux sampling. The roller-bottle procedure for this research was designed to be relevant for surface oil slick interactions in the days following a tanker spill (t = 5 d; oil slick = 5 µm; headspace in bottle simulating surface slick rather than dispersed droplets).

Three identical roller-bottle incubation experiments were conducted. Surface water was collected in a 5 gallon carboy, rinsed with surface water three times using a research vessel from the laboratory. For each experiment, 1 L of surface water was filtered using GF/F for surface water characterization of Total Particulate Matter (TPM) and Total Volatile Solids (TVS). Eight 0.95 L (32 oz.) glass bottles each received 800 mL of surface water. The inside of each glass bottle's plastic lid was lined with Teflon to limit oil sorption. Four bottles each received 80 µl of Alaska North Slope (ANS) crude oil from a positive displacement pipette. The resulting nominal oil concentration was 80 µl oil/ 800 mL seawater or 0.01% oil by volume (100 ppm) but is more appropriately characterized by a surface sheen thickness of ~5 µm.

Table 1. Experimental set-up for roller-bottle incubations

Experimental Condition	***Seawater Volume (mL)***	***ANS Oil (µl)***	***Sediment (mg)***
W	800	-	-
W	800	-	-
W + S	800	-	160
W + S	800	-	160
W + O	800	80	-
W + O	800	80	-
W + S + O	800	80	160
W + S + O	800	80	160

Sediment from Nikiski, AK (middle Cook Inlet), was added to four of the bottles at a nominal concentration of 200 mg/L, which is a realistic concentration of suspended matter found in the middle and upper inlet (Hein et al., 1979). The dried sediment sample used was characterized by Khelifa et al. (2008) to be 49% fine content (weight of grains less than 5.3 μm in diameter), with a density of 2.58 ± 0.11 g/mL and an organic matter content of 3.3 ± 0.1%.

The bottles were then placed on the rolling apparatus at a speed of 4 rpm under constant light (two 40W bulbs) in a 10°C temperature controlled room. After 120 h, the bottles were removed from the apparatus and set upright on the laboratory bench to allow contents to settle. Qualitative visual estimates of relative aggregate abundance, size, and settling rates were recorded for each treatment condition as bottles were removed from the rolling apparatus. After 1-3 h of settling, the surface oil sheen was manually removed with a sorbent pad and five aggregates were extracted from one bottle of Water (W) and Water + Sediment (W+S) and five aggregates were extracted from both replicate Water + Oil (W+O) and Water + Oil + Sediment (W+S+O) bottles. Aggregates were analyzed using 10x ocular and 10x objective lenses with a Nikon Eclipse 80i microscope Fluorescent light was used to distinguish the oil droplets. Photomicrographs were taken of aggregates with and without fluorescence for image analysis with the program ImageJ (Rasband, 1997; Bethesda, MD) to estimate the quantity of oil associated with aggregates. Then, the contents of the bottles were filtered on GF/F filters to determine TPM and TVS. All filters were dried for 24 h at 60°C to obtain the dry weight of particles (TPM). Then, the filters were ignited for 6 h at 500°C and weighed to estimate the organic content (TVS) (Traiger and Konar, 2017).

RESULTS AND DISCUSSION

The surface water used in the incubations had TPMs and TVSs of ~30 and ~10 mg/L, respectively (30-35% organic matter), typical of this season and area of Cook Inlet. The bottles had no noticeable NMS or large particles at the start of the experiments. Oil negative controls, W and W+S, exhibited no fluorescence, confirming no oil in aggregates after rolling. Once the bottles were taken off the roller table, most aggregates in them settled to the bottom over 30 minutes (**Figure 1**). The addition of oil to high sediment concentrations did lead to reduced overall TPM at the bottom of the bottle after settling, which was also observed by Stoffyn-Egli and Lee (2002), who noted that oil-mineral aggregates are less dense and often buoyant.

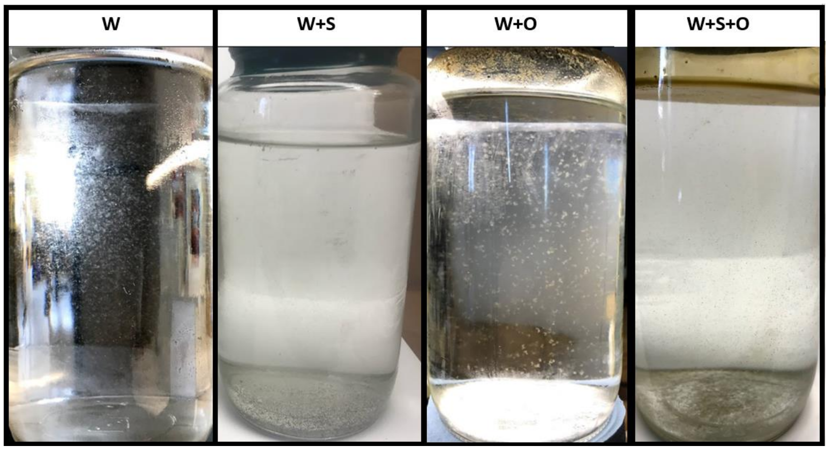


Figure 1. Photographs of bottles at the end of the third experiment prior to filtration.

Oil fluoresced on considerable percentages of the aggregates observed at the end of the incubations for W+O and W+S+O treatments (see **Figure 2** for W+O photomicrographs). The total oil volumes estimated in each aggregate were between 0.01 to 0.4 µl. These ranges were used to extrapolate the total volume of oil associated with the submerged MOS. Estimates of total oil volume associated with the aggregates ranged from 0.6 to 9.3 % ± 1.4% of the total oil volume (80 µl) that was added to the bottles at t=0.

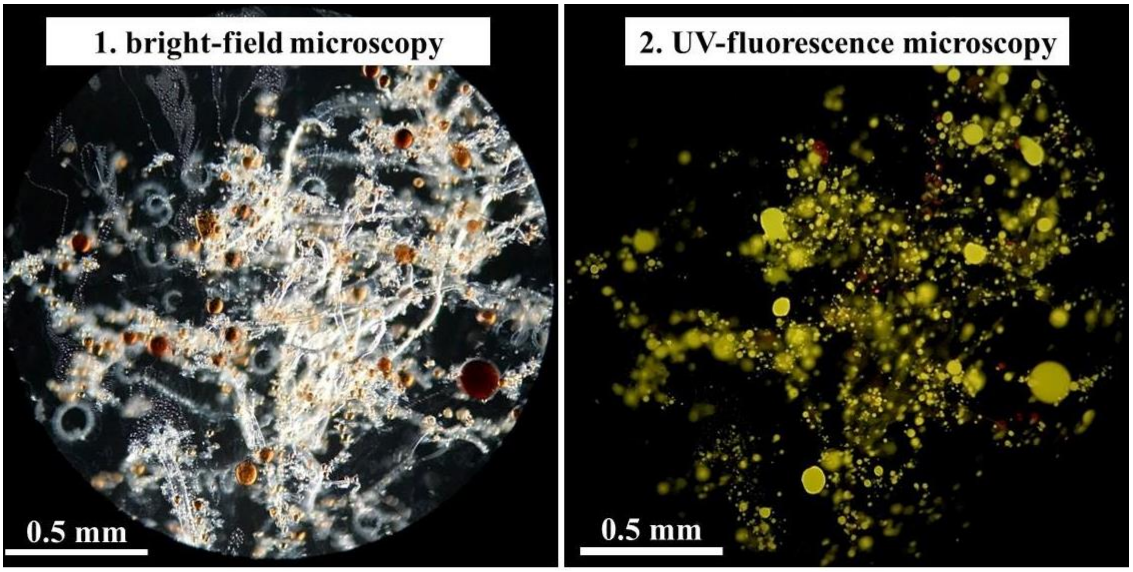
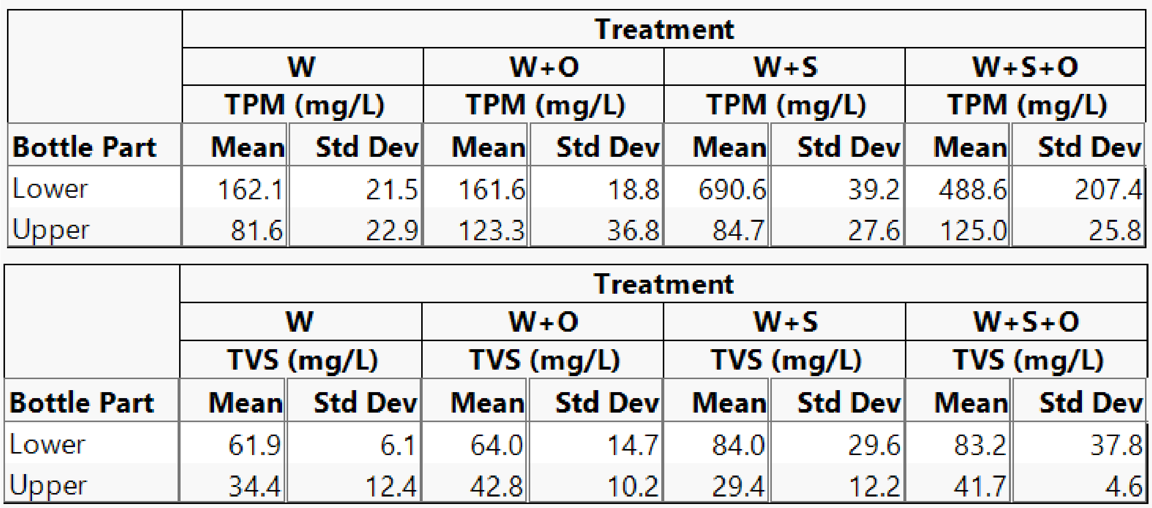


Figure 2. Photomicrograph of a W+O aggregate under direct light and fluorescence.

The TPM and TVS results for the experiments are shown in **Table 2**. Student's t-tests and ANOVAs showed a significant difference driven by the addition of oil was an increase in suspended material. Increased TPM concentrations with oil were likely due to enhanced aggregation, or integration of oil in clusters, which has been observed in other MOS studies (Passow et al., 2012; Brakstad et al., 2015; Passow et al., 2017).

Table 2. Results summary TPM and TVS means from all three experiments.



	Treatment							
	W		W+O		W+S		W+S+O	
	TPM (mg/L)		TPM (mg/L)		TPM (mg/L)		TPM (mg/L)	
Bottle Part	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**
Lower	162.1	21.5	161.6	18.8	690.6	39.2	488.6	207.4
Upper	81.6	22.9	123.3	36.8	84.7	27.6	125.0	25.8

	Treatment							
	W		W+O		W+S		W+S+O	
	TVS (mg/L)		TVS (mg/L)		TVS (mg/L)		TVS (mg/L)	
Bottle Part	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**
Lower	61.9	6.1	64.0	14.7	84.0	29.6	83.2	37.8
Upper	34.4	12.4	42.8	10.2	29.4	12.2	41.7	4.6

The MOS formed in this study was similar to previous laboratory observations of formation from surface waters within similar timescales (days) in the presence of oil: enhanced growth resulting in consolidated, non-sinking aggregates (Passow et al., 2012; Ziervogel et al., 2014). Oil negative controls also showed that diatom laden surface waters formed small aggregates, but did not grow larger overtime, which supports findings of Passow (2014). The addition of sediment created ballast weight and led to higher masses of organics sinking.

The mechanisms of MOS formation have been identified in past studies, but little is known about underlying drivers of these processes (Quigg et al., 2016). MOS formation in this study was likely due to a combination of microbial and physical drivers. EPS plays a significant role in the stickiness of aggregates and was likely abundant in the surface water samples from Cook Inlet, although not directly measured (Alldredge et al., 1993). *Chaetoceros sp.*, consistently the most abundant diatom in blooms in lower Cook Inlet (Holderied et al., 2018), is closely linked to the production of EPS in surface waters during blooms (Passow et al., 2002). In the presence of oil, more EPS may have also been produced by microbes and bacteria as a result of oil degradation or by diatoms for protection from the toxic components of oil (Gutierrez et al., 2013; Passow et al., 2016).

The marine snow that formed in the roller-bottles interacted with the thin sheen of ANS crude oil and oil was incorporated in non-floating aggregates. Without the presence of sediment, biologically-based aggregates formed within 5 days, and did not rapidly sink. The estimates of oil association with MOS range from 0.6 to 9.3% of the oil added and were within the 0.5 – 14% estimated range of DWH oil that was deposited on the seafloor, measured by various methods (Chanton et al., 2015; Valentine et al., 2014). They are also bracket the estimated 6.8 to 7.2% of the total DWH oil that reached the surface and was not recovered or burned and was measured subsequently in sediment traps at 450 m (Stout and German, 2018).

Oil in the roller-bottle experiments likely enhanced aggregation through multiple mechanisms such as increasing stickiness and production of EPS. These aggregates clearly incorporated oil and looked fluffier and more consolidated than diatom aggregates in oil negative treatments. The neutrally buoyant state of most MOS aggregates was an interesting finding. Other marine oil snow studies that employed roller-bottles have found that aggregates become dense enough to sink rapidly when the bottle is put upright (Ziervogel et al., 2012). The results suggest that more suspended sediment than what was in the water sample is necessary to induce rapid sedimentation.

There are interdependent conditions of oil-aggregate interactions that were not explored in this study which may have significant impacts on aggregation and associated impacts: the effects of photo-oxidation, weathering, the possibility that the roller-bottle may have gone anoxic, the role of chemical dispersants, and the origin of bacteria populations (crude oil or the natural seawater). Some of these factors have been investigated in other studies (e.g., Ziervogel et al., 2012; Ziervogel et al., 2014; Passow et al., 2012; Passow, 2016; Passow et al., 2017), and should be addressed in future work in this region.

CONCLUSIONS

To the best of our knowledge, this was the first study to examine the potential formation of biological oil aggregates in Cook Inlet or other Alaskan waters. The DWH oil spill demonstrated the potential for biological aggregates to carry surface oil to depth and critical habitats. While laboratory studies cannot perfectly simulate *in-situ* conditions and processes, the results suggest that MOS would form in the upper water column of Cook Inlet and similar waters during a spill but may not sink to the bottom rapidly except in areas with high sediment load and/or relatively quiescent conditions. The results of this research corroborate previous findings from other regions showing enhance aggregation of NMS, forming MOS in the presence of oil (Passow et al., 2012; Ziervogel et al., 2012; Passow, 2017), and suggest that MOS would likely form in an oil spill in lower Cook Inlet. It is also likely that similar percentages of oil could be associated with marine snow and potentially transported to the benthos if there is enough suspended sediment in the surface waters to induce sinking. The ultimate fate of MOS in Cook Inlet would likely be a function of oil type, composition of the bloom, and the suspended sediment load at the time of an event. MOSSFA has the potential to impact the marine ecosystem through different mechanisms than OMAs.

This study has shown distinct differences in aggregate formation with and without high sediment loads. Daly et al. (2016) highlighted the potential toxic exposure routes associated with MOS: ingestion, smothering, and bioaccumulation. Pelagic and benthic food webs are more susceptible to oil exposure through MOS as organic aggregates are a natural food source (van Eenennaam et al., 2019). Bacterial degradation of oil may also move toxic components of oil higher in the food web (Almeda et al., 2014; Ziervogel et al., 2016).

The formation and sinking of MOS should be further studied and considered in oil spill response and damage assessment in the lower Cook Inlet region, as well as other areas exhibiting similar seasonally high productivity, riverine inputs, and strong connectivity from surface to benthos. Better understanding of MOS formation and fate in the environment will help inform response decision-making to more accurately manage injury and optimize response efforts. This is especially important because unlike oil mineral aggregates (OMAs), MOS can serve as a food source for benthic organisms.

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