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Marine snow increases the adverse effects of oil on benthic invertebrates

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ABSTRACT

After the Deepwater Horizon oil spill, a MOSSFA (Marine Oil Snow Sedimentation and Flocculent Accumulation) event took place, transporting an estimated 14% of total released oil to the sediment, and smothering parts of the benthic ecosystem. This microcosm study describes the effects of oiled artificial marine snow on benthic macroinvertebrates. *Corophium volutator* survival was reduced by 80% in oil-contaminated snow. *Hydrobia ulvae* survival was reduced by 40% in oil-contaminated snow, possibly due to consumption of oiled snow. *Macoma balthica* was sensitive to marine snow, addition of oil slightly decreased survival. This study reveals trait-dependent sensitivity to oil with or without marine snow. The main drivers for organismal response to marine snow and oil are motility, sensitivity to hypoxia and oil toxicity, and feeding habits. Adverse effects of MOSSFA events on benthos will have consequence for the benthic-pelagic habitat and food chain, and should receive more attention in oil spill management.

1. Introduction

The Deepwater Horizon (DWH) oil spill in the Gulf of Mexico in 2010 was one of the largest marine oil spills in US history. Over a three month time period, 4.9 million barrels of oil leaked into the sea (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011). The main spill response was the use of 6.8 million liters of dispersants (BP Gulf Science Data, 2017a, 2017b, 2017c), both applied at the sea surface as well as injected in the sub-sea at the well head.

One of the unexpected events seen during the DWH spill was the unprecedented formation of marine snow, which was probably due to an increased production of extracellular polymeric substances (EPS) by the microbial and phytoplankton community as a biological stress response to dispersants and oil (Passow et al., 2012; Ziervogel et al., 2012; van Eenennaam et al., 2016). Laboratory-induced EPS made by phytoplankton-associated bacteria was found to contain substantial amounts of alginate-like exopolysaccharides (van Eenennaam et al., 2016), and a peak of polysaccharides in sediment from the Gulf of Mexico coincided with the marine snow formation during the spill (Hollander et al., 2016). During the oil spill, there was a large amount of suspended solids due to the flushing of the Mississippi river as a spill response (Bianchi et al., 2011) as well as a phytoplankton bloom (Hu et al., 2011; O'Connor, 2013). Marine snow aggregates, made of dispersed oil, organic debris, phytoplankton, and suspended particles, glued together by the sticky EPS, settled on the ocean floor in a process called MOSSFA: Marine Oil Snow Sedimentation and Flocculent Accumulation (Daly et al., 2016). MOSSFA increased sedimentation rates and caused a downward flux of oil to the sediment (MOSSFA Steering Committee, 2013; Brooks et al., 2015; Hastings et al., 2016). Estimates vary, but as much as 14% of the total oil released during the DWH oil spill may have ended up on the sediment due to MOSSFA (Daly et al., 2016). Estimates of the total area of sedimentary oil deposition range from 3200 km^2 (Valentine et al., 2014) to 24,000 km^2 (Chanton et al., 2015). A review of large historical oil spills has indicated that the MOSSFA process may have occurred during other spills as well, such as the IXTOC-I blowout (Vonk et al., 2015).

The MOSSFA related oil contamination sparked interest in the potential long-term effects of sedimented oil for benthic habitats (Kinner et al., 2014). Benthic organisms, especially those that are sedentary, are particularly at risk for the MOSSFA related oil contamination (Fisher et al., 2016), since they cannot easily escape the contamination. Moderate to severe reduction of macro- and meiofaunal abundance and diversity was found over an area of 172 km^2 around the wellhead (Montagna et al., 2013). Persistent reducing conditions in the sediment and the 2–3 fold increase in PAHs reduced benthic foraminiferal

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Table 1
Test treatments for the Microcosm Experiment. Artificial marine snow was composed of alginate, phytoplankton biomass, and kaolin clay.

	Treatment description	Treatment code	Oil	Kaolin clay	Alginate and phytoplankton biomass
Set 1:	Control	Control			
In vivo observations	Kaolin clay	Clay		✓	
	Marine snow	Snow		✓	✓
	Oil and kaolin	Oil	✓	✓	
	Marine snow and oil	Snow + Oil	✓	✓	✓
Set 2:	Oil and kaolin	Oil/B	✓	✓	
Biodegradation sampling	Marine snow and oil	Snow + Oil/B	✓	✓	✓

diversity and density (Schwing et al., 2015; Schwing et al., 2016).

MOSSFA could affect benthic ecosystems via two mechanisms: 1) direct toxicity of the oil and 2) reduced oxygen availability caused by the microbial degradation of the marine snow. Direct oil toxicity to benthic organisms is widely reported, both in experimental studies (e.g., (Foekema et al., 1996) and (Bhattacharyya et al., 2003)) and in oil spill observations (e.g., (Teal and Howarth, 1984), (Lee and Lin, 2013), and (Jewett et al., 1999)). The availability of oxygen in deeper layers of the sediment can be increased by the bioturbation activity of many benthic organisms (Pelegrí and Blackburn, 1994). Bioturbation enhances the sediment oxygenation, solute transport, and remineralization of organic matter, and mixes horizontal sediment layers (Levin, 2003). Oxygen consumption by biodegradation of marine snow, the second mechanism, can impact benthic organisms after a MOSSFA event. The accumulation of organic material on the sea floor increased microbial respiration, resulting in decreased oxygen in sediment pore waters (Hastings et al., 2016). In laboratory studies, artificially produced marine snow was found to consume oxygen at a rapid rate (Rahsepar et al., 2017) and this lower oxygen concentration in the sediment is detrimental to benthic invertebrates living in the top layers of the sediment.

Benthic organisms in general are an important part of the marine food web, and many pelagic species are dependent on the benthic ecosystem for feeding or reproduction while some have benthic life stages. Most oil effect studies are performed with pelagic species, and without marine snow or sediment. This article describes a microcosm experiment revealing the additional effect of marine snow on the toxicity of oil on marine benthic invertebrates. The effect of the presence of invertebrates on bioturbation and oil biodegradation is also presented. The experiment simulated the effect of marine snow and oil in small scale microcosms with sediment, natural sea water, and four representative species of benthic invertebrates. These invertebrates reflect four different benthic lifestyles and feeding strategies (traits): an infaunal deposit and suspension feeding amphipod, an epifaunal deposit feeding and grazing gastropod, an infaunal deposit and suspension feeding bivalve, and epi- and infaunal deposit and suspension feeding foraminifera.

2. Materials and methods

2.1. Experimental setup

The basic experimental design was similar to (Rahsepar et al., submitted), with the inclusion of benthic macroinvertebrates. In short, 21 microcosms of 25 × 25 × 25 cm were placed in a climate controlled room at 14 °C, with a day-night light regime of 16 h light and 8 h dark. Full glass microcosms were used to avoid the presence of silicone rubber.

Sediment was collected during low tide from the top 10 cm of an intertidal mudflat in the Dutch Wadden Sea, at approximate location N 52° 56.112 E 004° 59.976, and transported directly to the laboratory of Wageningen Marine Research, Den Helder, The Netherlands. Sediment was subsequently sieved over a 1 mm sieve to remove large organisms and particles, and thoroughly mixed by hand to create a homogeneous sediment.

The microcosms were filled with a 5 cm thick layer of sediment, and a 15 cm layer of natural sea water (0.45 μm filtered Eastern Scheldt water) on top. The sediment was left to settle for one day prior to the start of the experiment.

The next day, day 0, the treatments as described in Section 2.3 were added to the microcosms. One day later (day 1), 40 *Corophium volutator* (amphipod), 20 *Macoma balthica* (bivalve), and 20 *Hydrobia ulvae* (gastropod) were randomly added to each microcosm. These test organisms were collected at the same time and location as the sediment, and were kept in the lab at 14 °C with natural sea water and aeration. The natural sediment already contained foraminifera.

Air was bubbled via two tubes with glass Pasteur pipette tips per microcosm in the top 5 cm of the water column, using an aquarium air pump. Microcosms were covered with acrylic plastic covers to minimize evaporation. Approximately once per week the water level was adjusted with demineralized water to compensate for evaporation.

In order to stimulate biodegradation of the oil, 1 mL at optical density 0.98 of *Rhodococcus qingshengii* TUHH-12 (DSMZ No. 46766), an alkane-degrading bacterial culture, and 1 mL at optical density 0.30 of *Pseudomonas putida* F1, an aromatic-degrading bacterial culture, were added to the water column of each microcosm at day 0. See (Rahsepar et al., 2016) for the culturing procedure.

2.2. Treatments

Five treatments were tested in triplicate (Table 1). The Control treatment consisted of only a sediment layer. The Oil treatment consisted of oil mixed with clay particles to let the oil settle on top of the sediment. As a control for this treatment, a Clay only treatment was included with the same amount of suspended clay. The Snow + Oil treatment included artificial marine snow plus oil mixed with clay. As a control, the Snow treatment was the same but without oil. The clay was needed to make the marine snow and oil negatively buoyant. With the clay no floating oil and/or marine snow were visible neither on the surface nor in the water column.

In addition to the five treatments in triplicate as mentioned above, in which invertebrate survival was tested for 16 days, a second set of 6 microcosms was run in parallel with only the Oil and Snow + Oil treatments, used for oil biodegradation sampling. These microcosms in Set 2 had to be separate from the microcosms in Set 1, since taking sediment samples for oil biodegradation analysis from the same microcosms would interfere with the final observations of invertebrate survival. The microcosms in Set 2 ran for 42 days, and samples were taken on day 1, 8, 16, 30, and 42 as described by (Rahsepar et al., submitted).

2.3. Test treatment preparation

Artificial marine snow was used as an approximation of natural marine snow, since it was not feasible to create natural marine snow in the amounts needed for this study. To mimic the lab-induced EPS containing alginate-like exopolysaccharides (van Eenennaam et al., 2016), we used commercially available alginate, a gelling and nontoxic