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Short Communication

Adhesion of mechanically and chemically dispersed crude oil droplets to eggs of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*)

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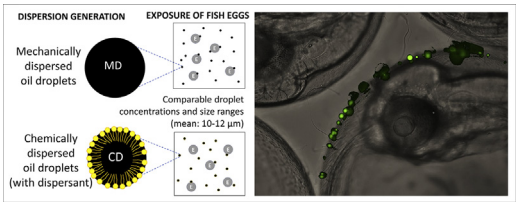
HIGHLIGHTS

- Dissolved oil components are considered the most important driver for oil dispersion toxicity, but oil droplets may represent an additional route of exposure and toxicity.
- Dispersed crude oil droplets readily adhere to the surface of fish eggs.
- Oil droplet adhesion may be an important exposure route for oil components to fish embryos.
- Chemical dispersants reduce crude oil droplet adhesion to fish eggs.
- Oil droplet adhesion capacity of eggs can vary between fish species.

GRAPHICAL ABSTRACT



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ABSTRACT

Crude oil accidentally spilled into the marine environment undergoes natural weathering processes that result in oil components being dissolved into the water column or present in particulate form as dispersed oil droplets. Oil components dissolved in seawater are typically considered as more bioavailable to pelagic marine organisms and the main driver of crude oil toxicity, however, recent studies indicate that oil droplets may also contribute. The adhesion of crude oil droplets onto the eggs of pelagic fish species may cause enhanced transfer of oil components via the egg surface causing toxicity during the sensitive embryonic developmental stage. In the current study, we utilized an oil droplet dispersion generator to generate defined oil droplets sizes/concentrations and exposed Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) to investigate if the potential for dispersed oil droplets to adhere onto the surface of eggs was species-dependent. The influence of a commercial chemical dispersant on the adhesion process was also studied. A key finding was that the adhesion of oil droplets was significantly higher for haddock than cod, highlighting key differences and exposure risks between the two species. Scanning electron microscopy indicates that the differences in oil droplet adhesion may be driven by the surface morphology of the eggs. Another important finding was that the adhesion capacity of oil droplets to fish eggs is significantly reduced (cod 37.3%, haddock 41.7%) in the presence of the chemical dispersant.

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1. Introduction

Formation of mechanically dispersed oil droplets in the water column following an oil spill may be caused by many factors, including the nature of the spill (e.g. an underwater blowout) and turbulence caused by wave-action. This formation of oil droplets is often seen as beneficial in spill scenarios, as the higher oil-water surface area increases the rate of oil compound dissolution and subsequently biodegradation (Brakstad et al., 2015; NRC, 2005). In some cases, dispersion of spilled oil is encouraged through intentional application of chemical dispersants to a slick or underwater plume (Brandvik et al., 2013). Mechanically dispersed oil droplets are typically <100 μm (Muschenheim and Lee, 2002), while chemically dispersed droplets are typically smaller (Khelifa et al., 2008; Li et al., 2007). Produced water emissions also contains dispersed oil droplets, and the legislations on the Norwegian continental shelf is that produced water should not exceed 30 mg/L produced water.

It is generally considered that the dissolved fraction of crude oil is the most bioavailable to marine organisms, and therefore contributes most to bioaccumulation. However, oil droplets present in dispersions have the potential to significantly affect filter-feeders, which ingest oil droplets that match the size of their natural prey and coat feeding apparatus reducing feeding efficiency (Almeda et al., 2014; Hansen et al., 2012; Hansen et al., 2009). Importantly, in a crude oil dispersion in seawater, most of the oil component mass is present in the droplet phase. For oil dispersions in seawater with oil concentrations in the range 0,1–10 mg/L this also applies to the larger PAHs (MW > 230 Da) and high logK_{OW} (>6) and their dissolved concentrations are generally very low. On the other hand, lighter components such as naphthalenes (e.g. naphthalene: logK_{OW} = 3.17, MW = 128,171 Da) are mostly found in the dissolved phase and only a small mass fraction is retained in the oil (Nordtug et al., 2011a) Previous studies have shown that oil droplets do not appear to contribute to the observed toxicity of oil dispersions to fish larvae (Carls et al., 2008; Nordtug et al., 2011b; Olsvik et al., 2011; Olsvik et al., 2010), or the uptake of PAHs by other marine species (Viaene et al., 2014). However, recent studies have suggested that adhesion of oil droplets onto the chorion of fish eggs may be an important route of entry for oil components to the developing fish embryos (Sørhus et al., 2015; Sørhus et al., 2016).

It has been reported that cod and haddock eggs exposed to similar doses of mechanically dispersed crude oil were exhibiting significantly differences in PAH accumulation resulting in more severe toxicity (cardiotoxicity and larvae deformation) in the latter(Sørensen et al., 2017; Sørhus et al., 2015). The studies showed that dispersed oil droplets adhered to the chorion of haddock eggs, while the same phenomenon was not observed for cod eggs. The adhesion appeared to correlate with an increase in body residue of polycyclic aromatic hydrocarbons (PAHs) in the haddock eggs, as well as more severe malformations (Sørensen et al., 2017). At the embryo stage, the haddock and cod eggs are nearly identical in terms of size, colour and embryonic development (Fridgeirsson, 1978; Hall et al., 2004). Like most pelagic species, both cod and haddock eggs have a thin, homogenous, lamellated chorion (Lønning et al., 1988; Morrison et al., 1999). Therefore, the differences in oil droplet adhesion observed between eggs of the two species could be driven by variations in the chemistry and/or surface morphology of egg chorions facing the surrounding water.

Despite oil production and transport within spawning areas, both in Norwegian waters and globally, there is currently a lack of data on how dispersed crude oil droplets affect the early life stages of fish (Olsen et al., 2013). The areas around the Lofoten Islands of northern Norway, as well as the Barents Sea, and the Atlantic Arctic area, are considered especially vulnerable to oil spills since they are spawning and larval-drift areas for several commercially important species of marine fish, including Atlantic haddock (*Melanogrammus aeglefinus*) and cod (*Gadus morhua*) (Hauge et al., 2014; Misund and Olsen, 2013; Olsen et al., 2010). A more detailed understanding of the effects of dispersed crude oil on different fish species is therefore necessary to identify both species and regions that represent highest risks for oil spill impacts.

In the present study, the capacity for dispersed oil droplets to adhere to the chorion of cod and haddock eggs were estimated and compared. Furthermore, the potential differences in the adhesion properties of mechanically (MD) and chemically dispersed (CD) crude oil to the eggs was also assessed. Identical oil dispersions, in terms of concentrations and oil droplet sizes, of mechanically and chemically dispersed (added chemical dispersant) crude oil were prepared and eggs of both species were exposed for 24 h. Body burden analyses of high logK_{OW} PAHs were used as a proxy for oil droplet adhesion estimation. Differences in cod and haddock egg surface morphology were investigated using scanning electron microscopy (SEM) imaging. The study should provide important information about potential species-differences in oil adhesion capacity and how chemical dispersants affect this process. For net environment benefit analyses and resource damage assessment processes in the event of an accidental oil spill, these results are key to decide on oil spill responses and to estimate exposure and toxicity to native fish populations.

2. Materials and methods

2.1. Eggs

Fertilized eggs were collected from the stock fish facility at the Institute of Marine Research at Austevoll, Norway. The eggs were transported by air freight to Trondheim and kept at 5 °C in incubator tanks until used for experiments and analyses.

2.2. Dispersion generation, egg exposure, sampling and analyses

Uniform oil dispersions were generated using an oil droplet generator (Nordtug et al., 2011a), where crude oil (Heidrun blend) was dispersed in filtered (0.22 μm cartridge filter) sea water through a series of nozzles yielding a constant flow of dispersion with a homogeneous droplet size. To generate the chemically dispersed oil (CD), the commercially available oil spill dispersant Dasic NS was premixed into the oil (4% w/w dispersant) prior to dispersion. Oil dispersions generated without the use of chemical dispersant are termed mechanically dispersed (MD). The two dispersions were generated with identical set-up, but to achieve a similar oil droplet size distribution and concentration in the two treatments, the energy input (water flow and thus turbulence) for generating the dispersion with Dasic NS was reduced compared to the purely mechanically generated dispersion (Nordtug et al., 2011a). Both dispersions were generated at a nominal concentration of 1 mg oil/L. Droplet size distributions were verified by a Coulter Counter (Multisizer 3, with 100 μm aperture).

Freshly prepared dispersions were transferred to 2 L borosilicate bottles ($N = 4$ for each treatment), 200 cod or haddock eggs (14 days post fertilization) were added, and the bottles capped with Teflon-lined caps (VWR International). The bottles were filled completely (no headspace) and mounted on a custom-built carousel incubation system, as previously described (Brakstad et al., 2015). Bottles filled only with filtered sea water and eggs (no oil) were used as negative controls ($N = 4$). The carousel system maintained a constant clockwise rotation at a velocity of 0.75 rpm at 5°. After 24 h rotation, the bottles were taken off the carousel and sampled immediately.

The fish eggs were separated from the water phase by sieving the dispersions through a 100 μm size mesh. An aliquot of the water sample was also analysed for droplet size distribution and concentrations using Coulter Counter Multisizer. A sub-sample (40 mL) was removed for analysis of volatile organic compounds using purge and trap gas chromatography coupled to mass spectrometry (Faksness et al., 2015). The remaining volume of water was acidified (pH ~ 2, HCl) and extracted by serial liquid-liquid extraction using dichloromethane (45-30-30 mL) for analysis of semi-volatile organic components (SVOC).