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Effects of low crude oil chronic exposure on the northern krill (*Meganyctiphanes norvegica*)

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ABSTRACT

Chronic oil pollution related to gas and oil drilling activities is increasing in the sea due to the rising offshore petroleum industry activity. Among marine organisms, zooplankton play a crucial role in the marine ecosystem and therefore understanding the effects of crude oil chronic exposure on zooplankton is needed to determine the impact of oil in marine environments. The present study reports on the effect of crude oil on adult northern krill, *Meganyctiphanes norvegica*, collected during three seasons. Their sensitivity to oil was examined with oil concentration of 0.01 versus 0.1 mg oil L^{-1} and photo-modified oil in flowing seawater maintained in the dark for 2 weeks at in situ temperature. Oil (polycyclic aromatic hydrocarbons, PAHs) entered the krill (on average, 350 and 4400 $\mu g \cdot kg^{-1}$ wet weight in low and medium oil treatments respectively) and a larger fraction of the krill exhibited digestive gland pathologies (enhanced apoptosis and pathology of digestive tubules) in oil treatments (27–80%) compared to a significantly lower fraction (7–13%) in treatments that received no oil. However, 2-week oil exposure at these concentrations did not significantly decrease survivorship or impair basic functioning such as feeding and respiration rates. Similarly, there were only limited changes in the transcription of 7 selected genes from head tissue. Additionally, although there was significant seasonal variation in krill total lipid content and fatty acid composition, there was no treatment effect on both these parameters, which suggests limited oxidative stress under experimental conditions. Furthermore, there was no significant treatment effect on two direct measures of oxidative stress (MDA: malondialdehyde and AOPP: advanced oxidation protein products) in any of the seasons. Nevertheless, histology clearly revealed enhanced digestive gland pathologies in krill even at low concentrations. Although krill with such pathologies continue to survive, their accumulation of PAHs may be transferred up the food chain, impacting their predators and the wider ecosystem.

1. Introduction

Zooplankton play a pivotal role in marine food web dynamics and biogeochemical cycling (e.g. Banse, 1995; Alcaraz et al., 2010; Fujii, 2016) and it is therefore important to understand the factors that modulate the functioning of key species. In addition to examining fundamental biotic and abiotic factors, it is of growing urgency to examine the impact of anthropogenic (human-induced) forcing factors. These factors have had an increasing influence on the marine ecosystem during the last decades (e.g. Halpern et al., 2008).

Petroleum or crude oil is one of the most common pollutants released into the marine environment but our knowledge of the interactions between zooplankton and anthropogenic pollutants is very limited (e.g. Almeda et al., 2013a). The rise in human energy demand (but also the numerous petroleum related products) during the last decades has resulted in intense exploration, production and transportation of crude oil in the sea, increasing not only the risk of oil spill to the marine environment (e.g. Almeda et al., 2014) but also more discharge points of produced water (PW: the largest volume waste stream containing production chemicals in oil and gas production operations on most

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offshore platforms e.g. Johnsen et al., 2004, Neff et al., 2011) that could potentially lead to chronic low-level oiling. This is of particular importance in the sub-Arctic and Arctic seas, where there is increasing oil exploration (Bakke et al., 2013). Many studies address the effect of acute, high concentration (4–80 mg oil L^{-1}) oil exposure on different planktonic species and clearly demonstrate lethal or sub-lethal effects (e.g. Hansen et al., 2012, 2013; Jensen and Carroll, 2010; Almeda et al., 2013a). However, only a limited number of studies examine the effect of chronic low-level oil exposure on zooplankton which play a key role in marine food webs. Although the northern krill (*Meganyctiphanes norvegica*) is considered a key species in the sub-Arctic and boreal North Atlantic (e.g. Tarling et al., 2010; Agersted et al., 2014 and references therein), studies on the effects of pollution on this species are very limited (Spicer and Saborowski, 2010). Except for a study showing that high concentrations of crude oil affect feeding behavior (Hebert and Poulet, 1980) and a later study that showed that an oil concentration of 5 mg oil L^{-1} was directly lethal, while 0.5–1.6 mg oil L^{-1} significantly enhanced mortality and reduced moulting (Ingvarsdottir et al., 2016), there is limited data on the effects of oil or other contaminants on northern krill. Euphausiids (krill) are key prey for shrimps, fish, squid, baleen whales and seabirds (Fujii, 2016 and references therein); they are omnivores feeding on phytoplankton and *Calanus* spp., and they play an important ecological role in the carbon pump through the transport of faecal material to deeper layers (e.g. Tarling et al., 2010; Schmidt, 2010). Hence, it is important to increase our knowledge regarding potential effects of oil, especially low-level chronic exposures. Any direct or indirect adverse effects can have a cascading effect in the marine ecosystem (e.g. Langangen et al., 2016).

In addition to passive or indirect uptake of dissolved oil, zooplankton also ingest droplets of crude oil (e.g. Gyllenberg, 1981; Almeda et al., 2014; Nordtug et al., 2015). Crude oil droplets (1–100 μm) generated by natural mixing of oil (e.g. Delvigne and Sweene, 1988) are frequently in the prey size spectra of zooplankton (Hansen et al., 1984). Oil droplets are formed during PW discharge in the field so we used mechanically produced oil droplets (oil mechanically dispersed in water, hereafter referred as oil) in the present study (Sanni et al., 1998; Bechmann et al., 2010). In the only chronic oil exposure study with northern krill (2 weeks exposure), Ingvarsdottir et al. (2016) documented the high sensitivity of *M. norvegica* to high concentrations of oil (0.5–5 mg oil L^{-1}). However, adverse effects on pelagic organisms have been also observed for very low oil concentrations (0.01 mg oil L^{-1}, e.g. Taban et al., 2007, Bechmann et al., 2010) and, in the present study, concentrations that can be expected just before extreme dilution of PW effluent were examined (~0.1–1% of PW oil content < 2 km from PW discharge point, Bakke et al., 2013, see also Meier et al., 2010). Additionally, given that photo-modification or oxidation of crude oil can increase the toxicity of oil (e.g. Lee, 2003) and thereby its effect on organisms (e.g. Boese et al., 1997; Bechmann et al., 2010; Almeda et al., 2016 and references therein), a treatment with photo-modified oil was included. Furthermore, the sensitivity of *M. norvegica* to low-level chronic dispersed oil exposure was examined at different seasons because, related to the physiological state of the organism (e.g. lipid content and reproductive state), seasonal variation in tolerance to pollutants has been documented (e.g. for amphipods and mussels (Kater et al., 2000, Aarab et al., 2011). A high lipid content may leave the animals more disposed to oxidative stress, as lipids are preferred targets of reactive oxygen species (Obermüller et al., 2005).

In addition to documenting the sensitivity of this keystone species, this study attempts at identifying bio-monitors/biomarkers that can be used for monitoring oil pollutants in the field (e.g. Sanni et al., 2017). Trace concentrations of toxic compounds may be undetectable but may have adverse effects on organisms (e.g. Brooks et al., 2011). Being an abundant group with a key function in northern marine ecosystems and with high abundance in current and future oil producing areas in the northern hemisphere (Tarling et al., 2010; Fujii, 2016), the northern krill is a potential candidate as an indicator species of oil exposure (Ingvarsdottir et al., 2016). However, this calls for a better understanding of the effects of oil exposure on krill basic functioning and identification of sensitive biomarkers of oil exposure. Promising and relevant biomarkers examined in the present study were: 1) gene expression of selected genes (to help reveal changes in physiology in response to various stresses at an early stage, before the effects can be observed phenotypically (e.g. Hansen et al., 2008; Seear et al., 2010, 2012), 2) different direct measures of oxidative stress (when pollutants such as hydrocarbons enter an organism, it can lead to oxidative stress that can cause cell damage through e.g. lipid or protein oxidation (Lesser, 2006, and references therein), 3) total lipid FA composition patterns (polyunsaturated FAs (PUFAs) are primary targets for lipid oxidation (e.g. Ayala et al., 2014) and therefore lipid peroxidation may be reflected in altered FA profiles, 4) digestive gland histopathology (exposure to pollutants can lead to histopathologies, e.g. Lowe et al., 1981, Stentiford and Feist, 2005) and 5) concentration of accumulated PAHs (bioconcentration of PAHs can be calculated from the concentration of PAHs in the water and krill, e.g. Baussant et al., 2009). Direct sensitivity to oil exposure was documented by studying the effects of oil on krill feeding, respiration and survival. The effects of three oil treatments (0.01 mg oil L^{-1}, 0.1 mg oil L^{-1}, and UV irradiated 0.01 mg oil L^{-1}) were examined and a duration of 2 weeks exposure was chosen to be able to compare to an earlier similar exposure study (Ingvarsdottir et al., 2016) that documented enhanced mortality and reduced functioning under higher oil concentrations (0.5–5 mg oil L^{-1}).

The objectives of the present study were to (1) examine the effects of chronic low oil exposure on northern krill feeding, respiration and survival and (2) identify potential krill biomarkers of low-level chronic oiling. Understanding biological impacts and identifying biomarkers of low-level chronic oiling is key to sustainable development in the oil and gas industry.

2. Material & methods

2.1. Krill collection

Collection and maintenance of krill were conducted based on methods described in Ingvarsdottir et al. (2016). Sampling was done using a local fishing vessel (Vassøygutt) in Hidlefjorden (north of Åmøy, Norway; 59° 04′ 00″ N; 5° 45′ 00′ E), close to Stavanger. The animals were collected with a modified shrimp trawl (netting replaced with a netting with ~2 mm opening (Manufactured by Karmøy Traalbøtterie, Åkrahavn, Norway) and a closed cod end (100 L hard plastic barrel). Trawling (30 min at a speed of 1 knot) was during darkness between 03:00 and 04:00 h because of krill high sensitivity to light (Gaten et al., 2010; Ingvarsdottir et al., 2016). Sampling site water depth was 90–100 m and the trawl for the collection of the krill was at 50 m depth, not only because of their higher position in the water column during the night (following diel vertical migration behavior, e.g. Kaartvedt, 2010) but also because this depth provided the “cleanest” krill samples, free of surface-water debris and larger bentho-pelagic organisms. Upon retrieval, krill were gently transferred from the cod end container, always completely submerged in water, into transport containers, (25 L carboys and 20 L buckets), each filled with the local fjord water (oxygenation monitored and maintained > 80% air saturation by purging with oxygen). Containers with krill were transported to the laboratory within 2 h of capture and kept in darkness. Krill were collected during three seasons (Spring, Autumn & Winter) and maintained at in situ temperature in the laboratory (Table 1). A planned Summer collection was unsuccessful (krill were absent at the collection site). Field krill samples (T-field) were fixed within 1–3 h after collection (either on ship or in the laboratory): individual krill were transferred to cryovials, flash frozen in liquid nitrogen and then stored at − 80 °C.