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Bioaccumulation of oil compounds in the high-Arctic copepod *Calanus hyperboreus*

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ABSTRACT

Oil and gas exploration in the Arctic will increase the risk for accidental oil spills and thereby have a potential impact on the ecosystem and the organisms inhabiting these areas. Lipid rich copepods are an important food source for higher trophic levels in Arctic marine ecosystems. However, high lipid content and a slower metabolism increase the risk for bioaccumulation in Arctic species. Here we exposed three late development stages of the lipid rich high-Arctic copepod species *Calanus hyperboreus* to two different ^{14}C-marked crude oil model compounds, the alkane dodecane (log K_{ow} 6.10) and the polycyclic aromatic hydrocarbon (PAH) phenanthrene (log K_{ow} 4.46) on a short-term scale of 4 days. Exposure was followed by a depuration phase of 3 days. We observed a difference in estimated bioaccumulation of the two model compounds between stages and found a slower depuration of dodecane than of phenanthrene in the two largest and most lipid rich stages. However, depuration of dodecane and phenanthrene was non-significant for all three stages. The results indicate that even short-term exposure may result in long-term bioaccumulation and internal exposure of oil compounds in the lipid rich high-Arctic copepods *C. hyperboreus*. Slow elimination and depuration of oil components indicate a risk for transfer of oil component up the food web to pelagic fish, seabirds and baleen whales.

1. Introduction

A future increase in shipping and off-shore oil activity in Arctic areas will increase the risk for accidental oil spills into the Arctic sea. Toxic effects of oil components in the sea may have short-term and long-term lethal and sub-lethal effects on organisms such as copepods (*e.g.* Bejarano et al., 2006; Calbet et al., 2007; Hansen et al., 2017; Saiz et al., 2009; Seuront 2011), which are linking lower and higher trophic levels in the food web. In general, there is a lack of knowledge on bioaccumulation and toxic effects of oil components for high-Arctic organisms. Data for temperate species are often extrapolated onto Arctic species when making environmental risk assessments (ERA) of *e.g.* oil spills. However, as an adaptation to the seasonal availability of food, high-Arctic organisms have higher lipid content than temperate species, and consequently bioaccumulation of lipophilic contaminants is more prone to take place in polar organisms (AMAP, 1998; Chapman and Riddle, 2005; Goerke et al., 2004). Oil contains several lipophilic components with high affinity for accumulation in lipids (National Research Counsil, 1985), which therefore can accumulate in organisms in the event of an oil spill.

Copepods of the genus *Calanus* are the primary grazers during spring and summer in Arctic marine ecosystems, and have an important role in transferring lipid-based energy from primary production to fish, seabirds and baleen whales (Conover, 1988; Falk-Petersen et al., 2009; Falk-Petersen et al., 2007; Hirche and Mumm, 1992). Three species of *Calanus* are present in Arctic areas; *C. finmarchicus* (of Atlantic origin and the smallest species), *C. glacialis* and *C. hyperboreus* (both true Arctic species, with the latter being the largest) (Conover, 1988; Falk-Petersen et al., 2009; Hirche and Mumm, 1992). Especially the two Arctic *Calanus* species have a high lipid content (> 50% of their dry weight), and are particularly rich in wax esters, a high energy storage lipid (Lee et al., 2006; Lee et al., 1971; Scott et al., 2000; Swalethorp et al., 2011).

Toxicity tests and tolerance levels have been investigated in *Calanus* spp., and consequently, lethal concentrations (LC_{50}) has been established for some oil compounds and chemical substances (*e.g.* Hansen et al., 2014; Hansen et al., 2012; Hansen et al., 2016; Jager et al., 2017). Due to slow biodegradability, high lipophilicity and high toxicity, polycyclic aromatic hydrocarbons (PAHs) is a group of substance in oil of high environmental concern. Exposure of adult *Calanus* to the PAH pyrene indicates negative effects on feeding and egg production (Hjorth and Nielsen, 2011; Jensen et al., 2008; Nørregaard et al., 2014). Other

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studies has shown that PAHs may affect naupliar development and feeding (Grenvald et al., 2013; Jørgensen, 2017; Utne, 2017), and several studies have found bioaccumulation of PAHs in adult *Calanus* (Bergentz 2016; Jager et al., 2017; Jensen et al., 2012; Nørregaard et al., 2015). However, knowledge regarding bioaccumulation as a result of uptake, elimination and depuration of different types of crude oil components is still scarce for the largest and most lipid rich high-Arctic *Calanus* species, *C. hyperboreus*.

Thus, the aim of the study is to increase knowledge on bioaccumulation of oil components in the high-Arctic copepod *C. hyperboreus* copepodite stages CIV, CV and in adult females, respectively. Bioaccumulation is estimated for two oil model compounds, respectively dodecane as model compound for alkanes in oil and phenanthrene for PAHs.

2. Material & methods

2.1. Experimental animals

Copepods of the species *Calanus hyperboreus* were sampled in Disko Bay, West Greenland in late June 2016. A 300-µm WP3-net with a closed cod-end was hauled vertically from 100 m to the surface at 0.5 m/s to collect live animals. The mean temperature in the upper 100 m was 1.4 ± 1.9 °C. After retrieval, the cod-end was emptied into several thermo boxes containing seawater (SW) to dilute the sample and keep the animals in good condition. The containers were brought back to the laboratory where animals were sorted out under microscope while kept cold. They were allowed to acclimatize to 2 °C for a minimum of two days while kept in *in situ* seawater from fluorescence maximum.

2.2. Accumulation of ^{14}C marked dodecane and phenanthrene in Calanus hyperboreus

Uptake, elimination and depuration rates of two model compounds found in crude oil: dodecane and phenanthrene were investigated in *C. hyperboreus* copepodite stages CIV, CV, and for adult females. ^{14}C dodecane and ^{14}C phenanthrene were added in non-toxic concentrations. Uptake expressed as clearance (mL g lipid^{-1} h^{-1}) is the rate at which an individual remove (or clear) a given volume of compound from the surrounding seawater. Elimination rate constant (k_e, h^{-1}) estimates the loss of compound from the individual while the individual is still exposed to the oil substances, and depuration rate (k_d, h^{-1}) is the elimination of compound from the individual in clean water while no longer being exposed (Landrum et al., 1992). Average prosome lengths of the copepods were CIV: 3700.2 µm ± 126.1 SD (n = 48), CV: 4920.3 ± 159.4 SD (n = 26) and adult females: 6384.6 µm ± 231.5 (n = 118). CIV and CV stages were defined by range measurements following Hirche et al. (1994). Dry weight was estimated from a length:DW regression in Hirche and Mumm (1992), including all stages of *C. hyperboreus*. Lipid weight was estimated by using an average value of 60.5% lipid:DW ratio for the three stages (Scott et al., 2000). Lipid weight of the individuals (g lipid ind^{-1}) was used to calculate specific tissue concentrations of dodecane and phenanthrene (µg (g lipid^{-1})).

Adult females (n = 36) were incubated individually in 20 mL glass scintillation vials filled with *in situ* 200-µm filtered SW sampled from fluorescence max, whereas one CIV (n = 36) and one CV (n = 36) were added the same vial. 100 µL of either ^{14}C dodecane or ^{14}C phenanthrene was added each vial (n = 36 for each compound). After adding ^{14}C dodecane and ^{14}C phenanthrene to the respective vials, 1 mL from 6 vials (per compound) was sampled and saved for later analysis. Experimental vials were incubated in dark at 2 °C. The exposure phase lasted for approximately four days (89 h), where water was not changed, nor was additional ^{14}C compound added. During the exposure phase, three vials from each treatment (dodecane and phenanthrene, respectively) were sampled after 22, 44, 66 and 89 h. Here, 1 mL of the water in each vial was saved before the rest of the content in the vial was filtered onto a 240-µm sieve to collect the animals. While on the sieve, the animals were thoroughly washed in SW, put onto a filter paper to remove excess SW and saved in individual vials. Furthermore, the experimental vials were emptied and saved. To investigate depuration rates of dodecane and phenanthrene, the remaining animals (n = 6 of each stage from each exposure experiment) from both treatments were, after 89 h of exposure, transferred to new vials containing *in situ* 200-µm filtered SW without ^{14}C dodecane and ^{14}C phenanthrene where they were incubated for approximately three days (75 h). These vials were then sampled after 134 and 164 h after beginning of experiment and treated as described above (n = 3 at each time for each compound), and 10 mL of SW from the vials was saved when sampled. All saved samples were stored at −20 °C until analysis. After 5 months all samples were analyzed in a scintillation counter. ^{14}C dodecane and ^{14}C phenanthrene was quantified by liquid scintillation counting on a Perking Elmer Tri-Carb 2910TR. By using the molecular weight (dodecane: 170.34 g/mol; phenanthrene: 178.234 g/mol) and specific activity of the compounds (55 mCi/mmol for dodecane (American Radiolabeled Chemicals Inc. (ARC)); 8.2 mCi/mmol for phenanthrene (Sigma Aldrich)), the concentrations of dodecane and phenanthrene in copepods (µg (g lipid^{-1})) and in the experimental seawater (µg L^{-1}) were calculated.

We assume that the copepods used for the experiments, which all were sampled *in situ*, do not contain ^{14}C-marked phenanthrene and dodecane, and therefore we did not measure any background concentrations of these compounds in 'background' individuals. As the copepods were incubated in a small volume and the *in situ* Chl a was very low (~0.5 µg L^{-1}), it is furthermore assumed that the amount of available food was not sufficient for the copepods to sustain growth during the experimental period.

2.3. Data analysis

To estimate uptake and elimination rates during exposure phase (up to 89 h), a first-order rate coefficient model (Jensen et al., 2012; Landrum et al., 1992) was used on the measured accumulation data for each copepod stage:

$$C_a = \left(k_u C_w / k_e\right)\left(1 - exp^{-k_e t}\right) \quad (1)$$

Where C_a = concentration of the model substance measured in copepods (µg model substance g lipid^{-1}), k_u = conditional uptake clearance (mL g lipid^{-1} h^{-1}), C_w = concentration (dose) of the model substance in the seawater (µg mL^{-1}) (average of initial water concentrations and water concentrations during the exposure phase), k_e = conditional elimination rate constant (h^{-1}), and t = time (h^{-1}). The model assumes that at time 0 h the individuals contain 0 µg dodecane/phenanthrene g lipid^{-1}.

We wanted to investigate whether steady state was reached within the accumulation phase of the two model compounds. Thus, measured body content in the individuals at times 44, 66 and 89 h were used in a linear model, which was tested by a one-way ANOVA to see whether the slope of the linear model was different from zero (Honkanen and Kukkonen, 2006).

To estimate depuration rate constant (k_d, h^{-1}), *i.e.* elimination of the model compound from the tissue taking place when the animal is no longer exposed to the substance (Landrum et al., 1992), a linear model was used to fit body content data (µg compound g lipid^{-1}) over time (h) after animals had been transferred to clean seawater (accumulation data during depuration phase (> 89 h)). Body content data was log-transformed, and the slope of the fitted line was the depuration rate constant k_d (h^{-1}). One-way ANOVA was used to test whether the slope of the line was different from zero. The depuration half life ($T_{½}$, d), *i.e.* when half of the model substance would be eliminated from the copepods, was calculated as $\ln(0.5)/k_d$ following Jensen et al. (2012).

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