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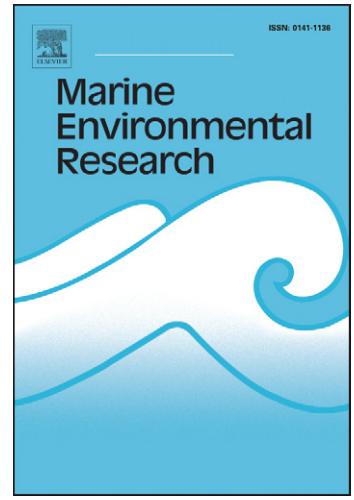

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Cellular energy allocation in the Arctic sea ice amphipod *Gammarus wilkitzkii* exposed to Water Soluble Fraction of oil

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Abstract

Increasing offshore oil and gas activities in the European Arctic has raised concerns of the potential anthropogenic impact of oil-related compounds on the polar marine ecosystem. We measured cellular energy allocation (CEA) in the sea ice amphipod *Gammarus wilkitzkii* after exposure for one month to the water soluble fraction (WSF) of oil. The CEA biomarker measures the energy budget of organisms by biochemically assessing changes in carbohydrates, protein and lipid content as well as the electron transport system activity. A significantly higher protein content was observed in the medium dose compared to controls, while the total energy budget was not affected in *G. wilkitzkii*. This indicates that parts of the energy budget of *G. wilkitzkii*, which is a key species in the Arctic ecosystem, is affected by a WSF of oil.

Keywords: Arctic; Amphipods; Oil; Cellular energy allocation; *Gammarus wilkitzkii*

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Oil and gas activity in the Barents Sea is increasing as is the risk of accidental discharges, which underscores the need for more information on the effects of oil on Arctic organisms. PAHs have been shown to be some of the most toxic components of crude oil, bioaccumulating in organisms and disrupt cell and tissue function (French-McCay, 2004). To our knowledge no studies have focused on effects of oil-related compounds on sea ice species. In sea ice, amphipods are the dominant organisms and they represent a direct link between lower and higher trophic levels (Lønne and Gulliksen, 1991).

Exposure to pollutants may increase the energy requirement of animals and hence result in reduced energy available for growth and reproduction (Calow and Sibly, 1990). The cellular energy allocation methodology measures energy available (Ea) (protein, lipid and carbohydrate content) and energy consumed (Ec) (electron transport system activity, ETS). Ea and Ec can be combined into a value expressing the net energy budget (De Coen and Janssen, 1997).

In this study, we exposed ovigerous females of the sea ice amphipod *G. wilkitzkii* to the water soluble fraction of crude oil to investigate whether the energy budget of *G. wilkitzkii* was altered by sub-lethal concentration of oil. This study is the first application of CEA technique on an Arctic sea ice species.

Ovigerous female specimens of *G. wilkitzkii* were sampled in August 2006 in the Arctic Ocean (ca. N81°43' E15°46'). To simulate oil spill conditions, an exposure system was set-up according to Carls et al. (1999). After one month exposure Ea, Ec and Cea analyses and calculations were performed on each individual (De Coen, 1997). Dissolved PAH concentrations were determined by the modified standard US Environmental Protection Agency (EPA) procedure 3510 C. Total PAH concentrations in the water were as follows at day 0 and day 30: high dose, 55-8 ppm; medium dose, 10-2ppm; and low dose, 5-1ppm. The concentrations of PAHs decreased with time and also the relative PAH composition changed, a higher proportion of soluble compounds were found in the beginning and less soluble compounds in the end of the experiment. Ten females per treatment were used, and no replicates were done owing to a limited number of animals. Embryos developmental stages were different between females.

The protein levels were significantly higher in the medium dose compared to controls (Fisher's LSD: $p= 0.02$) (Fig. 1), indicating that WSF altered the energy budget related to protein metabolism in *G. wilkitzkii*. There were no significant differences in any of the other Ea parameters, Ec and CEA (Fig. 2) among treatments (ANOVA $p>0.05$), indicating that WSF did not affect the other energy parameters.

The higher protein level observed in organisms from the medium dose demonstrates that the WSF affected parts of the energy budget in *G. wilkitzkii*. A higher protein value can reflect sub lethal stress that induced compensatory changes in the organisms' energy metabolism. This is in accordance with a study from Verslycke et al. (2004) showing protein induction in *Neomysis integer* from a polluted area. Increased protein level could be caused by an increased lysosomal activity as lysosomes have shown to be a target of toxic stress induced by PAHs and PCBs (Gagnaire et al., 2006). Lysosomal enzymes are considered to play an important role in invertebrate defense reaction systems. Exposure to WSF may therefore have triggered increased production of lysosomal activities, explaining higher protein level in exposed group compared to controls.

Higher protein levels were observed in the medium dose only. Explanations for this may be that high levels of PAHs may have reduced the physiological response systems (gene and protein expression) in the amphipods receiving high dose.

Negative CEA values were observed in all treatments, including the controls. This indicates that animals used more energy than they were able to allocate to their reserves. It is likely that a reduced food uptake and stress caused these negative values. In all cases, a higher Ec value compared to Ea were measured. This can be explained by the fact that it is assumed that ETS activity is an overestimation of the actual respiration (Båmstedt, 1980).

In the Arctic Ocean, the sea ice amphipod *G. wilkitzkii* is a key species and provide a link between primary production, detritus and higher trophic levels. Our results show that parts of the energy metabolism of the ice-amphipod were affected by WSF of oil. More time points are needed to definitively link the responses of energy budget parameters to petroleum hydrocarbons, but the results show evidence for disturbed protein metabolisms in *G. wilkitzkii* exposed to WSF of oil.

Acknowledgements

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Figure caption

Fig.1. Protein content (mg/g wwt) in *Gammarus wilkitzkii* in control (C) , low dose (LD), medium dose (MD) and high dose (HD) following a 30 day exposure to water-soluble fractions of crude oil. Median ( ), 25 and 75 percentiles (lower and upper bar, respectively) are shown.

Fig. 2. Cellular energy allocation (CEA) in Gammarus wilkitzkii in control (C) , low dose (LD), medium dose (MD) and high dose (HD) following a 30 day exposure to water-soluble fractions of crude oil. Median ( ), 25 and 75 percentiles (lower and upper bar, respectively) are shown.

Figure 1

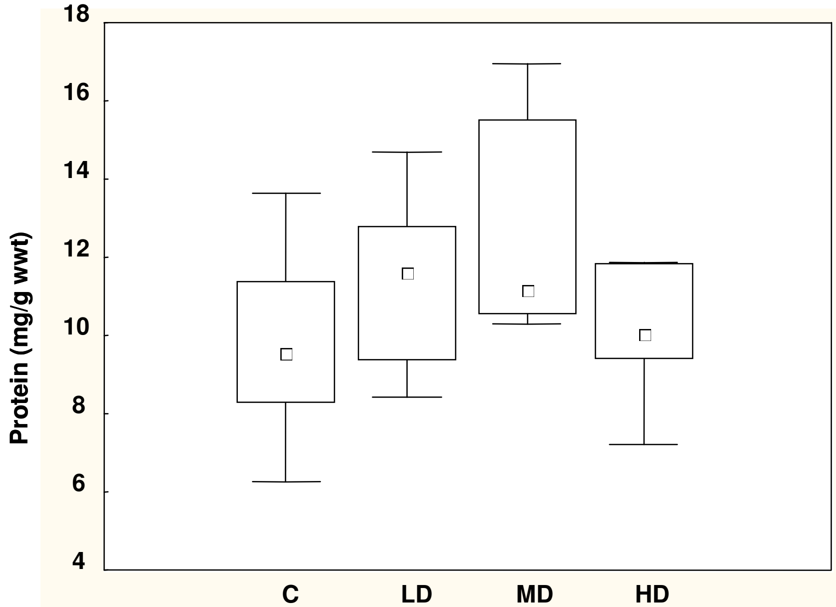

Figure 2