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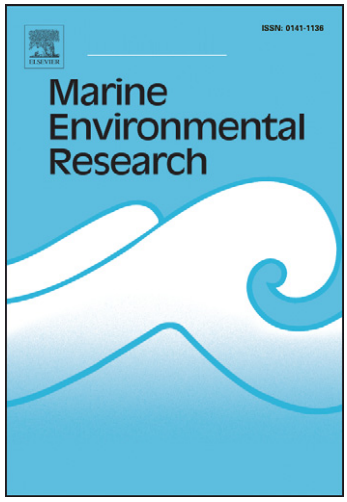

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Embryo aberrations in sea ice amphipod *Gammarus wilkitzkii* exposed to water soluble fraction of oil

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Abstract

Offshore oil and gas activities have gained momentum in the European Arctic, raising concerns of the potential impact of oil-related chemicals on the polar marine ecosystem, notably on sea ice communities. Herein, malformations on embryos of the Arctic sea ice amphipod *Gammarus wilkitzkii* exposed to the water soluble fraction of oil were studied. The females ranged from development stage three to nine. No differences in reproductive stage were observed among the different treatments after 30 days of exposure. Frequency of embryo aberrations was significantly higher in the high dose compared to controls, indicating that the embryos of *G. wilkitzkiii* were affected by oil.

Keywords: Arctic; Sea ice; Amphipod; Crude oil; Embryo; Malformation

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As the oil industry expands in the Barents Sea, there are increasing risks of accidental oil spill with potential drift and incorporation into sea ice for several years. Animals in the sea ice, notably amphipods (Lønne and Gulliksen, 1991), may be exposed chronically to polycyclic aromatic hydrocarbons (PAH) and other oil-related compounds. More data are needed on the toxicity the toxicity of PAH on Arctic amphipods (Chapman and Riddle, 2005). In addition, the amphipod population (*Ampelisca sp*) has yet to fully recover studies of the 'Amoco Cadiz' oil spill in Northern coast of France (>20 years ago) (Poggiale and Dauvin, 2001). A potential explanation is that amphipods are brooders (female amphipod carries her young in the brood pouch until they hatch as fully developed immature specimens); hence, pollutants affecting adults will also affect offspring since they are released into the same local environment. Furthermore re-colonisation is much slower since amphipods do not have pelagic larvae. In this study, we exposed ovigerous females of the sea ice amphipod *Gammarus wilkitzkii* to the water soluble fraction (WSF) of crude oil to investigate the effects on the developmental stages and malformations of *G. wilkitzkii*.

Divers collected female individuals of *G. wilkitzkii* (3cm length) in late August 2006 in the Arctic Ocean (ca. N81^{o}43' E15^{o}46') from the research vessel of the University of Tromsø (RV JanMayen).

The principle of a rock column was used to simulate oil spill (Carls et al 1999). The WSF of crude oil was extracted by passing continuously cold seawater through a column filled up with glass beads (3mm) coated with crude oil. Four doses (including control) were made with the extract to expose the ovigerous females for 30 days using a continuous flow system. Concentration of PAH decreased with time, in addition, relative composition changes, with a higher proportion of soluble compounds at the beginning and less soluble in the end

(Carls et al., 1999). Ten females per treatment were used, and no replicates were done owing to a limited number of animals. Embryo developmental stages were different between females.

Embryo were scored separately for abnormalities by 2 scientists using stereo microscopes (see Sundelin and Eriksson, 1998). The frequency of malformations/aberrations per brood per female was counted, and no attempt was made to distinguish between the different types of aberrations due to small sample sizes.

The PAH concentrations in water were analyzed according to the modified standard US Environmental Protection Agency (EPA) procedure 3510 C. PAH analyses were performed at UNILAB, Tromsø, Norway, accredited laboratory. Total PAH concentrations in the water were as follow at day 0 and day 30: high dose, 55-8 ppm; medium dose, 10-2ppm; and low dose, 5-1ppm.

The reproductive stages of *G. wilkitzkii* were similar to the stages described in the Baltic Sea amphipod *Monoporeia affinis* (Sundelin and Eriksson, 1998). Across treatments, the females in this study ranged from stage three to nine. No females had embryos in the first development stages (stage 1 and 2). Three of the females were empty (no embryos or juveniles already released) and four females died during the course of the experiment. Average number of eggs/juveniles per female was 84 (Min: 1, Max 201). The different observed aberrations were also the same as described in Sundelin and Eriksson (1998); an example of malformed embryos is shown in Fig. 1. There were no significant differences in reproductive stage, eggs per female or weight among the different treatments (Unequal N post-hoc $p \geq 0.64$), indicating that the females in the different treatments were in similar stages, had similar weight and similar amounts of embryos. The frequency of embryo aberrations was significantly higher in the high dose compared to controls (Unequal N post hoc test; $p = 0.025$; Fig. 2). The significant higher number of aberrations observed in the high

dose demonstrated that 30 days exposure to the WSF of crude oil cause severe damages to the embryo of *G. wilkitzkii*. This finding is in agreement with Sundelin and Eriksson (1998) that reported elevated frequencies of malformed embryos among females collected near an aluminum smelter where high concentrations of PAHs were measured. In this study, no attempt was made to distinguish between the different types of malformations due to small sample size. The percentage of total embryo aberrations was 10.4 % in control, 4% in low dose, 12.2 % in medium and 29 % in high dose. In a four-year study in the Baltic proper and Bothnian Sea with a large sample size, Sundelin and Eriksson (1998) reported that gravid females showed a frequency of 2 to 6% malformed embryos, 0 to 5% undifferentiated and 0 to 6% dead eggs. A high proportion of enlarged embryos had a relatively wide gap between the membrane and the fetus. This may be a direct impact of the PAH on the embryo cell membrane, allowing water to enter. No differences in developmental stages were seen among treatments, indicating that WSF did not alter the period of embryogenesis as observed by (Lawrence and Polter, 2001) in the amhipod *Chaetogammarus marinus* exposed to benzo (a) pyrene. Additionally, a higher proportion of smaller embryos, indicating a reduced development, seem to correlate the smaller hatched juveniles from exposed group versus control observed by Lawrence and Polter (2001). Finally, the lack of a membrane delimiting the pouch in *G. wilkitzkii*, contributes to enhanced effects of water borne contaminants on the embryos. To conclude, embryo development of the sea ice amphipod can be impaired by WSF. In addition, it would be interesting to expose adult amphipods prior and during mating and additionally to investigate the survival and fitness of the released offsprings.

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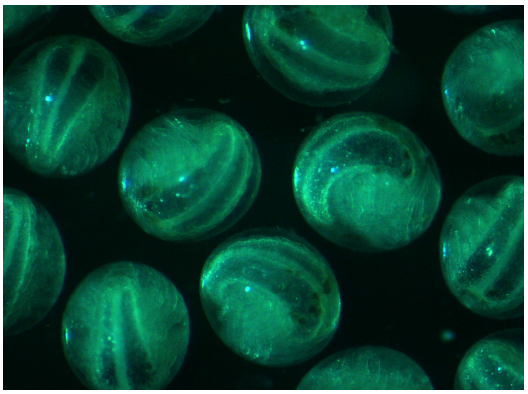
Figure captions

Fig. 1. Example of healthy (A) and malformed embryos (B) at stage 7 observed after 30 days of exposure to WSF in the control and medium dose, respectively.

Fig. 2. Aberration frequencies (total number of malformations/total number of eggs per female) in *Gammarus wilkitzkii* from control, low-, medium- and high dose of water-soluble fractions of oil (quantile plot). There were no significant differences in reproductive stage, eggs per female or weight among the different treatments (Unequal N post-hoc $p \geq 0.64$), indicating that the females in the different treatments were in similar stages, had similar weight and similar amounts of embryos. The frequency of embryo aberrations was significantly higher in the high dose compared to controls (Unequal N post hoc test; $p = 0.025$).

Figure 1

A



B

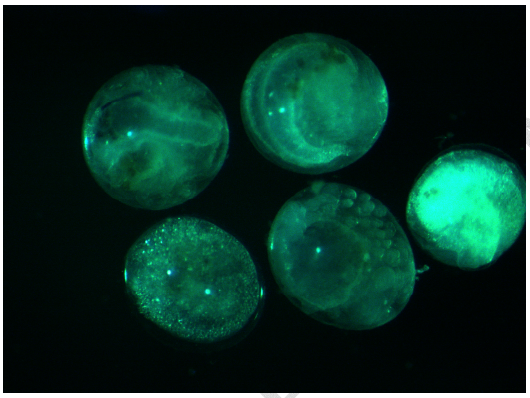


Figure 2