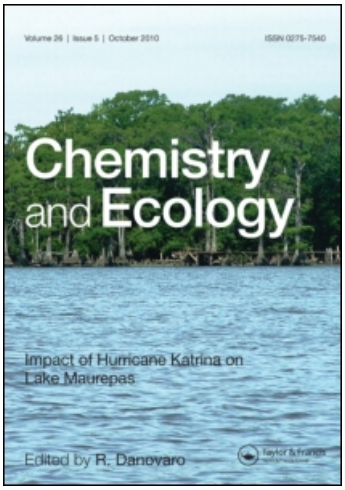

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

Biological effects of water soluble fraction of crude oil on the Arctic sea ice amphipod *Gammarus wilkitzkii*

K. Hatlen[abc]; L. Camus[a]; J. Berge[b]; G. H. Olsen[a]; T. Baussant[d]
[a] Akvaplan-Niva AS, Polar Environmental Centre, Tromsø, Norway [b] The University Centre on Svalbard, Longyearbyen, Norway [c] Institute of Biology, University of Tromsø, Norway [d] IRIS - Biomiljø, Stavanger, Norway

To cite this Article Hatlen, K. , Camus, L. , Berge, J. , Olsen, G. H. and Baussant, T.(2009) 'Biological effects of water soluble fraction of crude oil on the Arctic sea ice amphipod *Gammarus wilkitzkii*', Chemistry and Ecology, 25: 3, 151 — 162
To link to this Article: DOI: 10.1080/02757540902964978
URL: http://dx.doi.org/10.1080/02757540902964978

Chemistry and Ecology
Vol. 25, No. 3, June 2009, 151–162

Taylor & Francis
Taylor & Francis Group

Biological effects of water soluble fraction of crude oil on the Arctic sea ice amphipod *Gammarus wilkitzkii*

K. Hatlen[a,b,c]*, L. Camus[a], J. Berge[b], G.H. Olsen[a] and T. Baussant[d]

[a]*Akvaplan-Niva AS, Polar Environmental Centre, N-9296 Tromsø, Norway;* [b]*The University Centre on Svalbard, Longyearbyen, Norway;* [c]*Institute of Biology, University of Tromsø, Norway;* [d]*IRIS – Biomiljø, PO Box 8046, N-4068 Stavanger, Norway*

(*Received 16 March 2009; final version received 9 April 2009*)

With increasing petroleum related activity in the Barents Sea and the subsequent risk of accidents, there is a demand for knowledge about the effect of oil pollution in Arctic ecosystems. In the present study, the sea ice amphipod *Gammarus wilkitzkii* was exposed to a water soluble fraction (WSF) of oil in two experiments, using a rock column system. First, three groups of adult females were exposed for 36 days for control ($n = 9$), low dose ($n = 10$) (initial and final oil concentration: 14, 115 and 120 μg/l) and high dose ($n = 10$) (initial and final oil concentration: 78, 764 and 395 μg/l). Total oxyradical scavenging capacity (TOSC), malondialdehyde (MDA), catalase activity (CAT), respiration rate and mortality were measured. In the second experiment, moulting rate was measured in immature individuals of two groups, control ($n = 10$) and exposed ($n = 20$), over 113 days. No mortality was observed in either of the experiments. There was a dose-related significant increase in respiration rate (40.69 ($\pm$22.82), 55.63 ($\pm$20.98), 94.57 ($\pm$22.80)) mg O_2 h^{-1}g ww^{-1} in control, low dose and high dose, respectively. A higher MDA level was detected in the low dose group (25.04 ($\pm$6.00) nmol g^{-1} of tissue) compared to both control (20.44 ($\pm$2.62) nmol g^{-1} of tissue) and high dose groups (20.93 ($\pm$4.79) nmol g^{-1} of tissue). Likewise, the low dose group had the highest value of TOSC towards hydroxyl (727.74 ($\pm$475.58), 1157.58 ($\pm$278.62), 1067.30 ($\pm$369.22) TOSC unit value mg^{-1} of protein for control, low and high dose, respectively). Although no difference in the catalase activity between control and exposed groups was detected, higher activity was measured at 0 °C (average: 248 μmol $^{-1}$ min^{-1} mg^{-1} of protein) than at the standard temperature for catalase measurement of 25 °C (average: 140 μmol $^{-1}$ min^{-1} mg^{-1} of protein), indicating a need to optimise the standard operational procedure when working with Arctic organisms. No effect of WSF exposure on moulting rate was detected. In conclusion, little mortality was observed during the experiments, indicating some degree of tolerance levels. However, the biomarker results indicated sub-lethal effects in *G. wilkitzkii* after exposure to WSF.

Keywords: oil pollution; *Gammarus wilkitzkii*; water soluble fraction; polycyclic aromatic hydrocarbons; Arctic; sea ice; amphipod

1. Introduction

The petroleum age is commencing in the Arctic as the availability of oil and gas is increasing due to advancing technology and retreating sea ice. As a consequence, the risk of pollution from ship accidents, leaks and blowouts is increasing [1], and a gap in knowledge on the response

*Corresponding author. Email: lc@akvaplan.niva.no

ISSN 0275-7540 print/ISSN 1029-0370 online

DOI: 10.1080/02757540902964978
http://www.informaworld.com

of Arctic species to oil is currently being recognised. Oil spilled in temperate and tropic areas will immediately and relatively effectively be affected by evaporation and degradation by light and bacteria. In the Arctic, however, ice cover, light regime and low temperatures constrain these processes [1–4], resulting in longer exposure periods and a changing composition of the oil than in the more investigated southern habitats. The marine populations' responses to contaminants also differ between arctic and temperate areas, as detected by [5].

Certain petroleum compounds, such as some polycyclic aromatic hydrocarbons (PAHs), are bioavailable and tend to bioaccumulate [6]. In the process of degrading PAHs, reactive oxygen species (ROS) are formed [7]. All aerobic organisms are constantly exposed to ROS, by partial reduction of molecular oxygen [8], and in normal situations the antioxidant forces of the cell will balance the prooxidant forces. However, when exposed to pollution, the antioxidant system is overwhelmed by ROS production, resulting in oxidative stress [9,10]. The consequence is damage to proteins, lipids and DNA and is expressed as cell disfunctionality, malformations, mutation and cancer [11].

Due to its few and specialised species, the ecosystem in the marginal ice zone (MIZ) is thought to be vulnerable to changes [12], and little is known about how it may react to an oil spill. Amphipods of more southern areas have been shown to be particularly susceptible towards oil spills and populations have a long recovery time [13–15]. However, polar marine invertebrate species are characterised by a more effective antioxidant system compared to temperate species [16,17].

The autochthonous sympagic sea ice amphipod *Gammarus wilkitzkii* is a long-lived species, which reproduces by brooding. It is the dominant amphipod in the pack ice with regard to biomass [4,18] and provides a link between the lower and higher trophic levels in the area [19,20]. An alteration in the population of this species could therefore have cascading effects. In this study, two groups of *Gammarus wilkitzkii* were exposed for one month and 113 days, respectively, to the water soluble fraction of oil (WSF). The ability of the animal to handle ROS generated during oil compound metabolism was investigated by measuring the total oxyradical scavenging capacity (TOSC) toward hydroxyl and peroxyl radicals, and the catalase activity (Cat), which converts the ROS H_2O_2 into water and oxygen. Cellular damage caused by ROS was measured as malondialdehyde concentration (MDA), which is an end product of lipid oxidation. Information on the health of the animals at individual level was measured by recording whole body respiration and moulting frequency.

2. Materials and methods

2.1. *Sampling and maintenance of the amphipods*

Ice amphipods (*Gammarus wilkitzkii*) were sampled with RV Jan Mayen, operated by the University of Tromsø, in the Barents and Greenland Seas in July and August 2005. The sampling was done under ice flows with baited traps, and by scuba divers with hand nets and a suction pump [21]. Additional sampling was done with pelagic trawls off the ice edge. All animals were grouped together, independently of their origin, and stored in darkness, in permanently aerated seawater at ca. 0 °C.

2.2. *Experimental design*

In the field, following an oil spill, organisms are exposed to a decreasing concentration of oil over time. To simulate this process, a rock column system were designed [22]. Four dl of North Sea crude oil was mixed with 4 kg of glass beads and left to evaporate for 24 h before the oil and

Table 1. Water samples were analysed for ΣPAH (16 EPA and 12 additional PAH compounds).

16 EPA PAH	Additional PAH
Naphthalene	C1-naphtalene
Phenantrene	C2-naphtalene
Antracene	C3-naphtalene
Acenaphylene	C1-phenantrene/antracene
Acenaphtene	C2-phenantrene/antracene
Fluorene	C3-phenantrene/antracene
Fluorantene	Dibenzotiophene
Pyrene	C1-dibenzotiophene
Benzo[a]antracene	C2-dibenzotiophene
Krysene	C3-dibenzotiophene
Benzo[b]fluorantene	Benzo[e]pyrene
Benzo[k]fluorantene	Perylene
Benzo[a]pyrene	
Indeno[1,2,3-cd]pyrene	
Benzo[ghi]perylene	
Dibenzo[a,h]antracene	

glass were poured into a column of plexiglas (diameter: 7 cm, height: 83 cm). The column was rinsed with running seawater for 4 days to allow evaporation of the most volatile compounds, before being connected to three aquariums. Due to the small flow and a buffer tank, no oil droplets could enter the aquariums; hence the animals were exposed to WSF of oil only. Throughout the experiment, WSF was sampled in dark 2-litre bottles and stored in darkness at 0–5 °C for ca. 24 h. The samples were analysed for a total amount of 28 types of polycyclic aromatic hydrocarbons at Unilab Analyse AS (Table 1).

2.3. *Exposure experiments*

In Experiment A the biological responses of *G. wilkitzkii* to oil pollution was measured on the molecular, cellular and physiological level. Ovigerous females were exposed for 36 days to a low dose of North Sea crude oil (LD) ($n = 10$) and a 5.58 times higher dose (HD) ($n = 10$), as well as control (C) with sea water only ($n = 10$). Mortality was monitored throughout the exposure period, while respiration rate was measured on day 33. Thereafter amphipods were frozen in liquid nitrogen and analysed for catalase activity (Cat), total oxyradical scavenging capacity (TOSC) and malondialdehyde (MDA). In Experiment B, the same exposure system was utilised to study the effect of WSF of North Sea crude oil on moulting frequency of immature individuals. *G. wilkitzkii* was exposed for 113 days to high dose ($n = 20$), and control ($n = 10$). Immature amphipods were separated from mature individuals by absence of oostegites and by approximate size, determined as all individuals smaller than the smallest female [23].

3. Biological effect parameters

3.1. *Respiration*

Respiration rate was measured using a closed bottle method [24]. Oxygen consumption of the amphipods was determined by measuring (O_2) after 5 h of incubation in separate bottles. (O_2) in a parallel set of bottles, containing seawater only, was measured to extract the microbial respiration.

3.2. *Biochemical analysis*

Before biochemical studies, the samples were prepared as follows: animals were thawed on ice and the whole body of the amphipod was weighed and placed in a potter with phosphate buffer (50 mM KH_2PO_4+ 2.5% NaCl, pH 7.5) (volume/weight ratio of 4) and homogenised with a Potter-Elvhjem glass/Teflon homogeniser. The homogenate was centrifuged for 1 h at 100,000 *g* and the resulting supernatant (cytosol) was stored at −80 °C [25] . The TOSC assay measures the organisms' total ability to process ROS [9,26]; buffers were adjusted for marine organisms, as described earlier [27]. The enzyme catalase degrades H_2O_2 into oxygen and water, as in the equation $2H_2O_2 \rightarrow O_2 + 2H_2O$. The rate of this degradation gives the catalase activity [28]. We measured the activity at 0 °C in addition to the normal procedure of 25 °C. Malondialdehyde was measured according to [29,30]. Protein concentration in the samples was determined according to the Bradford method [31].

3.3. *Moulting rate*

Exuvia of each amphipod were counted and removed from the aquarium cups of the amphipods every 4th day.

3.4. *Statistics*

The data analyses were performed in the statistical open source software R (http://cran.r-project.org), with the effect parameters as response variables, testing for treatment differences. The distributions were tested with the Shapiro–Wilk test and the homogeneity of variance with the Bartlett test when normally distributed, and with the Levenes test when not normally distributed. Normally distributed data with homogenous variance were analysed with analysis of variance (ANOVA, $p < 0.05$). The non-normally distributed data and the data without homogenous variance were analysed with a Kruskal–Wallis test ($p < 0.05$). Subsequently, results were tested with a Tukey HSD and studied in a diagnostic plot. The difference in catalase activity at 0 and 25 °C was checked for normality with the Shapiro–Wilks test and analysed by a paired t-test. The moulting data were analysed with a Kruskal–Wallis test ($p < 0.05$) and a generalised linear model with a Poisson family function was applied to study the influence of wet weight and length, but no dependence of these parameters was detected ($p > 0.05$).

4. Results

4.1. *Mortality*

There was no mortality in control, low or high dose group in either of the two experiments; however, one amphipod of the control group of Experiment A was lost posterior to the experiment. Hence the control group contained one less animal than the two other groups.

4.2. *Experiment A*

4.2.1. *Concentration of PAH in water samples*

The initial concentrations of $\sum$PAH in the water samples in Experiment A for high dose, low dose and control were 78,764, 14,115 and 274 μg/l, respectively. They then followed a steep decrease

in both high and low dose before flattening out at around day 22, with concentrations of 343, 62 and 136 μg/l for high dose, low dose and control, respectively (Figure 1). The presence of each compound in the beginning and end of the experiment is presented in Figure 2. The low molecular weight naphtalenes dominated the first day, while the medium molecular weight compounds C3-phenantrene/anthracene and dibenzothiophenes dominated day 36. This indicates a weathering process occurring in our experiment.

4.2.2. *Respiration rate*

The mean respiration rate of animals exposed to control, low and high dose of WSF, was 40.69 (±22.82), 55.63 (±20.98) and 94.57 (±22.80) mg O_2 g^{-1} (w.w.) h^{-1}, respectively. Animals

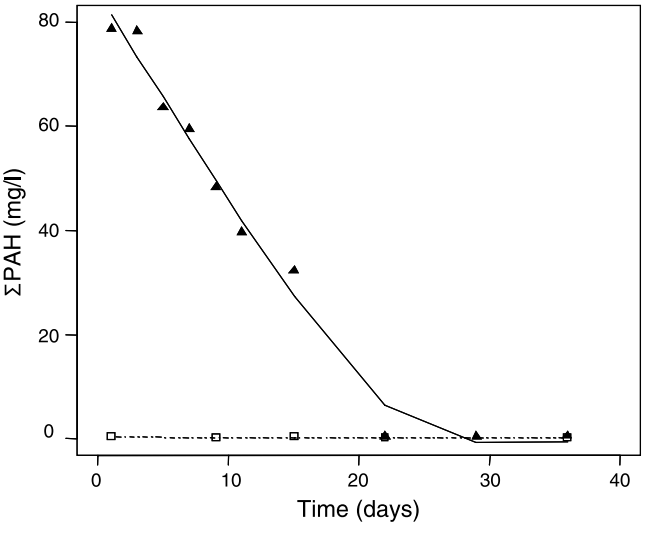

Figure 1. Concentration of ΣPAH (see Table 1) over time, measured throughout the experiment of exposing oovigerous animals to WSF. High dose (black triangles) and control (white squares). Low dose was calculated to be 5.58 times lower than the high dose.

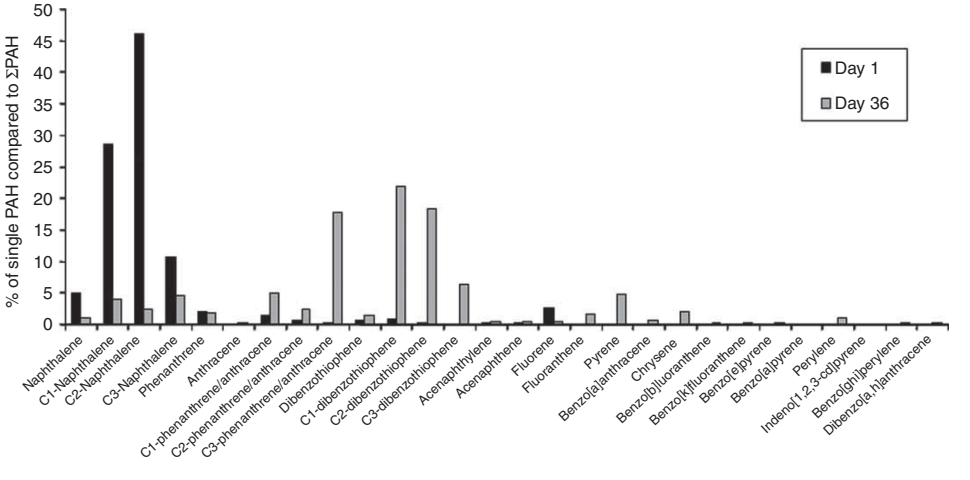

Figure 2. Comparison between the relative proportions expressed as % of single PAH relative to the sum of ΣPAH at day 1 (black) and day 36 (grey).

exposed to high dose had a significantly higher respiration rate than the animals in the control group (ANOVA: $p < 0.05$), while animals in the low dose did not (ANOVA: $p = 0.16$) (Figure 3A).

4.2.3. *Protein concentration*

The mean concentration of protein measured in animals exposed to control, low and high dose of WSF, was 2.39 (±0.45), 2.49 (±0.66) and 3.03 (±0.84) mg l^{-1}, respectively. The control group

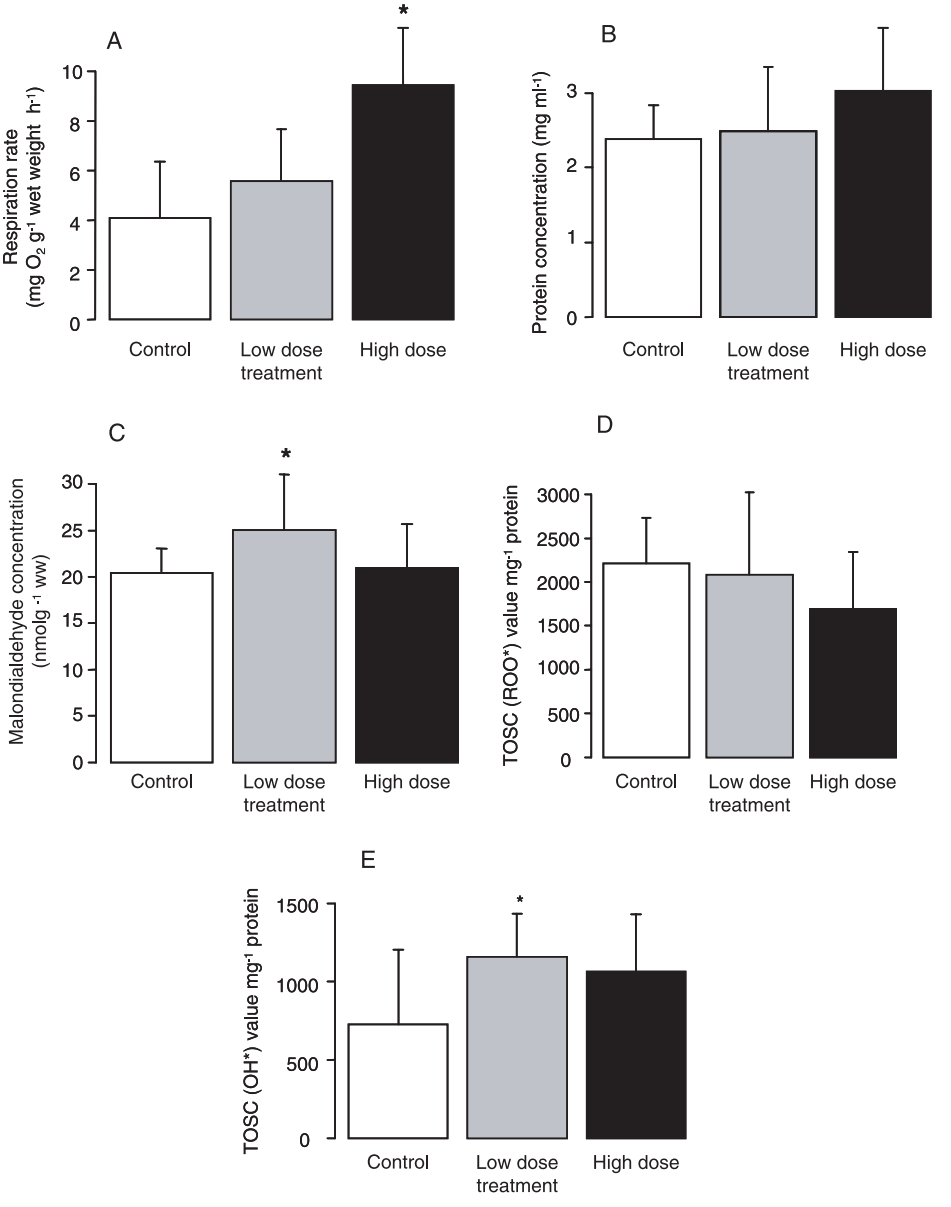

Figure 3. (A) Respiration rate, (B) protein concentration, (C) concentration of malondialdehyde (MDA), (D) total oxyradical scavenging capacity towards peroxyl (TOSC ROO*) and (E) total oxyradical scavenging capacity towards hydroxyl (TOSC OH*) measured in *Gammarus wilkitzkii* exposed to clean water (control), low dose and high dose of WSF. The error bars represent the standard deviation around the mean. Asterisks symbolise significant difference from control.

did not differ significantly from the two other groups (ANOVA: low dose $p = 0.77$, high dose $p = 0.07$) (Figure 3B).

4.2.4. *Concentration of malondialdehyde*

The mean concentration of MDA of animals exposed to control, low and high dose of WSF, was 20.44 (±2.62), 25.04 (±6.00) and 20.93 (±4.79) nmol g^{-1} of tissue, respectively. There was a significantly higher concentration of MDA in animals of the low dose group compared to the control group (ANOVA: $p < 0.05$), while no significant difference was found in the high dose group (ANOVA: $p = 0.82$) (Figure 3C).

4.2.5. *Total oxyradical scavenging capacity*

TOSC-ROO, with means of 2212.13 (±517.59), 2083.18 (±940.45) and 1687.05 (±657.15) for control, high and low dose, respectively, did not show any significant difference between treatments (ANOVA: low dose $p = 0.71$, high dose $p = 0.13$) (Figure 3D). The mean TOSC-OH measured in animals exposed to control, low and high dose of WSF, was 727.74 (±475.58), 1157.58 (±278.62) and 1064.30 (±369.22) TOSC unit mg^{-1} of protein, respectively. A significantly higher mean of the low dose group compared to the control group was detected (ANOVA: $p < 0.05$), while no significant difference was seen in the high dose group (ANOVA: $p = 0.07$) (Figure 3E).

4.2.6. *Catalase activity*

The mean catalase activity of animals exposed to control, low and high dose of WSF, measured at 0 °C, was 229 (±144), 270 (±135) and 245 (±253) μmol min^{-1} mg^{-1} proteins, respectively. No significant difference was discovered between the three groups (ANOVA: low dose vs. control $p = 0.49$, high dose vs. control $p = 0.89$). Similarly, no significant difference was revealed in catalase activity measured at 25 °C with means of 118 (±62), 137 (±110) and 164 (±116) μmol min^{-1} mg^{-1} proteins for control, high and low dose (ANOVA: low dose $p = 0.69$, high dose $p = 0.38$). However, there was a significant difference between catalase activity measured at 25 and 0 °C, with the activity at 0 °C being a mean of 108 μmol min^{-1} mg^{-1} proteins higher than at 25 °C (paired t-test: $p < 0.05$) (Figure 4).

4.3. *Experiment B*

4.3.1. *Moulting rate*

The moulting rate did not differ between the control and the exposed group, with means of 1.4 and 1.5 moults per animal, respectively (Kruskal–Wallis: $p = 0.90$). 14 individuals (of 30) went through two moults, with an inter moult period ranging from 39 to 92 days (average: 66 days).

5. Discussion

Concentrations of WSF were initially high, with a consecutive rapid drop occurring in the first few days, and a steady flattening after a prolonged period. This is similar to the behaviour of oil when being released into the ocean, indicating a successful imitation of a weathering process.

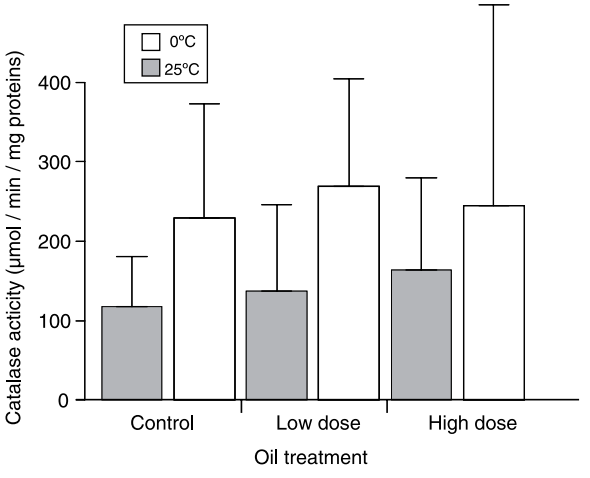

Figure 4. Catalase activity, measured in *Gammarus wilkitzkii* exposed to clean water (control, $n = 9$), low dose PAH ($n = 10$) and high dose PAH ($n = 10$) of WSF, at 0 (light grey) and 25 °C (dark grey). The bars represent mean ± standard deviation.

5.1. *Mortality*

No mortality was detected throughout the experiments, despite high initial concentrations of WSF and relatively long-term exposure periods. This is in contrast to another study that reported that 3 out of 10 and 1 out of 10 of ovigerous females of *G. wilkitzkii* died when being exposed to high and low doses of WSF, respectively [32]. The study was of comparable duration and concentrations of WSF with our Experiment A. Moreover, 50% mortality was seen in *G. oceanicus*, exposed to 100 mg/l of North Sea crude oil (similar oil type as the one used in the present study) for 96 h [33], and the authors comment on an indication of significant mortality at even lower concentrations. In contrast, similar to our study, other authors detected no mortality of the temperate amphipod *C. volutator* after 10, 28 and 75 days of exposure to 3 mg/l WAF of crude oil [34]. It appears that the sensitivity of marine amphipods towards oil pollution varies both within and between species. No mortality in the present study suggests a certain degree of resistance of *G. wilkitzkii* to WSF of North Sea crude oil. However, more sub-lethal effects are indicated due to differences in respiration, MDA and TOSC between control and some treatment groups.

5.2. *Experiment A*

Animals of the high dose group had a higher respiration rate compared to the control group. Several studies have demonstrated similar physiological responses. The crab *Carcinus maenas* showed increasing respiration rates when being exposed to WSF of crude oil [35]. An increased electron transport system (ETS) activity was detected in the Arctic benthic amphipod *G. setosus* exposed to water accommodated fraction of oil (WAF) indicating increased respiration [36]. A prolonged increase in respiration rate may indicate increased metabolic activity, in this case most likely due to metabolism of harmful compounds taken up in the body. On the other hand, no response in respiration rate was seen in the low dose group of our experiment. Similarly, no effect on respiration in the benthic Arctic amphipod *G. oceanicus* exposed to sub lethal concentrations of WSF of North Sea crude oil was observed [33]. However, a reason for the lack of response could be the short exposure period, as the experiment only lasted for 96 h [33]. The lack of an effect on respiration rate in the low dose group of our experiment may be due to the lower concentration compared to the high dose. However, an effect in other parameters was detected in this group, such

as TOSC towards hydroxyl and MDA (see discussion below), suggesting an increased metabolism that was not reflected in the respiration rate. Similarly, the increased respiration rate of the high dose group is not reflected by an increase in TOSC, MDA nor catalase. Hence we do not see a direct correlation between respiration rate and efficiency of antioxidant defences related to the degradation of PAH.

No difference from the control was seen in low and high dose groups of TOSC values towards peroxyl (TOSC-ROO). Similarly, no effect on TOSC-ROO in the Arctic crab *Hyas araneus* and the Arctic clam *Mya truncata*, exposed through sediments to North Sea crude oil was found [37,38]. The lack of response of TOSC-ROO in *G. wilkitzkii* can be a result of poor metabolism of oil compounds resulting in relatively low production rate of peroxyl radicals by the cells. On the other hand, if no increase in TOSC-ROO is detected despite a presence of peroxyl radicals in the cells demonstrated by an increased MDA, the reason could be an actual limited ability to degrade peroxyl.

TOSC values towards hydroxyl (TOSC-OH) were significantly higher in the low dose group compared to the control group. This indicates an induced antioxidant system, following exposure to ROS generated by PAH metabolism. However, no divergence was detected in the high dose group of TOSC-OH. Similarly, MDA concentration was significantly higher in the low dose group while no effect was seen in the high dose group. Antioxidant defence responses are known to be transient and each parameter will vary differently with time [39]. As we sampled at one time point only, we may therefore have missed an effect in the high dose group. *G. wilkitzkii* exposed to WSF of North Sea crude oil, and showed a dose dependent response except in the high dose [40]. This lack of effect seen at high doses in both studies could be a result of a malfunctioning antioxidant system owing to narcosis of the whole organism, organs or cells.

Animals in our low dose group showed an increased production of MDA, while others detected an even higher MDA concentration in mussels (*Mytilus galloprovincialis*), transplanted in the channels of Venice, an area influenced by intense anthropogenic pollution [29]. MDA is produced through lipid peroxidation and a subsequent effect is cell destruction [30]. This is a sign of a dysfunctional or overwhelmed antioxidant system, possibly as a result of PAH metabolism [41]. It is noteworthy that owing to reduced number of animals, all the biological effect parameters reported in this study indicated about the status of the oxidative metabolism of the amphipod after one month of exposure when the oil concentrations were low. Considering that the levels of oil were exponentially declining and that antioxidant defence responses are transient, it can be expected that a totally different response profile would have been observed if we have had several sampling points during the experiment.

In the present study, catalase activity was significantly higher at 0 compared to 25 °C. The optimal temperature for catalase activity was measured to be 45 °C in the fresh water prawn *Macrobrachium malcolmsonii* [42] and the bivalve *Mytilus edulis* [28]. The higher activity at low temperatures observed in our study could be a result of an enzyme system adapted to cold environments, where the optimal temperature is closer to 0 than 25 °C. Based on our results, we recommend an adaptation of the methods for measuring enzyme activities related to the habitat temperature of the organism. Catalase activities of low and high dose groups of animals did not differ significantly from the control, suggesting no induction nor inhibition. However, our values indicate the catalase activity of *G. wilkitzkii* to be 10 times higher than in the temperate amphipod *G. locusta* [43]. These results support previous findings [17], suggesting that Arctic organisms have a more effective antioxidant defence than temperate counterparts.

5.3. *Experiment B*

During the period of moulting, crustaceans are particularly fragile towards environmental stressors [44]. However, our experiment revealed no mortality related to moults, nor any difference

in moulting frequency between the exposed and control group. Little information is available on moulting frequency of crustaceans related to toxic exposure, but a longer intermoult period has been detected in several studies of crustaceans exposed to aromatic hydrocarbons [44]. Our experiment lasted 113 days, while the moulting time of the animals was 66 days (average inter moult period for the 14 individuals going through two moults). Hence the animals moulted only once or twice during the whole period, making it difficult to detect any divergence between the two groups. Younger individuals grow faster and consequently moult more often. In a future study it would therefore be recommended to utilise younger individuals and/or a longer lasting experiment.

6. Conclusion

In this study, mature and immature specimens of the species *G. wilkitzkii* were demonstrated to have no mortality towards WSF of North Sea crude oil within concentrations ranging from 78,746–395 μg/l (high dose). But indications of oxidative stress were detected through increased respiration rates and concentrations of MDA. However, the presence of an active catalase enzyme and the capacity of the amphipod to increase its TOSC-OH indicate that *G. wilkitzkii* can handle and control the oxidative metabolic processes. Future studies should implement natural stress factors like salinity variation, seasonal differences in the antioxidant defence system should be considered and more sampling points would provide more extensive information of the antioxidant defence system working against ROS.

Acknowledgements

This study was financed by the Norwegian Research Council under the programme PROOF N° 164407. We would like to thank Paul Renaud and Arntraut Gotsch for helping with the experiment. Additionally, we are grateful for the work done by the crew of RV Jan Mayen during the amphipod sampling.

References

[1] L.K. Sydnes, *Oil, water, ice and light*, Polar Res. 10 (1991), pp. 609–618.
[2] R. Siron, E. Pelletier, D. Delille, and S. Roy, *Fate and effects of dispersed crude oil under icy conditions simulated in mesocosms*, Mar. Environ. Res. 35 (1993), pp. 273–302.
[3] P.M. Chapman and M.J. Riddle, *Polar marine toxicology: Future research needs*, Mar. Pollut. Bull. 50 (2005), pp. 905–908.
[4] I. Werner and R. Gradinger, *Under-ice amphipods in the Greenland Sea and Fram Strait (Arctic): Environmental controls and seasonal patterns below the pack ice*, Mar. Biol. 140 (2002), pp. 317–326.
[5] G.H. Olsen, M.L. Carroll, P.E. Renaud, W.G. Jr. Ambrose, R. Olssøn, and J. Carrol, *Benthic community response to petroleum-associated components in Arctic versus temperate marine sediments*, Mar. Biol. 151 (2007b), pp. 2167–2176.
[6] Bakke T., Hameedi J., Kimstach V., Macdonald R., Melnikov S., Robertson A., Shearer R., and Thomas D. (2002) *Chapter 10: Petroleum hydrocarbons*, in *AMAP assessment report*, pp. 661–701.
[7] D.R. Livingstone, *Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms*, Mar. Pollut. Bull. 42 (2001), pp. 656–666.
[8] F. Regoli and G.W. Winston, *Applications of a new method for measuring the total oxyradical scavenging capacity in marine invertebrates*, Mar. Environ. Res. 46 (1998), pp. 439–442.
[9] G.W. Winston, F. Regoli, A.J. Dugas, J.H. Fong, and K.A. Blanchard, *A rapid gas chromatographic assay for determining oxyradical scavenging capacity of antioxidants and biological fluids*, Free Radical Bio. Med. 24 (1998), pp. 480–493.
[10] B. Halliwell and J. Gutteridge, *Free Radicals in Biology and Medicine*, 4th ed., Oxford Science, Oxford, 2007.
[11] D.J. Hoffman, A.R. Barnett, G.A. Burton, and J. Cairns, *Handbook in Ecotoxicology*, CRC Press, New York, 2002.
[12] P.M. Chapman and M.J Riddle, *Missing and needed: Polar marine ecotoxicology*, Mar. Pollut. Bull. 46 (2003), pp. 927–928.

[13] G.J.L. Gesteira and J.C. Dauvin, *Amphipods are good bioindicators of the impact of oil spills on soft-bottom macrobenthic communities*, Mar. Pollut. Bull. 11 (2000), pp. 1017–1027.
[14] G.J.L. Gesteira and J.C. Dauvin, *Impact of the Aegan sea oil spill on the subtidal fine sand macrobenthic community of the Arez-Betanzos Ria (Northwest Spain)*, Mar. Environ. Res. 60 (2005), pp. 289–316.
[15] C.C.S. Nikitik and A.W. Robinson. *Patterns in benthic populations in the Milford Haven waterway following the 'Sea Empress' oil spill with special reference to amphipods*, Mar. Pollut. Bull. 46 (2003), pp. 1125–1141.
[16] F. Regoli, M. Nigro, S. Bompadre, and G.W. Winston, *Total oxyradical scavenging capacity (TOSC) of microsomal and cytosolic fractions from Antarctic, Arctic and Mediterranian scallops: Differentiation between three potent oxidants*, Aquat. Toxicol. 49 (2000), pp. 13–25.
[17] L. Camus, B. Gulliksen, M.H. Depledge, and M.B. Jones, *Polar bivalves are characterized by high antioxidant defences*, Polar Res. 24 (2005), pp. 111–118.
[18] H. Hop, M. Poltermann, O.J. Lønne, S. Falk-Petersen, R. Korsnes, and W.P. Budgell, *Ice amphipod distribution relative to ice density and under-ice topography in the northern Barents Sea*, Polar Biol. 23 (2000), pp. 357–367.
[19] I. Werner, H. Auel, and C. Friedrich, *Carnivorous feeding and respiration of the Arctic under-ice amphipod* Gammarus wilkitzkii, Polar Biol. 25 (2002), pp. 523–530.
[20] O.J. Lønne and G.W. Gabrielsen, *Summer diet of seabirds feeding in sea-ice-covered waters near Svalbard*, Polar Biol. 12 (1992), pp. 685–692.
[21] O.J. Lønne, *A diver operated electric suction sampler for sympagic (= under ice) invertebrates*, Polar Res. 6 (1988), pp. 135–136.
[22] M.G. Carls, S.D. Rice, and J.E. Hose, *Sensitivity of fish embryos to weathered crude oil: Part 1. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval pacific herring* (Clupea pallasi), Environ. Toxicol. Chem. 18 (1999), pp. 481–493.
[23] F. Beuchel and O.J. Lønne, *Population dynamics of the sympagic amphipods* Gammarus wilkitzkii *and* Apherusa glacialis *in sea ice north of Svalbard*, Polar Biol. 25 (2002), pp. 241–250.
[24] I. Werner, H. Auel, C. Garrity, and W. Hagen. *Pelagic occurrence of the sympagic amphipod* Gammarus wilkizkii *in ice free waters of the Greenland Sea – dead end or part of life cycle?* Polar Biol. 22 (1999), pp. 56–60.
[25] F. Regoli, G.W. Winston, V. Mastrangelo, G. Principato, and S. Bompadre, *Total oxyradical scavenging capacity in mussel* Mytilus *sp. as a new index of biological resistance to oxidative stress*, Chemosphere 37 (1998), pp. 2773–2783.
[26] F. Regoli and G.W. Winston, *Quantification of total oxidant scavenging capacity of antioxidants for peroxynitrite, peroxyl radicals, and hydroxyl radicals*, Toxicol. Appl. Pharm. 156 (1999), pp. 96–105.
[27] L. Camus and B. Gulliksen, *Antioxidant defence properties of Arctic amphipods: comparison between deep-, sublittoral and surface-water species*, Mar. Biol. 146 (2005), pp. 355–362.
[28] D.R. Livingstone, E. Lips, P. Garcia Martinez, and R.K. Pipe, *Antioxidant enzymes in the digestive gland of the common mussel* Mytilus edulis, Mar. Biol. 112 (1992), pp. 265–276.
[29] D.M. Pampanin, L. Camus, A. Gomiero, I. Marangon, E. Volpato, and C. Nasci, *Susceptibility to oxidative stress of mussels* (Mytilus galloprovincialis) *in the Venice Lagoon* (*Italy*), Mar. Pollut. Bull. 50 (2005), pp. 1548–1557.
[30] H. Esterbauer, R.J. Schaur, and H. Zollner, *Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes*, Free Radical Biol. Med. 11 (1991), pp. 81–128.
[31] M.M. Bradford, *A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding*, Anal. Biochem. 72 (1976), pp. 248–254.
[32] G.H. Olsen, J. Carrol, E. Sva, and L. Camus, *Cellular energy allocation in the Arctic sea iceamphipod* Gammarus wilkitzkii *exposed to the water soluble fraction of oil*, Mar. Environ. Res. 66 (2008), pp. 213–214.
[33] T. Aunaas, A. Olsen, and K.E. Zachariassen, *The effect of oil and oil dispersants on the amphipod* Gammarus oceanicus *from Arctic waters*, Pol. Res. 10 (1991), pp. 619–630.
[34] A. Scarlett, S.J. Rowland, M. Canty, E.L. Smith, and T.S. Galloway, *Method for assessing the chronic toxicity of marine and estuarine sediment-associated contaminants using the amphipod* Corophium volutator, Mar. Environ. Res. 63 (2007), pp. 457–470.
[35] M.H. Depledge, *Changes in cardiac activity, oxygen consumption and perfusion indices in* Carcinus maenas *(L.) exposed to crude oil and dispersants*, Comp. Biochem. Phys. C 78 (1984), pp. 461–466.
[36] G.H. Olsen, E. Sva, J. Carroll, L. Camus, W. De Coen, R. Smolders, H. Øveraas, and K. Hylland, *Alterations in the energy budget of Arctic benthic species exposed to oil-related compounds*, Aquat. Toxicol. 83 (2007a), pp. 85–92.
[37] L. Camus, M.B. Jones, J.F. Børseth, F. Regoli, and M.H. Deplegde, *Heart rate, respiration and total oxyradical scavenging capacity of the Arctic spider crab*, Hyas araneus, *following exposure to polyaromatic compounds via sediment and injection*, Aquat. Toxicol. 61 (2002), pp. 1–13.
[38] L. Camus, S.R. Birkely, M.B. Jones, J.F. Børseth, B.E. Grøsvik, B. Gulliksen, O.J. Lønne, F. Regoli, and M.H. Depledge, *Biomarker responses and PAH uptake in* Mya truncata *following exposure to oil-contaminated sediment in an Arctic fjord (Svalbard)*, Sci. Total Environ. 308 (2003), pp. 221–234.
[39] L. Camus, D.M. Pampanin, E. Volpato, E. Delaney, S. Sanni, and C. Nasci, *Total oxyradical scavenging capacity responses in* Mytilus galloprovincialis *transplanted into the Venice lagoon (Italy) to measure the biological impact of anthropogenic activities*, Marine Pollut. Bull. 49 (2004), pp. 801–808.
[40] L. Camus and G.H. Olsen, *Embryo aberrations in the sea ice amphipod* Gammarus wilkitzkii *exposed to the water soluble fraction of oil*, Mar. Environ. Res. 66 (2008), pp. 221–222.
[41] D.R. Livingstone, E. Lips, P. Garcia Martinez, X. Michel, J.F. Narbonne, S. O'Hara, D. Ribera, and G.W. Winston, *Oxyradical production as a pollution-mediated mechanism of toxicity in the common mussel* Mytilus edulis *L., and other molluscs*, Funct. Ecol. 4 (1990), pp. 415–424.

[42] S. Arun, R. Thirumurugan, R. Visakan, S. Balamurugan, V. Arunachalam, and P. Subramaniam, *Optimal analytical conditions for catalase in fresh water prawn*, Macrobrachium malcolmsonii, Biotech. Histochem. 78 (2003), pp. 1–4.
[43] A.D. Correia, M.H. Costa, O.J. Luis, and D.R. Livingstone, *Age-related changes in antioxidant enzyme activities, fatty acid composition and lipid peroxidation in whole body* Gammarus locusta (*Crustacea: Amphipoda*), J. Exp. Mar. Biol. Ecol 289 (2003), pp. 83–101.
[44] J.S. Weis, A. Cristini, and K.R. Rao, *Effects of pollutants on moulting and regeneration in crustacean*, Am. Zool. 32 (1992), pp. 495–500.