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Antioxidant responses in the polar marine sea-ice amphipod *Gammarus wilkitzkii* to natural and experimentally increased UV levels

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ABSTRACT

Polar marine surface waters are characterized by high levels of dissolved oxygen, seasonally intense UV irradiance and high levels of dissolved organic carbon. Therefore, the Arctic sea-ice habitat is regarded as a strongly pro-oxidant environment, even though its significant ice cover protects the ice-associated (= sympagic) fauna from direct irradiation to a large extent. In order to investigate the level of resistance to oxyradical stress, we sampled the sympagic amphipod species *Gammarus wilkitzkii* during both winter and summer conditions, as well as exposed specimens to simulated levels of near-natural and elevated levels of UV irradiation. Results showed that this amphipod species possessed a much stronger antioxidant capacity during summer than during winter. Also, the experimental UV exposure showed a depletion in antioxidant defences, indicating a negative effect of UV exposure on the total oxyradical scavenging capacity. Another sympagic organism, *Onisimus nanseni*, was sampled during summer conditions. When compared to *G. wilkitzkii*, the species showed even higher antioxidant scavenging capacity.

1. Introduction

During the last decades, levels of stratospheric ozone have been decreasing over both polar areas (Kerr and McElroy, 1993), allowing an increased amount of ultraviolet radiation (UVR) to reach the Earth's surface (Madronich et al., 1998). Since UVR can penetrate to ecologically important depths in water, a large number of studies on the effects of UVR on aquatic environments have been carried out. UV radiation creates oxidative stress, and causes cellular damage and increased mortality (Browman et al., 2000; Lesser, 2006). The Arctic sea-ice environment is characterized by high dissolved oxygen concentrations, seasonally high levels of dissolved organic carbon (DOC) (Belzile et al., 2000), as well as a seasonally extended photoperiod and high primary productivity. These factors, especially the co-existence of high DOC levels under significant UVR levels (Scully et al., 1996), lead to the production of reactive oxygen species (ROS) (Lesser, 2006; Häder et al., 1998). The deleterious effects of ROS on aquatic organisms have been covered in reviews by Di Giulio et al. (1989) and Winston and Di Giulio (1991), and include lipid peroxidation, DNA strand breaks, cyclobutane pyrimidine dimers (CPD's), and enzyme inactivation (Livingstone, 2001; Browman et al., 2003; Karentz et al., 2004). To cope with these and other pro-oxidant challenges, aquatic organisms can deploy a number of antioxidant defence mechanisms, which rely on enzymatic as well as nonenzymatic components. These include superoxide dismutase (SOD) (McCord and Fridovich, 1969), catalase (CAT), and glutathione peroxidases as enzymatic defences, while vitamin C (ascorbic acid), glutathione (GSH), vitamin E (tocopherol), carotenoids (Davenport et al., 2004) and several other small-molecule antioxidants like dimethyl sulfide (DMS) and mycosporine-like amino acids (MAA's) function as nonenzymatic antioxidants (Lesser, 2006). Another adaptation to UVR-induced stress is the photoenzymatic repair (PER) mechanism, where the enzyme photolyase breaks down CPD's in the presence of photosynthetically active radiation (PAR, 400–700 nm) and ultraviolet radiation (UVR) in the UVA range (320–400 nm). Grad et al. (2001) and Lacuna and Uye (2001) have published studies on PER in zooplankton which showed that PER can significantly alleviate the UVR-induced stress.

The importance of UVR as a pro-oxidant force in polar surface waters has been recognized by a number of papers, see Belzile et al. (2000) and Browman et al. (2000) for Arctic marine habitats, Karentz and Lutze (1990) and Karentz and Bosch (2001) on

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Antarctic marine habitats, Hessen (1996) and Hessen et al. (2002) on Arctic freshwater ponds, and Kepner et al. (2000) and Rocco et al. (2002) on Antarctic freshwater habitats. In order to address the complex issue of oxyradical stress and antioxidant responses, Regoli and Winston (1998) and Winston et al. (1998) developed and proposed the total oxyradical scavenging (TOSC) assay as an integrative approach to the problem of quantifying the level of oxyradical defence in a given tissue or organism sample (Regoli et al., 2002a). Rather than studying one antioxidant component at a time which can be induced through several pathways, this assay quantifies the total antioxidant response to a specific oxyradical challenge. It has since been applied to a number of polar studies (Camus et al., 2003; Corsolini et al., 2001; Regoli et al., 2000, 2002b, 2005) including studies on polar amphipods (Camus and Gulliksen, 2004, 2005; Obermüller et al., 2005).

Lipid peroxidation is a well-known mechanism of cellular injury induced by oxidative stress in cells and tissues. Malondialdehyde (MDA) is a decomposition product of lipid peroxides derived from polyunsaturated fatty acids. This reactive carbonyl compound is the most abundant lipid peroxidation product and therefore widely used as an indicator of lipid peroxidation.

The polar sympagic amphipod *G. wilkitzkii* plays a central role in the ecosystem of Arctic sea-ice-covered waters (Steele and Steele, 1975; Poltermann, 2000; Poltermann et al., 2000), since it consumes sea-ice algae and sympagic fauna (Gulliksen and Lønne, 1989; Scott et al., 2001; Werner, 1997) and thus makes this sympagic biomass available to higher trophic levels (Lønne and Gulliksen, 1989) as well as to the underlying waters (Werner, 2000).

In this study, we measured natural UVR in the sympagic habitat and studied the effects of natural versus artificial UVR exposure on *G. wilkitzkii* to determine whether this species is susceptible to oxyradical stress. As a supplement to the TOSC assay, we also measured the content of malondialdehyde (MDA) as an indicator of lipid peroxidation (Marnett, 1999). We also compared the effects of natural UVR exposure in *G. wilkitzkii* and another sympagic species, *Onisimus nanseni*.

2. Materials and methods

2.1. Fieldwork

2.1.1. Spectral measurements of UVR

Spectral measurements in the field were performed at 78°65′ N, 21°25′ E during winter and at 81°59′ N, 11°50′ E during summer, using a TriOS Ramses ACC UV/PAR spectrophotometer (range 280–550 nm, 2.2 nm resolution) which was deployed by scuba-divers under the sea-ice, where it was affixed to the ice underside with a L-shaped arm and ice screws during measurements. Ice thickness in the field ranged from 1.5 to 2 m at the winter station and from 2 to 2.5 m at the summer station, with a snow cover of >1 dm during winter and 2–5 cm during summer. All ice floes in the respective areas were established to be multi-year ice.

2.1.2. Sampling of amphipods

During a winter sampling campaign in March/April 2004, sympagic amhipod samples of *G. wilkitzkii* were collected under consolidated pack ice at 78°65′ N, 21°25′ E near Heleysundet, on the east coast of the Svalbard Archipelago. More amphipods of this species as well as another sympagic species, *O. nanseni*, were collected during two research cruises to the ice edge north of Svalbard in August and September 2004 at 81°59′ N, 11°50′ E (compare Fig. 1: study area 1). A battery-driven suction sampler or hand-held dip net, operated by scuba-divers, were used to collect the animals from the sea ice underside. Species, sex and developmental stages as well as total body length were determined immediately with stereomicroscopes. Animals intended for laboratory exposure were kept alive in temperature-controlled aquaria (0 °C) at low white light intensities until transfer to the laboratory. Organisms intended for determination of *in situ*-TOSC and MDA status were frozen immediately in liquid nitrogen and later transferred and stored at −80 °C.

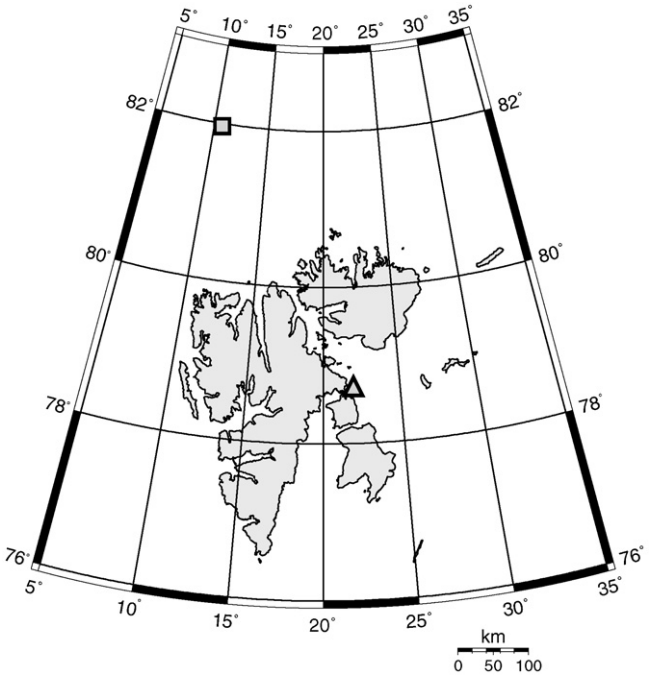

Fig. 1. Sampling areas for winter (March 2004, triangle) and summer (August 2004, square) sea ice stations. Locations were chosen based on their accessibility and sufficient cover of multi-year ice.

2.2. Laboratory work

2.2.1. Exposure setup

This laboratory exposure was designed to detect whether there was a direct relationship for the sample organisms towards near-natural or increased radiation levels. A daily irradiation period, followed by a recovery period, was programmed to simulate the total radiation received by an organism per day during summer conditions. At the laboratory facilities of IRIS-Akvamiljø, a climate-controlled and light-controlled room was prepared with a controlled sea water supply. Room and water temperatures were set to 0 °C, and were constantly monitored throughout the experimentation period. Five aquaria were setup, of which two were equipped with a high-intensity UVA + B fluorescent lamp array (UVB), another two with a low-intensity UVA fluorescent lamp array (UVA), and the last was kept as control group in near-darkness (DC). All aquaria were supplied from the same climatised water pump with a flow of 1.8–2 l/min. and constant water circulation was maintained by a filtered overflow.

The high-intensity UV fluorescent lamp array consisted of one Q-Panel 340 lamp (40 W tube, 120 cm length) together with two Philips TL-12 lamps (20 W tube, 60 cm length), while the low-intensity UV fluorescent lamp array was composed of only one Q-Panel 340 lamp (40 W tube, 120 cm length) only. The high-intensity array was aimed at modelling Arctic summer sea surface levels of UV radiation (0.7 W m^{-2} UV-B, 8.5 W m^{-2} UV-A), while the low-intensity array was aimed at modelling typical Arctic under-ice levels of UV radiation (0.2 W m^{-2} UV-B, 3.5 W m^{-2} UV-A). Lamps were encased in UV-transparent plexi glass tubings to decrease the