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Response of oxidative stress parameters and sunscreening compounds in Arctic amphipods during experimental exposure to maximal natural UVB radiation

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Abstract

The paper investigates tolerance to UV radiation (UVR) in 3 amphipod species from the Arctic Kongsfjord, Spitsbergen: the herbivore *Gammarellus homari* (0- to 5-m water depth), the strictly carnivore scavenger *Anonyx nugax* (2- to 5-m water depth) and the detritivore/carnivore *Onisimus edwardsi* (2- to 5-m water depth). In previous radiation exposure experiments, both carnivore species displayed elevated mortality rates already at moderate UVR levels. Therefore, the concentrations of sunscreening compounds (mycosporine-like amino acids, MAAs, and carotenoids) and two antioxidant enzymes (superoxide dismutase, catalase) were studied in the animals under control conditions and following moderate as well as high UVR exposure.

In both carnivore amphipods elevated sensitivity to experimental UVR exposure went along with a degradation of the tissue carotenoid and MAAs and a decrease of the enzymatic antioxidant defence, which resulted in increased lipid peroxidation in exposed animals. In contrast, the herbivore *G. homari* seems well protected by high concentrations of MAAs absorbed from its algal diet, and no oxidative stress occurred under experimental UVR. The species-specific degree of UV tolerance correlates well with the animals' typical vertical distribution in the water column.

Keywords: Amphipods; MAAs; Oxidative stress; Polar coastal ecosystems; UVB

1. Introduction

Arctic coastal ecosystems experience strong seasonal changes of day length and light climate. Underwater solar irradiation, absent throughout polar winters and rapidly increasing after sea ice break-up in spring, plays a fundamental role in water column processes (Williamson et al., 1994) and affects community structure and productivity of benthic macroalgal assemblages and associated animals (Wiencke et al., 2000; Hoyer, 2003).

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Northern high latitude shallow water ecosystems are currently threatened by a selective increase in ambient UVB radiation (280–320 nm) due to ozone depletion (Madronich et al., 1998). Although highly variable, Arctic total column ozone losses in winter/spring 1997–2001 amounted to 25% relative to 1980 means, peaking at 70% ozone reduction in 1999/2000 (Executive Summary of the UNEP/WMO, 2002). This corresponds to an estimated increase in erythermal irradiation of up to 40% at the earth's surface. Biologically relevant UVB doses reach subtidal shallow water depths in Northern mid- and high latitudes, and 1% depth of surface UVB was located at 9 m in Arctic coastal areas (Bischof et al., 1998). This is still low compared to offshore North Atlantic waters, where 10 times more surface UVB penetrates to the same depths (Wängberg et al., 1996; Bischof et al., 1998). For the Arctic Kongsfjord, Hanelt et al. (2001) showed that UV radiation was high enough under clear spring conditions to affect macroalgal primary productivity already in 5–6 m depth and, moreover, to cause DNA damage already in 1–3 m depth on summer days with low water transparency (measured with a biological dosimeter). UVB radiation can have direct deleterious effects in marine animals and damage biomolecules such as nucleic acids and proteins, which absorb in the UV-range. Physiological disorder and death in response to direct UVB exposure were reported for northern anchovy populations (Hunter et al., 1979), northern temperate zooplankton and ichthyoplankton (Browman et al., 2000), temperate Cladoceran *Daphnia* (Williamson et al., 2001), as well as for Patagonian crustaceans (Helbling et al., 2002).

UVB radiation further induces generation of reactive oxygen species (ROS) in surface waters. Here hydrogen peroxide (H_2O_2) forms and accumulates in temperate and polar regions as a consequence of DOC photo-activation (Abele-Oeschger et al., 1997a; Abele et al., 1999). Uncharged H_2O_2 passes soft body surfaces by diffusion and can cause depression of respiration and filtration rates in invertebrates. Further, H_2O_2 induces formation of highly reactive hydroxyl radicals ($OH^{\cdot}$) that induce oxidative damage within cells and tissues and induce lipid peroxidation chain reactions (Abele-Oeschger et al., 1997b; Abele et al., 1998; Abele and Puntarulo, 2004 for review). Under non-stressed conditions, well-developed antioxidant defence mechanisms scavenge ROS before critical concentrations build up and thus establish a balance between pro-oxidant and antioxidant processes. Among other enzymes, superoxide dismutase (SOD), converting $O_2^{\cdot}-$ to H_2O_2, and catalase (CAT), converting H_2O_2 to water, represent a strong enzymatic defence system (Boveris, 1998; Viarengo et al., 1998). Other small molecule ROS scavengers (vitamins C, E, β-carotene, glutathione and other redox active thiols) function as free-radical chain braking agents and singlet oxygen ($^1O_2$) quenchers. β-Carotene and other carotenoids have photo-protective functions by absorbing energy of excited reaction products of visible and UV-light (Halliwell and Gutteridge, 1999; Montenegro et al., 2002).

Further, sunscreening compounds such as mycosporine-like amino acids (MAAs) absorb UV-photons and are widely distributed among aquatic organisms (Karentz, 2001; McClintock and Karentz, 1997). They efficiently absorb UVR in the range between 309 and 360 nm and dissipate the energy thermally without showing fluorescence or generating oxygen radicals (Shick and Dunlap, 2002). In addition, mycosporine–glycine, the dominant MAA in various marine organisms, is also ascribed a moderate antioxidant potential (Dunlap and Yamamoto, 1995). Pigmentation is a foremost protection against harmful radiation, but as shown by melanin pigmented and transparent *Daphnia* coexisting in clear water lakes, does not uniquely render the animal UV-resistant (Borgeraas and Hessen, 2002). By its increasing effect on UVB surface radiation, ozone depletion has become a major environmental stressor, and UVB-induced effects are contributing synergistically with other environmental hazards (e.g., chemical contamination, rising temperature and CO_2 levels) to oxidative stress conditions and damage of key biomolecules in transparent marine organisms (Livingstone, 2001; Häder et al., 1995).

Amphipods are abundant and widely distributed crustaceans in Arctic and subarctic regions (Jazdzewski et al., 1995; Legezynska et al., 2000; Poltermann, 2001; Weslawski and Legezynska, 2002). Herbivorous amphipods are associated with the rich macroalgal communities (Hop et al., 2002). Carnivorous and omnivorous amphipods form an important food web link between small zooplankton and detritic material and the higher trophic levels (Falk-Petersen et al., 1988; Hop et al., 2002 and references therein).

Amphipod crustaceans are UV-transparent (Obermüller and Abele, 2004) and intertidal species are prone to suffer stress as the irradiation climate changes. Further, cell membranes of polar amphipods are rich in polyunsaturated fatty acids (PUFA) (Graeve et al., 1997; Nelson et al., 2001) as an adaptation to life in cold climates (Clarke et al., 1985; Storelli et al., 1998). These homeoviscous adaptations render the animals even more susceptible to UV-mediated oxidative damage and lipid peroxidation chain reactions (Abele and Puntarulo, 2004).

We investigated potentially damaging direct and ROS-mediated effects of UVB on three abundant amphipod species from shallow water depths of the Kongsfjord (Spitsbergen). Irradiation experiments were carried out simulating natural UVB doses, and concentrations of MAAs, as well as tissue antioxidant potential and lipid peroxidation were investigated. The study forms part of a project which compares amphipods with comparable lifestyle and food chain positioning from Arctic and Antarctic habitats. All experimental work was carried out under Arctic field conditions.

2. Material and methods

2.1. Animals

Three species of Gammarid amphipods occurring in the Arctic Kongsfjord were studied: the herbivore *Gammarellus homari* (Gammarellidae) as well as the carnivorous/necrophagous *Anonyx nugax* (Lysianassidae) and *Onisimus edwardsi* (Lysianassidae). *G. homari* was collected by divers with a handnet at 0- to 5-m water depth at various stations along the coastline of Kongsfjord with medium to dense macroalgal canopy (e.g., Nansen Bay, Hansneset, see Lippert, 2003). Adult *G. homari* were mainly associated with the red alga *Devaleraea ramentacea*, and could be found at the base of algal thalli. This habitat preference is reflected in the food spectrum of *G. homari*, which preferentially feeds on red and, to a minor extent, on brown seaweeds (H. Wessels, personal communication). Freshly collected specimens varied highly in colouration. Pigmentation was either patchy or more even on the carapace and ranged from light grey–green or beige–orange to darker red–brownish colour. Despite these differences, all amphipods were more intensely coloured on the dorsal compared to less exposed ventral side. *A. nugax* and *O. edwardsi* were collected at 2–5 m depth with baited traps at London, a sampling site on the southern side of the island Blomstrandhalvøya (central Kongsfjord), where macroalgae are restricted to single drop stones and boulders. All *A. nugax* specimens exhibited a similar and more uniform colouration in the range of milky yellow to light orange, the back being darker compared than the ventral side. The pigmentation of *O. edwardsi* was bright orange to light brown, more intense than in *A. nugax* but equally uniform throughout collected specimens and also darker on the dorsal side. *A. nugax* is considered to be strictly carnivore/necrophage and able to consume large quantities of bait efficiently in short times. Whereas *O. edwardsi* exhibits more generalistic feeding habits and is also believed to ingest detritus of plankton, macroalgal or other animal origin. Animals were immediately transferred to the aquarium and kept at 6–8 °C and 34 psu salinity for up to 3 days maximum prior to experimentation. Running seawater was steadily supplied directly from the fjord. Only adult amphipods were used in the experiments.

3. Radiation measurements and experimental set-up

Solar UVB radiation was measured continuously with a 32-channel single-photon counting spectroradiometer installed on the roof of the NDSC-building at Koldewey station. Underwater light climate (0–5 m) in the fjord was recorded on July 10 and July 13, 2001, with an underwater UVB-spectroradiometer according to Hanelt et al. (2001).

3.1. Experimentation

In two series of laboratory experiments, amphipods were exposed to a moderate and a high UVB dose. Experimental irradiation was carried out using white light and UV-tubes (Q-Panel, type UVA 340) for moderate UVB exposure and a sunshine simulator (SONSI), providing a solar-like spectrum (developed in the AWI Physics Department by H. Tüg and Fa. IsiTEC, Bremerhaven) for the high UVB dose.

3.1.1. Moderate UVB treatment

In each experimental set-up, 20–33 adult *G. homari* and *A. nugax* and 60–130 *O. edwardsi* were exposed to UV and visible radiation emitted by Q-Panel and white light tubes in small aquaria (2 l volume, 10 cm depth) for 5 h daily, over 20 days. Experiments were run in a constant temperature room at 6–8 °C in July–August. Irradiances were 0.4 W m^{-2} UVB, 3.7 W m^{-2} UVA and 5.7 W m^{-2} PAR (surface level), resulting in a dose of 1.44 kJ m^{-2} h^{-1} experimental daily dose of 7.2 kJ m^{-2} day^{-1}, which represents 40% of the average atmospheric daily UVB dose (17.8 kJ m^{-2} day^{-1}). Between each 5-h irradiation interval, the animals received dim light only (equivalent to control set-up, see below). Amphipods in our experiments close to surface-level experienced 100% of subsurface in-situ UVB dose if transmittance of 41% is assumed. As attenuation of UVB takes place already in the first 10 cm of the water column, those amphipods, which remained at the bottom of the aquaria throughout the exposure time received a reduced and thus lower than in-situ UVB dose. A 400-nm cut-off filter (400-nm cut-off, Folex PR, Folex, Dreieich, Germany) was used for the PAR only waveband.

3.1.2. High UVB treatment

In each experimental set-up, 20–33 adult *G. homari* and *A. nugax* and 60–130 *O. edwardsi* were exposed in the SONSI (5l sample chamber, 20 cm depth) for 4 h daily over 20 days. Experimental water temperature was controlled between 6.7 and 6.9 °C during the first series of experiments and between 7.8 and 8.0 °C for experiments started at the end of July 2001. This was necessary because the temperature in the fjord increased and therewith the temperature of maintenance during irradiation pauses and in control aquaria. Irradiance was 1.30 W m^{-2} UVB, 21.84 W m^{-2} UVA and 117.66 W m^{-2} PAR (surface level), resulting in a dose of 4.68 kJ m^{-2} h^{-1} UVB and an experimental daily dose of 18.72 kJ m^{-2} day^{-1}. Between each 4-h irradiation interval, the animals were maintained at dim light only (equivalent to control set-up, see below). This represents a 5.2% increase over the average atmospheric UVB dose in July 2001. Amphipods in the experiments close to surface level experienced a 1.5-fold increase (41% transmittance in 10–20 cm depth) over the average in-situ UVB dose between the surface and 1 m depth in Kongsfjord. In contrast, amphipods which remained at the bottom of the aquaria (20 cm) throughout the exposure time received a reduced and thus closer to in-situ UVB dose.

Control animals received dim light only. Herbivores were exposed to experimental irradiation without macroalgae to avoid shading effects. One group of herbivore amphipods received algal food between irradiations, while the other group was not fed. Food consisted in a mixture of MAA-containing *D. ramentacea*, and MAA-free red macroalgae (*Odonthalia dentata*, *Coccolythus truncatus*) (Karsten et al., 1998). Similarly, one group of each carnivore species (*A. nugax, O. edwardsi*) was fed little pieces of fish, while the other group was starved throughout 20 days of experimental duration. Dead animals were removed and counted daily for mortality records.

In both experimental UVB treatments (low and high dose), subsamples of surviving animals were taken after 7, 10, 12, 14 and 20 days and deep frozen in liquid nitrogen for screening of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), lipid peroxidation status measured as content of TBARS (thiobarbituric reactive substances), carotenoid content, and content and composition of mycosporine-like amino acids (MAAs).

Superoxide dismutase (SOD) activity in homogenates (1:5–1:8; w/v) of 1–4 pooled whole body amphipods, which had been deep-frozen in liquid nitrogen was measured according to Livingstone et al. (1992). 1 ml reaction volume contained 43 mM KH_2PO_4/K_2HPO_4 buffer with 0.1 mM EDTA, pH 7.8, 5.0 mM xanthine, 100 μM cytochrome *c*, and 1.8 mU xanthine oxidase. Assay temperature was 20 °C. Data were normalised to tissue fresh mass and expressed as (U mg^{-1} FM). 1 U (unit) SOD reduces the reduction rate of oxidised cytochrome *c* by 50%.

Catalase (CAT) activity in homogenates (1:5 w/v) of 1–4 pooled whole body amphipods, which had been deep-frozen in liquid nitrogen was measured after Aebi (1985). 1 ml reaction volume contained 50 mM KH_2PO_4/Na_2HPO_4 buffer, pH 7.00, and 10.5 mM H_2O_2. CAT activity was calculated from the turnover time of hydrogen peroxide H_2O_2 resulting in an absorbance decrease at 240 nm. Assay temperature was 20 °C. Data were normalised to tissue fresh mass (U mg-1 FM). 1 U CAT consumes

1.150 μM H_2O_2 min^{-1}, starting at an initial concentration of 10.5 mM resulting in an absorbance decrease by 0.05 E.

Determination of TBARS (thiobarbituric reactive substances) concentration in amphipod tissues was carried out according to Uchiyama and Mihara (1978). 1–4 deep-frozen whole body amphipods were homogenised in liquid nitrogen and diluted with 0.2% H_3PO_4 (1:6–1:10 (w/v)). Subsequently the same volume of 2.0% H_3PO_4 was added resulting in a final H_3PO_4 concentration of 1.1%. Subsamples (0.4 ml) of tissue homogenate were treated with 0.4 ml TBA solution (sample) or 3 mM HCl (blank) and adjusted to pH 1.6. The method was modified with respect to heating time of the samples, which was 1 h at 100 °C. After cooling, 1.5 ml *n*-butanol was added, samples and blanks vortexed and centrifuged successively at 1000 and 14,000×*g*, and the absorbance measured in the supernatants at 532 and 600 nm. TBARS concentration was quantified using the molar extinction coefficient (ε: 156 l $mmol^{-1}$ cm^{-1}). Concentration is expressed as μmol TBARS g^{-1} fresh mass (FM).

3.1.3. Carotenoid concentration

The butanolic supernatant from the TBARS measurements was scanned photometrically between 400 and 500 nm to measure carotenoid content. The carotenoid absorption spectrum was identified by comparison with different concentrations of β-carotene standard (Sigma, MW: 536.88 g) in *n*-butanol (Jeffrey, 1997). Carotenoid concentration was calculated from a 4-point calibration curve of β-carotene in butanol at 455 nm (ε 138×10^3 l $mmol^{-1}$ cm^{-1}). Concentration is expressed as μmol β-carotene equivalents g^{-1} fresh mass (FM).

Extraction and analysis of mycosporine-like amino acids (MAAs) was carried out according to Karsten and Garcia-Pichel (1996) and Newman et al. (2000) with the following modifications: pooled samples of 10- to 40-mg whole animal dry weight from freeze-dried samples were homogenised and extracted twice into 1 ml 100% methanol for 1.5 h at 45 °C. Samples were centrifuged at 10,000×*g* for 5 min and supernatants combined. Pooled extracts were evaporated to dryness under vacuum and re-dissolved in 500 μl 25% aqueous methanol (v/v). Extracts were then passed through a Strata™ C_8-SPE cartridge (Phenomenex) to remove interfering lipids and subsequently mycosporines eluated with 2 ml of 5% aqueous methanol (v/v). Extracts were again evaporated to dryness, taken up in 500 μl 25% aqueous methanol (v/v) and analysed with an Agilent high-performance liquid chromatography system (HPLC) modified after Karsten et al. (1998). MAAs were separated on a stainless-steel Phenomenex Sphereclone RP-8 column (5 μm, 250 × 4 mm I.D.). The mobile phase consisted of 2.5% methanol (v/v) and 0.1% acetonitrile (v/v) in water run isocratically at a flow rate of 0.7 ml min^{-1}. MAAs were detected online with a photodiode array detector at 330 nm, and absorption spectra (290–400 nm) were recorded each second directly of the HPLC-separated peaks. Identification was done by spectra, retention time (RT) and by co-chromatography with standards, extracted from the marine red macroalgae *Chondrus crispus* Stackhouse (Karsten et al., 1998) and *Porphyra umbilicalis* (L.) Kützing, the lichen *Lichina* spec., as well as from ocular lenses of the coral trout *Plectropomus leopardus* Lacepède, kindly provided by Dr. David Bellwood, James Cook University, Townsville, Australia. Molar extinction coefficients were obtained from Karsten et al. (1998). Concentrations are expressed as μg ml^{-1} and μg g^{-1} DM (dry mass).

3.1.4. Statistics

Effects of irradiation duration on UV–stress parameters were tested for statistical significance using a Student's *t*-test. Significance level was ($p < 0.05$).

4. Results

4.1. Radiation climate

In July and August 2001, maximal atmospheric UVB ranged between 0.8 and 1.2 W m^{-2}. The average atmospheric daily UVB dose amounted to 17.8 kJ m^{-2} day^{-1} in July 2001 (29.9 kJ m^{-2} day^{-1} peak and 9.0 kJ m^{-2} day^{-1} minimum dose) and to 9.2 kJ m^{-2} day^{-1} in August 2001 (19.7 kJ m^{-2} day^{-1} maximal and 3.0 kJ m^{-2} day^{-1} minimal dose).

UVB at the surface and transmission into the water column were recorded on 2 sunny days without cloud cover. Surface irradiances were 0.49 W m^{-2} on July 10, and 0.56 W m^{-2} on July 13 and decreased to 0.07

and 0.11 W m^{-2} in 1 m depth. K_d was 0.97 m^{-1} on July 10 and 0.79 m^{-1} on July 13. The fjord water allowed transmission of 41% of atmospheric UVB in the subsurface layer (10–20 cm) and 15.2 and 19.3% in 1 m depth. The 1% depth for surface UVB was around 4 m on both days.

4.2. Comparison between fed and non-fed animals

Since no significant differences were detected between all fed and starved control and UV-irradiated animals in any parameter the results were pooled.

4.3. UV-induced mortality

In this study, survival of herbivorous *G. homari*, carnivorous *A. nugax* and the detritivore/carnivore *O. edwardsi* from the Arctic Kongsfjord during high-dose exposure experiments is presented as new data set, whereas (only) survival data from low-dose irradiation experiments were published earlier (Obermüller and Abele, 2004). Both experiments were run during the same time, and animals did not differ with respect to developmental or reproductive state.

Like low-dose treatment, high-dose experimental UVB exposure did not affect survival of *G. homari* during 20 irradiation days. In contrast, mortality from high-dose experimental UVB irradiation in *A. nugax* was 22% after 12 days and was therefore lower than the 81% under low-dose UVB exposure. Mortality in *O. edwardsi* under high-dose UVB exposure was 39% after 12 days and also significantly lower than under low-dose exposure (90% after 12 days, Obermüller and Abele, 2004).

4.4. Activities of antioxidant enzymes (AOX) and content of TBARS

4.4.1. Gammarellus homari (herbivore)

Animals maintained under laboratory conditions for 1–3 days before starting the irradiation experiments had high and highly variable CAT (Fig. 1b), but low SOD (Fig. 1a) activities with little individual variability. Whereas CAT activity decreased to stable and very low values, SOD activity rose significantly during 20 days of maintenance under control conditions. Different irradiation treatments had no effect on *G. homari* catalase, but SOD activities were significantly elevated over controls following 10 days of high UVB+UVA+PAR irradiation. Prolonged exposure to low-dose UVB+UVA+PAR over 20 days resulted in a further increase of SOD activities (significant over control level after 10–14 days), however, 20 days irradiated SOD activities were not significantly different from animals under control conditions. TBARS, as marker for lipid oxidative damage, increased mildly ($p=0.048$) during 10 days of maintenance under control conditions but were back to initial levels after 20 days (Fig. 1c). No alteration of TBARS was found when animals were exposed to low-dose UVR (UVB+UVA+PAR), but a significant increase occurred when amphipods were treated with high-dose UVR. However, no statistical comparison was possible due to only two replicate samples.

4.4.2. Anonyx nugax (carnivore)

Enzyme activities under control conditions were two (SOD, Fig. 2a) and three times (CAT, Fig. 2b) higher than in *G. homari*. Animals maintained shortly ($\leq$ 3 days) under laboratory conditions (start value) again had highly variable CAT activity, whereas SOD activity was less variable between individuals. Under control conditions, CAT activity was not affected, whereas SOD activity rose significantly during 12 days of maintenance. Exposure to experimental high-dose irradiation had no significant effect on either SOD or CAT activities in *A. nugax*. Only under low-dose UVB exposure, we found significantly lower SOD activity after 7 days and also significantly reduced CAT activity after 10–14 days compared to the respective control groups. TBARS concentrations (Fig. 2c) were again very variable in animals measured on start day and far more homogenous after 12 days of control maintenance. Exposure to high and low irradiation doses provoked no clear change of TBARS after 7 days. Twelve days of low-level UVB radiation resulted in higher TBARS concentrations compared to controls ($p=0.215$), while animals exposed to high doses had significantly reduced TBARS levels.

4.4.3. Onisimus edwardsi (detritivore/carnivore)

While SOD activities (Fig. 3a) were in the range of those from the larger scavenger *A. nugax*, CAT (Fig.

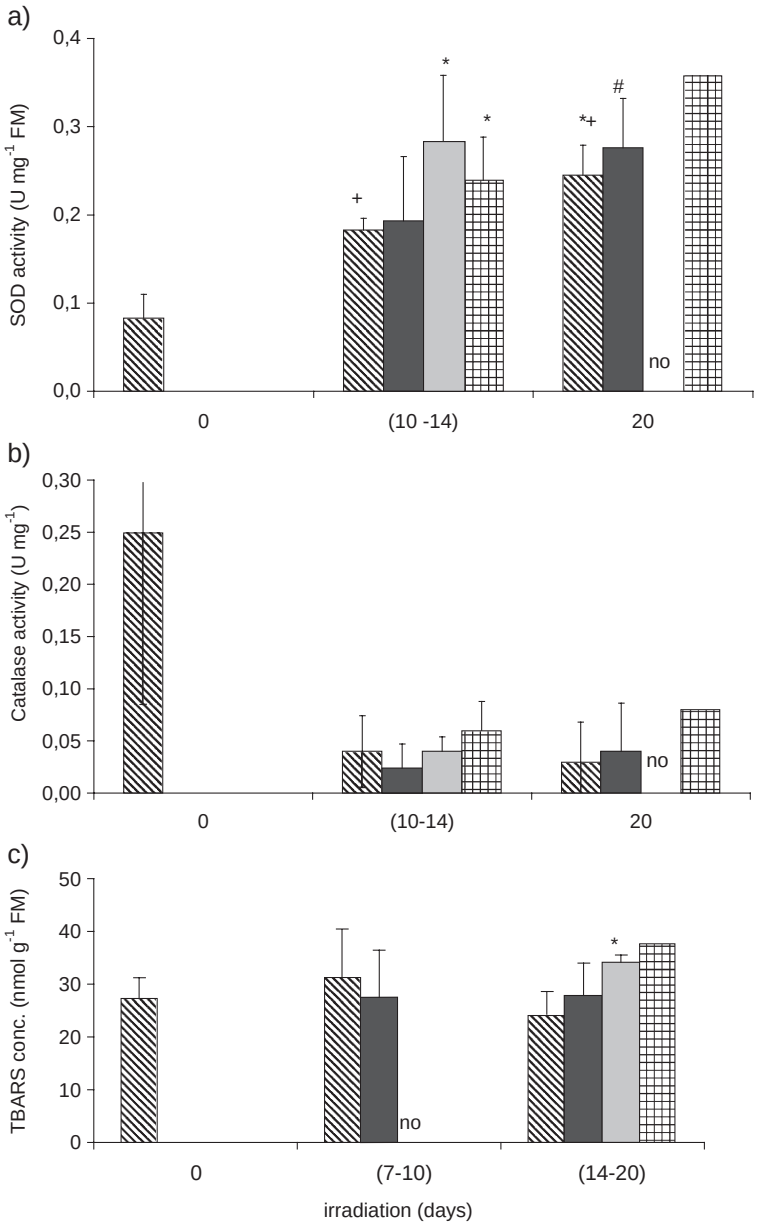

Fig. 1. *Gammarellus homari*: (a) SOD activity, (b) CAT activity, (c) TBARS concentration. Values are means ± SD, $n = 4$–10. Striped—control; black—UVB + UVA + PAR low dose (ld); grey—UVA + PAR low dose; checked—UVB + UVA + PAR high dose (hd), no—no data. Symbols of significance: *significantly different from controls on the same date, +significantly different from 0 controls, #significantly different from 10-day UVB (ld).

3b) activities were three times lower and comparable to values found in *G. homari*. As in the other species, CAT activity was very variable in animals maintained shortly (< 3 days) under laboratory conditions prior experimentation. Contrasting the other species, CAT activities in *O. edwardsi* remained highly variable through all treatments. SOD activities were less variable between control individuals and rose significantly

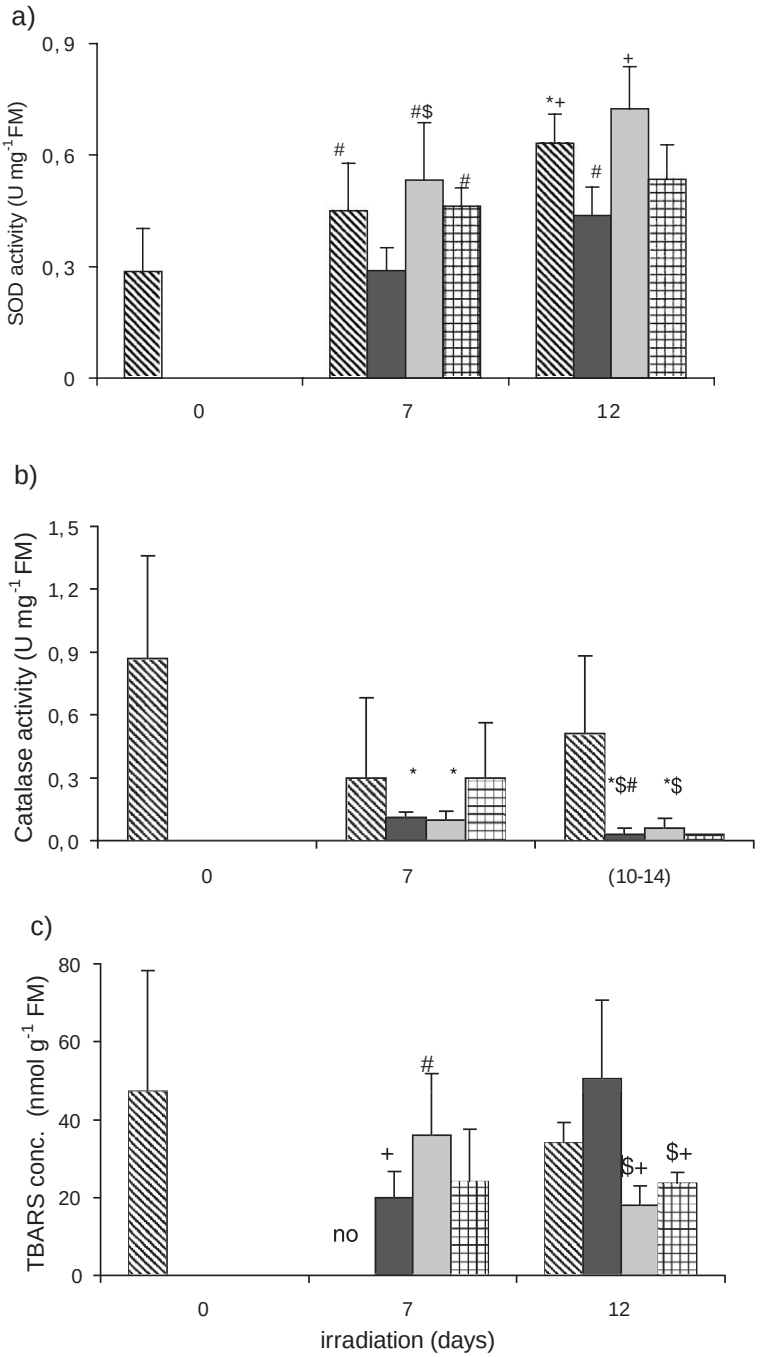

Fig. 2. *Anonyx nugax*: (a) SOD activity, (b) CAT activity, (c) TBARS concentration. Values are means ± SD, $n = 5–10$. Striped—control; black—UVB+UVA+PAR low dose (ld); grey—UVA+PAR low dose; checked—UVB+UVA+PAR high dose (hd), no—no data. Symbols of significance: *significantly different from 0-day controls, $significantly different from controls on the same date, #significantly different from 7-day UVB (ld), +significantly different from 12-day UVB (ld).

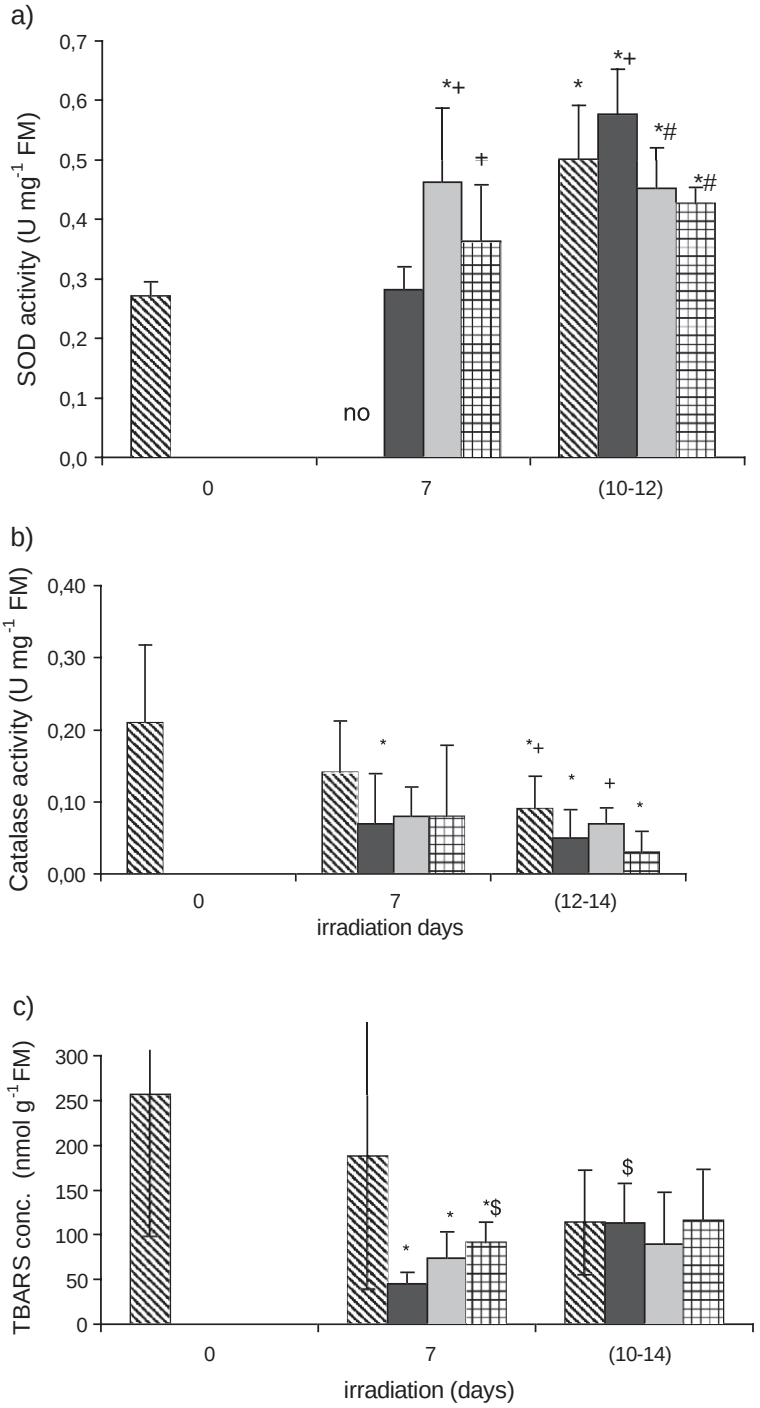

Fig. 3. *Onisimus edwardsi*: (a) SOD activity, (b) CAT activity, (c) TBARS concentration. Values are means ± SD, $n = 3–10$. Striped—control; black—UVB + UVA + PAR low dose (ld); grey—UVA + PAR low dose; checked—UVB + UVA + PAR high dose (hd), no—no data. Symbols of significance: *significantly different from 0-day controls, +significantly different from 7-day UVB (ld), #significantly different from 12-day UVB (ld), $significantly different from 12-day high-dose UVB (hd).

during 12 days of maintenance. In contrast CAT activity decreased significantly in control animals over 12 days. There was no significant effect of low- or high-UVR on antioxidant enzyme activities in *O. edwardsi*, except for a significant reduction in CAT activities after 12–14 days of high-dose UVB exposure. TBARS concentrations (Fig. 3c) in *O. edwardsi* measured on the start day were about 5 times higher than in the two other species and highly variable between individuals. TBARS remained high and variable during 14 days of maintenance under control conditions. In no irradiation treatment, TBARS concentrations increased over controls. After 1 week, TBARS in all irradiation treatments were significantly reduced compared to control values on start day, but only insignificantly compared to controls on day 7 due to high individual variance. After 2 weeks, TBARS were roughly the same in all groups.

4.5. Concentrations of carotenoid and Mycosporine-like Amino Acids (MAAs)

Carotenoid contents in each species and treatment are given in Table 1. The herbivore amphipod maintained carotenoid concentrations in controls as well as in moderately irradiated specimens on a constant level. No samples were taken for MAA analyses from high-dose irradiation experiments with *G. homari*. In *A. nugax*, carotenoid levels decreased within the first 7 days in controls and UVB-irradiated animals to about half the start value and did not change significantly thereafter. However, only 4 out of 6 irradiated animals were strongly bleached after 1 week high- and low-dose irradiation so there was no statistical significance. In the carnivore/detritivore *O. edwardsi*, the carotenoid content increased (insignificantly) during the first 7 days under control conditions, while it decreased mildly but significant under low-dose UV-irradiation if compared to controls. After 2 weeks of experimentation, carotenoid contents in all treatments were back to initial and control levels.

Four different MAAs, mycosporine–glycine, porphyra-334 (P-334), palythine, and shinorine, were detected in all three amphipod species in more than 60% of investigated specimens. Asterina-330 was present in *G. homari* and *O. edwardsi* in more than 60% of all investigated animals, but only two specimens of *A. nugax* showed small amounts of this MAA. Further, an unknown substance with an absorption maximum (λ_{max}) at 332 nm (unkn-332) and a retention time (RT) of 3.73–3.89 min was detected in all three species. Another unknown substance with λ_{max} 308–310 nm (unkn-310) at RT of 2.81–2.88 min was only present in *O. edwardsi*.

Table 1
Total carotenoid concentrations (μmol β-carotene equivalents g^{-1} FM) in amphipods from Kongsfjord (Spitsbergen) exposed to low-dose and high-dose irradiation treatment in July 2001

Gammarellus homari			
Irradiation (days)	0 d	7–10 d	14–20 d
Control	0.092 ± 0.027 (4)	0.114 ± 0.019 (8)*	0.081 ± 0.018 (12)
UVB + UVA + PAR low dose		0.095 ± 0.020 (5)	0.077 ± 0.026 (3)
Anonyx nugax			
Irradiation (days)	0 d	7 d	12 d
Control	0.069 ± 0.030 (3)	–	0.051 ± 0.028 (4)
UVB + UVA + PAR low dose		0.033 ± 0.006 (6)	0.045 ± 0.008 (3)
UVB + UVA + PAR high dose		0.032 ± 0.011 (6)	0.021 ± 0.026 (4)
Onisimus edwardsi			
Irradiation (days)	0 d	7 d	(10–14) d
Control	0.069 ± 0.017 (6)	0.097 ± 0.020 (3)	0.081 ± 0.022 (6)
UVB + UVA + PAR low dose		0.051 ± 0.007 (4) #	0.076 ± 0.007 (8)
UVB + UVA + PAR high dose		0.063 ± 0.008 (5)	0.078 ± 0.009 (3)

Values are means ± SD, with numbers in brackets indicating replicates per value. *Significantly different from 14- to 20-day controls; #significantly different from 7-day controls, UVB + UVA + PAR high dose (7 days), UVB + UVA + PAR low dose (10–14 days), d—duration of irradiation in days.

Table 2
MAA concentrations (μg g^{-1} dry mass) in amphipods from the Kongsfjord (Spitsbergen)

Species	Control (7 d)	Control (14 d)	Low-dose UVB (14 d)	High-dose UVB (14 d)
Gammarellus homari	761 ± 467 (4)	594 ± 240 (4)	831 ± 305 (6)	836 ± 338 (4)
Anonyx nugax	30 ± 23 (3)	89 ± 54 (8)	76 (2)	39 (2)
Onisimus edwardsi	138+30 (4)	93+31 (4)	70+32 (7)	55 ± 62 (3)

Values are means ± SD, with numbers in brackets indicating the replicates per value, d—duration of irradiation in days.

The red alga *D. ramentacea*, which was used as food, contained the following rank order of major MAAs: Palythine, P-334, mycosporine–glycine, asterina-330 and shinorine, resulting in a mean total MAA content of 641.09 ± 201.75 μg g^{-1} dry mass (n = 7). Table 2 gives MAA concentrations after 7 and 14 days of control maintenance, as well as under 14 days of low and high UVB treatment. MAA content in *G. homari* controls, fed and maintained under dim light conditions, did not change significantly during 2 weeks of maintenance. Overall MAA content was not affected by either high- or low-dose UVB exposure during 14 days. Fig. 4 shows stacked mean concentrations for individual, clearly identified MAAs for each radiation treatment (note: column sum does not equal MAA concentration in Table 2). Exposure to mild UVB irradiation led to a significant reduction in the concentrations of the major MAA components (P-334 and mycosporine–glycine) and also of shinorine when compared to control animals after 20 days of irradiation. Exposure to a high UVB dose did not result in a significant difference of MAA composition compared to controls. High individual variability of the unknown substance unkn-332 from 31.91 to 117.12 μg g^{-1} DM was found with no significant trend.

Both scavenging amphipod species had far lower total MAA concentrations compared to the herbivore *G. homari* (Table 2). MAA content and distribution in the strictly carnivore *A. nugax* was highly variable between individuals and consequently between treatments and also within control groups (Fig. 5). Inspite of the low overall MAA content in the carnivore (Table 2), significant differences of individual MAAs emerged between treatments. Thus, P-334 was significantly reduced during 7 days of low-dose UVB + UVA + PAR exposure when compared with control animals. Only one out of each two specimens exposed to low-dose UVB and high-dose UVB respectively had some P-334 left. Shinorine and

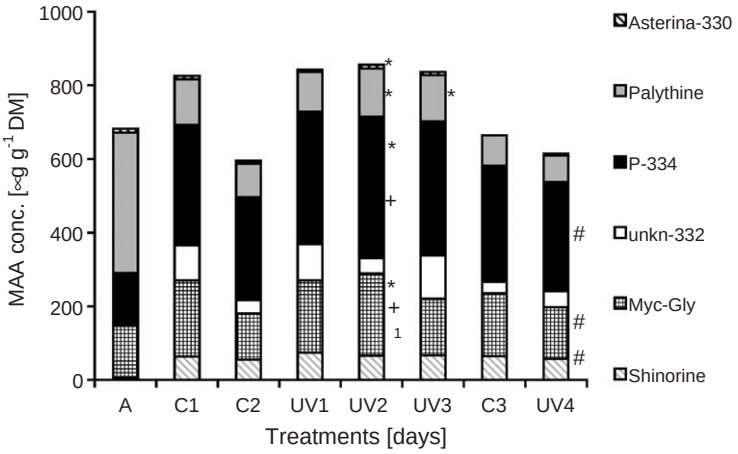

Fig. 4. *Gammarellus homari*: Variation of individual MAA content during low- and high-dose UV treatment and control maintenance over 20 days. Values are means ± SD given as stacked columns. C1—control at (0–7) days, C2—control at (8–14) days, UV1—UVB + UVA + PAR low dose at (8–14) days, UV2—UVA + PAR low dose at (8–14) days, UV3—UVB + UVA + PAR high dose at (8–14) days, C3—control at (20) days, UV4—UVB + UVA + PAR low dose at (20) days. *Indicates significant difference from UV1, +indicates significant difference from C2, #indicates significant difference from C3. Myc-Gly—mycosporine–glycine.

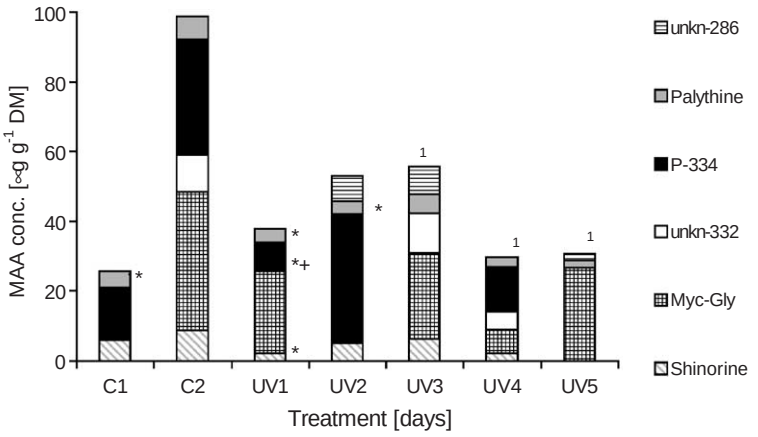

Fig. 5. *Anonyx nugax*: Variation of individual MAA content during low- and high-dose UV treatment and control maintenance over 14 days. Values are means ± SD given as stacked columns. C1—control at (0) days, C2—control at (7–14) days, UV1—UVB+UVA+PAR low dose at (7) days, UV2—UVA+PAR low dose at (7) days, UV3—UVB+UVA+PAR low dose at (12) days, UV4—UVA+PAR low dose at (14) days UV5—UVB+UVA+PAR high dose at (12) days. *Indicates significant difference from C2, +indicates significant difference from UV2,[1] only 2 replicate samples. Myc-Gly—mycosporine–glycine.

palythine were also significantly lower in low-dose UVB treated amphipods than in controls.

The MAA composition in control *O. edwardsi* resembled those in algae. Overall MAA concentration in *O. edwardsi* decreased rapidly within the first week of UVR exposure (significantly under low dose) and further on, however insignificantly, during the second week in irradiated animals compared to non-irradiated controls (Fig. 6). P-334 and shinorine decreased significantly over 7 days of low-dose UVA exposure, whereas mycosporine–glycine was only significantly reduced when a high UVB dose was applied or the low UVB dose persisted over 14 days. Interestingly, mycosporine–glycine was constant in con-

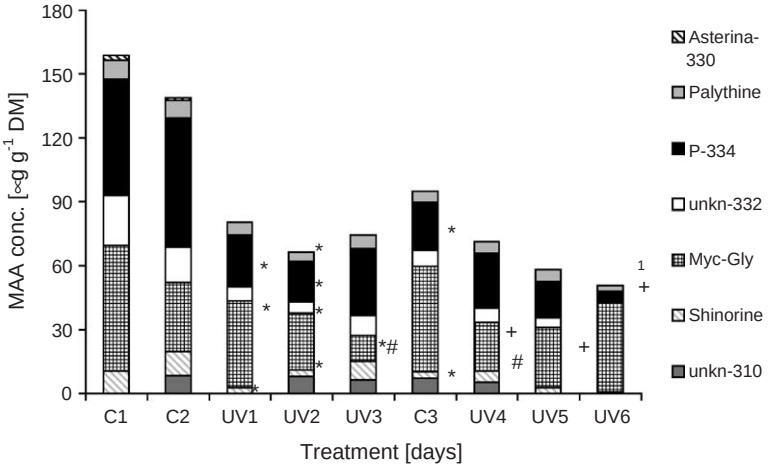

Fig. 6. *Onisimus edwardsi*: Variation of individual MAA content during low- and high-dose UV treatment and control maintenance over 14 days. Values are means ± SD given as stacked columns. C1—control at (0) days, C2—control at (7) days, UV1—UVB+UVA+PAR low dose at (7) days, UV2—UVA+PAR low dose at (7) days, UV3—UVB+UVA+PAR high dose at (7) days, C3—control at (14) days, UV4—UVB+UVA+PAR low dose at (14) days, UV5—UVA+PAR low dose at (14) days, UV6—UVB+UVA+PAR high dose at (14) days. *Indicates significant difference from UV1, +indicates significant difference from UV5, #indicates significant difference from C3. Myc-Gly—mycosporine–glycine.

trols throughout the experiment, whereas shinorine and P-334 were significantly lower after 14 days of maintenance.

5. Discussion

5.1. Radiation conditions

The experiments were carried out during a typical Kongsfjord late summer situation. The mean atmospheric daily UVB dose (17.8 kJ m^{-2} day^{-1} in July 2001) during our experimental period was slightly lower than under spring (19.1 kJm^{-1} day^{-1}, May 2001) and early summer conditions (26.5 kJm^{-1} day^{-1}, June 2001). Additionally, in the warmer month of July, glacial melt water run-off causes elevated sediment discharge into surface waters and, therewith, a considerable shading effect. In July 2001, surface waters at the sampling site were already very turbid and transmittance was low. Comparing our K_d values (0.8–0.97 m^{-1}) with values from other studies demonstrates that underwater radiation conditions were among the lowest also of the preceding years. Before the onset of glacial melting in June 1997, transmittance was significantly higher and surface UVB was attenuated by 22% in 1 m depth (Hanelt et al., 2001). K_d values were 0.42 m^{-1} on June 15, 1997, and increased to 0.68–0.75 m^{-1} in July 1997. Minimal transmittance on July 16, 1998, resulted in a K_d of 1.34 m^{-1} and a 1% depth at 3.4 m (Bischof et al., 1998). For our experimental irradiations, a set-up was chosen, which was calibrated to match the situation in the environment at the time when the experiments were run. Hence, the animals received more than the incident daily environmental dose, but less than the dose they had supposedly experienced earlier in spring.

5.2. Amphipod mortality

In an earlier paper, we reported significantly accelerated mortality under moderate experimental UVB exposure (7.2 kJ m^{-2} day^{-1}) in the carnivore amphipod *A. nugax* compared to the herbivore *G. homari*. This was in line with higher carapace UV-transparency in *A. nugax* (41%) compared to a slightly lower transparency of 37% for UVA and UVB in *G. homari* (Obermüller and Abele, 2004). The present study adds the mortality data under high-dose experimental UVB exposure (18.7 kJ m^{-2} day^{-1}), showing that also stronger UVB exposure did not affect survival in *G. homari* amphipods. On the other hand, mortality was accelerated in *A. nugax* and *O. edwardsi*, however not as severe as under the low UVB-conditions. This raises two questions, (a) what exactly leads to a higher UVB susceptibility and accelerated mortality in not exclusively herbivore amphipods, and (b) why is low dose more detrimental than a high-dose irradiation in these animals? One possible explanation is related to an unfavourable waveband ratio between damaging UVB and UVA+PAR (photoreactivating radiation) from two Q-Panel lamps and only one white light tube in our low-dose experimental set-up. In a study especially designed to monitor photorepair, Williamson et al. (2001) used a separate UVB source together with two white light and two Q-Panel UVA tubes, emitting a total of 89 kJ m^{-2} of photoreactivating radiation (PRR) in the UVA range (320–400 nm). Although the ratio of UVB:UVA in this experimental set-up was far lower (1: 3) than in the solar spectrum, photoenzymatic repair of DNA damage in *Daphnia* was substantial already with only two PRR lamps (1 white light, 1 Q-Panel) and was fully saturated with four PRR lamps (2 white light, 2 Q-Panel). In comparison, we applied 66.6 kJ m^{-2} in the UVA range (320–400 nm) and were thus within the PRR range defined by Williamson et al. (2001), so that photoenzymatic repair should already be active. However, the applied PRR was obviously not saturating for damage repair in the considerably larger amphipods in our study, and survival was severely compromised at least in the carnivore species. The UVB:UVA:PAR ratio in our low-dose experimental set-up was 1:10:15 and differed largely from atmospheric measurements in the Kongsfjord during summer, where it was 1:17:257 (Bischof et al., 1998). By contrast, in the high-dose treatment, the ratio was 1:17:90 and obviously allowed for nearly saturating photorepair in the amphipods.

As the small difference in carapace transparency (38% UVB-transmission in *G. homari* versus 41% UVB-transmission in *A. nugax*, Obermüller and Abele, 2004) seemed unlikely to produce so much more UV protection in the herbivore, we hypothesized that there should be a complementing protective effect

from sunscreening pigmentation in *G. homari*. Indeed, high MAA concentrations were present in the Arctic herbivore (Table 2), when compared to both carnivore/detritivore species, and also carotenoid concentrations were 30% higher in herbivore than carnivore control animals (Table 1). High MAA concentrations seem characteristic of herbivore crustaceans and equally high amounts were observed in Antarctic herbivore amphipods (Obermüller et al., 2003) and Antarctic krill (Karentz et al., 1991). Also carotenoid levels were comparable to other crustaceans (krill: Yamaguchi et al., 1983, shrimps: Negre-Sadargues et al., 2000). The carnivorous/detritivore amphipods had only 20% of herbivore MAA concentrations and were comparable to the large Antarctic carnivore *Bovallia gigantea* (Karentz et al., 1991).

Loss of tissue carotenoids, irrespective of the applied UVB dose (high and low), was found in *A. nugax*, whereas in *O. edwardsi*, only mild bleaching of carotenoids (low-dose treatment) and MAAs (both treatments) was observed. Only the herbivore *G. homari* kept both types of sunscreens high and constant throughout the entire experimental irradiation. MAAs and carotenoids endow the amphipods with physical sunscreening protection against direct UV insult (Shick and Dunlap, 2002; Roy, 2000). Moreover, carotenoids are strong antioxidants and also active quenchers of singlet molecular oxygen (Montenegro et al., 2002). They absorb excited energy of singlet oxygen or the radical electron of ROS (reactive oxygen species) onto the carotenoid chain, a process leading to the degradation of the carotenoid, but preventing other molecules from being damaged. Thereby they confer radical chain-braking antioxidant activity to lipid rich membranes and tissues. As carotenoids become depleted, radiation penetrates into deeper layers of a tissue, propagating formation of detrimental ROS, now unbalanced by the carotenoid antioxidant effect. Thus, a lower basal sunscreening protection and the UV-driven bleaching of carotenoids in the carnivore amphipods can, at least in part, effect the higher mortality rates observed under high and especially under low-dose UV exposure. In contrast, a better and UV-resistant pigmentation yields a powerful UV-shield and supports zero mortality in the herbivore *G. homari*.

Highest absolute TBARS levels were observed in *O. edwardsi* (260 nmol TBARS g^{-1}), with TBARS in *G. homari* and *A. nugax* controls reaching only 10% and 20% of the values measured in the small detritivore. Irradiation treatments did not exacerbate *O. edwardsi* lipid peroxidation rates (unaltered TBARS levels), but caused a significant decline of CAT and a concomitant increase of SOD activity. As CAT activities were even more suppressed under high- than under low-dose UVB exposure, they can only in part be held responsible for the extremely elevated UVB induced mortality (90%) at low-dose UVB exposure over 12 days. A clearer picture was obtained in the strictly carnivore, *A. nugax*, where high mortality (81%) in low-dose irradiated animals went along with increased TBARS tissue concentration and a combined reduction of both SOD and CAT activities compared to controls. In contrast, high UVB doses may have supported survival of *A. nugax* by stimulating SOD and CAT activity over 7 days (but not over 2 weeks).

Again, the strictly herbivore *G. homari* seems well protected against the effects of irradiation by its carapace and the intense tissue pigmentation. The animals display only low CAT activities and neither of both antioxidant enzymes responded to experimental UVB exposure. With low and only mildly increasing TBARS under UV treatment, the herbivore species seems well protected against the potential threat of oxidative stress arising under maximal natural UVB exposure and, thus, highly adapted to survive periods of extreme natural radiation in the Arctic.

5.3. MAA composition

MAA composition in *G. homari* and in *O. edwardsi* clearly reflects the pattern in the red alga *D. ramentacea* used as food for the herbivore species (Figs. 4 and 6). The three most abundant algal MAAs were also the main components in the amphipods, demonstrating non-selective MAA uptake by *G. homari* and by *O. edwardsi*. The latter obviously consumed macroalgal detritus as part of its natural diet. None of the individual MAAs were selectively reduced during maintenance under dim light control conditions and UVB exposure in both taxa. *A. nugax* was the only species in which we observed an effect of UVB exposure, leading to a decrease in P334 content within 12 days (Fig. 6). In contrast, mycosporine–glycine was relatively better conserved in

UVB treated groups. This indicates that UVR does not induce elevated and selective uptake of MAAs in the investigated Arctic amphipods. However, in *A. nugax* MAA levels, especially P-334, as well as tissue carotenoids became depleted already during low UVB exposure, a process which might be causal for the high UVB induced mortality observed in this species.

In addition to the spectrum of algal MAAs, all amphipods carried unidentified components, which were absent in algae and could therefore be metabolic derivatives of original algal sunscreens.

5.4. Antioxidative enzymes and TBARS

CAT appeared more susceptible to photoinactivation in the two carnivore species than SOD. Indeed the enzyme was shown to be highly susceptible to photodamage from direct absorption of radiation with a prominent maximum at 405 nm, suggesting inactivation by light absorption in the heme-groups (Gantchev and van Lier, 1995; Grotjohann et al., 1997). UVA, rather than UVB, seems to be the active spectral range for this phenomenon (Shindo and Hashimoto, 1997; Zigman et al., 1998). Likewise, oxidative mechanisms involving membrane peroxidation and singlet oxygen formation are involved in CAT photoinactivation in human and animal lenses and play a role in lens opacification (Zigman et al., 1998). Interestingly, in human skin two medical doses (MED) of UVR caused gradual decrease in CAT activity and expression in the epidermis until 48 h following radiation, whereas after 72-h CAT activity was back to control levels (Rhie et al., 2001). In contrast the authors found that chronic exposure to elevated UVR increased CAT activity in human skin. Among other small molecular antioxidants, carotenoids have been shown to confer photoprotection to human skin. Therefore, it is reasonable to assume that carotenoid bleaching in *A. nugax* and in *O. edwardsi* (only under low-dose UVB) may have stimulated CAT photoinactivation under UVB exposure.

Initial analyses were carried out with animals after capture, which had been maintained between 1 and 3 days under laboratory conditions. These animals had high and variable CAT activities and TBARS concentrations, whereas SOD activities were low and increased significantly in all 3 species during control maintenance under dim light. In contrast, CAT activities were reduced and TBARS levels unaffected during control maintenance.

High inter-individual variability of in-situ enzyme activities or after only short maintenance under laboratory conditions have already been reported in other studies. Elevated CAT activities in situ were observed in the Arctic pteropode *Margarites helicinus* and declined to significantly lower levels within 11 days of maintenance (Philipp, 2000). We found high and extremely variable TBARS (then termed MDA) concentrations in the Antarctic mud clam *Yoldia eightsi*, which levelled off after 2 days of maintenance under control conditions in an aquarium (Abele et al., 2001). Highly variable in-situ values of malate dehydrogenase were observed in the oyster, *Crassostrea virginica* (P. Ulrich, A. Marsh, personal communication) and this heterogeneity was interpreted as a reflection of the eco-physiological capacity of a population to cope with fluctuations of environmental variables. Indeed, laboratory-reared herbivore *Gammarus locusta* displayed very low inter-individual variation of biochemical parameters, including antioxidant enzymes, in response to very constant laboratory conditions (Correira et al., 2003).

5.5. Conclusive remarks

Elevated mortality rates especially of fed specimens of *A. nugax* under experimental UVB exposure (Obermüller and Abele, 2004) document accelerated susceptibility of the carnivore to UVR. Carotenoid bleaching represents the clearest indication of irradiation induced damage and was accompanied by a diminishment of the antioxidant defense, including CAT photoinhibition, and increased lipid oxidative damage (TBARS) in the present study. *A. nugax* has relatively high total lipid content of 16% of body dry mass, whereas lipid content in the herbivore *G. homari* ranged as low as 6% body dry mass (H. Wessels, personal communication). High lipid stores are typical for carnivore/ necrophage invertebrates, dependent on episodic food supply, but may also render the animals prone to direct and indirect radiation damage, as lipids are preferred targets of ROS attack. Additionally, *A. nugax* accumulates only rela-

tively low amounts of algal MAAs via the food chain and MAA loss under experimental UVB exposure was considerable. Given their carnivore diet, the animals can avoid detrimental UV exposure in situ by seeking greater depths during hours of maximal daily radiation around noon, and indeed the animals were retrieved from depths between 2 and 5 m and rarely encountered in shallower areas. In contrast, the herbivore *G. homari* proved highly tolerant of subsurface UV radiation in the macroalgal canopy of shallow Kongsfjord habitats and this tolerance persisted under experimental exposure without macroalgal shading. The animals have acquired strong photoprotection from assimilation of algal MAAs and sustained high carotenoid levels. These sunscreening compounds obviously prevent severe photo-induced ROS and singlet oxygen formation in the animals. However, comparably variable CAT activity in newly captured specimens documents that the animals have to cope with environmental oxidative stress, presumably from photo-produced H_2O_2 in their subsurface habitat. The small detritivore/carnivore species *O. edwardsi* is also adapted to deeper subtidal habitats with lower enzymatic and MAA levels than the herbivore species. Carotenoids were bleached significantly during low-dose UV exposure, again accompanied by CAT photoinactivation. Thus *O. edwardsi* seems to be of moderate UV tolerance, only, and, similar to the carnivore *A. nugax*, confined to deeper water depths in the Arctic.

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