8 Effects of UV-radiation on oxidative stress parameters in polar marine amphipods, and the role of UV-absorbing mycosporine-like amino acids (MAAs) in their diet

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ABSTRACT

This study investigates the impact of elevated UVB radiation on UV-transparent Antarctic shallow water amphipods with respect to UV-induced oxidative stress in animal cells. Transfer of UV absorbing macroalgal-derived mycosporine-like amino acids (MAAs) was studied, to assess the importance of the algal diet for UV and antioxidant protection in crustaceans.

Animals were exposed to artificial UVB light up to four weeks in the laboratory. Mortality increased to roughly 10% of exposed amphipods under moderate UVB dose (42-54% of shallow water in-situ dose, i.e. 21-27% of natural atmospheric dose over exposure time) and to a maximum of 28% under high UVB exposure (50-93% increase of shallow water in-situ dose, i.e. 75-97% of natural atmospheric dose over exposure time) in animals not fed with macroalgae over 14 days. By contrast, the state of nutrition does not seem to affect antioxidant protection and lipid peroxidation directly as no significant differences between fed and non-fed animals could be detected. Variability of in-situ values was high for superoxid-dismutase (SOD) and malondialdehyde (MDA) accumulation. SOD activity increased under moderate, and decreased during high dose UVB exposure. Lipid peroxidation (MDA) decreased after three weeks of low dose UVB exposure and exhibited a slight increase under high dose UVB. Batch uptake of MAAs from algal food was not increased under experimental high dose UVB exposure over 3 weeks, and mycosporine-glycine and P-334 were the only compounds that accumulated in exposed animals. Without UVB (cut-off filter) mycosporine-glycine as well as P-334 and palythine decreased during the experiment compared to initial values. This suggests a better conservation of individual MAAs under UVB exposure and a faster degradation of these sunscreening compounds in UVB non-irradiated amphipods.

Key Words: UVB, amphipods, Antarctica, oxidative stress, mycosporines

INTRODUCTION

Solar ultraviolet radiation (UVR) penetrates marine and fresh waters and produces detrimental effects in tissues of shallow water organisms. Direct effects are based on interaction with UV-absorbing compounds in animal tissues and lead to genetic damage and physiological disorder. Moreover, UVR creates reactive oxygen species (ROS), especially the cell permeant hydrogen peroxide (H_2O_2) in surface waters, containing UV-absorbing dissolved organic matter (Abele-Oeschger *et al.* 1997, Abele *et al.* 1999). This active oxygen species can penetrate soft-bodied animals and cause major damage in the affected tissues through interaction with biomolecules such as proteins, nucleic acids and fatty acids, which can be altered or destroyed. Further, superoxide anion radicals together with H_2O_2 can generate highly aggressive hydroxyl radicals ($^{\cdot}OH$) in a reaction catalysed by iron ($O_2^{\cdot-} + H_2O_2 \rightarrow {}^{\cdot}OH + OH^- + O_2$). Finally, UVR can lead to ROS production inside irradiated tissues, and oxidative stress has been recognised as one of the major hazards involved in UV-induced radiation injury in marine organisms (Dunlap *et al.* 2000). Thus, most aerobic organisms have developed protective mechanisms, amongst which the antioxidant enzymes superoxide-dismutase (SOD: $2\,O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$) and catalase ($2\,H_2O_2 \rightarrow O_2 + 2\,H_2O$) form an efficient detoxifying system.

The polar environments of the Antarctic are prone to UVR exposure especially under ozone hole conditions during the austral spring (Karentz 1991, Vincent & Roy 1993), when deleterious UVB surface radiation between 280 and 320 nm can dramatically increase. Low temperatures may exacerbate oxidative stress owing to a cold induced loss in enzymatic antioxidants like superoxide-dismutase in ectothermal animals, which leads to reduced ROS scavenging capacities. A generally higher susceptibility to UVR and internal ROS attacks may also relate to higher unsaturation of membrane lipids in polar ectotherms adapted to permanent cold. Thus, rapidly occurring mortality upon exposure to high UVB irradiation with an LT50 within

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2 to 4 days of Antarctic krill has been attributed to disruption of cellular membranes, causing detrimental disturbance of physiological processes (Newman *et al.* 1999).

Most shallow water marine invertebrates are reasonably well protected against detrimental UVR effects by shells or exoskeletons. Additionally, UV and PAR absorbing substances are sequestered from the algal diet by herbivores and incorporated into the cuticula or other susceptible tissues as sunscreens (Carefoot *et al.* 2000). Among those, the class of UV-absorbing mycosporine-like amino acids have received considerable interest during the last 10 years, while other substance classes like carotenoids have long been known for their UV-protecting and also radical scavenging properties. Other small-molecule antioxidants like tocopherol have been found at far higher concentrations in tissues of Antarctic fishes (Gieseg *et al.* 2000) and bivalves (Estevez *et al.* 2002), when compared to related temperate species.

Here we are presenting the first results of an experimental study of the effects of elevated UVB radiation on Antarctic shallow water amphipods. Measurements of carapace transparency of North Sea amphipods for different ranges of solar radiation had shown that 42-58% of UVB, 52-59% of UVA and 62-74% of PAR passed through the chitinous exoskeleton to the inner animal tissue. The study is centred around the question whether the protection against UVB radiation and the antioxidant defence in these UV-transparent herbivorous crustaceans depend to any major extent on transfer of sunscreening MAAs from their algal diet, and whether the state of nutrition is crucial for maintenance of the enzymatic antioxidant status in the amphipods.

MATERIAL AND METHODS

Radiation measurements

Depth profiles (0-20 m) of under water light climate in Potter Cove, King-George Island, South Shetland Islands (62° 14' S; 58° 40' W) were recorded with a spectroradiometer (Construction by M. Kruse, Germany) in the range between 320 to 700 nm on sunny days between October 20 and December 20, 2000 to obtain information on maximal under water UV intensities in Potter Cove. These measurements indicated that traceable UVA penetrates to water depths up to 10 m and more (e.g. maximal values in November: 9,14 W m^{-2} at 0.5 m and 1,22 W m^{-2} at 9.5 m depth). Atmospheric solar UVB was measured using a 32-channel single-photon counting spectroradiometer installed on the roof of the Dallmann Laboratory (developed in the AWI Physics Department). Maximal atmospheric UVB irradiation ranged between 1.4 W m^{-2} and 1.8 W m^{-2} during the experimental period (Oct. 2000 – Jan. 2001). Underwater UVB could not be measured directly but according to Dr. Tüg (AWI Physics Department. pers. comm.), atmospheric UVB can be reduced by up to 50% in the upper 10 cm of the water column in Potter Cove in summer due to attenuation while entering the water column and penetrating to further depths. Therefore, average in-situ UVB intensities of 0.7–0.9 W m^{-2} could have been expected during the experimental period in the amphipods natural habitat. Daily sunshine hours ranged between 13 hrs at the beginning of October and 20 hrs in mid December.

Animal sampling and maintenance

Gammarid amphipods of the species *Gondogeneia antarctica* (Calliopiidae, Eusiroidea) and *Djerboa furcipes* (Eusiridae, Eusiroidea) were collected with a handnet from the shallow intertidal rocky shores of Potter Cove, between October 2000 and February 2001. The sampling area at Peñon 1 is covered with a dense macroalgal community (Quartino *et al.* 1998). Water depth was below 50 cm at the sampling area during low tides with a monthly minimum <10 cm. Animals were immediately transferred to an aquarium system at the Dallmann Laboratory and kept at 0°C and 34 PSU prior to experimentation. Species were sorted into different experimental aquaria and maintained for between 2 days and 2 weeks with and without red macroalgal food of the following species supplied as intact thalli: *Iridaea cordata*, *Neuroglossum ligulatum*, *Palmaria decipiens*, *Porphyra endivifolium* and *Sarcothalia papillosa* collected at the sampling site.

Experimental UV-irradiation

Experimental UV-irradiation was carried out using white light- and UV-tubes (Q-Panel, type UVA 340) for mild UVB exposure. Aquaria (volume: 2 l, depth: 10 cm) containing the exposed animals and algae were cooled to 0°C in a constant temperature room. For high dose UVB irradiation, we used a laboratory sunshine simulator, which provides a solar-like spectrum (developed in the AWI Physics Department by Dr. H. Tüg). Irradiated samples were thermostated to 0°C in a 5 l sample chamber (depth: 20 cm).

Experimental settings

In a first experiment (Exp. I), amphipods were exposed daily to 5 hrs UVR (Q-Panel) and PAR over 4 weeks. Exposure time was chosen to simulate a low tide situation. Irradiances at surface level were: 0.38 W m^{-2} UVB, 3.68 W m^{-2} UVA and 5.73 W m^{-2} PAR resulting in a daily dose of 6.82 kJ m^{-2} d^{-1} UVB over 4 weeks amounting to a total of 191.09 kJ m^{-2} in 28 days. This is a mild dose, compared to maximal natural atmospheric UVB radiation of 1.40 – 1.80 W m^{-2} and a daily dose of 25.20–32.40 kJ m^{-2} (within 5 hrs) during the experimental period. Considering a 50% attenuation of the UVB radiation in the upper water layer, animals experienced approximately half of the possible daily in-situ dose (which would amount to 12.60 – 16.20 kJ m^{-2}) in the sampling area. In the natural habitat, two to three hours of exposure to maximal UVB intensities with no cloud cover would be necessary to gain the same dose. In the experimental set-up 'fed' animals were exposed and kept together with small red macroalgal thalli to simulate natural conditions and to provide food as well as substrate for the amphipods. Whereas in the 'non-fed' set-up, animals were exposed without macroalgal food over the entire experimental period. Algal substrate was replaced by plastic gauze to allow attachment, as amphipods do not swim continuously. Therefore, some shading effects could not be excluded in the 'fed' set-up. Pooled samples of whole animals of at least 50 mg fresh weight were taken after 1, 2, 3, and 4 weeks and analysed for antioxidant enzymes or frozen to –30°C for analyses of malondialdehyde (MDA). Exp. II: Fed and non-fed animals (as exp. I) were exposed in the sunshine simulator for 4 hrs per day over 3 weeks to: 1.35 W m^{-2} UVB, 15.67 W m^{-2} UVA and 134,08 W m^{-2} PAR (surface level) amounting to a daily dose of 19,48 kJ m^{-2} d^{-1} UVB or a total of 409.09 kJ m^{-2} in 21 days. This is a high dose compared to the intensities in exp. I and constitutes 75 – 97% of the possible atmospheric UVB climate (within 4 hrs). Considering a 50% reduction of the UVB radiation in the upper water layer, animals experienced a 50-93% increase of the possible in-situ 4-hrs-dose in the sampling area. Six to eight hours of permanent exposure would be necessary in the natural habitat to result in the same UVB-dose if no shading effects of clouds would occur. Pooled samples were taken and analysed accordingly to exp. I. Exp. III was directed to the investigation of MAA uptake from algal diet: amphipods were exposed to UV-radiation in the sunshine simulator, applying the same conditions as in exp. II. A 320 nm long pass cut-off filter (Schott, Germany) was used for UVB-free incubations. Controls were exposed to full UVR without algae. Pooled samples of at least 10 mg dry weight were taken after 1, 2 and 3 weeks and frozen to –30°C for MAA analysis.

Measurements of superoxide-dismutase (SOD) activities

SOD activity in crude homogenates was measured using the xanthine

oxidase/cytochrome c assay according to Livingstone *et al.* (1992). Assays were carried out using freshly sacrificed animals at 20°C and data normalised to tissue wet weight (expressed as U mg^{-1} FW).

Determination of the lipid peroxidation product malondialdehyde (MDA)

MDA quantification in amphipod tissues was carried out according to Uchiyama & Mihara (1978). The method was modified with respect to heating time of the samples, which was 1 h at 100°C. Quantification was done according to a 5-point calibration curve, between 0.5 and 50 μM. Concentration is expressed as μmol g^{-1} wet weight (FW).

Analysis of mycosporine-like amino acids

MAA extraction and analysis were carried out according to Karsten & Garcia-Pichel (1996) and Newman *et al.* (2000) with the following modifications: pooled samples of 10-40 mg whole animal dry weight were homogenised manually 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 redissolved in 500 μl 2.5% aqueous methanol (v/v). Extracts were then passed through a Strata™ C_8-SPE cartridge (Phenomenex) to remove interfering lipids and subsequently mycosporines were eluted with 2 ml of 5% aqueous methanol (v/v). Extracts were again evaporated to dryness, taken up in 500 μl 2.5% aqueous methanol (v/v), and analysed with a Waters high-performance liquid chromatography system according to Hoyer *et al.* (2001). Mobile phase: 10% aqueous methanol (v/v) containing 0.1% acetic acid (v/v). Molar extinction coefficients were obtained from Karsten *et al.* (1998). Concentrations are expressed as μg g^{-1} DW (dry weight).

Statistics. Effects of irradiation duration on UV-stress parameters were tested for statistical significance using a one-way analysis of variance (ANOVA) as well as Student's t-test (employing SigmaStat 2.0 software).

RESULTS

Observation of mortality

Low mortality between 2% and 11% of the initial number of exposed animals occurred under Q-Panel light climate (mild UVB dose, 21-27% of natural atmospheric daily dose) over 4 weeks. Under high UVB doses (75-97% of natural atmospheric dose) in the sunshine simulator mortality was 16% with highest losses in week 2. High dose UVB exposure of non-fed animals caused mortality to rise to between 15% and 28% of the initial number of experimental animals within 2 weeks, which is a 12% increase relative to the fed group. No significant mortality was observed in fed non-irradiated animals.

UVR effects on superoxide-dismutase and malondialdehyde (exp. I).

Fig. 1 depicts irradiation of *Gondogeneia*. Starvation (open triangles and circles) did not cause significant changes of either SOD (solid line) or MDA (dotted line) compared with the fed group (filled triangles and circles).

In-situ values (0 weeks) displayed high standard deviation, reflecting high natural variability for both parameters. Lowest SOD activities were found after 1 week of mild UVB exposure followed by a significant increase between week 1 and week 3 ($p=0.014$, ANOVA). After 4 weeks of UVB exposure activities were higher than the in-situ values ($p=0.054$) and significantly different from the group after 1week of irradiation ($p=0.012$, for number of replicates per data point see Tab. 1)

Lipid peroxidation (MDA) increased during the first week of irradiation, where after a significant decrease ($p=0.002$, ANOVA) occurred to lowest values at the end of week 3. After 3 weeks, MDA values were different from in-situ ($p=0.050$), as well as from the value after 1 week of mild UVB exposure ($p<0.001$). At the end of the experiment after 4 weeks, MDA tissue concentrations were back to in-situ concentrations (for number of replicates per data point see Tab. 1).

Results from the high dose UVB exposure of fed (filled symbols) and starved (open symbols) *G. antarctica* in the sunshine simulator are presented in Fig. 2 (SOD and MDA, exp. II). Due to high mortality and requirements of a minimum sample size, only single measurements of starved animals were possible and therefore values are plotted but not considered in the statistical evaluation. Due to a high variability of the in-situ samples, the decrease in SOD activities (solid line) was steady while insignificant throughout the experiment ($p=0.330$, ANOVA). A comparison between activities after 1 and 3 weeks of UVB exposure yielded a significant decrease ($p=0.014$, t-test), however sample size was small. In contrast to SOD activities, MDA tissue concentrations (dotted line) increased, however insignificantly between 0 and 3 weeks of exposure to high UVB dose ($p=0.153$, ANOVA, for number of replicates per data point see Tab. 1)

In-situ MAA tissue concentrations

Analyses of in-situ MAA composition in both amphipod species yielded 5 different MAAs, which were identified as mycosporine-glycine, shinorine, porphyra-334 (P-334), palythine and asterina-330. These MAAs accounted for over 85% of all detectable compounds. Additionally, small amounts of unknown UV-absorbing substances could be detected (see Fig. 3: HPLC-chromatogram). In both species, shinorine and P-334 (*G. antarctica* > 300 μg g^{-1} DW; *D. furcipes* >

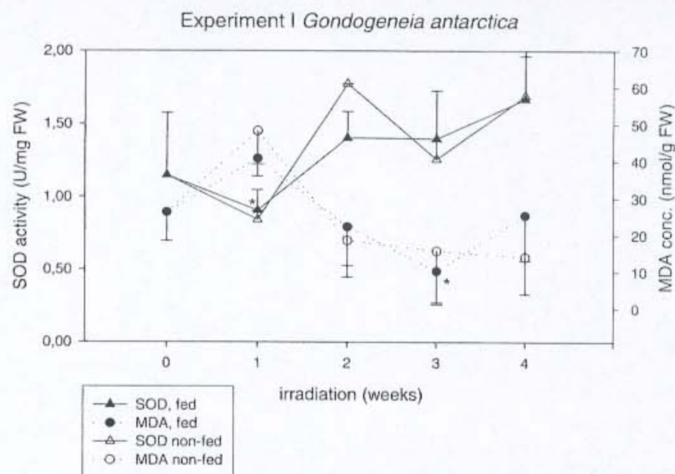

Fig. 1. UVR effects on SOD activity (triangles, solid line) and MDA concentration (circles, dotted line) under Q-Panel light climate (mild UVB dose) in *G. antarctica* (exp. I) presented as means and standard deviation. For number of replicates per data point see Tab. 1. Filled symbols indicate fed, open symbols represent non-fed animals. * indicates significant difference of 1st week SOD activity from following values, second * indicates significantly lower MDA concentration after 3 weeks of UVB exposure (both ANOVA).

Tab. 1: Number n of replicates per data points in exp. I, II and III shown in Fig. 1, 2 and 4 a, b, c, d (SOD = superoxid-dismutase, MDA = malondialdehyde, without UVB = 320 nm cut-off filter employed).

Experiment	Analysis/ treatment	week 0 initial	week 1 fed / not fed	week 2 fed / not fed	week 3 fed / not fed	week 4 fed / not fed
I	SOD	12	5 / 2	4 / 2	4 / 2	5 / 2
	MDA	10	4 / 2	4 / 2	4 / 2	6 / 2
II	SOD	12	2 / 1	4 / 1	3 / -	
	MDA	10	3 / 1	3 / 1	4 / -	
III	with UVB	5	3 / 2	3 / 2	4 / -	
	without UVB	5	2 / -	6 / -	- / -	

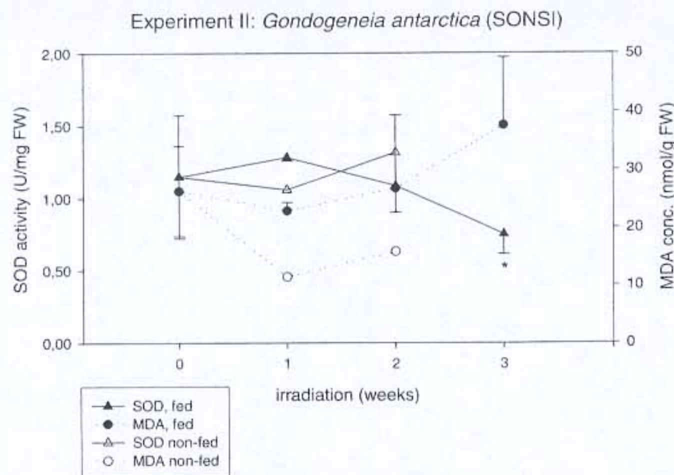

Fig. 2. UVR effects on SOD activity (triangles, solid line) and MDA concentration (circles, dotted line) under high UVB dose in the sunshine-simulator in *G. antarctica* (exp. II) presented as means and standard deviation. For number of replicates per data point see Tab. 1. Filled symbols indicate fed, open symbols represent non-fed animals. * indicates significantly lower SOD activity after three weeks compared to in-situ and week 1 values (t-test).

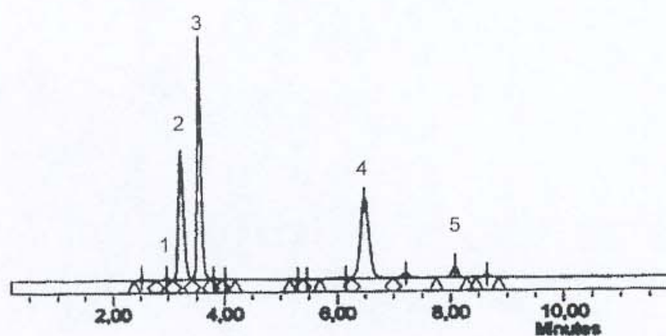

Fig. 3. HPLC-chromatogram of separated peaks recorded at 330 nm of UV-exposed *G. antarctica* with identified MAAs: 1 mycosporine-glycine, 2 shinorine, 3 Porphyra 334, 4 Palythine, 5 Asterina 330 (retention time in minutes).

150 µg g^{-1} DW) as well as palythine (*G. antarctica* < 200 µg g^{-1} DW in, *D. furcipes* < 100 µg g^{-1} DW) were the dominating MAAs. Mycosporine-glycine and asterina were always below 10 µg g^{-1} DW.

UVB effects on MAA tissue concentrations (exp. III)

Experimental UVB exposure of *G. antarctica* did not result in a significant increase of total MAAs in the fed group. Starved animals did not show significantly different MAA levels after UVB exposure compared to fed amphipods ($p>0.05$, ANOVA, Fig. 4a). Elimination of UVB radiation with a 320nm cut-off filter had no effect on MAA levels compared to amphipods, which received the full solar spectrum. Insignificant accumulation of mycosporine-glycine in continuously fed animals occurred only after 3 weeks of UVB exposure ($p=0.057$, ANOVA, Fig. 4b). By contrast, fed animals, which received the solar spectrum, minus UVB were found to have significantly decreased mycosporine-glycine concentrations after 1 week already ($p=0.015$, ANOVA) and throughout the experiment ($p>0.05$, ANOVA). Neither UVB exposure nor feeding had a significant effect on shinorine concentrations ($p>0.05$, ANOVA, data not shown). P-334 was unchanged in the UVB irradiated group with only a slight but insignificant accumulation after three weeks but decreased significantly when UVB was cut-off ($p=0.023$, t-test, Fig. 4c). Palythine concentrations after one week were significantly lower when compared with in-situ values ($p=0.013$, ANOVA, Fig. 4d), in both UVB irradiated and non-UVB irradiated amphipods.

In *D. furcipes* a trend towards higher MAA concentrations in UVB exposed specimens was observed, compared with exposure under a 320 nm cut-off filter. Due to the limited amount of samples this trend is, however, not significant ($p>0.05$). Additionally, mycosporine-glycine, shinorine and palythine accumulated over 3 weeks of UVB exposure, whereas P-334 concentration decreased under the same conditions (data not shown).

DISCUSSION

Marine amphipods were chosen for this study, as they constitute a dominating, highly abundant and diverse component of Antarctic shallow water fauna. The herbivorous forms provide a direct link between algal primary production and higher trophic food chain levels. Amphipods are rich in storage lipids, UV transparent and thus presumably susceptible to UVR and oxidative stress.

Experimental set-up: Irradiation intensities and exposure time were chosen in order to simulate a natural situation with low and high UVB intensities (ozone hole conditions). As direct UVB measurements were only possible in the atmosphere, underwater UVB had to be approximated from underwater UVA measurements and via attenuation factors of atmospheric UVB transmittance in the water column. Spectra and intensities of Q-Panel tubes could not be altered and supplied the low dose treatment. High dose was achieved by simulating maximal atmospheric UVB dose under cloudless conditions in the sunshine simulator and accordingly adjusting its solar-like spectra. This yields an elevated UVB dose when compared to 50 cm water depth, while it was lower than maximal daily doses at surface level, and thus resembled a realistic simulation of high UVB intensities under field conditions. Intensities employed in these experiments are comparable to those of Newman *et al.* (1999) applied in irradiation experiments with Antarctic krill.

Exposure to such a high in-situ UVB dose (75–97% of natural atmospheric dose over exposure time) produced detrimental effects on both exposed Antarctic amphipod species causing elevated and fast mortality. Even a mild UVB dose (21–27% of natural atmospheric dose over exposure time) caused death after 5 hours of continuous daily radiation without algae. Deprivation of algal food was found to exacerbate the detrimental UVB effect, which was related to the lack of direct UVR shelter as well as to the impact of starvation. However, compared to krill (Newman *et al.* 1999), amphipods displayed lower death rates and longer survival under UVB, indicating adaptation to high radiation levels in intertidal habitats.

Under moderate UVB exposure, SOD activities increased steadily, indicating enhanced gain of antioxidant protection. Levels of lipid peroxidation declined accordingly. By contrast, a high UVB dose caused SOD activities to fall below in-situ levels and MDA accumulation to increase. It seems unlikely that SOD activity only would be disturbed as a direct effect of the applied radiation. The observed reduction of SOD activity might reflect UV induced protein damage, however, more likely, relate to a general impairment of protein synthesis under high UVB stress. MDA, which represents a transient marker for lipid peroxidation, accumulated in the crustaceans. However, high natural variability of both parameters did not permit clear trends. The state of nutrition does not seem to affect the antioxidant system or the extent of lipid peroxidation in the investigated amphipods. This could also relate to the high natural variability and the small number of replicates, especially of non-fed animals. Additionally, starvation time might have been too short and amphipods like many polar organisms are able to buffer this period of food deprivation without a major decline of enzymatic functions.

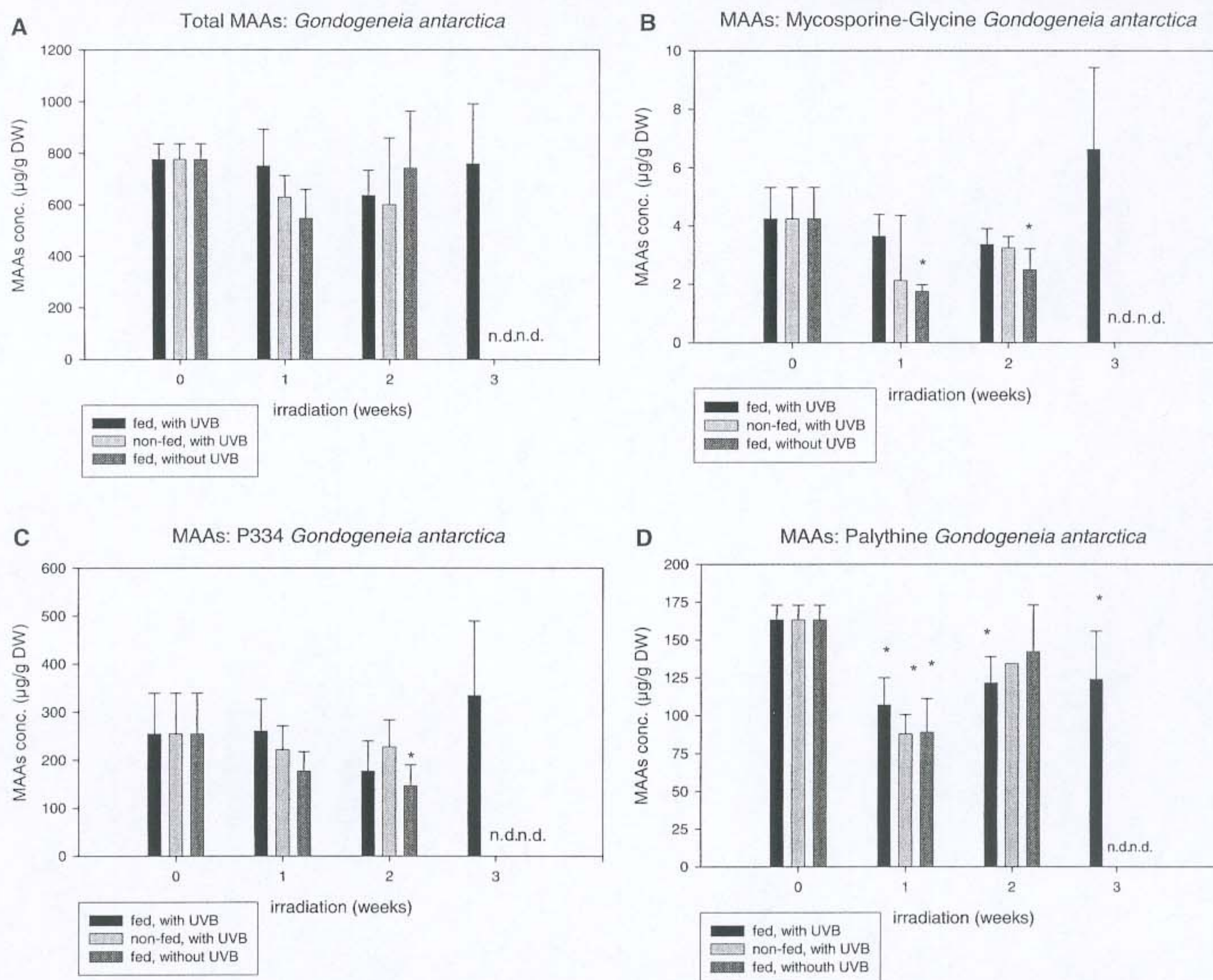

Fig. 4. UVR effects on the concentrations of a) total MAAs as well as b-d) individual MAAs in *G. antarctica* presented as means and standard deviation in fed and non-fed UVB-irradiated animals and in fed but UVB-non-irradiated (320nm cut-off filter) animals. b) mycosporine-glycine. * indicates significantly lower concentration after 1 and 2 weeks without UVB radiation (p=0,015, ANOVA), c) P-334. * indicates significantly lower concentration after 2 weeks without UVB exposure (p=0.023, t-test), d) Palythine. * indicate significantly lower values after 1 week of all three treatments and 2-3 weeks of UVB exposure compared to initial concentrations (p=0.013, ANOVA).

MAA composition in the amphipods reflects the dominating MAAs present in their algal food (for macroalgal MAA composition see Hoyer *et al.* 2001). As shown by Newman *et al.* (2000) and Adams *et al.* (2001), MAA content in herbivorous animals is a direct function of their algal diet and radiation induced variations of algal MAA content.

Accumulation of MAAs was neither selective nor enhanced under UVB, if compared with animals exposed to UVA + PAR only. This indicates that MAA loading under in-situ conditions was already maximal, and that MAAs were conserved if UVB irradiation was cut off and not digested even under starvation. Likewise, Newman *et al.* (2000) observed no decomposition of MAAs in krill tissues during experimental UV (and PAR) exposure. These authors showed that the microalgae *Phaeocystis antarctica*, which was used as food source for *Euphausia superba*, produced MAAs even under PAR-only. The same holds true for various species of red macroalgae, which accumulate MAAs independently of the prevailing radiation conditions (PAR only, PAR + UVA and/or UVB, Hoyer *et al.* 2001). No significant differences between MAA contents in fed and non-fed amphipods indicate that the starvation period might have been too short. Newman *et al.* (2000) showed that even after the prolonged period of 63 days without algal food, MAA content in krill did not significantly differ from initial values. Further, starvation of 35 days after feeding of 63 days with food of defined MAA composition did not lead to significant changes in mycosporine-glycine and palythine in krill. No feeding experiments with MAA containing pellets of defined composition and levels of individual MAAs were carried out by us with these Antarctic amphipods but would probably lead to a better understanding of processes involved in MAA accumulation and conservation. Interestingly, P-334 and mycosporine-glycine, were better preserved in amphipods that received UVB, as compared with UVA and PAR exposed animals. According to Dunlap and Shick (1998), accumulation of mycosporine-glycine might reflect metabolic transformation of shinorine or P-334 by marine bacteria in the amphipods' guts. It is interesting to note that this transformation shifts the maximal absorption from 333 nm of shinorine and P-334 to 310 nm in mycosporine-glycine and therewith into the UVB range.

Our findings suggest that antioxidant defence (SOD) can be increased under mild UVB exposure, while high radiation levels have an adverse effect on this antioxidant enzyme. Short periods (< 4 weeks) of high UVB exposure do not induce increased accumulation of sunscreening MAAs in investigated amphipods.

Further experimental studies are necessary to clearly elucidate the role of nutrition in antioxidant defence and UVB protection in polar amphipods.

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