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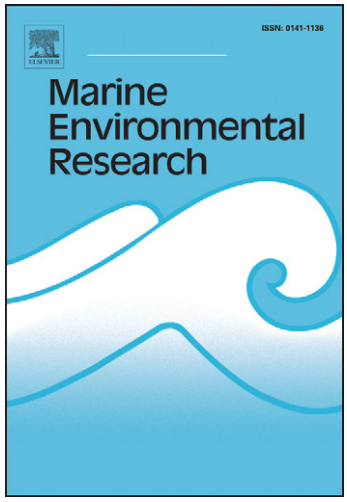

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UV-tolerance and instantaneous physiological stress responses of two Antarctic amphipod species *Gondogeneia antarctica* and *Djerboa furcipes* during exposure to UV radiation

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Abstract

We investigated the shielding against solar ultraviolet radiation and inducible damage, as well as the short-term response of whole animal metabolic rate in two Antarctic shallow water amphipod species. Light absorbance by the carapace of *Gondogeneia antarctica* and *Djerboa furcipes* was higher in the UVR (UVB+UVA) range (42.1 and 54.5% on average respectively) compared to the PAR (photosynthetically active radiation) range (38.1 and 50.1% respectively) of the solar spectrum. Bands of higher absorbance correlated with maximal absorbance ranges of sunscreening compounds indicating mycosporine- like amino acids (MAAs) and carotenoids to be innate compounds of the exoskeleton of these species. Though the antioxidant enzyme catalase was photoinhibited, protein damage products did not accumulate under experimental exposure to a daily dose of 6.84 $kJm^{-2}d^{-1}$ UVB, 66.24 $kJm^{-2}d^{-1}$ UVA and 103.14 $kJm^{-2}d^{-1}$ PAR. Animal oxygen consumption during UV-exposure was measured as an indicator of immediate behavioural and physiological stress response. UVB as well as UVA induced a response with altered and highly variable respiratory intensity. Our findings indicate that sub-lethal UVR exposure causes increased oxygen consumption in polar amphipods due to radiation avoidance, shelter seeking behaviour, and presumably also from cellular repair processes.

Keywords: Amphipods, UV-radiation, photoprotection, stress response, oxygen consumption

1. Introduction

Direct exposure to natural sunlight, particularly UV-photons, has been shown to cause damages in the tissues and cells of aquatic animals and plants inhabiting shallow waters (Rozema et al. 2002, Williamson et al. 2001). More specifically, UVB (280-320 nm) but also the UVA- (320-400 nm) photons can damage biomolecules with chromophoric, UV-

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absorbing groups. These molecules can be metabolites or stored pigments, which literally exert the function as "internal UV-photon absorbers", a defence system that can be overruled under extreme irradiative exposure, resulting in "bleached" plants and animals (Dunlap & Shick 1998). Longer wavelength UVA and shorter wavelength PAR can also affect haem-containing enzymes such as the hydrogen peroxide (H_2O_2) detoxifying catalase, which absorbs around 405 nm. Photodestruction of the haem-group causes direct loss of function in the affected molecule and physiological disorder (Gantchev & van Lier 1995). On the other hand, on penetrating animal tissues, UVB-photons exert indirect effects by propagating formation of reactive oxygen species (ROS) through activation of photosensitizer molecules (Abele & Puntarulo 2004, Lesser 2006). The released electrons are individually transferred to highly electro-positive molecular oxygen, forming superoxide ions ($O_2^{-\bullet}$), which then dismutate to H_2O_2 or react with nitric oxide (NO) to form the deleterious peroxynitrite. H_2O_2 formation yields the aggressive hydroxyl radicals (OH•) via Fenton reactions of transition metals and, moreover, gives rise to radical forming chain reactions in lipid rich tissues, causing damage to biomembranes especially in lysosomal organelles (Halliwell & Gutteridge 1999, Abele & Puntarulo 2004).

By producing a selective increase in the ambient UVB-radiation, the massive ozone depletion observed in southern, and recently also in northern polar regions, strongly affects high latitude shallow water ecosystems (Madronich et al. 1998, WMO 2002). Oxidative tissue damage is frequently observed in organisms when the balance of pro-oxidant, ROS-forming and antioxidant processes is biased. This can occur under severely elevated UV-exposure and when animals are deprived of the sheltering macroalgal canopy or trapped in shallow tide pools with no escape to deeper dim light environments (Zellmer et al. 2004). As animals age (Philipp et al. 2006) or are severely stressed, deprived of adequate food, or lose the

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endosymbionts that supply them with algal antioxidants and sunscreens (Dunlap et al. 2000, Lesser 2006), they lose antioxidant defence and photodamage repair capacity.

We have observed high mortality under experimental UVB-exposure over three weeks in carnivore amphipods. This related to either insufficiently induced or partly photoinhibited antioxidant (AOX) defence systems with both, superoxide dismutase (SOD) and catalase activities reduced after UV-exposure in animals from Arctic and Antarctic environments (Obermüller & Abele 2004, Obermüller et al. 2003, 2005). Impairment of SOD and catalase, which neutralise $O_2^{-\bullet}$ and H_2O_2, then allows formation of toxic photoproducts and propagation of free radical chain reactions, damaging lipids, proteins and DNA. These ROS-induced injuries cause functional changes in affected tissues, which influence the animal's metabolic performance, growth and reproduction and may finally limit survival (Abele & Puntarulo 2004, Boveris 1998). In the present study we measured UVB- and UVA-absorbance of the amphipods' chitinous exoskeleton (carapace) as a first protective body barrier against penetrating UV-photons. Tissue carotenoids, antioxidants quenching singlet oxygen, and catalase activity were investigated in amphipods exposed to low-dose UVB, UVA and PAR for up to 10 days. As catalase itself is vulnerable to photodamage this enzyme is also a tool to evaluate radiation stress as well as protective capacity. Previous experiments with *G. antarctica* exposed to low-dose UVR did not show elevated levels of peroxidised lipids (Obermüller et al. 2003). Here, we measured the degree of oxidative stress and damage to proteins, likewise targets of ROS-attacks in the tissues of amphipods exposed to UVR over the short period of 10 days.

Further we examined immediate effects of direct UVA- and UVB-exposure on whole animal aerobic metabolic rates. Previous work from our laboratory demonstrated exposure to elevated levels of reactive oxygen species (H_2O_2), leading to concentration dependent

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alterations of whole animal metabolic rates in various marine invertebrates. Specifically, lower H_2O_2-concentrations than those present in the animals' own haemolymphatic fluid caused an increase, whereas concentrations, elevated over haemolymph levels, caused a depression of isolated tissue oxygen consumption (Storch et al. 2001), as well as of whole animal respiration (Abele-Oeschger et al. 1997, Abele et al. 1998). We ventured that direct UVB-exposure should produce a similar effect on metabolic rates via its ROS inducing potential and, therefore, recorded the aerobic metabolic rate in two species of Antarctic amphipods before, during and after several hours of UVA- and UVA+UVB-exposure.

2. Material and methods

2.1 Sampling and maintenance of experimental animals

The Gammarid amphipods *Gondogeneia antarctica* (Calliopiidae, Eusiroidea) and *Djerboa furcipes* (Eusiridae, Eusiroidea) were collected on the intertidal rocky shore of Potter Cove, King George Island, South Shetland Islands (Antarctica) at low tide, using a handnet. Animals of between 1-2 cm size were taken from areas of 10-50 cm water depth, where they colonised the canopy of intertidal macroalgae. The amphipods also swam freely between algae, receiving full natural radiation. Prior to experimentation, the amphipods were maintained between one and two weeks in the Dallmann Laboratory in a constant temperature room at 0°C ± 0.5°C and a salinity of 34. The windows in the maintenance room were not shielded, resulting in dim daylight conditions inside, following the outside light-dark-cycle. Small thalli of red macroalgae collected at the same sampling site were placed into the aquaria as substratum and food source.

2.2 Atmospheric UVB-measurement and radiation climate at the Antarctic Peninsula during the experimental period

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Solar UVB-radiation was measured continuously with a 32-channel single-photon counting spectroradiometer installed on the roof of Dallmann laboratory at Jubany Station, King George Island. For comparison, during a previous campaign in 2000 solar UVA and PAR had been recorded with an additional fast scanning double monochromator spectroradiometer (Instrument Systems, Germany). Maximal ambient solar radiation intensities between October and December were 1.3 – 1.8 Wm^{-2} UVB (2002), 1.4 – 1.8 Wm^{-2} UVB (2000), 16.5 – 27.9 Wm^{-2} UVA (2000), and 133.2 – 140.7 Wm^{-2} PAR (2000). The ozone layer over the South Shetland Islands area had reached its minimum in September 2002 (159 Dobson Units, DU). In November 2002 mean column ozone was at 281 DU (minimum 198 DU) and 324 DU (minimum 300 DU) in December (Source: AWI-Physics Department based on NASA TOMS data, http://toms.gsfc.nasa.gov/teacher/ozone_overhead.html).

2.3 Carapace absorbance

Three amphipods per species, which had been maintained in the constant temperature room for up to 1 week, were dissected and the chitinous exoskeleton cleaned from residual tissue. Carapaces were spread onto a UV-transparent filter (295 nm cut-off) and the transmission spectrum recorded from 295 to 700 nm in the solar simulator (SONSI). We used a Zeiss Monolithic Miniature Spectrometer MMS UV-VIS (Zeiss, Germany), combined with electronics by M. Kruse (Germany). Mean carapace absorbance expressed in (%) was calculated as follows:

$$\text{Carapace absorbance } Abs_c = \frac{1}{T_c} = \frac{T_f}{T_s * T_{c+f}}$$

T_c: Transmission of SONSI light source through carapace only (no filter)

T_f: Transmission of SONSI light source through filter

T_s: Transmission of SONSI light source only (no filter, no carapace)

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T_{c+f}: Transmission of SONSI light source through carapace placed on filter

2.4 UVR-exposure experiments in aquaria (Q-Panel-tubes)

Adult *G. antarctica* and *D. furcipes* were exposed to artificial low-dose PAR and UVR using white light and Q-Panel-tubes (type UVA 340, Cleveland, USA) in small aquaria (2 l volume, 10 cm depth, 20-25 animals per aquarium). Exposure was carried out for 5 h daily over 10 days at 0°C in a constant temperature room. Between each irradiation interval amphipods were maintained at dim daylight only, which was equivalent to lighting conditions for control specimens. Amphipods were not fed during the experiments to avoid shading effects from algal thalli. Also, a previous study had not revealed significant differences in antioxidant enzyme activities and oxidative tissue damage between fed and starved specimens during 4 weeks of differential feeding (Obermüller et al. 2003).

Two cut-off filters were employed, 320 nm and 400 nm, to selectively shield amphipods in selected aquaria from UVB and UVB+UVA spectral ranges in order to determine wavelength dependent effects of UVR. In animals that received the maximal radiation of all 3 spectral ranges experimental intensities were 0.38 Wm^{-2} UVB, 3.68 Wm^{-2} UVA and 5.73 Wm^{-2} PAR, amounting to a daily dose of 6.84 $kJm^{-2}d^{-1}$ UVB, 66.24 $kJm^{-2}d^{-1}$ UVA and 103.14 $kJm^{-2}d^{-1}$ PAR. This is a low-dose compared to maximal natural atmospheric radiation, which may amount to 27 kJm^{-2} UVB, 400 kJm^{-2} UVA, and 2465 kJm^{-2} PAR during 5 hours continuous irradiation at the surface without shielding effects by clouds. Pooled samples of at least 50 mg fresh weight were taken after different irradiation intervals and frozen in liquid nitrogen prior to analyses of carotenoid content, antioxidant catalase activity and protein oxidation. Mortality was also monitored throughout the exposure experiments.

Carotenoid concentration was measured at the start and after 10 days of exposure in whole animal butanolic extracts of *G. antarctica* and *D. furcipes* as described in Obermüller et al.

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(2005). An ε of 141 x 10^3 l $mmol^{-1}$ cm^{-1} for ß-carotene in ethanol was taken from Jeffrey (1997). Concentrations are expressed in µmol ß-carotene equivalents g^{-1} fresh weight (FW).

Catalase activity was measured in both species at the start and after 4 and 7 days (samples pooled from 3-4 and 5-7 days) of exposure in whole animal homogenates (1:5) as described in Obermüller et al. (2003). Catalase activity is expressed in U mg^{-1} whole animal fresh weight (FW).

Oxidative damage to proteins was measured as the presence of carbonyl groups in amino acid residues of proteins, according to Levine et al. (1990) at the start and after 7 and 10 days of exposure. The detection and quantification is possible through reaction of the carbonyl groups with the carbonyl-specific reagent 2,4-dinitrophenylhydrazine (DNTP). Briefly, supernatants of whole animal homogenates were incubated with 1.4 ml 10 mM DNTP in 2 M HCL for 1 h at room temperature and vortexed every 15 min during incubation (blanks run without DNTP). Then, 0.2 ml 100% TCA were added, centrifuged at 10 000 g for 10 min, and the precipitated protein pellet was washed three times with 1 ml ethanol:ethylacetate (1:1), resuspended in 0.6 ml 6 M guanidine hydrochloride in 20 mM potassium phosphate and incubated at 37°C until complete resuspension. The carbonyl content was measured spectrophotometrically at 360 nm (molar extinction coefficient ε = 22 x 10^3 M^{-1} cm^{-1}) and expressed in nmol mg^{-1} protein.

2.5 Oxygen consumption measurements under UV-exposure in the solar simulator (SONS I)

Irradiation experiments were carried out with live animals (5-6 specimens per experiment) directly within the solar simulator (SONSI), in which a solar-like spectrum can be simulated using a 400 Watt discharging lamp containing rare elements (type Philips MSR 400 HR) and a three layered liquid filter with $CuSO_4$, $KCrO_4$, and KNO_3 (developed in the AWI Physics Department by Dr. H. Tüg and Fa. IsiTEC, Bremerhaven, Germany, see Dethlefsen et al.

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2001). For simultaneous exposure and respiration measurements we used a flow-through system with special UV-transparent respiration chambers of adjustable volume thermostated in a water bath. Water oxygen concentration was continuously recorded at the outflow of the chamber with a fiber optic oxygen sensor system (Mops-4, Fa. COMTE, Hannover, Germany). The sensor consisted in a silicone coated fiber optode, containing an oxygen-sensitive fluorophor, introduced into the gas tight respiration system directly behind the respiration chamber in a separate, UV-opaque measurement chamber. The optode was calibrated in air-saturated (normoxic) and N_2-saturated (anoxic) sea water. Before each new experiment the filtered (0.2 µm) sea water was completely exchanged and the optode re-calibrated. The overall experimental volume of respiration chamber, measurement chamber and tubing amounted to 30 ml.

In the solar simulator amphipods were exposed to 1.5 Wm^{-2} UVB, the mean maximal atmospheric UVB-intensity (1.3 – 1.8 Wm^{-2} UVB) measured at Jubany during the experimental period. The solar-like experimental spectrum in the SONSI was adjusted to 39.7 Wm^{-2} UVA and 117.7 Wm^{-2} PAR. Up to 6 similarly sized specimens of 73.9 ± 11.6 mg mean fresh weight (FW) in *G. antarctica* and 56.5 ± 11.1 mg mean fresh weight (FW) in *D. furcipes* per experiment were placed carefully into the respiration chamber and allowed to acclimate for at least two hours to the experimental conditions. Subsequent irradiation experiments lasted 26 hours. Chambers were maintained at 0.5 ± 0.3°C and air-saturated, filtered sea water was pumped at a flow rate of 0.79 ml min^{-1}, exchanging the overall experimental volume once every 38 min. Little pieces of plastic mesh were placed into the chamber as substratum to restrict movements within the chamber to obtain resting metabolic rate (RMR) without shading the amphipods from irradiation. RMR was used as defined by Chapelle et al. (1994) and Chapelle & Peck (1995) as a state in which animals have settled to the bottom of the respiration chamber without vigorous or locomotive activity or swimming.

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However, animals could and did move slightly in the chamber. As reported by Chapelle & Peck (1995) Antarctic amphipods of the species *Waldeckia obesa* (Chevreux 1905) and *Bovallia gigantea* (Pfeffer 1888) if offered substratum of nylon mesh decreased oxygen consumption by factors ranging between 1.1 and 3.9 with respect to the actively swimming animals.

The radiation routine for the experiments is briefly explained here: Each experiment started with a 6 h recording of "low light respiration" (RMR, phase 1). Animals (5-6 specimens per experiment) were shielded from irradiation emitted by the SONSI lamp by a sheet of black plastic placed over the respiration chamber system. The animals received only diffuse dim daylight from the sides but no UV-radiation. The "low light" phase was followed by a 4 h "irradiation" (phase 2) with UVA+PAR under a 320 nm cut off filter wrapped around the respiration chamber. Subsequently, the chamber was shielded again for 6 h with a black sheet (phase 3: "recovery"). During the second 4 h "irradiation" (phase 4) animals received UVB+UVA+PAR. A final 6 h "low light respiration" (phase 5) ended the experiment. Oxygen concentration was recorded throughout the entire experiment with a 2-channel chart recorder. Before and after each 26 h experiment, microbial oxygen consumption was recorded in the flow-through system without amphipods (blank), and animal respiration was corrected accordingly. Respiration rates as well as respiratory amplitudes of oxygen consumption were calculated for each 30 min interval during all 5 phases of each experiment. Amplitudes represent the difference between maximal and minimal oxygen consumption during each of these 30 min intervals and are depicted as bars (width) of the 30-min mean oxygen consumption in the respective figures (Fig. 2 and 3). We hypothesised that under non-stressed conditions differences between maximal and minimal oxygen consumption and thus amplitudes should be small and respiration regular, while stressful irradiation should cause large amplitudes from irregular respiration. Further, an overall mean oxygen consumption ±

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standard deviation as well a mean overall amplitude ± standard deviation was calculated for each phase.

Under these conditions the animals were exposed to an experimental dose of 21.60 kJm^{-2} total UVB during 1 x 4 h, 1143.4 kJm^{-2} total UVA during 2 x 4 h and 3389.8 kJm^{-2} total PAR during 2 x 4 h, yielding a ratio of UVB:UVA:PAR as 1:26:78 during phase 4 exposure. The experimental UVB-dose amounts to 95% on average of the possible maximal natural UVB-dose at the water surface. Typical K_d values (K_d: diffuse vertical attenuation coefficients of downward irradiance) for Potter Cove in November are 0.7 on average at 10 cm and 0.5 on average at 1 m water depth with transmission values of surface UVB-radiation of approx. 55% and 27% respectively (pers. comm. AWI Physics Department). Thus, between 7 and 15 h of continuous irradiation and cloud-free conditions would be necessary to yield the same dose in the natural shallow water environment down to 1 m depth.

2.6 Statistics

Differences in carapace absorbance, carotenoid concentrations, catalase activity, proteinoxidation, and survival rates were tested for statistical significance using a Student's t-test at a significance level of $p<0.05$. Differences in respiration rates and UV-induced changes in oxygen consumption within each experiment were tested for statistical significance using a Student's paired t-test at a significance level of $p<0.05$. Where data were not normally distributed a Wilcoxon rank test was performed at a significance level of $p<0.05$. Significant differences in oxygen consumption between all experiments within one species were tested using a Kruskal-Wallis one-way ANOVA on ranks followed by a multiple comparison post-hoc test (Dunn's Procedure). Data are given as means ± SD if not stated otherwise.

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3. Results

3.1 Carapace absorbance and tissue UV- and antioxidant protection

Carapace absorbance (mean ± SD) was more effective in the UVR-range (UVB+UVA) than in the PAR-range in both species, with 43.0 ± 2.56 % UVB and 41.4 ± 0.53 % UVA as compared to 38.1 ± 0.2. % PAR in *G. antarctica* and 52.0 ± 9.43 % UVB, 57.0 ± 0.75 % UVA, and 50.1 ± 1.41 % PAR in *D. furcipes* (number of replicate carapaces n=3 for each species). On average, carapace UVR-absorbance was 12.5% lower in *G. antarctica* than in *D. forcipes*, with a significant difference in the UVA ($p<0.01$) but not in the UVB-range ($p=0.28$). Figure 1 shows carapace absorbance spectra of *G. antarctica* and *D. furcipes.* In both species bands of higher absorbance are located between 305-340, 430-450 and 480-530 nm (see arrows in Fig. 1), corresponding to the ranges of absorbance of several sunscreening compounds: mycosporine-like amino acids (MAAs) absorb between 305-340 nm and carotenoids between 430-450 nm. Carotenoid tissue concentration was significantly higher in *G. antarctica* (0.174 ± 0.02 µmol g^{-1} FW) compared to *D. furcipes* (0.131 µmol g^{-1} FW) ($p<0.05$). In neither species did medium-term exposure to low-dose UVB, UVA or PAR radiation over 10 days result in any observable bleaching of carotenoids, compared to non-irradiated controls (Table 1). *G. antarctica* specimens retained their dark brown-black, and *D. furcipes* specimens their bright orange-brown carapace coloration.

Catalase activity decreased in both species during several days of repeated UVR+PAR-exposure, but the decrease reached significance only in *G. antarctica* after 4 and 7 days of irradiation (Table 2). Differences between species were significant only on the start day with higher catalase activity in *D. furcipes*.

Proteinoxidation did not differ significantly between species or within either species between control and UVR-treatments over 10 days of repeated exposure. Only in *G. antarctica*

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carbonyl content was significantly lower after PAR-exposure over 4 to 7 days than in controls ($p<0.05$) (Tab. 3).

In *G. antarctica* mortality of low-dose UVR-exposed specimens was not significantly different from controls. Survival rates amounted to 87.3 ± 6.7 % of initially exposed animals as compared to 90.4 ± 11.0 % in controls after 10 days. In *D. furcipes* mortality was much higher and differed significantly from the control treatments already after 5 days. Survival rates decreased to 23.1 ± 13.4 % of initially exposed animals in the UVR-treatment compared to 98.7 ± 1.9 % after 5 and 93.5 ± 9.1 % after 7 days in control animals. Therefore, experiments with *D. furcipes* specimens were stopped after 7 days.

3.2 Oxygen Consumption

Oxygen consumption (means) and respiratory amplitudes (bars) calculated for each 30 min interval within the different experimental phases are plotted in Figure 2 for *G. antarctica* (Ga) and Figure 3 for *D. furcipes* (Df). Under non-stressed conditions (phase 1) mean resting metabolic rate (RMR) was 6.06 ± 0.98 µmol O_2 g^{-1} FW h^{-1} with an amplitude around the mean calculated for all 30 min intervals of 0.23 ± 0.35 µmol O_2 g^{-1} FW h^{-1} for *G. antarctica* (Tab. 4). Mean RMR in *D. furcipes* amounted to 4.57 ± 2.20 µmol O_2 g^{-1} FW h^{-1} with an amplitude of 0.73 ± 0.83 µmol O_2 g^{-1} FW h^{-1} (Table 5). Both species displayed regular respiration in this initial experimental phase under low light without UVR, indicated by the small respiratory variability (amplitude) around the mean value.

G. antarctica: UV-induced stress in irradiated amphipods became apparent in significant changes of mean oxygen consumption as well as increased respiratory amplitudes under UVB and UVA, compared to low light conditions during the first control phase and in both

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recovery phases (Table 4). UVR effects were not consistent within the 4 experiments. In experiments Ga 1 and 3 (Fig. 2) UVA+PAR-exposure alone (phase 2) caused a respiratory increase over resting levels (phase 1), significantly higher ($p<0.01$) than subsequent exposure to UVB+UVA+PAR (phase 4). The opposite occurred in experiments Ga 2 and 4 (Fig. 2), where exposure to UVB+UVA+PAR caused an increase over low light RMR (phase 1) to significantly ($p\leq0.01$) higher respiration than under UVA+PAR alone. In all experiments an increase of respiration after exposure was followed by a sometimes rigid decline in oxygen uptake. This pronounced decrease in respiration was mostly observed in the second irradiation phase (4) which included UVB light. In 2 out of 4 experiments, the rigid decline was delayed to the dark phase following UVB+UVA+PAR-exposure (experiments Ga 1 and 4). In all four experiments, respiratory variation (amplitude) was higher under full spectral irradiation with UVB (1.45 ± 1.10 µmol O_2 g^{-1} FW h^{-1} on average) than under UVA+PAR alone (0.92 ± 0.86 µmol O_2 g^{-1} FW h^{-1} on average) or when compared to low light respiration. Especially, when irradiation started, high amplitudes were recorded, which decreased again as irradiation continued. In the dark recovery phases the respiratory irregularity was steadily reduced to nearly constant respiration of pre-irradiation levels in all experiments. Only one of all *G. antarctica* individuals died within 4 experimental runs, resulting in a mean survival of 95.8 ± 8.3 % of the initially exposed amphipods.

According to Kruskal-Wallis one-way ANOVA on ranks and the multiple comparison post-hoc test (Dunn's Procedure), all experiments except Ga 2 vs Ga 3 were significantly different from one another with respect to the absolute levels of specific oxygen consumption (µmol g^{-1} fresh weight) during the entire experiment ($p<0.05$).

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Djerboa furcipes: The animals' mean oxygen consumption and respiratory amplitudes increased above resting levels (RMR: phase 1) during UVR-exposure in all five experiments (Table 5). Addition of UVB to the irradiation spectrum (phase 4) caused significantly larger respiratory increases compared to UVA+PAR-exposure (phase 2) in experiment Df 1 (Fig. 3, $p=0.01$), Df 3 (Fig. 3, $p=0.02$), Df 4 (Fig. 3, $p<0.01$), and Df 5 (Fig. 3, $p<0.01$), whereas UVB- and UVA-effects on respiration were not significantly different in experiment Df 2 (Fig. 3, $p=0.93$). Oxygen consumption decreased slowly to reach RMR-levels (phase 1) during both recovery phases (3 and 5) in all experiments, except Df 1 and Df 4 (phase 5). Sometimes the instantaneous respiratory increase and subsequent decrease were very rigid as seen in experiments Df 2, Df 4, and Df 5. Amplitudes remained clearly higher and more variable during the final, compared to preceding low light phases. The final recovery phase in experiment Df 5 was stopped after 2.5 h due to problems with the optode sensor. Respiratory amplitudes of irradiated compared to low light exposed amphipods were about the same during UVB+UVA+PAR (2.58 ± 2.75 µmol O_2 g^{-1} FW h^{-1} on average) and UVA+PAR-exposure (2.62 ± 2.47 µmol O_2 g^{-1} FW h^{-1} on average) (Table 5) and, clearly, more variable in range between maximal and minimal respiration compared to recovery phases. *D. furcipes* had a mean survival rate of 92.7 ± 10.1 % for the 5 oxygen consumption experiments, with two specimens dying of all experimental animals.

The Kruskal-Wallis one-way ANOVA on ranks revealed significant differences in specific oxygen consumption (µmol g^{-1} fresh weight) between all five experiments with different animals ($p<0.01$). According to the multiple comparison post-hoc test (Dunn's Procedure), the following experiments were significantly different ($p<0.05$): Df 1 from Df 4, Df 1 from Df 3, Df 1 from Df 5, Df 5 from Df 3, Df 5 from Df 2.

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4. Discussion

Carapace as physical absorbance barrier against UV-photons in Antarctic amphipods

Higher carapace absorbance in the UV-range indicates slightly better (12.5%) UV-shielding in *D. furcipes* than in *G. antarctica*. Carapace absorbance of both Antarctic shallow water amphipods was lower than in the Arctic littoral species *Gammarellus homari*, which proved to be extremely UVR-tolerant (Obermüller & Abele 2004, Obermüller et al. 2005). Carapace absorbance spectra of *G. antarctica* and *D. furcipes* (Fig. 1) indicated MAAs and carotenoids to be incorporated into the chitinous exoskeleton matrix. Both types of sunscreening metabolites and pigments are known to be incorporated into the cuticle (Schiedt et al. 1993) and tissues of aquatic organisms (Karentz et al. 1991, Carefoot et al. 2000, Newman et al. 2000). Sagi et al. (1995) detected 66% on average of total tissue carotenoids, mainly astaxanthin, in the cuticle of crayfish *Cherax quadricarinatus*, suggesting a photo-protective role of the exoskeleton. Maximal absorbance of astaxanthin (485 nm) is within the third, undefined range of the carapace absorbance spectra in our amphipods (480-530). In an earlier study (Obermüller et al. 2003) we showed that MAA tissue concentrations were several times higher in *G. antarctica* than *D. furcipes* and similar to those of highly UV-tolerant Arctic *G. homari*. Contrasting, differences between *G. antarctica* and *D. furcipes* in carapace absorbance in the "MAA-range" (respective UVB-range) were not significant. Table 6 summarises data of tissue MAA content in Arctic and Antarctic species. Tissue MAAs were significantly reduced during 14 days of high-dose UVR and PAR exposure only in one Antarctic amphipod (*G. Antarctica* with $p<0.05$) but not in the other two species. Photobleaching of MAAs also occurred in the freshwater copepod *Boeckella titicacea*, with a decrease in total MAAs concentration already after 3 days exposure to surface level solar UVR (Helbling et al. 2002). In contrast, several authors demonstrated photostability of MAAs during prolonged UVR-exposure, which is fundamental for an effective sunscreening role

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(Conde et al. 2000, Newman et al. 2000, Shick & Dunlap 2002). In addition to higher total MAA content, *G. antarctica* had 1.3 – 1.6 times higher tissue carotenoid levels than *D. furcipes*, and catalase activity did not decrease as severely in *G. antarctica* as in *D. furcipes* during UVR+PAR-exposure. Catalase is highly susceptible to light exposure with damage actually being more prominent in the UVA- and adjacent blue range PAR (λ max 405 nm) than in the UVB part of the spectrum (Gantchev & van Lier 1995, Zigman et al. 1998). Accordingly, in our study also PAR alone inhibited amphipod catalase activity (decrease down to 55 and 36% of initial activity in *G. antarctica* and *D. furcipes*, respectively). Possibly the differences in sunscreening (MAAs) and antioxidant (catalase) potential between the two Antarctic species can in part explain better survival in *G. antarctica* than in *D. furcipes*. Contrary, in neither species did exposure to UVR cause significant bleaching of tissue carotenoids. Further, tissue protein carbonyl content, indicative of oxidative protein damage, did not increase in irradiated amphipods. Apparently, the antioxidant defence system in the amphipods' cells was sufficiently protective to prevent protein damage in spite of impaired catalase activity. Besides, protein turnover is efficient in removing carbonylated proteins (Levine 2002), and elevated respiration under UVR-exposure may also have included elevated rates of protein new synthesis.

Metabolic rate and response to UVR-exposure

Little variation of respiratory amplitudes during the initial low light phase indicates that the amphipods had adjusted to the experimental conditions and were at rest (RMR). Opalinski & Sicinski (1995) measured oxygen consumption in three intertidal amphipod species from Admiralty Bay, King George Island, adjacent to Potter Cove where our samples were taken. They found three times higher oxygen consumption for *G. antarctica* (16.06 ± 0.71 µmol O_2 g^{-1} FW h^{-1} at 1°C) than our values and lower respiration rates in the other two species:

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Bovallia gigantea 4.46 ± 0.49 µmol O_2 g^{-1} FW h^{-1} and *Eurymera monticulosa* 5.26 ± 0.27 µmol O_2 g^{-1} FW h^{-1} at 1°C. These latter rates were similar to what we recorded for *G. antarctica* and *D. furcipes.* Amphipods in Opalinski & Sicinski's study received natural daylight and were adapted for 36 h in an aquarium containing macroalgal food prior to the measurements. In contrast, our animals were acclimated for between one and two weeks to aquarium maintenance conditions. As respiration in the other study was recorded without offering substratum to settle, higher locomotive activity may account for the higher oxygen consumption of *G. antarctica*, an effect also described for other Antarctic amphipods by Chapelle & Peck (1995). Also, unclear nutritional history prior to sampling as well as the reproductive state may have caused the difference in respiration rates of *G. antarctica* in the two studies.

Amphipods showed an immediate stress response when acutely exposed to UVR+PAR light, reflected in increased respiration rates. Although swimming and more vigorous movements were restricted, minor movements were possible and observed when the black cover was removed and animals were UVR exposed. A corresponding increase in oxygen consumption was observed for an interval of 60 to 120 min before respiration rates decreased again, indicating that animals were resettling to the gauze. A pronounced decrease in mean oxygen consumption with high and variable amplitudes during UVB+UVA+PAR-exposure reflects exhaustive activity followed by depression of metabolic performance to control levels or even lower under UVR. UVA and PAR blue- and green-light photoreceptors have been described in a variety of marine and fresh water organisms (Shashar 1994, Leech & Johnsen 2003) and crustaceans such as *Daphnia* and are supposed to play a role in negative phototaxis (Storz & Paul 1998). Elevated oxygen consumption forms part of an avoidance and flight response during which the animals seek shelter underneath algal thalli. Following the initial high stress

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respiration periods, the animals become metabolically depressed, which parallels H_2O_2 exposure experiments we performed earlier with polychaetes and mudshrimp (Storch et al. 2001, Abele-Oeschger et al. 1997). This provides a first hint that ROS, formed in tissues during exposure to intense UVR could also lead to metabolic slow down in irradiated animals. Juvenile Antarctic krill (*Euphausia superba*, Newman et al. 1999) exposed to experimental irradiation (PAR, PAR+UVA, PAR+UVA+UVB) exhibited less swimming activity (visual activity scoring), compared to the dark controls. No significant differences were seen between UVA (5.01 Wm^{-2}) and high (0.92 Wm^{-2}) or low (0.38 Wm^{-2}) UVB treatments. Krill perceives UVA, but no UVB light (Denys 1982, Quetin et al. 1998), and the slowdown of swimming causes these pelagic animals to sink passively out of the photic zone to greater depths. Further, Newman et al. (2003) found that krill avoids tank areas with high UVA and PAR, which under natural radiation exposure simultaneously reduces UVB-exposure and photo-damage. The behavioural response (passive sinking) is indicative of decreased respiration under UVB-exposure in krill. In our shallow water amphipods accelerated swimming activity was observed only when animals were initially exposed to UVR in aquaria. After a couple of hours this effect ceased. In long term UV-experiments lasting up to 3 week we observed accelerated activity in response to repeated UVR exposure only during the first 3-4 days. After 2 weeks, the amphipods appeared altogether apathetic and metabolically exhausted.

Increased respiration under exposure to strong UVR may reflect a stress response directed towards repair of ROS damage occurring under direct radiation exposure in nature, where ROS are generated in animal tissues but also in the surrounding sea water (Abele et al. 1999). Increased mean respiration of more than 200% over RMR during irradiation in *G. antarctica* and more than 500% over RMR in *D. furcipes* in the experiments reflect increased energy demand due to radiation avoidance, but could also involve costs of repair processes (light

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dependent photoenzymatic repair) of direct DNA-damage as shown for Antarctic juvenile fish species and adult krill by Malloy et al. (1997). In these species, DNA-damage and repair are simultaneous processes and UVB-induced cyclobutane pyrimidine dimers (CPDs) are repaired very rapidly within one day. This is also the case for northern anchovy eggs and larvae, where UV-induced DNA-damage (CPDs) and photoenzymatic repair happen very fast (within hours) and the daily cycle of UVR damage and photorepair follow the solar intensity, with a peak in CPD accumulation at noon, followed by a decline in CPD content until sunset, due to photorepair (Vetter et al. 1999). Dark repair during low light recovery phases in our experiments could explain oxygen consumption, 200% elevated over initial non-stressed rates. However, studies of Malloy et al. (1997) showed that dark repair was considerably slower and of minor importance than photoenzymatic repair, with less than 15% of UV-induced CPDs removed after 24 h. This holds true also for *Daphnia* (Williamson et al. 2001), in which photoenzymatic repair was the most important strategy of UVR defence and dark repair contributed only very little to survival. Goncalves et al. (2002) measured significantly higher survival rates for the copepod *Metacyclops mendocinus* from a Patagonian lake in animals exposed to UVB in the presence of photoreactivating radiation (PAR+UVA) as compared to exposure to UVB alone, indicating UVA and PAR to play a role in photo-enzymatic repair.

Simulated irradiation conditions are rarely an exact reflection of the natural habitat with respect to spectral ratios (UVB:UVA:PAR) important for photoenzymatic repair capacity (Williamson et al. 2001). Spectral ratios (UVB:UVA:PAR) in our experiments were (1:27:78) in the SONSI and (1:10:15) in the Q-Panel and white light setting as compared to (1:15:91) measured in the field during the experimental period (October - December 2002). Thus, artificial SONSI light should provide sufficient photoreactivating radiation to activate photo-

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repair in exposed amphipods in our experiments (Goncalves et al. 2002, Williamson et al. 2001). In contrast, the Q-Panel light is probably insufficient for photoreactivation, which may explain the higher mortality of *D. furcipes* in low-dose exposure.

Is UVB-exposure more stressful for physiological processes than UVA?

While UVB's detrimental influence has been widely demonstrated (e.g. Holm-Hansen et al. 1993, Vincent & Roy, 1993), UVA can have beneficial (e.g. photoenzymatic repair, Williamson et al. 2001) and adverse effects. The freshwater calanoid copepod *Diaptomus* was sensitive to UVB exhibiting increased mortality when exposed to natural solar radiation, while survival in UVB-shielded animals, which received natural UVA+PAR-radiation, was not affected (Williamson et al. 1994). By contrast, in the same study, in situ UVA+PAR-exposure significantly reduced survival in two cladoceran species (*Daphnia* and *Diaphanosoma*) with equally high mortality in UVB-shielded and unshielded *Diaphanosoma* over all depth ranges (0-6 m), and better survival in UVB-shielded *Daphnia* only below 2 m water depth. In our *G. antarctica* experiments, UVB as well as UVA seemed to be effective stressors, causing significant increases in oxygen consumption, whereas *in D. furcipes* the UVB-effect on respiration was clearly dominating the UVA effect. Respiratory amplitudes however were more affected by UVB in *G. antarctica* than *D. furcipes*, where both UVB and UVA-exposure increased variability of oxygen consumption.

Conclusion

Both Antarctic amphipod species are herbivores, feeding on macroalgae of the intertidal zone, and incorporating algal metabolites as sunscreening compounds into tissues and into the exoskeleton to different proportions. Both strategies obviously confer sufficient protection from UVR, as the animals colonise shallow intertidal environments. However, in our

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experiments *G. antarctica* seemed to possess a more efficient physical and chemical defence compared to *D. furcipes*, protecting the species from direct UV-damage and promoting better survival. Exposure to UVR caused a direct stress response in locomotive activity, reflected in the increased respiration rates and, specifically, in the UVA- and UVB-induced instantaneous changes of respiratory amplitudes. The observed metabolic increase on exposure to UVR may involve elevated oxygen demand due to repair of UV-induced and ROS-mediated oxidative damage.

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Tables for manuscript Obermüller et al.

Table 1: Total carotenoid concentration (µmol ß-carotene equivalents g^{-1} fresh weight FW) in whole animal homogenates from Antarctic amphipods from Potter Cove exposed to low-dose irradiation (Q-Panel-tubes: 0.38 Wm^{-2} UVB, 3.68 Wm^{-2} UVA, 5.73 W m^{-2} PAR). Values are mean ± SD. Numbers in brackets indicate replicates per value. T-tests carried out between controls vs. UVB+UVA+PAR and controls vs. PAR at 10 days.

Table 2: Catalase activity (U mg^{-1} FW) in whole animal homogenates from Antarctic amphipods from Potter Cove exposed to low-dose irradiation (Q-Panel-tubes, see Tab. 1). Values are mean ± SD. T-tests carried out between controls vs. radiation treatments at each time interval. * indicates significant differences at a level of $p<0.05$.

Table 3: Protein carbonyl content (nmol mg^{-1} protein) in whole animal homogenates from Antarctic amphipods from Potter Cove exposed to low-dose irradiation (Q-Panel-tubes, see Tab. 1). Values are mean ± SD. T-tests carried out between controls vs. radiation treatments at each time interval. * indicates significant differences at a level of $p<0.05$.

Table 4: Oxygen consumption (µmol O_2 g^{-1} FW h^{-1}) of *G. antarctica* (Ga) expressed as M O_2 and amplitudes and given as the overall mean ± SD of all 30 min values for each experimental phase in the solar simulator (1.5 Wm^{-2} UVB, 39.7 Wm^{-2} UVA, 117.7 Wm^{-2} PAR). [1)] Wilcoxon rank test carried out instead of paired t-test because normality test failed.

Table 5: Oxygen consumption (µmol O_2 g^{-1} FW h^{-1}) of *D. furcipes* (Df) expressed as M O_2 and amplitudes and given as the overall mean ± SD of all 30 min values for each experimental

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phase in the solar simulator (1.5 Wm^{-2} UVB, 39.7 Wm^{-2} UVA, 117.7 Wm^{-2} PAR). [1)] n.d. not determined due to sensor problems, experiment stopped after 2.5 h.

Table 6: Total MAA concentration (µg g^{-1} dry weight DW) in herbivorous amphipod species *G. antarctica* and *D. furcipes* from Potter Cove (Antarctic) and *G. homari* from Kongsfjord (Arctic). Values are mean ± SD. [1] data from Obermüller et al. 2003, high-dose exposure 1.35 Wm^{-2} UVB, 15.67 Wm^{-2} UVA, 134.08 Wm^{-2} PAR. (+UVB)-treatment: UVB+UVA+PAR, (-UVB)-treatment: UVA+PAR. [2] data from Obermüller et al. 2005, high-dose exposure for [A] 1.30 Wm^{-2} UVB, 21.84 Wm^{-2} UVA, 117.66 Wm^{-2} PAR, low-dose exposure for [B] 0.40 Wm^{-2} UVB, 3.70 Wm^{-2} UVA, 5.70 Wm^{-2} PAR. T-tests carried out between [C] controls vs. radiation treatments and between [D] (+UVB)- vs. (-UVB)-treatments. * indicates significant difference at a level of $p<0.05$.

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Figure 1: Carapace absorbance spectra between 295 and 700 nm of *G. antarctica* (Ga, lower grey curve) and *D. furcipes* (Df, upper black curve) recorded in the solar simulator (SONSI). Arrows indicate bands of higher absorbance corresponding to maximal absorbance ranges of sunscreening substances (MAAs: mycosporine-like amino acids).

Figure 2: *G. antarctica*. Oxygen consumption (µmol O_2 g^{-1} FW h^{-1}) for experiments Ga 1-4 given as means (dots) during 30 min time intervals in each experimental phase and amplitudes depicted as bars around each 30 min mean.

Figure 3: *D. furcipes*. Oxygen consumption (µmol O_2 g^{-1} FW h^{-1}) for experiments Df 1-5 given as means (dots) during 30 min time intervals in each experimental phase and amplitudes depicted as bars around each 30 min mean.

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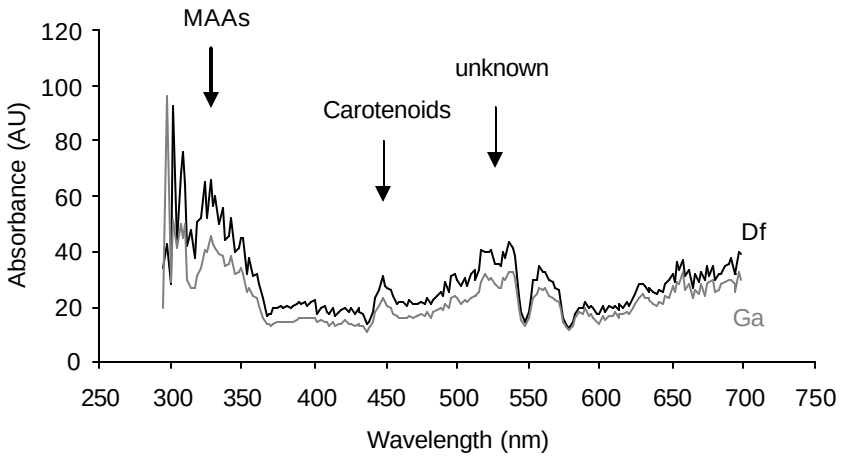

Figure 1

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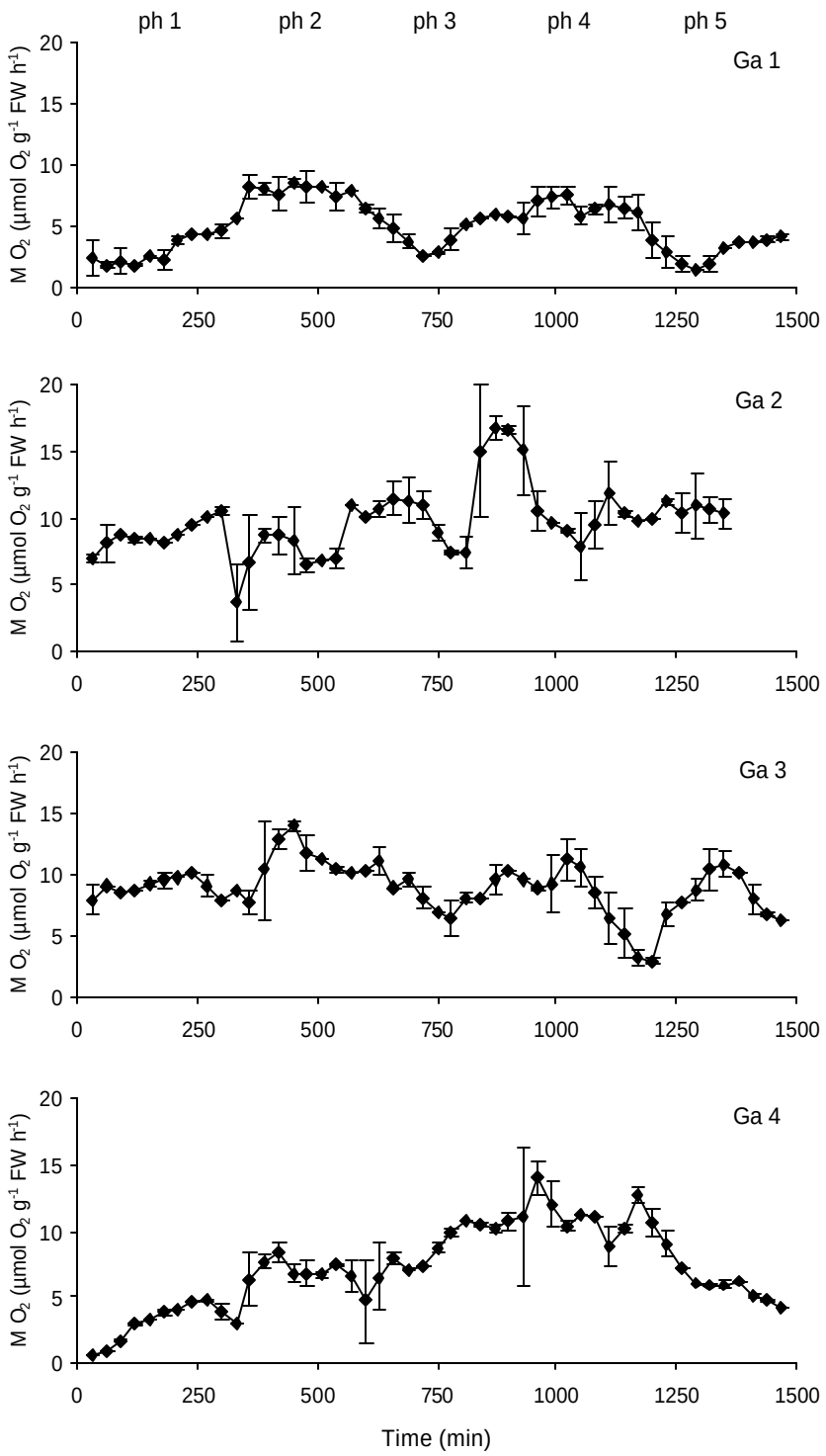

Figure 2

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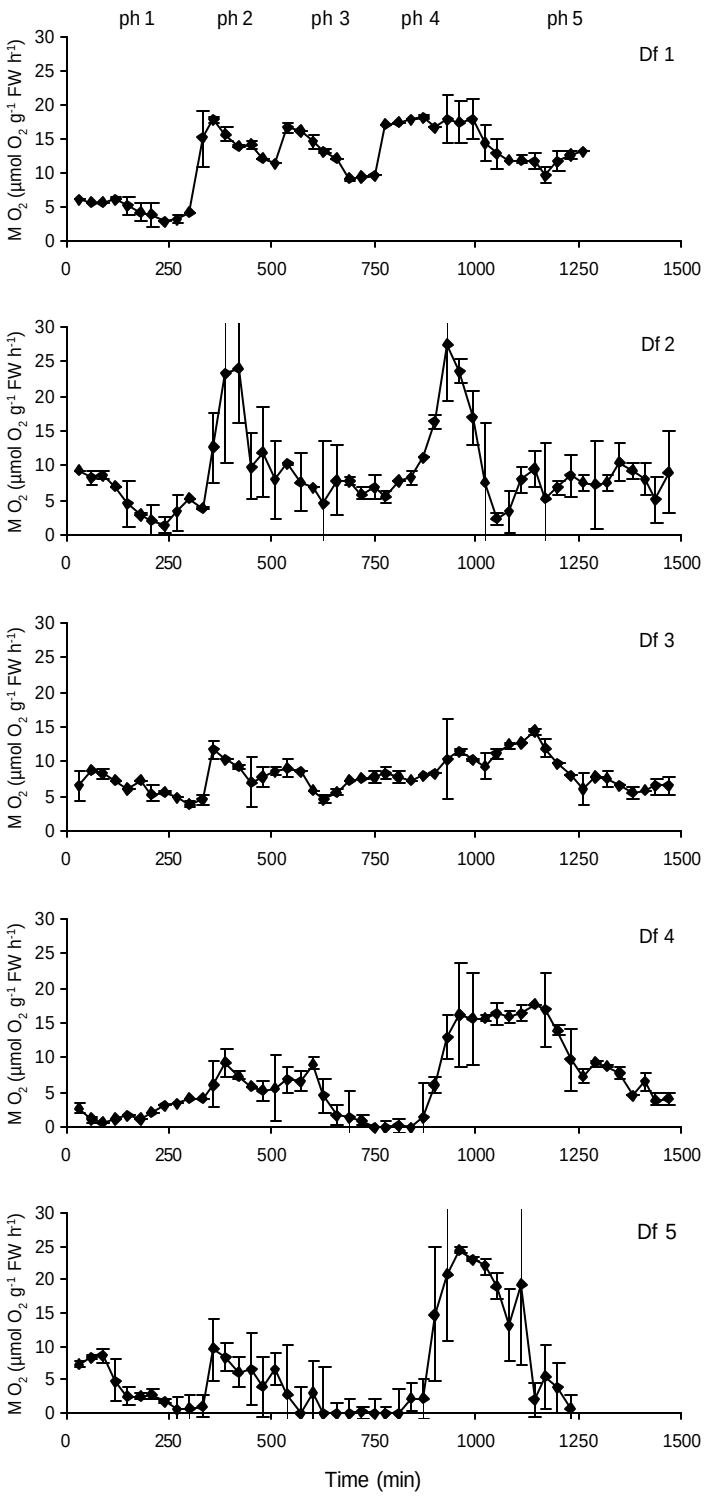

Figure 3