Effects of UV-radiation on crustaceans from polar and temperate coastal ecosystems

Effekte der UV-Strahlung auf Crustaceen aus polaren und temperaten Küstenökosystemen

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List of frequently used abbrevations

BWF	biological weighting function
DNA	desoxyribonucleic acid
DOC	dissloved organic carbon
DOM	dissolved organic matter
DU	Dobson Unit
e.g.	for example, abbrevation for "*exemple gratia*" (Latin)
H_2O_2	hydrogen peroxide
HPLC	high-performance liquid chromatography
i.e.	that is, abbrevation for "*id est*" (Latin)
K_d	downwelling diffuse attenuation coefficient
MAAs	mycosporine-like amino acids
MDA	malondialdehyde
PAR	photosynthetically active radiation
PER	photoenzymatic repair
Publ.	Publication
PUFAs	polyunsaturated fatty acids
ROS	reactive oxygen species
SOD	superoxide dismutase
SONSI	sunshine simulator
TBARS	thiobarbituric-acid-reactive substances
TOMS	total ozone mapping spectrometer
UV	ultraviolet
UVA	ultraviolet A
UVB	ultraviolet B
UVR	ultraviolet radiation

Summary

Solar radiation is a fundamental physical force, modulating the earth's ecosystems. The visible range of the solar spectrum most notably comprises beneficial effects, promoting processes such as photosynthesis, production of organic matter and oxygen generation. The ultraviolet (UV) portion of the solar spectrum, however, induces various detrimental effects in both terrestrial and aquatic organisms on all systemic levels. Over millions of years species have evolved mechanisms to tolerate, avoid, and repair UV-induced damage. This balance of damage and repair could be obstructed by recent ozone depletion, which causes a selective increase in harmful UVB radiation reaching the earth's surface.

I studied the effects of UV-exposure on different UV- and oxidative stress parameters and defence systems against direct and indirect UV-damage in polar and temperate shallow water amphipods (crustaceans). The UV-tolerance was compared in species from two different polar regions, the Antarctic Potter Cove (King George Island) and the Arctic Kongsfjord (Spitsbergen), currently undergoing different degrees of ozone depletion. This comparison was carried out in relation to a reference species from a temperate North Sea coast (Helgoland), which displays higher natural UV-impact, however, lower ozone depletion compared to the polar areas. I distinguished between dose- and wavelength-dependent effects and also considered the possible influence of nutrition on UV-protective capacities by comparing herbivorous and carnivorous/necrophagous amphipods.

The investigated species were classified into three different UV-tolerance categories, according to their sunscreening and antioxidant defence capacities. Herbivorous *Gammarellus homari* (Kongsfjord) proved to be the most UV-tolerant of all investigated species. High concentrations of dietary derived mycosporine-like amino acids and carotenoids combined with inducible increases of antioxidant superoxide dismutase activity promoted highest survival rates under all UVR-exposure experiments. Its high UV-tolerance threshold allows *G. homari* to successfully colonise shallow water habitats in Kongsfjord.
Herbivorous Antarctic *Gondogeneia antarctica* and *Djerboa furcipes* as well as carnivorous/necrophagous Arctic *Onisimus edwardsi* had deficiencies in their sunscreening and antioxidant defence, which led to accumulation of lipid peroxidation products in the amphipods' tissues. As survival rates were still above 50% under most irradiation treatments, these species were classified as moderately UV-tolerant. The species-specific UV-tolerance

thresholds resembled thereby depth gradients of occurrence in the field, with *G. antarctica* shriving in shallower, intertidal and *D. furcipes* and *O. edwardsi* in deeper, sub-tidal habitats. Arctic carnivorous/necrophagous *Anonyx nugax* was the most UV-sensitive of all investigated species, exhibiting increased mortality, bleached tissue pigmentation, impaired antioxidant enzyme activities and increased lipid peroxidation already under exposure to a moderate UVB-dose. Under the current UV-radiation regime, *A. nugax* is confined to deeper water during midday peak irradiance and rarely found in subsurface depths in Kongsfjord.

Zusammenfassung

Solare Strahlung beeinflusst als fundamentale Kraft die Ökosysteme der Erde. Der sichtbare Bereich des Sonnenspektrums wirkt vor allem positiv und fördert Prozesse wie Photosynthese, Produktion organischen Materials und Sauerstoffbildung. Der ultraviolette (UV) Anteil des solaren Spektrums induziert jedoch unterschiedliche schädliche Effekte in terrestrischen wie auch aquatischen Organismen auf allen systemischen Ebenen. Im Laufe von Jahrmillionen haben die Arten Schutzmechanismen ausgebildet, um die ausgelösten UV-Schäden tolerieren, vermeiden oder reparieren zu können. Aber dieses Gleichgewicht von Schaden und Reparatur wird womöglich in der jüngsten Zeit gestört durch die Verminderung der Ozonschicht, die selektiv einen Anstieg der schädlichen UVB-Strahlung an der Erdoberfläche verursacht.

Ich untersuchte die Effekte der UV-Bestrahlung auf unterschiedliche Stressparameter und Verteidigungsmechanismen gegen direkte und indirekte UV-Schädigung in Amphipoden (Krebsen) aus dem Flachwasser polarer und gemäßigter Breiten. Dabei wurde die UV-Toleranz der Arten aus zwei unterschiedlichen polaren Regionen, der Antarktischen Potter Cove (King George Island) und dem Arktischen Kongsfjord (Spitzbergen), miteinander verglichen, die momentan unterschiedlich starker Zerstörung der Ozonschicht unterworfen sind. Dieser Vergleich wurde in Relation zu einer Referenzart aus einem Küstenbereich der gemäßigten Breiten (Helgoland, Nordsee) durchgeführt, die zwar eine höhere natürliche UV-Einstrahlung aufweisen, aber eine schwächere Ozonverminderung im Vergleich zu den Polargebieten. Ich unterschied zwischen Dosis- und Wellenlängen-abhängigen Effekten und berücksichtigte ausserdem den Einfluss der Ernährungsweise auf die Kapazität des UV-Schutzes in herbivoren und carnivoren/necrophagen (Aas-fressenden) Amphipoden.

Die untersuchten Arten wurden in Abhängigkeit ihres Sonnenschutzpotentials und ihrer antioxidativen Kapazitäten in drei unterschiedliche Kategorien der UV-Toleranz klassifiziert. Die herbivore Art *Gammarellus homari* erwies sich als UV-toleranteste aller untersuchten Arten. Die hohe Konzentration an aus der Nahrung aufgenommenen Mycosporin-ähnlichen Aminosäuren und Carotenoiden, kombiniert mit einem induzierbaren Anstieg der Aktivität der antioxidativen Superoxiddismutase bewirkte die höchsten Überlebensraten unter allen experimentellen Bestrahlungsbedingungen. Seine hohe UV-Toleranz-Schwelle ermöglicht *G. homari*, Habitate im Flachwasser des Kongsfjords erfolgreich zu besiedeln.

Die herbivoren Antarktischen Arten *Gondogeneia antarctica* und *Djerboa furcipes*, sowie die carnivore/necrophage Arktische Art *Onisimus edwardsi* wiesen dagegem Defizite in ihrem Sonnenschutzpotential und ihrer antioxidativen Verteidigung auf, die dazu führten, dass sich Lipidperoxidationsprodukte in den Geweben der Amphipoden anreicherten. Da jedoch die Überlebensraten unter den meisten Bestrahlungsbedingungen über 50% lagen, wurden diese Arten als moderat UV-tolerant klassifiziert. Hierbei spiegelt die artspezifische UV-Toleranz-Schwelle den Tiefengradienten im Vorkommen dieser Arten im Feld wider: *G. antarctica* besiedelt den flacheren (litoralen) Bereich der Gezeitenzone, *D. furcipes* und *O. edwardsi* eher den tieferen (sub-litoralen) Bereich.

Die carnivore/necrophage Arktische Art *Anonyx nugax* war die UV-sensitivste aller untersuchten Arten und zeigte bereits nach Bestrahlung mit einer moderaten UVB-Dosis eine erhöhte Mortalität, ausgebleichte Pigmentierung des Gewebes, geminderte Aktivität der antioxidativen Enzyme und erhöhte Lipidperoxidation. Unter dem momentanen UV-Strahlungsklima im Kongsfjord ist *A. nugax* während der stärksten Sonneneinstrahlung am Mittag auf größere Wassertiefen beschränkt und tritt nur selten im Flachwasser auf.

1 Introduction

"Solar radiation is the fundamental ecosystem modulator" (Wetzel 2003). The different components of solar radiation provide the necessary energy for the generation of organic matter (visible range: photosynthesis) and its degradation (ultraviolet range: photolysis and oxidation, bio-availability for microbial degradation). Consequently, solar radiation contributes to photosynthetic oxygen evolution into the atmosphere at levels sufficient for respiration of heterotrophic organisms, and also adds heat to the Earth and maintains the temperature within boundaries required for the functioning of biological processes (Diamond 2003). Besides these beneficial effects, the ultraviolet (UV) portion of the solar spectrum in particular induces various detrimental effects in both terrestrial and aquatic organisms. These are all naturally occurring processes and over millions of years of evolution species have developed mechanisms to avoid harmful effects and to repair damage, at least at natural intensity and dose levels. However, recent decreases in ozone concentrations, which result in an increase in harmful UVB radiation (280-315 nm), could exacerbate the situation and obstruct the balance between damage and repair, the organism's UV-tolerance. The tolerance of polar marine crustaceans towards these UV-induced effects is the main focus of this study.

1.1 Solar spectrum composition and determinants

Solar radiation reaching the Earth's surface constitutes of light in the ultraviolet, the visible and infrared range (700-2000 nm). UVC radiation (wavelength λ<280 nm) is extremely energy-rich and hence biologically harmful, but entirely absorbed by stratospheric gases (e.g. ozone). UVB radiation (280-315 nm) is also highly energetic and a very important photoactivating agent in aquatic processes, with many detrimental effects to biomolecules. Only part of the UVB passes through the stratosphere reaching the Earth's surface. The less energetic UVA radiation (315-400 nm) transmits almost freely through the atmosphere, and also penetrates deeper into the water column. Additionally, the blue portion of the visible spectrum (400-500 nm), which is part of the photosynthetically active radiation (PAR 400-700 nm), is functionally similar to the near UVA radiation in many important photochemical reactions (Wetzel 2003).

The Commission Internationale d'Eclairage (CIE) define UVB as ranging from 280-315 nm and UVA as from 315-400 nm. In this thesis however the more 'pragmatic' ranges for UVB (280-320 nm) and UVA (320-400 nm) are applied in all experiments, as commonly found in

literature. In addition, all the radiometers used in the experiments are calibrated to these spectral ranges. The solar spectrum 'flattens out' around 320 nm, causing only very small response in biological action spectra (Blumthaler & Webb 2003).

Factors determining UV radiation (UVR) at the Earth's surface are solar elevation, ozone, aerosol and pollutant gases content, altitude, season, albedo, and cloudiness (Blumthaler & Webb 2003). Penetration of UVR in aquatic ecosystems is strongly influenced by dissolved organic matter (DOM), especially chromophoric dissolved organic carbon (DOC) and other mainly humic substances (="Gelbstoffe") (Hargreaves 2003). DOM is not only important in regulating the distribution, attenuation and absorption of UVR and visible blue light (400-500 nm) in marine and freshwater ecosystems but also the direct and indirect UV-effects on metabolism, growth and reproduction of the organisms living therein. Physical processes are influenced through photochemical modifications of organic macromolecules (partial or complete photolysis), resulting in alteration in biological availability of DOC (Wetzel 2003). Not only dissolved but also particulate matter such as phytoplankton cells, as well as nucleic acids, proteins and pigments of living cells absorb UVR and PAR with various beneficial and adverse effects (Williamson et al. 2001).
Underwater solar radiation is commonly measured as irradiance (Wm^{-2}), which defines the energy striking a unit of surface area (Hargreaves 2003). In marine and fresh water two factors describe the radiation transmittance through the water column: the percent attenuation depth and the downwelling diffuse attenuation coefficient (K_d), which is proportional to the concentration of absorbing or scattering substances in the water and wavelength-specific (Smith & Baker 1978).

1.2 Thirty years of ozone depletion: implications for long-term UVR trends

2005 was the year of the "20th anniversary" of the discovery of the Antarctic 'ozone hole'. The concentration of stratospheric ozone has been declining since the mid-1970s (Molina & Rowland 1974) and in 1985 Farman and colleagues first reported annual ozone depletion over Antarctica (Farman et al. 1985). The last decades have seen over 20 years of ozone research, making the 'ozone hole' and induced increases in solar UVB radiation to one of the best studied, human-made global environmental problems and challenges. Stratospheric ozone serves as a protective shield against short wavelength, high energetic UVC and UVB radiation in this upper atmospheric region (15-25 km). UVR is absorbed and scattered by the ozone-air

mixture in the stratosphere containing cloud droplets and aerosols. The transmitted absorption energy results in heating or breaking of chemical bonds (Blumthaler & Webb 2003 and references therein). Contrasting, tropospheric ozone in the lower atmosphere (0–13 km on average) with origins in industrialised and polluted regions (smog) is detrimental to humans and terrestrial life, causing lung and heart diseases when formed. As a powerful oxidising agent ozone can react with proteins, lipids and DNA (Hermes-Lima 2004).

Ozone is produced in a two-stage process by the action of radiation (with wavelengths $\lambda<242$ nm) on molecular oxygen. This yields two oxygen atoms, which then react with molecular oxygen to form ozone (equation 1 and 2):

(1) $O_2 + h\nu \rightarrow O + O$

(2) $O + O_2 \rightarrow O_3$

Ozone loss occurs through various different reactions (equations 3 to 6) also involving catalytic cycles of free radicals (X) such as NO, HO and halogen radicals in presence of light ($\lambda<800$ nm).

(3) $O_3 + h\nu \rightarrow O + O_2$

(4) $O + O_3 \rightarrow 2\ O_2$

(5) $O_3 + X \rightarrow O_2 + XO$

(6) $O + XO \rightarrow O_2 + X$

The ozone concentration is expressed as Dobson Units (DU), where 100 DU correspond to an ozone layer of 1 mm thickness at standard surface pressure and temperature. Most ozone is formed over the equator and transported poleward through atmospheric circulation (Whitehead et al. 2000). Ozone formation and destruction create a dynamic balance and the ozone layer naturally fluctuates in thickness through the year. This dynamic photochemical equilibrium can be disturbed by extra species of destructive catalytic molecules (especially chlorine) from anthropogenic releases of pollutive gases into the atmosphere (Blumthaler & Webb 2003). Especially the catalytic action of chlorofluorocarbons (CFCs) leads to severe decreases in ozone concentration, but also halogen source gases containing bromine, e.g. methyl bromide, an agricultural fumigant, are efficient ozone destructive substance. Total column ozone of around 290 DU has been quite similar at both poles in the last 15 years during summer/autumn, however, winter and spring time series have differed severely (Rex & von der Gathen 2004 and references therein). While measurements over the Antarctic

Neumayer Station revealed an annual 'ozone hole' formation with a decline of total column ozone down to around 100-150 DU, measurements at the Arctic Koldewey Station showed highly interannual variability in total column ozone, with overall increases in average values in March (300-500 DU, Rex & von der Gathen 2004). When chemical ozone destruction occurred in the Arctic, ozone loss ranged between 0-100 DU in certain years, e.g. March 2000, and rather balanced out the annual increase through dynamic poleward ozone supply than to create an 'ozone hole' situation. However, in February/March 2005 approximately 30% of total column ozone over the Arctic was destroyed (WMO press release 04/2005). Ozone depletion is highest over the Antarctic (Southern spring), followed by the Southern hemisphere mid-latitudes (Southern spring) and finally the Arctic (Northern winter/spring). It is less severe over Northern hemisphere mid-latitudes (Madronich et al. 1998). No changes in ozone concentration have been reported over the tropics so far.

Antarctic and Arctic ozone losses differ because the different distribution of land and sea in the Northern hemisphere allows for only a weak polar vortex (prevailing winds) over the Arctic. The polar vortex is very strong over the Antarctic in winter and acts as a trap for ozone as well as ozone destroying species and polar stratospheric clouds (PSC). PSC form due to low temperatures (below -80°C), providing suitable surfaces (droplets, aerosol particles) for rapid ozone destruction cycles and chain reactions with returning sunlight (Whitehead et al. 2000). Variability of Arctic chemical ozone loss is almost exclusively driven by the variation of stratospheric temperatures during the Arctic winter (Rex & von der Gathen 2004). PSC formation is higher in cold winters, thus large ozone losses are likely during cold winter as shown for the year 1999/2000, when local ozone loss reached 70% at about 19-20 km altitude, and total column ozone loss exceeded 80 DU (approx. 20-25% of total column ozone) by early spring (WMO 2002). Warmer, more disturbed Arctic winters show hardly any chemical ozone loss, such as in 1998/1999. If cooling of the Arctic stratosphere continues, a process possibly linked to increasing greenhouse gas concentrations in the atmosphere, ozone losses may become worse in the next decades. Although, extreme 'ozone holes' like over the Antarctic are highly unlikely (Salawitch 1998, WMO 2002, Rex & von der Gathen 2004).

Recent ozone depletion has brought up an increase of the harmful UVB-portion of the solar spectrum, with significantly higher UVR values in the Southern hemisphere compared to the North at same solar elevations and the corresponding summer time (Seckmeyer & McKenzie 1992). Even though the Montreal Protocol (1987), which limits and bans the CFCs-emissions, is under force and ozone losses seem to have reached maxima in consecutive years (records 2000, 2002/2003, 2005) with minimum values around 100 DU, and e.g. 50% reduction of

normal ozone levels over the Antarctic Peninsula and the Weddell Sea region in 2005 (source: WMO, World Climate News 2001-2005, WMO Scientific Assessment of Ozone Depletion 2002, British Antarctic Survey: www.antarctica.ac.uk), total recovery will probably not be reached before the year 2050 (Madronich et al. 1998, WMO 2002). Long-term UVR trends however are difficult to evaluate as ozone is not the only determinant, cloudiness for example has a stronger modulating impact on UVR (Booth et al. 1997). Also aerosols, water vapour, pollution gases and ground albedo all influence UV-irradiation and atmospheric penetration. Ground-based ozone measurements and satellite data (TOMS, total ozone mapping spectrometer) reveal trends of erythema weighted UVR of around 5-10% increase per decade in the last two decades for Northern Europe (Kaurola et al. 2000). Further severe increases (>20%) in UVB-radiation are not likely to occur but current natural peak UVB doses are already biologically effective, leading e.g. to alteration in phyto- and zooplankton assemblages with implications for higher trophic levels (Browman et al. 2000, Vincent & Roy 1993, Williamson et al. 1994).

1.3 UV radiation effects: direct and indirect stress – damage – death

UVR is very harmful to biological processes causing a broad array of direct and indirect (oxidative) genetic and cytotoxic damage to DNA, proteins and pigments with alteration of structure and function of important biomolecules (Malloy et al. 1997, Buma et al. 2003). Inhibition of important cellular processes has negative effects on tissue and organ levels and increased energetic costs of protection and repair may lead to decreased metabolic activity, growth or reproduction, also to immunosuppression and finally increased mortality (Aguilera et al. 1999, Häder et al. 1998, Knowles 1992, Lesser et al. 2001 and 2003, Newman et al. 1999, Steeger et al. 2001). At a population level UVR can alter plankton or macroalgal assemblages and potentially affect distribution and survival of other aquatic taxa such as associated crustacean species (Holm-Hansen et al. 1993, Hop et al. 2002, Madronich et al. 1995, Wiencke et al. 2000).

1.3.1 *Direct effects are induced by absorption of photon energy*

Nucleic acids strongly absorb radiation energy in the UVB range causing formation of photoproducts and mutations, which disrupt DNA replication and translation. Photoinduced DNA-damage comprises cyclobutane pyrimidine dimers (CPDs), exclusively in the UVB range, and also other lesions formed at lower rates such as pyrimidine 6-4 pyrimidone

photoproducts (6-4 PP) (Buma et al. 2003). Generally, wavelengths below 302 nm cause more damage than higher wavelengths. Melanomas (skin damage) and cataracts (eye damage) are not only present in humans but also shown for fish (Nairn et al. 1996) and amphibians (Hofer 2000, Little & Fabacher 2003). Ultimately, accumulated DNA damage will result in increased mortality (Kouwenberg et al. 1999 I and II, Lesser et al. 2006). Targets are key proteins e.g. antioxidant catalase (Butow et al. 1994, Cheng et al. 1981, Zigman et al. 1996), lens proteins (Weinreb & Dorvat 1996), and rubisco, the key enzyme of photosynthesis (Hidema et al. 1996). Bleaching of protein-based pigments such as phycobilliporteins in cyanobacteria (Lao & Glazer 1996,) and other pigments such as ß-carotene in skin of humans and animals as well as carotenoids in algae (Biesalski 1996, Vincent & Neale 2000). Under favourable natural conditions UVR damage and repair are balanced, however ozone depletion (UVB increase) or insufficient energy supplies can bias this equilibrium. Also, non photoenzymatic repair of CPDs and 6-4 PP is susceptible to errors and may lead to point mutations in the genome, resulting in impairment or even complete loss of biological function (Buma et al. 2003 and references therein).

1.3.2 *Indirect effects are mediated via photosensitising agents*

Absorption of photon energy by intermediate compounds (photosensitising agents) leads to formation of reactive oxygen species (ROS) inside or outside cells, including free radicals and non-radicals (Halliwell & Gutteridge 1999, Whitehead et al. 2000). Besides, ROS are continuously produced during natural cellular processes such as photosynthesis (chloroplasts), during mitochondrial respiration, at the endoplasmatic reticule, and during autoxidation inside cells (Asada & Takahashi 1987, Halliwell & Gutteridge 1999, Brookes 2005). ROS are also produced in the surrounding aquatic medium during geochemical cycling under visible light, however, the rate of production is greatly accelerated in the presence of UVR (Hermes-Lima 2004, Kieber et al. 2003).

ROS are generated externally through absorption of solar energy by DOM (equation 7), followed by a series of photochemical transformation, where excited state DOM* reacts with oxygen resulting in various ROS (e.g. superoxide $O_2^{-\bullet}$, singlet oxygen $^1O_2$, hydrogen peroxide H_2O_2, hydroxyl radical $^\bullet OH$) and recycling DOM to ground state (Kieber et al. 2003). H_2O_2 photoproduction was demonstrated for Antarctic surface waters in Potter Cove (King George Island, South Shetlands), where 90 ± 40 nmol H_2O_2 h^{-1} l^{-1} were produced in natural sea water under experimental conditions (Abele et al. 1999). This rate, however, is low because DOC concentrations of 121 ± 59 µmol Cl^{-1} in Potter Cove were low in comparison to 50-fold

higher DOC levels in for example the temperate German Wadden Sea coast, where H_2O_2 accumulates to between 1000 and 4000 nmol l^{-1} in intertidal pools during summer (Abele et al. 1997b).

Internally, sources for ROS are the mitochondrial respiratory chain, the endoplasmatic reticule as well as several enzymes and pigments or cellular compounds (P in equation 8, e.g. aromatic amino acids such as tryptophan, hemes, retinal), primarily generating superoxide and recycling the excited state photosensitising agent (P*) to ground state (P) (Halliwell & Gutteridge 1999). Once formed, ROS can initiate various fast reactions resulting in photoproducts that might be more vulnerable than their precursors or the direct UVR effects.

Externally (7) $DOM + h\nu \rightarrow DOM^*$

$DOM^* + O_2 \rightarrow O_2^{-\bullet} + DOM$

Internally (8) $P + h\nu \rightarrow P^*$

$P^* + O_2 \rightarrow P + O_2^{-\bullet}$

The impact of external ROS is a function of permeability through cell membranes and inversely related to reactivity in the external media. Their direct effect is minimal due to high reactivity and very short half-life times but they can produce longer-lived free radical species (Kieber et al. 2003). Diffusion of charged ROS inside a cell is limited to short distances but they can react with proteins and carbohydrates on the surface of cells and organisms (e.g. transport proteins) and thus inhibit vital processes. However, uncharged and stable H_2O_2 permeates easily through cell membranes, and in the presence of transition metals (Fe^{2+}, Cu^{+}) it can form toxic photoproducts (e.g. hydroxyl radical $^{\bullet}OH$) through (photo-)Fenton reactions (equation 9) if not readily scavenged (Hermes-Lima 2004). Fe^{3+} catalyses the quenching of superoxide in the Haber-Weiss reaction (equation 10).

(Photo-)Fenton reaction (9) $H_2O_2 + Fe^{2+} \rightarrow {}^{\bullet}OH + Fe^{3+} + OH^-$

Haber-Weiss reaction (10) $O_2^{-\bullet} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$

The hydroxyl radical in turn, probably the most reactive species able to attack almost any cellular part, damages cell membranes through propagation of free radical chain reactions. Lipids, proteins and pigments as well as DNA are all targets of ($^{\bullet}OH$-induced) oxidative modification and damage such as formation of toxic peroxidation products (thiobarbituric-acid-reactive substances TBARS, carbonyl derivatives), cross-links between membrane proteins, amino acid side chains, DNA cross-links and single stand breaks, and DNA base

modifications (Boveris 1998, de Zwart et al. 1999). Peroxidation of membrane phospholipids (especially polyunsaturated fatty acids PUFAs are prone to ROS attack) reduces membrane fluidity and disrupts integrity and may lead to severely altered cell function and cell death if membrane bound proteins (enzymes) are involved (Halliwell & Gutteridge 1999). Consequently, ROS-induced cell injuries which lead to functional changes in cells and tissues can decrease overall metabolic performance, growth and reproduction and finally increase mortality (Abele & Puntarulo 2004).

Further, UVR can dramatically increase toxicity of many natural (acetylenes, furans) and anthropogenic organic compounds (esp. polycyclic aromatic hydrocarbons PAHs), which act as sensitizers of cellular and tissue oxidative damage (photodynamic photosensitization). Hereby, the UVA range is of greater concern as e.g. PAHs absorb radiation more effectively in the UVA range compared to UVB (Diamond 2003).

Contrasting to the above negative effects, ROS are also important in signal transduction processes e.g. as mediators and second messengers of cell damage and programmed cell death (Schrek & Baeuerle 1991, Lesser 2006).

1.4 UV-Tolerance and photoprotective responses

The balance between damage and repair processes determines the tolerance of an organism towards UVR, and the far reaching success of plants and animals in aquatic and terrestrial ecosystems indicates the evolution and existence of various and efficient strategies to minimise UV and oxidative damage. Not only organisms inhabiting generally high UV intensity environments such as coral reefs or alpine lakes, but also organisms from habitats with fluctuating UV impact, such as polar marine animals, have evolved UV-protective mechanisms: avoidance, screening, quenching, and repair (Dunlap et al. 1999, Sommaruga 2001). Viruses, bacteria and heterotrophic protists, however, seem to be highly sensitive towards UV-induced DNA-damage (Sommaruga et al. 1997 and 1999, Helbling et al. 2001).

1.4.1 *Avoidance*

Avoidance of UVR such as seeking of shelter underneath macroalgal canopy or by downward migration is restricted to motile benthic and planktonic organisms, resulting e.g. in pronounced diurnal vertical migrations with origins also in predator avoidance (Hessen 1993). This results in a trade-off between duration in the photic zone, where primary consumers profit from phytoplankton primary production and where warmer temperatures accelerate

growth e.g. of planktonic larvae, and the protection from harmful UVR and predators utilising UV vision for hunting deeper in the water column. Pigmentation (see below) also plays a key role in this trade-off as it enables the organism to stay in the photic zone, however visibility may enhance the predation risk (Hessen 2003).

1.4.2 *Screening*

Screening can be achieved with physical (morphological) features, such as shells, spines, cuticle, chitinous carapace, mucous and multi-layered cell walls. Mycosporine-like amino acids (MAA), scytonemin and melanin, for example, constitute chemical UV-absorbing intracellular and extracellular compounds (Banaszak 2003).

Mycosporine-like amino acids (MAAs) are widely spread in organisms from all latitudes from the poles to the tropics and found in most marine organisms ranging from bacteria to vertebrates (Arai et al. 1992, Karentz et al. 1991, Karentz 2001, Dunlap & Shick 1998). MAAs are imino carbonyl derivates of mycosporines, compounds identified in the mycelia of fungi. MAAs absorb maximally between 309-360 nm, and dissipate the energy thermally without showing fluorescence or generating oxygen radicals (Shick & Dunlap 2002). They consist of a cyclohexenone or cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid. MAAs derive most probably from the shikimic acid pathway, thus de-novo synthesis is only present in bacteria, fungi and algae, while other organisms, such as invertebrates and vertebrates acquire MAAs through their nutrition or via translocation from symbionts (Banaszak & Trench 1995). Some heterotrophic organisms are, however, capable of chemical conversion of specific MAAs in their gut system, by which they acquire a whole suite of specific MAAs providing a broader protection range over the solar spectrum (Dunlap & Shick 1998, Whitehead et al. 2001). Animals such as echinoderms are able to accumulate specific MAAs in certain tissues, e.g. ovaries and spawns, and they also occur in ocular tissues of reef fish (Bandaranayake & Des Rocher 1999, Dunlap et al. 1989, Shick et al. 1992). In crustaceans, for example, 10 different MAAs and the chemical precursor gadusol have been identified so far, with shinorine, porphyra 334, mycosporine-glycine, palythenic acid and palythine being the most dominant substances. UV-protection by MAAs in animals is clearly shown, though stimulation of uptake and accumulation still varies from species to species and results are sometimes contradictory (Banaszak 2003).

Melanin absorbs in all UVR and PAR wavelengths, which makes it beneficial to non-photosynthetic, heterotrophic organisms, e.g. fresh water cladocera (Zellmer 1998), and is also found e.g. in the skin of aquatic vertebrates such as fish (Fabacher & Little 1998), and in

the cuticle and carapace of zooplankton (Gouveia et al. 2005, Hessen 2003). Apart from UV-screening, melanin precursors and eumelanins (black melanins) may also act as a free radical scavenger (Halliwell & Gutteridge 1999, Nakano et al. 1993).

1.4.3 *Enzymatic and non-enzymatic antioxidant pathways*

Low molecular weight compounds such as carotenoids, ascorbic acid, α-tocopherol, uric acid, ubiquinol, and glutathione, scavenge singlet oxygen ($^1O_2$) and efficiently remove free radicals, thus breaking free-radical chain reactions (Halliwell & Gutteridge 1999). In animals such as polar marine crustaceans these non-enzymatic scavengers are mostly dietary derived (Siebeck 1978, Ringelberg et al. 1984). Carotenoids for example are found in various tissues in crustaceans, e.g. in the eyes of the horseshoe crab *Limulus* (arthropods), where they not only function as important precursors for vitamin a and rhodopsin but may also play a direct protective role in some species (Halliwell & Gutteridge 1999, Kirschfeld 1982). Antioxidant enzymes (e.g. superoxide dismutase SOD, catalase CAT, glutathione peroxidase GPX) neutralise ROS and other toxic reactive photoproducts (equations 11, 12) and form very effective, synergistic and compensatory defence systems against oxidative damage such as lipid peroxidation (Boveris 1998).

SOD (11) $\quad 2\ O_2^{-\bullet} + 2\ H^+ \rightarrow H_2O_2 + O_2$

CAT (12) $\quad 2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$

These enzymes constitute of complexes with metals such as Fe, Cu, Zn, Mn, and non-metal elements such as Se (e.g. CuZn-SOD, FeMn-CAT, Se-GPX). Thus iron and copper are both, essential as well as detrimental for life as catalysts for formation and detoxification of ROS. Marine crustaceans provide a set of these enzymes (Hermes-Lima 2004).

Also, some MAAs, e.g. mycosporine-glycine present in polar marine crustaceans and 4-deoxygadusol in algae-invertebrate symbioses have been shown to posses moderate and high antioxidant potential (Dunlap & Yamamoto 1995, Dunlap et al. 1999).

1.4.4 *DNA-Repair*

Repair mechanisms comprise two forms of DNA-repair: Photoreactivation is a light-dependent (385-450 nm) repair system based on the single enzyme photolyase (Sancar & Sancar 1988). Photoreactivation is present in various organisms including bacteria, algae, zooplankton, and fish larvae (Goncalves et al. 2002, Grad et al. 2003). Nucleotid excision repair (dark repair) is light independent, requiring the production of a series of DNA

replication enzymes, and present in all types of pro- and eukaryotes and is the major mechanism in mammalian cells (often due to lack of photolyase enzyme) (Mitchell & Karentz 1993, Mitchell et al. 1993, Banaszak 2003).

1.5 Ecological role of amphipods in Polar coastal ecosystems

Amphipods are widely distributed crustaceans in polar regions. They are often abundant and generally diverse and together with polychaets outnumber other major meso- and macrobenthos groups (Jazdzewski et al. 1996, 2001a, De Broyer & Jazdzewski 1996). A comparison of two polar fjord ecosystems, Admiralty Bay (King George Island, South Shetland Islands) in the West Antarctic sub-region (as defined by Hedgpeth 1969, Knox & Lowry 1977) and Hornsund (south-west Spitsbergen) in the Arctic, showed that amphipods were more abundant and richer in taxa at all levels in the Antarctic compared to the Arctic fjord (Jazdzewski et al. 1996). It is suggested by Gray (2001) that generally biodiversity is higher in the Antarctic due to its longer cold water history and isolation allowing for longer periods of adaptation and speciation (c. 15-20 million years) compared to the Arctic (c. 1-2 million years). In the North as well as in the South amphipods occur from shallow to deep water ecosystems, inhabiting comparable ecological niches: Benthic herbivores and omnivores constitute an important direct link between primary producers (macroalgae, microphytobenthos) and higher trophic levels such as benthic and demersal fish and some invertebrates and contribute highly to energy fluxes (Kock 1992, McClintock 1994, Olaso et al. 2000). Carnivores hunt animal prey and as scavengers feed also on carrion and detritus, and, besides serving as food themselves for fish, birds and seals, they play an important role in the recycling of organic material (De Broyer & Jazdzewski 1996, Hop et al. 2001, Iken 1996, Jazdzewski et al. 1991). In the littoral and upper sublittoral, amphipods are often associated with dense macroalgal communities, utilising the algae as food source and/or shelter. Predation is suggested to be low in the Arctic (Gulliksen 1979) and the impact of mesograzers such as herbivore amphipods is considered to be low, at least in Spitsbergen fjord systems, as they occur regularly but generally in low abundance (Wessels et al. 2004). Contrasting, influence of herbivores on macroalgal communities may be considerable in Antarctic habitats, as shown for a snail but assumed to be similar for the studied amphipod species *Gondogeneia antarctica* at King George Island, which is regarded a generalistic herbivore feeding preferably on red macroalgae if available (Iken 1996, Huang et al. 2006). Scavengers such as lysianassid amphipods are important and often dominant members of

benthic communities in both polar regions and can accumulate to high numbers around carrion also in very shallow depths (Legezynska et al. 2000, Sainte-Marie et al. 1989). It is noteworthy, of course, that changes in macroalgal assemblages or nutritional components of the algae caused by elevated UVB levels under ozone depletion could have far reaching feed-back mechanisms on the associated amphipod species and also on higher trophic levels. This was already shown for freshwater cladocerans *Daphnia* fed with UV-treated food (Zellmer et al. 2004).

Polar and sub-polar amphipods possess a high level of storage lipids, which enable the animals to overcome long starvation periods with seasonally limited food supply (Graeve et al. 1997, Nyssen et al. 2005, Sainte-Marie et al. 1989). Some Antarctic and Arctic amphipod species have been shown to possess high levels of unsaturated membrane lipids (PUFAs) as an adaptation to life in cold climates (Clarke 1983, Clarke et al. 1985, Graeve et al. 1997, Nyssen et al. 2005). However, higher unsaturation of membrane lipids may render the amphipods more susceptible to peroxidation processes (Halliwell & Gutteridge 1999). Further, low temperatures may exacerbate oxidative stress owing to higher solubility of oxygen in cold sea water, low oxygen consumption rates, and cold induced loss in enzymatic antioxidants like SOD in ectothermal animals. This leads to reduced ROS scavenging capacities, while half-life times of radical species might be prolonged (Abele and Puntarulo 2004). Herbivorous amphipods derive a suite of protective and antioxidant substances directly from their macroalgal diet (e.g. MAAs, carotenoids, vitamins) but also carnivore, omnivore or detritivore species are able to obtain certain screening and quenching compounds through dietary translocation from lower to higher trophic levels.

1.6 UV-inducible stress potential within the climate change context

UVB has been shown to contribute synergistically with other environmental hazards (e.g. temperature rise, chemical contamination, increasing CO_2 levels, increased metal input with glacier / river run-offs) to oxidative stress conditions and damage to key biomolecules in transparent marine organisms. Exposure to UVR can increase the sensitivity towards a single, sub-lethal factor and render the animals more vulnerable to detrimental effects and finally increase mortality (Liess et al. 2001, Winckler & Fidhiany 1996a and b).

While current ozone depletion and probably UV-inducible stress potential is higher in the Antarctic compared to the Arctic, expected further biologically effective temperature rises in both polar regions may affect the animals in two ways: directly through temperature extremes

in shallow water areas with limited water exchange (e.g. tidal pools), which may be critical for cold adapted species. And indirectly through increased melt water inflow containing contaminants, which had been stored in ice and snow over years or decades (ACIA 2004). Antarctic species have evolved for more than 20 million years in a relatively stable ecosystem dominated by cold temperatures, very little terrestrial input and isolated from adjacent oceans by the Circumantarctic current (Dayton et al. 1994). The Arctic cold water ecosystem on the other hand is much younger, probably only 2 million years, with alternating glacial and warm periods (Dayton et al. 1994). The Arctic Ocean, being a mediterranean sea surrounded by continental land masses, has a strong fresh water input, is less isolated, and in the case of Spitsbergen strongly influenced by adjacent Atlantic currents (West Spitsbergen current), leading to a very small number of endemic species and more disturbance compared to the Antarctic (Arntz et al. 1994 and 1997, Crame & Clarke 1997). This inherent variability may increase the adaptive potential to global climate change in Arctic and sub-arctic species compared to their Antarctic counterparts and facilitate colonisation in the North with temperate species reaching northward.

In the predicted global change scenario increasing temperature will lead to severe glacial melting circumpolar in the Arctic and also in the Antarctic Peninsula and Western Antarctic region, as well as to severe reduction of Arctic sea ice during summer months (Thompson & Solomon 2002, ACIA 2004, Haas 2006, Leckebusch et al. 2006). Increases in river run-off and material transport, as well as in precipitation might enhance input of heavy metals (e.g. from volcanic origin as in Potter Cove, King George Island, Ahn et al. 2003) and pollutants (e.g. from old waste disposal sites, Casey Station, Antarctic, Duquesne & Liess 2003), and thus accelerate ROS cycling and oxidative stress especially in the Arctic Ocean, with its high terrestrial influence of surrounding land masses. Contrasting, increased turbidity due to increased material load limits penetration and propagation of harmful UVR into the water column and thus could counterbalance UV-induced impacts in the photic zone.

1.7 Objectives and aims of the present study

The aim of the present study was to compare the effects of UV-exposure on various oxidative stress parameters and defence systems against direct and indirect UV-damage in polar marine amphipods from two different coastal regions, one Antarctic and one Arctic fjord, currently undergoing different degrees of ozone depletion. This comparison was carried out in relation

to a temperate reference species from a North Sea coast. Different doses of UV radiation (high and low) as well as different exposure times (hours to weeks) were chosen to evaluate a threshold level, which the amphipods could tolerate, compensate or repair induced damage, or beyond which the UV-sensitivity leads to increased mortality. In particular, the following questions were addressed:

1. Does the atmospheric and underwater light climate in the Antarctic Potter Cove impose a more severe UV-stress impact on shallow water amphipods due to higher seasonal ozone depletion compared to the Arctic Kongsfjord?

2. Do amphipods from both polar coastal systems possess efficient physical and chemical UV-protection (e.g. carapace absorbance, MAAs, carotenoids, antioxidant enzymes) against natural and artificial UVR and oxidative stress? Are there species, habitat or hemisphere specific differences? Is the type of nutrition (herbivory, carnivory, omnivory, detritivory) and the state of nutrition (fed, starved) important?

3. Can sub-lethal or lethal effects of UV-exposure be distinguished on different time scales (instantaneous to medium-term) and on various systemic levels? Does UVR affect the animals' metabolism?

4. Is the UV-tolerance threshold different in Antarctic and Arctic species? Are polar amphipods in comparison to temperate species endangered by current natural UVR (and ozone) levels on an individual as well as population level?

2 Material & Methods

2.1 Study sites & radiation climate

Potter Cove

King George Island belongs to the South Shetland Islands (Antarctic, 62° 14' S, 58° 40' W), which are situated approximately 100 km westwards of the Antarctic Peninsula, divided from the continent by the Bransfield Strait. Potter Cove is a small cove of approximately 7.5 km^2 adjacent to Maxwell Bay in the South of King George Island, opening south-eastward into Bransfield Strait. The sampling area Peñon 1 is situated at the southern side of Potter Cove towards the outer bay (Fig. 2.1). The Argentinean Jubany Station with the Dallmann Laboratory also in the South lies further inside the cove (Fig. 2.1). Temperature and salinity in Potter Cove range between –1.8 and 2.2°C and 30.6 and 34.5 psu (practical salinity units) in the inner sector, with typically cooler and less saline water in spring, warmer and more saline water in summer, and cooler and more saline water in winter (Klöser et al. 1994b, Schloss et al. 1998). However, during snow and glacier melting and fresh-water input in summer, salinity can be reduced below 28 psu in the vicinity of streams. Also, in rock pools in the intertidal, such as sampling site Peñon 1, temperature and salinity vary sometimes extremely within a few hours during summer (3-12°C, 28.4-34.3 psu, Iken 1996). Peñon 1 lies within a wide intertidal area with steadily decreasing plateaus, rocky bottom with stones and large boulders, and is characterised by rich macroalgal assemblages with different zonational species distribution related to water depth, turbulence and ice impact (Klöser et al. 1996, Quartino et al. 1998). Semidiurnal tides predominate with a tidal range around 1.4 m and a tidal minimum below 10 cm water depth in rock pools. Exposed areas dry out completely during extreme low tide. More detailed descriptions of algal zonation and environmental factors are given in Iken (1996), Klöser et al. (1994a, b, 1996), and Wiencke et al. (1998).

Figure 2.1: King George Island (South Shetland Islands, Antarctic) with Potter Cove (*) and Dallmann Laboratory (°) at Argentinian Jubany Station. (Source: http://www.kgis.scar.org/, SCAR, King George Island GIS Project, Institute for Physical Geography, University Freiburg, Germany).

Kongsfjord

Kongsfjord, situated at the north-western side of Spitsbergen (Arctic, 78° 55‘ N, 11° 56’ E, Fig. 2.2), is 26 km long and between 3 and 8 km wide. Despite its high Arctic location Kongsfjord should rather be regarded as sub-Arctic, because it is influenced by North Atlantic water masses, which are transported northward with the West Spitsbergen Current (Svendsen et al. 2002). Water temperature is generally around 0°C (annual mean, Ito & Kudoh 1997), however, in summer it can rise up to 6-8°C at the surface (Hanelt et al. 2001, and own measurements in August 2001). Salinity averages at 34 psu, however, freshwater input from strong glacier and river run-offs reduce salinity locally at the surface down to 20 psu (Hanelt et al. 2001). With this freshwater input there is also a high material and sediment discharge into the fjord, which locally increases water turbidity severely. Kongsfjord is maximally 400 m deep and the coastline is dominated by steep and rocky shores more towards the outer sector and shallower parts characterised by soft mud glacier deposits mainly in the inner sector. Sampling sites with medium and dense macroalgal communities were located at various stations along the coastline. A more detailed description is given in Publication II and III, as well as in Lippert (2003).

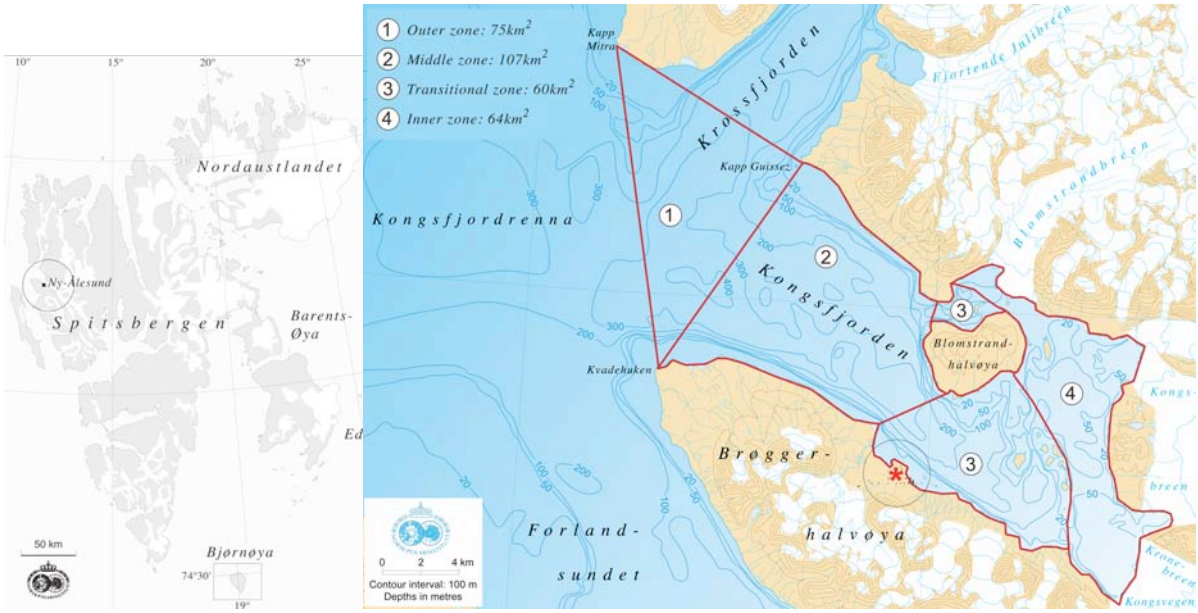


Figure 2.2: Kongsfjord (area within red line) with Koldewey Station (*) at Ny-Ålesund (Spitsbergen, Arctic). Maps kindly provided by Stein Tronstad, cartography by Anne Estoppey, Norwegian Polar Institute, Tromsø, Norway, http://www.npolar.no).

Helgoland

The sampling area at Biological Station Helgoland, island of Helgoland (North Sea, 54° 05' N, 07° 53' E), is located north of the island and characterised by a broad, steadily declining rocky bottom intertidal with rich macroalgal assemblages (Bartsch & Kuhlenkamp 2000). Mean temperature and salinity measured at "Helgoland-Reede Kabeltonne" vary between 3°C in winter (February) and 19°C in summer (August), and between 30.2 and 35.0 psu, with minimum salinity in spring (April) and maximum in summer (July) (source: http://www.bsh.de/de/Meeresdaten/Beobachtungen/MURSYS-Umweltreportsystem/).

Table 2.1 gives a short overview of the different expeditions carried out.

Tab. 2.1: Summary of expeditions and study sites with information on location and duration of experimental periods.

Expedition	Location	Experiments carried out between:
Antarctic	Potter Cove, King George Island 62° 14' S, 58° 40' W	I: October 2000 - January 2001 II: October 2002 - December 2002
Arctic	Kongsfjord, Spitsbergen 78° 55' N, 11° 56' E	July - August 2001
Helgoland	Island of Helgoland, North Sea 54° 05' N, 07° 53' E	July - August 2002

Radiation measurements

1) Atmospheric light climate: Solar UVB-radiation was measured continuously with a 32-channel single-photon counting spectroradiometer (AWI Physics Department) installed on the roof of Dallmann Laboratory at Jubany Station, King George Island (Antarctic), and on the roof of the NDSC-building at Koldewey station, Kongsfjord, Spitsbergen (Arctic). Solar UVR and PAR were also measured with an additional fast scanning double monochromator spectroradiometer (Instrument Systems, Germany) at Kongsfjord as well as at Helgoland.

2) Underwater light climate: During Antarctic Expedition I (2000) only underwater UVA and PAR (320-700 nm) but not UVB in Potter Cove (0–20 m) could be recorded with the specific spectroradiometer (Construction by M. Kruse, Germany) on sunny days. Additionally, in 2003 underwater UVB irradiance in Potter Cove (0-20 m) was recorded for the first time with an underwater UVB-spectroradiometer, which was also used in Kongsfjord (0-5 m) in July 2001 (according to Hanelt et al. 2001). I calculated underwater UVB-profiles for Potter Cove from the 2003 data sets, which are included in section 4) Additional Results for a better comparison of underwater UVB-parameters between Antarctic Potter Cove and Arctic Kongsfjord.

Total column ozone at the study sites

In 2000, the year of Antarctic Expedition I, the ozone layer over the South Shetland Islands area reached its minimum in two consecutive months: September (126 DU) and October (129 DU). Mean column ozone was back to 312 DU by December. In 2002, the year of Antarctic Expedition II, the total column ozone minimum was 159 DU in September, and mean column ozone was back to 324 DU in December. Over the Kongsfjord area (Arctic Expedition July-August 2001) total column ozone in July 2001 ranged between 287 and 370 DU. In March 2001, when destruction of ozone can peak in the Arctic, concentrations ranged between 381 and 443 DU. Over Helgoland (Helgoland Expedition July-August 2002) column ozone values in July 2002 spanned from minimal 286 DU to maximal ozone of 388 DU (Source: NASA TOMS data, http://toms.gsfc.nasa.gov/teacher/ozone_overhead.html).

A summary of study sites, atmospheric and underwater radiation climate and investigated species is given in Table 8.1 in the Appendix.

2.2 Investigated species

In Antarctic Potter Cove the gammarid amphipods *Gondogeneia antarctica* Cevreux, 1906 (Calliopiidae/Gammarellidae, Eusiroidea) and *Djerboa furcipes* Chevreux, 1906 (Eusiridae, Eusiroidea) were investigated. Both species occurred in the inter- and upper sub-tidal at Peñon 1 between 0 and 4 m depth. While *G. antarctica* is reported from very shallow depths around King George Island (Iken 1996, Jazdzewski et al. 1996, 2001b), *D. furcipes* also typically inhabits the lower subtidal and has even been collected from 150 m depth (overview De Broyer et al. 2001, Jazdzewski et al. 1991, Nyssen et al. 2005). Both species are further distributed throughout the West Antarctic and the Subantarctic Islands sub-region, and *G. antarctica* occurs also in the Magellanic sub-region (De Broyer & Jazdzewski 1993).

a) b)

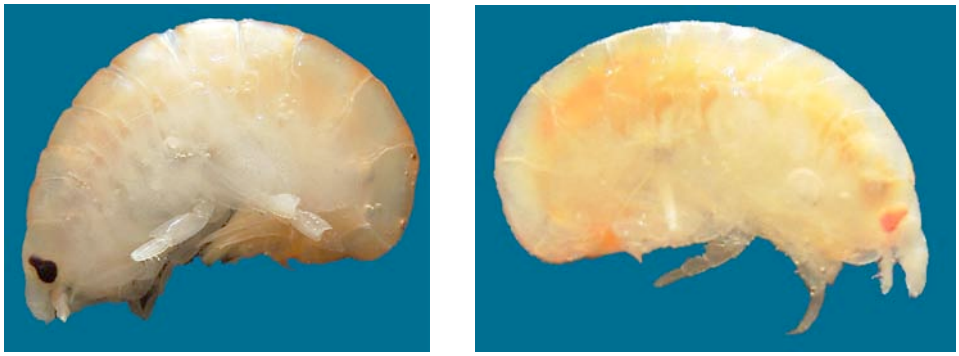
Figure 2.3: *Gondogeneia antarctica* (a) and *Djerboa furcipes* (b)

G. antarctica is regarded a herbivore, feeding preferably on red macroalgae if available, however, switching to a broad generalistic nutrition in response to food availability and season, including green and brown macroalgae, diatoms, ice algae, and small crustaceans (Richardson & Whitaker 1979, Momo 1995, Momo et al. 1998, Iken 1996, Huang et al. 2006, all studies carried out in the West Antarctic sub-region including South Shetland and South Orkney Islands). *D. furcipes* is also supposed to be herbivorous, feeding on macroalgae, however, nutrition is less well studied (overview De Broyer et al. 2001). Nyssen et al. (2005) measured a high content of C_{18} and C_{20} polyunsaturated fatty acids (PUFAs) in *D. furcipes* from South Shetland and South Orkney Islands, indicating a herbivorous life style, which is supported by stomach content (mainly brown macroalgae residues of *Desmarestia mensiezii*) and isotopic carbon to nitrogen ratios resembling primary producers. Coloration of freshly collected *G. antarctica* specimens from Potter Cove was dark brown to grey with a white stripe along the entire dorsal side of the carapace or only on a few segments. *D. furcipes* was

brightly orange-brown with uniform coloration. Size of adults ranged between 1 and 2 cm in both species.

At Kongsfjord three species of gammarid amphipods were studied: *Gammarellus homari* Fabricius, 1779 (Gammarellidae), *Anonyx nugax* Phipps, 1774 (Lysianassidae) and *Onisimus edwardsi* Krøyer, 1846 (Lysianassidae). All species were collected between 0-5 m water depth. The herbivore *G. homari* is widely distributed in the boreal Atlantic and Arctic realm, associated with macroalgae, preferably red, which provide food and shelter. The scavengers *A. nugax* and *O. edwardsi* are restricted to Arctic and sub-arctic regions, and while carnivore/necrophage *A. nugax* feeds predominantly on carrion or injured animals such as fish, molluscs or other crustaceans, *O. edwardsi* ingests also detritus of algal origin, such as planktonic algae or macroalgae (Saint-Marie et al. 1989, Legezynska et al. 2000, Lippert 2003, Tromsø Museum, Norway: http://www.imv.uit.no/crusticon/Amphipoda). A detailed description of sampling sites, nutrition and carapace coloration is given in Publication II and III. Size of adults was up to 3 cm in *G. homari* and *A. nugax* and up to 1 cm in *O. edwardsi*.

Figure 2.4: *Gammarellus homari*



a) b)

Figure 2.5: *Anonyx nugax* (a) and *Onisimus edwardsi* (b)

At Helgoland the gammarid amphipod *Chaetogammarus marinus* Leach, 1815 (Gammarellidae) was collected in the intertidal. This temperate herbivore species occurs along the North Sea and Atlantic coast (source: Zoological Museum, University of Amsterdam, http://ip30.eti.uva.nl/bis/amphipoda.php?menuentry=soorten&id=50) and is an important link between primary producers and algal detritus, and higher trophic levels in coastal and estuarine areas (McLusky 1989, Lawrence & Poulter 2001). Prior to experimentation, animals were maintained in an aquarium system with running sea water at the Biological Station Helgoland at 15°C and 34 psu salinity.

2.3 Experimental conditions

To test for dose-dependent UV-effects, amphipods were exposed in two series of laboratory experiments to a low/moderate and a high UVB-dose with respect to maximal atmospheric UVB-intensities:

2.3.1 *UV-tubes: low/moderate UVB-dose*

In a first series experimental irradiation at Potter Cove and Kongsfjord was carried out using white light and UV-tubes (Q-Panel, type UVA 340, Cleveland, USA) for low/moderate UVB-exposure. Irradiation intensities were adjusted by the distance between tube and water surface and were 0.38 W m^{-2} (Potter Cove) and 0.40 Wm^{-2} UVB (Kongsfjord). Experimental total daily UVB-dose amounted to 24% (Potter Cove) and 41% (Kongsfjord) on average of maximal atmospheric dose at the respective sampling site, and was thus defined as low and moderate. Assuming an average attenuation of for example 50% per meter for UVB in polar coastal areas during summer, amphipods in my experiments, which remained close to the surface, experienced 48% (Potter Cove) and 82% (Kongsfjord) of the *in-situ* UVB-dose at 1 m water depth on sunny days. This dose was applied over days and up to 4 weeks.

2.3.2 *Sunshine simulator: high UVB-dose*

In a second series experimental irradiation was carried out using a sunshine simulator (SONSI) for the high UVB-exposure at all study sites. The SONSI has a 400 W 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). The irradiation intensities of the solar-like spectrum could be adjusted by altering the liquid filter width and by placing 1-3 metal grades between

lamp and filter unit. Experimental UVB-intensities were 1.30 W m^{-2} (Potter Cove, Antarctic Expedition I in 2000), 1.35 W m^{-2} (Kongsfjord), and 1.0 W m^{-2} (Helgoland), and experimental total daily UVB-dose amounted to 86% (Potter Cove), 105 % (Kongsfjord), and 50% (Helgoland) on average of possible maximal atmospheric dose at the respective sampling site, and was thus defined as high for Potter Cove and Kongsfjord. Amphipods in my experiments, which remained close to the surface, experienced a 50 to 100 % increase on average over *in-situ* 1 m UVB-dose if an average attenuation of for example 50% per meter for UVB is assumed. However, attenuation in the polar regions can be locally far higher (e.g. up to 58 and 70 % in upper 10 cm in Potter Cove and Kongsfjord) during summer months in the vicinity of melt water inflow, and is generally very high (90-99% in upper 1.5 m) in waters around temperate Helgoland (Tab. 8.1 Appendix). This also classifies the experimental UVB-exposure at Helgoland as high compared to natural conditions, although irradiation intensity applied in the laboratory was half-maximal. These doses were applied over days and up to 3 weeks.

To determine wavelength dependent UV-effects, different cut-off filters were employed: 320 nm cut-off glass filter (long pass Schott, Germany) and filter foil (Ultraphan URUV, Digefra, München, Germany), and 400 nm cut-off filter foil (Folex PR, Folex, Dreieich, Germany). Amphipods were selectively fed or starved to test for nutritional influence on UV-impacts. Experimental radiation parameters for each area are summarised in Tab. 2.2 and detailed descriptions of experimental routines are given in Publication I - IV.

Table 2.2: Radiation parameters applied in experimental series with Q-Panel tubes and with sunshine simulator SONSI at Antarctic Potter Cove in 2000 (1, 2), at Antarctic Potter Cove in 2002 (1, 3), and at Arctic Kongsfjord in 2001 (4, 5). For comparison, maximal atmospheric light climate (Atmosphere) is given for each study site during experimental periods. UVB-intensities in SONSI experiments (2) and (5) are close to maximal atmospheric UVB-intensities at each respective study site during start of experimental series. SONSI UVB-intensity (3) during oxygen consumption measurements in Antarctic Expedition II in 2002 represents mean maximal atmospheric UVB-intensity of the entire period as experiments commenced later at the end of November due to extensive set-up and calibration of the respiration measurement system. Further details are given in Publication I to IV. SONSI UVB-intensity (6) during exposure experiments at Helgoland represents 50% of maximal atmospheric UVB-intensity, however, attenuation in the natural sea water *in-situ* (K_d of 3.3 m^{-1} at 305 nm, Dring et al. 2001) is far higher than in the filtered sea water (K_d 0.1-0.2 m^{-1}, Hargreaves 2003) used in laboratory experiments, thus the resulting artificial UVB-dose is high. The total daily UVB-dose is calculated form the maximal UVB-intensity at surface level and the respective exposure time. Atmospheric total daily UVB-dose is the possible maximal UVB-dose calculated from the mean maximal UVB-intensities, assuming optimal conditions in the field (no shading by clouds).

Study Site	max UVB (Wm^{-2})	max UVA (Wm^{-2})	max PAR (Wm^{-2})	Exposure time ($h\ d^{-1}$)	Total daily UVB-dose ($kJ\ m^{-2}\ d^{-1}$)	Ratio UVB:UVA:PAR	Publication No.
Potter Cove							
Atmosphere Oct-Jan 2000	1.4 (Oct) 1.8 (Dec)	16.5 - 27.9	133.2 - 176.0	5, 4	mean: 28.8 (5h), 23.0 (4h)	1:14:97	Publ. I
Atmosphere Oct-Dec 2002	1.3 (Oct) 1.8 (Dec)	not determined	not determined	5, 4	mean: 27.9 (5h), 22.3 (4h)		Publ. IV
(1) Q-Panel tubes							
low dose	0.38	3.68	5.73	5	6.84	1:10:15	Publ. I, IV
(2) SONSI (Exp. I 2000)							
high dose	1.35	15.67	134.08	4	19.5	1:12:99	Publ. I
(3) SONSI (Exp. II 2002)							
high dose	1.5	40	118	4	21.6	1:26:78	Publ. IV
Kongsfjord							
Atmosphere (July-August 2001)	0.8 - 1.2	15 - 21	170 - 200	5 4	mean: 18.0 (5) mean: 14.4 (4)	1:24:247	Publ. II, III
(4) Q-Panel tubes							
moderate dose	0.4	3.7	5.7	5	7.2	1:9:14	Publ. II, III
(5) SONSI high dose	1.3	21.8	117.6	4	18.7	1:17:90	Publ. II, III
Helgoland							
Atmosphere (July-August 2002)	1.8 – 2.2	60 - 85	200-268	4	28.8	1:36:117	
(6) SONSI high dose	1.0	27.3	215	4	14.4	1:27:215	

2.4 Methods

After certain time intervals of days to weeks, subsamples of exposed and control amphipods were taken from the experiments, the entire animals frozen in liquid nitrogen and stored at -80°C prior to analysis. Different defence and damage parameters for direct UVR and oxidative stress were investigated to determine the amphipods' UV-tolerance (Fig. 2.5). The methods and techniques used are described in Publication I – IV and are briefly summarised here.

Screening

Absorbance spectra of the chitinous carapace cleaned of all body tissues were recorded from 295 to 700 nm in the sunshine simulator to evaluate the degree of physical protection of the outer body barrier in the UVB and UVA range.

Whole body homogenates were investigated for content and composition of mycosporine-like amino acids (MAAs), compounds which absorb radiation energy between 309-360 nm and maximally at 320 nm without generating oxygen radicals. Extraction and analysis of MAAs was carried out according to Karsten & Garcia-Pichel (1996) and Newman et al. (2000) on two high-performance liquid chromatography systems (HPLC): Waters (Waters Corporation, USA), modified after Hoyer et al. (2001; Publ. I this study) and Agilent (Agilent Technologies, USA), modified after Karsten et al. (1998; Publ. III this study). For clarification, all extracts, which had been evaporated to dryness, were re-dissolved in 2.5% aqueous methanol (v/v) before analyses in the respective HPLC system. Detailed descriptions are given in Publication I and III.

Enzymatic and non-enzymatic antioxidant defence

Carotenoids are known ROS scavengers and the carotenoid concentration in whole animal butanolic extracts was determined photometrically between 400 and 500 nm as an indicator of non-enzymatic antioxidant defence potential.

Biochemical assays of antioxidant superoxide dismutase (SOD) and catalase (CAT) activities were run in whole body homogenates according to Livingstone et al. (1992) and Aebi et al. (1985). These enzymes efficiently quench various reactive species and their activity status reveals the amphipods' antioxidant capacity.

Oxidative damage

The thiobarbituric-acid-reactive substances (TBARS) assay according to Uchiyama & Mihara (1978) was used to determine the degree of oxidative damage to lipids in the amphipods' tissues. When exposed to heat and acid pH, thiobarbituric acid (TBA) forms a pink fluorescent adduct with malondialdehyde (MDA), one of the end products of lipid peroxidation reactions, and the concentration can be measured photometrically. Lipid peroxidation is supposed to be the dominant cause of cell injury and death (Hermes-Lima 2004).

Proteins form carbonyl derivatives as products of ROS attacks on proteins. The degree of oxidative stress and damage to proteins was measured photometrically according to Levine et al. (1990), as the presence of carbonyl groups in amino acid residues of proteins.

Survival

Death upon exposure to UVR is the most obvious indicator for lethal damage caused by direct (radiation) and indirect (ROS) effects. The number of surviving and dead amphipods was counted during the experiments for survival ratios.

UV-radiation stress

Changing environmental factors (temperature, salinity, pH, oxygen concentration, H_2O_2 concentration) impose stress on animals adapted to previous habitat conditions. This often leads to an increase in respiration and overall metabolic rates (Aarset et al. 1991, Muskó et al. 1995, Storch et al. 2001) for avoidance reactions, but also for biochemical stress defence and repair. Measurement of oxygen consumption during UV-exposure was used as a non-invasive tool to determine immediate effects of UV-radiation on whole animal metabolic rates. I used a flow-through system with special UV-transparent respiration chambers of adjustable volume for simultaneous UV-exposure and respiration measurements. Non-stressful conditions should result in regular respiration with little variation between maximal and minimal oxygen consumption (amplitudes) and represent resting metabolic rate of non-starved animals (Chapelle & Peck 1995). Contrasting, stressful irradiation conditions should cause visible changes in oxygen consumption resembling an immediate stress response. A detailed description of the radiation routine applied and the respiration measurements is given in Publication IV.

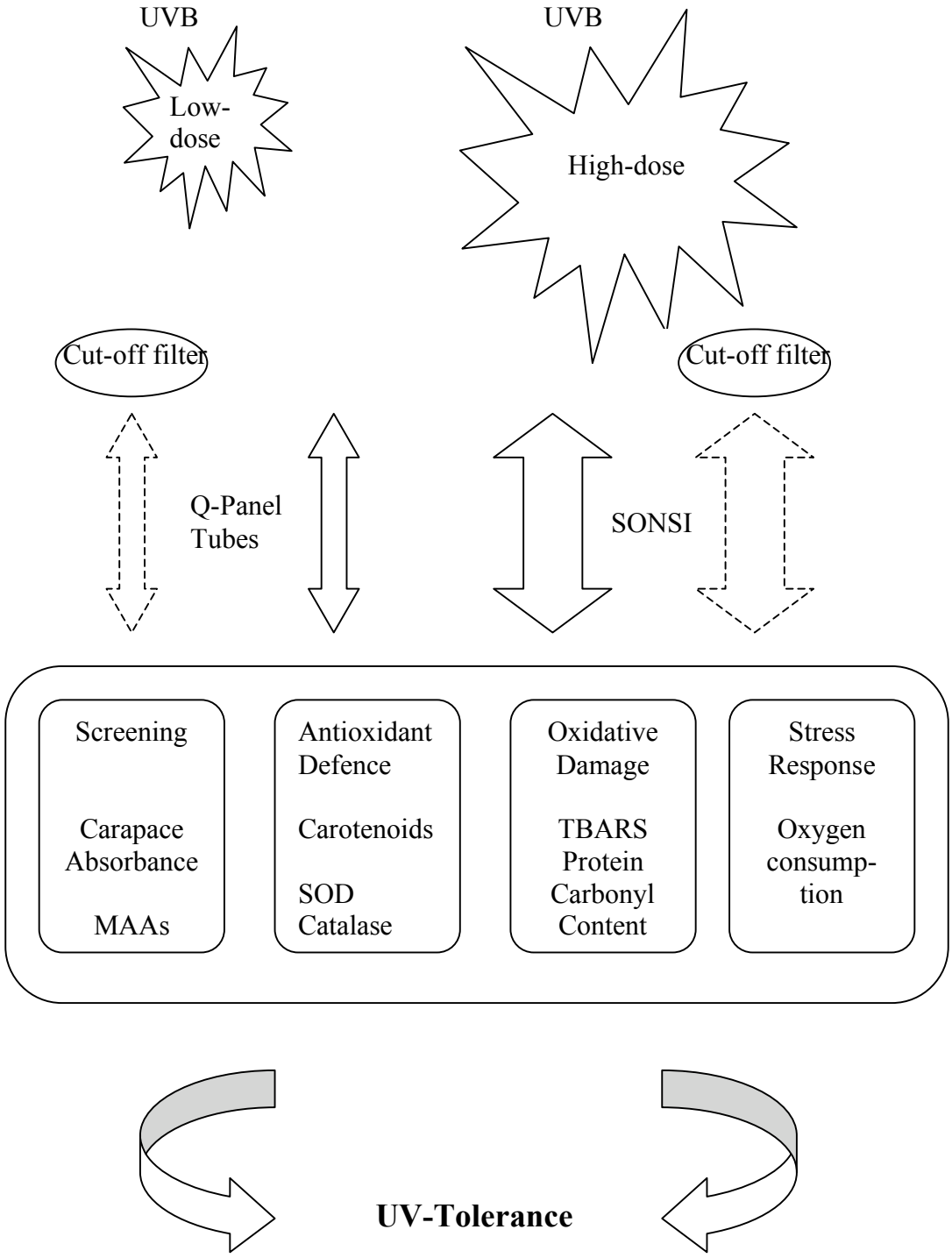


Fig. 2.5: Diagram summarising the different defence and damage parameters against UVR and oxidative stress investigated to evaluate the amphipods' UV-tolerance.

3 Publications

List of publications and declaration of my contributions towards them

Publication I

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

Birgit Obermüller, Ulf Karsten, Hans-Otto Pörtner & Doris Abele

Antarctic Biology in a Global Context (2003): 63-68

I developed the concept of this study together with the second and fourth author. I carried out the experimental work at the Argentinian Jubany Station and the Dallmann Laboratory on King George Island in Antarctica. Biochemical analysis was done by myself at the Alfred-Wegener-Institute in Bremerhaven, Germany. I further analysed the data at the Alfred-Wegener-Institute in Bremerhaven, Germany and wrote the first manuscript draft. This was improved in discussion with all the co-authors.

Publication II

Different UVB-tolerance in herbivorous versus carnivorous amphipods from Kongsfjorden

Birgit Obermüller & Doris Abele

Reports on Polar and Marine Research (2004), 492: 222-230

The co-author and myself developed the scientific idea for the experiments carried out in this study. All experimental work was carried out by myself at the Koldewey Station at Arctic Kongsfjord (Spitsbergen). I analysed the data at the Alfred-Wegener-Institute in Bremerhaven, Germany. The first manuscript was written by myself and revised in cooperation with the co-author.

Publication I:

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

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Publication III:

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

Birgit Obermüller, Ulf Karsten, & Doris Abele

Journal of Experimental Marine Biology and Ecology (2005), 323: 100-117

Publication III

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

Birgit Obermüller, Ulf Karsten, & Doris Abele

Journal of Experimental Marine Biology and Ecology (2005), 323: 100-117

The concept for these investigations was planned together with the second and third author and myself. I carried out all experimental work at the Koldewey Station at Arctic Kongsfjord (Spitsbergen) and part of the biochemical analyses at the University of Rostock. The second author supported the practical work at the University of Rostock. I analysed all data and discussed it with both co-authors. The first manuscript draft, which was written by myself, was improved in cooperation with both co-authors.

Publication IV

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

Birgit Obermüller, Susana Puntarulo & Doris Abele

Marine Environmental Research, submitted

Together with the third author, I elaborated the ideas for this study. I carried out all experimental work at the Argentinian Jubany Station and the Dallmann Laboratory on King George Island in Antarctica. Part of the biochemical analyses was executed at the University of Buenos Aires in Argentina. This practical work was carried out by myself and was supported by the second author. I conducted the rest of the biochemical analyses and all data analyses at the Alfred-Wegener-Institute in Bremerhaven, Germany. I wrote the first manuscript, which was then discussed together with the second and third author.

Publication I

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 ($^{\bullet}OH$) in a reaction catalysed by iron ($O_2^{\bullet-} + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- + O_2$). 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: $2\ O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$) 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 I 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 eluated 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

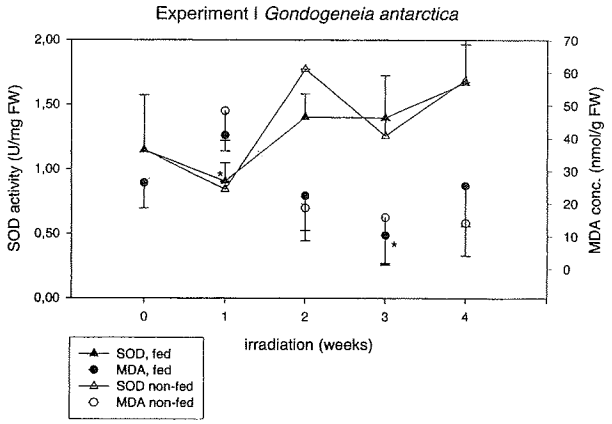

Fig. 1. UVR effects on SOD activity (triangles, solid line) and MDA concentration (circles, dotted line) under Q-Panel light climate (mild UVB dose) in *G. antarctica* (exp. I) presented as means and standard deviation. For number of replicates per data point see Tab. 1. Filled symbols indicate fed, 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 / -	- / -	

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* >

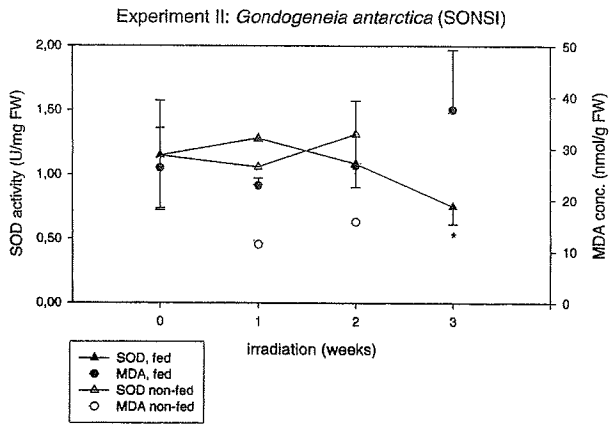

Fig. 2. UVR effects on SOD activity (triangles, solid line) and MDA concentration (circles, dotted line) under high UVB dose in the sunshine-simulator in *G. antarctica* (exp. II) presented as means and standard deviation. For number of replicates per data point see Tab. 1. Filled symbols indicate fed,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

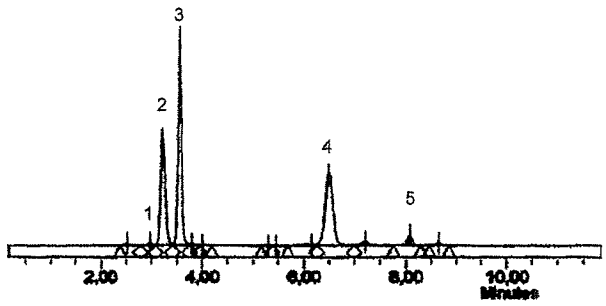

Fig. 3. HPLC-chromatogram of separated peaks recorded at 330 nm of UV-exposed *G. antarctica* with identified MAAs: 1 mycosporine-glycine, 2 shinorine, 3 Porphyra 334, 4 Palythine, 5 Asterina 330 (retention time in minutes).

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.

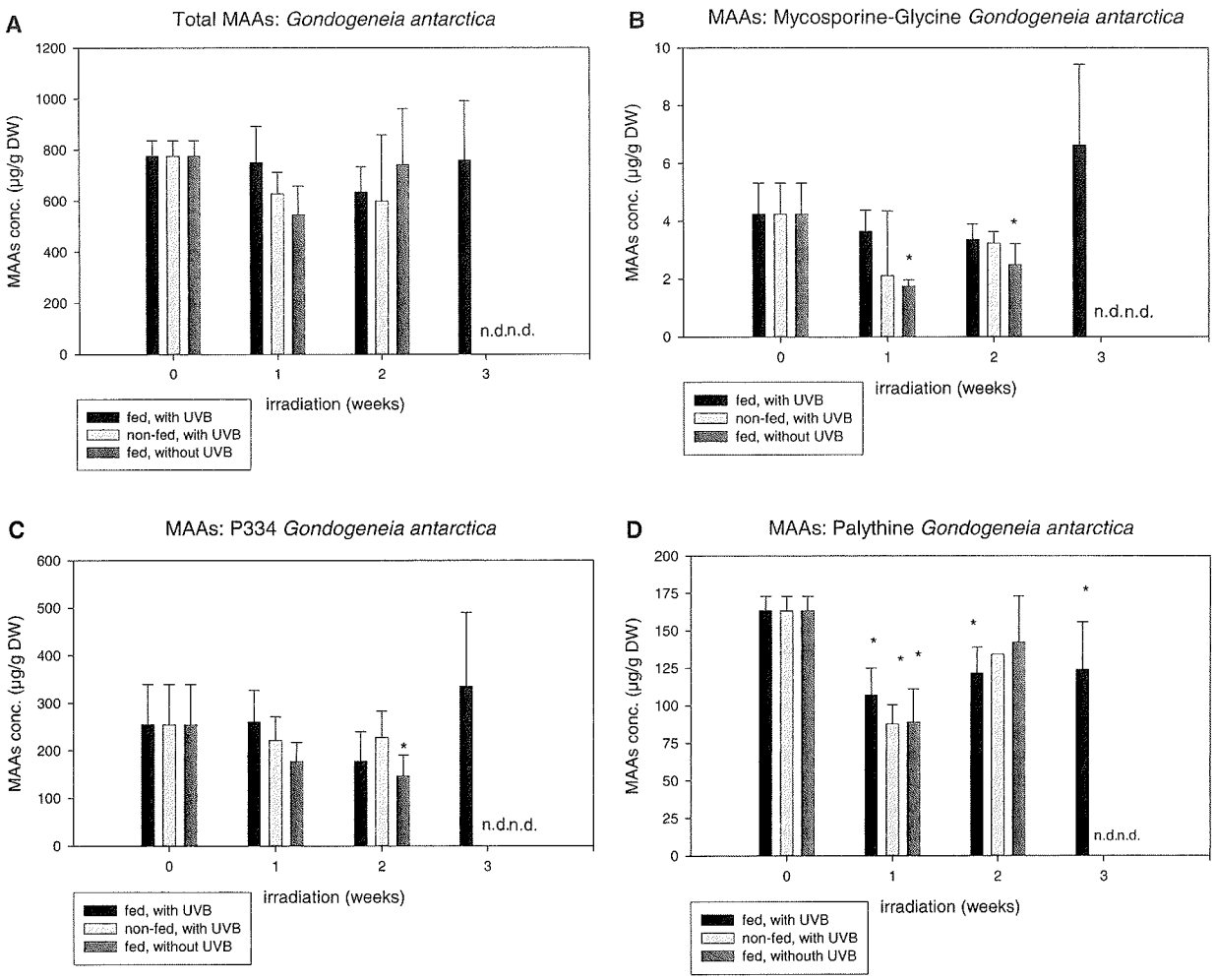

Fig. 4. UVR effects on the concentrations of a) total MAAs as well as b-d) individual MAAs in *G. antarctica* presented as means and standard deviation in fed and non-fed UVB-irradiated animals and in fed but UVB-non-irradiated (320nm cut-off filter) animals. b) mycosporine-glycine. * indicates significantly 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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Publication II

Different UVB-tolerance in herbivorous versus carnivorous amphipods from Kongsfjorden

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Introduction and objective

Over the last 30 years, polar environments, not only of the Antarctic but also the Arctic have received elevated levels of UV surface irradiation (UVR) due to stratospheric ozone depletion (Kerr & McElroy 1993, Groß et al. 2001). Although more prominent in the South, this is also an issue in the Northern hemisphere. Madronich et al. (1998) predict a maximal increase of erythemal (sun burning) UV-radiation to 22% over 1970s levels for Northern high latitudes. An increasing number of publications documents UVB-radiation to reach biologically relevant levels in Northern mid and high latitudes (Hunter et al. 1979, Björn et al. 1999, Browman et al. 2000, Williamson et al. 2001), which are expected to impact temperate and Arctic ecosystems, as elevated UV-levels are anticipated to persist over the next decades (IASC 1995, Madronich et al. 1998). Direct UV-photon interaction alters the chemical structure of biomolecules, and elevated oxidative stress in marine organisms has been detected following intense UVB-irradiation and is endangering terrestrial and aquatic organisms (Dunlap et al. 2000, Rozema et al. 2002). Mildly elevated UVB-irradiation increased mortality of Antarctic shallow water amphipods and caused elevated rates of lipid-peroxidation and impaired antioxidant enzyme activities (Obermüller et al. 2003). Exposure to natural surface UVB-levels increased mortality in Cladoceran populations (*Daphnia pulicaria*) from a temperate lake (Williamson et al. 2001) and reduced the reproductive success in *D. pulicaria* from subarctic fresh water ponds (Zellmer 1998). Moreover, natural surface UVB was shown to rupture the transparent animals' intestinal system (Zellmer et al. 2004).

The aim of the present study was to explore effects of atmospheric and elevated UVB-radiation levels on herbivorous and carnivorous/necrophagous (carn/necr) amphipods from Arctic Kongsfjorden. Carapax transmission was measured to approximate the impact of environmental UV-radiation on the animals' soft tissues. Irradiation experiments with a mild and a high UVB-dose, as compared to *in-situ* light climate, were performed to study UV-sensitivity of exposed animals. In particular, mortality rates and damage to biomolecules like lipids, proteins and DNA as well as protective mechanisms against photo-induced oxidative stress (antioxidant enzymatic systems, sunscreening substances) were investigated. A first set of results on UVB carapax transparency and photo-induced mortality of UV-exposed amphipods is presented here.

Material and methods

Solar UVB-radiation was measured 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 with a UVB-spectroradiometer. Data are still being calibrated and processed and therefore not available to date. With maximal atmospheric UVB-intensities ranging between 0.8 and 1.2 W m^{-2} in July and August 2001 a moderate (0.4 W m^{-2}) and a high UVB-treatment (1.3 W m^{-2}) were chosen for laboratory experiments with amphipods. 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.

Two species of Gammarid amphipods were studied: the herbivorous *Gammarellus homari* (Gammarellidae) and the carn/necr *Anonyx nugax* (Lysianassidae). *G. homari* were collected between algae with a handnet between 0-5 m water depth at various stations along the coastline of Kongsfjorden (e.g. Nansen Bay, Hansneset, see Lippert 2003). The original habitats at the Southern coastline decline gradually to 12 m depth, and are colonized with medium and dense macroalgal communities. Hansneset, situated on the Western side of the island Blomstrandhalvøya in central Kongsfjorden is characterised by gradually (inner part) to steeply (outer part) declining rocky bottom, with mostly dense macroalgal communities (M. Assmann, pers. comm.). Adult *G. homari* were mainly associated with red algae (e.g. *Devaleraea ramentacea*), occasionally with brown algae, and could be found at the base of algal thalli. *A. nugax* were collected between 2-5 m depth with baited traps at London, a sampling site on the Southern side of Blomstrandhalvøya, where macroalgae are restricted to single drop stones and boulders. Animals were immediately transferred to the aquarium and kept at 6-8°C and 34 ‰ salinity prior experimentation, seawater being directly supplied from the cove. Only adult amphipods were used in the experiments.

Carapax UVB-transparency of *G. homari* and *A. nugax* was measured. Animals were dissected and the chitinous carapax cleaned from remaining tissue. The carapax was placed on a UV-transparent filter foil (295 nm cut-off filter) and transmission spectra were recorded in the sunshine simulator.
In a first series of experiments, UV-induced mortality was studied in irradiation experiments with Q-Panel-tubes (low dose), only. In each experimental set-up 20 – 33 adult amphipods were exposed in small aquaria (2l volume, 10cm depth) for 5 hrs daily, over 20 days to light intensities of: 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 $m^{-2}h^{-1}$ UVB and an experimental daily dose of 7.2 kJ m^{-2} d^{-1}. Between each 5 h irradiation interval the animals received dimmed laboratory light, only (as the control set-up, see below). Over the entire 20 days of experimentation the animals were exposed to a maximal total dose of 144.0 kJ m^{-2} UVB during 100-irradiation hrs. This represents a mild dose (41% on average of atmospheric) compared to maximal surface UVB-doses of 2.88 – 4.32 kJ m^{-2} h^{-1} at noon during the experimental period when maximal UVB-intensities of 0.8 – 1.2 Wm^{-2} were measured. Average atmospheric daily doses for June and July 2000 were 36.6 and 22.3 kJ m^{-2} d^{-1} UVB, respectively (Hoyer et al. 2003). Assuming an attenuation of 53% for UVB per meter water column during the summer months (after Hanelt et al. 2001), the resulting average daily UVB-dose between 0

and 1m depth would range between 17.2 and 10.5 kJ m^{-2} d^{-1}. 100-irradiation hrs in the field would yield similar to higher doses between 135.4 and 203.0 kJ m^{-2} UVB in 1m depth than under experimental conditions. Thus, amphipods in our experiments experienced 88% on average of 1m *in-situ* UVB-dose. In the laboratory, different cut-off filters settings for different wavelength ranges were employed: UVB+UVA+PAR (no filter), UVA+PAR (320 nm cut-off), and PAR (400 nm cut-off). Control animals received dimmed laboratory light only and no additional radiation. Where 3 replicate experiments were run means ±SD (standard deviations) are given. Where 2 or 1 replicate experiments were run single values are given (see legends of figures for details).
Herbivores were exposed without macroalgae to avoid shading effects. One group of herbivorous amphipods received algal food between irradiations, while the other was not fed. One group of carn/necr were fed little pieces of fish, while the other group was starved throughout the experimental duration of 20 days. Experiments were checked daily and dead animals counted.

In a second series of experiments, animals were exposed to a high UVB-treatment (1.30 W m^{-2} UVB, daily dose 18.72 kJ m^{-2} d^{-1}) as compared to maximal natural UVB-radiation (see above) amounting to a 35% increase on average of atmospheric UVB-dose. In both experimental UVB-dose-settings sub samples were taken after 7, 12, 14 and 20 days for further analyses of the antioxidant enzymes superoxide dismutase and catalase, the lipid peroxidation status, ß-Carotene content, and content and composition of mycosporine-like amino acids (MAAs).

Results and discussion

Carapax UVB-transparency

Carapax transmission of adult *G. homari* and *A. nugax* was measured to approximate the impact of environmental UV-radiation on the animals' soft tissues. Figure 1 and 2 show transmission spectra recorded in the sunshine simulator. The following settings were compared: lamp spectrum without filter, spectrum below filter foil without carapax, and spectrum below filter foil plus carapax. Differences in carapax transparencies (as % of filter transmission) between herbivorous and carn/necr amphipods from Kongsfjorden are shown in Table 1.
Lower transparency, i.e. better shading against UVB and UVA was found in the Arctic herbivore *G. homari* compared to the carn/necr *A. nugax*. A higher degree of physical sunscreening may be necessary due to the preference of amphipods to associate with macroalgae, which means they are restricted to a certain water depth and light climate and dependent on the shading effect provided by the algae. Carn/necr amphipods can minimise UV-exposure time in shallow water by actively migrating to greater depths at noon. Carapax material was not further analysed for content of UV-protective substances or pigments.
In both amphipod species from Kongsfjorden carapax transmission is balanced in the UVB and UVA range. This holds as well for the Antarctic herbivorous species *Gondogeneia antarctica* and *Djerboa furcipes* (Tab. 2), which were investigated for UV-tolerance during two Antarctic expeditions in 2000 (Obermüller et al. 2003) and 2002 (Tab. 2).

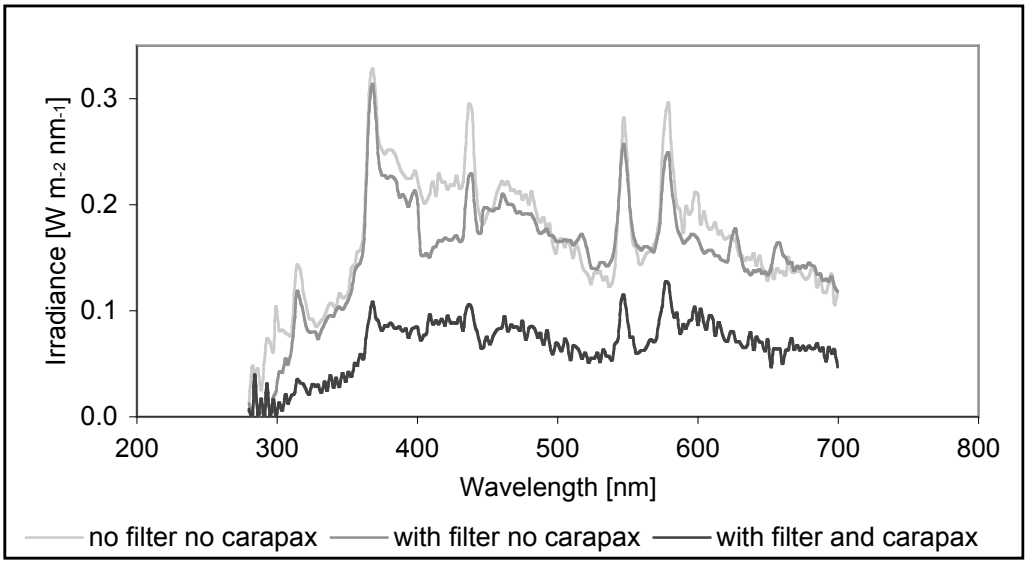

Fig. 1. Carapax transmission spectrum (295-700 nm) of herbivorous *G. homari* (black line) recorded in the sunshine simulator.

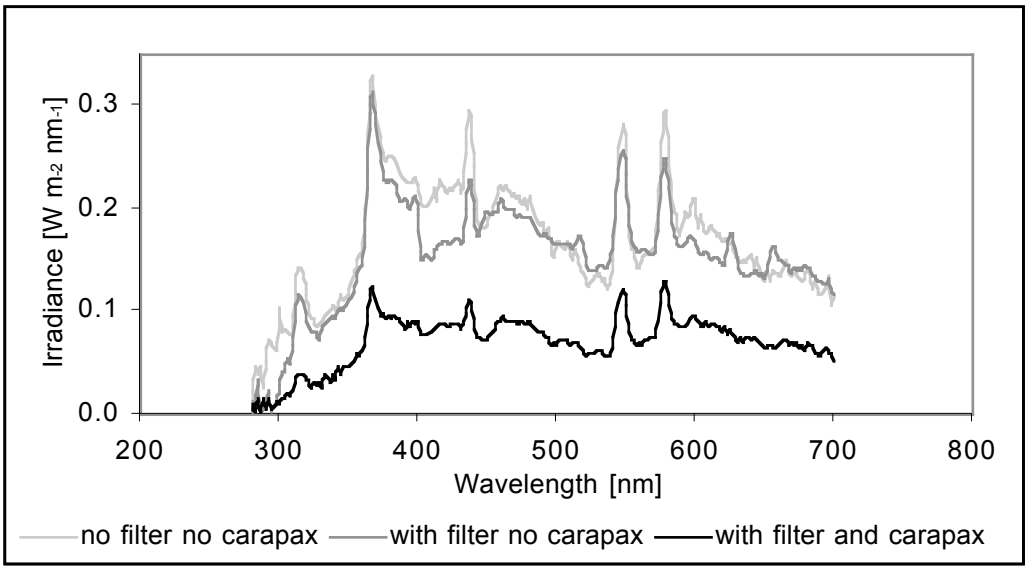

Fig. 2. Carapax transmission spectrum (295-700 nm) of carn/necr *A. nugax* (black line) recorded in the sunshine simulator.

Tab. 1. % Carapax transmission of lamp spectrum light between 295 and 700 nm of amphipods from Kongsfjorden, Arctic.

	% Transmission		
	UVB range (295-320nm)	**UVA** range (320-400nm)	**PAR** range (400-700nm)
G. homari, Arctic herbivore	37.5	36.3	45.6
A. nugax, Arctic *carn/necr*	41.4	41.3	47.4

Tab. 2. % Carapax transmission of lamp spectrum light between 295 and 700 nm of amphipods from Potter Cove, Antarctica.

	% Transmission		
	UVB range (295-320nm)	**UVA** range (320-400nm)	**PAR** range (400-700nm)
G. antarctica, Antarctic herbivore	56.4	58.5	61.2
D. furcipes, Antarctic herbivore	42.4	43.0	49.4

Carapax transparencies of *G. antarctica* are 20% higher in the UV-range compared to *G. homari* from Kongsfjorden, whereas transmission of *D. furcipes* is only slightly higher than in the Arctic herbivore and within the range of the carn/necr *A. nugax*. Both Antarctic species were collected at the same sampling site in the shallow rocky intertidal (0-2m) in Potter Cove, King George Island, where they are associated with macroalgae, which form moderate to dense communities. Despite higher carapax transparency (i.e. lower protection), *G. antarctica* is more agile than *D. furcipes* and actively swimming in the water column above and around the algae even during peak radiation at noon, thus being fully exposed to UV-radiation whilst active. Similar transmission values as Antarctic *G. antarctica* were also measured for the temperate North Sea amphipod *Chaetogammarus marinus*, where transmission was 9-22% higher (i.e. lower protection) in all spectral ranges in comparison to amphipods from Kongsfjorden (Obermüller et al. 2003).

UV-induced mortality

No mortality (100% survival) was found in herbivorous *G. homari* under almost any condition (Tab. 3). Together with the low UV-carapax transmission this reflects a high UV-tolerance of this species and supports its occurrence in various intertidal and subtidal habitats with moderate to dense macroalgal communities. In similar radiation experiments the intertidal Antarctic herbivore *G. antarctica* was slightly more sensitive. 98 and 89% of exposed animals survived a mild UVB-dose (Q-Panel-tubes: 0.38 W m^{-2} UVB, daily dose 6.82 kJ m^{-2} d^{-1}, i.e. 48% on average of *in-situ* dose) (Obermüller et al. 2003). Starvation further reduced survival of *G. antarctica* by 12% whereas in Arctic *G. homari* starvation had no effect on UVB-survival rates at the applied dose and data of starved and fed animals are shown together in Table 3.

Tab. 3. *G. homari*: Survival of amphipods exposed to a mild UVB-dose (7.2 kJ m^{-2} d^{-1}) during 20 days (20-33 individuals per experiment). Survival rate (%) of all individuals initially exposed. Data as means (±SD) for treatments "control" (3 replicates) and "UVB+UVA+PAR" (3 replicates). 1 experiment for treatment "UVA+PAR". Fed and non-fed animals shown together.

	0 days	10 days	20 days
control	100.0 (±0)	100.0 (±0)	100.0 (±0)
UVB+UVA+PAR	100.0 (±0)	100.0 (±0)	98.8 (±2.5)
UVA+PAR	100.0	100.0	100.0

In carn/necr Arctic amphipods of the species *A. nugax* both, UVB and UVA, led to reduced survival in fed animals (Fig. 4). Various authors have reported UVA to have positive and negative effects on plants and animals by stimulating photoenzymatic repair (PER), but at the same time contributing to the damaging effects of UVR (Williamson et al. 2001 and therein). Until day 7 UVA and UVB appeared equally damaging, where after subtraction of UVB clearly increased survival. As UVA was not selectively cut off, we cannot determine the respective contribution of PER, dark repair and photoprotection to overall UV-tolerance, as defined by Williamson et al. 2001. As a matter of fact, fed amphipods appeared more vulnerable to UVB than starved ones (Fig. 4).

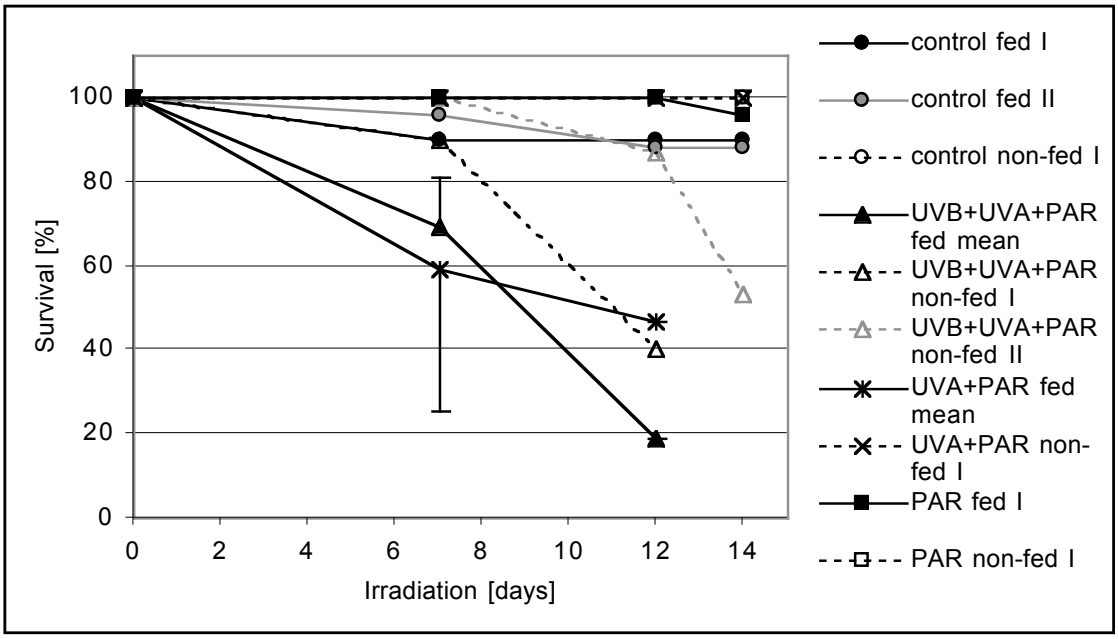

Fig.4. *A. nugax:* Survival of fed (filled symbols) and non-fed (open symbols) amphipods exposed to a mild daily UVB-dose (7.2 kJ m^{-2} d^{-1}) during 14 days (20-30 individuals per experiment). Survival rate of all individuals initially exposed. Data as means ±SD for treatments "UVB+UVA+PAR fed" (3 replicates) and "UVA+PAR fed" (3 replicates). 1 experiment for other treatments: "control fed I", "control fed II", "control non-fed I", "UVB+UVA+PAR non-fed I", "UVB+UVA+PAR non-fed II", "UVA+PAR non-fed I", "PAR fed I", "PAR non-fed I". Experiments "UVB+UVA+PAR fed", "UVB+UVA+PAR non-fed I", and "UVA+PAR fed" were stopped after 12 days due to poor condition of exposed animals.

Polar and sub-polar carn/necr amphipods have been reported to survive between one and several months (Sainte-Marie et al. 1989: *A. nugax*, St. Lawrence estuary, Canada, Chapelle et al. 1994: *Waldeckia obesa*, King-George Island, Antarctica) of starvation mobilising storage lipids, but also inducing metabolic reduction (Chapelle et al. 1994). This is an adaptation to fluctuating food supply in their natural habitat. Chapelle et al. (1994) recorded a decrease of oxygen consumption in *Waldeckia obesa* during starvation over 65 days and a dramatic increase to even higher consumption rates than those, measured in control animals prior to starvation, when the amphipods were fed again. Elevated post starvation rates lasted for 8-10 days. We conjecture, that a possible reason for the higher UVB-vulnerability of *A. nugax* might be the ad libitum feeding with fish and the ensuing high metabolic activity of the animals, which is bound to increase the metabolic production of reactive oxygen

species, and obviously has rendered the animals more susceptible to UVB damage. This is in keeping with the general finding that metabolic reduction can ameliorate production of oxygen free radicals and thereby confers higher stress resistance and longevity to an animal (further reading: Yoon et al. 2002). UVB-exposure of adult females of the copepod *Sinocalanus tenellus* significantly reduced gut pigment content, suggesting radiation to impact on feeding or digestion processes (Lacuna and Uye 2000). On the contrary, in *Daphnia pulex*, a frequently occurring cladoceran crustacean in shallow alpine and Arctic lakes, increasing the quantity of algal food had a positive effect on UV-tolerance, leading to increased survival (Zellmer 1996). In our experiments differences in mortality between non-fed and fed control amphipods amounted to 10% after 12 experimental days, where after no more fed control animals died. 100% survival was found only in non-fed *A. nugax* under all irradiation condition, except when UVB was included. We hypothesize that carn/necr amphipods are more sensitive to abiotic stress during intensive feeding and digestion processes.

Our preliminary results of the high UVB-dose experiments and UVB-induced effects on oxidative stress parameters support the findings presented in this study. The investigated carn/necr amphipods are more vulnerable to UVB-exposure than their herbivorous kin, which was indicated in the clearly differing survival rates of herbivores and carn/necrs (this study). Antioxidant enzyme activities of exposed amphipods seem to be maintained at the same level as in controls (catalase) or induced (SOD) in herbivorous *G. homari* under both high and low UVB-dose, whereas in carn/necr *A. nugax* antioxidant enzyme activities fail to be induced (SOD) or collapse (catalase). This reflects a high UV-tolerance of the herbivore species. The results of the two experimental series will be completed by a detailed analyses and comparison of the amphipods' biochemical defence systems to ameliorate photo-induced oxidative damage.

Our investigations will further evaluate the question whether carn/necr lack UV-absorbing sunscreens (MAAs), which herbivores extract from their macroalgal diet, (Obermüller et al. 2003), and thereby lack a vital part of protective defence against UV-induced damage.

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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.

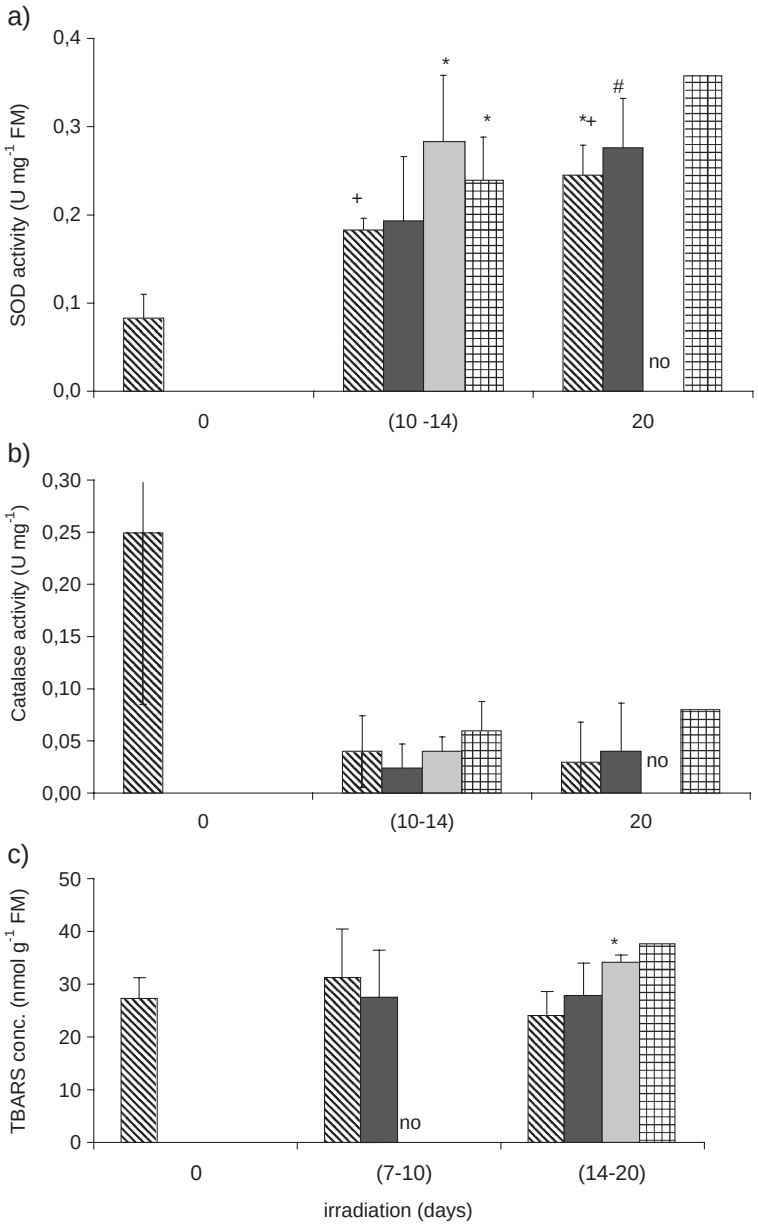

Fig. 1. *Gammarellus homari*: (a) SOD activity, (b) CAT activity, (c) TBARS concentration. Values are means ± SD, $n = 4–10$. Striped—control; black—UVB + UVA + PAR low dose (ld); grey—UVA + PAR low dose; checked—UVB + UVA + PAR high dose (hd), no—no data. Symbols of significance: *significantly different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

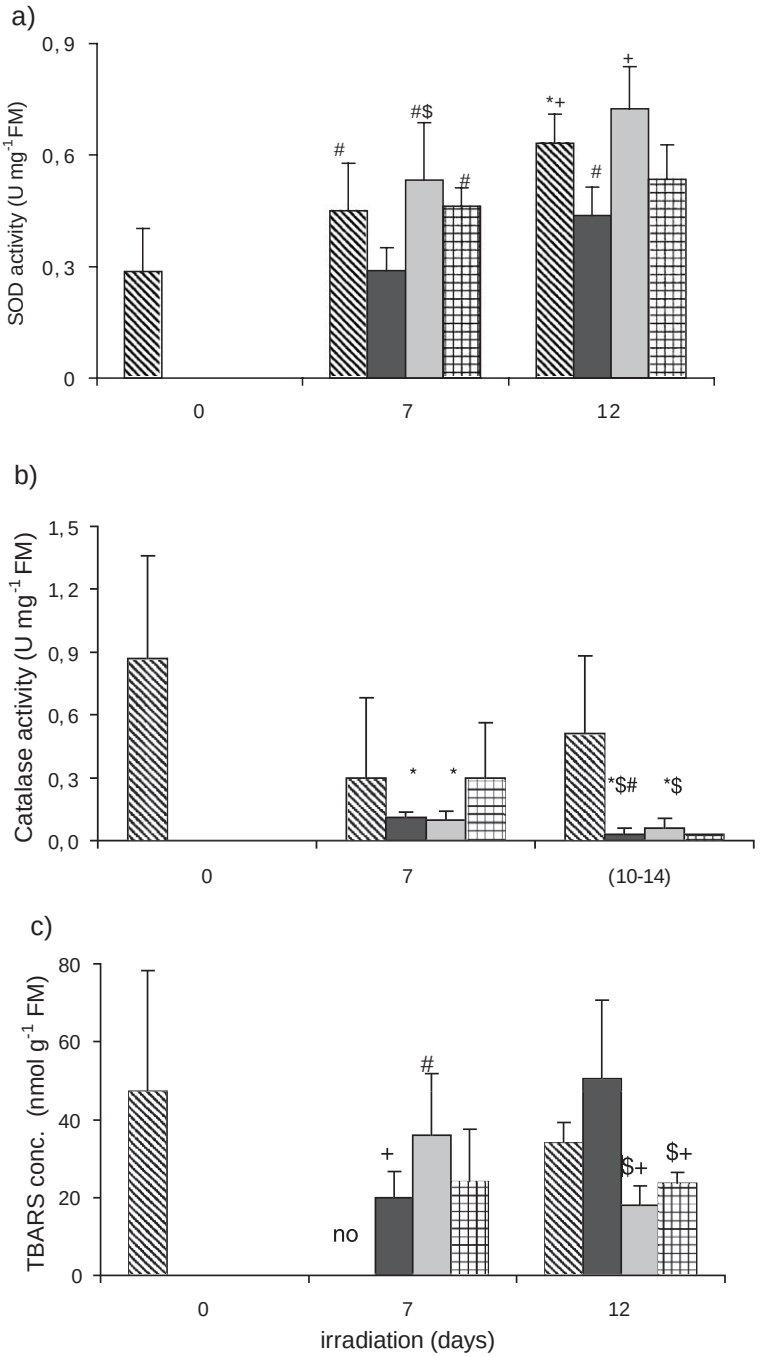

Fig. 2. *Anonyx nugax*: (a) SOD activity, (b) CAT activity, (c) TBARS concentration. Values are means ± SD, $n = 5–10$. Striped—control; black—UVB+UVA+PAR low dose (ld); grey—UVA+PAR low dose; checked—UVB+UVA+PAR high dose (hd), no—no data. Symbols of significance: *significantly different from 0-day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

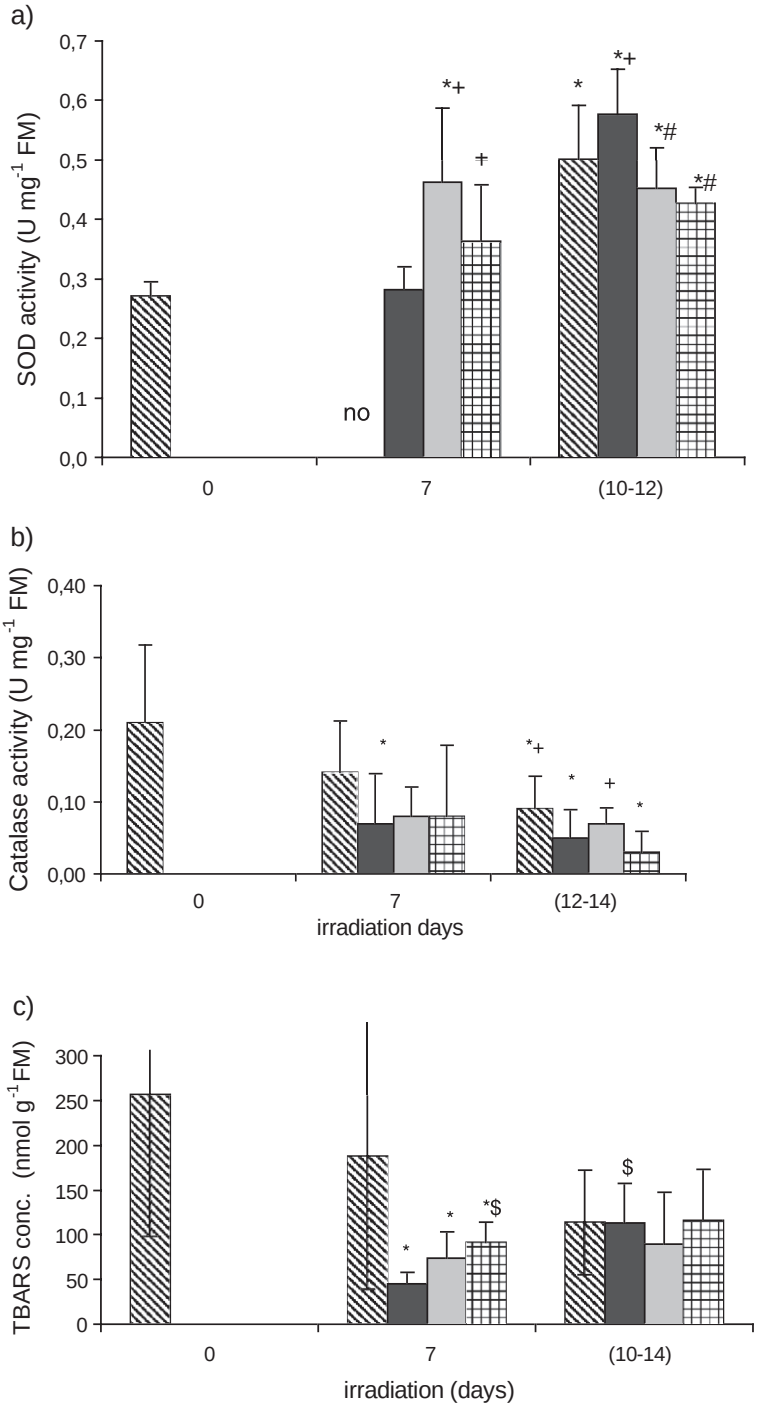

Fig. 3. *Onisimus edwardsi*: (a) SOD activity, (b) CAT activity, (c) TBARS concentration. Values are means ± SD, $n = 3–10$. Striped—control; black—UVB + UVA + PAR low dose (ld); grey—UVA + PAR low dose; checked—UVB + UVA + PAR high dose (hd), no—no data. Symbols of significance: *significantly different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

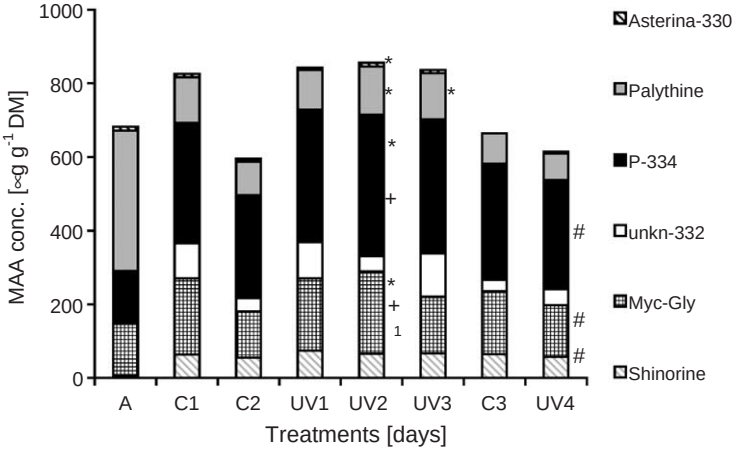

Fig. 4. *Gammarellus homari*: Variation of individual MAA content during low- and high-dose UV treatment and control maintenance over 20 days. Values are means ± SD given as stacked columns. C1—control at (0–7) days, C2—control at (8–14) days, UV1—UVB + UVA + PAR low dose at (8–14) days, UV2—UVA + PAR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

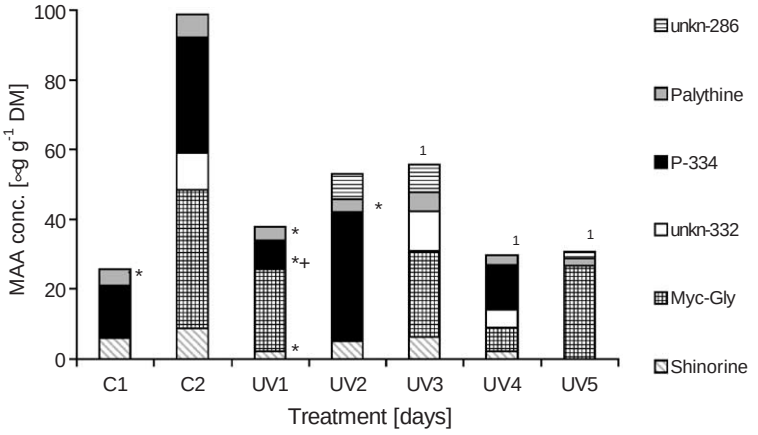

Fig. 5. *Anonyx nugax*: Variation of individual MAA content during low- and high-dose UV treatment and control maintenance over 14 days. Values are means ± SD given as stacked columns. C1—control at (0) days, C2—control at (7–14) days, UV1—UVB+UVA+PAR low dose at (7) days, UV2—UVA+PAR low dose at (7) 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-

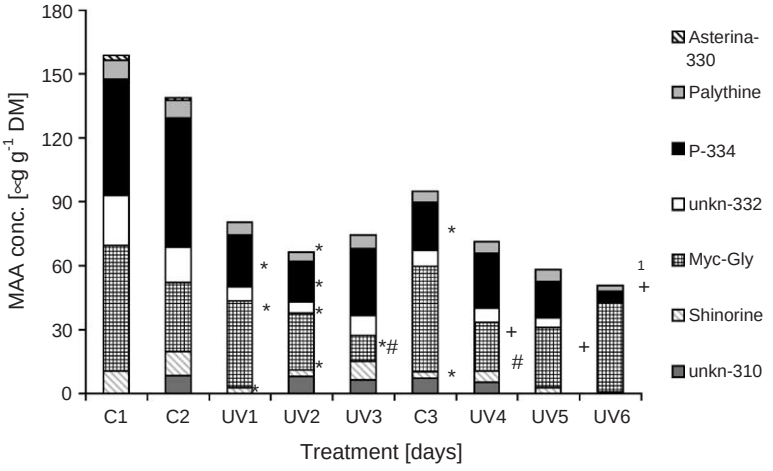

Fig. 6. *Onisimus edwardsi*: Variation of individual MAA content during low- and high-dose UV treatment and control maintenance over 14 days. Values are means ± SD given as stacked columns. C1—control at (0) days, C2—control at (7) days, UV1—UVB+UVA+PAR low dose at (7) days, UV2—UVA+PAR low dose at (7) 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.

Acknowledgements

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Publication IV

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 UV (42.1 and 54.5% on average respectively) compared to the PAR range (38.1 and 50.1% respectively) of the solar spectrum. Bands of higher absorbance correlated with maximal absorbance ranges of sunscreening compounds indicating MAAs and carotenoids to be innate components of the exoskeleton of these species. Though the antioxidant enzyme catalase was photoinhibited, protein damage products did not accumulate under experimental UVR-exposure. 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, UV-protection, stress response, oxygen consumption

1. Introduction

Direct and unshielded exposure to UV-photons reaching the earth's surface with the natural sunlight has been shown to cause damage in tissues and cells of aquatic animals and plants already under natural shallow water irradiation conditions (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-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). But the effects can be reaching also at heme-containing enzymes, absorbing around 405 nm, e.g. the hydrogen peroxide H_2O_2-detoxifying catalase. Photodestruction of the heme 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 the highly electro-positive molecular oxygen, forming superoxide ions, which then

dismutate to hydrogen peroxide 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 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 the Antarctic environments (Obermüller et al. 2003, 2005, Obermüller & Abele 2004). In the present paper we studied 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 alterations of whole animal metabolic rates in various marine invertebrates. Specifically, lower H_2O_2-concentrations than those present in the animals' own hemolymphatic fluid caused an increase, whereas concentrations, elevated over hemolymph 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 were taken from areas of 10-50 cm water depth, where they colonised the canopy of intertidal macroalgae, but also swam freely, 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 $\pm$ 0.5°C and 34 psu salinity. 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

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. Solar UVA and PAR (photosynthetically active radiation) were measured in 2000 during a previous campaign 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), 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

Animals were dissected and the chitinous exoskeleton cleaned from residual tissue. The carapace was spread onto a UV-transparent filter foil (295 nm cut-off) and the transmission spectrum recorded from 295 to 700 nm in the sunshine simulator (SONSI), using a Zeiss Monolithic Miniature Spectrometer MMS UV-VIS (Zeiss, Germany), combined with electronics by M. Kruse (Germany). 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 foil)

T_f: Transmission of SONSI light source through filter foil

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

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

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

Adult *G. antarctica* and *D. furcipes* were exposed to artificial low-dose PAR+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.

Two cut-off filter foils were employed, 320 nm and 400 nm, to selectively shield amphipods in selected aquaria from UVB and UVA+UVB 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.

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. (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) and in *G. antarctica* also after 10 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 $\varepsilon = 22 \times 10^3$ M^{-1} cm^{-1}) and expressed in nmol mg^{-1} protein.

2.5 Oxygen consumption measurements under UV-exposure in the sunshine simulator (SONSI)

Irradiation experiments were carried out with live animals (5-6 specimens per experiment) directly within the sunshine 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. 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 oxygen-saturated (100%) and N_2-saturated (0%) 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 sunshine simulator amphipods were exposed to 1.5 Wm^{-2} UVB, which was 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 $\pm$ 0.3°C and oxygen 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. 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 given in Figure 1. Briefly, each experiment started with a 6 h recording of "low light respiration" (RMR, phase 1). Animals were shielded from irradiation emitted by the SONSI lamp by a black plastic foil 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 foil wrapped around the respiration chamber. Subsequently, the chamber was shielded again for 6 h with black foil (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 within the different experimental phases. Amplitudes represent the difference between maximal and minimal oxygen consumption during each of these 30 min intervals and are depicted as bar widths of mean oxygen consumption in Figures 5 and 6. 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.

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 carotenoid concentrations 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 the five experiments within one species were tested using a Kruskal-Wallis one-way ANOVA on ranks followed by a multiple comparison post test (Dunn's Procedure). Data are given as means $\pm$ SD if not stated otherwise.

3. Results

3.1 Carapace UV-absorbance and tissue UV- and antioxidant protection

Carapace UV-absorbance of *G. antarctica* and *D. furcipes* was measured to approximate the impact of environmental UV-radiation on the animals' soft tissues. Carapace absorbance was more effective in the UV-compared to the PAR-range in both species, but 9% (UVB), 16% (UVA), and 12% (PAR) lower in *G. antarctica* than in *D. furcipes* (Tab.1). Figure 2 depicts a carapace absorbance spectrum of *D. furcipes*. Bands of higher absorbance are located between 305-340, 430-450 and 480-530 nm (see arrows in Fig. 2), and correspond to the ranges of

absorbance of several types of sunscreening compounds: mycosporine-like amino acids (MAAs) between 305-340 nm and carotenoids between 430-450 nm (e.g. ß-carotene). The third range (480-530 nm, unknown) was not investigated in detail but might reflect additional carotenoids (e.g. astaxanthin at 485 nm). This indicates incorporation of these metabolites and pigments into the chitinous exoskeleton matrix. *G. antarctica's* carapace absorbance spectrum was similar to the spectrum in Fig. 2 and is therefore not shown.

Carotenoid tissue concentration was significantly higher in *G. antarctica* (0.174 $\pm$ 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 (Tab. 2). *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 differences reached significance only in *G. antarctica* after 4 and 7 days of irradiation (Ga: Fig. 3, Tab. 3, Df: Fig. 4, Tab. 3). Differences between species were significant only on the start day with higher catalase activity in *D. furcipes*.

Proteinoxidation did not differ significantly between species, nor within either species between control and UVR-treatments over 10 days of repeated exposure. Only in *G. antarctica* carbonyl content was significantly lower after PAR-exposure over 4 to 7 days than in controls ($p<0.05$) (Tab. 4).

3.2 Oxygen Consumption

Oxygen consumption (mean $\pm$ SD) as well as respiratory amplitudes (mean $\pm$ SD) calculated for each 30 min interval (bar width) within the different experimental phases are depicted in Figures 5 a-d for *G. antarctica* (Ga) and Figures 6 a-e for *D. furcipes* (Df). The total number of successful experiments was 4 for *G. antarctica* and 5 for *D. furcipes*. 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 $\pm$ 0.35 µmol O_2 g^{-1} FW h^{-1} for *G. antarctica* (Tab. 5). Mean RMR in *D. furcipes* amounted to 4.57 $\pm$ 2.20 µmol O_2 g^{-1} FW h^{-1} with an amplitude of 0.73 $\pm$ 0.83 µmol O_2 g^{-1} FW h^{-1} (Tab. 6). 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 in the first control phase and in both recovery phases (Tab. 5). UVR effects were not consistent within the 4 experiments. In experiments Ga 1 and 3 (Fig. 5a, c) 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 the other two experiments Ga 2 and 4 (Fig. 5b, d), where exposure to UVB+UVA+PAR caused an increase over low light RMR (phase 1), significantly higher ($p\leq0.01$) than 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. Irradiation with both UV spectral ranges caused high amplitudes when light exposure commenced. The variability was reduced 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.

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 ($p<0.05$).

Djerboa furcipes: The animals' mean oxygen consumption and respiratory amplitudes increased above resting levels (RMR: phase 1) during UVR-exposure in all five experiments (Tab. 6). 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. 6a, $p=0.01$), Df 3 (Fig. 6c, $p=0.02$), Df 4 (Fig. 6d, $p<0.01$), and Df 5 (Fig. 6e, $p<0.01$), whereas UVB- and UVA-effects on respiration were not significantly different in experiment Df 2 (Fig. 6b, $p=0.93$). Oxygen consumption decreased slowly to reach RMR-levels (phase 1) during both recovery phases (3 and 5) in all experiments, except Df 1 (Fig. 6a) and Df 4 (Fig. 6d, phase 5). Sometimes, instantaneous respiratory increase and subsequent decrease were

very rigid as seen in experiments Df 2 (Fig. 6b), Df 4 (Fig. 6d), and Df 5 (Fig. 6e). 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, already, due to problems with the optode sensor. Respiratory amplitudes of irradiated compared to low light amphipods were about the same during UVB+UVA+PAR (2.58 $\pm$ 2.75 µmol O_2 g^{-1} FW h^{-1} on average) and UVA+PAR-exposure (2.62 $\pm$ 2.47 µmol O_2 g^{-1} FW h^{-1} on average) (Tab. 6) and, clearly, more variable in range between maximal and minimal respiration compared to recovery phases.

The Kruskal-Wallis one-way ANOVA on ranks revealed significant differences in specific oxygen consumption (µmol g^{-1} fresh weight) levels 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 vs. Df 4, Df 1 vs. Df 3, Df 1 vs. Df 5, Df 5 vs. Df 3, Df 5 vs. Df 2.

4. Discussion

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

Higher carapace absorbance in the UV-range indicates slightly better (10%) 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. 2) indicated MAAs and carotenoids to be innate compounds of the exoskeleton. 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*. Tab. 7 summarises the data from Arctic and Antarctic species. In addition to better carapace shielding, *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). Contrary, in neither species did exposure to UVR cause significant bleaching of tissue MAAs (Tab. 7) and carotenoids. Further, tissue protein carbonyl content indicative of oxidative protein damage did not increase in irradiated amphipods. Apparently, the antioxidant defence system of the investigated amphipods was sufficient to prevent protein damage in spite of the impaired catalase activity, which could be compensated by (glutathione) peroxidases, which are efficient scavengers of small H_2O_2 levels (Hermes-Lima 2004). 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 $\pm$ 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: *Bovallia gigantea* 4.46 $\pm$ 0.49 µmol O_2 g^{-1} FW h^{-1} and *Eurymera monticulosa* 5.26 $\pm$ 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. The increase in oxygen consumption lasted between 60 and 120 min before it 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 respiration periods, the animals became metabolically depressed and in this paralleling our H_2O_2 exposure experiments with polychaetes and mudshrimp (Storch et al. 2001, Abele-Oeschger et al. 1997). This provides a first hint that ROS, formed in tissues during intense UVR could also lead to metabolic slow down in irradiated animals. In juvenile Antarctic krill (*Euphausia superba*, Newman et al. 1999) animals, 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 seized and animals resettled to the gauze. During long term exposure of up to 3 weeks we observed accelerated activity 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 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 diel 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 as compared to 1:15:91 measured in the field during the experimental period (October - December 2002). Thus, basically artificial SONSI light provides sufficient photoreactivating radiation to activate photo-repair in exposed amphipods of our experiments (Goncalves et al. 2002, Williamson et al. 2001).

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 level 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. Physical and chemical defence should sufficiently protect the amphipods from direct UV-damage. 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

Table 1: Carapace absorbance (%) of SONSI lamp spectrum light between 295 and 700 nm of Antarctic amphipods from Potter Cove (South Shetland Islands).

% Absorbance			
	UVB (295-320nm)	**UVA** (320-400nm)	**PAR** (400-700nm)
G. antarctica	43.0	41.4	38.1
D. furcipes	52.0	57.0	50.1

Table 2: Total carotenoid concentration (µmol ß-carotene equivalents g^{-1} FW) in whole animal homogenates from Antarctic amphipods from Potter Cove (South Shetland Islands) 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. n = number of replicate samples, with numbers in brackets indicating the replicates per value. T-tests carried out between values from controls vs. UVB+UVA+PAR* at 10 days and controls vs. PAR for *G. antarctica* and *D. furcipes*.

Total carotenoid concentration (µmol g^{-1} FW)			
	0 days	10 days	t-test
G. antarctica			Control vs +UVB*
Control	0.174 ± 0.02 (3)	0.199 ± 0.019 (3)	p = 0.33
UVB+UVA+PAR*		0.177 ± 0.041 (5)	
UVA+PAR (320nm cut-off)		0.173 (2)	Control vs PAR
PAR (400 nm cut-off)		0.199 ± 0.027 (4)	p = 0.99
D. furcipes			Control vs +UVB*
Control	0.131 (2)	0.117 ± 0.012 (4)	p = 0.74
UVB+UVA+PAR*		0.114 ± 0.007 (3)	
UVA+PAR (320nm cut-off)		0.118 (2)	Control vs PAR
PAR (400 nm cut-off)		0.112 ± 0.011 (3)	p = 0.62

Table 3: Catalase activity (U mg^{-1} FW) in whole animal homogenates from Antarctic amphipods from Potter Cove (South Shetland Islands) exposed to low-dose irradiation (Q-Panel tubes: 0.38 Wm^{-2} UVB, 3.68 Wm^{-2} UVA, 5.73 Wm^{-2} PAR). Values are mean ± SD. n = number of replicate samples, with numbers in brackets indicating the replicates per value. * Significant differences in this irradiation interval between control values and respective treatment ($p<0.05$).

Catalase activity (U mg^{-1} FW)			
	0 days	3-4 days	5-7 days
G. antarctica			
Control	0.85 ± 0.30 (15)	0.79 ± 0.09 (4)	0.61 ± 0.51 (9)
UVB+UVA+PAR		0.48 ± 0.26 (5)	0.15 ± 0.16 (6) *
UVA+PAR (320nm cut-off)		0.45 ± 0.21 (6) *	0.28 ± 0.29 (5)
PAR (400nm cut-off)		0.34 ± 0.06 (3) *	0.47 ± 0.32 (3)
D. furcipes			
Control	1.67 ± 0.97 (18)	0.89 ± 0.63 (4)	0.86 ± 0.88 (6)
UVB+UVA+PAR		0.36 ± 0.32 (6)	0.17 (1)
UVA+PAR (320nm cut-off)		0.16 + 0.22 (5)	0.28 ± 0.38 (5)
PAR (400nm cut-off)		0.88 ± 0.80 (7)	0.60 ± 0.49 (4)

Table 4: Protein carbonyl content (nmol mg^{-1} protein) in whole animal homogenates from Antarctic amphipods from Potter Cove (South Shetland Islands) exposed to low-dose irradiation (Q-Panel tubes: 0.38 Wm^{-2} UVB, 3.68 Wm^{-2} UVA, 5.73 Wm^{-2} PAR). Values are mean ± SD. n = number of replicate samples, with numbers in brackets indicating the replicates per value. * Significant difference between PAR-exposure and control animals at 4 – 7 days ($p<0.05$).

Protein carbonyl content (nmol mg^{-1} protein)			
	0 days	4 - 7 days	10 days
G. antarctica			
Control	4.76 + 2.02 (6)	8.18 + 3.63 (3)	4.53 + 2.32 (4)
UVB+UVA+PAR		5.15 + 2.31 (5)	4.69 + 1.90 (4)
UVA+PAR (320nm cut-off)		6.21 + 1.08 (3)	6.44 (2)
PAR (400nm cut-off)		* 3.42 + 1.33 (6)	6.08 + 3.01 (5)
D. furcipes			
Control	6.04 (2)	5.27 + 2.63 (9)	5.68 (2)
UVB+UVA+PAR		3.67 + 2.64 (5)	
UVA+PAR (320nm cut-off)		4.56 + 2.51 (4)	
PAR (400nm cut-off)		4.71 + 2.74 (9)	6.34 + 2.22 (4)

Table 5: Respiration rates of *G. antarctica* (Ga) during irradiation experiments in the sunshine simulator (1.5 Wm^{-2} UVB, 39.7 Wm^{-2} UVA, 117.7 Wm^{-2} PAR). Values given as mean ± SD and amplitudes (i.e. short-term changes in oxygen consumption around the means during a 30 min time interval in an experimental phase) given as mean ± SD. Oxygen consumption expressed as M O_2 (µmol O_2 g^{-1} fresh weight FW h^{-1}). [1)] Wilcoxon rank test instead of paired t-test because normality test failed.

Table 6: Respiration rates of *D. furcipes* (Df) during irradiation experiments in the sunshine simulator (1.5 Wm^{-2} UVB, 39.7 Wm^{-2} UVA, 117.7 Wm^{-2} PAR). Values given as mean ± SD and amplitudes (i.e. short-term changes in oxygen consumption around the means during a 30 min time interval in an experimental phase) given as mean ± SD. Oxygen consumption expressed as M O_2 (µmol O_2 g^{-1} fresh weight FW h^{-1}). [1)] n.d. not determined due to sensor problems, experiment stopped after 2.5 h.

Table 5

Oxygen consumption (µmol O_2 g^{-1} FW h^{-1}) *G. antarctica*										
phase 1 low light		phase 2 UVA+PAR		phase 3 low light		phase 4 UVB+UVA+PAR		phase 5 low light		experiment figure
M O_2	amplitude	M O_2	amplitude	M O_2	amplitude	M O_2	amplitude	M O_2	amplitude	
3.27 ± 1.33	0.42 ± 0.49	8.11 ± 0.36	0.70 ± 0.55	4.84 ± 1.32	0.34 ± 0.42	6.69 ± 0.67	0.97 ± 0.33	3.39 ± 1.30	0.54 ± 0.61	Ga 1, 5a
8.75 ± 1.04	0.23 ± 0.45	7.03 ± 1.65	1.51 ± 1.29	9.89 ± 1.61	0.73 ± 0.60	12.56 ± 3.65	1.71 ± 1.78	10.48 ± 0.71	1.06 ± 0.94	Ga 2, 5b
8.89 ± 0.72	0.31 ± 0.45	11.31 ± 1.42	0.86 ± 1.34	8.81 ± 1.30	0.47 ± 0.54	7.19 ± 3.20	1.46 ± 0.71	8.43 ± 1.67	0.64 ± 0.62	Ga 3, 5c
3.04 ± 1.39	0.11 ± 0.20	7.16 ± 0.72	0.83 ± 0.63	8.62 ± 2.03	0.75 ± 1.09	11.14 ± 1.50	1.29 ± 1.71	7.10 ± 2.76	0.29 ± 0.39	Ga 4, 5d

t-test	t-test	t-test	experiment figure
ph 1 vs ph 3	ph 1 vs ph 3	ph 2 vs ph 4	
$p < 0.01$	[1] $p = 0.77$	$p < 0.01$	Ga 1, 5a
$p = 0.29$	$p < 0.01$	$p < 0.01$	Ga 2, 5b
$p = 0.89$	$p = 0.19$	$p < 0.01$	Ga 3, 5c
$p < 0.01$	[1] $p < 0.01$	$p < 0.01$	Ga 4, 5d

Table 6

Oxygen consumption (µmol O_2 g^{-1} FW h^{-1}) *G. antarctica*										
phase 1 low light		phase 2 UVA+PAR		phase 3 low light		phase 4 UVB+UVA+PAR		phase 5 low light		experiment figure
M O_2	amplitude	M O_2	amplitude	M O_2	amplitude	M O_2	amplitude	M O_2	amplitude	
4.84 + 1.21	0.50 + 0.66	14.39 + 2.15	0.85 + 1.49	12.69 + 3.05	0.30 + 0.40	17.62 + 0.50	0.98 + 1.62	12.88 + 2.15	1.28 + 1.09	Df 1, 5a
5.61 + 2.90	1.33 + 1.62	13.49 + 6.50	5.19 + 4.12	7.31 + 1.81	2.00 + 2.85	13.27 + 9.35	3.85 + 2.90	7.94 + 1.63	3.37 + 2.29	Df 2, 5b
6.29 + 1.55	0.49 + 0.68	9.11 + 1.42	1.09 + 1.21	7.23 + 1.20	0.33 + 0.40	11.58 + 1.61	1.23 + 1.94	7.52 + 1.94	0.73 + 0.76	Df 3, 5c
2.35 + 1.29	0.10 + 0.25	6.67 + 1.29	1.93 + 1.49	2.36 + 3.01	1.60 + 1.58	15.90 + 1.37	2.68 + 2.90	7.96 + 4.02	1.53 + 1.80	Df 4, 5d
3.75 + 4.07	1.23 + 0.93	6.32 + 2.34	4.05 + 1.97	0.77 + 1.19	2.97 + 1.79	19.70 + 4.27	4.16 + 4.37	6.26 + 7.50	4.99 + 3.29	Df 5, 5e

t-test	t-test	t-test	experiment figure
ph 1 vs ph 3	ph 1 vs ph 3	ph 2 vs ph 4	
$p < 0.01$	$p < 0.01$	$p = 0.01$	Df 1, 5a
$p = 0.04$	$p = 0.06$	$p = 0.93$	Df 2, 5b
$p = 0.25$	$p = 0.05$	$p = 0.02$	Df 3, 5c
$p = 0.99$	$p < 0.01$	$p < 0.01$	Df 4, 5d
$p = 0.01$	[1] n.d.	$p < 0.01$	Df 5, 5e

Table 7: Total MAA concentrations (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, with numbers in brackets indicating the replicates per value. [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, in (-UVB)-treatment a 320 nm cut-off filter was employed resulting in UVA+PAR-exposure, [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. * Significant differences ($p<0.05$) only between control values and 14 days (+UVB)-exposure (UVB+UVA+PAR) in *G. antarctica*.

Antarctic	Control 0 days	14 days +UVB	14 days -UVB
G. antarctica [1]	776 ± 60 (5)	*636 ± 97 (3)	742 ± 220 (6)
D. furcipes [1]	53 (1)	211 ± 28 (3)	117 ± 61 (4)
Arctic			
G. homari [2]	761 ± 467 (4)	[A]836 ± 338 (4)	[B]856 ± 189 (3)

Figures

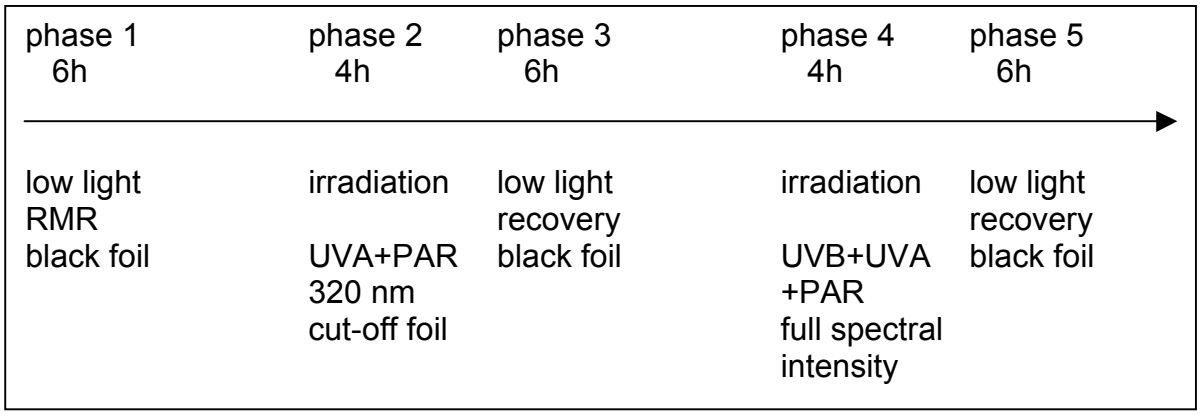

Figure 1: Flow chart showing succession of experimental phases and alternating radiation and recovery phases.

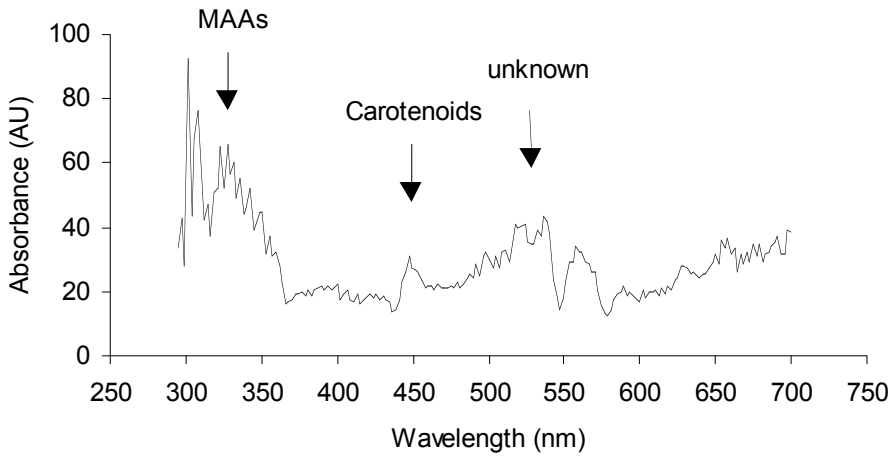

Figure 2: *D. furcipes* carapace absorbance spectrum between 295 and 700 nm recorded in the sunshine simulator (SONSI). Arrows indicate bands of higher absorbance corresponding to maximal absorbance ranges of sunscreening substances (MAAs: mycosporine-like amino acids).

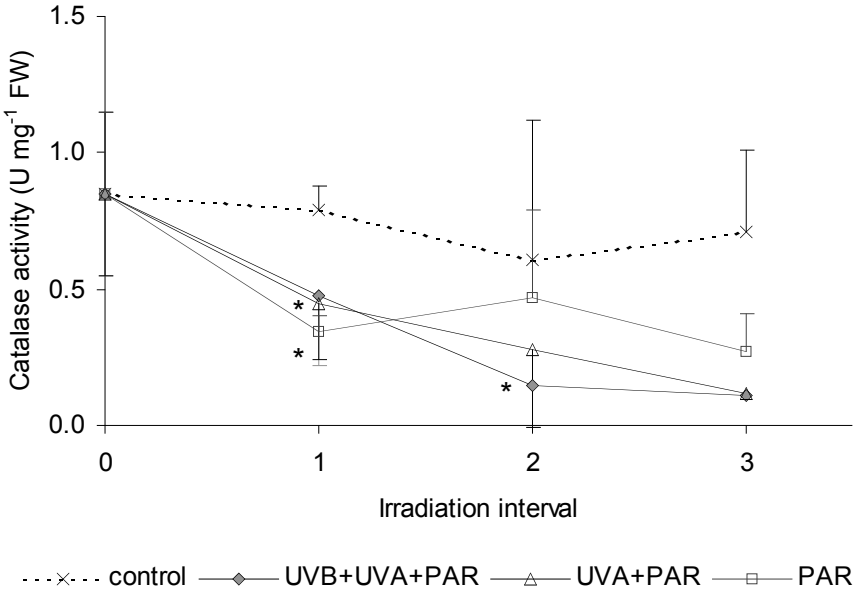

Fig. 3: Catalase activity (U mg^{-1} FW) in *G. antarctica* during low-dose irradiation. Irradiation intervals are 0 days, 1: 3-4 days, 2: 5-7 days, 3: 10 days. * Significant differences between control and UVA+PAR, and PAR exposure at 3-4 days and between control and UVB+UVA+PAR at 5-7 days.

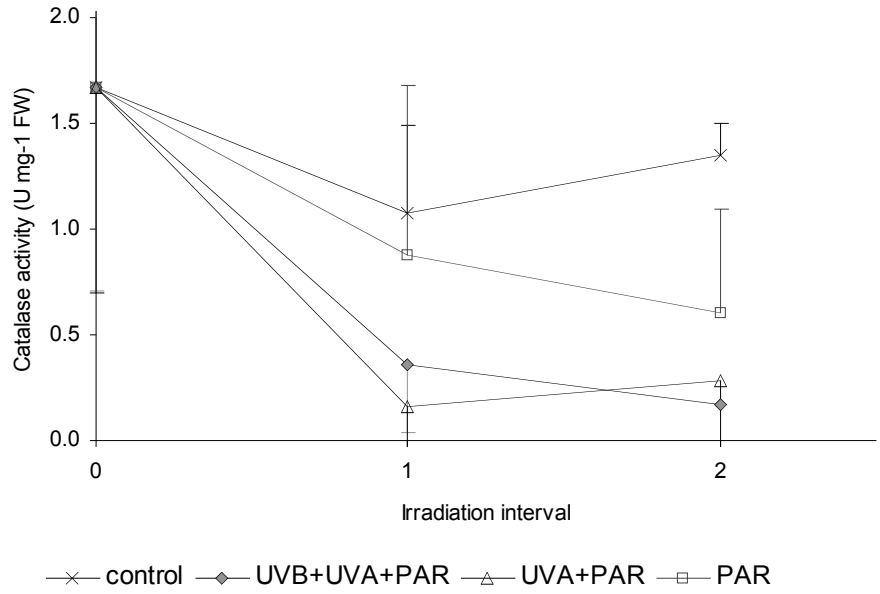

Fig. 4: Catalase activity (U mg^{-1} FW) in *D. furcipes* during low-dose irradiation. Irradiation intervals are 0 days, 1: 3-4 days, 2: 5-7 days.

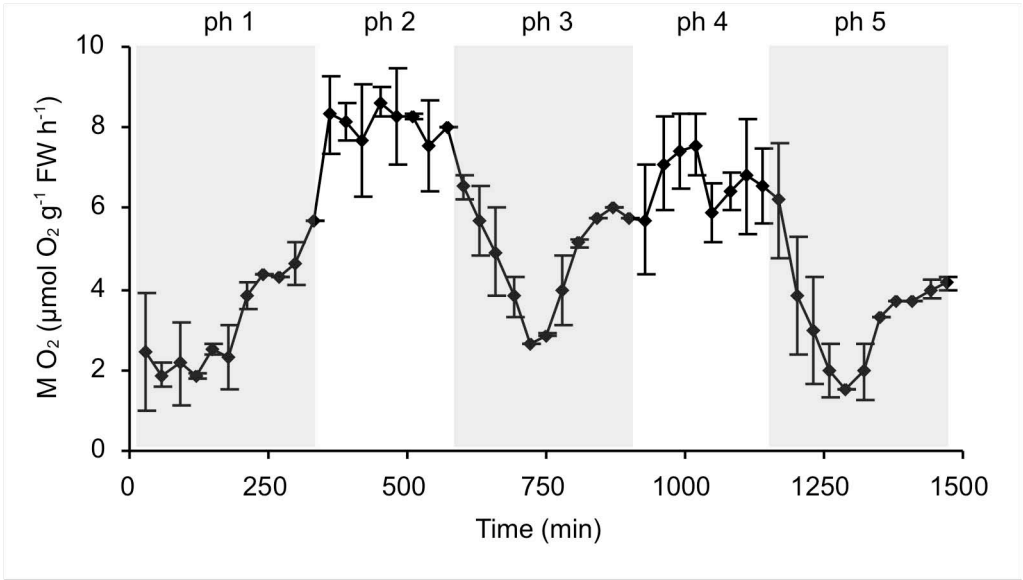

Figure 5a: *G. antarctica*: Respiration experiment Ga 1 expressed as MO_2 (µmol O_2 g^{-1} FW h^{-1}). Values are given as means and bars express amplitudes (short-term changes) of oxygen consumption during a 30 min time interval within an experimental phase (ph) and not standard deviation (SD).

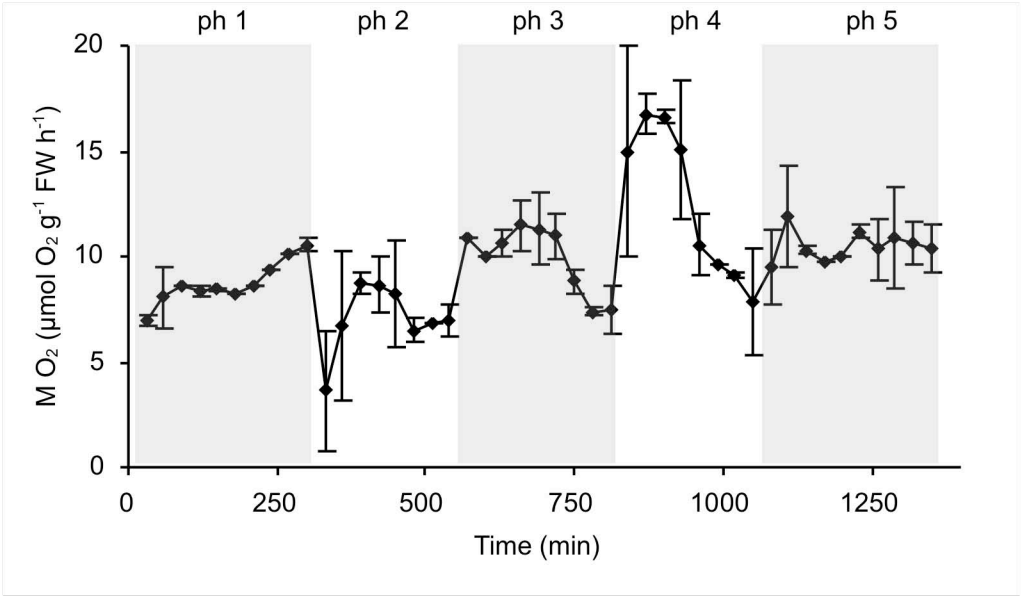

Figure 5b: *G. antarctica*: Respiration experiment Ga 2 expressed as MO_2 (µmol O_2 g^{-1} FW h^{-1}). Values are given as means and bars express amplitudes (short-term changes) of oxygen consumption during a 30 min time interval within an experimental phase (ph).

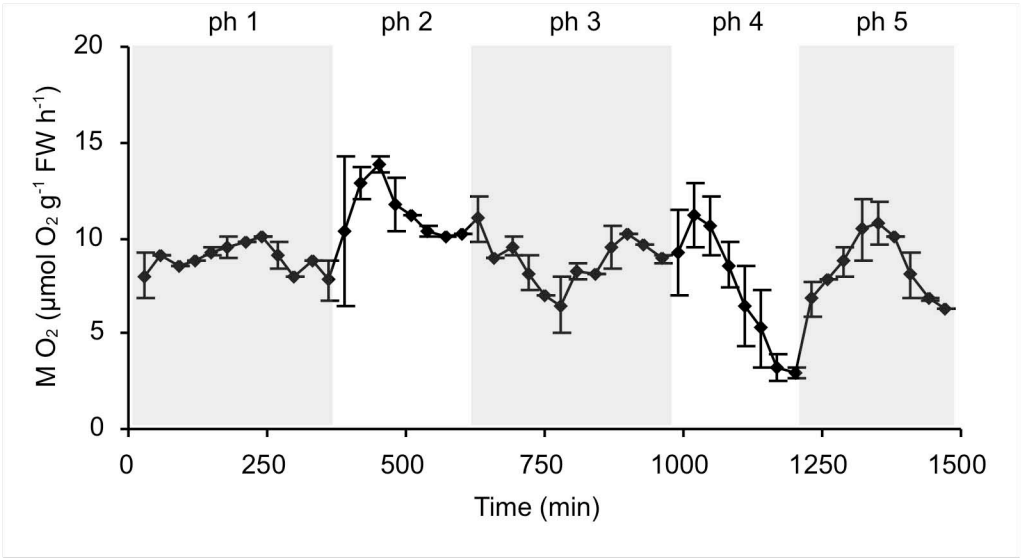

Figure 5c: *G. antarctica*: Respiration experiment Ga 3 expressed as MO_2 (µmol O_2 g^{-1} FW h^{-1}). Values are given as means and bars express amplitudes (short-term changes) of oxygen consumption during a 30 min time interval within an experimental phase (ph).

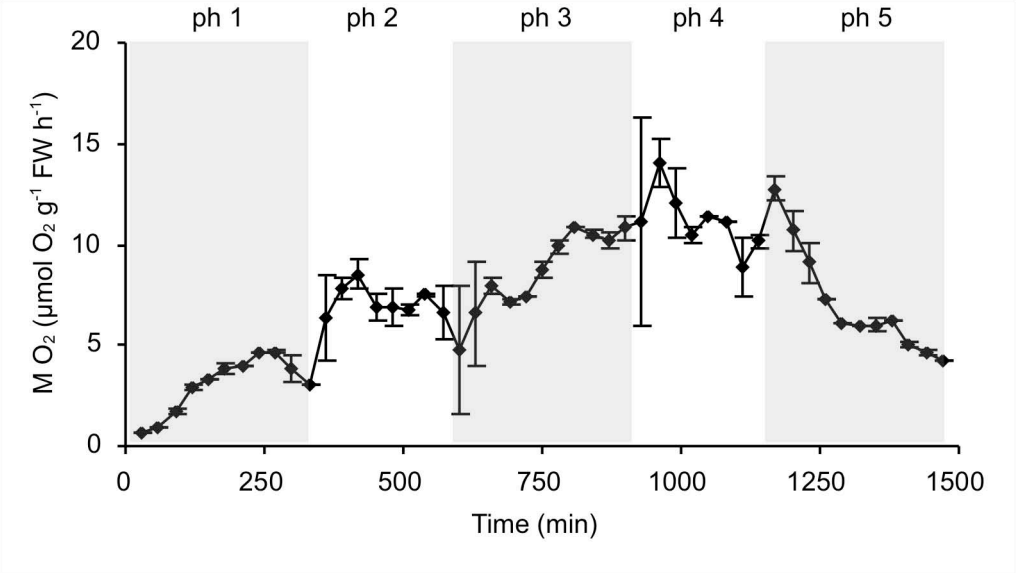

Figure 5d: *G. antarctica*: Respiration experiment Ga 4 expressed as MO_2 (µmol O_2 g^{-1} FW h^{-1}). Values are given as means and bars express amplitudes (short-term changes) of oxygen consumption during a 30 min time interval within an experimental phase (ph).

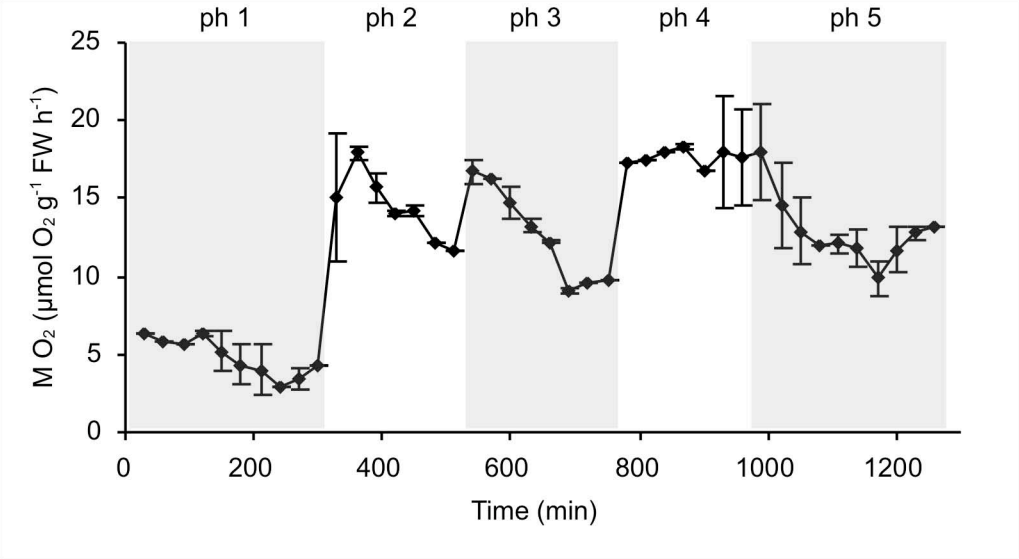

Figure 6a: *D. furcipes*: Respiration experiment Df 1 expressed as MO_2 (µmol O_2 g^{-1} FW h^{-1}). Values are given as means and bars express amplitudes (short-term changes) of oxygen consumption during a 30 min time interval within an experimental phase (ph).

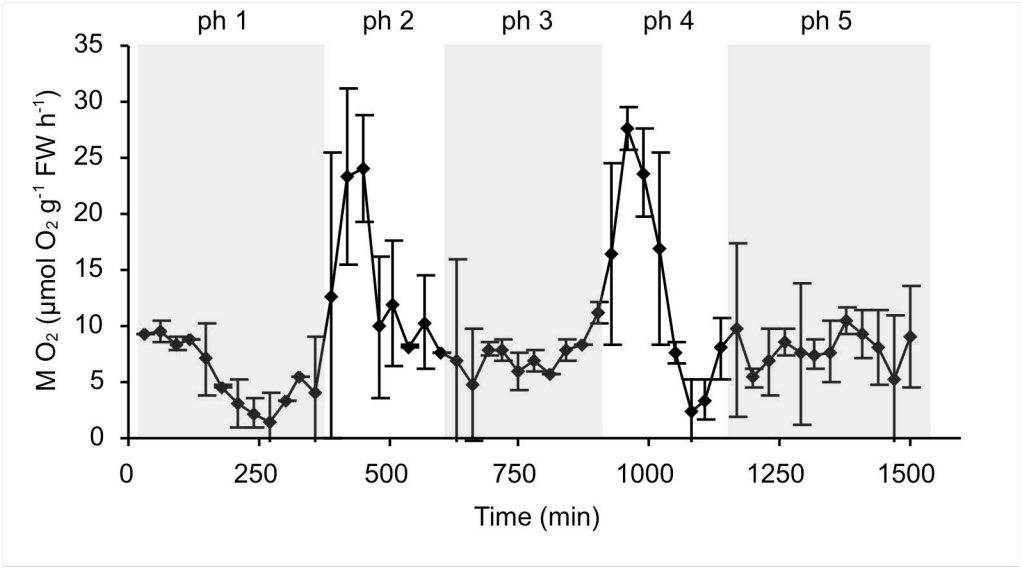

Figure 6b: *D. furcipes*: Respiration experiment Df 2 expressed as MO_2 (µmol O_2 g^{-1} FW h^{-1}). Values are given as means and bars express amplitudes (short-term changes) of oxygen consumption during a 30 min time interval within an experimental phase (ph).

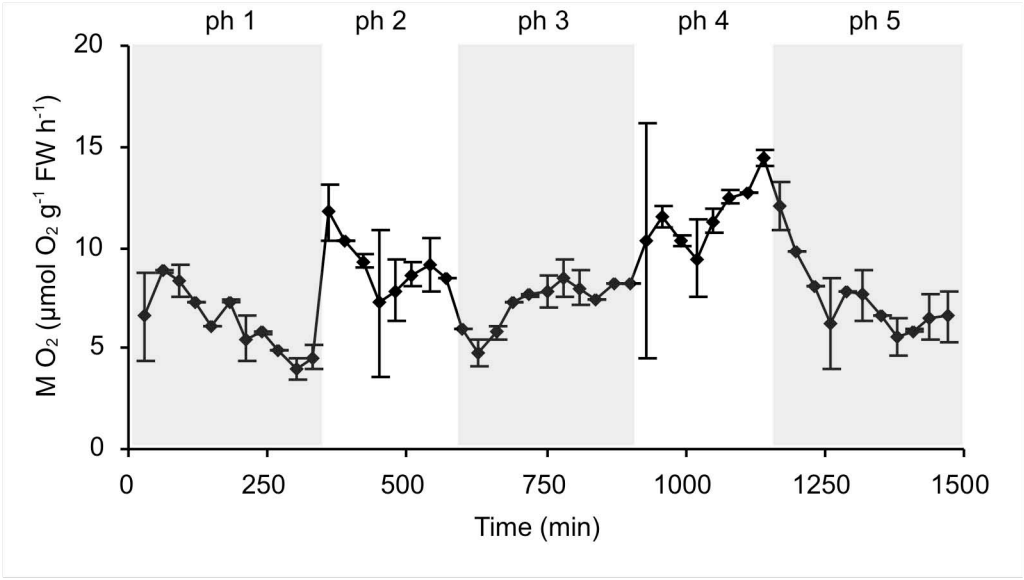

Figure 6c: *D. furcipes*: Respiration experiment Df 3 expressed as MO_2 (µmol O_2 g^{-1} FW h^{-1}). Values are given as means and bars express amplitudes (short-term changes) of oxygen consumption during a 30 min time interval within an experimental phase (ph).

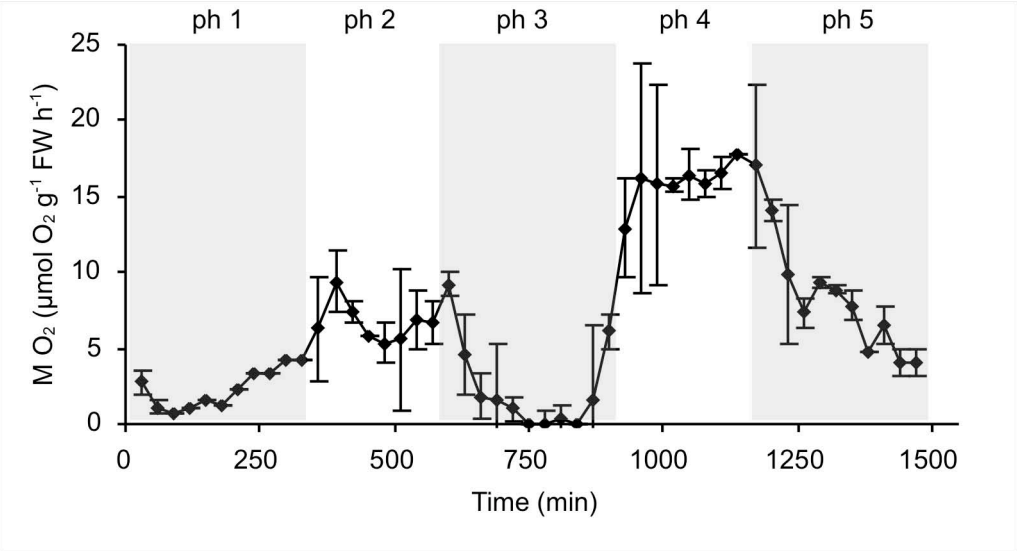

Figure 6d: *D. furcipes*: Respiration experiment Df 4 expressed as MO_2 (µmol O_2 g^{-1} FW h^{-1}). Values are given as means and bars express amplitudes (short-term changes) of oxygen consumption during a 30 min time interval within an experimental phase (ph).

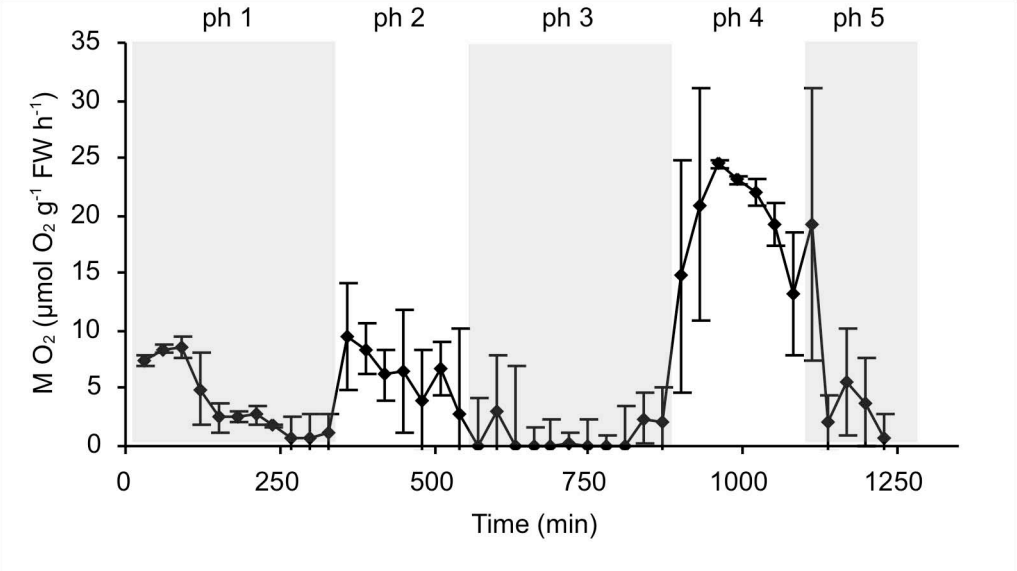

Figure 6e: *D. furcipes*: Respiration experiment Df 5 expressed as MO_2 (µmol O_2 g^{-1} FW h^{-1}). Values are given as means and bars express amplitudes (short-term changes) of oxygen consumption during a 30 min time interval within an experimental phase (ph).

4 Additional Results

4.1 Underwater UVB-climate: Comparison between Potter Cove and Kongsfjord

The following section displays parameters of underwater radiation climate and profiles of downwelling irradiation measured during Antarctic Expedition I (2000), Kongsfjord Expedition (2001), and an additional measurement campaign with continuous automatic recordings at Antarctic Potter Cove in 2003. Parameters summarised in Table 4.1 include the UVB-transmission, the downwelling diffuse attenuation coefficient K_d, which is proportional to the concentration of absorbing or scattering substances in the water and wavelength-specific, as well as the 1% depth of the UVB-intensity measured at surface level.

Table 4.1: Parameters defining underwater UVB-light climate in Antarctic Potter Cove (King George Island) and Arctic Kongsfjord (Spitsbergen): Transmission (%) of surface UVB-irradiance in two depth levels, 1% depth of surface UVB-irradiance, and downwelling diffuse attenuation coefficient K_d in two depth levels. n.d. not determined, only recorded down to 6 m water depth due to sensor problems.

	(%) Transmission	1% depth	K_d (cm^{-1}, m^{-1})
Potter Cove			
19.11.2003	55 (10 cm), 29 (1 m)	n.d., below 6 m	0.73 (10 cm), 0.54 (1 m)
26.11.2003	49 (10 cm), 34 (1 m)	14.0 m	0.40 (10 cm), 0.45 (1 m)
28.11.2003	39 (10 cm), 24 (1 m)	11.5 m	0.56 (10 cm), 0.54 (1 m)
29.12.2003	51 (10 cm), 26 (1 m)	14.0 m	0.73 (10 cm), 0.21 (1 m)
Kongsfjord			
10.07.2001	41 (20 cm), 15 (1 m)	4.0 m	1.16 (20 cm), 0.97 (1 m)
13.07.2001	41 (20 cm), 19 (1 m)	4.0 m	0.91 (20 cm), 0.79 (1 m)
01.08.2001	31 (20 cm), 10 (1 m)	2.5 m	1.35 (20 cm), 0.93 (1 m)

UVB-Transmission was higher in Potter Cove in austral early summer (November 2003) than in Kongsfjord in summer (July-August 2001), thus, UVB radiation penetrated more than three times deeper into the water column in Potter Cove.

Further, underwater UVB-profiles and UVA- and PAR-profiles are given for Antarctic Potter Cove (Fig. 4.1.1, 4.1.2), as well as UVB-profiles for Arctic Kongsfjord (Fig. 4.1.3).

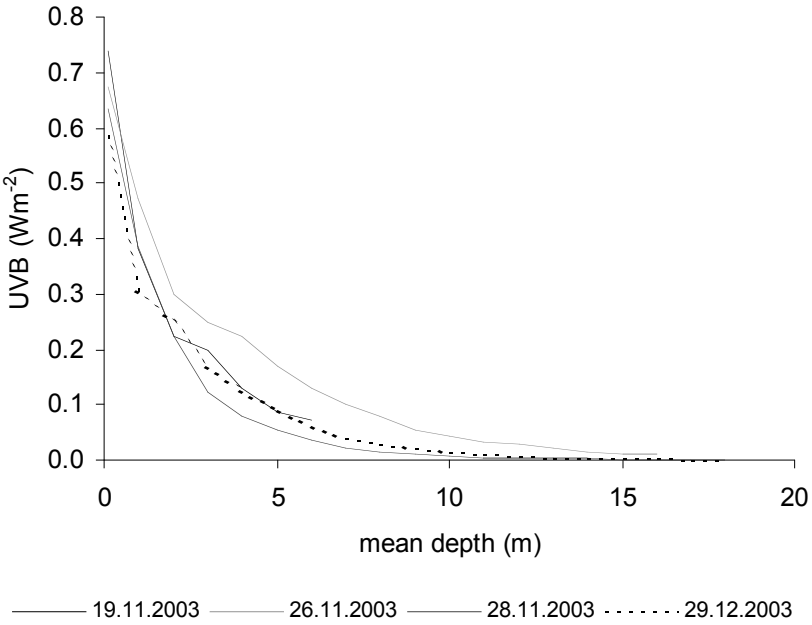

Figure 4.1.1: Underwater UVB-profiles (280-320 nm) recorded in Potter Cove (King George Island) on different days in November and December 2003. On 19.11.2003 (black line) the profile was only recorded down to 6 m water depth due to sensor problems.

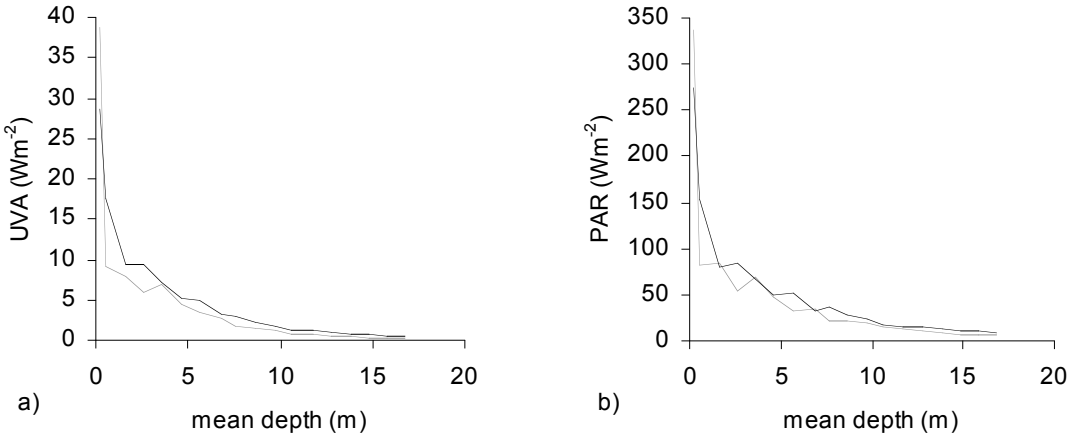

Figure 4.1.2: Underwater profiles of a) UVA (320-400 nm) and b) PAR (400-700 nm) recorded in Potter Cove (King George Island) on 13.11.2000 (grey line) and 17.11.2000 (black line).

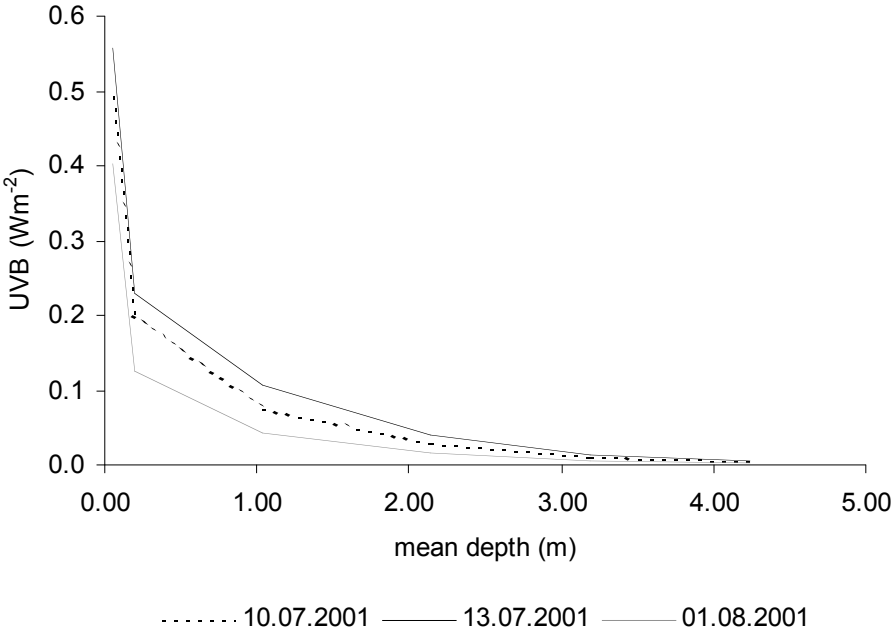

Figure 4.1.3: Underwater UVB-profiles (280-320 nm) recorded in Kongsfjord near the harbour of Ny-Ålesund (Spitsbergen) on different days in July and August 2001.

4.2 Temperate amphipod *Chaetogammarus marinus* from Helgoland (Island of Helgoland, North Sea)

Additional measurements and UV-exposure experiments in the sunshine simulator (SONSI) were carried out with *Chaetogammarus marinus* from Helgoland to test for UV- and antioxidant defence and induced damage in this temperate reference species.

4.2.1 *Physical UVR-screening*

Carapace absorbance in the UV- and PAR-range of *Chaetogammarus marinus* is shown in Table 4.2.1. Specimens collected during summer 2000 and 2002 had approximately the same shielding capacity in the UVB-range, but *C. marinus* from 2002 exhibited a 15 and 18% higher carapace absorbance in the UVA- and PAR-range compared to animals from 2000.

Table 4.2.1: Carapace absorbance (%) of SONSI lamp spectrum light between 295 and 700 nm of temperate amphipod species *Chaetogammarus marinus* (Helgoland) measured with specimens collected in July-August 2002 and July 2000.

	UVB (295-320 nm)	**UVA** (320-400 nm)	**PAR** (400-700 nm)
C. marinus 2002	50.1	59.5	51.1
C. marinus 2000	49.7	44.2	32.4

Figure 4.2.1 shows a carapace absorbance spectrum of *C. marinus* from July 2002 recorded in the sunshine simulator (SONSI) between 295 and 700 nm. Arrows indicate ranges of increased absorbance, which correspond to maximal absorbance ranges of mycosporine-like amino acids (MAAs, 309-360 nm) and carotenoids (430-490 nm). These are known sunscreening and oxygen radical quenching substances. The third region remains unknown as the carapace sample was not investigated to further detail.

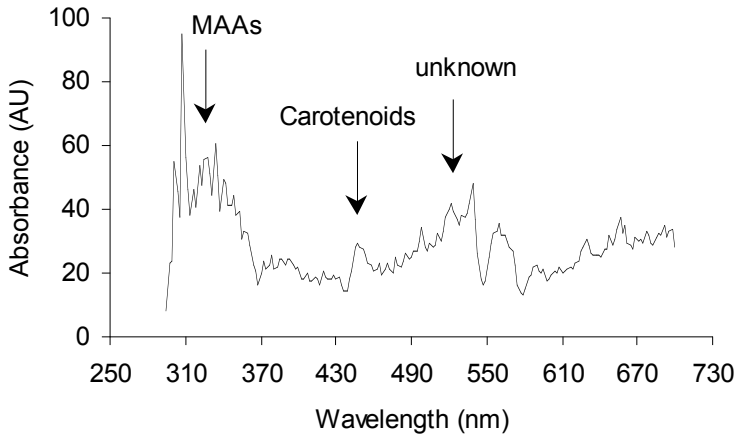

Figure 4.2.1: Carapace absorbance spectrum in temperate *Chaetogammarus marinus* (collected in 2002) recorded between 295 and 700 nm in the sunshine simulator (SONSI). Arrows indicate bands of higher absorbance corresponding to maximal absorbance ranges of known sunscreening and antioxidant substances (mycosporine-like amino acids MAAs, carotenoids) and unknown substances.

4.2.2 *Enzymatic and non-enzymatic antioxidant defence and oxidative damage*

Exposure to experimental high-dose UVB over 5 to 10 days in the sunshine simulator (SONSI) caused activity of antioxidant superoxide dismutase (SOD) to increase significantly compared to control animals at start day (Fig. 4.2.2. left). However, differences between the treatments at the end of the experiment were not significant. Activity of antioxidant catalase

was lower in UV-exposed compared to control animals, however, insignificantly due to high individual variability (Fig. 4.2.2. right).

High-dose UVB-exposure did not have any significant effect on whole animal carotenoid concentration (Fig. 4.2.3a). The concentration of lipid peroxidation marker thiobarbituric-acid-reactive substances (TBARS) was significantly lower in exposed compared to non-exposed amphipods (Fig. 4.2.3b).

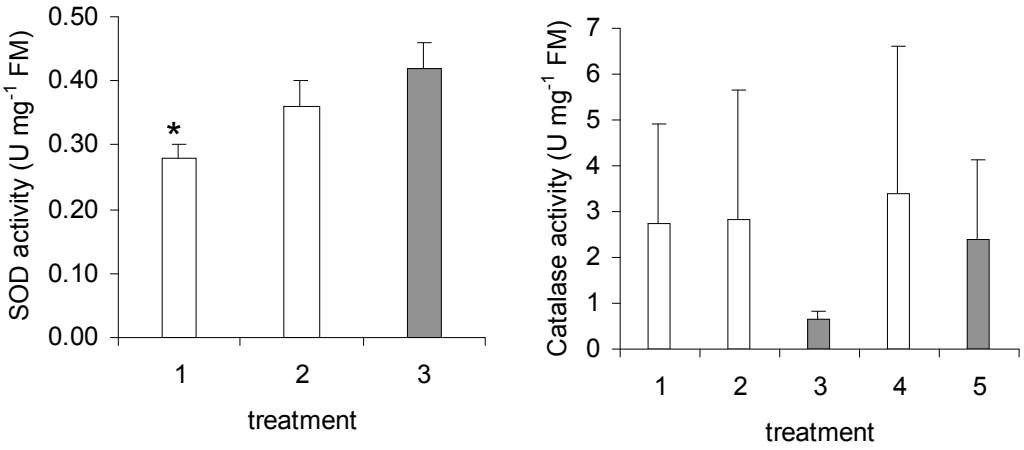

Figure 4.2.2: Superoxide dismutase (SOD) activity (left) and Catalase activity (right) in temperate *Chaetogammarus marinus*. Values are means + SD. Number of replicates n = 3-5. Treatments are 1: control 0 days, 2: control 5-10 days, 3: high-dose UVB-exposure 5-10 days, 4: control 14 days, 5: high-dose UVB-exposure 10-14 days. * significant difference between SOD start value (1) and both control (2) and UVB-exposure (3) after 5-10 days.

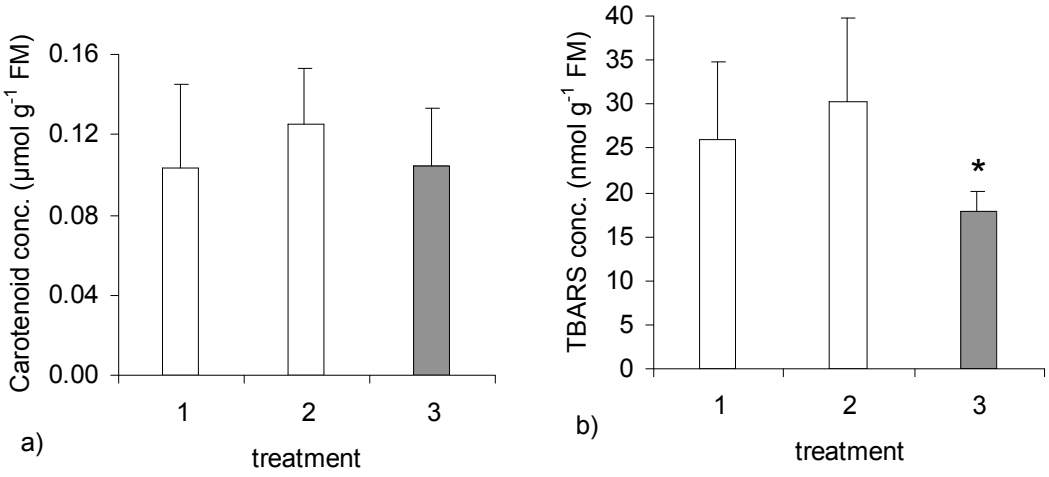

Figure 4.2.3: a) Total carotenoid concentration (µmol ß-carotene equivalents g^{-1} fresh mass FM) and b) Concentration of thiobarbituric-acid-reactive substances TBARS (nmol g^{-1} fresh mass FM) in temperate *Chaetogammarus marinus*. Treatments 1: control 0 days, 2: control 5-10 days, 3: high-dose UVB-exposure 5-10 days. * significant difference between TBARS concentration of UVB-exposed (3) and control animals (2) after 5-10 days.

4.3 Survival of Arctic and Antarctic amphipods: Is there a dose-dependent effect?

Survival rates of Arctic and Antarctic amphipods were plotted against total UVB-dose received to evaluate dose-dependent effects and to test for reciprocity (i.e. the response to a given total UVR-dose is independent of the time over which the exposure occurs (dose rate)).

Carnivorous/necrophagous *Anonyx nugax* from Arctic Kongsfjord was more vulnerable to the moderate-dose than to the high-dose UVB-treatment, resulting in lower survival at the same total UVB-dose received by the amphipod (Fig. 4.3.1). Starvation (open symbols) did not alter survival rates during high-dose UVB-exposure, contrasting to the moderate-dose UVB-treatment, where more specimens died, which received little pieces of fish (filled symbols) than those, which were starved at the same total UVB-dose level.

Similar effects were found for the second Arctic scavenger *Onisimus edwardsi*. The moderate-dose UVB-treatment caused higher mortality of animals, which had received the same total UVB-dose as in the high-dose UVB-treatment. Starvation clearly supported survival, remaining rates within control values of non-exposed amphipods (Fig. 4.3.2).

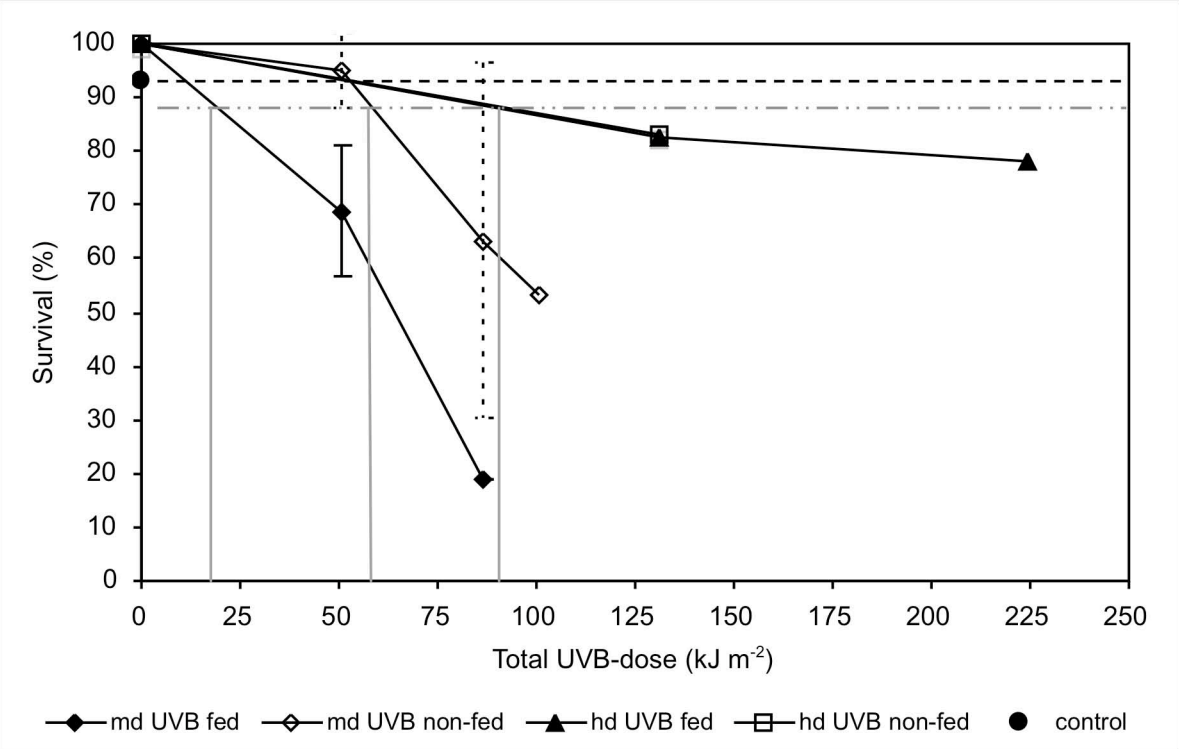

Figure 4.3.1: Survival of *Anonyx nugax* during moderate-dose (md, Q-Panel tubes, 7.2 kJ m^{-2} d^{-1}) and high-dose (hd, SONSI, 18.7 kJ m^{-2} d^{-1}) UVB-exposure experiments plotted against total UVB dose (kJ m^{-2}) received by exposed animals. Number of replicate experiments n=3 (md UVB fed), n=2 (md UVB non-fed), n=1 (hd UVB fed and non-fed). Specimens either received little pieces of fish (fed, filled symbols) or were starved (open symbols) over the entire experimental duration. Upper regularly dotted line ----- resembles mean survival of control animals, lower irregularly dotted line ─ -- ─ -- resembles lower standard deviation range of survival of control animals. Vertical grey lines depict UVB dose-response thresholds for decreasing survival.

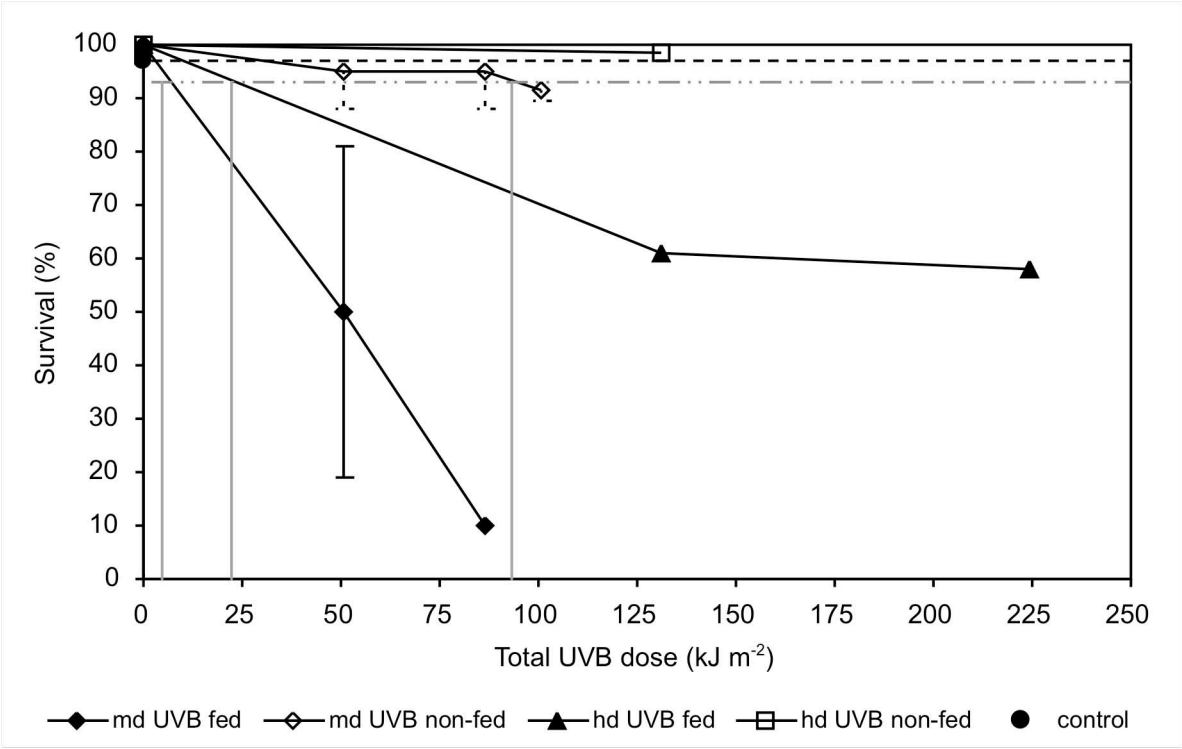

Figure 4.3.2: Survival of *Onisimus edwardsi* during moderate-dose (md, Q-Panel tubes, 7.2 kJ m^{-2} d^{-1}) and high-dose (hd, SONSI, 18.7 kJ m^{-2} d^{-1}) UVB-exposure experiments plotted against total UVB dose (kJ m^{-2}) received by exposed animals. Number of replicate experiments n=3 (md UVB fed), n=2 (md UVB non-fed), n=1 (hd UVB fed and non-fed). Specimens either received little pieces of fish (fed, filled symbols) or were starved (open symbols) over the entire experimental duration. Upper regularly dotted line ----- resembles mean survival of control animals, lower irregularly dotted line ¯ -- ¯ -- resembles lower standard deviation range of survival of control animals. Vertical grey lines depict UVB dose-response thresholds for decreasing survival.

Figure 4.3.3 and Figure 4.3.4 show high survival rates of Antarctic herbivorous *Gondogeneia antarctica* and *Djerboa furcipes* during low- and high-dose exposure experiments during Antarctic Expedition I in 2000. Contrasting, the low-dose treatment during Antarctic Expedition II in 2002 seemed to be more detrimental than the high-dose treatment in 2000, causing higher mortality in *D. furcipes* at the same total UVB-dose. This effect appears more clearly in Figure 4.3.4 and is less severe in *G. antarctica*.

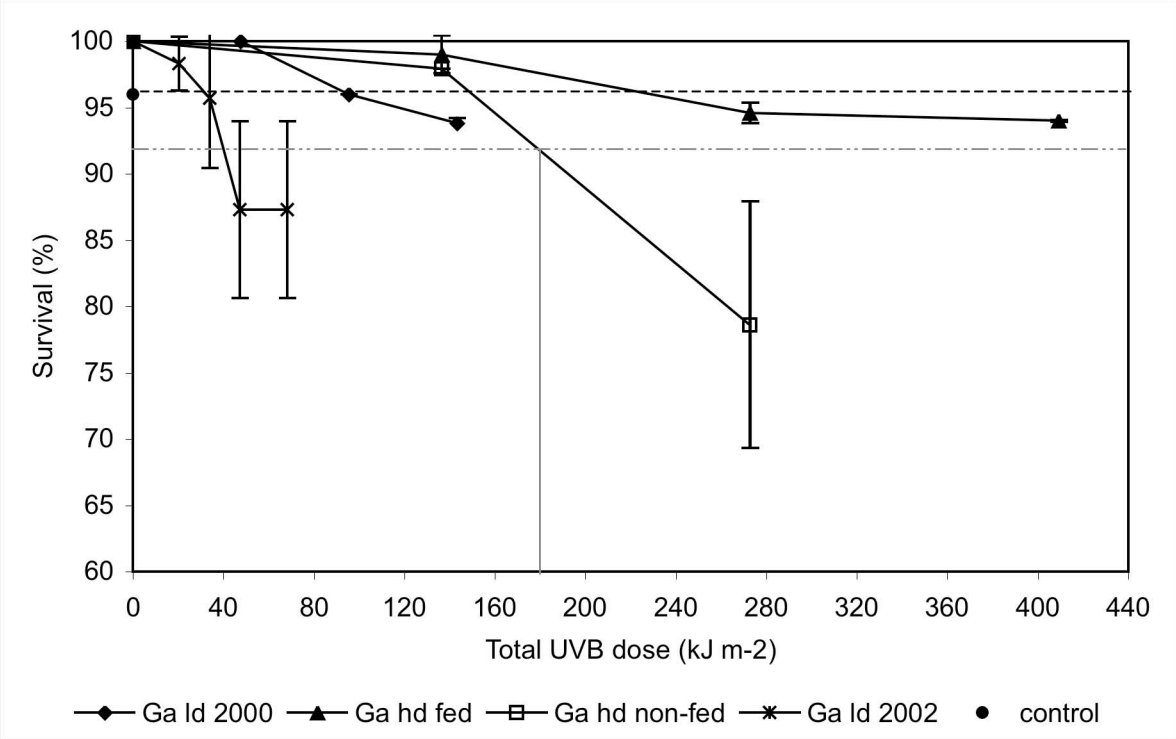

Figure 4.3.1: Survival (%) of *Gondogeneia antarctica* (Ga) during low-dose (ld, Q-Panel tubes, 6.82 kJ m^{-2} d^{-1}) and high-dose (hd, SONSI, 19.48 kJ m^{-2} d^{-1}) UVB-exposure experiments plotted against total UVB dose (kJ m^{-2}) received by exposed animals during expedition I in 2000 and II in 2002. Number of replicate experiments n=4 (Ga ld 2002), n=3 (Df ld 2002), n=2 (all others), control mean for both species n=4. All specimens in low-dose experiments were fed macroalgae. Specimens in high-dose experiments either received macroalgae (fed) or were starved (non-fed). Upper regularly dotted line ----- resembles mean survival of control animals, lower irregularly dotted line ─ -- ─ -- resembles lower standard deviation range of survival of control animals. Vertical lines depict UVB dose-response thresholds for decreasing survival.

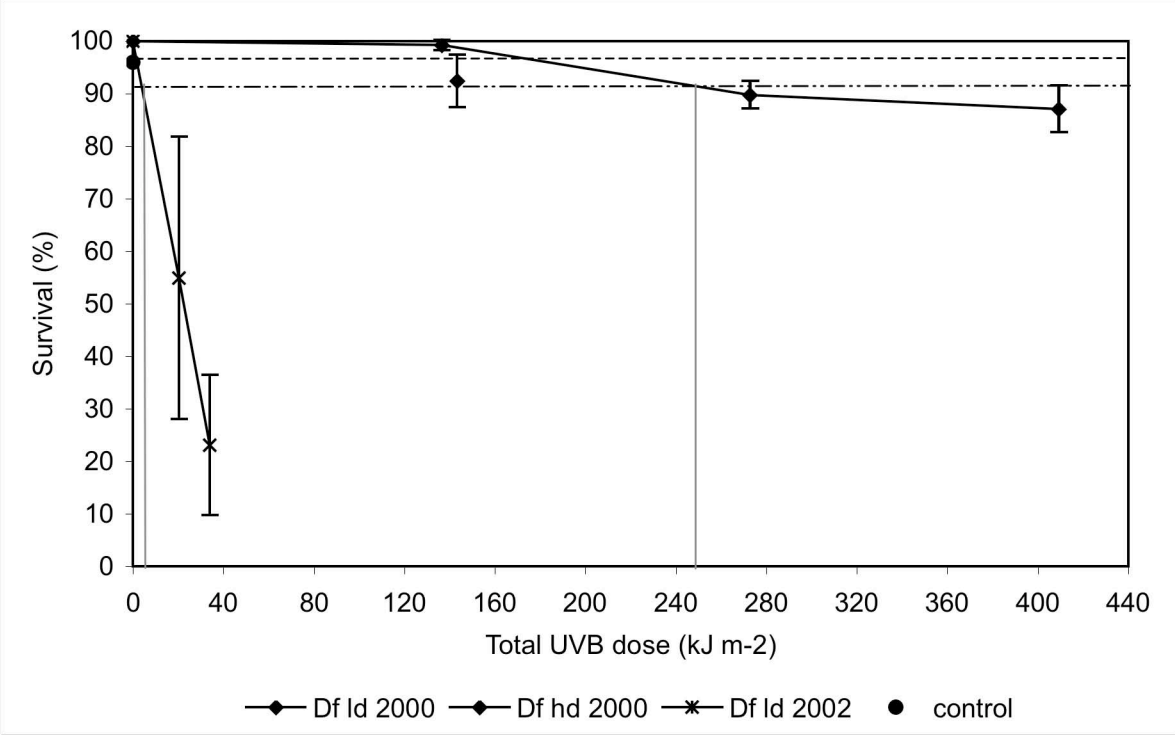

Figure 4.3.2: Survival (%) of *Djerboa furcipes* (Df) during low-dose (ld, Q-Panel tubes, 6.82 kJ m^{-2} d^{-1}) and high-dose (hd, SONSI, 19.48 kJ m^{-2} d^{-1}) UVB-exposure experiments plotted against total UVB dose (kJ m^{-2}) received by exposed animals during expedition 2000 and 2002. Number of replicate experiments n=4 (Ga ld 2002), n=3 (Df ld 2002), n=2 (all others), control mean for both species n=4. All specimens in low-dose experiments were fed macroalgae. Specimens in high-dose experiments either received macroalgae (fed) or were starved (non-fed). Upper regularly dotted line ----- resembles mean survival of control animals, lower irregularly dotted line — -- — -- resembles lower standard deviation range of survival of control animals. Vertical lines depict UVB dose-response thresholds for decreasing survival.

5 Discussion

In my doctoral dissertation I studied the effects of exposure to ultraviolet radiation (UVR) on different UV- and oxidative stress parameters and defence systems against direct and indirect tissue damage in shallow water amphipods from polar and temperate coastal regions. In the following section, I will discuss the results presented in Publication I to IV and in the section "Additional results".

5.1 Is the potential for UV- and oxygen radical stress higher in the Antarctic or the Arctic?

A comparison of atmospheric and underwater light climate parameters shows higher UVB-intensities and less attenuation i.e. higher transmission in Antarctic waters in Potter Cove than in Arctic Kongsfjord during the respective months the experiments were carried out (Publ. I; II; III; Fig. 4.1.1-4.1.3 Additional results; Tab. 8.1 Appendix). Varying maximal UVB-intensities are not only a result of different solar elevation angles at different latitudes, but are also related to different ozone concentrations. Spring-time ozone depletion was highest over the Antarctic during this study (>50%), whereas no ozone destruction was recorded over the Arctic in spring and summer in 2001. However, in more recent years, 30% depletion of total column ozone was recorded for the first time in Spitsbergen in February/March 2005 (M. Rex, personal contribution, WMO press release 04/2005). Consequently, the effect of 50% ozone reduction in the Antarctic (spring) and 15% in the Arctic (spring) is estimated to cause an increase of erythemal (sunburn) UVR of 130% in the Antarctic and 22% in the Arctic (Madronich et al. 1998). Turbidity was less in Potter Cove than in Kongsfjord during both experimental periods due to a smaller input of terrestrial and glacial material from river run-off into Potter Cove compared to Kongsfjord. But earlier studies have measured low K_d values (<0.4 m^{-1}), i.e. high transmittance, in both polar coastal areas before the onset of glacial melting (Hanelt et al. 2001, Hoyer et al. 2003). Overall, this creates a higher potential for UV-induced stress for shallow water animals in Potter Cove compared to Kongsfjord.

UV-driven H_2O_2 production in the habitat as an external source for ROS stress was not determined in this study, but Abele et al. (1999) measured maximal H_2O_2 concentrations of up to 2 µmol l^{-1} in surface waters in Potter Cove, deriving mainly from wet deposition from snow on the sea surface. Dissolved organic carbon (DOC) was low (121 µmol C l^{-1}), indicating only

minor contribution of direct UV-induced H_2O_2 photoproduction. To my knowledge, no H_2O_2 measurements exist so far for Kongsfjord. Kongsfjord receives a high amount of mineral particles with glacier and river run-off, resulting locally in very turbid water layers (Jerlov water Type 9, K_d up to 0.75 m^{-1} and more, Hanelt et al. 2001), but the concentration of dissolved organic material (DOM) is believed to be generally low (Svendsen et al. 2002). Thus, the potential for H_2O_2 accumulation through direct DOC photo-oxidation and resulting oxidative stress should be of minor importance, but the influence of atmospheric wet deposition from snow like in Potter Cover is unknown. For comparison, in the Arctic Beaufort Sea (70°51'N, 133°39'W) 385 nmol l^{-1} H_2O_2 were measured at a DOC content of 62 µmol l^{-1} (O'Sullivan et al. 2005). However, ozone depletion may enhance H_2O_2 production in certain years, as demonstrated by Abele-Oeschger et al. (1997b) imposing a potential for oxidative stress on the organisms in the upper water column.

5.2 Does the artificial radiation in the laboratory reflect *in-situ* conditions?

Steeger et al. (2001) and others discussed that artificial radiation simulation can only be an approximation of natural conditions as in the field many factors control the underwater radiation climate, e.g. turbidity, tidal range, wind and wave action apart from clouds, aerosols and other atmospheric features. Contrasting, in the laboratory all conditions can be controlled in order to evaluate the influence of a specific factor and these often mimic cloudless sky and calm weather for several consecutive days, which is rarely found in the polar field. Thus, the artificial conditions resemble a worst case scenario.

Q-Panel tubes Antarctic

Spectral composition of lamp spectra emitted by the Q-Panel tubes could not be altered and radiation intensities could only be changed by varying the distance between tubes and water surface of experimental aquarias. Resulting artificial UVB-intensity (0.38 Wm^{-2}, Publ. I; Tab 2.2 Material & Methods) and simulated daily UVB-dose (6.84 kJ m^{-2} d^{-1}, Publ. I; Tab. 2.2 Material & Methods) was approximately a fourth of possible maximal atmospheric daily UVB-dose in the field within the same 5 hours exposure time and thus a good resemblance of "low-dose field conditions". UVB attenuation in the filtered sea water used in the experiments compared with natural water from Potter Cove is lower and, thus, transmittance higher, however, I did not determine the K_d for the filtered sea water. Literature K_d values for "pure" or "clear sea water" (as listed in Hargreaves 2003) are generally below 0.10 m^{-1} at 320 nm

and around 0.15 m^{-1} at 310 nm and very turbid water has values of 1.0 m^{-1} or higher (Hanelt et al. 2001). In Potter Cove a mean K_d of 0.49 m^{-1} was measured in November 2003 ranging between 0.41 and 0.54 m^{-1} (integrated from 280 to 320 nm). Tidal ranges in the field regularly include depths below 10 cm, and amphipods were observed in these very shallow areas swimming freely, thus receiving full solar radiation. The beakers of 10 cm depth used for experimentation provide a realistic simulation of shallow water field conditions. However, animals in my experiments could not escape from exposure by seeking greater depths or shading algal canopy. This situation occurs in the field probably only during trapping in shallow tidal pools during low tide or very clear sky conditions with no cloud cover and calm sea state. In addition, radiation intensities were maintained constant over the entire exposure time and did not fluctuate due to diurnal cycles as in nature, where highest intensities occur around mid day. To compensate diurnal changes the daily exposure time was limited to 5 hours in the laboratory, in contrast to maximal 20 hours of variable sunshine levels in the field during summer.

The spectral composition of white-light and Q-Panel tubes with a ratio of 1:10:15 (UVB:UVA:PAR) is quite different from that of the sun with 1:14:97 (Tab. 2.2 Material & Methods) with respect to the UVB:PAR ratio and fits better in the UVA-range. PAR in the field measurements in Potter Cove might have been underestimated, as other studies from the same area recorded higher PAR-levels between 163 and 380 Wm^{-2}, resulting in a UVB:PAR ratio of between 1:105 and 1:245, exacerbating the ratio difference between artificial and solar radiation even further (October – November 1993, Gomez et al. 1998). As a result, the proportion of UVB was selectively increased in the spectrum under laboratory conditions, a scenario corresponding to spring-time ozone depletion in the field, which increases the risk for UVB-damage. Differences in spectral composition may become important for repair processes of UV-induced DNA-damage, which in part rely on radiation in the UVA-and near blue PAR-range and will be discussed later.

Sunshine simulator Antarctic

The light spectrum emitted in the sunshine simulator (SONSI) resembled much more of the natural radiation conditions than that emitted by Q-Panel tubes. The UVB-intensity was adjusted to match the maximal UVB-intensity recorded in the field during the start of the experimental series, in this case 1.4 Wm^{-2} in October 2000. In 2002 the experiments commenced later at the end of November, therefore the applied UVB-intensity resembles the mean maximal atmospheric UVB-intensity during the entire period (1.5 Wm^{-2}). Respective

UVA- and PAR-intensities resulted from the adjustments of the liquid filter and metal grades in the SONSI. Thus, this was a direct simulation of a high *in-situ* UVR-scenario and the amounting daily UVB-dose within 4 hours was regarded as "high" as it resembled approximately 87% of the possible maximal atmospheric daily UVB-dose in the field within the same exposure time. Apart from the spectral composition, all other factors discussed above (e.g. different K_d values, continuous exposure, exposure time) apply also to the SONSI system, resulting in a higher simulated dose in comparison to the natural shallow water *in-situ* dose. The spectral composition in the SONSI (UVB:UVA:PAR 1:12:99, Tab. 2.2 Material & Methods) is close to the solar ratio measured in the field.

Q-Panel tubes Arctic

The distance of the white-light and Q-Panel tubes from the water surface was chosen in order not to loose too much irradiation intensity in the UVA- and PAR-range while aiming for a low UVB-intensity compared to the atmospheric level. This resulted in UVB-intensities and doses similar to those applied in the Antarctic experiments. However, as maximal atmospheric radiation intensities in Kongsfjord are lower than in Potter Cove, the simulated dose was higher and amounted to 40% of the mean total daily UVB-dose of 17.8 kJ m^{-2} recorded in Kongsfjord at surface level in July 2001. K_d values can be highly variable in Kongsfjord due to local melt water input, and in July 2001 the K_d ranged between 0.79 and 0.91 m^{-1} in the coastal vicinity of Koldewey Station at Ny-Ålesund. Thus, resulting UVB-transmittance in Kongsfjord in summer is even lower than in Potter Cove. The solar spectral composition in Kongsfjord amounted to 1:24:247 (UVB:UVA:PAR), and the simulated ratio (1:9:14, Tab. 2.2 Material & Methods) in the Arctic experiments, which was similar to that in the Antarctic experiments, differed even more from the solar spectrum than the laboratory levels at Potter Cove. Thus, the selective increase in the UVB-range is accelerated.

Sunshine simulator Arctic

The simulated dose was 5% on average higher than the mean total daily UVB-dose measured in Kongsfjord in July 2001 (Tab. 2.2 Material & Methods). The simulated spectral ratio of 1:17:90 (UVB:UVA:PAR) resulted in higher relative elevation in UVB compared to Potter Cove. Like Antarctic experiments, the SONSI simulation was a better resemblance of the atmospheric light climate in Kongsfjord than that of the Q-Panel tubes, but overall UVB-impact was slightly higher (5%) than the natural UVB-regime in Kongsfjord.

For comparison, radiation spectra recorded with each type of artificial light source and solar radiation spectra recorded at each experimental site (region) are shown in the following figures: Figure 5.2.1 delineates the simulated radiation spectrum recorded in the SONSI and the solar radiation spectrum both recorded in the range from 280 to 700 nm at Arctic Kongsfjord with a fast scanning double monochromator spectroradiometer (Instrument Systems, Germany).

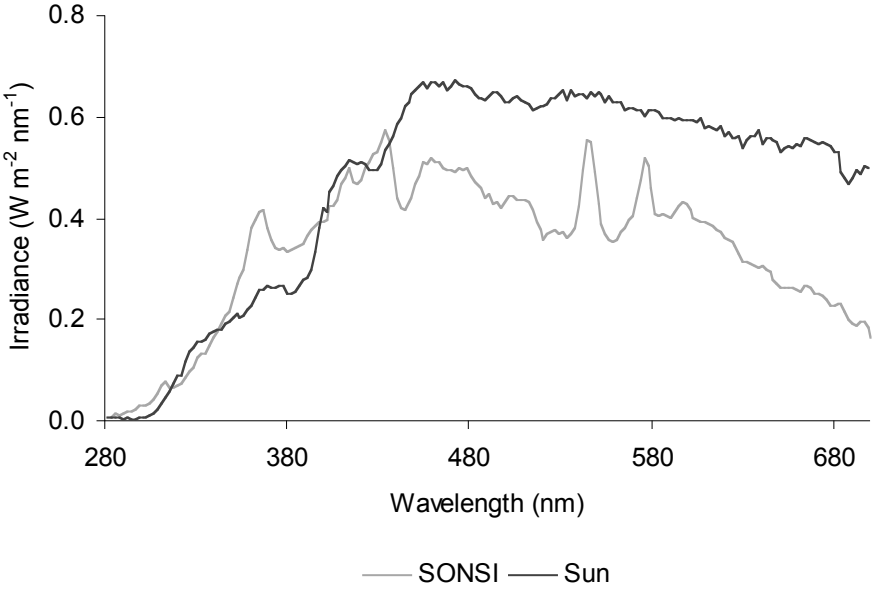

Figure 5.2.1: Comparison of simulated SONSI spectrum with solar radiation spectrum recorded at Kongsfjord (Spitsbergen, Arctic) in the range from 280 to 700 nm.

The solar radiation spectrum in Antarctic Potter Cove was recorded with a different instrument, a diode-array spectroradiometer equipped with a cosine diffuser (Construction by M. Kruse, Germany), between 320 and 700 nm and cannot be combined with the SONSI spectrum, as the distance between each nm range is different. Therefore, the solar spectrum is shown in Figure 5.2.2a and the simulated spectrum recorded in the SONSI in Figure 5.2.2b.

The radiation spectrum recorded below the Q-Panel and white light tubes during the Antarctic exposure experiments is shown in Figure 5.2.3. The Arctic Q-Panel lamp spectrum is similar and, therefore, not shown.

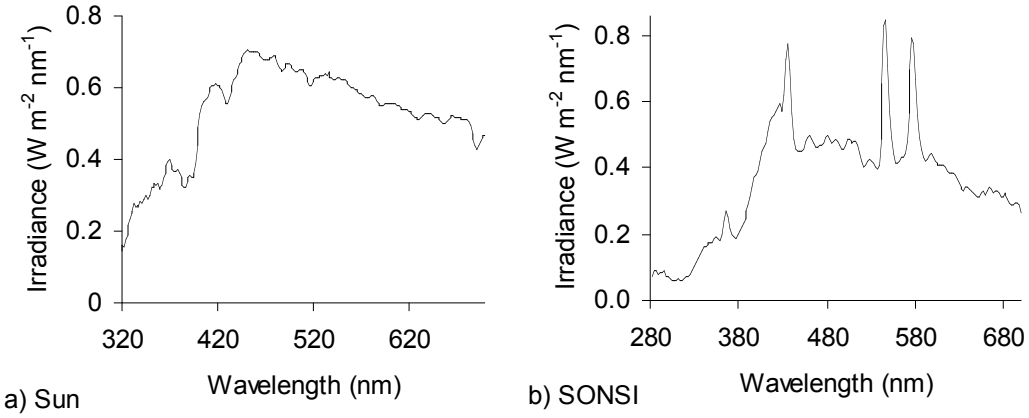

Figure 5.2.2: a) Solar radiation spectrum recorded between 320 and 700 nm and b) simulated SONSI spectrum recorded between 280 and 700 nm at Potter Cove (King George Island, Antarctic).

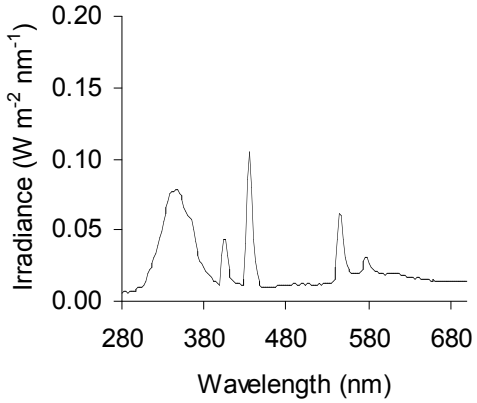

Figure 5.2.3: Radiation spectrum of Q-Panel and white light tubes recorded at Potter Cove (King George Island, Antarctic).

5.3 Is the UVR- and antioxidant protection of polar and temperate amphipods efficient to prevent elevated stress, damage and death?

In the following section UVR-protection will be discussed and comparisons will be made between Antarctic and Arctic species, and also where possible to temperate species to evaluate the efficiency of different protective mechanisms in the respective region.

5.3.1 *Screening by carapace as a pre-requisite for physical UV-protection*

The *degree of absorbance* of the outer chitinous body barrier determines the amount of damaging UV-photons reaching inner soft tissues and is thus a measure for efficiency of physical UV-protection. In all investigated species, carapace absorbance was slightly higher (6% on average) in the UVR than the PAR-range. The Arctic herbivorous *Gammarellus homari* had the highest absorbance in the UVB- and UVA-range (63%, λ 295-400 nm) and hence the best physical UV-shield of all investigated species. This was followed by the Arctic carnivorous/necrophagous *Anonyx nugax* (59%, λ 295-400 nm) and the Antarctic herbivorous *Djerboa furcipes* (55%, λ 295-400 nm), and finally the Antarctic herbivorous *Gondogeneia antarctica* (42 %, λ 295-400 nm) thereafter (Publ. II, Tab. 1; Publ. IV, Tab. 1). The absorbance did not differ between the UVB- and UVA-range. The temperate herbivorous *Chaetogammarus marinus* exhibited physical UV-protection within the range of the Antarctic species (47-55%, λ 295-400 nm, Tab. 4.2.1 Additional results). The measurements revealed interannual differences in carapace absorbance levels of UVB and UVA, which could be due to differing morphotypes of lighter and darker coloration (number of carapaces measured n=3 in 2000, n=1 in 2002). Further, the absorbance depends not only on colour, brightness, and thickness of the chitinous carapace but also on the nature and amount of innate compounds. All carapace absorbance spectra recorded for each of the above species showed bands of higher absorbance, which correspond to the maximal absorbance ranges of various mycosporine-like amino acids (MAAs) and carotenoids (Publ. II, Fig. 1, 2; Publ. IV, Fig. 2; Fig. 4.2.1 Additional results). Although the chitinous carapace was not analysed further, several studies demonstrated the occurrence of sunscreening MAAs and antioxidant carotenoids in cuticle and tissues of marine organisms (Bandaranayake & Des Rocher 1999, Carefoot, et al. 2000, Shick & Dunlap 2002).

Higher shielding extends exposure time to UVR in shallow water habitats, as observed in *G. homari*, which successfully colonise macroalgal communities in the littoral and sublittoral zone (0-5 m) in Kongsfjord. However, *G. antarctica*, which has approximately 20% less carapace screening capacity, also shrives successfully in shallow water areas (0-4 m) of Potter Cove, receiving full radiation while actively swimming between algal thalli on tidal platforms. This indicates that physical (carapace) UV-protection together with other strategies, e.g. behavioural avoidance, confers UV-resistance. Carnivorous/necrophagous *A. nugax*, which is generally not restricted to certain water depths concerning food availability, is able to migrate to greater water depths during mid day peak irradiance and low tide in order to compensate lower physical sunscreen and to avoid direct prolonged UV-

exposure. Vertical migrations have been shown for several crustacean zooplankton species (Lampert 1989, Leech & Williamson 2001).

In some Arctic ponds 2 morphotypes of *Daphnia* coexist: animals with melanin pigmentation of carapace and clear (transparent) carapace. Zellmer (1995) observed that transparent animals were restricted to the bottom of the ponds during daylight hours, while melanic specimens swam actively in all water depths. Apart from UV-protection, these vertical migrations also decrease predation risk in upper water layers during daylight hours, e.g. from fish with UV-orientation (Leech & Johnsen 2003, and references therein, Rautio et al. 2003). This may also apply to amphipods, as they constitute a main food source for various fish in Kongsfjord (Hop et al. 2001). Also, low UVB water transparency in summer in Kongsfjord and especially in intertidal water at Helgoland (North Sea) may in part compensate for lower carapace UV-absorbance in Arctic *A. nugax* and temperate *C. marinus* (Hanelt et al. 2001, Dring et al. 2001, Tab. 8.1 Appendix).

5.3.2 *Survival as a measure of overall UV-protection*

If we define efficiency of protection as *sustained survival* of exposed animals, Arctic herbivores were highly efficient with nearly 100% survival of exposed *Gammarellus homari* under any treatment, showing a dose and wavelength *in*dependent response (Publ. II; III). Contrasting, Arctic carnivorous/necrophagous *Anonyx nugax* and *Onisimus edwardsi* exhibited elevated mortality under moderate- as well as high-dose UVR over 14 days. The influence of nutrition on UV-protective mechanisms and survival favoured starved above fed animals, which will be discussed in section 5.3.3. Survival of *A. nugax* and *O. edwardsi* decreased extremely to minimal values of 19% (*A. nugax*) and 10% (*O. edwardsi*) under full spectral irradiance and to 47% (*A. nugax*) and 69% (*O. edwardsi*) when UVB was cut off at 320 nm, animals receiving only UVA+PAR (Publ. II, Fig. 4, data shown for *A. nugax*). This supports the general finding that in the UV-spectrum the shorter wavelengths (UVB) are the more damaging, while UVA can even have beneficial effects (Williamson et al. 2001, Buma et al. 2003, Hessen 2003, Little & Fabacher 2003).

Effects of UVR on biological systems strongly depend on wavelength. Therefore, spectral weighting functions should be created for experimental data in order to correctly interpret results gained with artificial light sources or experimental manipulations of solar UVR and to be able to predict possible effects in nature (Cullen & Neale 1997). Biological weighting functions (BWFs) demonstrate, which wavelength range is potentially the most damaging.

Williamson et al. (2001) developed a model to calculate the BWF for *Daphnia pulicaria* survival in the range from 280 to 500 nm exposed to solar and artificial radiation:

$$(1) \qquad H^* = \sum_{\lambda\,280}^{500} \varepsilon_H(\lambda) \text{ X } H(\lambda) \text{ X } \Delta\lambda$$

with $\underline{H^*}$ being the total biologically weighted UVR exposure (unitless), as a function of $\underline{H(\lambda)}$ being the total cumulative energy exposure (= total dose received) at each wavelength (λ) ($J\ m^{-2}\ nm^{-1}$), and $\underline{\varepsilon_H(\lambda)}$ being the biological weighting coefficient for each wavelength ($(J\ m^{-2})^{-1}$), integrated over the desired wavelength range (λ 280-500 nm). Necessary for the integration process is $\Delta\lambda$ being the difference between each wavelength step of the specific broadband spectroradiometer used for the radiation measurement. The X symbolises multiplication.

Williamson and co-workers' (2001) model was based on the standard exponential equation for population growth:

$$(2) \qquad N_t = N_0 \text{ X } e^{-H^*}$$

with N_0 being the number of animals at the start and N_t being the number of surviving animals at the end of each experiment. Assuming that the natural logarithm of survivorship is inversely proportional to total biologically weighted UVR exposure (H*), and assuming further that reciprocity holds (i.e. the response to a given total UVR dose is independent of the time over which the exposure occurs (dose rate), Cullen & Neale 1997) equation (2) can be transformed the following way:

$$(3) \qquad H^* = -\ln (N_t \text{ X } N_0^{-1})$$

N_t and N_0 can also be used for values in percent. The resulting biologically weighted exposure H* is approximately equal to "total dose received", however, used in equation (3) in the sense of a "predicted effect" of exposure. Williamson et al. (2001) used this H* to predict (model) survival of *Daphnia pulicaria* at different wavelengths and to compare it with survival rates recorded in their experiments. To generate the BWF the authors used several cut-off filters in the UVR-range to be able to differentiate between small wavelength bands. In contrast, I used only two cut-off filters, at 320 nm (UVR-range) and at 400 nm, to differentiate between UVR and PAR in general, as well as between UVB- and UVA-induced damage in particular. The emphasis of the present study lay on biochemical analysis of *surviving* specimens to evaluate physiological processes of damage and repair. That is why

the number of replicate experiments included into survivorship ratings is rather small (n=1-3). Nevertheless, to approximate UVB-and UVA-effects, I calculated the respective biologically effective exposure H* for the UVB- and UVA-range from survival rates (in percent) of *A. nugax* after 12 days of UVR-exposure with Q-Panel tubes using equation (3). 19% (N_{tUVB}) on average of initially exposed amphipods survived the UVB and 47% (N_{tUVA}) on average the UVA-exposure (Publ. II, Fig. 4), resulting in a H_{UVB}* of 1.66 and H_{UVA}* of 0.76. Subsequently, I calculated the biological weighting coefficients ($\varepsilon_{H(280\text{-}320nm)}$, $\varepsilon_{H(320\text{-}400nm)}$) with the following equation (4) (Williamson et al. 2001):

$$\varepsilon_{H(280\text{-}320nm)} = (H^* \times H_{(UVB)}^{-1}) \quad (4)$$

with $H_{(UVB)}$ being the cumulative dose received (0.38 J m^{-2} nm^{-1}) integrated between 280 to 320 nm, and respective for $H_{(UVA)}$ being the cumulative dose received (3.68 J m^{-2} nm^{-1}) integrated between 320 and 400 nm. In the UVB-range the resulting $\varepsilon_{H(280\text{-}320\ nm)}$ was 4.37, and in the UVA-range the $\varepsilon_{H(320\text{-}400\ nm)}$ was 0.21. Finally, I weighted (= multiplied) the cumulative energy spectrum integrated over the 12 days exposure period from the Q-Panel and white light tubes, multiplying each UV-dose at each respective wavelength in the UVB-range with $\varepsilon_{H(280\text{-}320\ nm)}$ and each dose in the UVA-range with $\varepsilon_{H(320\text{-}400\ nm)}$. I plotted the resulting energy spectrum against wavelength. The resulting weighted energy spectrum shown in Figure 5.3.1 can only be an approximation for the total biologically effective exposure, as only two weighting coefficients $\varepsilon_H(\lambda)$ do not allow for a good regression fit.

It has to be taken into account, that Williamson et al. (2001) weighted the cumulative energy spectrum differently. They multiplied the spectrum with the BWF gained from a non-linear model algorithm based on several $\varepsilon_H(\lambda)$ in the UV-range. However, as an approximation for biologically effective exposure, the weighted energy spectrum shown in Figure 5.3.1 clearly distinguishes between UVB- and UVA-effects (red line). While weighting in the UVB-range increased the biologically effective dose 4.4 fold, weighting in the UVA-range decreased the effective dose 4.8-fold. This means, that radiation emitted by the Q-Panel tubes in the UVB-range is biologically more effective to cause lethal damage in *A. nugax* and to decrease its survival in this study than UVA. Although, the shortfalls of only two different weighting coefficients over a broad UV-range are visible as the drop within the red line at 320 nm. The black continuations (black line) of the red line resemble “false” or “masked” effects, which would result from extrapolation with the wrong weight in that respective UV-range ($\varepsilon_{H(280\text{-}320\ nm)}$ at UVA and $\varepsilon_{H(320\text{-}400\ nm)}$ at UVB), leading to wrong predictions of UVR effects, as discussed by Cullen & Neale (1997).

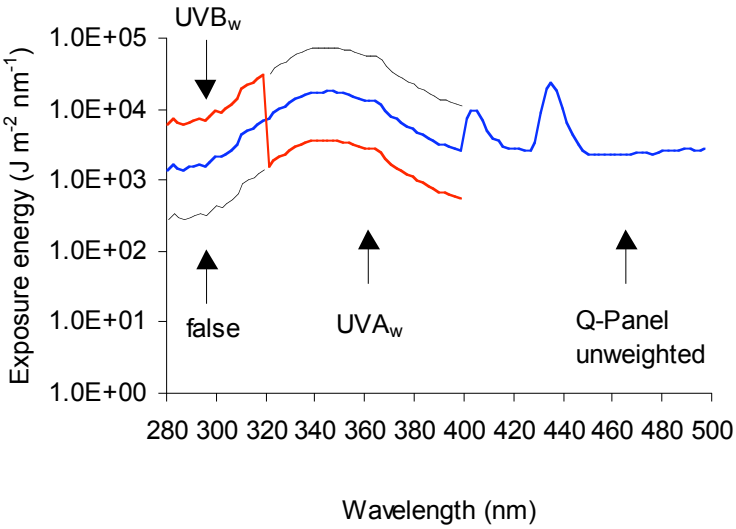

Figure 5.3.1: Approximation of biologically effective exposure (red line, unitless) for *Anonyx nugax* as calculated from the exposure energy spectrum (J m^{-2} nm^{-1}) with the Q-Panel and white light tubes over 12 days (blue line, unweighted). Black line resembles "false" or "masked" effects and results from extrapolation with the wrong weight in that respective UV-range ($\varepsilon_{H(280\text{-}320\ nm)}$ at UVA and $\varepsilon_{H(320\text{-}400\ nm)}$at UVB).

Interestingly, in *Daphnia pulicaria* the potential damage in nature predicted from exposure experiments to solar radiation was greatest between 305-322 nm, including longer wavelength UVB and shorter wavelength UVA (Williamson et al. 2001). This might also apply for *A. nugax* as the biologically effective exposure (red line in Figure 5.3.1) still rises in the long-wavelength UVB-range. As species respond differently to applied UVR-doses, BWFs have to be developed separately and cannot be transferred between species or taxonomic groups or responses (Tartarotti et al. 2000). So far, in most studies responses were weighted in phyto- and zooplankton species (Cullen & Neale 1997, Franklin & Neale 2002, Helbling et al. 2002a, Browman et al. 2003, Kouwenberg et al. 1999 I and II), and to my knowledge, this is the first attempt to weigh UV-effects in shallow water amphipods.

Besides wavelength specific effects, survival rates of carnivorous/necrophagous *A. nugax* and *O. edwardsi* also seemed to depend on different dose levels applied. However, the moderate-dose treatment was more damaging than the high-dose UVR impact, which caused survival to drop only to 78% (*A. nugax*) and 61% (*O. edwardsi*), in contrast to 19% (*A. nugax*) and 10% (*O. edwardsi*) survival at moderate UVB (Publ. III, section 4.3 Results). In Figure 4.3.1 and 4.3.2 (section 4.3 Additional results) survival is plotted against total UVB-dose received,

which clearly elucidates the detrimental effect of the moderate-dose treatment, causing higher mortality at the same total UVB-dose received than the high-dose treatment. This is contrasting to what one would expect and, also, at first sight, seems to contrast the findings from the Antarctic species. Here, UV-protection seemed to be more efficient under low-dose UVR, maintaining survival during the experiments in 2000 between 98 and 89% in *Gondogeneia antarctica* and *Djerboa furcipes* (Publ. I), and within the range of non-UV-exposed controls. High-dose UVR decreased survival slightly to between 94% (*G. antarctica*) and 84% (*D. furcipes*) after three irradiation weeks (Publ. I, section Results).

However, if survival of *G. antarctica* and *D. furcipes* is plotted against total UVB-dose received and not against exposure time (days), then the low-dose treatment also seems to lead to higher mortality at the same total UVB-dose received by the amphipods (Fig. 4.3.3 and 4.3.4 Additional results). This effect is obvious in *D. furcipes* from the second Antarctic expedition in 2002, with higher mortality under the low- than under the high-dose treatment at the same total dose received. Overall, this indicates a dose-*in*dependent response, and decreased survival is probably a result of other factors such as the differing spectral composition of the artificial light sources (Q-Panel tubes, SONSI) contributing differently to UV-induced damage as well as repair.

As discussed above in section 5.2, the spectral composition (UVB:UVA:PAR ratio) of the sunshine simulator resembles that of the sun more closely than the Q-Panel tubes. This wavelength ratio is in part responsible for induced repair mechanisms, such as photoenzymatic repair (PER), which is light-dependent enzymatic DNA-repair in the longer wavelength UVA- and near blue PAR range (385-450 nm) (Mitchell & Karentz 1993, Sinha & Häder 2002). Williamson et al. (2001) showed, that survival of UVR-exposed *Daphnia* depends predominantly on PER. Exposure experiments with and without PER-stimulating radiation (UVA+PAR) showed increasing survival of this small fresh water crustacean in the presence of PER-stimulating radiation. PER followed a saturation curve in *Daphnia* with increasing number of UVA+PAR tubes (Williamson et al. 2001). This suggests, that in the low-/moderate dose experiments of the present study, PER-stimulating radiation might have been insufficient for saturating repair of UV-induced sub-lethal damage in amphipods. Accumulating sub-lethal damage may cause elevated and fast mortality as seen in exposed amphipods. Contrasting, in the high-dose experiments in the SONSI, PER-stimulating radiation was obviously high enough to sustain survival. PER was not directly measured in exposed amphipods in this study, but presence of PER has been demonstrated in several

zooplankton species (cladocera: Grad et al. 2001, copepods: Zagarese et al. 1997, ice fish eggs: Malloy et al. 1997).

However, in the case PER is present, the principle of reciprocity, essential for a biological weighting function (BWF), does not hold. This means that a specific BWF cannot be applied effectively to exposure conditions, which differ from those of that respective BWF. In *Daphnia pulicaria* exposure experiments with artificial UVR (UV lamp phototron) reciprocity failed as low-dose UVB-exposure over a long period lead to lower survival than high-dose UVB-exposure over a short period. Failure of reciprocity also seems to be the case in the experiments presented here for *A. nugax*, *O. edwardsi* and *D. furcipes*. This means, that in the present study the transfer of the biologically effective exposure (Fig. 5.3.1) for *A. nugax* gained with artificial radiation (Q-Panel tubes) to the natural solar radiation in Kongsfjord is difficult, and predictions of UV-effects *in-situ*, if at all, have to be done with caution (Cullen & Neale 1997, Williamson et al. 2001).

In the future, the sunshine simulator rather than the Q-Panel tubes should be used for exposure experiments addressing the animal's survival. Additionally, outdoor experiments with *in-situ* exposure to solar radiation should accompany artificial exposure in order to develop biologically relevant weighting functions with ecological implications in the field.

5.3.3 *Influence of nutrition on UVR defence and damage*

As indicated by the survival rates, UV- and antioxidant protection seem to be dependent on the *type of nutrition*, favouring herbivory and discriminating carnivory. In carnivores, the *state of nutrition* is also important favouring starved over fed animals.

A) Mycosporine-like amino acids (MAAs)

Of all UV-protective substances, the concentration and composition of *mycosporine-like amino acids (MAAs)* in animals depend mainly on nutrition, as only algae, fungi and bacteria are able to synthesise MAAs de-novo. Animals, such as amphipods, have to take-up and accumulate MAAs via the food chain, either directly from macro- and microalgal food or animal prey (Banaszak et al. 1998, Newman et al. 2000, Adams et al. 2001, Whitehead et al. 2001). MAAs were studied in whole animal homogenates of all polar amphipod species (Publ. I, III). Herbivorous *Gammarellus homari* (Arctic) and *Gondogeneia antarctica* (Antarctic) exhibited highest and similar total MAA levels of around 770 µg g^{-1} dry weight (Publ. III, Tab. 2; Publ. IV, Tab. 7). Such high MAA content was also measured in the Antarctic amphipod *Pontogeneia* sp., a nutritional generalist also ingesting sea ice microalgae

and coastal macroalgae (Karentz et al. 1991). The MAA composition in the present study reflected the macroalgal food species: for *G. homari* the red alga *Devaleraea ramentacea* (Publ. III, A in Fig. 4), and for *G. antarctica* a combination of *Porphyra endivifolium*, *Palmaria decipiens*, *Neuroglossum ligulatum*, and other red algae (Publ. I). Hence, dominating MAAs differed in Arctic and Antarctic amphipods. While in *G. homari* (Kongsfjord) porphyra P-334, mycosporine-glycine and palythine were most abundant in this rank order, shinorine, P-334, and palythine were dominant in *G. antarctica* (Potter Cove). Concentrations of mycosporine-glycine were one to two magnitudes lower in *G. antarctica* than in *G. homari*. Investigations of several Antarctic red macroalgal species showed generally very low mycosporine-glycine concentrations below 40 μg g^{-1} DW, with only trace levels in most algae (Hoyer et al. 2001), whereas high concentrations of 160 and 122 μg g^{-1} DW were measured in Arctic *D. ramentacea* and *Palmaria palmata* from Spitsbergen (Karsten et al. 1998). This may indicate metabolic transformation of e.g. shinorine or P-334 by ubiquitous marine gut bacteria as shown for *Vibrio harveyi* isolated from the holothuroid *Thelenota ananas* yielding mycosporine-glycine (Dunlap & Shick 1998). Another possible pathway during digestion is the production of mycosporine-glycine by acid hydrolysis of palythenic acid, which itself derives from P-334 via a simple dehydration reaction (Whitehead et al. 2001). Additionally, mycosporine-glycine may also derive from animal food or detritus, as *G. antarctica* is able to switch from preferably macroalgae to a broader nutritive spectrum (Iken 1996).

Maximal spectral absorption of shinorine and P-334 is at 333-334 nm and of palythine at 320 nm in the UVA range. Mycosporine-glycine absorbs maximally at 310 nm in the UVB range. Thus, Arctic *G. homari* has a broad protective range in both the UVB and UVA region, whereas Antarctic *G. antarctica* has more shielding potential in the UVA part of the radiation spectrum (Banderanayake 1998, Dunlap & Shick 1998). In addition, Adams & Shick (2001) observed half-maximal absorption of shinorine (partially purified red alga methanol extracts) and of sea urchin eggs extracts (containing mainly shinorine and P-334) at 312 nm, which could partially compensate lower mycosporine-glycine levels found in *G. antarctica* in my study. Besides sunscreening, mycosporine-glycine is also ascribed a moderate antioxidant activity (Dunlap & Yamamoto 1995).

Hoyer (2003) showed that several Antarctic and Arctic macroalgae (e.g. *P. decipiens*, *D. ramentacea*) are able to adjust their MAA concentrations in response to prevailing radiation conditions. This constitutes another advantage for herbivores, facilitating adaptation

to changing radiation climate within days or weeks (Newman et al. 2000). However, UV-exposure might also negatively affect algae, altering quality (e.g. lipid content and composition) or digestibility (e.g. cell morphology and structure) and consecutively accelerate negative UV-effects in consumers (Häder et al. 1995, van Donk & Hessen 1995). This was shown for Subarctic herbivorous UVR-exposed *Daphnia*, in which feeding UV-treated phytoplankton increased gut damage in comparison to specimens fed non-irradiated algae (Zellmer et al. 2004). Accumulating sub-lethal damage leads to increased mortality with possible effects for entire populations as demonstrated by decreased reproductive success in *Daphnia*, feeding on UV-treated algae (De Lange & van Donk 1997, De Lange & Reeuwijk 2003).

Overall, total MAA content in herbivorous amphipods from both polar regions did not change significantly during various UVR-exposure experiments, neither with nor without feeding over three weeks. But in *G. homari* (Kongsfjord) composition of individual MAAs changed after prolonged exposure to moderate-dose UVB over 20 days. The major MAAs P-334 and mycosporine-glycine as well as shinorine decreased significantly ($p<0.05$) compared to UVB-shielded controls (Publ. III, Fig. 4). In contrast, P-334 and mycosporine-glycine did not change significantly ($p>0.05$) in *G. antarctica* from Potter Cove exposed to low-dose UVB over three weeks (Publ. I, Fig. 4B and C). The photostability of MAAs, as pre-requisite for their sunscreening role, was demonstrated by various authors even under prolonged starvation (65 days) or UV-exposure (10 days) (Conde et al. 2000, Newman et al. 2000, Adams & Shick 2001, Shick & Dunlap 2002). However, Helbling et al. (2002a) also measured a decrease in total MAA content in freshwater copepod *Boeckella titicacae* already during 3 days of exposure to surface level solar UVR, suggesting successive bleaching of MAAs. Such contradictory findings of photostability on the one hand and loss of MAAs on the other were also discussed by Hoyer (2003), who classified different physiological response types to UVR-exposure in Arctic and Antarctic macroalgae: firstly algae with the ability to increase MAA content, secondly algae with a constant high MAA level without further induction, and thirdly algae, in which MAAs decrease under enhanced UVR. The author suggests, that a loss of MAAs may occur through photodestruction or leakage from damaged algal thalli. Reasons for loss of MAAs from amphipod tissues, however, remain unclear.

Contrasting to above herbivorous amphipod species, carnivorous/necrophagous *Anonyx nugax* and *Onisimus edwardsi* from Arctic Kongsfjord had several times lower total MAA

concentrations around 60 to 116 µg g^{-1} dry weight (Publ. III, Tab. 2). Their contents were in the range of other Antarctic carnivore and scavenging amphipods (Karentz et al. 1991). This deficiency in sunscreening compounds might in part be responsible for the lower survival compared to the herbivores. Likewise, *B. titicacae* specimens with low MAA levels had significantly lower survival (approximately 28%) during UVR-exposure than specimens with high MAA concentrations (Helbling et al. 2002a). The MAA composition in *O. edwardsi* revealed that this species probably consumes macroalgal detritus in addition to animal derived food. The amphipods' MAA composition was similar to that of the investigated red alga, including e.g. asterina-330, which was completely lacking in most investigated *A. nugax* specimens (Publ. III, Fig. 5 and 6). Whitehead et al. (2001) demonstrated trophic transfer of MAAs from primary producers (Antarctic algae) to primary consumers (Antarctic herbivorous pteropod *Limacina helicina*) and to secondary consumers (Antarctic predatory pteropod *Clione antarctica*). MAAs were further concentrated and modified in the consumers.

Contrasting to all other species in the present study, concentrations of total MAAs and of individual MAA classes decreased in *O. edwardsi* in exposed animals as well as in controls shielded from UVR (Publ. III, Tab. 2, Fig. 6). A decrease in exposed specimens might have been due to beginning photodestruction of MAAs after prolonged UVR-exposure and the lack to re-supply MAA content with macroalgal detritus. Animals in our experiments were either fed pieces of fish or starved. However, in my study, MAAs declined also in control animals not receiving any UVR. This could be explained by a certain natural turn-over of dietary derived ("senescent") MAAs. Newman et al. (2000) showed that dietary derived MAAs were not maintained indefinitely in Antarctic krill. They measured a decrease in shinorine, P-334 and mycosporine-glycine:valine after 35 days of starvation in the laboratory without any additional UVR stress.

Overall, this demonstrates that even an incomplete UV-protection - low MAA levels - constitutes at least an initial defence, reducing UV-damage for up to 7 days, or up to a threshold of total UVB-dose received by the amphipod. Freshwater copepod *Boeckella titicacae* containing high MAA levels had a high UVR dose-response threshold for a beginning decrease of survival of around 300 kJ m^{-2} (Helbling et al. 2002a). Whereas in the Patagonian amphipod *Amphitoe valida* survival decreased significantly in animals with low MAA levels when the total UVB-dose received exceeded 40 kJ m^{-2} (Helbling et al. (2002b). Interestingly, in Antarctic herbivorous *G. antarctica* from the first Antarctic expedition (in

2000), the UVB dose-response threshold for decreasing survival did not reach saturation in fed *G. antarctica* specimens with high MAA levels. Starvation decreased this threshold to approximately 180 kJ m^{-2}. But as there was no significant difference in MAA concentration between fed and starved amphipods, this effect was caused be different factors, such as limited energy supply for repair processes of UV-induced damage due to food shortage. In *D. furcipes*, which had 5 times lower MAA concentrations than *G. antarctica* (Publ. IV, Tab. 7), the UVB dose-response threshold for decreasing survival was approximately 240 kJ m^{-2} in fed animals. The UVB dose-response thresholds are derived from the intersection of survival within the high-dose treatments with the lower standard deviation range of control animals depicted as vertical lines in Figure 4.3.3 and 4.3.4 (section 4.3 Additional results).

In Arctic herbivore *G. homari* with high MAA concentrations (Publ. III, Tab. 2; Publ. IV, Tab. 7) UVB dose-response threshold for decreasing survival did not reach saturation under any treatment. Contrasting, in Arctic carnivorous/necrophagous amphipods, which had low MAA levels (Publ. III, Tab. 2), UVB dose-response thresholds for decreasing survival of fed animals were approximately 18 kJ m^{-2} for moderate and 88 kJ m^{-2} for high-dose UVB-exposed *A. nugax*, and 8 kJ m^{-2} and 25 kJ m^{-2} in moderate and high-dose UVB-exposed *O. edwardsi*. Contrasting to Antarctic herbivorous *G. antarctica*, starvation elevated the threshold levels in both Arctic scavengers between 3 and 12 fold. However, explanation is difficult as no significant differences in MAA concentrations between fed and starved animals occurred. As for Antarctic amphipods, UVB dose-response threshold derived from intersection of survival with lower standard deviation range of control animals depicted in Figure 4.3.1 and 4.3.2 (section 4.3 Additional results).

> Concluding, this demonstrates that high MAA levels promote survival in amphipods, increasing the maximal exposure time as well as the total UVB-dose, which the animals can endure.

B) Carotenoids

Carotenoids functioning as antioxidants are also dietary derived. They are passed on well through the food chain, and amphipods sequester and modify carotenoids either from fresh material, or from algal/animal derived detritus (Edge et al. 1997, Halliwell & Gutteridge 1999). In the present study, carotenoid tissue concentration of 0.174 $\pm$ 0.020 µmol ß-carotene equivalent g^{-1} fresh mass FM was highest in Antarctic herbivores (Publ. IV, Tab. 2), followed by 0.092 $\pm$ 0.027 µmol g^{-1} FM in the Arctic herbivore (Publ. III, Tab. 1), and 0.010 $\pm$ 0.042

µmol g^{-1} FM in the temperate herbivore (Fig. 4.2.3a Additional results). Results for the temperate reference species *Chaetogammarus marinus* (Helgoland, North Sea) show that a higher natural solar UVR impact at lower latitudes does not necessarily lead to a higher accumulation in tissue carotenoids.

None of the polar and temperate herbivorous species showed any bleaching of tissue carotenoids upon experimental UVR-exposure. Contrasting, Arctic scavengers exhibited lower initial carotenoid values of 0.069 $\pm$ 0.030 µmol g^{-1} FM in *Anonyx nugax* and 0.069 $\pm$ 0.017 µmol g^{-1} FM in *Onisimus edwardsi* (Publ. III, Tab. 1). In addition, bleaching of 35% of total carotenoids occurred in *O. edwardsi* after 7 days, and more severely (59%) in *A. nugax* after 14 days of high UVB-impact (Publ. III, Tab. 1). There are two possible mechanisms of carotenoid photoprotection against singlet oxygen and other ROS during oxidative stress: Chemical quenching leads to the degradation of the carotenoid (Farmillo & Wilkinson 1973), whereas in the electron exchange energy transfer the carotenoid acts as a catalyst quenching the excited molecule and being recycled itself either by energy dissipation as heat or physical quenching (Liebler 1993). Photoprotection can only be sustained if electron exchange energy transfer predominates over chemical quenching (Edge et al. 1997). This would explain maintenance of carotenoid concentration during UVR-exposure in herbivorous amphipods from all regions. By contrast, in carnivorous/necrophagous *A. nugax* and *O. edwardsi* carotenoid levels decrease possibly due to a higher degree of chemical quenching of ROS.

Moreover, UVR-screening potential of carotenoids is very low in comparison to that of MAAs. The UVR-screening potential was calculated from the absorption of the carotenoid extract from echinoid eggs at 334 nm in the UVA-range (Lamare et al. 2004). As a consequence, herbivorous amphipods, which had a high content of sunscreening MAAs may have generally had a lower oxidative stress level. In contrast, carnivores/necrophages had lower MAA levels, allowing more UVR-photons to pass deeper into the tissue, thereby inducing production of detrimental ROS (Zagarese & Williamson 2000, Helbling et al. 2002a, Lamare et al. 2004). Carotenoids belong to the group of low molecular weight antioxidants such as ascorbic acid and α-tocopherol. Tissues depleted in this first borderline defence depend on other mechanisms to prevent oxidative damage, for example by the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase (Camus & Gulliksen 2005). Direct transfer from primary producers (algae) to primary consumers (herbivorous amphipods) might replenish carotenoids faster or more efficiently, whereas dietary conversion in secondary consumers (scavenging amphipods) may take longer. In addition, herbivores may accumulate different and more efficient carotenoids from their macroalgal diet as

compared to scavengers. For example, astaxanthin quenches singlet oxygen more efficiently than ß-carotene and canthaxanthin (Tinkler et al. 1994, Edge et al. 1997).

> Concluding, low carotenoid levels in combination with low MAA concentrations possibly render the animals prone to oxidative stress. This may lead to elevated mortality rates as observed in carnivorous/necrophagous amphipods in the laboratory. In the field, amphipods lacking other sources of protection (e.g. melanin, antioxidant enzymes, photoenzymatic repair PER) would be restricted to deeper depths or diurnal migration to avoid peak irradiance during midday (Zagarese et al. 1997).

C) Starvation

In herbivorous amphipods from Potter Cove, starvation decreased survival in UV-exposed *Gondogeneia antarctica* by 12% after 14 days relative to the fed group under the same UV-treatment (Publ. I, Results). This is also visible in the lower UVB dose-response threshold for decreasing survival of approximately 180 kJ m^{-2} as compared to the non-saturating response in fed animals. Supporting my findings, survival in UVR-exposed *Daphnia* increased significantly with amount of food, providing the necessary energy for UV-defence and repair processes (Zellmer 1996).

Contrasting, starved carnivorous/necrophagous amphipods from Kongsfjord displayed 44% (*Anonyx nugax*) and 85% (*Onisimus edwardsi*) higher resistance to UVB-exposure over 12 days than specimens fed *ad libitum* with little pieces of fish (Publ. II, Fig. 4; Publ. III, section 4.3 Results; Fig. 4.3.1 and 4.3.2 Additional results). This was also visible in the 3-fold (*A. nugax*) and 12-fold (*O. edwardsi*) higher UVB dose-response threshold for decreasing survival in starved compared to fed amphipods (Fig. 4.3.1 and 4.3.2 Additional results). Carnivorous/necrophagous *A. nugax* and *O. edwardsi* belong to the group of lysianassid amphipods, which are reported to be voracious feeders, consuming high amounts of prey in short time and accumulating in extremely high numbers on carrion (Sainte-Marie et al. 1989, Legezynska et al. 2000). Competition for space on food surface and for amount of food intake most likely imposes stress to actively feeding animals. Exposure to UVR could have been an additional factor converting sub-lethal stress into lethal damage, thereby increasing mortality. On the contrary, starvation probably decreased stress levels in amphipods, leading to higher survival observed in this study. Prolonged food limitation reduces the oxygen consumption and metabolic rate in amphipods (Chapelle et al. 1994). These reductions are believed to

ameliorate ROS production and subsequently prolong life (Yoon et al. 2002, Abele & Puntarulo 2004, Philipp et al. 2006, Stuart & Brown 2006).

Radiation has an impact on feeding or digestion processes in crustacean zooplankton, leading e.g. to reduced gut pigments in a copepod and damage in the gut system of a cladoceran (Lacuna and Uye 2000, Zellmer et al. 2004). Contrasting, in *Daphnia pulex* there exists a positive relationship between the amount of food and percentage of survival during UVR-exposure (Zellmer 1996). Perhaps, the herbivorous amphipod species in this study have an advantage above the carnivores, as they are generally not food limited in Potter Cove and in Kongsfjord after the onset of primary production in spring until the decrease in solar radiation in autumn. Thus, the herbivores experience a more constant energy supply during the light period, maintaining their metabolism relatively constant. By contrast, the carnivorous/necrophagous amphipods might undergo prolonged periods of starvation due to absence of prey or carrion regardless of season, leading to a continuous up and down in metabolic rate and (oxidative) stress potential.

In contrast to survival, no significant differences between feeding or starvation in antioxidant superoxide dismutase (SOD) activity, accumulation of lipid peroxidation marker thiobarbituric-acid-reactive substances (TBARS, i.e. equivalent MDA), and MAA concentration were measured in Antarctic herbivorous *Gondogeneia antarctica* exposed to UVR over two weeks. (Publ. I, Fig. 1, 2, 4). This holds true for antioxidants (SOD, catalase, carotenoids), lipid peroxidation marker TBARS, and MAAs of Arctic herbivorous and carnivorous/necrophagous amphipod species over three weeks (Publ. III, section 4.2 Results). It has to be considered that all biochemical analyses were run on freshly sacrificed specimens *surviving* the exposure experiments. Energy reserves in these animals were obviously sufficient to maintain UV-induced damage on sub-lethal levels, regardless supply with or deprivation from food. Although, the high natural variability and small number of replicates might have camouflaged existing differences. Another explanation could be that the starvation time of up to 3 weeks might not have been long enough to cause significant differences in the amphipods studied. Like many polar organisms, amphipods have a large amount of storage lipids to overcome long periods of food limitation during polar winter season (darkness). Polar amphipods exhibit a pronounced decline of metabolic functions during starvation, however, not critical in terms of survival (Clarke et al. 1985, Clarke 1988, Hagen 1988, Sainte-Marie et al. 1989, Chapelle et al. 1994, Graeve et al. 1997, Nyssen et al. 2005, Hagen et al. 2001).

In future exposure experiments, amphipods should be starved for several days and weeks *prior* to experimentation to elucidate possible differences due to limited energy supply *during* the experiments.

5.3.4 *Time scales of antioxidant defence mechanisms and sub-lethal effects*

Enzymatic antioxidant defence mechanisms and accumulating sub-lethal damage were investigated on different time scales to evaluate instantaneous (4 hours), short-term (1 to 7 days) and medium-term (2 to 4 weeks) effects of UVR.

A) Instantaneous responses to UV-exposure (4 hours)

Instantaneous responses to UV-induced stress in behaviour and physiology were measured as changes in whole animal oxygen consumption in Antarctic amphipods during 4-hours exposure intervals over the experimental period of 26 hours (Publ. IV). In both species, exposure to UVB and UVA caused immediate and high increases in mean oxygen consumption over initial resting metabolic rates (RMR). In *Gondogeneia antarctica* these immediate increases reached extremes between 150 and 300% of initial RMR (e.g. Publ. IV, Fig. 5a phase 2, Fig. 5d phase 4), and in *Djerboa furcipes* extreme increases even exceeded 500% over initial RMR (e.g. Publ. IV, Fig. 6d phase 4, Fig. 6e phase 4). Such peak respiration was followed by sometimes pronounced declines. The resulting oxygen consumption at the trough was 3-5 fold lower than the preceding peak in *G. antarctica* (e.g. Publ. IV, Fig. 5a phase 3 and 5) and 2 to 10 fold lower *D. furcipes* (Publ. IV, Fig. 6b phase 2 and 4, Fig. 6e phase 5). High UV-induced stress caused irregular respiration and was visible in highly variable respiratory amplitudes, resembling large differences between maximal and minimal oxygen consumption within 30 minute time intervals. UV-induced changes in oxygen consumption were wavelength specific in *D. furcipes* (higher specific response to UVB-exposure), but not in *G. antarctica*. In contrast, higher respiratory amplitudes in response to UVB-exposure were more pronounced in *G. antarctica* than in *D. furcipes*.

Increased respiration is a sign of increased energy turnover during reactions of avoidance and shelter seeking from irradiation. Rigid decreases reflect metabolic depression upon exhaustive initial high-stress period. Firstly, avoidance and shelter seeking from UVR-exposure has been well documented in literature (Adams 2001, Leech & Williamson 2001, Newman et al. 2003). For example, Antarctic krill reduces its swimming activity during exposure to UVB and UVA, which will cause the animals to sink out of the photic zone to greater, less exposed depths (Newman et al. 1999). Further, increased respiration also reflects energy consuming

repair processes of UV-induced DNA- and cellular damage. Photoenzymatic repair of DNA-damage is fast and occurs simultaneously to ongoing UV-induced damage within hours of UVR-exposure as shown for various marine and freshwater zooplankton species (Malloy et al. 1997, Williamson et al. 2001, Goncalves et al. 2002). Accelerated metabolic activity leads to increased formation of detrimental ROS, causing oxidative tissue damage and possibly damage directly to respiratory organelles, the mitochondria (Halliwell & Gutteridge 1999). Elevated, stress-induced ROS formation may have finally caused respiratory depressions during irradiation (Publ. IV, Fig. 5b, 5c, 6b) and dark recovery phases (Publ. IV, Fig. 5a, 5d, 6c-e), similar to the effects of H_2O_2 exposure observed in the shrimp *Crangon crangon* (whole animal and isolated tale muscle) and the polychaetes *Arenicola marina* (whole animal and isolated body wall tissue) and *Nereis diversicolor* (whole animals) within 6 hours (Abele-Oeschger et al. 1994, 1997a, Storch et al. 2001).

Gills of marine crustaceans (e.g. *Carcinus maenas*, *Gammarus pulex*) can be damaged through oxidative stress and direct toxicity of ROS, in this case caused by for example exposure to heavy metals such as copper, and not by UVR as in my experiments. But both stressors function through similar mechanisms through metal-mediated Fenton reactions (Halliwell & Gutteridge 1999, Kedwards et al. 1996, Camus et al. 2004). ROS-induced gill damage occurred yet on longer time scales of 3 (*Carcinus*) to 10 days (*Gammarus*). This suggests possible detrimental effects of prolonged UVR-exposure over days and weeks.

The respiratory exhaustion was reversible during dark recovery phases, showing that amphipods in this study were not critically damaged by the irradiation stress. In half of the experiments oxygen consumption of both species did not return to initial resting metabolic rate (RMR) in the final recovery phase. This may be caused by cumulative effects of repeated exposure intervals and may overlay the more effective (damaging) UVB- than UVA-exposure observed in *D. furcipes*. Future experiments may solve this problem by alternating UVB- and UVA-exposure phases in replicate measurements. In addition, the measure time during this final phase could be prolonged. Alternatively, oxygen consumption could be measured again after a certain time gap of 6 or more hours additional recovery period.

Significance of instantaneous stress response on longer time scales:

My observations showed that during UVR-exposure for up to 3 to 4 weeks, amphipods, especially carnivores/necrophages, were active during the first 3 to 4 days of irradiation with a pronounced immediate activity response (shelter seeking) upon onset of UV-irradiation. After

2 weeks of continued experimental UVR-exposure for 5 hours daily, exposed specimens did not respond to the onset of UV-irradiation any longer and appeared altogether apathetic and metabolically exhausted. Although, short- to medium-term responses in oxygen consumption were not measured, these observations indicate that the initial instantaneous metabolic increase is abrogated due to exhaustion of energy reserves and accumulating damage during prolonged exposure to UVR, or gives way to behavioural adaptation. In experiments addressing behaviour and learning, the giant marine snail *Aplysia* withdrew the gills upon a tactile stimulus and developed a memory for this reflex. Upon prolonged repeated stimulation, *Aplysia* "learned" to ignore the tactile stimulus and not to respond with the withdrawal reflex any longer, known as habituation (Kandel 2001). This may also apply to the amphipods in this study during prolonged daily UVR-exposure.

Steeger et al. (1999) also measured lowered metabolism indicated by significantly reduced heart rates during medium-term exposure with a moderate UVB-dose (8.64 kJ m^{-2} d^{-1} UVB daily) in plaice embryos (*Pleuronectes platessa*) from the North Sea. Whereas instantaneous high- and low-dose, as well as short-term low-dose exposure (4.86 kJ m^{-2} d^{-1} total UVB daily) did not lead to significant differences in heart rate in plaice embryos. In contrast to a prolonged life span discussed above, a reduced metabolic activity can finally lower the organism's fitness if this reduction is caused by detrimental UV-induced damage to important metabolic tissues such as the digestive tract. Zellmer et al. (2004) found that intestinal damage and damage to the digestive gland in *Daphnia* was already measurable after 12 hours of UVR exposure, and clearly visible after two days. Partially or completely impaired digestion will be detrimental in a medium to long-term time scale by reducing the organism's energy supply, thus *lowering* the metabolism. Reduced fitness and productivity may lead to increased mortality with possible effects on a population level.

B) Short-term responses to UV-exposure (1-7 days)

In Antarctic herbivorous *G. antarctica* low-dose UVB-exposure (6.84 kJ m^{-2} d^{-1}) over 7 days caused activity of antioxidant superoxide dismutase (SOD) (Publ. I, Fig. 1) and catalase (Publ. IV, Fig. 3) to decrease significantly as compared to start values, accompanied by an insignificant increase of the lipid peroxidation marker thiobarbituric-acid-reactive substances TBARS (equivalent to MDA Publ. I, Fig. 1). This demonstrates beginning accumulation of oxidative lipid damage due to insufficient antioxidant activity. The overall antioxidant defence was, however, still high enough to prevent elevated protein damage as no increase in protein carbonyl content during UVB-exposure compared to non-irradiated control animals

occurred (Publ. IV, Tab. 4). In addition, carbonylated proteins might have been readily removed and replaced by newly synthesised proteins (Levine 2002). This was also the case for *D. furcipes*, where no significant increase in protein nor in lipid peroxidation damage occurred, despite significant impairment of SOD activity during 3 to 4 days of low-dose UVB-exposure (Publ. IV, Tab. 4, protein carbonyl content shown). Impairment of SOD activity was reversible in *G. antarctica* during the experimental course, however, catalase was photodamaged, and activities failed to recover in the course of the experiment. Catalase directly absorbs radiation energy and is thus easily damaged by UVR, especially by long-wavelength UVA and short-wavelength PAR (λ_{max} 405 nm) (Gantchev & van Lier 1995, Zigman et al. 1996, 1998). This happened in both Antarctic herbivores, *G. antarctica* and *D. furcipes*, already after 3 to 4 days of UVA+PAR exposure (Publ. IV, Fig. 3 and 4). Deficiency in catalase may be compensated by peroxidases (e.g. glutathione peroxidase) at small H_2O_2 levels. In addition, other non-enzymatic antioxidants such as α–tocopherol in combination with carotenoids and ascorbic acid quench ROS efficiently (Palozza & Krinsky 1991, Hermes-Lima 2004). Especially one α–tocopherol derivative, the "marine-derived-tocopherol" MDT widely distributed in cold-water fish, is a very efficient quencher of lipid radicals at low temperatures. Thus, MDT prevents peroxidation chain reactions in cold-adapted animals, which contain a high potential for peroxidation damage due to their high degree of polyunsaturated fatty acids (PUFAs, Yamamoto et al. 2001, Nyssen et al. 2005).
High-dose UVB-exposure (19.5 kJ m^{-2} d^{-1}) did not have any significant effect on antioxidant SOD activity or TBARS accumulation in Antarctic *G. antarctica*, demonstrating higher resistance despite higher UV-impact possibly also due to efficient low-molecular weight scavenging. These non-enzymatic antioxidants (e.g. carotenoids, α-tocopherol, ascorbic acid) constitute a primary defence in many organisms and, thus, protect protein enzymes such as SOD from oxidative damage (Regoli & Winston 1999, Camus & Gulliksen 2005).

In Arctic carnivorous/necrophagous *A. nugax* and *O. edwardsi* exposure to moderate-dose UVB (7.2 kJ m^{-2} d^{-1}) over 7 days also caused a significant impairment of SOD activity compared to the other irradiation treatments (Publ. III, Fig. 2). Catalase was only damaged in *A. nugax* under moderate-dose impact, but not in *O. edwardsi* under any treatment, providing the latter species with a better antioxidant defence potential (Publ. III, Fig. 3). In neither species was the decrease of TBARS lipid peroxidation marker significant due to high individual variability. Interestingly, under high-dose UVB-exposure (18.7 kJ m^{-2} d^{-1}) over 7 days activities of both antioxidant enzymes remained higher than under moderate-dose UVB,

indicating better stimulation of antioxidant defence under high as compared to moderate UVB-impact. Camus & Gulliksen (2005) measured susceptibility of *A. nugax* to oxidative stress as the total oxyradical scavenging capacity (TOSC) and suggested a generally very high ROS sensitivity combined with a minor role of catalase in antioxidant defence. However, *A. nugax* in their study displayed a high ROS scavenging efficiency of low molecular weight antioxidants such as carotenoids, α-tocopherol, ascorbic acid and others.

> Overall, this indicates that short-term low/moderate-dose UVB was more detrimental in all investigated species than high-dose UVB-exposure. In amphipods under low/moderate UVB-dose treatment antioxidant enzymes were reversibly impaired (SOD) or irreversibly damaged (catalase). Antioxidant defence was better stimulated during short-term high-dose UVB-exposure of Arctic scavenging amphipods, efficiently preventing accumulation of lipid peroxidation products.

C) Medium-term responses to UV-exposure (2-4 weeks)

In Antarctic herbivorous *G. antarctica* prolonged exposure to low-dose UVB over 3 weeks caused activity of antioxidant SOD (Publ. I, Fig. 1) to increase (insignificantly) and TBARS content (equivalent to MDA Publ. I, Fig. 1) to decrease significantly as compared to start values. This demonstrates reversibility of initial SOD impairment (see 5.3.4 section B) and induction of enzymatic antioxidant function possibly due to protein new-synthesis as a secondary defence mechanism efficient to prevent propagation of lipid peroxidation damage. The opposite occurred during high-dose UVB-exposure over 3 weeks, where significantly impaired SOD activity went along with increased levels of TBARS, indicating that primary and secondary antioxidant defence systems were insufficient to prevent accumulation of oxidative damage to lipids. Impairment of SOD activity might have been caused by photodamage as indicated for catalase, or pathways in protein new-synthesis might have been disrupted.

In Arctic *A. nugax* SOD activities increased over the course of 2 weeks under all treatments. However, SOD activity remained slightly impaired under moderate UVB-exposure compared to the other treatments. Correspondingly, TBARS level was highest under moderate-dose and significantly lower under high-dose UVB-impact. Contrasting, in *O. edwardsi* SOD activity was significantly higher under moderate- as compared to high-dose UVB-exposure. This demonstrated also in this scavenging species that induction of SOD activity was dose-dependent, and higher antioxidant protection could be induced during prolonged UV-

exposure, which prevented elevated lipid peroxidation damage. Catalase activity decreased further in *A. nugax* almost below detection level under all treatments apart from non-irradiated controls, indicating severe UV-induced damage to the enzyme. Catalase activity decreased in irradiated and non-irradiated *O. edwardsi*, so the effect of UVR was not clear in this species.

In herbivorous *G. homari* from Arctic Kongsfjord SOD activities increased, whereas catalase activities decreased in irradiated as well as non-irradiated control animals in comparison to start values over 20 days. Antioxidant defence was sufficient to prevent a significant increase in lipid peroxidation in low-dose UVB-exposed specimens. High-dose UVB-impact seemed to have the greatest effects on antioxidant enzymes and TBARS accumulation, however, low number of replicates prevented statistical significance.

In temperate reference species *Chaetogammarus marinus* SOD activity increased significantly in high-dose UVB-exposed (14.4 kJ m^{-2} d^{-1}) animals over 10 days compared to start values. This increase was accompanied by a significant decrease in TBARS level, demonstrating efficient quenching of lipid peroxidation reactions by the antioxidant enzyme (Fig. 4.2.2 and Fig. 4.2.3 Additional results). Catalase activity decreased in comparison to non-irradiated controls during 10 days high-dose UVB-impact, but was not damaged completely. Thereby, this temperate herbivore amphipod possesses a higher resistance to UV-induced damage compared to the polar species (Fig. 4.2.3 Additional results). The higher catalase photostability could be an adaptation of *C. marinus* to generally higher UVR and PAR intensities at this latitude.

> Overall, regulation of antioxidant defence in response to medium-term moderate- and high-dose UVB-exposure was species specific. High-dose UVB-exposure caused a better stimulation of and less damage to enzymatic antioxidant defence in herbivore *G. homari* and *C. marinus*. By contrast, low-dose UVB-exposure induced a better protection in herbivore *G. antarctica* and carnivorous/necrophagous *O. edwardsi*. In carnivorous/necrophagous *A. nugax* effects of moderate or high-dose UVB on enzymatic antioxidant defence were approximately the same, however lipid peroxidation was significantly higher under moderate UVB-dose.

Interestingly, all investigated species (Arctic, Antarctic and temperate) exhibited high initial values of catalase activities with high individual variability in freshly collected or only shortly laboratory acclimatised specimens (Tab. 5.3.1).

Table 5.3.1: Comparison of catalase activity (U mg^{-1} fresh mass FM) in Antarctic, Arctic and temperate amphipod species (freshly collected or only shortly laboratory acclimatised specimens) resembling *in-situ* values. Numbers in brackets indicate number of replicates per value.

Study site, Region	Investigated species	Type of nutrition	Catalase activity (U mg^{-1} FM)	Taken from
Potter Cove, Antarctic	*Gondogeneia antarctica*	herbivore	0.85 ± 0.30 (15)	Publ. IV, Tab. 3
	Djerboa furcipes	herbivore	1.67 ± 0.97 (18)	Publ. IV, Tab. 3
Kongsfjord, Arctic	*Gammarellus homari*	herbivore	0.25 ± 0.17 (9)	Publ. III, Fig. 1b
	Anonyx nugax	carnivore[1)]	0.87 ± 0.49 (8)	Publ. III, Fig. 2b
	Onisimus edwardsi	carnivore[1)]	0.21 ± 0.10 (4)	Publ. III, Fig. 3b
Helgoland, Temperate	*Chaetogammarus marinus*	herbivore	2.75 ± 2.15 (4)	Fig. 4.2.3 Additional Results

[1)] Type of nutrition is carnivore/necrophage/scavenging.

In Arctic, but not in Antarctic and temperate species, catalase activities decreased significantly in non-UV-exposed animals during laboratory maintenance. The elevated *in-situ* enzyme activities and heterogeneity in combination with high and variable *in-situ* TBARS level indicate that the amphipods were exposed to variable levels of oxidative stress *in-situ* possibly caused by UV-induced H_2O_2 photoproduction in their natural habitats. Oxygen diffusion is lower at cold temperatures than in warmer water, but may be overcome with higher degrees of unsaturated fatty acids, especially PUFAs, in polar animals, which increase the risk for ROS-mediated oxidative damage and might explain higher oxidative stress potentials observed (Regoli et al. 1997, Sidell 1998, Abele & Puntarulo 2004). By contrast, in warmer water oxygen solubility and thus dissolved oxygen-drived stress is lower. Here, high *in-situ* catalase levels in temperate *C. marinus* (North Sea), which are 2 to 11 times higher than in polar amphipods (Tab. 5.3.1), might be a response to H_2O_2 exposure in the shallow intertidal at Helgoland in summer. Field studies showed that exposure to elevated levels of H_2O_2 may occur on sunny days in the intertidal during low tide, e.g. in rock pools or sand flats from high to low latitudes (Abele-Oeschger et al. 1997b, Abele et al. 1999).

> Individual-specific modulation of antioxidant defence may increase the antioxidant capacity and thus promote survival in unstable intertidal habitats with fluctuating environmental parameters at high as well as lower latitudes.

D) Response reinforcement through combination of stressors

Contrasting to artificial exposure in the laboratory, natural conditions in the field and especially at intertidal shore levels present complex interactions of various environmental factors the animal has to cope with (e.g. changing temperature and salinity, variable oxygen availability, tide-dependent desiccation and rehydration, seasonal changes in food availability). Additionally, allochthonous stressors (such as ozone depletion and induced changes in underwater irradiation climate and UVR-intensities / input of pollutants and metal contaminants with melt water / more frequent occurrence of temperature extremes induced by global warming) can synergistically decrease the shallow water organism's tolerance threshold to a single or a combination of these factors (Verslycke & Janssen 2002). In laboratory experiments, Liess at al. (2001) measured that in the Antarctic amphipod *Paramoera walkeri* a combination of three environmental stressors (exposure to UVB, starvation, and copper) increased the amphipods' sensitivity to environmentally realistic concentrations of copper 30fold, and about 15fold with two stressors (UVB plus copper). *In-situ* bioassays also revealed a synergistic detrimental effect of solar UVR-exposure in combination with heavy metal input from an old waste disposal site near Antarctic Casey station (Duquesne & Liess 2003). Survival of *P. walkeri* decreased by 22% significantly more under exposure to stress in combination than with only one of the stressors applied.

At low concentrations of the toxicant (e.g. copper, iron, lead, chromium) the energy use is increased to metabolise the toxicant (Liess et al. 2001). But at higher concentrations metabolic functions are disrupted (e.g. respiration, osmoregulation, regular heartbeat) and organs damaged (e.g. gills, kidney, liver, heart, digestive gland) (Kedwards et al. 1996, Viarengo et al. 1990, Romeo et al. 2000, Livingston et al. 2001, Camus et al. 2004). Toxicity is induced by multiple mechanisms, including bioaccumulation of the contaminant in the amphipod's tissues, thus stimulating ROS production, and resulting in oxidative damage to proteins, lipids and DNA (reviewed by Livingston 2001). Subsequently, this may lead to the animal's death. Simultaneous exposure to UVB stimulates the energy demand due to accelerated repair and replacement processes, thus, only well fed animals have a higher tolerance level to sustain survival (Liess at al. 2001). In the vicinity of Antarctic Casey station, *P. walkeri* was only absent from contaminated shallow water habitats, even though concentrations of heavy metals were below guideline values (Duquesne & Liess 2003). This indicates that polar amphipods, such as *P. walkeri*, have a low tolerance-response threshold towards contaminants, and stress in combination (e.g. UVR) could affect entire populations.

In growing Cichlid fish *Cichlasoma nigrofasciatum* a combination of UVA-exposure and elevated temperature increased mortality and induced avoidance behaviour, whereas each single stressor did only have sub-lethal or not measurable effects (Winckler & Fidhiany 1996a and b). Prolonged exposure to sub-lethal UVA at constant temperature reduced oxygen consumption significantly, indicating UVA-induced respiratory impairment. However, mortality in exposed compared to non-exposed fish was not significantly different (Winckler & Fidhiany 1996a). In non-irradiated amphipods temperature stress did not alter behaviour nor increase mortality. At the upper temperature tolerance limit, oxygen consumption was significantly lower in the +UVA group. Interestingly, beyond this upper temperature tolerance limit, oxygen consumption of surviving animals was significantly elevated over the non-UVA-irradiated control group. Additionally, mortality increased significantly, thereby indicating severe physiological stress (Winckler & Fidhiany 1996b). This is of special interest in the context of global climate change and the recent as well as the predicted warming (air temperature) over the Antarctic peninsula and the Arctic. Shallow water temperatures and occurrence of temperature extremes may increase, possibly beyond critical tolerance limits, especially in tidal pools and intertidal habitats (Abele et al. 1998, Kaiser 2002, Kerr 2002, Pörtner 2002, Thompson & Solomon 2002).

6 Conclusions and Perspectives

The aim of my doctoral thesis was to study the effects of UV-exposure on a broad array of defence systems and oxidative stress parameters against direct UV-induced and indirect ROS-mediated damage in polar marine amphipods. I compared UV-tolerance in species form two different coastal regions, the Antarctic Potter Cove (King George Island) and the Arctic Kongsfjord (Spitsbergen), currently undergoing different degrees of ozone depletion in relation to a reference species from a temperate North Sea coast (Helgoland) with higher natural UV-impact, however lower ozone depletion.

During instantaneous and short-term exposure to artificial UVB and UVA radiation, sub-lethal doses cause an increase in respiration (metabolism) due to induced stress responses (avoidance behaviour, quenching of generated ROS, repair of UV-induced and oxidative damage). In UV-tolerant amphipods, pro-oxidant and anti-oxidant processes are balanced, and sunscreening (carapace absorbance, tissue MAAs) and antioxidant defence (tissue carotenoids, SOD, catalase) systems prevent accumulation of significant sub-lethal damage to lipids and proteins, thus, sustaining survival. During medium- to long-term UVR-exposure, the antioxidant defence becomes depleted (carotenoids) or damaged (catalase, SOD) in UV-sensitive amphipods and ROS reach critical, perhaps lethal concentrations. As a consequence, oxidative damage to lipids and proteins, and possibly UV-induced damage to DNA, accumulates when repair mechanisms (e.g. photoenzymatic repair, dark repair, protein turn-over) in UV-sensitive amphipods can no longer balance the damage, leading to significantly decreased survival. In contrast, in UV-tolerant amphipods, perhaps only a combination of various environmental stressors may impair the UV and oxidative defence systems and lead to accumulation of sub-lethal damage.

The observed short-term metabolic stimulation could convert into a metabolic depression during medium to long-term UV-exposure as energy demands might not be sufficiently met due to high costs for repair and defence. This could lead to decreased fitness, which might result in reduced productivity and recruitment and could affect the amphipods on an individual as well as population level with consequences for higher trophic levels. However, conditions applied in the laboratory resemble a “worst-case-scenario”. In the field, multiple abiotic environmental factors influence penetration of UVR into shallow water habitats and

thus control UV-induced direct and oxidative stress. Transfer of results gained with artificial light sources to natural assemblages has to be done with caution.

According to the efficiency of their sunscreening and antioxidant defence to prevent UV-induced and ROS-derived damage and to sustain survival, the investigated amphipod species could be classified into three categories differing by their UV-tolerance thresholds (summarised in Fig. 6.1).

1) High UV-tolerance

Gammarellus homari from Arctic Kongsfjord has the highest UV-tolerance threshold of all investigated shallow water amphipod species. Its herbivorous nutrition provides this species with a high sunscreening and antioxidant potential and maintains survival at highest rates under all UVR-dose levels and irradiation treatments applied. This high UV-tolerance threshold correlates well with its typical vertical distribution and occurrence in shallow water habitats in Kongsfjord. Judging these laboratory results, there should be a very low risk for UV-and oxidative damage in the field. The literature defines *G. homari* as an "Arctic-boreal " species, and possibly its distribution range well into temperate areas provides not only currently an "adaptive potential" for higher UV-radiation levels, but also under a future ozone depletion scenario.

2) Moderate UV-tolerance

Gondogeneia antarctica and *Djerboa furcipes* from Antarctic Potter Cove, as well as *Onisimus edwardsi* from Arctic Kongsfjord have moderate UV-tolerance thresholds. Their nutritive flexibility provide the Antarctic herbivorous and the Arctic carnivorous/ necrophagous generalists with sufficient protection to maintain survival rates clearly above 50% under the majority of artificial irradiation treatments. However, impairment of and damage to antioxidant enzymes in combination with lipid peroxidation damage indicates accumulating sub-lethal damage due to deficiencies in either screening, quenching or photoenzymatic repair capacity. Currently, the Antarctic species successfully colonise shallow intertidal and deeper subtidal areas in Potter Cove and are probably only endangered by UVR during low tide when trapped in tide pools on sunny and calm days. Ozone hole formation has been persisting for two decades now, and shallow water species might have already started to undergo efficient adaptational processes. However, if propagating climate change rises shallow water temperatures generally towards or beyond critical thresholds, then probably

synergistic effects of various stressors will accumulate damage faster and more severely, pushing the exposed animals towards their adaptive limit.

Arctic *O. edwardsi* most likely avoids elevated UV-exposure by downward migration during mid-day peak irradiation and should thus not be endangered under current irradiation conditions in its intertidal and preferred subtidal habitats in Kongsfjord. Moreover, turbidity in the fjord increases quickly after onset of snow-melt, and run-off from glaciers can locally reduce downwelling irradiance substantially. So far, periods of ozone depletion in the Arctic have maximally extended into spring-time March, which limits possible exposure to elevated UVB levels to a short period between sea ice break-up and onset of glacial and melt water discharge. The predicted temperature increase for the Arctic region will possibly impose a greater environmental stress than ozone depletion and induced increases in UVB radiation. However, in combination with pollutants tolerance thresholds of *O. edwardsi* might be crossed in anthropogenically influenced areas.

For the temperate reference species *Chaetogammarus marinus* from Helgoland (North Sea), criteria for evaluation of a UV-tolerance threshold are limited, however, if *C. marinus* possesses sunscreening MAAs in addition to efficient antioxidant defence then its tolerance should be at least moderate.

3) Low UV-tolerance

Anonyx nugax from Arctic Kongsfjord has the lowest UV-tolerance threshold of all investigated amphipod species. Its predominantly carnivorous/necrophagous nutrition does not provide a sufficient sunscreening and antioxidant potential to prevent damage to lipids and catalase. Its voracious feeding habit creates stressful conditions during mass occurrence on carrion, during which UVB-exposure acts as an additional stressor possibly reinforcing sub-lethal ROS-derived damage and causing reduced survival as observed in the laboratory. However, under current irradiation conditions *A. nugax* should not be at risk to undergo UV-induced damage in its natural habitat in Kongsfjord. Like *O. edwardsi*, *A. nugax* can avoid prolonged UV-exposure in the shallow intertidal by downward migration to greater depths. Although, a combination of climate change variables might aggravate the conditions for this Arctic/Subarctic scavenger in the future.

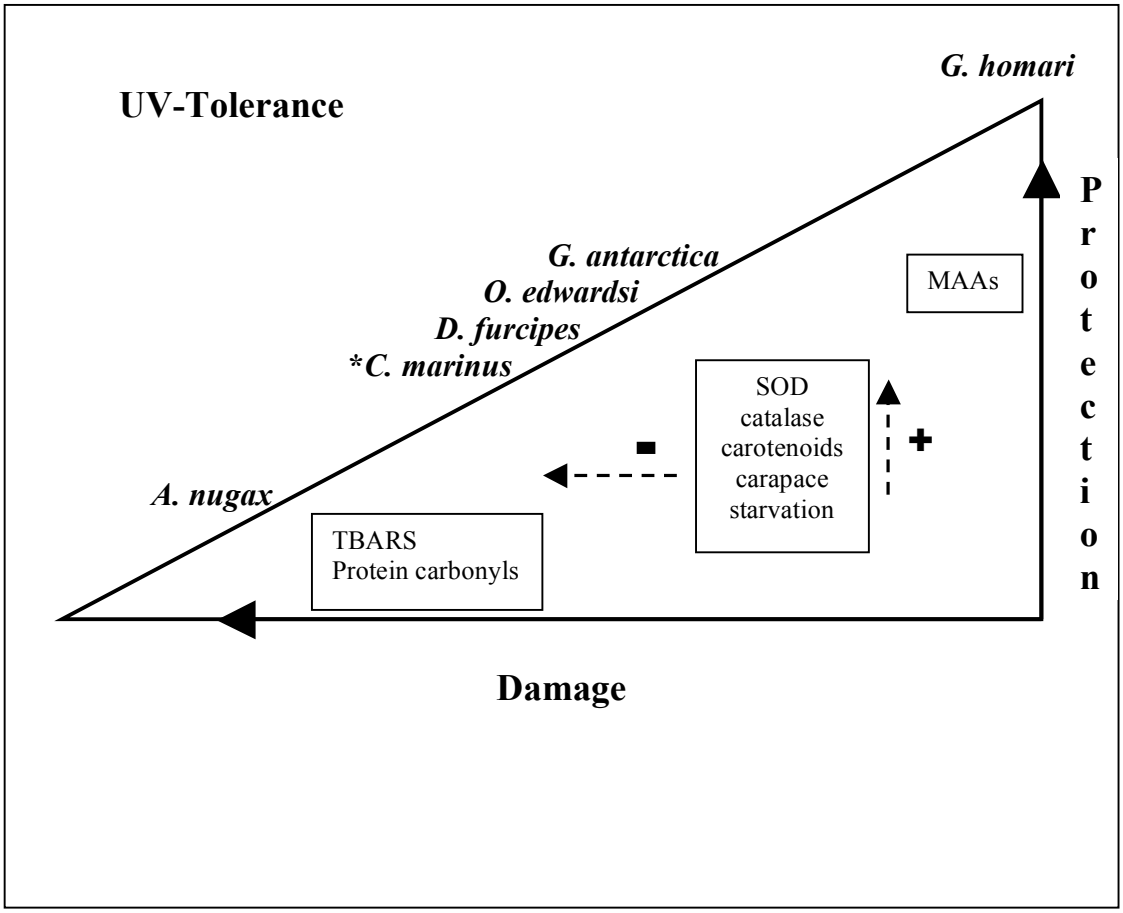

Figure 6.1: Different UV-tolerance thresholds of *Gammarellus homari* (high), *Gondogeneia antarctica* (moderate*), Onisimus edwardsi* (moderate), *Djerboa furcipes* (moderate), *Chaetogammarus marinus* (*possibly moderate), and *Anonyx nugax* (low). While MAAs constitute certain protection, TBARS and protein carbonyls indicate oxidative damage, regardless concentrations. The other factors, antioxidant SOD, catalase and carotenoids, as well as carapace UV-absorbance and starvation can promote protection or increase damage, depending on activity level, tissue concentration, degree of absorbance, and type of nutrition.

Future Perspectives

The presented results allow approximation of biologically effective UV-exposure and evaluation of the amphipods' UV-tolerance under simulated radiation conditions. It would be very interesting to compare laboratory with *in-situ* experiments under natural sunlight. Biological weighting functions (BWFs) and weighted UV-spectra should be generated for both light regimes to allow predictions and assessment of possible effects of increasing UV-radiation. A combination of stressors should be applied to simulate closer to natural conditions and to account for climate change impact.

In addition, further studies should allow a prolonged period of recovery to test for lasting sub-lethal effects on growth, reproduction and survival. The consequences are sometimes delayed in these processes, however, they affect the amphipod on an individual as well as population level.

The proportion of photoenzymatic repair (PER) in UV-tolerance should be determined to evaluate the effectiveness of the amphipods' UV-protective and antioxidant capacity in more detail. It is important in this context to measure not only the repair, for example indirectly in exposure experiments with and without PER-stimulating radiation, but also the extent of induced DNA-damage in different tissues, and possibly the molecular mechanisms mediating this damage. In combination with above factors this may enable elucidation of the ultimate cause of UV-induced death.

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Table 8.1: Summary of study sites with location, sampling area, radiation climate, and investigated amphipod species in Antarctic, Arctic and at Helgoland. Type of nutrition: H = herbivorous, C = carnivorous/necrophagous/scavenging.

	Antarctic	**Arctic**	**Temperate**
Study site Position	Potter Cove, King George Island 62° 14' S, 58° 40' W	Kongsfjord, Spitsbergen 78° 55' N, 11° 56' E	Helgoland, North Sea 54° 05' N, 07° 53' E
Experimental period	Antarctic Expedition I: Oct-Jan 2000 Antarctic Expedition II: Oct-Dec 2002	Arctic Expedition July-August 2001	Helgoland Expedition July-August 2002
Sampling site Habitat Tidal range Sampling	Penon 1 broad rocky shore littoral gradual decline rich macroalgal communities 1.4 m with handnet <50 cm at low tide	Nansen Bay, Hansneset, London mainly rocky shore littoral, few soft bottom gradual to steep decline medium to dense macroalgal communities 2 m by divers 0-5 m and baited traps 2-5 m	west of North Beach broad rocky shore littoral gradual decline rich macroalgal communities 2.5 m with handnet <50 cm at low tide
Atmospheric light climate	UVB: 1.3-1.8 Wm^{-2} (Oct-Dec 00,02) UVA: 16.5-27.9 Wm^{-2} (Oct-Dec 00) PAR: 133-176 Wm^{-2} (Oct-Dec 00) [a]	UVB: 0.8-1.2 Wm^{-2} (July-Aug 01) UVA: 15-21 Wm^{-2} (July-Aug 01) PAR: 170-200 Wm^{-2} (July-Aug 01) [a]	UVB: 1.8-2.2 Wm^{-2} (July-Aug 02) UVA: 60-85 Wm^{-2} (July-Aug 02) PAR: 200-268 Wm^{-2} (July-Aug 02) [a]
Literature Comparison	UVB: 1.8 Wm^{-2} (Dec 97) [b] PAR: 163-380 Wm^{-2} (Oct-Dec) [b, c]	UVB: 1.1 Wm^{-2}, UVA: 19 Wm^{-2} (June 97) [d] PAR: 283 Wm^{-2} (June 97) [d]	UVB: 40 mW m^{-1} nm^{-1} (305 nm) [e] PAR: 389 Wm^{-2} (July 94-99) [e]
Underwater UVB-light climate Absorption (UVB) 1 % depth (UVB)	0.74 to 0.38 Wm^{-2} UVB (10cm to 1m, 11/03) K_d 0.54 m^{-1} (19.11.03) up to 58% in upper 10 cm approx. 12 m	0.56 to 0.11 Wm^{-2} UVB (10cm to 1 m) 13.07.01 K_d 0.79 m^{-1} (13.07.01) up to 70% in upper 10 cm in summer approx. 4 m	no data 3.3 and 2.1 m^{-1} (305, 320nm) [e] 1 % depth 1.1 and 1.5 m (305, 320 nm) in summer [e]
Sunshine hours	13 - 20 h (Oct-Dec)	10 - 24 h (July-Aug)	13 - 16 h (July-Aug)
Ozone minimum Ozone mean Ozone mean Ozone maximum Ozone depletion	126 DU (09/00), 159 DU (09/02) 198 - 312 DU (Oct - Dec 00) 329 - 324 DU (Oct - Dec 02) 339 DU (12/00), 434 DU (10/02) 50-61% spring time ozone depletion in 2000 and 2002 [f]	287 DU (July 2001) 426 DU (07.-15.03.2001) 320 DU (July 2001) 370 DU (July 2001) high interannual variability no ozone destruction in March 2001[g]	286 DU (July 2002) 326 DU (July 2002) 388 DU (July 2002) no trend, reduction variable
Investigated Amphipod species and type of nutrition	*Gondogeneia antarctica* (Chevreux, 1906) H *Djerboa furcipes* (Chevreux, 1906) H	*Gammarellus homari* (Fabricius, 1779) H *Anonyx nugax* (Phipps, 1774) C *Onisimus edwardsi* (Kroyer, 1846) C	*Chaetogammarus marinus* (Leach, 1815) H

Footnotes for Table 8.1

a) Underestimation of PAR-range at high intensities with fast scanning double monochromator spectroradiometer (Instrument Systems, Germany)
b) Hoyer et al. 2003
c) Gomez et al. 1998
d) Bischof et al. 1998
e) Dring et al. 2001
f) Compared to average of about 320 DU before 1980s (WMO 1998)
g) Compared to total ozone variability of 300-500 DU from 12-years time series (Rex & von der Gathen 2004). Source for ozone data: NASA TOMS data: http://toms.gsfc.nasa.gov/teacher/ozone_overhead.html.

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Bremerhaven, den 7. Juni 2006

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Erklärung gemäß § 5(1) Nr. 3 PromO

Ich erkläre hiermit,

1. dass ich mich vor dem jetzigen Promotionsverfahren keinem anderen Promotionsverfahren unterzogen habe

und

2. dass ich ausser dem jetzt laufenden Promotionsverfahren auch kein anderes beantragt habe.

Birgit Obermüller

Changes

The following changes have been made in agreement with the Prüfungsausschuss

- page III 2. Prüfer: *Prof. Dr. Ulrich Saint-Paul, Zentrum für Marine Tropenökologie, Fahrenheitstrasse 6, 28359 Bremen*

(original version from 07. June 2006: 2. Prüfer: *PD Dr. Thomas Brey, Alfred-Wegener-Institut für Polar- und Meeresforschung, Am Alten Hafen 26, 27568 Bremerhaven)*

- page 134 – 139 Header text: *Conclusions and Perspectives*

(original version from 07. June 2006: Header text: *Discussion*)

Veränderungen

Folgende Veränderungen wurden im Einvernehmen mit dem Prüfungsausschuss vorgenommen

- Seite III 2. Prüfer: *Prof. Dr. Ulrich Saint-Paul, Zentrum für Marine Tropenökologie, Fahrenheitstrasse 6, 28359 Bremen*

(ursprüngliche Version vom 07. Juni 2006: 2. Prüfer: *PD Dr. Thomas Brey, Alfred-Wegener-Institut für Polar- und Meeresforschung, Am Alten Hafen 26, 27568 Bremerhaven)*

- Seite 134 – 139 Kopfzeilentext: *Conclusions and Perspectives*

(ursprüngliche Version vom 07. Juni 2006: Kopfzeilentext: *Discussion*)