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UV-B induced mortality and antioxidant enzyme activities in *Daphnia magna* at different oxygen concentrations and temperatures

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Abstract. Survival of adult *Daphnia magna* was assessed after acute (<96 h) exposure to UV_{312} under various temperatures (6, 12 and 18°C) or oxygen concentrations (5.6, 8.5 and 14.1 mg O_2 l^{-1}) in the laboratory. The surviving animals were screened for the enzymes catalase (CAT) and glutathione transferase (GST), which may protect against UV-induced oxidative damage. In addition, the same two enzymes were assayed in separate experiments after acute exposure to UV_{312} (6 h, 0.014 mW cm^{-2}) and the different levels of temperature and oxygen. No differences were observed in either CAT or GST activity after exposure to the three levels of oxygen, but there was a tendency for decreasing specific activity with decreasing temperature for both enzymes. CAT activity was not influenced by UV radiation, whereas GST activity displayed a slight increase. Oxygen concentration did not influence survival during UV exposure but, contrary to expectations, survival tests at different temperatures clearly showed that reduced temperature increased survival. The results indicate that temperature effects must be considered when comparing dose effect relationships *in situ*, and suggest that low temperature is not a major cause of UV susceptibility in cold-adapted alpine and Arctic populations of *Daphnia*.

Introduction

The potential detrimental effects of current levels of short-wavelength solar radiation on cladocera, and thus the need for protective mechanisms, have been known for a long time (Brehm, 1938; Merker, 1940), but the various means of UV protection in zooplankton is scarcely known. Studies mainly on mammalian cells suggest that UV induces cell damage by direct absorption by DNA/proteins, and by formation of reactive oxygen species (ROS) which are detected in both plant and animal cells after UV irradiation (Black, 1987; Masaki *et al*., 1995; Hideg and Vass, 1996). Zooplankton may be exposed to ambient UV-induced ROS formed either in surface waters of lakes and ponds through UV absorption by dissolved organic carbon (Cooper *et al*., 1994), or inside the organism's body by interaction between sensitive molecules (e.g. flavins, reduced pyridine nucleotides) and photons. Interaction between excited sensitizers and triplet oxygen produces active oxygen intermediates, such as $^1O_2$, H_2O_2 or $O_2^-\bullet$, which in turn can lead to the production of an extremely reactive hydroxyl radical ($\bullet OH$). Biological damage caused by ROS includes oxidation of membrane fatty acids, resulting in lipid peroxidation, oxidation of proteins and DNA damage (Fuchs and Packer, 1991).

Well known protective mechanisms against UV radiation in *Daphnia* include vertical migration, the ability to repair damage by photoreactivation mechanisms, and the presence of protective pigments such as carotenoids and melanin (Siebeck, 1978; Hebert and Emery, 1990; Hessen and Sørensen, 1990; Hobæk and Wolf, 1991; Siebeck and Böhm, 1991; Hessen, 1994). Another possible mechanism of protection is the production of various antioxidants. For detoxification and

removal of ROS, every organism has evolved a complex defence system consisting of both low weight antioxidants (ascorbate, glutathione and tocopherol) and antioxidant enzymes (e.g. catalase and glutathione transferase). Catalase (CAT) catalyses the degradation of H_2O_2 into water and oxygen and therefore might offer protection against the deleterious effects of H_2O_2 which may be generated in the photooxidation reactions occurring during UV exposure. Glutathione transferase (GST) catalyses the conjugation of glutathione with various electrophilic substrates, and plays a role in preventing oxygen toxicity due to their activity towards organic hydroperoxides and hydroxyalkenals, products of oxidative stress (Ålin *et al*., 1985; Ketterer and Meyer, 1989). Both enzymes have been reported to be induced by H_2O_2 in various organisms (Morichetti *et al*., 1989; Rushmore *et al*., 1991; Wang and Schellhorn, 1995; Buchner *et al*., 1996) and are connected with increased resistance against UV radiation in bacteria and mammalian cells (Wang and Schellhorn, 1995; Bertling *et al*., 1996; Kerb *et al*., 1997). Except for studies in fish, very little is known about antioxidant protection in freshwater animals, and still less is known for zooplankton.

Temperature and oxygen concentration may both be major determinants of productivity and community structure in aquatic systems. Both parameters undergo long-term seasonal cycles in temperate lakes. In productive systems, oxygen concentration may also show pronounced diurnal fluctuations related to photosynthesis and respiration. *Daphnia* spp. inhabit a variety of freshwater habitats, ranging from deep lakes to highly UV-exposed localities such as coastal rock pools and alpine and Arctic ponds. These animals have to cope with changing and often extremely variable levels of UV radiation, temperature and oxygen. Both temperature and oxygen concentration would be expected to modify the effects of UV radiation of aquatic biota.

Studies on phototrophic organisms show that UV effects may be a balance between photochemical damage and biosynthetic repair. This balance would shift increasingly towards damage with decreasing temperature (Roos and Vincent, 1998). The most harmful effects of UV-B are not temperature-dependent, whereas both DNA repair and ROS detoxification, as well as excretory processes, are kinetic mechanisms which would be assumed to decrease in cold environments in poikilotherms (Hessen, 1996). Thus, if UV susceptibility increased with decreasing temperature, this could explain the apparently extensive need for photoprotection in alpine and Arctic *Daphnia* (Hebert and Emery, 1990; Hessen, 1996).

Ambient oxygen concentrations could influence UV-induced mortality in at least two ways. The first proposed mechanism of UV-induced mortality involves the interaction between a UV-induced excited chromophore inside the animal body and oxygen, resulting in internal ROS stress. If UV mortality is a process depending at least partially on oxygen, we would expect animals to be more sensitive to UV radiation at high oxygen concentrations. Many effects of UV radiation have been shown to be strongly dependent on oxygen (Graetzer, 1987; Andley and Clark, 1989). Secondly, the oxygen level itself may induce ROS production inside the animals (Jones, 1985) and thus, the protective mechanisms could be more easily overwhelmed. Both mechanisms are, however, dependent on a rise in internal oxygen tension during water oxygenation.

There is a general lack of data on antioxidants in zooplankton and therefore, this study aimed to provide some basic information. As oxidative stress should be reflected in antioxidant activity, we attempted, for the first time in *Daphnia*, to focus on antioxidant enzymes in relation to UV exposure. As a first step in this process, we assessed to what extent activities of CAT and GST in *D.magna* are influenced by UV radiation, temperature and oxygen concentration.

Method

Animals

The animals used in this study were a clone of *Daphnia magna* continuously maintained in our laboratory for several years. Animals were cultured in Elendt M7 medium (OECD, 1996) at 18°C and fed daily with the green alga, *Selenastrum capricornutum*.

Temperature experiment

The effects of temperature on UV-induced mortality and antioxidant enzymes were investigated in two experiments using two different temperature-regulated rooms. In both experiments, three temperatures were used (6, 12 and 18°C) and a 15 W Vilber-Lourmat lamp, with peak intensity at 312 nm (range 280–380 nm), was used as UV-B source. According to the manufacturer, no UV-C was emitted by the lamp and hence, we did not apply any UV-C cut-off filters. Also, a spectral (minor) tail in UV-C would eventually be screened off by the water surface film. In all experiments, blue-white light was provided in a 10:14 h light dark (LD) cycle to allow for photo-repair. In the first experiments (Exp. I), MBID400 400W metal halide lamps (GE Lighting) at integrated PAR intensities approaching daylight were used, while standard cool-white fluorescent tubes at far lower intensities (<50 µE m^{-2} s^{-1}) were used in the second experiments (Exp. II). The spectral distribution of the UV-B source and the visible light used in the two experiments are given in Figure 1. The survival tests were performed in six replicate 40 ml beakers (3 cm depth), each with five adult individuals, with a 10 h irradiation regime. Animals were exposed to two UV light intensities, 0.025 and 0.005 mW cm^{-2} (Exp. I) or 0.042 and 0.014 mW cm^{-2} (Exp. II), obtained by regulating the distance from the UV source. The doses were assessed using a Vilber-Lourmat VLX-3W radiometer, with peak sensitivity at 312 nm but integrating over the entire range emitted by the lamp. The area covered by the six beakers was sufficiently small to allow for homogenous light intensities for all beakers. Animals exposed to visible light only served as controls. In each of the two experiments, the animals were acclimated to the temperature for 72 h (Exp. I) or 48 h (Exp. II) before the first UV exposure. Food levels were kept low (<5 µg Chl *a* l^{-1}) during the experiment to minimize chlorophyll absorbance of UV-B. The number of live animals was counted twice per day and the experiments were terminated after 96 h (Exp. I) or 72 h (Exp. II), when surviving animals were frozen with liquid nitrogen for later examination of antioxidants.

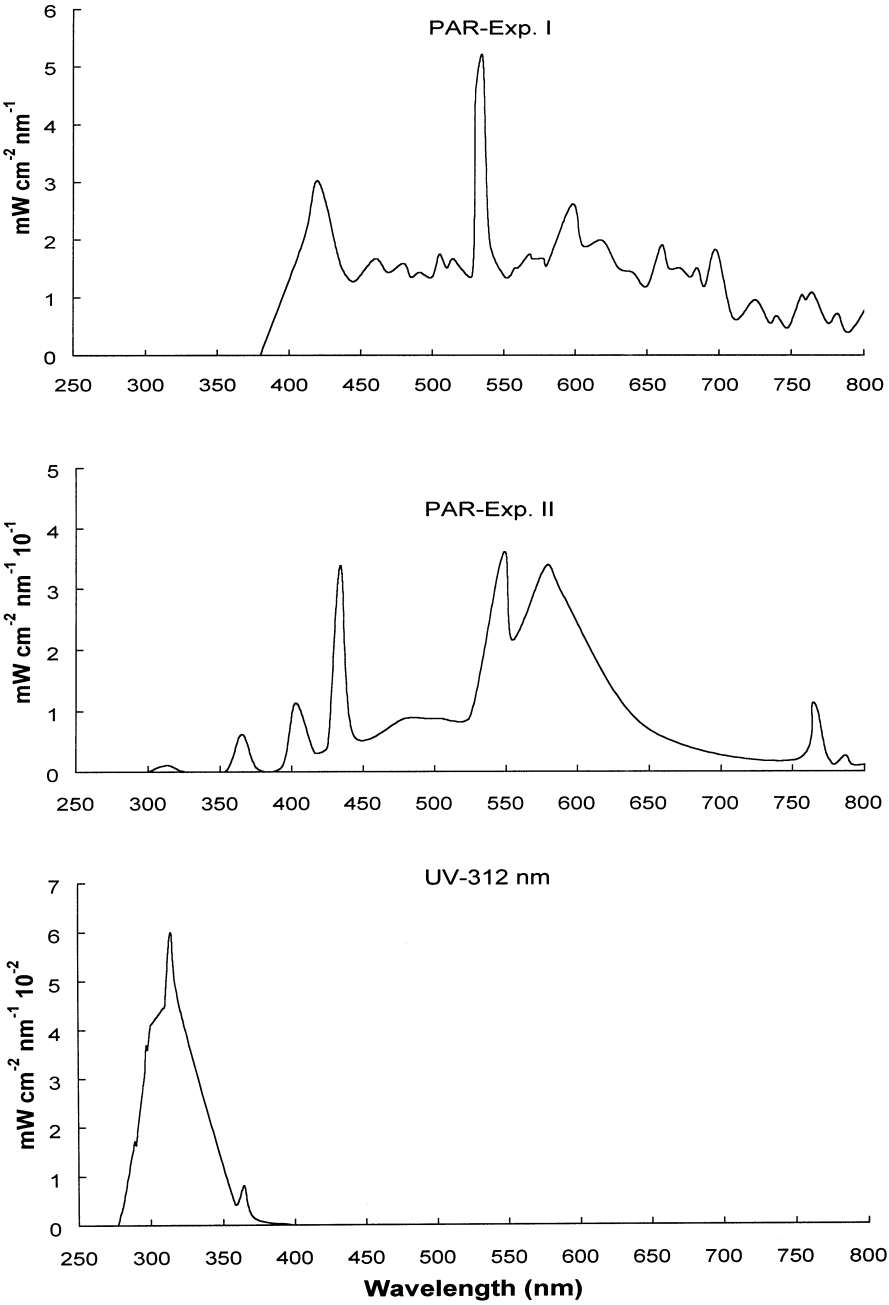

Fig. 1. Spectral distribution for the three light sources used in the experiments. **Upper**: MBID400 400W metal halide lamp. **Middle**: cool white fluorescent tubes. **Lower**: 15 W Vilber-Lourmat UV-B lamp.

Acute effects of single dose UV-B radiation on antioxidant enzymes

In order to assess the influence of a single dose of UV-B radiation on antioxidant enzymes, 240 adult animals were exposed for 6 h at an intensity of 0.014 mW cm^{-2}. A further 240 animals served as non-irradiated controls. The experiment was conducted at 18°C with continuous blue-white light as in Exp. II. The animals were exposed in 40 ml beakers (3 cm depth) containing five individuals as in the temperature experiment. Sub-samples of 80 animals (4 × 20) were obtained at 0, 6, 14 and 30 h after the onset of irradiation and frozen with liquid nitrogen for enzyme analysis.

Oxygen experiment

The oxygen experiments were performed at 18°C and with continuous cool-white light (~50 µE m^{-2} s^{-1}) to promote photorepair. The animals were exposed in an insulated flow-through apparatus consisting of one gas equilibration tank and two exposure chambers connected in series. The equilibrium tank was filled with M7-media and oxygen concentration was maintained by bubbling in gas mixtures of 10:90% O_2:N_2 to lower, or 40:60% O_2:N_2 to increase the oxygen level, respectively. Medium from the equilibration tank was transferred to the two exposure chambers at a rate of 1 ml min^{-1} and then back into the equilibration tank by means of peristaltic pumps. The exposure chambers consisted of either 150 ml quartz bottles (survival tests under UV radiation) or 250 ml glass bottles (antioxidant tests under varying O_2, without UV). Animals were exposed to three levels of oxygen (5.6, 8.5 and 14.1 mg O_2 l^{-1}) as measured in the exposure bottles using Winkler titration. The animals were fed immediately before, but not during, the exposure period. The survival tests were performed with 10 adult individuals and repeated 4–8 times. After an initial 16 h acclimation to the oxygen tension, the animals were exposed to 4 h day^{-1} of UV_{312} radiation at an intensity of 0.013 mW cm^{-2}, and mortality was recorded three times daily during the 96 h test period. The second exposure bottle (coupled in series) was shielded from the UV radiation and served as control. The animals in the control were thus not directly exposed to UV, but they received radiated water from the first, non-shielded exposure bottle. This meant that most of the short-lived free radicals would not be present in this second chamber, whereas more long-lived photoproducts, such as H_2O_2, CO_2, CO and free metals, would be. In order to assess the influence of oxygen concentration on levels of antioxidant enzymes, 15 adult individuals were exposed for 48 h using the same three oxygen levels but without UV radiation. For all three oxygen concentrations, the treatment was performed in duplicate and repeated 2–4 times.

Enzyme activities

Animals were immediately frozen with liquid nitrogen at the end of experiments and kept in a freezer (–80°C) for enzyme assays. Using a glass–Teflon Potter-Elvehjem homogenizer, 10–20 animals were homogenized in 900 µl ice-cold

50 mM potassium phosphate buffer, pH 7.7, containing 1 mM EDTA and 0.1% Triton X-100. Supernatant fluids (1000 *g* for 20 min at 4°C) of homogenates were used directly as enzyme sources. CAT activity was basically determined as described by Claiborne (Claiborne, 1985) using 20 mM H_2O_2 as substrate. One unit (U) is defined as µmol H_2O_2 decomposed min^{-1}, at pH 7.0 and 30°C. GST activity towards 1-chloro-2,4-dinitrobenzene (CDNB) was measured as described by Habig *et al*. (Habig *et al*., 1974), at pH 6.5, in a thermostatically-controlled (30°C) Beckman DU 62-spectrophotometer. One unit is defined as nmol CDNB conjugated min^{-1}. Protein concentrations were determined according to Bradford (Bradford, 1976) using dye reagent from Bio-Rad and with bovine serum albumin as standard.

Statistics

Differences in survival were analysed using the log-rank test. Enzyme activity data were analysed using one-way ANOVA followed by the Tukey–Kramer multiple comparison test.

Results

Temperature experiment

Exp. I clearly demonstrated the effect of temperature on UV-induced mortality (Figure 2). At 18°C, death began to occur within 48 h and all individuals were dead after 96 h. No deaths were observed in the control group. The response to the two applied intensities (0.005 and 0.025 mW cm^{-2}) was not significantly different as judged by the log-rank test at the highest temperature. On the other hand, at 12°C, the percentage survival after 96 h was 87, 57 and 3% at intensities of 0, 0.005 and 0.025 mW cm^{-2}, respectively. The survival tests conducted at 12°C thus yielded highly significant (log-rank test, $P < 0.01$) differences with respect to UV intensity, whereas at 6°C, none of the survival curves were significantly different from the control (no UV). The percentage survival after 96 h was 80, 73, and 57% at 0, 0.005 and 0.025 mW cm^{-2}, respectively. In order to assess the effect of temperature on expression of antioxidant enzymes during UV exposure, surviving animals were assayed for catalase and glutathione transferase. Without UV exposure, both enzymes showed a weak tendency for decreasing specific activity with decreasing temperature (Figures 3 and 4), but the differences were not significant. There were insufficient numbers of survivors to test for all groups at 18 and 12°C but at 6°C, CAT activity was not significantly changed by UV exposure for 96 h. GST activity showed a trend for increasing specific activity with increasing UV intensity at 6°C, but differences were not significant. Two problems arose during the performance of Exp. I. First, the temperature in the climate room varied diurnally (owing to heat emitted from the lamps) within the following ranges: 18 ± 3, 12 ± 2 and 6 ± 4°C. In addition, the survival tests indicated that the high intensity of the PAR light itself was having a negative impact on the animals, with the greatest effect at the lower temperatures (controls, Figure 2). In order to verify this, animals (5 × 3) were exposed at 12°C either to the full

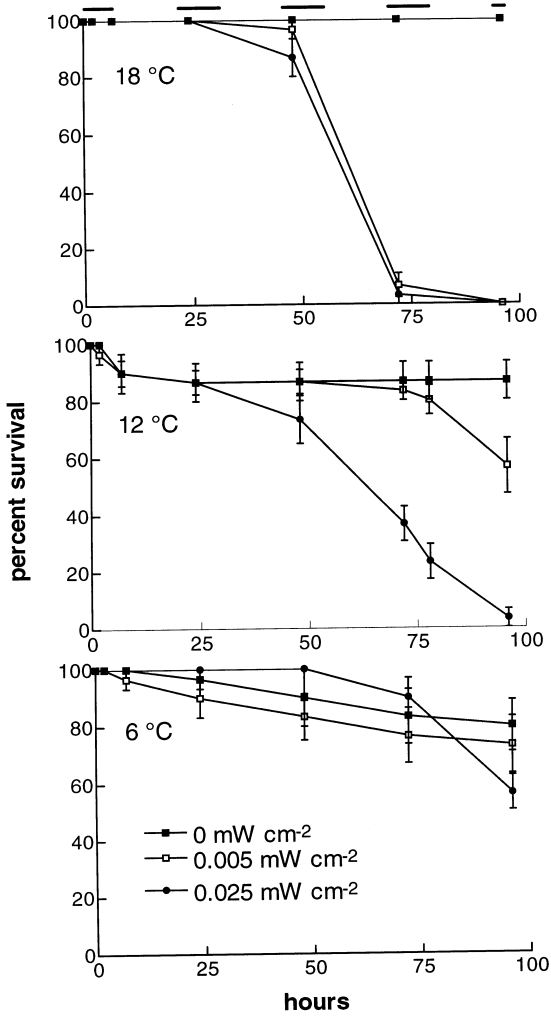

Fig. 2. Survival of *Daphnia magna* exposed to two levels of UV_{312} at 6, 12 and 18°C (Exp. I). Horizontal lines indicate UV exposure times (10 h day^{-1}). Average and SEM of six replicates.

PAR condition, or to a reduced light regime achieved by covering the beakers with a semi-transparent box. The animals given the full light treatment started to succumb from day 2 onwards and after 4 days, 13% of the animals were dead, whereas all animals receiving the reduced light treatment were alive at the end of the experiment. Therefore, a second series of experiments (Exp. II) was conducted in another climate room with stable temperature and a reduced level of PAR radiation (Figure 1). In Exp. II, no deaths were observed in the controls for 72 h, and the results confirmed the conclusions from Exp. I that reduced temperature increased survival. At the highest UV doses (0.042 mW cm^{-2}), death

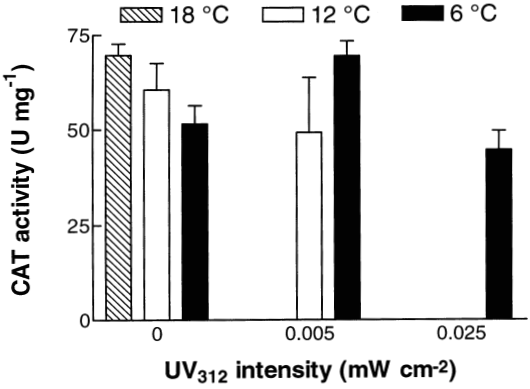

Fig. 3. Levels of catalase (U mg^{-1} protein) in surviving *Daphnia magna* after exposure at 6, 12 and 18°C to three levels of UV radiation for 96 h. Values are means ± SEM (n = 3).

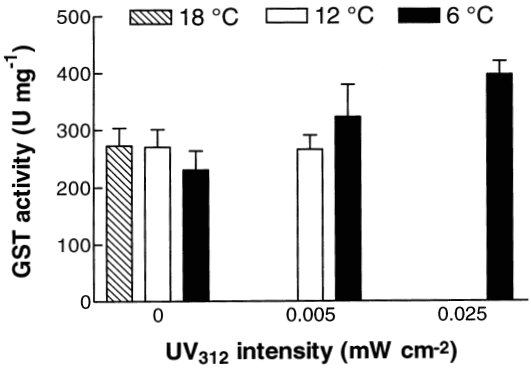

Fig. 4. Levels of glutathione transferase (U mg^{-1} protein) in surviving *Daphnia magna* after exposure at 6, 12 and 18°C to three levels of UV radiation for 96 h. Values are means ± SEM (n = 3).

began to occur within 32 h at 18°C whereas at 6°C, death was not observed until 54 h after the first irradiation (Figure 5). Percentage survival after 48 h was 17, 73 and 100 at 18, 12 and 6°C, respectively. At the lower dose (0.014 mW cm^{-2}), no deaths had occurred at 6°C after 72 h, whereas all animals were dead at 18°C. At both intensities, the effect of temperature was significant as judged by the log-rank test ($P \leq 0.03$). As in Exp. I, the results indicated a tendency towards decreasing specific activity with decreasing temperature for both enzymes (Figure 6). Moreover, specific activity of both enzymes was significantly ($P < 0.01$) decreased, in comparison with the 18°C control, at 120 h (acclimation plus exposure) of incubation at 6°C. Only animals incubated at 6°C survived in sufficient quantities to perform enzyme assays. GST activity was significantly ($P < 0.05$)

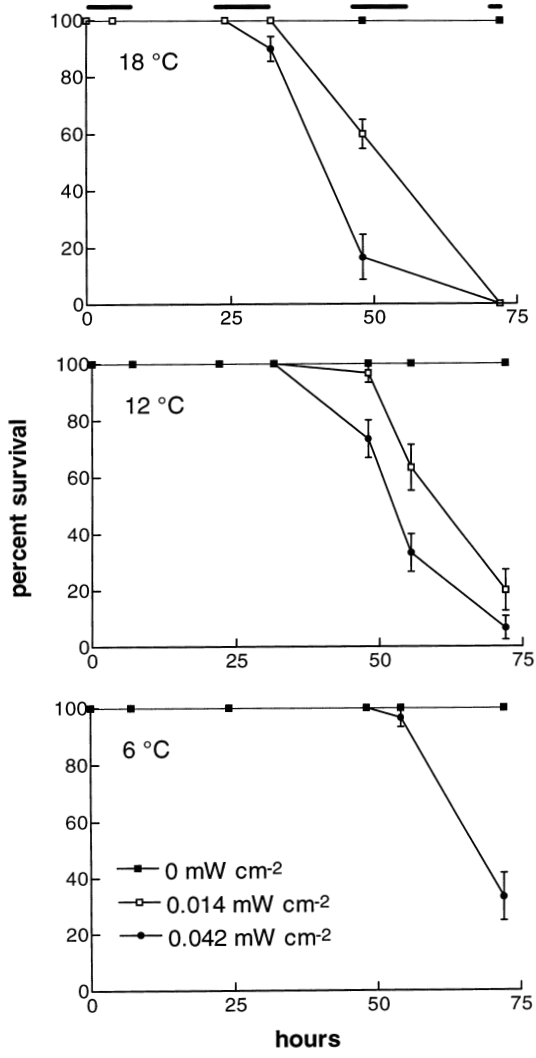

Fig. 5. Survival of *Daphnia magna* exposed to two levels of UV_{312} at 6, 12 and 18°C (Exp. II). Horizontal lines indicate UV exposure times (10 h day^{-1}). Average and SEM of six replicates.

increased in surviving animals exposed to UV intensity of 0.014 mW cm^{-2} compared with non-irradiated controls, whereas no significant differences were seen in CAT activity (Figure 7).

Acute effects of single dose UV-B radiation on antioxidant enzymes

Acute UV exposure at an intensity of 0.014 mW cm^{-2} for 6 h produced no changes in specific activity of CAT at any time following the treatment (Figure 8). GST

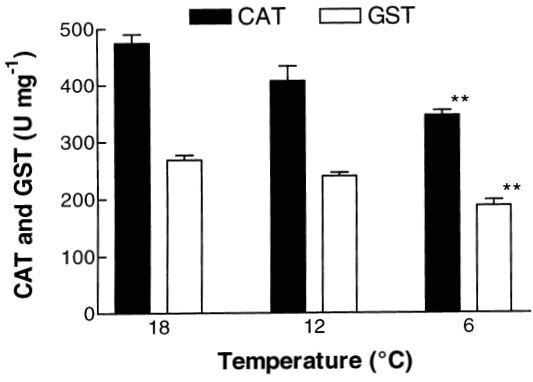

Fig. 6. Specific activity (U mg^{-1} protein) of catalase (CAT) and glutathione transferase (GST) from *Daphnia magna* exposed to 6, 12 and 18°C for 120 h. Values are means ± SEM (n = 3). **$P \leq 0.01$ significant difference to animals exposed to 18°C.

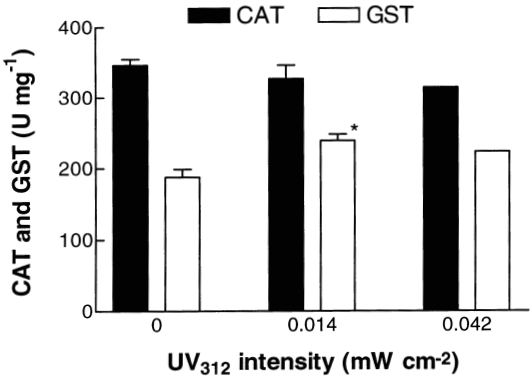

Fig. 7. Levels of catalase and glutathione transferase (U mg^{-1} protein) in surviving *Daphnia magna* after exposure at 6°C to three levels of UV radiation for 72 h. Average ± SEM of one to three replicates. *$P \leq 0.05$ significant difference to non-irradiated animals.

activity was significantly ($P < 0.01$) elevated 24 h post-irradiation compared with the control group. Additionally, there was an unexplained reduction of GST in both the exposed group and the control immediately after exposure, perhaps because of manipulation of the animals.

Oxygen experiment

The survival tests using different oxygen concentrations in water produced an identical pattern for all three concentrations. Using 4 h UV exposure per day, death began to occur from 44 h onwards and after 88 h, all individuals from all concentrations were dead (Figure 9). No death was observed in controls, i.e., in individuals receiving irradiated water without being directly exposed to UV. This

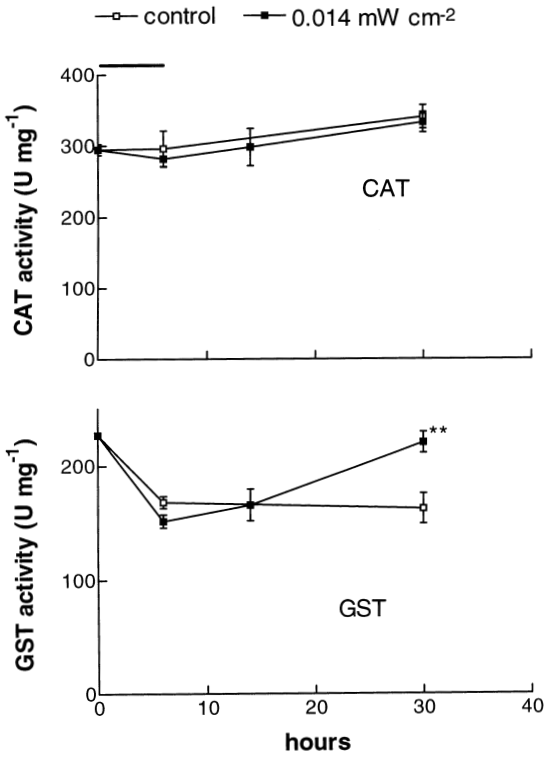

Fig. 8. Specific activity (U mg^{-1} protein) of catalase (CAT) and glutathione transferase (GST) from *Daphnia magna* after exposure to 0.014 mW cm^{-2} UV_{312} at 18°C for 6 h (horizontal line). Values are means ± SEM (n = 4). $^{**}P \leq 0.01$ significant difference to control.

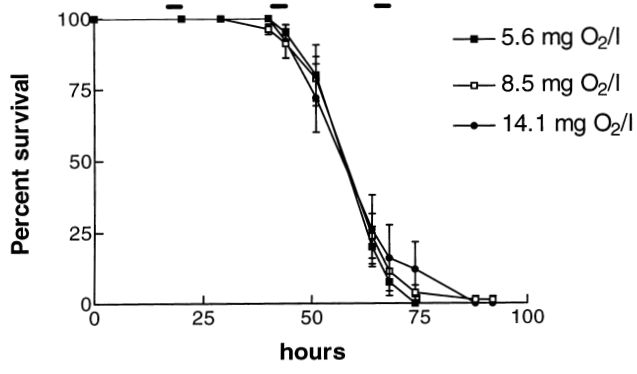

Fig. 9. Percentage survival of *Daphnia magna* exposed to three periods of 4 h (marked as horizontal lines) of UV_{312} at an intensity of 0.013 mW cm^{-2} and three levels of oxygen concentration in water. Average and SEM of four to eight replicates.

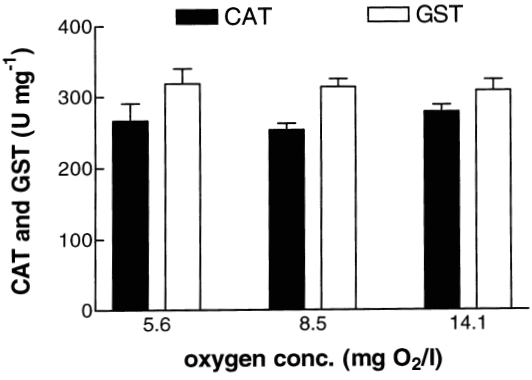

Fig. 10. Specific activity (U mg^{-1} protein) of catalase (CAT) and glutathione transferase (GST) from *Daphnia magna* exposed to three levels of oxygen concentration for 48 h. Values are means ± SEM (n = 4–8).

indicates non-lethality of the oxygen concentrations used and no indirect effect of UV-irradiated circulatory water. It should be remembered, however, that most free radicals and other photoproducts are extremely short-lived and would probably be largely eliminated before entering the control chamber. No differences were observed in CAT or GST activity after exposure for 48 h to the three levels of oxygen (Figure 10).

Discussion

To our knowledge, there is no information on the effects of temperature on UV-induced mortality in Cladocera. The present study indicates that low temperatures reduce sensitivity to acute exposure to UV-B radiation. Hairston found similar effects when studying copepods exposed to blue light (Hairston, 1979). This was somewhat surprising as the *a priori* assumption would be that direct UV-induced lesions would be temperature-independent, whereas the repair mechanisms should slow down at low temperatures. However, interactions involving temperature are complex because they affect the general metabolism of poikilotherms. Generally, reduced temperature leads to increased life span, generation time and body size, and decreased oxygen consumption, metabolic activity, heart rate, respiration rate, filtering rate and growth rate (MacArthur and Baillie, 1929; Goss and Bunting, 1980; Korpelainen, 1986; McKee, 1995; Paul *et al*., 1997). Most studies have shown that sensitivity of aquatic invertebrates to various toxic compounds increases with temperature (Persoone *et al*., 1989; Møller *et al*., 1994). Moreover, in aquatic organisms, toxic materials may be more toxic at higher temperatures when exposure time is short but with increased exposure time, the temperature effect on toxicity is gradually reduced (Schaefer and Pipes, 1973; Sprague, 1985). However, unlike UV radiation, temperature affects toxicity to chemicals because animals exposed at low temperature will take up less toxicant than those exposed at high temperature.

Low temperature may, however, slow down UV-induced mortality in several ways. First, even though the initial UV damage is temperature-independent, UV-induced mortality probably proceeds through a complex series of individual reactions that may be temperature-dependent. Secondly, not only are repair and detoxification processes slowed down at low temperatures, but also activation processes such as ROS metabolism and lipid peroxidation. Thirdly, low temperature may change the physiological state of the animal. It is possible that animals are able to acclimate to low temperatures by increasing their metabolic efficiency in order to achieve homeostatic control over enzyme reactions. The animals may respond by increased efficiency of damage repair or increased production of antioxidants.

Both temperature experiments showed a decrease in specific activities of GST and CAT with decreasing temperatures. This may indicate that the animals experience reduced ROS stress at lower temperatures and therefore respond by reducing synthesis of the enzymes. Lower temperature leads to reduced oxygen consumption and will entail reduced rates of normal ROS production, thus reducing the risk of oxidative damage. Moreover, the reduced activities indicate that these enzymes are not involved in the higher UV tolerance observed at low temperatures. However, the interpretation is not straightforward because all the activities were assayed at 30°C and not at the incubation temperature. Therefore, the standard activities given here represent the amount of catalytically-active enzyme per unit protein. However, the real activities (the true rates of antioxidant enzymes under specific temperatures) depend on the body temperature and generally decrease with decreasing temperature. Accordingly, measurements at 30°C will overestimate the real activity in animals experiencing low temperatures, although mammalian catalases are relatively temperature-insensitive (Aebi, 1984). The animals were apparently not acclimating to the colder environment by producing more CAT and GST, but we cannot rule out the possibility that during thermal acclimation, other forms of the enzymes were produced. Temperature-insensitive forms of CAT were observed in poikilotherm animals living in, or adapted to a cold environment (Gil and Barja de Quiroga, 1988; Regoli *et al*., 1997). The present study was performed by lowering the temperature for a warm-adapted *Daphnia* species (*D.magna*), and different results may have been experienced by increasing the temperature in cold-adapted species (Arctic *D.pulex* or alpine *D.longispina*).

The effects of UV on antioxidant enzymes have been almost exclusively investigated in bacteria, plants and mammalian skin. Acute exposure to UV leads to reduction in several antioxidants, including CAT activity in mammalian cells immediately after irradiation (Fuchs *et al*., 1989; Shindo *et al*., 1994). Several studies have indicated that CAT is destroyed both through direct absorbance of light and by the ROS created as a result of the UV radiation (Shindo *et al*., 1994; Shindo and Hashimoto, 1995; Zigman *et al*., 1996). Few studies have investigated the effect of UV radiation on GST, but this enzyme system is linked with protection against UV radiation-induced cutaneous damage in humans (Kerb *et al*., 1997). Punnonen *et al*. found a weak but insignificant increase in GST after chronic exposure to UV-B in human epidermis (Punnonen *et al*., 1995), whereas Nakano

et al. observed a decrease in GST mRNA levels by UV irradiation in rat keratinocytes (Nakano *et al*., 1997). In the present study, we measured CAT and GST in daphnids repeatedly exposed to UV for 96 h (Exp. I) or 72 h (Exp. II). We found no reduction in CAT activity, as observed in mammalian cells, after acute exposure, indicating that CAT is not destroyed by UV radiation in daphnids. Moreover, the results did not indicate that CAT was protecting the surviving animals against the effects of radiation. However, the results must be interpreted with caution because the doses were high and only the surviving animals were assayed. The results also indicated a dose-dependent increase in GST activity. Exposure to a single sub-lethal dose produced the same type of response: no effect on CAT activity but an increase in GST activity (Figure 8). It could be hypothesized that the need for detoxification of the toxic products of peroxidation processes induces GST, but the results are too preliminary to be conclusive.

Very little information is currently available concerning ROS and environmental oxygen concentration in aquatic invertebrates. Most originates from marine studies of anoxia-tolerant polychaeta, bivalves and gastropods (Abele-Oeschger and Oeschger, 1995; Abele *et al*., 1998; Pannunzio and Storey, 1998). Broadly speaking, because the rate of ROS production in animals is proportional to the rate of oxygen consumption (Barthelemy *et al*., 1981), an increase in ROS production and activity of antioxidant enzymes could be expected during hyperoxia, and the opposite situation during hypoxia. This is confirmed in several studies on vertebrate species, including fish (Radi *et al*., 1988) and amphibian tadpoles (Gil *et al*., 1987).

In the present study, we exposed the animals to dissolved oxygen concentrations ranging from moderate hypoxia (5.6 mg O_2 l^{-1}, 59% saturation) to moderate hyperoxia (14.1 mg O_2 l^{-1}, 149% saturation). These concentrations are not extreme. Jones, for example, measured a diurnal variation between 0.6 and 24 mg O_2 l^{-1} in a small pond (Jones, 1961). However, oxygen concentration did not have any effect on UV-induced mortality in our study. The explanation for this may be that *Daphnia* species are able to regulate their oxygen consumption and thus, prevent changes in their internal oxygen concentration. *Daphnia magna* shows a high degree of respiratory independence at declining oxygen concentrations and is able to maintain the rate of oxygen consumption, independent of the ambient oxygen concentration, down to a critical concentration of about 1 mg l^{-1} (Kobayashi and Hoshi, 1984). Unlike many other water breathers, oxyregulation in *D.magna* at hypoxia is not controlled by changes in ventilation but rather, by an increase in heart rate (Paul *et al*., 1997) and by producing multiform haemoglobin, thus improving their ability to extract oxygen molecules from the water (Kobayashi *et al*., 1988; Kobayashi and Tanaka, 1991). In contrast to hypoxia, few studies have examined the physiological effects of hyperoxia on *Daphnia*. However, most studies on other crustaceans show that oxygen consumption is independent of oxygenation at far higher concentrations than those used in the present study (Sinha and Dejours, 1980; Morris and Taylor, 1985). Moreover, Paul *et al*. found a decrease in heart rate during hyperoxia in *D.magna* (Paul *et al*., 1997). Apparently, the oxygen concentrations used in the present study were unable to raise the internal concentration of oxygen in the animals to a level

where increased UV-induced mortality could be observed. This was supported by the fact that CAT and GST activities were not affected by the concentrations used. Oxygen concentration could, however, exert a potential effect using a wider oxygen range than that used in this study.

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