Photochemistry and Photobiology, 2010, 86: 367–373

Effect of Ultraviolet Radiation on Acetylcholinesterase Activity in Freshwater Copepods

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Received 6 August 2009, accepted 20 October 2009, DOI: 10.1111/j.1751-1097.2009.00675.x

ABSTRACT

We analyzed the effects of UV radiation (UVR) effects on acetylcholinesterase (AChE) activity in two calanoid copepods, *Boeckella gibbosa* and *Parabroteas sarsi* that inhabit Patagonian shallow lakes. We studied the effect of experimental UVR (UV-B and UV-A) exposure on AChE activity in relation to basal antioxidant capacities of both copepods. Our experiments showed that UVR can effectively depress AChE activity, although with differences between species. In both copepods AChE was affected by UV-B, whereas UV-A only affected AChE in *B. gibbosa*. Both copepods also differed in body elemental composition (C:N:P), photoprotecting compound content (carotenoids and mycosporine-like amino acids) and enzymatic antioxidant capacity (glutathione *S*-transferase [GST]). Our results suggest that when exposed to UVR, AChE activity would depend more on the antioxidant capacity (GST) and P availability for enzyme synthesis than on the photoprotective compounds.

INTRODUCTION

In freshwater systems, UV radiation (UVR) is recognized as an important biological stressor (1). UVR can penetrate within the euphotic zone, affecting aquatic organisms' physiology to different extent (2,3), and in particular it is recognized that shorter wavelengths (UV-B, 280–315 nm) are more deleterious than longer ones (UV-A, 320–400 nm) (4). In aquatic organisms, harmful effects of UVR result from the direct absorption of specific wavelengths by macromolecules (DNA, protein and chlorophyll), and from the interaction of UVR with water, which forms a number of chemically reactive species and biologically toxic intermediates (5,6).

Planktonic organisms have developed a variety of photoprotection strategies, including behavioral avoidance of photodamage (7), production or incorporation of UV-absorbing compounds such as carotenoids, melanin, and mycosporine-like amino acids (MAAs) (8), enzymatic DNA repair (5) and in antioxidant defenses (9,10). Among antioxidants, glutathione *S*-transferase (GST) is a detoxifying enzyme involved in the removal of reactive organic hydroperoxides, such as the products of lipid peroxidation under oxidative stress (11). Antioxidant mechanisms are energetically demanding and may require additional elemental supplies, such as phosphorus (P) for antioxidant enzymes (12). Thus, the relative importance and efficiency of UVR defenses would depend on the availability of dietary factors, such as P and N.

Among the deleterious effect on proteins, UVR oxidant injuries could affect specific enzymatic endpoints such as acetylcholinesterase (AChE). AChE catalyzes the hydrolysis of acetylcholine (ACh), the primary neurotransmitter in sensory and neuromuscular systems in most species (13). The interaction between ACh and AChE is vital for normal behavior and muscular function. It represents a prime target on which some external agents, such as pesticides, can exert detrimental effects, and was used to monitor environmental health (13,14). In particular, AChE inhibition is an indicator of exposure to organophosphorus and carbamate pesticides, toxins, and toxic metals such as cadmium, lead and copper (15) and is considered as an early warning signal of ecotoxicological risk in marine zooplankton (16). However, natural factors such as UVR probably influence this enzyme activity, but so far the only available data come from an *in vitro* study which indicates that AChE was denatured by incident UVR with loss of enzyme activity (17). Based on this early evidence we can also postulate a direct effect of UVR on the enzyme activity in planktonic organisms.

Copepods are dominant members of planktonic communities and constitute an important link within food webs. In particular, calanoid centropagid copepods dominate aquatic environments of the Andean Patagonia region. Patagonia is affected by the Antarctic ozone depletion, exposing organisms to increasing UVR (18). Endemic species of Patagonian centropagids seem to be highly photoprotected (19,20), with high contents of carotenoids and MAAs as photoprotective compounds (21). The two studied species, *Boeckella gibbosa* Löffler and *Parabroteas sarsi* Daday, are inhabitants of Patagonian lakes. The difference in MAA content between these two copepods (19) lets us postulate that they would also have different tolerance to UVR. Defense mechanisms are not always fully successful in counteracting the UVR-induced oxidative stress, and when defense mechanisms fail, UVR may affect specific enzymatic endpoints such as AChE.

The aim of this study was to investigate the effects of UVR on AChE activity in *B. gibbosa* and *P. sarsi*. Photoprotective pigments reduce UVR damage in copepods (8,22), and elemental body composition affects antioxidant responses (12). Thus, we hypothesize that elemental body condition (C:N:P), photoprotecting compound content (carotenoids

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and MAAs) and enzymatic antioxidant capacity (GST) will interact to determine differential UVR damage to AChE activity. For this purpose we performed laboratory UVR exposure experiments, analyzing the AChE activity in relation to basal antioxidant and photoprotective capacities of both copepod species.

MATERIALS AND METHODS

The copepods. We carried out laboratory experiments with *P. sarsi* and *B. gibbosa*. *P. sarsi* was collected from Laguna Fantasma (41°05′S, 71°27′W, 780 m above sea level and 2.5 maximum depth, dissolved organic carbon [DOC] ~3 mg L^{-1}), and *B. gibbosa* from Laguna Los Cántaros (41°00′S, 71°49′W, 800 m above sea level and 12 m maximum depth, DOC ~0.7 mg L^{-1}). In Lake Los Cántaros, *B. gibbosa* remains at 8 m whereas *P. sarsi* in Laguna Fantasma, remains at 0.5–1 m depth. Due to different dissolved organic matter (DOM) content, both species are exposed to similar UVR level. In other natural habitats, neither *B. gibbosa* (Lake Tonček) nor *P. sarsi* (Lake Rivadavia) perform diel vertical migrations (23,24). Zooplankton samples were collected with a plankton net (200 μm mesh) by making horizontal tows in the pelagic central area of each lake. In the laboratory, we selected adults (males and females) of each species under a stereomicroscope. At least 30 individuals were measured, and another 100 individuals were immediately frozen (−20°C) for biochemical measurements: elemental composition (C:P), pigments (carotenoids and MAAs) and enzymatic activity (GST). We selected GST as antioxidant enzyme over catalase (CAT) because the latter has been found with negligible activity in calanoid copepods (10), and more recently it was observed that CAT mRNA levels were very low in response to oxidative stress in the calanoid *Calanus finmarchicus* (25).

Experimental design. Zooplankton was carried to the laboratory and maintained for 2 or 14 h in 2 L aquaria filled with natural lake water inside an incubator at 15°C and a 12:12 h light:dark cycle. For all the experiments we selected adults (males and females in 1:1 sex ratio) of each species under a stereomicroscope. Noninjured individuals of the same size were rinsed with filtered lake water (0.2 μm) and transferred to 20 mL quartz tubes with 0.2 μm filtered lake water (polycarbonate Osmonics™). Water was collected at 0.5 m depth from Laguna Fantasma and 4 m depth from Los Cántaros, immediately filtered and analyzed, and used for the experiments within the next 14 h (Los Cántaros) or 2 h (Laguna Fantasma). To prevent osmotic changes experiments were conducted in water of the corresponding lake. DOM concentration differed between the two lakes; therefore, we carried out spectrophotometric scans (250–790 nm) of 0.2 μm filtered lake water in 10 cm quartz cuvettes using a Shimadzu UV2450 double-beam spectrophotometer for estimating differences in UVR shielding due to DOM.

We carried out four experiments: Experiments 1 and 2 consisted of a single exposure to UV-B and UV-A, respectively, while Experiments 3 and 4 investigated the cumulative effect of UVR in both wavelength bands. In each experiment, at the initial time (T_0) and at the end of each incubation period, three groups of individuals (three replicates), were frozen (−20°C) until enzymatic activity measurements were made.

UV-B effects on AChE activity (Experiment 1): Thirty *B. gibbosa* or 10 *P. sarsi*, in three replicates each, were exposed in quartz tubes to UV-B for 90 min in an incubator at 15°C. The difference in copepod numbers is due to differences in size between species (Table 1). The UVR exposure time was adjusted in preliminary experiments that ensure detection of the sublethal UV-B effect. Another set of tubes were run under the same conditions but wrapped with aluminum foil to protect them from the UVR source (Dark Control, DC). Experiments were carried out inside an incubator at 15 ± 1°C with photosynthetically active radiation (PAR) intensity of 92 μmol m^{-2} s^{-1} (daylight fluorescent tubes). UV-B was provided by two UV-B 313 fluorescent tubes (Q-Panel Lab Products, Cleveland, OH) with maximum emission at 313 nm placed 30 cm from the tubes. The lamps were wrapped with acetate film to prevent any output less than 290 nm. During the incubation, animals received 510 J m^{-2} nm^{-1} of UV-B-305 nm band (measured with a PUV 500B Biospherical Instrument). This dose corresponds to 9.4 μW cm^{-2} nm^{-1} of the 305 nm band, a slightly higher irradiance than that at lake surface in North Patagonia (7 μW cm^{-2} nm^{-1}, clear summer mid-day, data from an intercalibrated GUV at Centro de Salmonicultura, Universidad Nacional del Comahue [26]).

Table 1. Features of the two studied adult copepods.

	Boeckella gibbosa	*Parabroteas sarsi*
Body length, mm	1.0 ± 0.03	4.8 ± 0.4
C:P (atomic)	257 ± 16	184 ± 5
C:N (atomic)	13 ± 1.4	4.74 ± 0.20
Total carotenoids, μg mg^{-1} DW	2.45 ± 0.02	0.56 ± 0.04
MAAs*, μg g^{-1} DW	8.6 ± 3.2	1.73 ± 0.50
GST, μmol mg^{-1} protein min^{-1}	1071 ± 82.1	3381 ± 296

*From Tartarotti *et al.* (19).

UV-A effects on AChE activity (Experiment 2): The same number of copepods was exposed to UV-A for 240 min, all other conditions remaining the same (temperature and PAR light). This exposure time was determined in preliminary experiments that ensure detection of the sublethal UV-A effect. We used two UV-A-340 fluorescent tubes (Q-Panel Lab Products) with maximum emission at 340 nm, placed 30 cm from the tubes. The UV spectrum of these light tubes closely resembles the solar spectrum between 290 and 350 nm (27,28). In this experiment, the animals received not only UV-A but also UV-B (27,28) but for simplicity we refer to the maximum emission of the lamp (340 nm) as UV-A. In this case animals received 35 μW cm^{-2} nm^{-1} of UV-A-340 nm band, and this irradiance level is equivalent to ground level noon summer sunlight in the area. The total dose in 4 h was 5000 J m^{-2} nm^{-1} of 340 nm band.

Cumulative effect of UV-B (Experiment 3): To analyze the AChE activity with increasing UV-B dose, the same number of copepods as in Experiment 1 was placed in 12 quartz tubes for different time exposures (30, 60, 120 and 240 min), which resulted in cumulative doses of 170, 340, 675 and 1350 J m^{-2} nm^{-1} of UV-B-305 nm band (UV-B irradiance same as in Experiment 1). Another set of three tubes were run under the same conditions during all the exposure (240 min) but wrapped with aluminum foil to protect them from the UVR source (DC). At each exposure time, three tubes (replicates) were removed for enzymatic activity measurements. All other experimental conditions were the same as in Experiment 1.

Cumulative effects of UV-A (Experiment 4): A similar assay design was used to evaluate the cumulative effect of UV-A. The same number of copepods as in Experiment 1 was placed in 12 quartz tubes for different time exposures (120, 240, 360 and 480 min), which resulted in cumulative doses of 2500, 5000, 7500 and 10 000 J m^{-2} nm^{-1} of UV-A-340 nm band (UV-A irradiance same as in Experiment 2). Another set of three tubes were run under the same conditions during all the exposure (480 min) but wrapped with aluminum foil to protect them from the UVR source (DC). At each exposure time, three tubes (replicates) were removed for enzymatic activity determinations. All other experimental conditions were the same as in Experiment 2.

Oxidative stress due to UVR (Experiment 5): To estimate the increase in oxidative stress, we performed an experiment to determine the reduced glutathione (GSH) levels during UVR exposition in *P. sarsi*. We performed this analysis only in *P. sarsi*, because the size of this species (Table 1) allowed us to have reliable readings. Fifteen copepods were placed in 12 quartz tubes for different time expositions (30, 60, 120 and 240 min), which resulted in cumulative doses of 170, 340, 675 and 1350 J m^{-2} nm^{-1} of UV-B-305 nm band (UV-B irradiances equal as in Experiment 3). Another set of three tubes were run under the same conditions during the whole exposure (240 min) but were wrapped with aluminum foil to protect them from the UVR source (DC). At each exposure time, three tubes (replicates) were removed for GSH determination. All other experimental conditions were the same as in Experiment 1.

Biochemical determinations. C:P and C:N ratios: Freshly collected specimens of each species were rinsed twice with 0.2 μm filtered lake water, and frozen immediately at −20°C until carbon and phosphorus measurements. In the laboratory, 30 adult *B. gibbosa* or 10 *P. sarsi* were placed on precombusted (450°C, 1.5 h) GF/F filters. Filters were

dried at 60°C for 48 h and stored at −20°C until analysis. All determinations were carried out on at least three replicates. Carbon and nitrogen concentration were determined on a Thermo Finnigan EA1112 elemental analyzer. Phosphorus was determined with persulfate digestion followed by molybdate reaction (29).

Pigment determinations: Adult copepods of approximately the same size were used. Egg-bearing females were excluded from pigment analysis. The determinations were carried out according to Hansson *et al*. (22). Ten individuals (*P. sarsi*) and 30 individuals (*B. gibbosa*) in three replicates each were used for carotenoid and MAA analyses. Briefly, samples for carotenoid analysis were placed in glass scintillation vials (6 mL) with 1 mL ethanol (95%) and were sonicated (20 kHz, 30 s, on ice) followed by extraction at room temperature for 12 h in darkness. The samples were centrifuged for 15 min at 15 700 ***g***. Quantification of the extracted red pigment in the supernatant was performed with a spectrophotometer (double-beam Shimadzu UV2450) at 474 nm, which is the absorption peak for common carotenoids in copepods. Absorbance of the samples was also scanned from 300 to 790 nm. No peaks were observed at the absorption maximum of chlorophyll (665 nm), indicating that gut evacuation had been effective and that chlorophyll did not interfere with the results. The concentrations of pigments were normalized to dry mass.

MAA concentrations were taken from the literature (19). However, we carried out MAA extractions of 10 individuals (*P. sarsi*) and 30 individuals (*B. gibbosa*) in three replicates in 25% methanol for 2 h at 45°C (19). Afterward spectrophotometric scans (250–790 nm) were performed in 1 cm quartz cuvettes using a double-beam spectrophotometer Shimadzu UV2450.

Enzyme activity determinations: Ten *P. sarsi* or 30 *B. gibbosa* (corresponding to one replicate of any of the experiments) were pooled to ensure reliable enzymatic determinations. Animals were homogenized using a glass-teflon homogenizer with ice-cold 50 mM potassium phosphate buffer, pH 7.7 containing 1 mM EDTA and 0.1% Triton X-100 according to Borgeraas and Hessen (30). Supernatants of homogenates, centrifuged at 10 000 ***g*** and 4°C for 10 min, were used as enzyme sources. From each treatment replicate, three subsamples were determined with a minimum of two measurements per subsample. Measurements of enzymatic activities were carried out using a Shimadzu UV2450 spectrophotometer.

AChE activity was determined in supernatants from homogenates of whole organisms. Total AChE activity was determined at 25°C following the colorimetric method proposed by Ellman *et al*. (31), using acetylthiocholine iodide as substrate and dithiobisnitrobenzoate (DTNB) as reagent. AChE activity is expressed as μmol product developed min^{-1} [g protein]$^{-1}$. Linearity of the reaction with protein quantity per assay was determined (*B. gibbosa* $r^2 = 0.952$; *P. sarsi* $r^2 = 0.932$).

Total GST activity was estimated in 10 individuals (*P. sarsi*) or 30 individuals (*B. gibbosa*) in three replicates. GST activity was determined following Habig *et al*. (32) in 100 mM phosphate buffer (pH = 6.5), with 1 mM of 1-chloro-2.4-dinitrobenzene in acetonitrile (1% vol/vol) and GSH 1.2 mM as substrates recording the absorbance at 340 nm. The specific activity of GST was expressed as μmol of product developed per minute per mg of protein (μmol prod min^{-1} [mg protein]$^{-1}$). Linearity of the reaction with protein quantity per assay was determined (*B. gibbosa* $r^2 = 0.975$; *P. sarsi* $r^2 = 0.983$).

Protein determination was performed according to Lowry *et al*. (33) with bovine serum albumin as the standard. The protein quantity per assay in *B. gibbosa* was 4.26 ± 0.001 μg protein per reaction, and in *P. sarsi* it was 34.82 ± 4.47 μg protein per reaction.

GSH levels: Three replicates of 15 individuals of *P. sarsi* from each exposure time were rinsed with cold (4°C) filtered lake water (0.2 μm) and homogenized in 5% trichloroacetic acid at 4°C. GSH was measured as acid-soluble thiols (34). The homogenates were centrifuged at 10 000 ***g*** for 10 min at 4°C and GSH content was immediately measured in triplicate in 0.1 mL of the supernatants, using 1 mL of 1.5 mM DTNB in 0.25 M sodium phosphate buffer (pH = 8.0). The mixture was incubated for 10 min at 23°C and the absorbance at 412 nm was measured in a double-beam Shimadzu UV2450 spectrophotometer. Acid-soluble thiols were quantified using a calibration curve with pure GSH as a standard. The GSH content was calculated from triplicate measurements and expressed as a function of protein concentration.

Data analysis. Results are expressed as mean ± standard error. Statistical significance was determined using a *t*-test and ANOVA. Homoscedasticity and normality were previously checked, and data were log transformed when needed to fulfill analysis requirements. Data analyses were performed using a Sigma Stat 3.5 package.

RESULTS

C:P, GST and pigments in *B. gibbosa* and *P. sarsi*

The two studied copepods differed greatly in body size, and photoprotective and antioxidant capacity. Comparing both species directly from the field, we observed that *P. sarsi* exhibits lower C:N and C:P ratios (Table 1). Regarding the photoprotective compounds carotenoids and MAAs, *B. gibbosa* is a well-protected species showing about five-fold higher values compared to those observed in *P. sarsi* (Table 1). However, in both species we obtained maximum peaks at 334 nm, which can probably be attributed to the MAA porphyra-334 (maximum absorbance at 334 nm) or shinorine (maximum absorbance at 332 nm). Additionally, we obtained distinctive GST activities in the two copepods (Table 1). GST activity was almost three-fold higher in *P. sarsi* than in *B. gibbosa*.

Experimental results

Our experiments were performed with natural lake water from Laguna Fantasma (*P. sarsi*) and Los Cántaros (*B. gibbosa*). Due to differences in DOM content between the lakes, spectrophotometric scans of filtered lake water (0.2 μm) showed different absorption in the UV and blue wavebands of the light spectrum. This results in differences in the net effect of our experimental UVR exposures. Due to these differences copepods of Laguna Fantasma received 12% less of the 340 nm band and 18% less of the 305 nm band during the experiments (estimates based on laboratory spectrophotometric measurements).

AChE inhibition by UVR exposures. UVR induced a significant inhibition of the AChE activity in *B. gibbosa* (Fig. 1A). After a relatively short exposure to UV-B (90 min) a significant decrease in enzyme activity (approximately 60%) was observed (*t*-test, $P = 0.001$). A similar inhibition was observed after the UV-A exposure (240 min) (*t*-test, $P = 0.002$). On the other hand, AChE activity in *P. sarsi* was inhibited only by UV-B exposure, decreasing by 36% (*t*-test, $P = 0.0018$) (Fig. 1B); however, in this species AChE activity was not affected by UV-A exposure (*t*-test, $P = 0.561$).

AChE inhibition in cumulative UVR exposures. We observed distinctive cumulative effects on AChE activity under increasing UVR (Fig. 2). UV-B (Experiment 3) resulted in a similar response pattern in both copepod species (Fig. 2A,B). The pattern consisted of a significant decrease from T_0 to 30 and 60 min of exposure (about 20–30% reduction); then there was a recovery to normal levels of activity after 120 min of exposure (Fig. 2A,B), and finally a new reduction in activity at 240 min (about 15–25% of reduction) (*B. gibbosa*: one-way ANOVA, $P = 0.002$; *P. sarsi*: one-way ANOVA, $P = 0.010$).

A similar temporal pattern was observed in Experiment 4 during UV-A exposure in *B. gibbosa* (Fig. 2C). The first

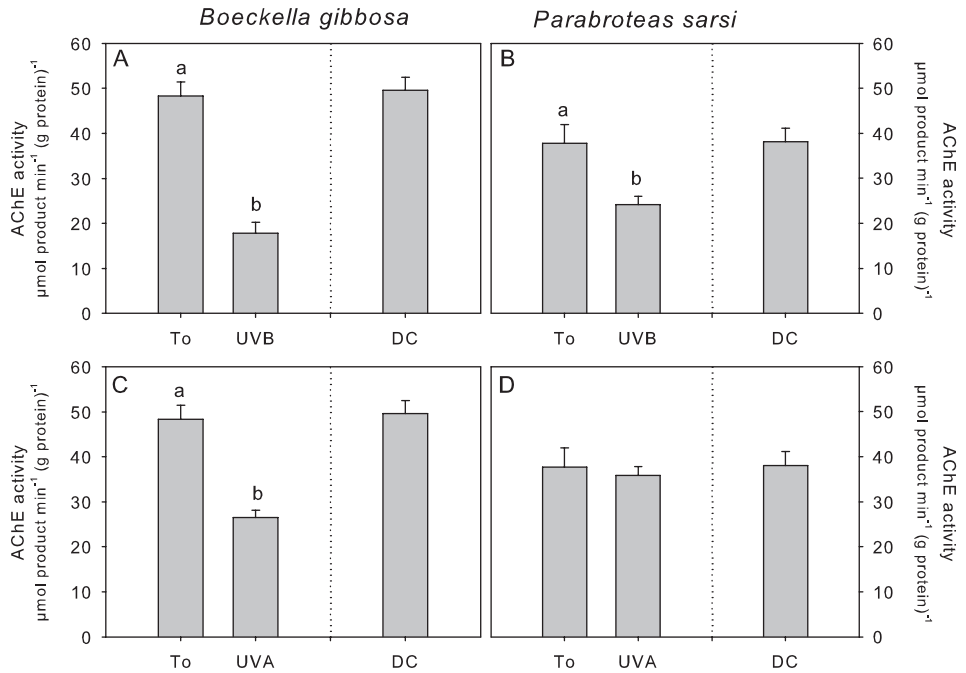

Figure 1. Acetylcholinesterase activity after UVR exposure in both copepods. (A) *Boeckella gibbosa* exposed to UV-B. (B) *Parabroteas sarsi* exposed to UV-B. (C) *Boeckella gibbosa* exposed to UV-A. (D) *Parabroteas sarsi* exposed to UV-A. T_0 = initial level; DC = dark control. Lower-case letters inside the graphs indicate homogeneous groups according to *t*-test results.

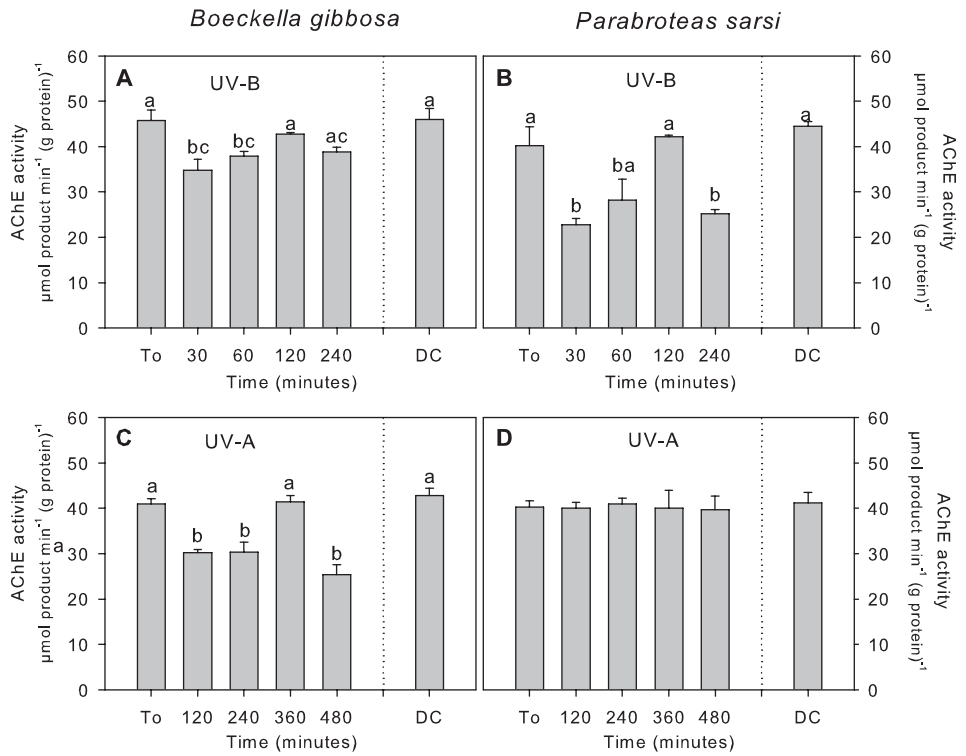

Figure 2. Effect of cumulative doses of UVR on acetylcholinesterase activity in both copepods. (A) *Boeckella gibbosa* exposed to UV-B. (B) *Parabroteas sarsi* exposed to UV-B. (C) *Boeckella gibbosa* exposed to UV-A. (D) *Parabroteas sarsi* exposed to UV-A. T_0 = initial level; DC = dark control. Lower-case letters inside the graphs indicate homogeneous groups according to ANOVA results.

decrease occurred from T_0 to 120 and 240 min, then a peak activity at 360 min and finally a new inhibition at 480 min (Fig. 2C) (one-way ANOVA, $P < 0.001$). *P. sarsi*, as observed in Experiment 2, showed no differences in enzyme activity during the UV-A exposure (Fig. 2D) (one-way ANOVA, $P = 0.546$).

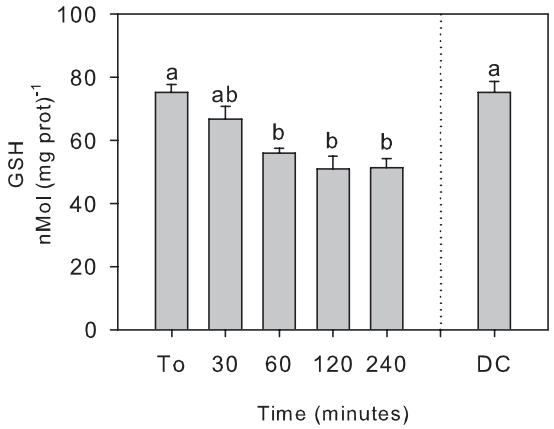

Figure 3. Effect of cumulative doses of UV-B on levels of reduced glutathione (GSH) in *Parabroteas sarsi*. T_0 = initial level; DC = dark control. Lower-case letters inside the graphs indicate homogeneous groups according to ANOVA results.

Measurement of the oxidative stress caused by UVR exposition. We observed a monotonic decrease pattern of GSH during the first 120 min, and then it remained at the same level from 120 to 240 min of UVR exposition. The decrease in GSH from T_0 to 240 min was statistically significant (one-way ANOVA, $P = 0.006$) (Fig. 3).

DISCUSSION

In the present study we show for the first time that UVR exposures (both UV-A and UV-B wavelength) can exert a detrimental effect on AChE activity in copepods, though with some differences between species. Even at the similar radiation levels that are found in Andean lakes (12,35), our experiments showed that UVR can effectively depress AChE activity, indicating that this deleterious effect may occur in natural environments. UVR has not yet been considered as a factor detrimental to AChE, although this enzyme activity has been used as a biomarker of the effects of neurotoxic contaminants in aquatic organisms (36,37). The observed UVR negative effect may arise either from a direct damage of UVR on the AChE molecule (17) or from an indirect effect caused by oxidative stress (38).

Exposure to UV-B leads to the generation of reactive oxygen species (ROS) that produce oxidative stress causing the disruption of cellular homeostasis (39). To counteract this oxidative stress, GSH plays a major role as a buffer of the cellular redox state, and an inverse relationship between the concentrations of ROS and GSH has been reported (40). The decrease in GSH observed in *P. sarsi* during the UVR exposure indicates that UVR effectively produces oxidative stress.

In our experiments, AChE activity was affected by UV-B in both copepods, whereas UV-A affected AChE activity only in *B. gibbosa*. This difference may arise from two main sources: (1) DOM content of the lake water that could create differential UVR shields and (2) differences in UVR photoprotective and antioxidant defenses of each species. In fact, we observed that lake water of Laguna Fantasma, from which *P. sarsi* was collected, has a higher DOM content, and individuals in the experiment received 12% less UV-A and 18% less UV-B. Nevertheless, the UV-B doses were high enough to affect AChE activity despite the higher shield effect at these wavelengths. On the other hand, photoprotective pigments and the antioxidant capacity would play a crucial role in preventing AChE damage by UVR as AChE is affected by oxidative stress (41,42). Regarding photoprotective compounds and antioxidant defenses, the two copepod species showed great differences. In *P. sarsi*, GST activity was almost three-fold higher than in *B. gibbosa*, and this enhanced protection may be responsible, at least partially, for the absence of UV-A effect on AChE activity. As demonstrated by Balseiro *et al*. (12), GST activity is dependent on the body C:P ratio; that is, on the P availability for protein synthesis (43). Our analysis of body elemental ratios of both species showed that *P. sarsi* has lower C:P ratios than *B. gibbosa*, and this would result in a higher availability of P for enzyme synthesis, hence more GST against oxidative stress damage. Finally, photoprotective compounds such as carotenoids and MAAs would also be important in the final balance of UV-induced damage in copepods (8,20). *B. gibbosa* seems much more photoprotected than *P. sarsi*, as the former has a five-fold higher concentration of both carotenoids and MAAs. Although in terms of survivorship *B. gibbosa* is considered one of the most resistant centropagid copepods to UVR (44), based on our experiments on AChE activity this species was more sensitive to other effects of UVR than *P. sarsi*. These results suggest that GST antioxidant capacity and P availability for enzyme synthesis would be more important for AChE activity than photoprotective compounds such as carotenoids and MAAs.

Our experiments showed that during relatively short exposure to UV-B (60 min) in both copepods, and to UV-A (120 min) in *B. gibbosa*, AChE activity decreased significantly. However, in Experiments 3 and 4 there was a transient recovery of its activity during longer exposures, with a final decrease. Similar transient recoveries have been observed in mice as a consequence of physical and chemical oxidative stress (41,42). These studies have shown that the molecular events switched on by the inhibition of AChE activity include responses involving a feedback mechanism that enhances the synthesis of new AChE (38,42,45).

Regardless of the mechanisms involved, dysfunctions in AChE are ecologically relevant. The reduction in AChE activity has been related to continuous and excessive stimulation of the nerve/muscle fibers, which leads to behavioral changes (46), tetany, paralysis and eventual death (13). Calanoid copepods depend on swimming for escape and for creating feeding currents (47), and a dysfunction of AChE would affect the copepod's fitness as it would affect their motility patterns. These organisms achieve velocities greater than 100 body lengths per second, with accelerations up to 30 G that allow them to reach these speeds in milliseconds (48). Motion is not a simple event, but results from the integrated function of several reaction components implying multiple nerve impulses that are precisely coordinated (49,50). Thus, any alteration in neurotransmitter mechanisms, such as AChe, will have a strong negative effect on this vital function.

Acknowledgements—We thank Nahuel Huapi National Park staff for help in the field work at Lake Cántaros. We thank the anonymous reviewers whose comments greatly improved the manuscript. This work was supported by FONCyT PICT 2007-01256 and PICT2007-01258 and Universidad Nacional del Comahue B141.

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