Limnol. Oceanogr., 55(3), 2010, 1024–1032

doi:10.4319/lo.2010.55.3.1024

Stoichiometric dietary constraints influence the response of copepods to ultraviolet radiation-induced oxidative stress

M. S. Souza,[a] B. E. Modenutti,[a] P. Carrillo,[b,c] M. Villar-Argaiz,[b] J. M. Medina-Sánchez,[b] F. Bullejos,[b] and E. G. Balseiro[a,*]

[a] Laboratorio de Limnología, INIBIOMA (CONICET-UNComahue), Bariloche, Argentina
[b] Departamento de Biología Animal y Ecología, Facultad de Ciencias, Universidad de Granada, Granada, Spain
[c] Instituto del Agua, Universidad de Granada, Granada, Spain

Abstract

We carried out field experiments in two clear mountain lakes of both hemispheres (Lake Los Cántaros, Patagonia, Argentina, and Lake La Caldera, Sierra Nevada, Spain) performing a full factorial design (light × nutrients: unfiltered sun light (ultraviolet radiation treatment [UVR]) and screened sunlight (> 380 nm; photosynthetically active radiation treatment), with and without nutrient enrichment. We analyzed the direct effect of UVR on enzymatic antioxidant responses (catalase [CAT], glutathione S-transferase [GST], and glutathione reductase [GR]) of two calanoid copepod species—*Boeckella gibbosa* and *Mixodiaptomus laciniatus*—and the indirect effects of food quality (carbon : nutrient ratio) potentially affecting body elemental compositions and hence enzymatic activities. Responses for the three enzymes were different: GST increased its activity under UVR exposure in the two copepods, CAT activity was null and showed no response, and GR activity differed between species. Light treatments also affected sestonic elemental ratios; UVR exposure lowered carbon : phosphorus (C : P) ratios, which in turn affected the C : P elemental compositions of the copepods. However, nutrient addition had different effects on the two species; it did not affect final somatic C : P ratio of *B. gibbosa* but had a substantial effect on body elemental composition of *M. laciniatus*. Finally, the relationship between grazer's C : P ratio and GST antioxidant enzyme activity was negative. UVR and nutrient inputs affected food quality, grazer somatic stoichiometry, and subsequently enzymatic responses. The ability of calanoid copepods to overcome increased UVR may depend, at least for GST, on the elemental nutrient balance of the food.

Transparent lakes with high light : phosphorus ratios have high elemental sestonic carbon : nutrient ratios (Sterner et al. 1997) that expose zooplankton to low food quality due to unbalanced elemental ratio (Boersma and Kreutzer 2002; Hessen et al. 2002*a*) and high ultraviolet radiation (UVR) (Kessler et al. 2008). Moreover, stoichiometric constraints are important in the pelagic environment to regulate organism growth and nutrient cycling in planktonic food webs (Sterner and Elser 2002). The "growth-rate hypothesis" proposes a positive relationship between body phosphorus (P) and ribosomal ribonucleic acid (rRNA) content and between rRNA and specific growth rate (Sterner and Elser 2002) since protein synthesis rate depends on the number of ribosomes rather than on their efficiency (Nomura et al. 1984). Moreover, rRNA is expected to increase together with specific growth rate as food concentration and/or quality increases (Hessen et al. 2002*b*). Proteins synthesized by the rRNA include enzymes needed for maintenance and repair, such as those involved in the response to UVR exposure. This suggests that P limitation may influence UVR response because rRNA shortage affects the ability of organisms to synthesize repair enzymes.

In aquatic systems, UVR is recognized as an important biological stressor generating reactive oxygen species (ROS) (Williamson and Neale 2009) that cause cell oxidative stress, damage to DNA, proteins, and lipids and apoptosis induction (Martindale and Holbrook 2002). Planktonic organisms have developed a number of different strategies to reduce these possible deleterious effects. Zooplankton defense mechanisms can include behavior (Leech et al. 2005; Kessler et al. 2008), photoprotective pigments or sunscreens (Hansson et al. 2007; Hylander et al. 2009), and antioxidant enzymes (Borgeraas and Hessen 2000; Balseiro et al. 2008). Antioxidant enzymes include enzymatic scavengers such as catalase (CAT), glutathione S-transferases (GST), and glutathione reductase (GR) (Hanzel et al. 2005), and the activity of these enzymes increase under UVR exposition (Borgeraas and Hessen 2000). Enzyme synthesis requires the additional supply of some dietary factors (Lesser 2006), with P and nitrogen (N) being essential building blocks for RNA and protein synthesis (Hessen et al. 2007; Hessen and Anderson 2008). Therefore, food quality or availability affect the responses of the organism to oxidative stress (Monaghan et al. 2009), and food quality for grazers depends on the resource availability for producers. According to the light-nutrient hypothesis, planktonic producers in lakes exposed to high photosynthetically active radiation (PAR) increase their carbon (C) : nutrient ratio, decreasing their quality as food for consumers (Sterner et al. 1997; Elser et al. 2002; Urabe et al. 2002). However, it was recently shown that UVR can also affect the elemental ratios of primary producers but in an opposite direction, decreasing the algae C : P ratio (Xenopoulos et al. 2002; Hessen et al. 2008). Moreover, Balseiro et al. (2008) showed that low food quality (high algal C : P ratio) was related to a decrease in antioxidant enzymes in *Daphnia*, linking the stoichiometric

* Corresponding author: balseiro@crub.uncoma.edu.ar

constraints of zooplankton to a response in UVR stress-induced antioxidant. However, copepods, especially large copepodites and adults, are expected to be less sensitive to P limitation than *Daphnia* (Andersen and Hessen 1991).

Spring UV doses are predicted to increase 14% and 40% over the next decade (2010–2020) in the Northern and Southern hemispheres, respectively (Taalas et al. 2000). Comparison of ecological processes between lakes in each hemisphere (e.g., north Patagonian Andes, Argentina, and Sierra Nevada, Spain) may contribute to a wider understanding of zooplankton responses to UVR in clear water. The two areas are affected differentially by atmospheric aerosol transport, which may modify nutrient balances in aquatic ecosystems. Atmospheric inputs of P in the Mediterranean area are especially intense in Sierra Nevada mountain lakes (Carrillo et al. 2008). In Sierra Nevada oligotrophic lakes, enhanced pulses of dust-aerosol loads from the Sahara, low precipitation, and longer and severe droughts are expected consequences of climatic warming (Escudero et al. 2005). In South American mountain areas of Patagonia, there have been reports of an increase in UVR (Villafañe et al. 2001) and a decrease in precipitation over the past century (Urrutia et al. 2005), but no increase in atmospheric nutrient precipitation has been recorded.

The present study was designed to test how food quality influences the enzymatic responses to UVR in two calanoid copepod species. We hypothesized that UVR and nutrient inputs would interact, thereby affecting food quality, grazer somatic stoichiometry, and subsequently the enzymatic defense battery of grazers against oxidative stress (CAT, GST, and GR). We predicted that UVR exposure would exert a direct effect increasing CAT, GST, and GR activities in clear lake copepods and that low food quality (high C : nutrient ratios) would indirectly decrease antioxidant enzymatic defenses of these consumers. These predictions were tested by conducting in situ experiments manipulating UVR and nutrients in mesocosm enclosures in two clear lakes: Los Cántaros (41°00′S, 71°49′W, north Patagonian Andes, Argentina) and La Caldera (37°03′N, 3°19′E, Sierra Nevada, Spain) measuring the stoichiometric changes in seston and its consequences for two calanoid copepods belonging to different families (*Boeckella gibbosa* [Brehm] [Centropagidae] in Los Cántaros and *Mixodiaptomus laciniatus* [Lilljeborg] [Diaptomidae] in La Caldera).

Methods

Experimental design—The study was performed in La Caldera from July to August 2007 (North Hemisphere summer) and in Los Cántaros from January to February 2008 (South Hemisphere summer). Six UVR-transparent polyethylene enclosures (height 5 m, diameter 0.7 m, volume 2 m^3) filled with 40 μm filtered lake water pumped from 3-m depth were incubated in each lake for 1 month. Three of the enclosures received the full spectrum of solar radiation (UVR treatment), and the other three received only the PAR treatment. In La Caldera, Plexiglas UF3 (Carrillo et al. 2008) was used to cut off UVR, covering the enclosures and extending horizontally 2 m beyond the setup. In Los Cántaros, a similar UVR cutoff was reached using a polyethylene bag. The optical features of this polyethylene, with cutoff at 380 nm and 85% transmittance above 400 nm, were checked before the experiment using a double-beam spectrophotometer (Shimadzu UV2450).

After 1 month, we applied a full factorial 2 × 2 design. Prior to this procedure, the enclosures were vertically homogenized using a round plastic disc fixed to a rope. Water from each of the six enclosures was used to fill two small closed containers of 20 L (height 0.2 m, diameter 0.4 m) constructed from same polyethylene as that of the enclosures (total number of containers = 12). Copepods (copepodite stages IV and V of *B. gibbosa* in Los Cántaros and *M. laciniatus* in La Caldera), obtained directly from the lake, were added to all containers to a final concentration around double that observed in each lake. Half of the containers (three UVR transparent and three screened) received an addition of P (Na_2HPO_4) and N (NH_4NO_3) to around double the total P concentration in the corresponding lake, maintaining a molar N : P ratio of ~ 30. These nutrient additions mimic the mean TN : TP ratios observed after natural atmospheric deposition events in the Northern Hemisphere lake and the potential nutrient input maintaining the N : P ratio in the Southern Hemisphere lake. The other six containers (three UVR transparent and three screened) received no nutrient addition. The result is a 2 (PAR or UVR light) × 2 (with or without nutrient addition) full factorial design with three replicates per treatment: PAR: screened sunlight (> 380 nm); PAR+NP: screened sunlight (> 380 nm) + nutrient addition; UVR: full sunlight; UVR+NP: full sunlight + nutrient addition. The 12 containers were then incubated for 1 week at 0.1 m below the surface, corresponding to 75% of surface solar radiation (light within the containers). The UVR was cut off by incubating the six containers (PAR treatments) under the same Plexiglas (UF3) in La Caldera and using the same polyethylene (cutoff at 380 nm) in Los Cántaros.

Underwater irradiance was measured with submersible radiometers (Biospherical Instruments, Profiling Ultraviolet Radiometer: PUV 500B in Los Cántaros; Biospherical Instrument Compact: BIC in La Caldera). At the surface, wavelength irradiances were similar between the lakes, for example, PAR 2195 and 2120 μmol photons m^{-2} s^{-1} in La Caldera and Los Cántaros, respectively (Table 1). La Caldera was more transparent than Los Cántaros (Table 1). However, as the UV-B irradiance was higher in Los Cántaros (305-nm band 40% higher in Los Cántaros; Table 1), the difference between container setups at 0.1 m below the surface was negligible. There were no differences in temperature between lake and enclosure water, and only slight temperature differences between lakes were observed (La Caldera: 17 ± 1°C; Los Cántaros: 19 ± 1°C).

Elemental C : N : P and biochemical determinations—The six enclosures were sampled (1 L) for the initial C, N, and P conditions in the containers and for their phytoplankton cell abundance and composition and chlorophyll *a* (Chl *a*) concentration. Samples for C, N, P, and Chl *a* were immediately delivered to the laboratory in acid-washed bottles. Phytoplankton samples were fixed in the field with acid Lugol's solution.

Table 1. Light conditions of Los Cántaros (Argentina) and La Caldera (Spain) during the experimental periods. λ = irradiance wavelength; K_d = light attenuation coefficient.

	Los Cántaros		La Caldera	
Wavelength (λ)	K_d	Irradiance	K_d	Irradiance
305 nm	1.740 m^{-1}	7 $\mu W\ cm^{-2}\ nm^{-1}$	0.648 m^{-1}	5 $\mu W\ cm^{-2}\ nm^{-1}$
320 nm	1.160 m^{-1}	29 $\mu W\ cm^{-2}\ nm^{-1}$	0.505 m^{-1}	28 $\mu W\ cm^{-2}\ nm^{-1}$
380 nm	0.580 m^{-1}	75 $\mu W\ cm^{-2}\ nm^{-1}$	0.215 m^{-1}	77 $\mu W\ cm^{-2}\ nm^{-1}$
PAR	0.290 m^{-1}	2120 $\mu mol\ m^{-2}\ s^{-1}$	0.124 m^{-1}	2195 $\mu mol\ m^{-2}\ s^{-1}$

After 1 week of the full factorial experiments, the water in each container was filtered through a 60-μm plankton net, and copepods were collected, rinsed (0.2 μm filtered lake water) and frozen immediately at $-20^{\circ}C$ until enzymatic analysis.

In the laboratory, Chl *a* was measured fluorometrically (excitation filter 10–113, 436 nm; emission filter 10–115, 680 nm; Turner AU 10, Turner Designs) and calibrated against spectrophotometric determinations. A volume of 300 mL was filtered on Whatman GF/F glass-fiber filter and extracted in 90% ethanol for 24 h. Lugol-fixed phytoplankton samples were settled in 50 mL Utermöhl chambers for at least 36 h and counted in a transmitted light inverted microscope (Olympus IX70, Olympus) at $\times$1000 magnification.

In addition, a volume of 300 mL from each sample was filtered onto precombusted GF/F Whatman filters (450°C for 1.5 h) for seston elemental analysis, and 30 copepods of each container replicate were placed on precombusted filters. In both cases, filters were dried at 60°C for 48 h and stored at $-20^{\circ}C$ until analysis. C and N were analyzed on a PerkinElmer 2400 (PerkinElmer) (Lake La Caldera) and Thermo Finnigan EA1112 (Thermo Finnigan) Carbon : Nitrogen CN (Lake Los Cántaros) elemental analyzers. P was analyzed with persulfate digestion followed by molybdate reaction (Eaton et al. 2005). In all cases, filters without samples were used as blanks, and for accuracy each treatment replicate was measured in two method replicates. Samples for P were centrifuged (3000 rpm) to prevent interference of filters in the absorbance measurements.

For the determination of enzymatic activity, 30–40 individuals of each replicate of treatment were pooled in each sample to ensure reliable results. Animals were homogenized using a glass-Teflon homogenizer with ice-cold 50 mmol L^{-1} potassium phosphate buffer (pH = 7.7), containing 1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA) and 0.1% Triton X-100 according to Borgeraas and Hessen (2000). Supernatants of homogenates centrifuged at 10,000 $\times$ *g* for 10 min at 4°C were used as enzyme sources. Three samples were analyzed from each lake, with a minimum of two replicated measurements per sample. Enzymatic activity was assessed with a Shimadzu UV2450 spectrophotometer at 23 $\pm$ 0.5°C. CAT enzyme activity was measured in 50 mmol L^{-1} phosphate buffer (pH = 7.0) containing H_2O_2 (0.6% v/v) by reduction in absorbance at 240 nm due to H_2O_2 consumption (Aebi 1984). GR enzyme activity was measured in 143 mmol L^{-1} potassium phosphate buffer containing 6.3 mmol L^{-1} EDTA (pH = 7.5); the activity was monitored by nicotinamide adenine dinucleotide phosphate–reduced (β-NADPH) oxidization as glutathione disulfide reduction according to Schaedle and Bassham (1977). The kinetics of β-NADPH consumption was obtained from the decay of the absorbance at 340 nm. Total GST activity was measured according to Habig et al. (1974) in 100 mmol L^{-1} phosphate buffer (pH = 6.5), with 1 mmol L^{-1} of 1-chloro-2.4-dinitrobenzene in acetonitrile (1% v/v) and 1.2 mmol L^{-1} reduced glutathione as substrates, recording the absorbance at 340 nm.

CAT and GR activities were expressed as μmoles of substrate hydrolyzed per minute per milligram of protein (μmol prod min^{-1} mg $prot^{-1}$), while GST activity was expressed in mmoles of product developed per minute per milligram of protein (mmol prod min^{-1} mg $prot^{-1}$). Protein concentration was measured according to Lowry et al. (1951), using bovine serum albumin as standard.

Statistical analysis—Significance levels of the light (UVR and PAR) effects on sestonic C : P ratio, Chl *a*, and phytoplankton cell abundance in the large enclosures were evaluated using *t*-tests. The experimental design for the small containers was a full factorial 2 $\times$ 2 design, using a two-way ANOVA to analyze the effect of light and nutrients on copepod C, N, and P contents, C : P and C : N ratios, and GST and GR enzymatic activities, applying a posteriori Holm–Sidak test when significant results were obtained. Homoscedasticity and normality were checked. Because the UVR screens differed between the lakes, no statistical comparisons were performed between the lakes. SigmaStat 3.5 was used for these data analyses. SMATR v.2 (http://www.bio.mq.edu.au/ecology/SMATR) was used to analyze the relationship among copepod C : P or C : N ratios and enzymatic activities, applying the Standard Major Axis (SMA) method and an ANCOVA to test differences between slopes (Warton et al. 2006).

Results

Seston elemental composition in large enclosures—Incubation of the enclosures for a month allowed us to generate different sestonic "food qualities" in terms of C : P and C : N ratios (Fig. 1a–d). In Lake Los Cántaros enclosures, seston had a significantly higher C : P ratio (*t*-test, $t = 2.85$, df = 4, $p = 0.046$) but similar C : N ratio (*t*-test, $t = 0.34$, df = 4, $p = 0.746$) in the PAR vs. UVR treatments. In Lake La Caldera enclosures, both C : P and C : N ratios were significantly higher in the PAR vs. UVR treatments (*t*-test, C : P, $t = 3.38$, df = 4, $p = 0.028$; C : N, $t = 3.17$, df = 4, $p = 0.033$). However, no significant differences in food

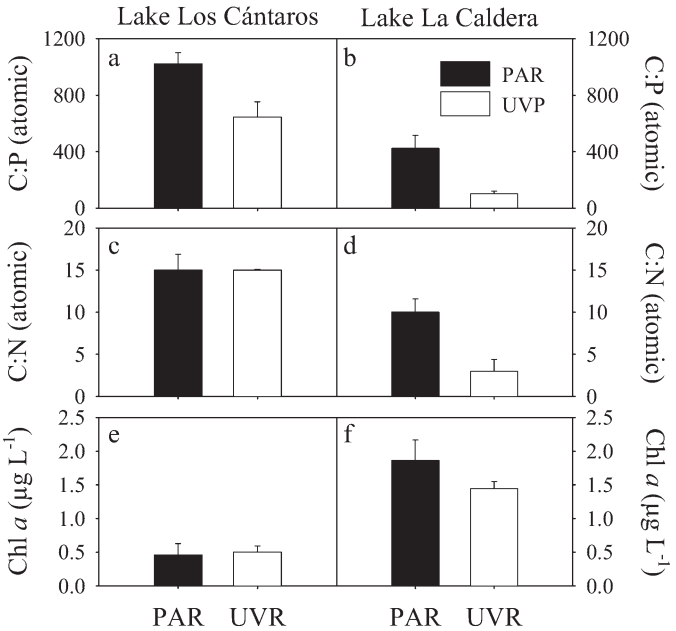

Fig. 1. Seston elemental ratios and chlorophyll *a* concentration under initial conditions (large enclosures) (a, c, e) in Lake Los Cántaros, and (b, d, f) Lake La Caldera. Ref: PAR: screened sunlight (> 380 nm); UVR: full sunlight. Error bars indicate 1 standard error.

quantity were registered in either lake, with no difference between treatments in Chl *a* (*t*-test, Los Cántaros, $t = 0.21$, df = 4, $p = 0.839$; La Caldera, $t = 1.59$, df = 4, $p = 0.187$) (Fig. 1e,f) or C content (*t*-test, Los Cántaros, $t = 0.74$, df = 4, $p = 0.497$; La Caldera, $t = 2.34$, df = 4, $p = 0.078$). Phytoplankton was dominated (> 80% in cell abundance) by Chrysophyceae and Dinophyceae in Los Cántaros and by Chlorophyceae and Chrysophyceae in La Caldera. Algal assemblages and abundances remained unchanged during the enclosure incubations (*t*-test, cell abundance, Los Cántaros, $t = 0.28$, df = 4, $p = 0.788$; La Caldera, $t = 1.67$, df = 4, $p = 0.170$). Mixotrophic small ciliates (Oligotrichida, < 80 μm) were present in Lake Los Cántaros although in very low abundances (< 2% algal cell abundance). There were no differences in ciliate abundance between treatments (*t*-test, ciliate abundance, Los Cántaros, $t = 1.36$, df = 4, $p = 0.243$). No ciliates were observed in Lake La Caldera.

Seston and copepod stoichiometry in small enclosures—Our experiment with a full factorial 2 × 2 design (light × nutrient treatments) showed significant differences in seston and copepod elemental composition (Figs. 2, 3). After 1 week of incubation with the copepods, the seston of the small containers exhibited significant differences in C : P ratio in both lakes (two-way ANOVA; Los Cántaros, light $F_{1,8} = 26.01$, $p = 0.001$; nutrient $F_{1,8} = 66.40$, $p < 0.001$; La Caldera, light $F_{1,8} = 16.08$, $p = 0.004$; nutrient $F_{1,8} = 48.15$, $p = 0.001$), lower C : P being in treatments with nutrient addition and UVR. In both lakes, we observed a significant interaction light × nutrient (Los Cántaros, $F_{1,8} = 13.9$, $p = 0.005$; La Caldera, $F_{1,8} = 46.08$, $p < 0.0001$). However, C : N ratios did not show any difference among treatments in Los Cántaros or in La Caldera (two-way ANOVA; Los Cántaros, $F_{1,8} = 1.69$, $p = 0.23$; La Caldera, $F_{1,8} = 0.036$, $p = 0.85$) (Fig. 2).

In Lake Los Cántaros, elemental compositions (C : P) of *B. gibbosa* differed significantly as a function of light treatment but not nutrient addition (two-way ANOVA; $F_{1,8} = 7.10$, $p = 0.003$ for UVR vs. PAR, $F_{1,8} = 0.06$, $p = 0.812$, for nutrient addition vs. nonaddition). Copepod C : P ratios were significantly lower in the UVR vs. PAR treatments (a posteriori Holm–Sidak test, $p = 0.028$)

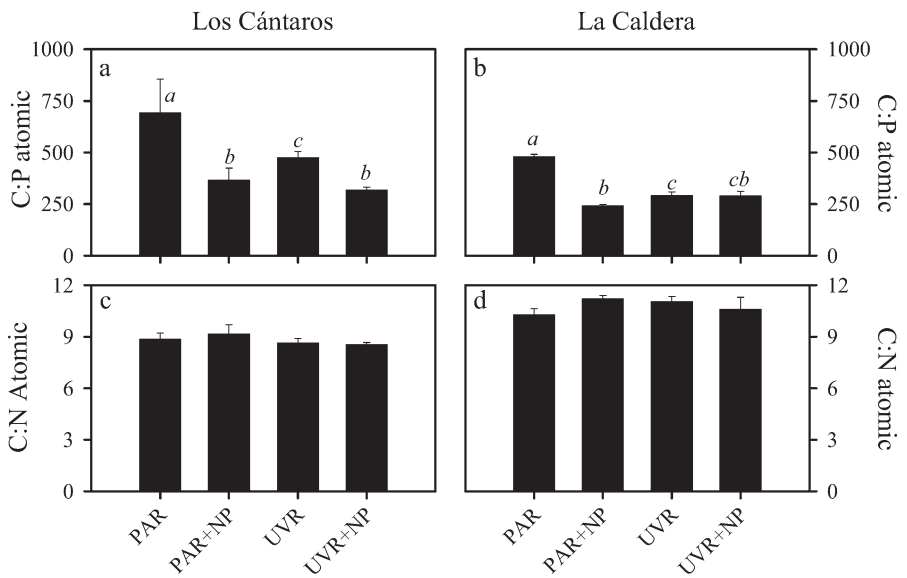

Fig. 2. Seston elemental ratios in the experimental treatments after 1 week of incubation with copepods, (a, c) in Lake Los Cántaros and (b, d) in Lake La Caldera. Ref: PAR: screened sunlight (> 380 nm); PAR+NP: screened sunlight (> 380 nm) + nutrient addition; UVR: full sunlight; UVR+NP: full sunlight + nutrient addition. Letters over bars indicate homogeneous groups according to ANOVA results. *See* Methods for experimental treatment details. Error bars indicate 1 standard error.

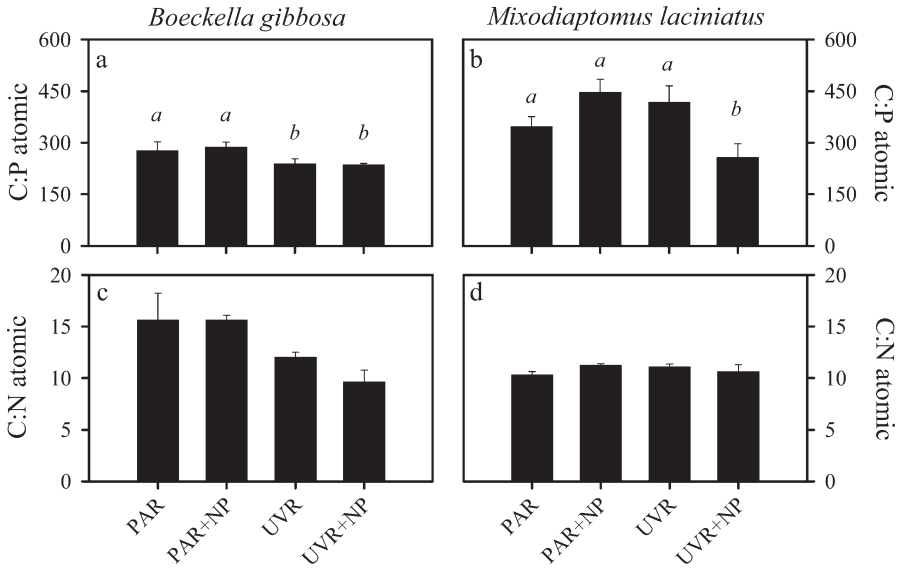

Fig. 3. Copepod elemental ratios in the experimental treatments carried out (a, c) in Lake Los Cántaros (*Boeckella gibbosa*) and (b, d) in Lake La Caldera (*Mixodiaptomus laciniatus*). Ref: PAR: screened sunlight (> 380 nm); PAR+NP: screened sunlight (> 380 nm) + nutrient addition; UVR: full sunlight; UVR+NP: full sunlight + nutrient addition. Letters over bars indicate homogeneous groups according to ANOVA results. *See* Methods for experimental treatment details. Error bars indicate 1 standard error.

(Fig. 3a), and the C : N ratio exhibited a similar pattern though only marginally significant (two-way ANOVA; $F_{1,8} = 4.98$, $p = 0.056$) (Fig. 3c). In Lake La Caldera, the *M. laciniatus* C : P somatic ratio showed significant differences only in the light × nutrient interaction (two-way ANOVA; light× nutrient, $F_{1,8} = 10.95$, $p = 0.011$). The a posteriori multiple-comparison test indicated significant differences for nutrient addition in UVR treatments (Holm–Sidak test, $p = 0.021$) and for light treatment in nutrient-added treatments (Holm–Sidak test, $p = 0.009$) (Fig. 3b). Again, copepod C : N did not differ between treatments (two-way ANOVA; $F_{1,8} = 0.038$, $p = 0.85$) (Fig. 3d).

The analyses of C content of the copepods showed that in both species, there were no effects of treatments, either light or nutrient addition, on somatic C contents, suggesting that the UVR effect did not generate variations in biomass in these short incubations (two-way ANOVA; *B. gibbosa*, light $F_{1,8} = 0.956$, $p = 0.35$; nutrient $F_{1,8} = 2.004$, $p = 0.19$; *M. laciniatus*, light $F_{1,8} = 0.057$, $p = 0.81$; nutrient $F_{1,8} = 0.869$, $p = 0.37$).

Enzymatic activity—Figure 4 depicts the responses in enzyme activities of the two copepods. For both species, CAT activity was negligible in all treatments (data not shown), while GST activity was higher under UVR exposure. The response of GR differed between species, showing increased activity under UVR only in *M. laciniatus*. Maximum GST activity levels were around threefold higher for *M. laciniatus* than *B. gibbosa*, and maximum GR activity was fourfold higher for *M. laciniatus* (Fig. 4).

In Lake La Caldera, GST activity in *M. laciniatus* differed among treatments and also showed a significant interaction between light and nutrient (two-way ANOVA; light $F_{1,8} = 20.65$, $p = 0.002$; nutrient $F_{1,8} = 7.80$, $p = 0.023$; light × nutrient $F_{1,8} = 11.91$, $p = 0.008$). GST activity was more than twofold higher for UVR+NP than UVR (a posteriori Holm–Sidak test, $p = 0.002$) (Fig. 4). Comparison between the two treatments with nutrient addition (UVR+NP and PAR+NP) showed a significantly higher GST activity under UVR (a posteriori Holm–Sidak test, $p < 0.001$). Comparison between the two treatments without nutrient addition showed no significant differences in GST activity (a posteriori Holm–Sidak test, $p > 0.050$).

In Lake Los Cántaros, GST activity in *B. gibbosa* showed significant differences between light treatments and in the interaction between light and nutrients but not between nutrient addition and nonaddition (two-way ANOVA; light $F_{1,8} = 62.15$, $p < 0.001$; nutrient $F_{1,8} = 0.32$, $p = 0.587$; light × nutrient $F_{1,8} = 6.88$, $p = 0.030$). Light quality significantly affected GST activity with and without nutrient addition (a posteriori Holm–Sidak test, $p < 0.001$ for UVR+NP vs. PAR+NP and $p = 0.006$ for UVR vs. PAR). Hence, the trend of increased enzyme activity in full sunlight was similar for both copepod species (Fig. 4a,b).

For *M. laciniatus*, GR activity was higher in the treatments exposed to full sunlight (two-way ANOVA; $F_{1,8} = 18.86$, $p = 0.003$) (Fig. 4d). Unexpectedly, there was no significant difference under UVR treatment between nutrient addition and nonaddition (Fig. 4d). The response pattern was complex in *B. gibbosa* from Los Cántaros (Fig. 4c), and there was a strong interaction between factors (two-way ANOVA; light × nutrient $F_{1,8} = 15.44$, $p = 0.004$). The addition of nutrients resulted in contrasting responses, with an increase in GR activity when nutrients were added to PAR treatments (a posteriori Holm–Sidak test, $p = 0.008$) but a decrease when they were added to UVR treatments (a posteriori Holm–Sidak test, $p = 0.001$).

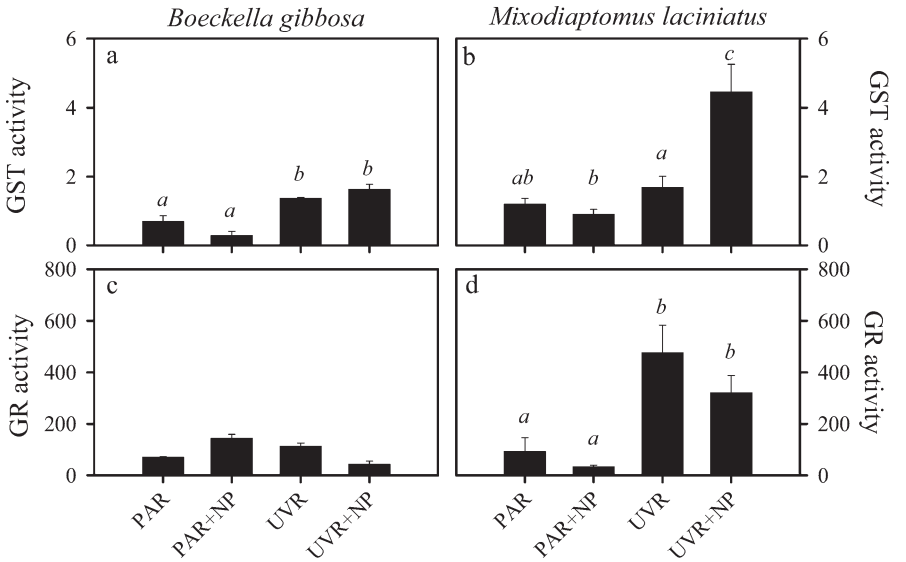

Fig. 4. Enzymatic activities of the copepods in the experiments. (a) Glutathione S-transferase (GST) activity in *Boeckella gibbosa* (Lake Los Cántaros), (b) GST activity in *Mixodiaptomus laciniatus* (Lake La Caldera), (c) glutathione reductase (GR) activity in *Boeckella gibbosa*, and (d) GR activity in *Mixodiaptomus laciniatus*. GST scale in mmol prod min^{-1} mg $prot^{-1}$; GR scale in μmol prod min^{-1} mg $prot.^{-1}$. Other references as in Fig. 2. Error bars indicate 1 standard error.

Finally, GST activity correlated the C : P ratio of the copepods. Both species showed the same trend, although this relationship was only statistically significant for *B. gibbosa* (SMA regression; *B. gibbosa*, $r^2 = 0.753$, $p = 0.002$; *M laciniatus*, $r^2 = 0.239$, $p = 0.127$). Nevertheless, the slope comparison test showed that the GST-C : P slopes were similar for both species (slope = -11.6 ± 2.6 [$\pm$ SE] for *B. gibbosa*; slope = -11.8 ± 3.6 for *M. laciniatus*, $F_{1,20} = 0.002$, $p = 0.96$) (Fig. 5).

Discussion

Antioxidant enzyme activities—Higher antioxidant enzyme activity is associated with greater stress (Balseiro et al. 2008); therefore, we predicted a higher activity for the three tested antioxidant enzymes under UVR vs. PAR treatment. However, we obtained different responses for the three enzymes; GST increased its activity under UVR exposure, while CAT activity was negligible in both copepods studied, and GR response differed between species.

CAT activity was undetectable in all treatments, and this was not a consequence of photoinhibition since similar null levels were observed in the UVR-protected treatments. Null CAT activity appears to be common in calanoid copepods since no effect on CAT activity was found in *Boeckella gracilipes* (Souza et al. 2007), and CAT mRNA levels were very low in response to oxidative stress in *Calanus finmarchicus* (Hansen et al. 2008). However, the GR activity response differed between the copepod species in our experiment; it was higher in the UVR treatments *in M. laciniatus*, whereas the pattern was complex in *B. gibbosa* from Los Cántaros, showing a strong interaction with nutrients.

The disparities in GST and GR levels between *M. laciniatus* and *B. gibbosa* under UVR may indicate a difference in defense mechanisms against UVR in these species. This difference may be related to the photoprotective

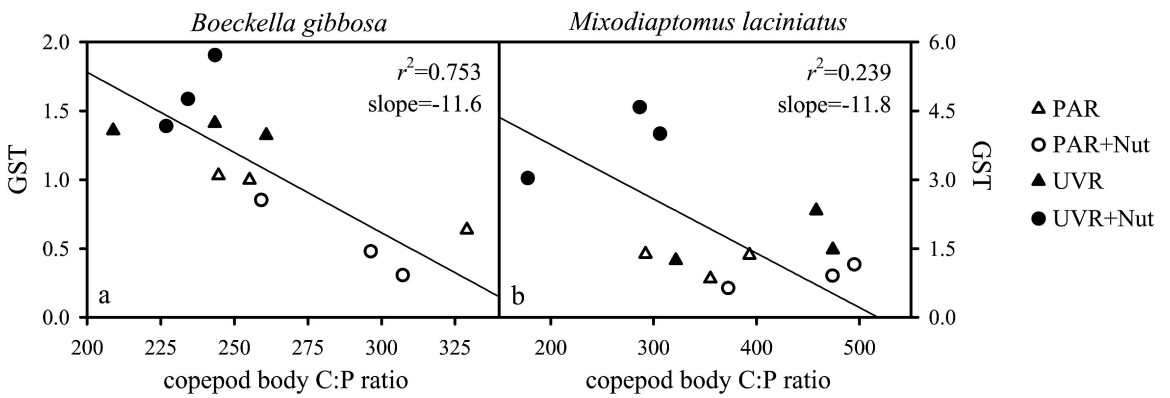

Fig. 5. GST-C : P relationship in (a) *Boeckella gibossa* and (b) *Mixodiaptomus laciniatus*. Regression lines estimated by SMA method. GST scale as in Fig. 4.

compound concentrations in each species. Recently, a negative relationship was observed in Arctic amphipods between body mycosporine-like amino acids (MAA) concentration and UVR-induced antioxidant enzymatic response (Obermüller et al. 2005). The MAA concentration is much higher in *B. gibbosa* (up to 9–10 mg MAAs g^{-1} dry wt) (Tartarotti et al. 2004; Persaud et al. 2007) than in *M. laciniatus* (up to 1.5 mg MAAs g^{-1} dry wt) (N. Korbee pers. comm.), and we found much higher antioxidant enzymatic activities in the latter. It has also been suggested that the MAA mycosporin-glycine and carotenoids have antioxidant properties (Dunlap and Yamamoto 1995). Carotenoids and this particular MAA are present in high concentrations in *B. gibbosa* (Tartarotti et al. 2004; Persaud et al. 2007). Hence, *B. gibbosa*, which can accumulate high amounts of photoprotective pigments, has less synthesis of antioxidant enzymes, compared to *M. laciniatus*, which must make a much greater investment in its enzymatic defense. Alternatively, it can be suggested that *B. gibbosa*, which experiences a more P-depleted food resource, with a stronger P limitation, has a potentially lower protein synthesis rate and so turns to photoprotective compounds, such as carotenoids and MAAs, to overcome the UVR-induced stress.

Stoichiometry and antioxidant response—It is known that UVR can decrease C : P ratio of algae (Xenopoulos et al. 2002; Carrillo et al. 2008; Hessen et al. 2008). We also found lower sestonic C : P ratios under UVR exposure, which had a relatively rapid effect on C : P elemental compositions of the copepods (at 1 week of experimentation). The threshold elemental ratio (TER) should be interpreted as a limiting point where zooplankton cannot compensate for elemental imbalance (Urabe and Watanabe 1992). Unfortunately, since there are no data on phosphorus TER values for copepods as there are for other crustaceans such as *Daphnia*, we are not able to evaluate if the sestonic C : P ratios were above or below it. However, elemental ratios of copepods were affected by seston composition in our experiments, implying that the elemental ratio was well above C : P TER.

However, the effect of nutrient addition differed between the species, having no effect on the final body ratio for *B. gibbosa* but a substantial effect for *M. laciniatus*, though in both cases the seston C : P after 1 week of incubation still showed the effect of nutrient addition. There may be two possible explanations: the ability to profit from nutrient pulses (as in La Caldera) may be an adaptive trait for the inhabitant copepods, which react rapidly to nutrient changes, and high UVR doses, as in Patagonia (Villafañe et al. 2001), provoke strong responses against this stressor. Lake La Caldera periodically receives the input of Sahara dust, which substantially increases nutrient concentrations (Villar-Argaiz et al. 2001; Carrillo et al. 2008). In a closed system, pulsed P addition would produce an immediate decrease in C : P ratio due to "luxury" uptake by the algae (Hessen and Anderson 2008), and these sestonic differences would be transferred to the body C : P ratios of copepods. In contrast, the study lake in the south Andes receives no type of nutrient pulses, and the response of *B. gibbosa* was influenced more by light quality than by nutrient addition. These differences may also be due to the different lineages (different families) of these two copepods. However, further laboratory experiments are needed to test these hypotheses.

We observed that GST in both copepods and GR in *M. laciniatus* are dependent on the somatic C : P ratio, supporting, at least in part, our prediction that low food quality (high C : nutrient ratios) would indirectly decrease antioxidant enzymatic defenses of these consumers. The negative relationships between GST activity and body C : P ratio observed in both copepods suggest that elemental stoichiometry plays an important role in the antioxidant response. Moreover, the comparable GST-C : P slopes for both species and the similar response previously reported for *Daphnia* (Balseiro et al. 2008) suggest that this relationship is not species dependent but is a common feature of planktonic crustaceans. Since P shortage also affects growth (Sterner and Elser 2002) and the synthesis of antioxidant enzymes would require additional P supplies, it could be expected that organisms under heavy P limitation and UVR-induced stress could need to switch from growth to enzyme synthesis to improve defense and repair. However, incubations in the small containers lasted only 1 week, and we did not detect significant changes in copepod's growth in this short period.

The negative relationship found between body C : P ratio and GST activity indicates that organisms under heavy P limitation would need to use a chemical pathway other than GST to overcome UVR-induced stress. The anabolic reduced glutathione pathway is known to act as reduced substrate for ROS, thereby protecting other possible and more sensitive substrates, such as DNA, proteins, and fatty acids (Meister 1994; Lesser 2006). Recovery of reduced glutathione (GH) implies an energetic cost to keep it operative by reducing oxidized glutathione (Amsellem et al. 1993); therefore, the UVR exposure would eventually deplete energy or elemental resources. Nevertheless, as the recovery may take place later, the GH pathway may remain operative as an antioxidant mechanism since it reduces ROS with no immediate energy or P cost (Amsellem et al. 1993). This is only a transient solution since GR is then needed to recover the reduced condition, which would be especially important in species receiving periodic nutrient pulses, such as the *M. laciniatus* in Lake La Caldera. However, it may allow organisms to overcome stress and then recover with some time lag but with no further damage. *M. laciniatus* of Lake La Caldera exposed to UVR without nutrient addition appeared to have oxidized glutathione while GST activity remained low, whereas the opposite was observed in the nutrient-enriched treatment, where the antioxidant mechanism operated via GST. Thus, the selection of one or the other pathway to reduce UVR-generated ROS would depend on the immediate availability of GST. On the other hand, *B. gibbosa* could not exploit the possibility of a quick GH recovery in a system with no nutrient pulses and did not show this type of response. *B. gibbosa* invests in accumulating photoprotective pigments and is the species of freshwater copepods with the highest MAA concentrations (Persaud et al. 2007).

The susceptibility of organisms to UVR and the different mechanisms to prevent oxidative stress will have important

consequences for population dynamics. Our experiments demonstrated that food stoichiometry interacts with cellular defense mechanisms, which would in turn affect planktonic copepod fitness. By generating ROS, UVR can also induce lipid peroxidation (Collinson and Grant 2003), which can reduce cholesterol, the basis for steroid production, thereby affecting steroidogenesis. Antioxidant enzymatic mechanisms would prevent negative effects on the reproduction of copepods by preserving cholesterol in particular and lipid stores in general, which are utilized primarily for egg and sperm production (Hansen et al. 2008). Hence, food stoichiometry would affect reproduction not only directly, via rRNA synthesis (Sterner and Elser 2002; Hessen et al. 2007), but also through other mechanisms that prevent damage in already synthesized molecules that may affect population fitness.

Acknowledgments

We thank Lars-Anders Hansson from Lund University for the valuable comments on an early version of this manuscript, Richard Davies for English writing assistance, and two anonymous reviewers whose comments improved this manuscript. This work was carried out in the framework of a collaboration project of the Agencia Española de Cooperación Internacional (AECI) between Universidad de Granada and Universidad Nacional del Comahue (Spain-Argentina). This work was supported by AECI A/056/06 and A/7926/07, FONCyT (Fondo para la Investigación Científica y Tecnológica) PICT 2008-01256 and PICT 2008-01258, Spanish Ministries of Environment (OAPN2009/067), Education and Science (GL2005/01564/BOS), Science and Innovation (CGL2008-01127/BOS), Junta de Andalucía (Excelencia P07-CVI-02598), and Universidad Nacional del Comahue (B141). We thank the authorities of Nahuel Huapi and Sierra Nevada National Parks for allowing us to carry out the experiments.

References

Aebi, H. 1984. Catalase in vitro. Methods Enzymol. **105:** 121–126, doi:10.1016/S0076-6879(84)05016-3

Amsellem, Z., M. A. K. Jansen, A. R. J. Driesenaar, and J. Gressel. 1993. Developmental variability of photooxidative stress tolerance in paraquat-resistant Conyza. Plant Physiol. **103:** 1097–1106.

Andersen, T., and D. O. Hessen. 1991. Carbon, nitrogen, and phosphorus content of freshwater zooplankton. Limnol. Oceanogr. **36:** 807–814.

Balseiro, E., M. S. Souza, B. Modenutti, and M. Reissig. 2008. Living in transparent lakes: Low food P : C ratio decreases antioxidant response to ultraviolet radiation in *Daphnia*. Limnol. Oceanogr. **53:** 2383–2390.

Boersma, M., and C. Kreutzer. 2002. Life at the edge: Is food quality really of minor importance at low quantities? Ecology **83:** 2552–2561.

Borgeraas, J., and D. O. Hessen. 2000. UV-B induced mortality and antioxidant enzyme activities in *Daphnia magna* at different oxygen concentrations and temperatures. J. Plankton Res. **22:** 1167–1183, doi:10.1093/plankt/22.6.1167

Carrillo, P., J. A. Delgado-Molina, J. M. Medina-Sanchez, F. J. Bullejos, and M. Villar-Argaiz. 2008. Phosphorus inputs unmask negative effects of ultraviolet radiation on algae in a high mountain lake. Glob. Change Biol. **14:** 423–439.

Collinson, E. J., and C. M. Grant. 2003. Role of yeast glutaredoxins as glutathione S-transferases. J. Biol. Chem. **278:** 22492–22497, doi:10.1074/jbc.M301387200

Dunlap, W. C., and Y. Yamamoto. 1995. Small-molecule antioxidants in marine organisms: Antioxidant activity of mycosporine-glycine. Comp. Biochem. Physiol. B **112:** 105–114, doi:10.1016/0305-0491(95)00086-N

Eaton, A. D., L. S. Clesceri, E. W. Rice and A. E. Greenberg [eds.]. 2005. Standard methods for the examination of water and wastewater. American Public Health Association, American Water Works Association.

Elser, J. J., P. Frost, M. Kyle, J. Urabe, and T. Andersen. 2002. Effects of light and nutrients on plankton stoichiometry and biomass in a P-limited lake. Hydrobiologia **481:** 101–112, doi:10.1023/A:1021217221004

Escudero, M., and others. 2005. Wet and dry African dust episodes over eastern Spain. J. Geophys. Res. **110:** 1–15, doi:10.1029/2004JD004731

Habig, W. H., M. J. Pabst, and W. B. Jakoby. 1974. Glutathione S transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem. **249:** 7130–7139.

Hansen, B. H., D. Altin, S. H. Vang, T. Nordtug, and A. J. Olsen. 2008. Effects of naphthalene on gene transcription in *Calanus finmarchicus* (Crustacea: Copepoda). Aquat. Toxicol. **86:** 157–165, doi:10.1016/j.aquatox.2007.10.009

Hansson, L.-A., S. Hylander, and R. Sommaruga. 2007. Escape from UV threats in zooplankton: A cocktail of behavior and protective pigmentation. Ecology **88:** 1932–1939, doi:10.1890/06-2038.1

Hanzel, C. E., M. S. Villaverde, and S. V. Verstraeten. 2005. Glutathione metabolism is impaired in vitro by thallium(III) hydroxide. Toxicology **207:** 501–510, doi:10.1016/j.tox.2004.11.002

Hessen, D. O., and T. R. Anderson. 2008. Excess carbon in aquatic organisms and ecosystems: Physiological, ecological, and evolutionary implications. Limnol. Oceanogr. **53:** 1685–1696.

———, J. Borgeraas, and J. B. Orbaek. 2002*a*. Responses in pigmentation and anti-oxidant expression in Arctic *Daphnia* along gradients of DOC and UV exposure. J. Plankton Res. **24:** 1009–1018, doi:10.1093/plankt/24.10.1009

———, P. J. Faerovig, and T. Andersen. 2002*b*. Light, nutrients, and P : C ratios in algae: Grazer performance related to food quality and quantity. Ecology **83:** 1886–1898.

———, T. C. Jensen, M. Kyle, and J. J. Elser. 2007. RNA responses to N- and P-limitation: Reciprocal regulation of stoichiometry and growth rate in *Brachionus*. Funct. Ecol. **21:** 956–962, doi:10.1111/j.1365-2435.2007.01306.x

———, E. Leu, P. J. Faerovig, and S. Falk Petersen. 2008. Light and spectral properties as determinants of C : N : P-ratios in phytoplankton. Deep-Sea Res. II **55:** 2169–2175, doi:10.1016/j.dsr2.2008.05.013

Hylander, S., N. Larsson, and L. A. Hansson. 2009. Zooplankton vertical migration and plasticity of pigmentation arising from simultaneous UV and predation threats. Limnol. Oceanogr. **54:** 483–491.

Kessler, K., R. S. Lockwood, C. E. Williamson, and J. E. Saros. 2008. Vertical distribution of zooplankton in subalpine and alpine lakes: Ultraviolet radiation, fish predation, and the transparency-gradient hypothesis. Limnol. Oceanogr. **53:** 2374–2382.

Leech, D. M., A. Padeletti, and C. E. Williamson. 2005. Zooplankton behavioral responses to solar UV radiation vary within and among lakes. J. Plankton Res. **27:** 461–471, doi:10.1093/plankt/fbi020

Lesser, M. P. 2006. Oxidative stress in marine environments: Biochemistry and physiological ecology. Annu. Rev. Physiol. **68:** 253–278, doi:10.1146/annurev.physiol.68.040104.110001

Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265–275.

Martindale, J. L., and N. J. Holbrook. 2002. Cellular response to oxidative stress: Signaling for suicide and survival. J. Cell. Physiol. **192:** 1–15, doi:10.1002/jcp.10119

Meister, A. 1994. Glutathione-ascorbic acid antioxidant system in animals. J. Biol. Chem. **269:** 9397–9400.

Monaghan, P., N. B. Metcalfe, and R. Torres. 2009. Oxidative stress as a mediator of life history trade-offs: Mechanisms, measurements and interpretation. Ecol. Lett. **12:** 75–92, doi:10.1111/j.1461-0248.2008.01258.x

Nomura, M., R. Gourse, and G. Baughman. 1984. Regulation of the synthesis of ribosomes and ribosomal components. Annu. Rev. Biochem. **53:** 75–117, doi:10.1146/annurev.bi.53.070184.000451

Obermüller, B., U. Karsten, and D. Abele. 2005. Response of oxidative stress parameters and sunscreening compounds in Arctic amphipods during experimental exposure to maximal natural UVB radiation. J. Exp. Mar. Biol. Ecol. **323:** 100–117, doi:10.1016/j.jembe.2005.03.005

Persaud, A. D., R. E. Moeller, C. E. Williamson, and C. W. Burns. 2007. Photoprotective compounds in weakly and strongly pigmented copepods and co-occurring cladocerans. Freshw. Biol. **52:** 2121–2133, doi:10.1111/j.1365-2427.2007.01833.x

Schaedle, M., and J. A. Bassham. 1977. Chloroplast glutathione reductase. Plant Physiol. **59:** 1011–1012, doi:10.1104/pp.59.5.1011

Souza, M. S., B. E. Modenutti, and E. G. Balseiro. 2007. Antioxidant defences in planktonic crustaceans exposed to different underwater light irradiances in Andean lakes. Water Air Soil Pollut. **183:** 49–57, doi:10.1007/s11270-007-9354-8

Sterner, R. W., and J. J. Elser. 2002. Ecological stoichiometry: The biology of elements from molecules to the biosphere. Princeton University Press.

———, J. J. Elser, E. J. Fee, S. J. Guildford, and T. H. Chrzanowski. 1997. The light : nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. Am. Nat. **150:** 663–684, doi:10.1086/286088

Taalas, P., and others. 2000. The impact of greenhouse gases and halogenated species on future solar UV radiation doses. Geophys. Res. Lett. **27:** 1127–1130, doi:10.1029/1999GL010886

Tartarotti, B., G. Baffico, P. Temporetti, and H. E. Zagarese. 2004. Mycosporine-like amino acids in planktonic organisms living under different UV exposure conditions in Patagonian lakes. J. Plankton Res. **26:** 753–762, doi:10.1093/plankt/fbh073

Urabe, J., M. Kyle, W. Makino, T. Yoshida, T. Andersen, and J. J. Elser. 2002. Reduced light increases herbivore production due to stoichiometric effects of light/nutrient balance. Ecology **83:** 619–627.

———, and Y. Watanabe. 1992. Possibility of N or P limitation for planktonic cladocerans: an experimental test. Limnol. Oceanogr. **37:** 244–251.

Urrutia, R., A. Lara, and R. Villalba. 2005. How did water availability varied in the Valdivian forest ecoregion in the last centuries? Revista Ambiente y Desarrollo **21:** 48–57. (in Spanish).

Villafañe, V., E. W. Helbling, and H. E. Zagarese. 2001. Solar ultraviolet radiation and its impact on aquatic systems of Patagonia, South America. Ambio **30:** 112–117.

Villar-Argaiz, M., J. M. Medina-Sanchez, L. Cruz-Pizarro, and P. Carrillo. 2001. Inter- and intra-annual variability in the phytoplankton community of a high mountain lake: The influence of external (atmospheric) and internal (recycled) sources of phosphorus. Freshw. Biol. **46:** 1017–1034, doi:10.1046/j.1365-2427.2001.00734.x

Warton, D. I., I. J. Wright, D. S. Falster, and M. Westoby. 2006. Bivariate line-fitting methods for allometry. Biol. Rev. **81:** 259–291, doi:10.1017/S1464793106007007

Williamson, C. E., and P. J. Neale. 2009. Ultraviolet light, p. 705–714. *In* G. Likens [ed.], Encyclopedia of inland waters. Elsevier.

Xenopoulos, M. A., P. C. Frost, and J. J. Elser. 2002. Joint effects of UV radiation and phosphorus supply on algal growth rate and elemental composition. Ecology **83:** 423–435.

Associate editor: Michael R. Landry

Received: 10 August 2009
Accepted: 29 November 2009
Amended: 17 December 2009