

Living in transparent lakes: Low food P : C ratio decreases antioxidant response to ultraviolet radiation in *Daphnia*

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Abstract

We experimentally tested the effect of food quality (phosphorus [P]:carbon [C] ratio) on the response of antioxidant enzymes to ultraviolet radiation (UVR) in *Daphnia commutata* fed with *Chlamydomonas reinhardtii*. Algal cultures were grown at different concentrations of phosphorus and light intensities, resulting in significant differences in the P : C ratios ($\mu\text{mol P} \cdot [\text{mmol C}]^{-1}$; 6.05, 1.70, and 0.83). After 12 d of *D. commutata* growth under these three food quality treatments, we observed significant differences in individual biomass and protein content of *Daphnia*. Subsequently, we carried out an ultraviolet exposure experiment to determine if stoichiometric constraints imposed would limit enzymatic defenses against UVR oxidative stress. The UVR-exposure experiment consisted of a factorial design with three levels of food P : C (low, medium, and high) and two levels of UVR (exposed and protected). The activities of glutathione S-transferases (GST) and catalase (CAT), enzymes involved in protection and repair of damage caused by UVR, were determined. Enzyme activities in the animals exposed to or protected from UVR showed a direct relationship with food P : C ratio that fit exponential models. Although GST and CAT differed slightly in their response to UVR, both enzymes were significantly affected by food quality: In low P : C treatments, there was significantly lower enzyme activity in response to UVR for both enzymes. Low food quality (less P for biosynthesis) may also impose a weaker antioxidant response on the organisms, a response of considerable ecological relevance in transparent Andean lakes which combine high UVR intensities with low seston P : C ratios.

The concept of “ecological stoichiometry” has been applied to describe the role of multiple chemical elements in controlling trophic processes and has even been proposed as a new branch of ecology (Sterner and Elser 2002; Andersen et al. 2004). It is now well established that stoichiometric constraints are important in regulating organism growth and nutrient cycling in food webs (Sterner and Elser 2002). In particular nitrogen (N) and phosphorus (P) are both structurally and functionally important in all organisms (Sterner 1995; Elser et al. 1996), often limiting primary and bacterial production (Vrede et al. 1999) and consumer growth (Gulati and DeMott 1997; Elser et al. 2000a; Ferrao-Filho et al. 2007). Moreover, it has been shown that carbon (C) : N : P stoichiometry is related to the elevated protein synthesis during rapid growth due to allocation to P-rich ribosomal ribonucleic acid (rRNA; Elser et al. 1996, 2000b). Since chemical reactions in living organisms are catalyzed by enzymes, the vast majority of which are proteins, it follows that stoichiometric constraints may be also crucial for enzymatic activities.

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Evolution has crafted thousands of enzymes that are efficient catalysts for a plethora of reactions. Among them, catalase (CAT) and glutathione S-transferases (GSTs) play an important role against oxidative stress caused by ultraviolet radiation (UVR; Borgeraas and Hessen 2000, 2002). Organisms are affected by UVR when key macromolecules (deoxyribonucleic acid [DNA], protein, chlorophyll) absorb specific wavelengths, altering important physiological or biochemical processes (Siebeck et al. 1994; Gonçalves et al. 2002). However, aquatic organisms can be also negatively affected by UVR through the generation of reactive oxygen species (ROS). The most long-lived ROS, hydrogen peroxide (H_2O_2), is of special interest because it is readily diffusible across cell membranes and functions as a signaling molecule in diverse cellular events. The generation of H_2O_2 is also associated with damage to DNA, proteins, and lipids and the induction of apoptosis (Martindale and Holbrook 2002). CAT, a widely distributed enzyme that reduces H_2O_2 , is important against oxidative stress (Barata et al. 2005) and, even if it is not essential, the lack or malfunction of catalases may lead to severe defects including high mutation (Cho et al. 2000). On the other hand, GSTs, a family of cytosolic multifunctional enzymes, are detoxifying enzymes that are present in all aerobic organisms (Hayes and Pulford 1995). They catalyze the conjugation of glutathione with a variety of reactive electrophilic compounds, thereby neutralizing their active electrophilic sites and subsequently making the parent compound more water soluble. Additionally, GST has been found to be involved in the removal of reactive organic hydroperoxides, such as the products of lipid peroxidation (Bartling et al. 1993).

Planktonic organisms are exposed to potentially harmful sunlight because of high intensities or damaging ultraviolet

wavelengths of light (UVA and UVB). Protection in planktonic organisms from the direct and indirect effects of UVR involves a variety of mechanisms, and, in case of damage, mechanisms of DNA repair (Gonçalves et al. 2002) and antioxidant enzymes (Hessen et al. 2002; Souza et al. 2007) may be important. The expression of CAT and GST have been previously reported in *Daphnia* species (Borgeraas and Hessen 2000, 2002) and in copepods (Souza et al. 2007).

In lake ecosystems, high sestonic C:P ratios (low P:C ratios) are associated with high light:phosphorus ratios (Sterner et al. 1997). Therefore, herbivore consumers living in transparent lakes would be constrained by poor stoichiometric food quality and would also be exposed to a potentially hazardous UVR regime. Early studies on *Daphnia pulex* have suggested that better nutritional status may contribute to a greater UVR tolerance (Zellmer 1996). Andean lakes are characterized by the high transparency and high UVR penetration (Morris et al. 1995). *Daphnia* species are considered to be highly P-demanding organisms (De Mott et al. 2001), and previous studies in Andean lakes have shown that seston P:C ratio would result in an important factor in the distribution of *D. commutata* (Balseiro et al. 2007). Based on this, we hypothesize that stoichiometric constraints would increase animal vulnerability to UVR by limiting enzymatic defenses against oxidative stress (CAT and GST). In this way, additional effects of low food quality are considered that may affect final fitness in very transparent lakes. Considering the high P requirement of *Daphnia*, we analyze this effect in a laboratory experiment with *D. commutata* examining the effect of food quality (in terms of P:C ratio) on the response of antioxidant enzymes to UVR. Additionally, we discuss the implications of our findings for the natural populations of *Daphnia* in transparent lakes.

Methods

Field survey—Eight large Andean lakes of glacial origin with deep basins ($Z_{\max} > 100$ m) located between 40°40'S and 42°49'S and 71°38'W and 71°44'W (North Patagonia, Argentina) were sampled during the summer seasons from 2000 to 2005. The lakes are included in the Nahuel Huapi National Park (Lakes Correntoso, Espejo, Nahuel Huapi, Moreno Oeste, Gutiérrez, and Mascardi), and Los Alerces National Park (Lakes Rivadavia and Futalaufquen). The region's climate is cold-temperate with an annual precipitation of 1500 mm and a mean annual temperature of 8.7°C. The surrounding vegetation consists of a mixed forest of *Nothofagus dombeyi* (Mirb.) Blume and *Austrocedrus chilensis* (D. Don) Florin et Boutleje. The lakes exhibit a warm-monomictic thermal regime, with stable thermal stratification during late spring and summer (December–March). Lakes are ultraoligotrophic (total P $< 6 \mu\text{g L}^{-1}$), and transparency is extremely high with very low diffuse attenuation coefficients ($K_{d\text{PAR}} = 0.10\text{--}0.16 \text{ m}^{-1}$; Morris et al. 1995).

Lakes were sampled under stable thermal stratification (January and February), at a central sampling point located at the deepest part of each basin. All sampling

was carried out at midday, 1 h before solar noon. Attenuation coefficients and nutrient concentrations did not change from previous sampling programs (e.g., summer 1993–1994; Morris et al. 1995). Based on these observations, the data from the different summer seasons (2000–2005) were pooled and averaged because they did not vary from year to year. Temperature and light vertical profiles (0 m to 50 m) of UV bands (305 nm, 320 nm, 340 nm, and 380 nm), and photosynthetic active radiation (PAR, 400–700 nm) were measured with a PUV500B submersible radiometer (Biospherical Instruments). Water samples of 12 L were obtained with a Schindler-Patalas trap from depths of 5 m, 10 m, and 30 m in all lakes except for Lake Nahuel Huapi, where the deep sample was collected at 40 m. Water samples were then transferred to acid-washed polypropylene containers, which were kept in darkness, thermally insulated, and immediately returned to the laboratory. Zooplankton of each lake were sampled with vertical tows from depths of 0–10 m, 10–30 m, and 30–50 m performed with a closing net (55- μm mesh size).

A volume of 300 mL of lake water from depths of 5 m, 10 m, and ~ 30 m (and in Nahuel Huapi at a depth of 40 m) was filtered through an 80- μm plankton net, in order to eliminate most of the zooplankton, and the filtrate was placed onto pre-combusted glass-fiber filters (GF/F Whatman filters) to assess the elemental composition of the seston of each lake. Filters were dried at 60°C for 48 h and stored at -20°C until analysis; they were analyzed for C, N, and P. Crustacean zooplankton were examined under a stereomicroscope in 5-mL Bogorov chambers.

Experimental conditions and experiment design—The experiment was designed as a factorial experiment with two factors (food P:C and UVR exposure) with three levels of food P:C and two levels of UVR exposure.

To obtain different levels of food P:C, algae were grown in three chemostats with different nutrient treatments and light intensities, following Hessen et al. (2002). Two chemostats were run with full Marine Biological Laboratory (MBL) medium (Guillard and Lorenzen 1972) and had 25 $\mu\text{mol P L}^{-1}$ and were exposed to 20 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ or 40 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (P:C level high [H] and medium [M], respectively; for values see Results). The other culture was grown with the same medium but with reduced P (1.25 $\mu\text{mol P L}^{-1}$) and exposed to 85 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (P:C level low [L]). For the three different treatments, actual concentrations of total particulate P and C were monitored by routine analysis (see Data analyses). A peristaltic pump supplied medium at a constant flow (0.25 d^{-1}) to each chemostat and the growing chamber was mixed by bubbling with air filtered through a 0.2- μm filter. *Chlamydomonas reinhardtii* were allowed to grow for at least 2 weeks before the start of *Daphnia* experiments in order to obtain a stable level of cell concentration number, volume, and particulate C and P. Algal cells were examined and measured microscopically using Image-Pro Plus (Media Cybernetics) software in order to assess differences in cell size or biovolume between treatments.

To obtain food for the *Daphnia* experiments, a known volume of algal culture from the three nutrient–light level chemostats was first centrifuged at $3000 \times g$, and the supernatant was discharged. The pellet was resuspended in Milli-Q water and subjected to particulate C and P analyses. Based on these measurements and with the same procedure, we collected and added chemostat algae in order to reach a concentration of $30 \mu\text{mol C L}^{-1}$ (similar C concentration to that of Lake Mascardi) in 500-mL experimental beakers containing *D. commutata*.

A clonal population of *D. commutata* was started from a single female isolated from the population of Lake Mascardi (Nahuel Huapi National Park, Patagonia, Argentina). The clone was maintained under laboratory conditions ($15 \pm 1^\circ\text{C}$ and $85 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ with a 14:10 light:dark photoperiod) fed with *C. reinhardtii*, for at least 20 generations prior to starting the experiment.

The experiment was begun by transferring 12 *Daphnia* neonates (<24-h old) to each flask with the corresponding food P:C level. We ran each food treatment with 20 replicates and every day; water from one half of the 500-mL beaker was replaced, and new food was added. Actual concentrations of total particulate P and C were monitored by routine analysis before adding new food to flasks. All glassware and pipettes were carefully cleaned and sterilized. Experiments were run in an incubator at 15°C (similar to lake temperature) with a 14:10 (light:dark, $60 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) photoperiod.

After 12 d of growth under these conditions and just before reproduction began, *D. commutata* adult individuals were subjected to the two UVR-exposure experiments (half of the replicates of each food P:C level were exposed, and the other half was protected). Prior to this, the body length and area of each animal were measured by taking lateral images and then processing the image via Image-Pro Plus, (Media Cybernetics) software, following Acharya et al. (2004) and Balseiro et al. (2007). These measurements were converted to dry weight (dry wt) based on our own length–weight regression obtained from the *Daphnia* culture and field-collected individuals. Seven to nine non-injured adults of approximately the same size were used in UVR treatments. Animals in 20-mL quartz tubes were exposed to UVR for 6 h in an incubator at 15°C . Another set of tubes was run in the same conditions but wrapped with aluminum foil to protect them from the UVR source. Each treatment was run in seven replicates. UVR was provided by two UVA-340 fluorescent tubes (Q-Panel Lab Products) placed 30 cm from the experimental tubes. The UV spectrum of these light tubes closely resembles the solar spectrum between 280 nm and 350 nm (Shick et al. 1999). During the incubation, animals received $35 \mu\text{W cm}^{-2} \text{nm}^{-1}$ of 340-nm wave band, an irradiance level of the wave band that was equivalent to surface sunlight in Andean lakes during summer. The total 340-nm wave-band dose was of $7600 \text{ J m}^{-2} \text{nm}^{-1}$. During this UVR exposure, no *Daphnia* mortality was observed.

Biochemical determinations—Carbon was analyzed on a Thermo Finnigan EA1112 CHN elemental analyzer. Total phosphorus (TP) and total particulate phosphorus (TPP) were analyzed with persulfate digestion followed by

molybdate reaction (APHA 1989). All determinations were carried out in at least three replicates.

After the 6 h of exposure (UVR-exposed or UVR-protected) *D. commutata* specimens were collected and immediately frozen at -20°C until enzymatic and protein determinations were made. 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} ethylene diamine tetra-acetic acid (EDTA) and 0.1% Triton X-100 according to Borgeraas and Hessen (2000). Homogenates were centrifuged at $10,000 \times g$ for 10 min at 4°C and the supernatants were analyzed for enzyme activity. Measurements of enzymatic activities were carried out using a Shimadzu 2450 spectrophotometer at $23 \pm 0.5^\circ\text{C}$.

Total GST activity was measured according to Habig et al. (1974) in 0.1 mol L^{-1} phosphate buffer, pH 6.5, with 0.1 mg mL^{-1} 1-chloro-2,4-dinitrobenzene (CDNB) in acetonitrile (1% v/v) and 0.75 mg mL^{-1} L-glutathione reduced (GSH) as substrates recording the absorbance at 340 nm. GST activity was expressed in nmoles of product developed per minute per individual and per mg of animal dry weight.

CAT activity was measured in 50 mmol L^{-1} phosphate buffer, pH 7.0, containing H_2O_2 (0.6% v/v) by the decrease in absorbance at 240 nm due to H_2O_2 consumption as described by Beers and Sizer (1952). Specific activity was expressed in μmoles of substrate hydrolyzed per minute per individual and per mg of *D. commutata* dry weight.

Protein concentration assay was performed according to Lowry et al. (1951) with bovine-serum albumin as the standard.

Data analyses—Differences in food P:C content, *Daphnia* biomass, and protein content were tested by one-way ANOVA. Differences in enzyme activities (CAT and GST) were tested by two-way ANOVA. An a posteriori Tukey test was applied when the overall ANOVA was statistically significant. Data were log-transformed when needed in order to fulfill assumptions of normality or homoscedasticity. Biomass, protein content, and enzyme activity data were fitted to an exponential rise to maximum model ($y = a[1 - e^{-bx}]$) where x is food P:C ratio. All statistical analyses were performed using SigmaStat 3.5.

Results

Field survey: *Daphnia* constraints in Andean lakes—Andean lakes are highly transparent with deep euphotic zones, reaching 47 m in the case of the large Lake Nahuel Huapi (Table 1, see $Z_{1\%PAR}$). The UVR bands also reach relatively deep layers. The depth of 1% penetration for UV-B (305 nm) ranges from 4 m to 10 m and from 5 m to 17 m for UV-A (340 nm; Table 1). This means that organisms living or migrating to the upper 17 m would be affected by these potentially hazardous bands or the indirect effect of UVR (i.e., ROS generation).

Sestonic P:C ratios were generally low, ranging from 0.68 in Lake Correntoso up to 2.08 in Lake Rivadavia (Table 1). In our eight-lake survey, the five lakes with lower P:C ratio lacked *Daphnia* (Table 1). Sestonic P:C ratio were significantly lower in lakes without *Daphnia* (Student

Table 1. Sestonic P:C ratio, light features, and *Daphnia commutata* presence in the eight sampled Andean lakes. Lakes were sampled in summer under stable thermal stratification (January and February), at a central sampling point located at the deepest part of each basin. Seston P:C corresponds to ranges of samples taken at depths of 5 m, 10 m, and 30 m (40 m for Nahuel Huapi).

Lake	P:C ($\mu\text{mol P} \cdot [\text{mmol C}]^{-1}$)	PAR	$Z_{1\%}$ (m)				<i>Daphnia</i> presence
			UVR (nm)				
			305	320	340	380	
Correntoso	0.68–0.91	41	9	10	12	18	No
Espejo	0.82–1.04	35	8	9	12	17	No
Gutiérrez	0.69–1.11	38	8	10	13	20	No
Nahuel Huapi	1.16–1.34	47	10	13	17	26	No
Moreno	1.37–1.45	38	7	8	11	19	No
Mascardi	1.39–1.82	30	5	6	9	13	Yes
Rivadavia	1.65–2.08	22	4	4	5	8	Yes
Futalaufquen	1.54–1.69	29	5	6	7	12	Yes

t-test, $t = -2.71$, $df = 8$, $p = 0.0299$). Previous studies have shown that *Daphnia commutata* was present in abundance up to 5 ind. m^{-3} in Lakes Mascardi, Rivadavia, and Futalaufquen exhibiting a maximum size of 2.68 ± 0.05 mm in Lake Rivadavia. During daytime, *D. commutata* remains at depths of 30 m, migrating near the surface during nighttime (Balseiro et al. 2007).

Food quality and UVR-exposure experiments—Algal-cell size distributions were almost identical among the three different nutrient and light treatments (ANOVA, $F_{2,102} = 2.4$, $p > 0.05$) indicating that food was given in a similar grazable range for *D. commutata*. In all the three P:C levels, *C. reinhardtii* cells were nearly spherical (maximum diameter = 7.32 ± 0.18 μm and minimum diameter = 6.05 ± 0.21 μm). The mean P:C ratio ($\mu\text{mol P} \cdot [\text{mmol C}]^{-1}$) of these cultures covered a 7.3-fold range: 6.05 $\mu\text{mol P} \cdot \text{mmol C}$ (atomic C:P 165; P:C level H), 1.70 $\mu\text{mol P} \cdot \text{mmol C}$ (atomic C:P 588; P:C level M), and 0.83 $\mu\text{mol P} \cdot \text{mmol C}$ (atomic C:P 1204; P:C level L; Fig. 1). The differences in the P:C ratios were highly significant (ANOVA: $F_{2,40} = 125.7$, $p < 0.001$; Tukey a posteriori test: all pairs $p < 0.001$).

After 12 d of experiment, just before reproduction began in the higher P:C food, *Daphnia* reared under high and medium food quality (P:C level H and M) attained similar biomass, while animals reared under low food quality (P:C level L) attained significantly lower biomass (one-way ANOVA: $F_{2,31} = 7.37$, $p = 0.002$; Tukey a posteriori test: low P:C vs. medium P:C, $p = 0.003$; low P:C vs. high P:C, $p = 0.015$; medium P:C vs. medium P:C, $p > 0.05$; Fig. 2). The data fit an exponential model (Table 2). The same response was observed in protein content although with a lower initial slope (Fig. 2, Table 2, see coefficient b). Therefore, the three food-quality conditions affected individual biomass and protein content of *D. commutata*, which were then subsequently exposed or not to UVR. The different P:C ratio of the food resulted also in individuals with differences in their P:C body content. High and medium P:C treatments (H and M) resulted in rather similar values of body P:C ratio (13.16 ± 0.789 $\mu\text{mol P} \cdot \text{mmol C}$ and 12.07 ± 0.675 $\mu\text{mol P} \cdot \text{mmol C}$, respectively) while low P:C treatment resulted significantly lower (7.88 ± 1.21 $\mu\text{mol P} \cdot \text{mmol C}$) than the other two treatments (one-way ANOVA: $F_{2,11} = 9.14$, $p = 0.006$; Tukey a posteriori test: low P:C vs. medium P:C, $p = 0.019$; low P:C vs. high P:C, $p = 0.006$; medium P:C vs. medium P:C, $p > 0.05$).

After the experimental irradiation with UVR we observed that all of the exposed and protected individuals were alive, and no differences in swimming behavior were detected, suggesting a sublethal effect of UVR.

Enzyme activities (GST and CAT; expressed on a per-individual or per-biomass basis) in the animals reared under the three food conditions showed a direct relationship with food P:C ratio (Fig. 3, black bars). Enzyme activity consistently increased due to UVR exposure (Figs. 3, 4, gray bars). Nevertheless, the two enzymes were significantly affected by the food quality (Table 3). We found that the activities of both enzymes were lower under

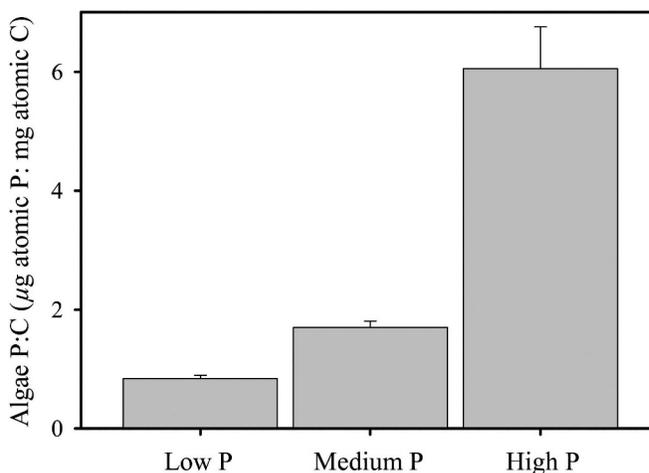


Fig. 1. Food P:C ratio ($\mu\text{mol P} \cdot \text{mmol C}$) in the three algal cultures with different P concentrations and light intensities. This algal biomass was then used as food for the *Daphnia* experiments. Error bars: 1 SE.

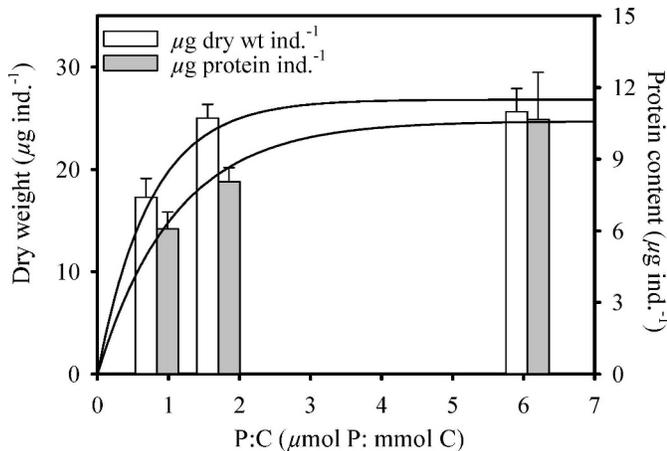


Fig. 2. Biomass (white bars) and protein content (gray bars) of *Daphnia commutata* after 12 d growth in each food P:C treatment. Fitted curves correspond to an exponential raise to a maximum model (see Table 2). Error bars: 1 SE.

a lower P:C ratio and that the obtained values also fitted exponential models (Fig. 3, Table 4).

While we observed differences in the response of each enzyme to UVR, the enzymes differed in the nature of their responses. GST activity increased in exposed animals by two-fold in almost all treatments (Tukey a posteriori test, UVR vs. protected: $p = 0.004$, $p = 0.015$, and $p = 0.004$ for low, medium, and high P:C, respectively, in terms of biomass) with a slight tendency to keep increasing towards the best food quality (high P:C). In contrast, CAT activity did not increase at low food P:C (Tukey a posteriori test, UVR vs. protected: $p > 0.05$ in terms of biomass); a significant increase in the activity of this enzyme was observed only at medium and high P:C ratios (Tukey a posteriori test, UVR vs. protected: $p = 0.033$ and $p = 0.042$, respectively, in terms of biomass).

Discussion

Food quality and UVR exposure—The C:N:P stoichiometry in invertebrate metazoa is driven by differences in allocation to P-rich ribosomal RNA (rRNA) to meet the protein-synthesis demands associated with differences in characteristic specific growth rates of particular taxa and/or life stages (Elser et al. 1996, 2000b). Ribosomes constitute a central part of the biosynthesis machinery in all cells since they are the structures where both structural and enzymatic proteins are synthesized. This “growth-rate hypothesis” (GRH; Sterner and Elser 2002) proposes that there is a

positive relationship between rRNA concentration and specific growth rate and that the P in rRNA makes up a significant fraction of the total P in invertebrate organisms. Since protein-synthesis rate depends to a large extent on the number of ribosomes rather than their efficiency (Nomura et al. 1984), a high growth rate should be closely related to the protein-production rate and to the amount of rRNA in a cell. Finally, the rRNA is expected to increase together with the specific growth rate with increasing food (substrate) concentration and/or quality. However, ribosomes also synthesize key protein enzymes needed for body maintenance and repair, such as the enzymes involved in response to UVR exposure. This suggests that P-limitation may affect UVR response if P-limitation impairs an animal’s ability to synthesize necessary repair enzymes. Our study on *Daphnia commutata* showed a decrease in the activity of two photoxidative enzymes because of dietary P limitation. The fact that in the L treatment level, the *Daphnia* P:C body content were significantly lower, indicates that the P limitation exerts an important effect in enzymatic response. This suggests that variation in *Daphnia*’s food P content, and consequently in body P content, resulted in differences in P allocation for enzyme synthesis. The decrease in enzyme activity per protein content indicates that not only were the proteins reduced under poor food quality, but also that the investment in these enzymes was proportionally lower than other enzymes. Our results indicate that P:C stoichiometry plays a central role in fitness response to UVR, an important ecological factor.

Early studies indicated that different species of zooplankton use diverse strategies for UVR protection, and some species have been shown to be more tolerant than others (Siebeck et al. 1994). At temperate latitudes, copepods are more UVR tolerant than cladocerans (Leech and Williamson 2000). However, comparing antioxidant protective mechanisms, the average CAT activity obtained in cladocerans was significantly higher (4–5-fold) than that of calanoid copepods under the same conditions (Souza et al. 2007). CAT activity has been associated with UVR response of freshwater cladocerans to harmful photochemicals such as H_2O_2 and free radicals (Barata et al. 2005). In addition, CAT contains four tightly bound molecules of reduced nicotinamide adenine dinucleotide phosphate (NADPH), and, although this dinucleotide is not essential for enzyme activity, its presence decreases the susceptibility of the enzyme to be inactivated when exposed to H_2O_2 (Kirkman and Gaetani 1984). NADPH is generated mostly in the pentose phosphate pathway (PPP), an alternative

Table 2. Results of the fitted model $y = a(1 - e^{-bx})$ of *Daphnia* biomass and protein content after 12 d growing under the three food P:C levels. Model coefficients a and b correspond to the constants origin and slope of the equation.

Variable	Model coefficients		R^2	df	F model	p model
	a	b				
Biomass ($\mu\text{g dry wt ind.}^{-1}$)	26.83	1.340	0.303	1,32	13.888	0.00075
Proteins ($\mu\text{g ind.}^{-1}$)	10.597	0.918	0.286	1,16	6.417	0.0221

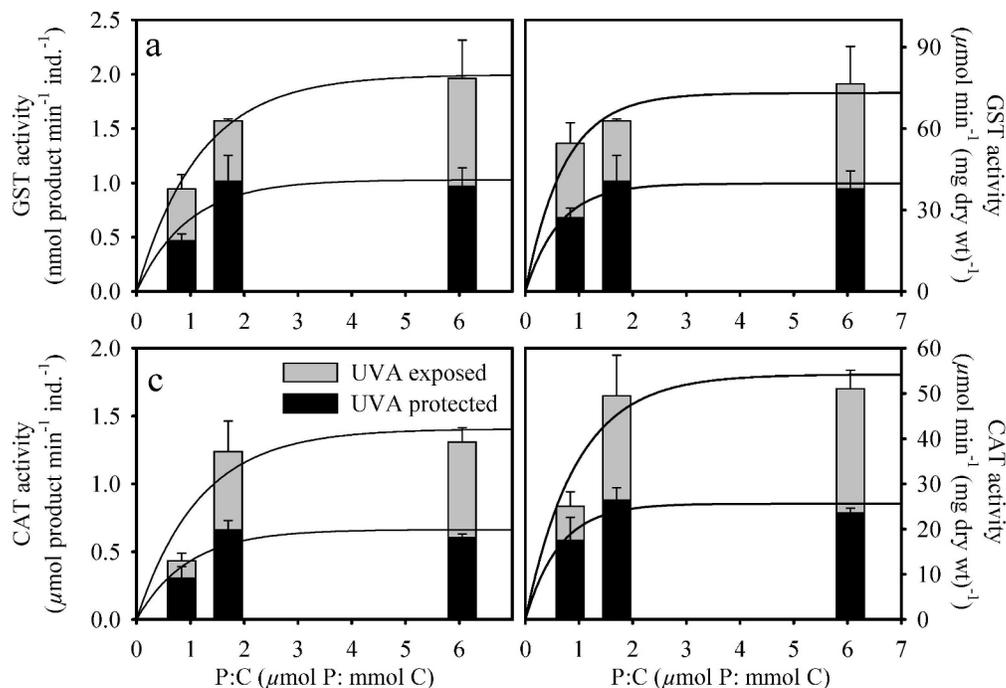


Fig. 3. Enzymatic activity of *Daphnia commutata* grown in each food P:C treatment and then protected (black bars) or exposed (gray bars) to UVR. (a) GST per individual, (b) GST per biomass, (c) CAT per individual, (d) CAT per biomass. Fitted curves correspond to an exponential raise to a maximum model (see Table 4). Error bars: 1 SE.

Table 3. Results of the two-way ANOVA test of the antioxidant enzyme (GST and CAT) activity under the different food qualities (P:C food) and UVR treatments.

Variable	Factor	df	F	p
GST ind. ⁻¹	P:C food	2,15	14.57	0.0003
	UV	1,15	30.15	<0.0001
CAT ind. ⁻¹	P:C food	2,15	5.79	0.0146
	UV	1,15	8.31	0.0120
GST (μg dry wt) ⁻¹	P:C food	2,15	5.60	0.0152
	UV	1,15	30.15	<0.0001
CAT (μg dry wt) ⁻¹	P:C food	2,15	6.68	0.0091
	UV	1,15	10.83	0.0053

pathway to glycolysis. While it does involve oxidation of glucose, its primary role is anabolic rather than catabolic. The PPP has both an oxidative and a non-oxidative arm. The oxidation steps, utilizing glucose-6-phosphate as the substrate, occur at the beginning of the pathway when NADPH is generated. The non-oxidative reactions of the PPP are primarily designed to generate ribose5P. Therefore, in our experiments with low food P:C, *Daphnia* was probably not able to react to a stress condition (i.e., UVR) both because of low enzymatic synthesis (low protein content, low rRNA) and low NADPH availability. In animals with low availability of P, a marked reduction in PPP can occur in order to increase glycolysis. On the other

Table 4. Results of the fitted model $y = a(1 - e^{-bx})$ of the enzymatic activities of *Daphnia* grown under the three food P:C levels and UVR treatments. References: +UVR = exposed; -UVR = protected. Coefficients a and b are the constants of the model $y = a(1 - e^{-bx})$.

Variable		Model coefficients		R ²	df	F model	p model
		a	b				
GST (ind. ⁻¹)	-UVR	1.029	1.046	0.377	1,7	4.251	0.0782
	+UVR	1.997	0.856	0.637	1,10	17.613	0.0018
CAT (ind. ⁻¹)	-UVR	0.663	1.055	0.520	1,7	6.506	0.0434
	+UVR	1.406	0.864	0.358	1,10	5.598	0.0396
GST (μg dry wt) ⁻¹	-UVR	39.77	1.568	0.201	1,7	1.763	0.2259
	+UVR	73.11	1.395	0.259	1,10	3.495	0.0910
CAT (μg dry wt) ⁻¹	-UVR	25.63	1.584	0.277	1,7	2.302	0.1799
	+UVR	54.206	1.057	0.283	1,10	3.955	0.0747

hand, it has been shown that low food quality (low P:C) increases respiration rate in *Daphnia* to compensate for the excess of C (Darchambeau et al. 2003; Jensen and Hessen 2007; He and Wang 2008). Additionally, Sterner and Elser (2002) indicate that herbivores can also increase DOC losses, and He and Wang (2008) showed a decrease in the P content of molts and excretion as a way to cope with the excess of C in the food. However, the increase in respiration directly affects enzymatic activity of CAT. In this way, glycolysis may be enhanced to cope with the excess of C, but this increased respiration also increases endogenous H₂O₂, which needs CAT to hydrolyze it. This means that almost all available CAT should be active, and a shortage may occur in response to an additional oxidative stressor such as UVR. Consistent with this, in the low P:C treatment, CAT did not increase when exposed to UVR.

Glutathione-associated metabolism is a major mechanism for cellular protection against oxidative stress, since it provides defenses not only against ROS but also against their toxic products. In particular, GSTs exhibit glutathione peroxidase activity toward lipid hydroperoxides generated during UVR oxidative stress (Collinson and Grant 2003). Lipid peroxidation products formed by the free-radical-mediated attack on membrane lipids can propagate an autocatalytic chain of lipid peroxidation in the presence of oxygen, eventually leading to membrane destruction (Cho et al. 2000). Lipid peroxidation products can also cause DNA damage. Hence, the prevention of lipid peroxidation is an essential process in all aerobic organisms. Our experiment showed that the activities of both enzymes (CAT and GST) are affected by animal nutrient status. Nevertheless, *Daphnia* GST showed greater differences under varying food P:C ratios, a result that may indicate the importance of GST in cell photooxidative response preventing peroxidation in this organism. The P shortage generated by low P:C food condition would also affect this enzyme through the NADPH generated in the PPP. This NADPH is essential to convert oxidized glutathione (GSSG) to reduced glutathione (GSH), which is a substrate for GST. Therefore, the increased glycolysis mentioned above would also affect GST activity. As a result, *Daphnia* grown under severe P limitation would be unprotected against further stressors.

Daphnia constraints in transparent lakes—High C:P ratios (low P:C) in lakes are associated with high light:phosphorus ratios (Sterner et al. 1997). The extremely transparent Andean lakes (all $K_d < 0.21 \text{ m}^{-1}$; Morris et al. 1995; Balseiro et al. 2007) would expose the large zooplankton both to broad levels of UVR exposure and to a low food quality due to an unbalanced elemental ratio (Balseiro et al. 2007; this study). The high transparency also implies increased visual predation risk (Gliwicz 2003). Consistent with this, a recent study on *Daphnia* distribution in Andean lakes has indicated that, in very transparent lakes with low food quality, species like large *Daphnia* are also exposed to higher visual predation (Balseiro et al. 2007).

In addition to low P:C, transparent lakes have low food concentration (in terms of autotrophic biomass, as C

content or Chlorophyll *a*). Although we did not carry out experiments with different food quantities (C concentration), the food levels used in our experiments are within the range observed in Andean lakes, sufficient to allow *D. commutata* growth and persistence in some Andean lakes (Balseiro et al. 2007; this study). Furthermore, it has been demonstrated that food quality in terms of P:C is still important at low food quantities (Boersma and Kreutzer 2002; Hessen et al. 2002). In our experiments, the medium and low P:C conditions resemble those of Andean lakes: medium P:C ratio coincides with those observed in Lake Mascardi where *D. commutata* population develops, while our low P:C treatment corresponds to those lakes where *D. commutata* growth is constrained (Balseiro et al. 2007). The higher P:C ratio in our experiments represents a condition not observed in Andean lakes but, based on theoretical and empirical evidence, it is above the threshold of P limitation for *Daphnia* (Urabe and Watanabe 1992).

Our study also showed a good correlation of food P content with grazer protein content. The fact that protein content, as well as enzyme activity, decreases under low food quality imposes another bottleneck for *Daphnia* colonization, particularly in highly transparent lakes where UVR contributes to the decrease in fitness. In agreement with previous studies our results indicate that food P:C ratio affects *Daphnia* growth. In the present study we analyzed the existence of a relationship between P limitation and enzymatic response to UVR. Additional studies on the effect of different levels of UVR would bring about clarity on this relationship in high P-demanding organisms.

However, our results highlight the ecological relevance of stoichiometric-UVR interactions affecting *Daphnia* success in transparent lakes. This finding could also have strong implications for our understanding of life in transparent aquatic environments where high P:C ratios and high UVR are likely to occur.

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