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Antioxidant Defences in Planktonic Crustaceans Exposed to Different Underwater Light Irradiances in Andean Lakes

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Abstract In freshwater environments direct biological effect of ultraviolet radiation (UVR) result from absorption of specific wavelengths by macromolecules and alteration of biochemical processes. Indirect effects are related to UVR interaction with water and dissolved organic carbon to form chemically reactive species (ROS: reactive oxygen species). Zooplankton photoprotection includes mycosporine-like amino acids, pigments, production of quenching agents and antioxidant enzymes. The relative importance of each mechanism would depend on the organisms. In this study, we determined the antioxidant enzyme activities Catalase (CAT) and Glutathione-*S*-transferase (GST) in the copepod *Boeckella gracilipes* and the cladoceran *Ceriodaphnia dubia* in three Andean lakes of the North-Patagonia region. There were differences in antioxidant enzymes expression between copepods and cladocerans. CAT was significantly higher in *C. dubia* than in *B. gracilipes* whereas GST was similar in both species. The comparison of *B. gracilipes* enzyme activity in the three lakes showed also differences in GST but not in CAT. DOC decreases the exposure by absorption of UVR but simultaneously acts as photosensitizer producing ROS and their successive toxic products in the surface waters. We discuss that comparisons among lakes of different DOC should be considered carefully because lake physico-chemicals parameters, as well as food web structure, will difficult any predictions on the net effect of DOC.

Keywords antioxidant enzymes · UV radiation · catalase · glutathione-*S*-transferase · copepods · cladoceran · oligotrophic lakes

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1 Introduction

Depletion of the stratospheric ozone due to the release into the atmosphere of industrial chemicals containing chlorine or bromine (CFCs, Halons, etc.) has led to increases in ambient levels of biologically damaging ultraviolet radiation (UVR) on a global scale (Perin & Lean, 2004). South America is a region of particular interest for the study of UVR effects because of its proximity to the Antarctic ozone hole (Díaz et al., 2006). This enhanced exposure to UVR is potentially deleterious to all living forms including aquatic freshwater organisms. Direct biological effects of UVR on organisms result from absorption of specific wavelengths by macromolecules (DNA, protein, chlorophyll) and alteration of physiological or bio-

chemical processes. In freshwater environments, indirect effects are related to UVR interaction with water and dissolved organic carbon (DOC) to form a number of chemically reactive species and biologically toxic intermediates. Such chemical species (i.e., superoxides, hydrogen peroxides, hydroxide radicals and carbon monoxide) resemble a xenobiotic redox imbalance (Choudhury & Panda, 2005) that may be potentially more damaging on aquatic organisms than direct UVR exposure (Perin & Lean, 2004).

Photoprotection in planktonic organisms from the direct and indirect effects of UVR includes a variety of mechanisms: vertical migration offers the potential for behavioural avoidance of photodamage (Alonso, Rocco, Barriga, Battini, & Zagarese, 2004; Modenutti, Balseiro, Callieri, Bertoni, & Queimaliños, 2005), production or incorporation of UV-absorbing compounds like mycosporine-like amino acids (MAAs) or pigments (Moeller, Gilroy, Williamson, Grad, & Sommaruga, 2005) and production of quenching agents (Borgeraas & Hessen, 2002a). In case of damage, mechanisms as DNA repair (Goncalves, Villafane, & Helbling, 2002; Zagarese, Feldman, & Williamson, 1997), and antioxidant enzymes (Hessen, Borgeraas, & Orbaek, 2002) may act.

The presence of diverse photoprotective compounds in crustacean zooplankton such as carotenoid pigments, cuticular melanin, and non-pigmented MAAs was observed to be a response to high irradiance (Siebeck et al., 1994). In particular, MAAs are believed to acts as sunscreens filtering out the most damaging UV wavelengths of solar radiation and releasing the excess of energy as harmless heat (Obermüller, Karsten, & Abele, 2005; Perez et al., 2005). However, not all crustaceans are able to accumulate these compounds, since the presence of MAAs has been well documented in copepods while were undetectable in cladocerans (Moeller et al., 2005).

On other hand, UVR also can negatively affect aquatic organisms through the generation of ROS (Reactive Oxygen Species). The most long-lived ROS, the hydrogen peroxide (H_2O_2), is of special interest because can be transported through lake mixing processes (Scully, Vincent, Lean, & Cooper, 1997) and is readily diffusible across cell membranes functioning as a signalling 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 & Holbrook, 2002). Catalase, which decomposes H_2O_2 to water and O_2, is a widely distributed enzyme and is an important member of the cellular defence system against oxidative stress (Barata, Varo, Navarro, Arun, & Porte, 2005). Even if it is not strictly essential, the lack or malfunction of catalases may lead to severe defects, such as an increased susceptibility to injury and high rates of mutations (Cho, Park, & Lim, 2000). On the other hand, Glutathione *S*-transferases (GSTs), a family of cytosolic multifunctional enzymes, are detoxifying enzymes that are present in all aerobic organisms (Hayes & 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 was found to be involved in the removal of reactive organic hydroperoxides, such as the products of lipid peroxidation (Bartling, Radzio, Steiner, & Weiler, 1993). The expression of these antioxidant enzymes (CAT and GST) has been previously reported in *Daphnia* species (Borgeraas & Hessen, 2000, 2002a, 2002b) however, these defences were not extensively studied in copepods.

Tartarotti, Baffico, Temporetti, and Zagarese (2004) found that the amount of photoprotective compounds as MAAs varied according to the lake light climate. Accordingly, it could be hypothesized that antioxidant enzyme activity would be enhanced in clear lakes. Besides, the fact that the presence of MAAs has been documented in copepods but not in cladocerans let us expect that the relative importance of these enzymes would depend on the clade.

Andean lakes of the North-Patagonia region (around 41°S) cover a wide range of concentration of dissolved organic matter (Morris et al., 1995) that strongly influences the underwater field of radiation to which organisms are exposed. The main objective of this study was to analyze the incidence of different UV exposure conditions in a natural gradient of dissolved organic carbon (DOC) and the relative expression of antioxidant enzyme protective mechanisms in two zooplankton species. The calanoid copepod *Boeckella gracilipes* is a widespread species in Andean lakes dominating all lakes in the natural gradient of DOC. On the contrary, the cladoceran *Ceriodaphnia dubia* was observed during summer only in clear and deep lakes (Balseiro, Modenutti, Queimaliños, & Reissig, 2006). We compare antiox-

idant enzyme activities in these two species in lake Moreno Oeste, a clear lake with low DOC concentration (Morris et al., 1995). We also compared the influence of underwater light climate on *B. gracilipes* enzyme activities in three lakes representing a natural gradient of DOC concentration (Lakes Moreno Oeste, El Trébol and Escondido).

2 Material and Methods

2.1 Study Area and Sampling

The three studied lakes (Moreno, El Trébol and Escondido) belong to the Nahuel Huapi system (Patagonia, Argentina) located at 41°S and 71°W and 760 m above sea level. The three lakes are located in a Protected Area and free of industrial and agrochemical activities. Lake Moreno Oeste has a surface area of 6 km^2 and a maximum depth of 90 m. The lake is warm monomictic, remaining stratified from late November through April (spring–summer months). During the period of direct stratification the lake develops a marked thermocline around 30 m depth (Modenutti, Balseiro, & Queimaliños, 2000). The other two lakes are smaller and shallower: Lake El Trébol has a surface area of 0.3 km^2 and a maximum depth of 12 m; while Lake Escondido is of 0.08 km^2 and 8 m deep. Both lakes exhibit homogenous temperature profiles all over the year although short period of stratification may be observed (Balseiro & Modenutti, 1990; Balseiro, Modenutti, Queimaliños, 1997). In the three lakes, oxygen concentration is at 100% of saturation level along the water column.

Sampling was carried out in a central sampling point located at the deepest part of each basin (Lake Moreno Oeste: z=70 m; Lake El Trébol: z=12 m and Lake Escondido: z=8 m). In order to compare, enzymes activities the three lakes were sampled once during southern summer (13–17 January 2006). For further comparisons between copepods and cladocerans Lake Moreno Oeste was also sampled on early autumn (10 May 2006). All samplings were carried out in sunny days (without clouds) at midday 1 h before astronomic noon. Temperature, PAR (400–700 nm) and UVR (305, 320 and 340 nm) profiles were measured in each lake from surface to the bottom with a PUV 500B submersible radiometer (Biospherical Instruments). Concurrently, water samples (2 replicates) for DOC and Chlorophyll *a* concentration determination were taken from 0 to 60 m at 10 m intervals from Lake Moreno Oeste and from 0 to 8 or 12 m each 3 or 4 m interval from Lake Escondido or El Trébol, respectively. Lake water was filtered through GF/F and DOC was estimated by spectrophotometry through a regression model based on Morris et al. (1995). Previously, a spectrophotometric intercalibration was performed against a Shimadzu TOC 5000 DOC determination (D. Morris pers. comm.). DOC (mg L^{-1}) was estimated as the mean of three regressions $y = a_\lambda + b^*_\lambda Abs^c_{\lambda\lambda}$, where Abs_λ is the absorbance at wavelength 305, 320 and 340, and a_λ, b_λ and c_λ are constants for each wavelength (a_{305}=−0.171, b_{305}=0.847 and c_{305}=0.622; a_{320}=−0.049, b_{320}=0.819 and c_{320}=0.660 and a_{340}=−0.0679, b_{340}=1.053 and c_{340}=0.655). Absorbance was estimated in 10 cm Quartz (Suprasil) cuvettes with MilliQ water as blank, in a Shimadzu 2450 UV-Vis Spectrophotometer.

Chlorophyll a was extracted with hot ethanol (Nusch, 1980) and measured with a 10-AU fluorometer (Turner Design).

In the two shallow lakes (Escondido and El Trébol), zooplankton sampling was performed from near bottom to surface with a 200 μm mesh size net with closing mechanism. Lake Moreno zooplankton was sampled with vertical tows from 30 to 0 m (epilimnion) performed with the same net. All samples were obtained with at least three replicates.

Solar radiation data was provided by a GUV radiometer (Biospherical Instruments) located at the Estación de Piscicultura from Universidad Nacional del Comahue, about 10 km from sampling sites. The data were kindly provided by Dr. Zagarese.

2.2 Biochemical Determinations

Freshly collected specimens of each species were twice rinsed (0.2 μm filtered lake water) and frozen immediately at −20°C until biochemical measurements. A total of 30–40 individual of each species were pooled in each sample to ensure reliable results. 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 (2000). Supernatants of homogenates centrifugation at 10,000×g during 5 min were used as enzyme sources. From each lake, three samples were

assayed with a minimum of two replicated measurements per sample. Measurements of enzymatic activities were carried out using METROLAB 1700 spectrofotometer at 23±0.5°C.

Total GST activity was measured according to Habig, Pabst, and Jacoby (1974) in buffer 0.1 M phosphate pH 6.5, with 0.1 mg/ml 1-chloro-2.4-dinitrobenzene (CDNB) in acetonitrile (1% v/v) and 0.75 mg/ml GSH as substrates recording de absorbance at 340 nm. GST activity was expressed in nmoles of product developed per minute per mg of protein (nMoles prod. $min^{-1}mg^{-1}$ prot.).

CAT activity was measured in buffer 50 mM phosphate pH 7.0 containing H_2O_2 (0.6% v/v) by 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 mg of proteins (μMoles prod. $min^{-1}mg^{-1}$ prot.).

Protein concentration assay was performed according to Lowry, Rosenbrough, Farr and Randall (1951) with bovine serum albumin as standard.

2.3 Data Analysis

The diffuse extinction coefficients (Kd) were estimated as regression coefficients from light profiles obtained with the radiometer (PUV 500B) in the field.

Statistical significance was calculated using ANOVA. Homocedasticity and normality was previously checked, data were log transformed when needed. Data analyses were performed using Sigma Stat 3.01 package.

3 Results

3.1 Lake Features

Lake Moreno presented a direct stratification with a thermocline depth at 30 m depth on both occasions (summer and early autumn). Epilimnetic temperature ranged from 16 (January) to 15°C (May) and hypolimnion was at 7.5°C in both occasions. Lake Escondido exhibited a temperature gradient from 17°C (surface to near 4 m) to 12°C (8 m). On the contrary, Lake El Trébol was thermally homogenous along the water column (16°C).

According to the GUV data, both maximum irradiances and daily integrated values during the 3 days of sampling in January were very similar (Table 1). Unfortunately we don't have GUV data from the early autumn sample in Lake Moreno. Therefore, in our January sampling differences among lake light climate cannot be attributed to changes in atmospheric irradiances. But differences in DOC concentration among the three lakes analysed could create different UVR exposure conditions. The three lakes represented a natural gradient of DOC concentration, the lower DOC concentration was observed in Lake Moreno and the highest value was registered in Lake Escondido (Table 2). Therefore, lakes have different UVR exposure conditions (see Kd of the different wavelengths in Table 2) being Lake Moreno the environment with the highest exposure (upper 10 m of the epilimnion) and Escondido with the lowest exposure (only 0.45 m of the water column).

Table 1 Irradiance values obtained with a GUV of the sampling site during the study (January 2006)

	13/01/06	16/01/06	17/01/06
Integrated values			
305 nm (J cm^{-2})	0.174	0.172	0.185
320 nm (J cm^{-2})	0.930	0.927	0.9265
340 nm (J cm^{-2})	2.002	1.999	1.976
PAR (400–700 nm) (mol m^{-2})	70.735	70.485	70.010
Max. values			
305 nm (J cm^{-2})	8.3	8.2	8.8
320 nm (J cm^{-2})	34.4	34.4	34.4
340 nm (J cm^{-2})	69.6	69.6	68.6
PAR (400–700 nm) (mol m^{-2})	2,381	2,380	2,365

Table 2 Diffuse extinction coefficient (Kd in m^{-1}), Depth of 1% of surface irradiance ($Z_{1\%}$ in m) of 305, 320, 340 nm and PAR (400–700 nm), Dissolved Organic Carbon (DOC in mg/l, mean ± s.e.) concentration, and chlorophyll *a* (Chl *a* in μg l^{-1}) in the three studied Andean lakes

Lake	$K_{d\ 305}$	$K_{d\ 320}$	$K_{d\ 340}$	$K_{d\ PAR}$	$Z_{1\%\ 305}$	$Z_{1\%\ 320}$	$Z_{1\%\ 340}$	$Z_{1\%\ PAR}$	DOC	Chl *a*
Moreno	0.73	0.60	0.45	0.14	6.24	7.63	10.05	32.89	0.64±0.04	1.02
El Trébol	4.60	3.95	2.90	0.46	1.00	1.16	1.59	10.01	1.58±0.05	8.48
Escondido	18.50	12.80	10.31	0.58	0.25	0.36	0.45	7.94	4.52±0.06	2.13

3.2 Inter and Intra-Specific Enzyme Activity Comparisons

Field samples from Lake Moreno indicated differences in antioxidant enzymes expression among the two crustacean groups tested in both samples (summer and early autumn) (Figs. 1 and 2). Mean CAT activity was significantly lower in the copepod *Boeckella gracilipes* (206±7 μmoles of product $min^{-1}mg^{-1}$) than in the cladoceran *Ceriodaphnia dubia* (1267±56 μmoles of product $min^{-1}mg^{-1}$) while the differences between seasons were not significant (Two way ANOVA $P<0.001$ between groups and $P>0.05$ between seasons) (Fig. 1). On the contrary, the difference in GST activity was not significant between both species and seasons (Two way ANOVA $P>0.05$ between groups and seasons) (Fig. 2).

The effect of the gradient in DOC concentration on antioxidant defences was tested in populations of *B. gracilipes* of the Lakes Moreno, El Trébol and Escondido since *C. dubia* was not present in El Trébol and Escondido lakes.

No significant differences were observed in CAT activity among *B. gracilipes* from lakes with different DOC concentrations (Fig. 3a). On the contrary, GST activity showed significant differences among lakes (ANOVA $P<0.05$). In particular, we observed the highest value in GST activity in the lake with the highest DOC concentration (Lake Escondido, Table 1) and the lowest value in the lake with intermediate DOC concentration (El Trébol, Table 1), showing these two lakes significant differences (*a posteriori* Tukey test Escondido vs El Trébol, $P<0.05$, Fig. 3b).

4 Discussion

4.1 Differential Antioxidant Enzymes Expression as UVR-against Defence

Organisms inhabiting Patagonian clear lakes can potentially be exposed to high levels of UV radiation. From previous studies (Morris et al., 1995) we did not observed substantial changes in underwater irradiances. Zooplankton may be directly affected by the UVR at the upper depths (Table 2) or indirectly by an increment in ROS induced by UVR, some of which can subsequently be transported deeper by vertical

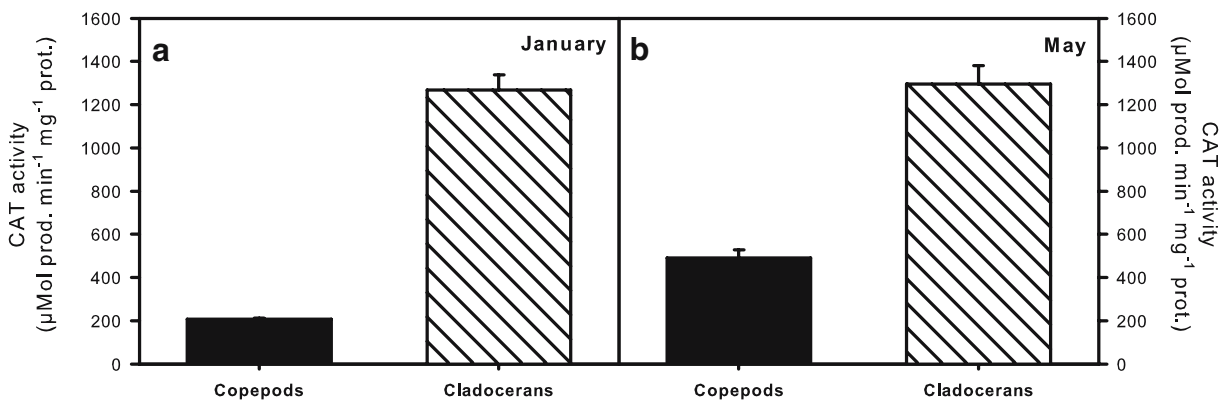

Fig. 1 Catalase activity from copepods and cladocerans of Lake Moreno Oeste. Mean catalase (*CAT*) activity was statistically significant lower in copepod *B. gracilipes* than cladoceran *C. dubia* as much in summer **a** as in autumn **b** sampling. Activity was expressed as mean of triplicate samples ± s.e.

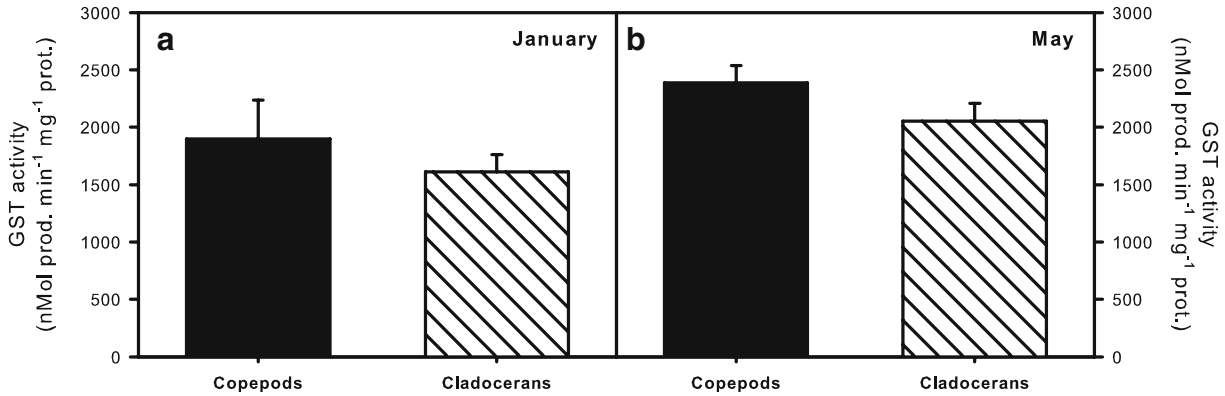

Fig. 2 Glutathione-*S*-transferase activity from copepods and cladocerans of Lake Moreno Oeste. No significant differences were detected in Glutathione-*S*-transferase (*GST*) mean activity between copepod *B. gracilipes* and cladoceran *C. dubia* neither in summer **a** nor in autumn **b**. Activity was expressed as mean of triplicate samples ± s.e.

mixing (for ROS like hydrogen peroxides that persist for a sufficient period of time, half-life 4–24 h) (Scully et al., 1997).

Planktonic organisms possess diverse defence mechanisms to avoid or reduce the damage. Previous studies indicate that different species of zooplankton use diverse strategies for UV protection and some species have been shown to be more tolerant than others. At intermediate latitudes copepods have been mentioned as more UV tolerant than cladocerans (Leech & Williamson, 2000). This situation can be explained by the presence of photoprotective compounds like MAAs, principals UV-screens compounds, in different freshwater cyclopoid and calanoid copepods (Edreva, 2005; Moeller et al., 2005; Tartarotti & Laurion, 2001). In addition, the role of carotenoids has been demonstrated in copepods but the levels in cladocerans are far lower and their function is not clear (Hessen & Sorensen, 1990). However, comparing antioxidant protective mechanism, the average CAT activity obtained in *C. dubia* (cladocerans) was significant higher (4–5 folds, Fig. 1) than those registered in *B. gracilipes* (copepods) under the same conditions. These results suggest a differential importance of this mechanisms against UVR and could be associated with the absent of MAAs in cladocerans. In contrast, no significant differences were detected in GST activity between both crustacean groups (Fig. 2). GSTs are a super family of enzymes that detoxify potentially hazardous reactive species from different origins, not necessarily associated with UV-exposure (metabolism, xenobiotic, etc).

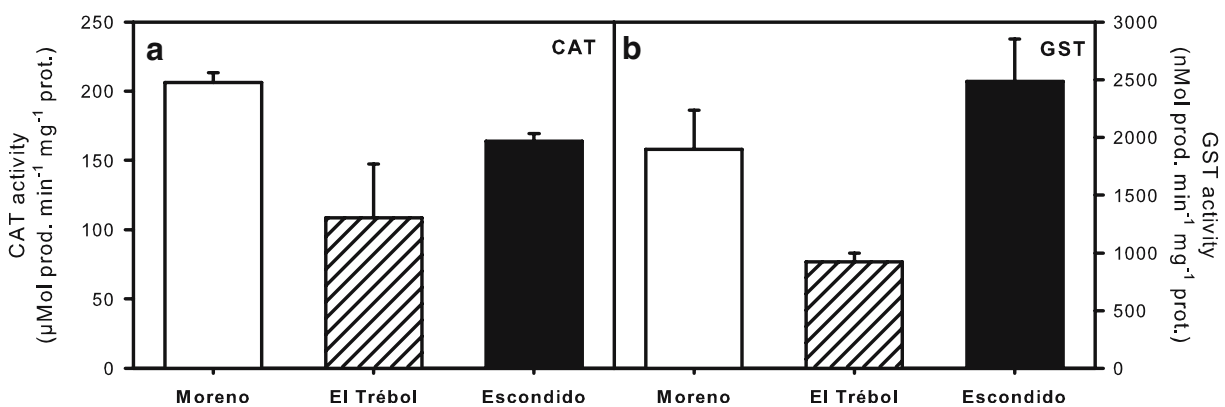

Fig. 3 Antioxidant enzymes activity from copepods of lakes with different DOC concentrations during summer sampling. **a** Catalase activity (CAT) expressed as mean of triplicate samples ± s.e. **b** Glutathione-*S*-transferase activity (GST) expressed as mean of triplicate samples ± s.e.

4.2 Intraespecific Antioxidant Expression with Different DOC Concentrations

We also evaluated enzyme activities among copepods of the same species in the three lakes with contrasting DOC concentration. The relationship between attenuation of UV and DOC is strong (Morris & Hargreaves, 1997; Morris et al., 1995). Moreover, it have been suggested that changes in DOC concentration in lake water could be even more important for underwater UV exposure than changes in stratospheric ozone concentrations (Williamson, Stemberger, Morris, Frost, & Paulsen, 1996). Thus, differences in DOC concentrations and, consequently in underwater UVR, would be related with distinctive enzyme activities. However, we observed significant differences only in GST activity, registering low values in the intermediate DOC lake El Trebol, and high in Moreno Oeste (low DOC) and Escondido (high DOC).

DOC, especially chromophoric dissolved matter, decreases the direct exposure by absorbing UVR but simultaneously can act as photosensitizer producing ROS in the surface waters (Borgeraas & Hessen, 2002b). ROS, including those generated from DOC-UVR interaction and water-UVR interaction as H_2O_2 and other free radicals, has been reported causing negative effect on planktonic populations (Hessen & Van Donk, 1994; Sinha & Hader, 2002). Glutathione-associated metabolism is a major mechanism for cellular protection against oxidative stress, providing defences not only against ROS but also against their toxic products. Particularly, Glutathione transferases exhibit glutathione peroxidase activity towards lipid hydroperoxides generated during oxidative stress as produced by UVR (Collinson & 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. We speculate that both low and high DOC concentration would drive to enhance GST activity because of direct UVR (e.g. increase in H_2O_2 in low DOC lakes) or through indirect effect of DOC-UVR (e.g., toxic derived products in high DOC lakes).

Nevertheless, lake El Trébol with intermediate DOC concentration but higher phytoplankton biomass (Table 2) showed an alternative situation. The high seston concentration produce a comparative higher light scattering that would decrease the direct and indirect effect of UVR. In fact, we observed that GST activity was lowered in this lake. Thus, as was also observed by Hessen et al. (2002)comparisons among lakes of different DOC should be considered carefully because lake physico-chemicals parameters, as well as food web structure, will difficult any predictions on the net effect of DOC.

Comparative studies on UV protection strategies raise a key issue in understanding organism distributions within water column or among lakes of contrasting UV transparency (Moeller et al., 2005). A differential use of these mechanisms would have an impact on the survival, growth, and reproduction of these zooplankters with consequences on food web interactions, community composition, biodiversity and ecosystem processes.

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