



J. Plankton Res. (2017) 00(00): 1–12. doi:10.1093/plankt/fbx028

Glacier melting and response of *Daphnia* oxidative stress

CECILIA LASPOUMADERES, MARÍA SOL SOUZA, BEATRIZ MODENUTTI AND ESTEBAN BALSEIRO*

LABORATORIO DE LIMNOLOGÍA, INIBIOMA, CONICET-UNCOMAHUE, QUINTRAL 1250, 8400 BARILOCHE, ARGENTINA

*CORRESPONDING AUTHOR: ebalseiro@comahue-conicet.gob.ar, ebalseiro@gmail.com

Received January 13, 2017; editorial decision April 20, 2017; accepted April 24, 2017

Corresponding editor: Karl Havens

We analysed the antioxidant response of *Daphnia commutata* in an oligotrophic North-Patagonian lake (Lake Mascaridi) that receives inputs of glacial clay in one extreme, which creates a plume with a consequent gradient in underwater light intensity (including ultraviolet radiation) and suspended solid material. This gradient in light intensity also affects the light:nutrient ratio and hence the C:P ratio of the food for planktonic herbivores. In the field, along a 9 km transparency gradient, we measured the activities of glutathione S-transferase (GST) and catalase (CAT) enzymes involved in protection against UVR. Through laboratory experiments, we tested the possible role of suspended sediment particles as an additional stressor for a filter feeding zooplankter. Our results indicate that the inputs of glacial clay into the lake have antagonistic effects on *Daphnia*. Glacial clay was a stress mitigating factor to UVR (decrease in the antioxidant response of GST activity), but was also a source of stress that generated feeding interference, increased respiration rates and consequently increased CAT activity. This light gradient also affected the C:P ratio of food and the maximum response in GST is also modulated by food quality (C:P ratio) that limits its activity in the transparent end of the gradient.

KEYWORDS: global change; glacial clay; turbidity; ultraviolet radiation; food quality; antioxidant enzymes

INTRODUCTION

Ecosystems around the world are affected by regional climate change, particularly due to temperature increases. The observed decreases in snow cover and the rapid retreat of mountain glaciers and ice sheets in the last 50 years are consistent with global warming (IPCC, 2014). Most glaciers are expected to shrink significantly over the next few decades, and eventually, many low

elevation glaciers will disappear. This glacier retraction, besides changing the glacier ecosystem itself, also affects the surrounding environments. Glacier melting implies a subglacial erosion of the bedrock, which produces high amounts of sediment loads that give origin to turbid meltwater proglacial lakes, rivers and streams (Zemp *et al.*, 2009). These sediments are transported to recipient aquatic ecosystems and modify several features of rivers and the receiving lakes, such as nutrient content,

optical properties and UV penetration in the water column, with diverse effects on benthic and planktonic populations (Laspoumaderes *et al.*, 2013; Martyniuk *et al.*, 2014; Rose *et al.*, 2014). During their ontogeny, proglacial lakes become less turbid until losing hydrological contact with the glacier and shifting to a clear condition with high water transparency (Sommaruga, 2015). Understanding the consequences of glacier retreat for glacier-fed lakes will be crucial for predicting ecosystem trajectories regarding changes in biodiversity, biogeochemical cycles and function.

Climate change is not only limited to the effects of increasing temperature, but anthropogenic emissions of ozone-depleting gases have also led to marked changes in surface UVR. In the past few decades, we have witnessed a massive loss of stratospheric ozone over Antarctica and the Arctic (Helbling *et al.*, 2003). In aquatic systems, UVR is recognized as an important biological stressor (Williamson and Neale, 2009; Hader *et al.*, 2015). Organisms are affected by UVR when key macromolecules (DNA, protein, chlorophyll) absorb specific wavelengths, altering important physiological or biochemical processes (Siebeck *et al.*, 1994; Gonçalves *et al.*, 2002). Zooplankton have developed a number of different strategies to counteract the damaging effects of UVR that include behavioural responses (Leech *et al.*, 2005; Kessler *et al.*, 2008), photoprotective pigments (Moeller *et al.*, 2005; Hansson *et al.*, 2007; Hylander *et al.*, 2009), and antioxidant enzymes (Borgeraas and Hessen, 2000; Souza *et al.*, 2007, 2010). However, aquatic organisms can also be negatively affected by UVR through the generation of reactive oxygen species (ROS). The longest-lived ROS, hydrogen peroxide (H₂O₂), is of special interest because it is readily diffusible across cell membranes and functions as a signalling molecule in diverse cellular events (Cooper and Lean, 1989). Enzymes such as catalase (CAT) and glutathione S-transferase (GST) play important roles against oxidative stress caused by UVR. The lack or malfunction of catalases may lead to severe defects, such as an increased susceptibility to injury and high rates of mutations (Cho *et al.*, 2000). GST is a detoxifying enzyme that neutralizes active electrophilic compounds making the parent compound more water soluble (Hayes *et al.*, 2005).

In the context of global change, high UVR and high rates of glacier recession may affect zooplankton in several ways. First, organisms inhabiting transparent lakes are exposed to high UV irradiance caused by ozone depletion, requiring the production of diverse UV protection strategies (i.e. pigments or enzymes) (Hansson *et al.*, 2007; Rautio and Tartarotti, 2010). But also, organisms that inhabit turbid lakes may receive protection from UVR that high loads of suspended sediment particles

offer, producing a shadowing effect (Martyniuk *et al.*, 2014). However, the benefits of the UV protection generated by glacial clay can be overshadowed by the problems that high loads of suspended solids might present for filter feeding zooplankton, as was observed for glacial clay and other suspended particles (Koenings *et al.*, 1990; Kirk, 1992; Wolinski *et al.*, 2013). Organisms living in lakes receiving these inputs might face a trade-off between inhabiting transparent high UVR areas, or protected turbid areas. Projections of ongoing global change (IPCC, 2014) highlight the importance of understanding these counteracting mechanisms, because some transparent alpine lakes will become turbid environments, and newly forming turbid lakes will eventually become clear (Sommaruga, 2015).

Patagonia is a region of particular interest in which to study the effects that UVR and glacier recession have on organisms, mainly due to its proximity to the Antarctic ozone layer depletion area (Thompson *et al.*, 2011) and because the Patagonian Andes is the largest glaciated area in South America (Masiokas *et al.*, 2008). In this region, there are many glaciers that have been showing continuous and maintained recession over the last 30 years (Masiokas *et al.*, 2008) and are contributing large amounts of sediment particles through rivers to the receiving lakes (Hylander *et al.*, 2011; Laspoumaderes *et al.*, 2013; Martyniuk *et al.*, 2014). We studied a transparent oligotrophic lake (Lake Mascardi) that receives inputs of glacial clay at one end of the lake, which creates a plume with a consequent gradient in underwater UVR and suspended solid material. Previous studies have shown that this gradient was related with significant differences in the light: nutrient ratio and stoichiometric food quality for zooplankton: *Daphnia commutata* dominance was observed in low carbon:nutrient stations (less transparent) while copepods (*Boeckella gracilipes*) dominate in high carbon:nutrient stations (transparent) (Laspoumaderes *et al.*, 2013). In addition, the enzyme response (GST and CAT) of *Daphnia* to oxidative stress was dependent on the food C:P ratio (Balseiro *et al.*, 2008; Wolinski *et al.*, 2016). Putting these constraints together, Lake Mascardi might represent a trade-off for *D. commutata* between clear areas with high UVR penetration and low quality food (high C:P) far away from the glacial plume, and less transparent areas with UVR protection due to glacial clay and higher food quality, but with a possible interference effect due to the high amount of suspended sediment particles. Here, we analysed the antioxidant enzymatic response of *D. commutata* (GST and CAT activity) in the light gradient (PAR and UVR) of Lake Mascardi. We hypothesize that, close to the glacial input, suspended sediment particles might act as UVR protection thus, antioxidant enzyme activity should be lower. At the same time, a higher food

quality can be expected due to a low light:nutrient ratio (low sestonic C:P ratio) so, food quality should not be limiting. Conversely, further away from the glacial plume, antioxidant enzymes should be higher but likely to be limited by food quality due to an increase in sestonic C:P ratio in the more transparent area. Finally, in the laboratory, we also tested the possible role of suspended sediment particles as a further stressor for a filter feeding zooplankton such as *D. commutata*.

METHOD

Field study

Lake Mascardi ($41^{\circ}15'–41^{\circ}25'S$; $71^{\circ}28'–71^{\circ}39'W$) is a V-shaped glacial lake with a western arm (Tronador arm, $Z_{\max} = 118$ m) and an eastern arm (Catedral arm, $Z_{\max} = 218$ m) (Fig. 1). The northernmost end of the Tronador arm receives meltwaters from glaciers of Tronador Mountain. Glacier fluctuations on Tronador Mountain have been observed since 1976 and show continuous and maintained recession (Masiokas *et al.*, 2008). Meltwater from the Ventisquero Negro and Castaño Overo glaciers is transported by the Upper Manso River to Lake Mascardi. The suspended sediment particles in the lake varied between 4.8 ± 0.56 to 0.35 ± 0.09 mg L^{-1} and caused a longitudinal light gradient ($Z_{1\%PAR}$ from 18.1 ± 1.5 m to 29.0 ± 2.3 m) along the 9 km western arm (Tronador) of Lake Mascardi (Modenutti *et al.*, 2000; Balseiro *et al.*, 2007). Conversely, the Catedral arm is clear with no glacial clay. The difference between the two arms arises from the lake outlet being located at the confluence between them.

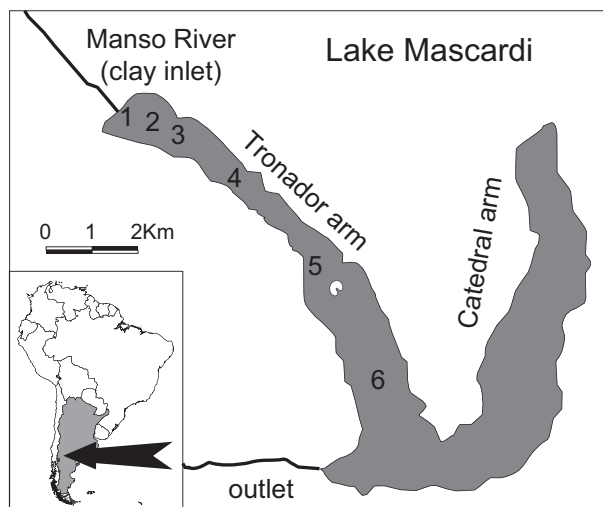


Fig. 1. Map of Lake Mascardi with sampling stations (1–6). The Manso River is the glacial clay input.

In four austral summers (January–February 2009, 2010, 2011 and 2015), we sampled a six-station transect across the Tronador arm of Lake Mascardi at increasing distances from the meltwater input of the Manso River, to include the entire underwater light gradient (Fig. 1). At each station, temperature and light vertical profiles (0–50 m) of UV bands (UVB-320 nm, UVA-340 nm) and photosynthetic active radiation (PAR, 400–700 nm) were measured with a PUV 500B submersible radiometer (PUV 500B; Biospherical Instruments, San Diego, CA, USA). Replicated lake water samples were obtained with a Schindler-Patalas trap from the epilimnion (0, 5 and 15 m depths) and hypolimnion (30 and 45 m depth) for sestonic carbon and nutrient analyses. The epilimnion was considered to be the top of the discontinuity layer according to the vertical temperature profiles obtained with the PUV radiometer, and the hypolimnion was considered to be all depths below the mixed layer, which technically includes the metalimnion.

Zooplankton was sampled with closing plankton nets from 0 to 15 m and 15 to 45 m (30 cm in diameter and 55 μm mesh size). All samples were transferred to acid-washed propylene bottles and transported to the laboratory in thermally insulated containers. Sampling was conducted during summer under full sunlight and windless days, starting at station 1 (nearest to the clay inputs; Fig. 1) at 12:00 and ending at approximately 14:00 at station 6 (farthest from the glacial inputs).

Laboratory determinations

Immediately after sampling, live zooplankton from each sampling station (*Daphnia commutata*) were frozen at -80°C until biochemical analyses. Because the abundance of individuals was very low in the upper level (0–15 m) at noon, zooplankton samples from the epilimnion and hypolimnion were combined for enzymatic analyses to ensure sufficient sample size. To determine enzymatic activity, we used three replicates of 20–30 individuals (adults of similar size) of *D. commutata* from each station. Individuals were rinsed in cold Milli-Q water, separated under a stereomicroscope and were homogenized using a glass Teflon homogenizer with ice-cold 50 mM potassium phosphate buffer, $\text{pH} = 7.7$, containing 1 mM EDTA and 0.1% Triton X-100 according to Borgeraas and Hessen (2000). Supernatants of homogenates were centrifuged at $10\,000 \times g$ for 10 min at 4°C and were used as enzyme sources. Measurements of enzymatic activities were carried out using a Shimadzu 2450 spectrophotometer (Shimadzu, Japan) at $23 \pm 0.5^{\circ}\text{C}$. CAT activity was measured according to Aebi (1984); in a buffer of 50 mM phosphate $\text{pH} = 7.0$ containing H_2O_2 (0.6% v/v), recording a decrease in

absorbance at 240 nm due to H_2O_2 consumption. The specific activity of CAT was expressed in μM of substrate hydrolysed per minute per mg of protein ($\mu\text{M prod. min}^{-1} \text{ mg prot.}^{-1}$). Total GST activity was measured according to Habig *et al.* (1974) in a buffer of 100 mM phosphate pH = 6.5, with 1 mM of 1-chloro-2,4-dinitrobenzene (CDNB) in acetonitrile (1% v/v) and GSH 1.2 mM as substrates, recording the absorbance at 340 nm. GST activity was expressed in nM of product developed per minute per mg of protein ($\text{nM prod. min}^{-1} \text{ mg prot.}^{-1}$). Protein determination was performed according to Lowry *et al.* (1951) using bovine serum albumin as standard curve. The protein quantity per assay was 5.2 ± 0.003 g protein per reaction.

Samples for zooplankton abundance quantification were stored in a freezer at -20°C . Individuals were identified, classified by species and counted under a stereomicroscope in 5 mL Bogorov chambers.

A volume of 200 mL of lake water from each station and depth was filtered through precombusted (450°C , 2 h) GF/F Whatman filters and analysed for particulate carbon (PC) using a Thermo Finnigan EA 1112 CN elemental analyzer (Thermo Scientific, Milano, Italy). Particulate phosphorus (PP) was calculated for each sample as the difference between total phosphorus (TP) and total dissolved phosphorus (TDP). TP was measured directly from each sample, and TDP was measured by filtering sample water through GF/F filters. Both fractions were digested with persulfate at 1.5 atm for 1 h, followed by a molybdate reaction (APHA, 2005). Sestonic C:P atomic ratios were calculated by averaging the depth-specific seston C:P ratios for the 5, 15 and 30 m depths.

Total suspended solids (TSSs) for each station were estimated by filtering 0.5 L of lake water onto pre-weighed GF/F filters that were then dried at 60°C for 48 h and reweighed. Dissolved organic carbon (DOC) was measured on lake water that had been filtered through precombusted GF/F glass fibre filters (WhatmanTM) and was quantified in a Shimadzu TOC VCSH Carbon analyzer (Shimadzu, Japan).

Laboratory experiment in a clay gradient

To test the role of suspended sediment particles as another stressor, we carried out an experiment analysing the enzymatic activity (CAT and GST) of *D. commutata* in a gradient of clay concentration, similar to the observed gradient in Lake Mascardi, under only PAR as light source (no UVR effect as stressor). At the end of the experiment we measured enzyme activities as well as respiration rates of *D. commutata* from the different clay treatments.

Clay for the experiments was collected directly from the bottom of the proglacial lake; Lake Ventisquero Negro, on Tronador Mountain. To prepare the clay for the experiments, 10 g of sterile clay (combusted at 450°C for 1 h) was placed in 1000 mL of sterile distilled water and stirred for 1 h, then allowed to settle for 1 h (to eliminate large particles). After that, we took only the supernatant 750 mL. TSS concentrations were measured to obtain the concentration of this stock suspension, and then dilutions were made to reach the desired concentrations. TSS was obtained by filtering a known volume of the supernatant suspended clay through previously-weighed glass fibre filters (GF/F Whatman filters). The filters were dried at 60°C for 48 h and then reweighed. The difference between weights (mg) and the filtered volume (L) was used to calculate TSS.

For these experiments, we used the same *D. commutata* clone as Balseiro *et al.* (2008) and Wolinski *et al.* (2016) when they demonstrated the effect of UVR and food quality on CAT and GST activities. In the present experiment, the aim was to isolate the effect of suspended sediment particles on CAT and GST activities, and on respiration rates. This clonal population of *D. commutata* has been maintained under laboratory conditions (15°C and $85 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ with a 14:10 light:dark photoperiod) and fed with *Chlamydomonas reinhardtii*. Egg-carrying females were carefully sorted under a dissecting microscope and placed in beakers with a freshwater culture medium COMBO (Kilham *et al.*, 1998) and food (*C. reinhardtii*). On the following day, the experiment began by transferring 15 neonates (<24 h old) to each flask with the corresponding food and clay concentration. Food concentration in the experiments was an average of 0.5 mg C L^{-1} and this C concentration was higher than that observed in Lake Mascardi (mean $0.15 \text{ mg C L}^{-1} \pm 0.01$) (see Results). The three clay concentrations were 0 (Control), 3 and 5 mg L^{-1} , corresponding to the observed concentrations at different stages of the gradient of Lake Mascardi (see Results). We ran the experiment in 18 Erlenmeyer flasks (150 mL) with ground glass stoppers with three clay levels in six replicates (15 individuals each), for 5 days in a half rotation device (2.5 rpm, half rotation every 2 min) to keep particles in suspension. On every second day, water from each beaker was replaced and new food and clay were added. The flasks were completely filled with medium to decrease the risk that individuals would be caught in the surface tension. The carbon concentrations in the *C. reinhardtii* cultures were estimated based on *in vivo* fluorescence on a Turner AU10 fluorometer (Turner Design, San Jose, CA, USA) against direct carbon measurements on a Thermo Finnigan EA1112 CHN elemental analyzer. Experiments were run in an

incubator at 15°C (similar to the lake's epilimnion temperature) with a 14:10 (light:dark, 85 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) photoperiod. After the 5-day experiment period, we determined CAT and GST activities, and respiration rates for the three treatments (0, 3 and 5 mg L^{-1} of glacial clay). To determine enzymatic activities, we removed seven individuals from each flask and determined enzymatic activity following the same procedure as for the field samples. With the remaining eight individuals from each flask, we determined respiration rates. We used a total of 18 respirometers divided amongst the three clay treatments, with six replicates. Respiration rates were assessed as oxygen consumption over time in 20 mL flasks (respirometers) with COMBO medium, without food and in the dark, stoppered to ensure that no air bubbles were present. We measured dissolved oxygen initially, and every 20 min for 4 h with an optical, fluorescence-based (Presens[®], Germany) DO metre that allows oxygen concentrations to be determined from the outside, without opening the flask. We calculated weight-specific respiration rates by standardizing oxygen consumption rates per hour to the mean C (μg) of each *D. commutata* in each treatment, estimated as the slope of a least squares regression of O_2 concentration vs time.

In another experiment, we compared feeding rates of *D. commutata* under the three different levels of clay concentrations, by feeding them with a mixture of *C. reinhardtii* and flavoured microspheres (DeMott, 1986; Wiedner and Vareschi, 1995). Flavoured spheres (FMP) were prepared by incubating Fluoresbrite[®] YG 6.00 μm (coefficient of variation 10%) microspheres for 20 h in a *C. reinhardtii* algal suspension on a shaking device. The microspheres were previously sonicated for approximately 1 min to break up aggregates. One *D. commutata* was transferred to each of 48 glass 60 mL beakers containing COMBO, *C. reinhardtii* at 0.1 mg C L^{-1} and three different levels of clay concentration (0, 3 and 5 mg L^{-1} , resulting in three treatments with 16 replicates each). After 1 h, we added the mixture of algae and flavoured spheres to reach a concentration of 0.5 mg C L^{-1} of algae (consistent among all experiments) and 800 flavoured spheres mL^{-1} . In each trial, zooplankters fed on these treatments for 10 min. To mix and avoid sedimentation of the spheres, the 60 mL beakers were everted two to three times at the beginning of each trial and at every 5 min. After the 10 min, zooplankters were anaesthetized with carbonated water and immediately frozen at -80°C . We determined the number of spheres ingested by each individual (FMP_I) placing them on microscope slides and counting the amount of FMP consumed under an Olympus BX 50 epifluorescence microscope (Olympus, Japan) fitted with a blue light excitation filter (U-MWB filter).

Data analysis

We calculated the mean light of the mixed layer (I_m) and the 1% depth ($Z_{1\%}$) (Sterner *et al.*, 1997; Balseiro *et al.*, 2007) for PAR, UVA and UVB according to the formulae:

$$I_m = \frac{1 - e^{(-K_d \times Z_m)}}{K_d \times Z_m},$$

where K_d indicates the attenuation coefficients of the corresponding light wavelengths that were determined from the slope of the simple linear regression of the natural logarithm of downwelling PAR, UVA or UVB irradiance vs depth. Z_m indicates the depth of the mixed layer. The mixed layer was estimated as described above. Depth of 1% surface light was estimated as:

$$Z_{1\%} = \frac{\ln(100)}{K_d}.$$

Based on the number of spheres consumed, we calculated, for each *Daphnia*, filtering and ingestion rates (IRs) of algae and clay following Wiedner and Vareschi (1995):

Filtering rates (FRs), (mL min^{-1}) were calculated according to the formula:

$$\text{FR} = \frac{\text{FMP}_I}{\text{FMP}_{\text{Total}}},$$

where FMP_I indicates the number of fluorescent particles ingested in the experiment (spheres min^{-1}). FMP_T indicates the concentration of fluorescent particles in the media (spheres mL^{-1}).

IRs ($\mu\text{g min}^{-1}$) of algae and clay were calculated according to the formula:

$$\text{IR}_x = \text{FR} \times x,$$

where x indicates μg of algae or clay in the media ($\mu\text{g mL}^{-1}$).

Correlation coefficients were calculated for TSS and I_m (UVB, UVA and PAR). GST and CAT in the light gradient of lake Mascardi were fitted to a regression model, which was selected by applying Akaike's information criterion (AIC). Experimental treatments in the clay gradient (GST and CAT activity and feeding rates) were compared with One-Way ANOVA. When this was significant, a Tukey *a posteriori* test was applied. Normality and homocedasticity were confirmed before the analyses. Respiration rates were fitted to linear regression. All data are expressed as mean \pm standard error.

RESULTS

Field study

We observed a gradient (TSS, Light, C:P) along the 9 km in the Tronador arm of Lake Mascaradi, with the same trend being present for the four year study period (Fig. 2a–d). Suspended solids were higher at the stations closer to the river input (in stations 1–3, TSS ranged within 2–4 mg L⁻¹) than in the farther ones (in stations 4–6, TSS were around 1 mg L⁻¹) (Fig. 2a). The attenuation coefficient of PAR and the different UV wavelengths (320 and 340 nm, UVB and UVA, respectively) decreased in the water column as a result of the declining effects of the glacial clay, with a steady decrease in TSS as distance from the river input increased (Fig. 2b and c) (TSS vs $I_{m(\text{PAR})}$: $r = -0.96$, $P < 0.001$; TSS vs I_{m320} : $r = -0.88$, $P < 0.001$; TSS vs I_{m340} : $r = -0.87$, $P < 0.001$, $n = 17$). Along the light gradient, we also observed that the sestonic C:P ratio increased: the lowest C:P ratios were recorded in the turbid area of the lake and increasing C:P ratios were recorded in the clearer water, as distance from the clay input increased (Fig. 3a). The particulate C was low, with a mean value, considering all sampling stations and years, of 0.15 mg L⁻¹, ranging from 0.07 to 0.31 mg L⁻¹.

Finally, DOC concentration was very low ($0.61 \text{ mg L}^{-1} \pm 0.02$) along the gradient and in the different years ($r^2 = 0.0001$, $df = 40$, $P = 0.94$). Consequently, no differential light absorption (mainly UVR) that could change the underwater specific waveband attenuation was observed. Therefore, the main factor of light attenuation, in addition to water itself, was scattering by TSS, which is not wavelength specific.

Zooplankton communities in Lake Mascaradi were dominated by the cladoceran *D. commutata* and the calanoid copepod *Boeckella gracilipes*, which together represented almost the entire zooplankton biomass (>95%) in the lake. The remaining zooplankton were small rotifers (such as *Polyarthra vulgaris*, *Keratella cochlearis* and *Synchaeta* spp). There was clear variation in the crustacean zooplankton species abundances along the gradient. Remarkably, the abundance of *D. commutata* decreased with increasing distance to the clay input ($\sim 1200 \text{ ind m}^{-3}$ to 160 ind m^{-3}), thus its absolute and relative abundances decreased along the Tronador Arm and this was consistent over all the sampled years (Fig. 3b and c).

We found different patterns for GST and for CAT activity of *D. commutata* along the light gradient (note that in Fig. 4, the abscissa is light gradient, i.e. I_m). GST activity in *D. commutata* showed a hump-shaped pattern along the gradient (increasing UV effect and decreasing food quality, i.e. high C:P). In areas of the lake under the influence of inputs of glacial clay through Upper

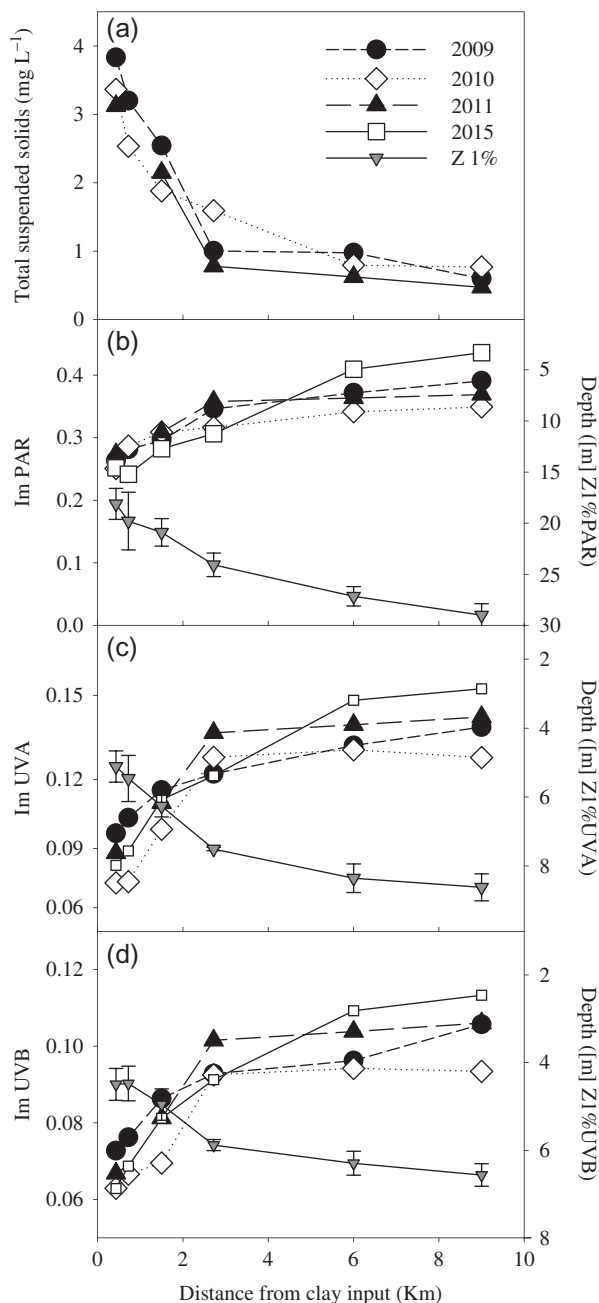


Fig. 2. (a) TSSs (mg L⁻¹) (data from 2015 are missing), (b), (c) and (d) mean light intensity of the mixed layer (I_m) and mean penetration depth ($Z_{1\%}$) (mean, SE for all sampling years) for PAR, UVA and UVB respectively, at increasing distance (A_m) from the river input (data from station 2, 2011 are missing).

River Manso (with high TSS and consequently low I_m of both UVA and UVB), GST activities were the lowest (Fig. 4a and b). While, as I_m increases (due to lower clay concentration), GST activities increased until a certain threshold, after which GST activity again diminished (Fig. 4a and b). In contrast, we recorded no defined

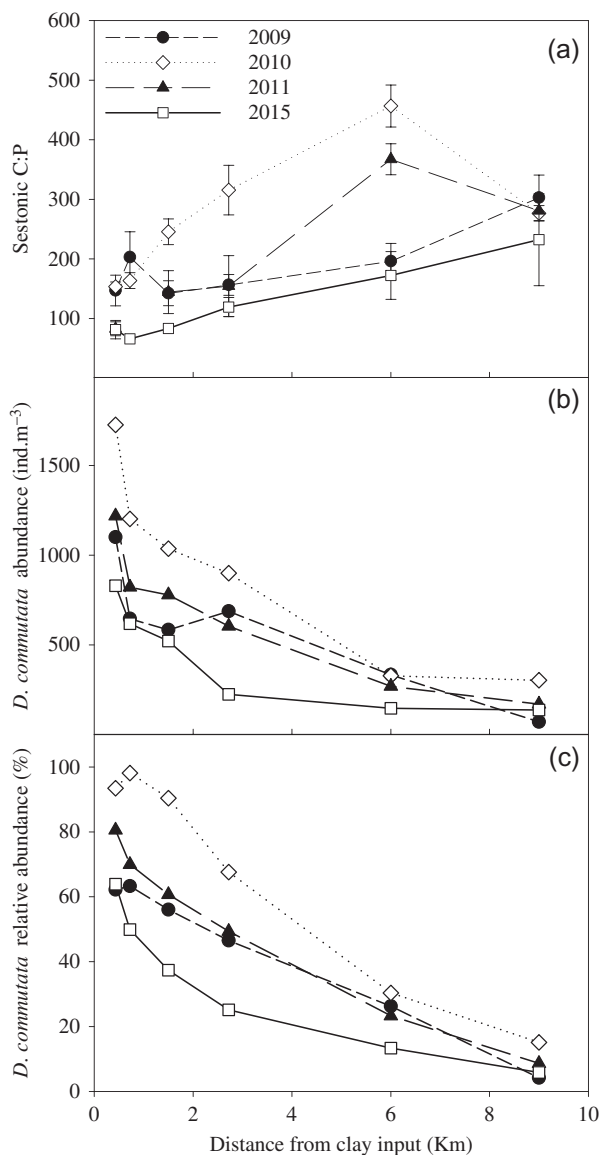


Fig. 3. (a) Sestonic C:P (atomic) (mean \pm SE), (b) abundance of *D. commutata* at increasing distance from the clay input, (c) relative abundance of *D. commutata* to total crustacean zooplankton at increasing distance from the clay input.

trend in CAT activity along the lake gradient and there was no correlation between CAT activity and I_{m320} ($r^2 = 0.039$, $df = 71$, $P = 0.09$) or I_{m340} ($r^2 = 0.045$, $df = 71$, $P = 0.07$) (Fig. 4c and d).

Laboratory experiments

We observed that the enzyme activities in the experimental clay gradient (clay concentration in the same range as Lake Mascardi) but without the UVR effect, showed a different pattern than the one obtained in the

lake. While CAT activity was higher when *D. commutata* was exposed to increasing clay concentrations (Fig. 5a), GST activity remained constant among treatments (Fig. 5b). We found that CAT activity from all clay treatments was higher than the control (3 and 5 mg L⁻¹ vs 0 mg L⁻¹) (One-Way ANOVA $F_{2,17} = 25.27$, $P < 0.001$, Tukey test multiple comparisons, control vs 3 mg L⁻¹ and 5 mg L⁻¹ $P < 0.001$) (Fig. 5a). GST was not affected by suspended clay, as GST activity was constant regardless of the presence of suspended clay, with no differences among treatments (One-Way ANOVA $F_{2,17} = 2.15$, $P = 0.147$) (Fig. 5b).

In addition, we found that clay concentration significantly affected the respiration of *D. commutata*, with increased respiration as clay concentrations increased ($r^2 = 0.89$, $df = 16$, $P < 0.001$) (Fig. 5c). Respiration rates of the organisms that were not exposed to clay were 0.24 ± 0.003 nmol O₂ h⁻¹ μ g C⁻¹, while for organisms exposed to 3 and 5 mg L⁻¹ of suspended clay had respiration rates of 0.27 ± 0.003 and 0.28 ± 0.001 , nmol O₂ h⁻¹ μ g C⁻¹, respectively.

In order to identify clay mediated changes in feeding rates, we fed *D. commutata* with a mixture of algae and fluorescent beads in the same clay gradient (0, 3 and 5 mg L⁻¹) and all treatments were significantly different (ANOVA $F_{2,47} = 20.20$, $P < 0.001$; Tukey test $P < 0.05$). The lowest feeding rates were recorded in the control group, the highest in the 3 mg L⁻¹ of clay, and intermediate in the 5 mg L⁻¹ clay concentration (Fig. 5d). *D. commutata* in the control group (with no glacial clay) ingested a mean of 0.11 ± 0.026 ng C of algae ind⁻¹ min⁻¹. When *D. commutata* was exposed to 3 mg L⁻¹ of glacial clay, its feeding rate was the highest, as it ingested a mean of 0.57 ± 0.081 ng C of algae ind⁻¹ min⁻¹. Intermediate feeding rates were observed for the animals that were exposed to the highest clay concentrations, where they ingested 0.26 ± 0.036 ng C of algae ind⁻¹ min⁻¹ (Fig. 5d). In addition, we estimated the amount of clay ingested in each treatment, and the results showed that *Daphnia* ingested 0, 3.4 ± 0.49 and 2.6 ± 0.37 ng of clay ind⁻¹ min⁻¹, respectively.

DISCUSSION

In mountain regions, changes in glacier length are widely recognized as the most reliable and easily observed indicators of climate change (Heinsbroek *et al.*, 2007). In this sense, Lake Mascardi is particularly interesting, as it presents a natural gradient that results from the input of glacial clay particles from glacier retreat (Modenutti *et al.*, 2000, 2013). At the first sampling stations, light penetration was drastically diminished by

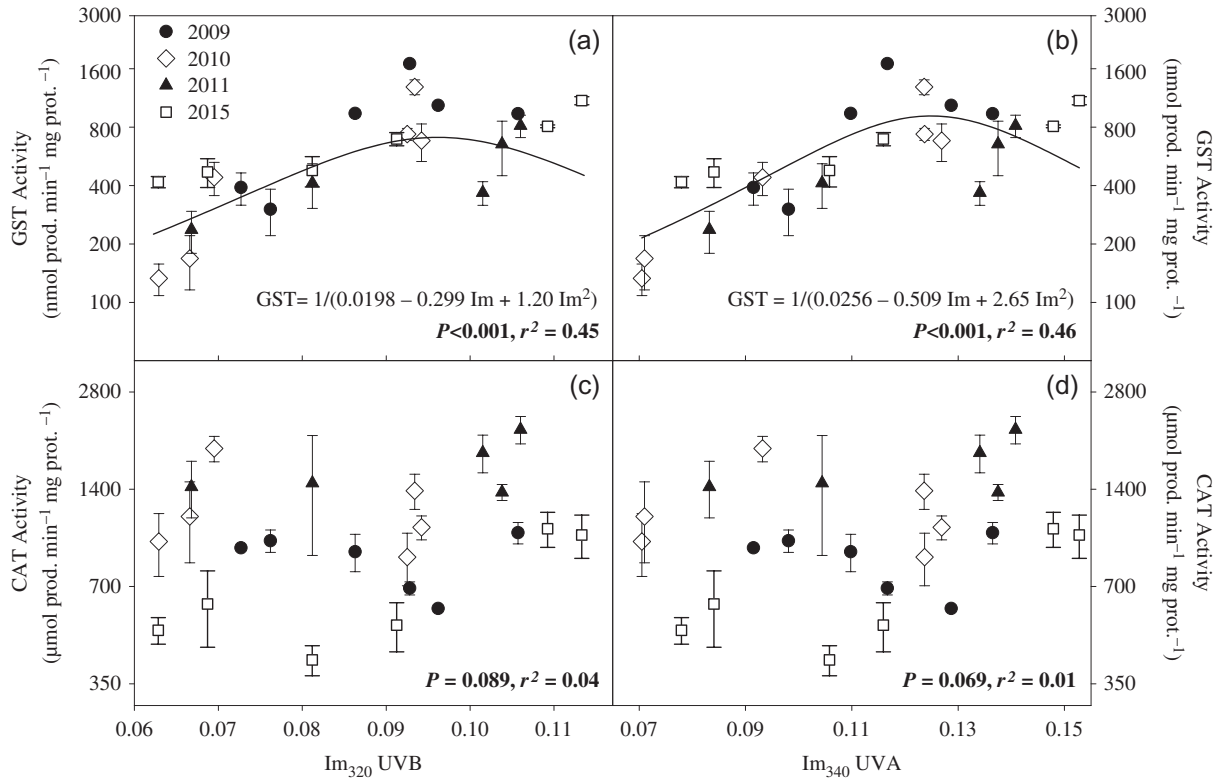


Fig. 4. Relationship between enzyme activities and mean light intensity of the mixed layer (I_m). (a) and (b) GST activity and (c) and (d) CAT activity. Left panels are UVB and right panels are UVA. Best fitted function for GST: inverse quadratic polynomial function. (lower AIC and higher r^2) I_{m320} : $GST = 1/(0.0198 - 0.299 I_{m320} + 1.20 I_{m320}^2)$; $r^2 = 0.45$, ($df = 61$, $P < 0.001$) and I_{m340} : $GST = 1/(0.0256 - 0.509 I_{m340} + 2.65 I_{m340}^2)$; $r^2 = 0.46$, ($df = 61$, $P < 0.001$).

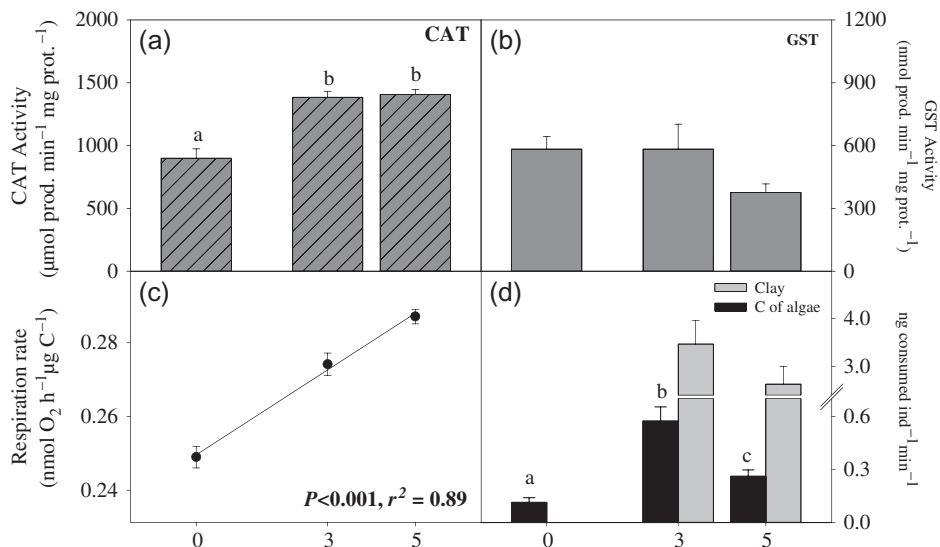


Fig. 5. Results of the experiments of *D. commutata* exposure to a glacial clay gradient (0, 3 and 5 mg L⁻¹ of glacial clay). (a) CAT activity, (b) GST activity, (c) respiration rate and (d) IRs of algae and clay calculated from the consumption of FMP (see Methods section for details).

suspended glacial clay, and inhabiting filtering species coexisted with particles that may have a positive effect (protection) and/or a negative effect (interference). Indeed, our data indicate that the inputs of glacial clay into the lake can have both effects on the cladoceran *D. commutata*: on the one hand, glacial clay diminishes underwater light intensity (both PAR and UVR), diminishing the potential of a hazardous effect of short wavelengths (low GST activity). On the other hand, we observed that glacial clay can be also a source of stress, as suspended particles caused an increase in respiration and FRs with a concomitant effect on CAT activity (Fig. 5). Finally, because the gradient in glacial clay changes the light:nutrient ratio and thus food quality (C:P ratio), this, in turn, affected the *Daphnia* enzymatic response to UVR.

An important factor associated with *Daphnia* decline along the light gradient is the possible shifting role of visual predation by fish. Because the higher predation risk is associated with higher water transparency (Kessler *et al.*, 2008), zooplankton in Lake Mascardi would be more strongly affected by visual predation at the more transparent end of the lake (Balseiro *et al.*, 2007; Laspoumaderes *et al.*, 2013). Therefore, predation risk may act in the same direction as the increase in UVR stress and the change in food quality (sestonic C:P ratio). In a previous study (Laspoumaderes *et al.*, 2013) *Daphnia* growth experiments strongly supported the stoichiometric hypothesis, i.e. changes in food quality (sestonic C:P). Along the gradient the *Daphnia* population experiences a stoichiometric constraint that lowers its growth rate. In addition, the study of Laspoumaderes *et al.* (2013) did not report changes in adult size which would be expected under moderate or high visual fish predation due to size selective feeding (Gliwicz and Pijanowska, 1989); even a decrease in size induced by fish chemical cues might be expected (Boersma *et al.*, 1999). In the present study, we observed changes in the GST antioxidant enzymatic response and this cannot be attributed to fishes since, to our knowledge, there are no references that report changes in enzymatic oxidative stress due to fish predation.

The antioxidant enzymes GST and CAT are both well-known defence mechanisms against oxidative stress, and their activities increase with UVR exposure (Dahms and Lee, 2010; Kim *et al.*, 2010). However, in our field analyses, only GST activity increased with increasing underwater UVR (increase I_m of both UVA and UVB), while CAT activities were similar in areas with high and low UVR (I_m) (Fig. 4). This flat pattern of CAT activity in the UVR gradient, suggests that another stressor could have affected CAT activity in the opposite way, with a net result of no change along the gradient.

Indeed, these two stressors should be negatively correlated (as are UVR [I_m] and TSS) in order to generate this response. In the laboratory experiment, we found that both respiration rates and CAT activity were higher when *D. commutata* was exposed to suspended clay (Fig. 5a and c). Also, we found an increase in feeding rates particularly at the intermediate clay concentration in our experiments (Fig. 5d). The increase in feeding rates generated by glacial clay was probably a result of compensatory feeding (Suzuki-Ohno *et al.*, 2012), since the relative concentration of food decreased as clay increases. However, this trend changed when clay concentration increased to 5 mg L^{-1} , where feeding rates decreased sharply. An increase in feeding rate, as observed at our intermediate clay concentration, implies higher activity of the thoracic limbs that are responsible for the water current involved in food uptake and gas exchange (Kirk, 1991). In consequence, CAT increases to deal with the excessive internal H_2O_2 that is produced by the increase in respiration rates (Góth *et al.*, 2001; Giorgio *et al.*, 2007). Nevertheless, we found a decrease in feeding rate at the highest clay concentration but still with an increase in respiration. This apparently controversial outcome could be the result of the increased limb and post-abdomen movements: since clay induces rejection of food boluses with a higher frequency of post-abdominal rejection, as collected clay particles clog the food groove immediately after clay exposure (Kirk, 1991). Consistently, our results showed that not only the ingested algae decreased, but also the ingested clay, though the clay concentration was higher. Consequently, respiration rates were elevated due to high appendage activity, and hence CAT activity, but feeding decreased due to the high frequency of post-abdomen movements and low feeding efficiency.

Our experimental high clay concentration (5 mg L^{-1}), which represents the highest clay concentration we observed in the lake, seems low in comparison with other clay interference studies (Koenings *et al.*, 1990; Kirk, 1991; Rellstab and Spaak, 2007). However, the effect of clay depends greatly on food concentration, since the effect decreases when food concentration is high (Kirk, 1991). Although Lake Mascardi has a low food concentration (0.15 mg C L^{-1}), even during summer, the *Daphnia* population can reproduce in this natural system. This indicates that the C concentration is above its food threshold level, as has been shown for other *Daphnia* species (Gliwicz, 1990; Rellstab and Spaak, 2007). Nevertheless, in our laboratory experiments with suspended clay, we used a much higher food concentration (0.5 mg C L^{-1}) than was present in the lake, therefore our *Daphnia* should be less food-limited than those in the natural population. In addition, food quality was also higher in our

experiments than in the field, as food consisted of *Chlamydomonas reinhardtii* from a batch culture with a C:P ratio of 100, in comparison with the highly variable C:P data of Lake Mascardi.

Interestingly, CAT activity in the *D. commutata* laboratory clone was higher than the values obtained directly from the field. This higher activity in the lab was observed in other *Daphnia* groups (Borgeraas and Hessen, 2002) and can be attributed to higher destruction of CAT by higher light and UVR levels in the field (Shindo and Hashimoto, 1995) and to nutritional status, which is a major determinant of antioxidant concentrations (Borgeraas and Hessen, 2000; Balseiro *et al.*, 2008). In our laboratory experiments, UVR was excluded in order to have only one stressor (clay gradient). Moreover, food quantity and quality were higher than in the lake. Thus, it seems to be reasonable to have higher CAT activity in the laboratory than in the field, suggesting that this parameter cannot be used as a nominal factor, but as a comparative factor amongst equivalent treatments.

The clay gradient in the lake causes not only an underwater UVR gradient, but also an underwater PAR gradient. This light gradient results in an increase of the light:nutrient ratio; thus, the phytoplankton C:P ratio increases, which implies a decrease in food quality and a gradual change in crustacean dominance from cladocerans to copepods (Laspoumaderes *et al.*, 2013). This gradient in food quality can affect *Daphnia* GST activity as a response to increased UVR. High C:P ratio food limits the activity of the antioxidant enzymes CAT and GST (Balseiro *et al.*, 2008; Wolinski *et al.*, 2016). In our experiment, we observed that GST was not affected by clay, whereas in the field, GST activity increased with increasing UVR (Fig. 4a and b, note the abscissa is I_m), but near the transparent end of the gradient, the trend changes and GST activity decreases (Fig. 4a and b). This response might be attributed to the effect of food quality on *Daphnia*'s antioxidant response. The turbid area is a high quality food area (regarding algal elemental composition, C:P < 200), while the transparent part exhibited low food quality (high C:P), as predicted by the light-nutrient hypothesis (Sterner *et al.*, 1997). The turbid area with high food quality allows *D. commutata* to produce antioxidant responses to UVR, but because of the protection by glacial particles, GST activity remains low. As the lake becomes more transparent, UVR increases, which induces stress leading to an increase in GST. This increase indicates that food quality remains sufficiently high to allow the increase in response. However, near the transparent end of the gradient, GST begins to decrease, though UVR is still increasing. This change in the GST trend reveals that the decrease in food quality becomes the limiting factor of GST activity.

In our experiments, we used a single clone acquired from this lake, though it could be argued that there are several clones along this clay-light-food quality gradient. However, we don't yet have data to support or reject this hypothesis. We did not use organisms from different areas of the lake, in order to retain only one changing factor, i.e. clay concentration. This allowed a clear experimental setup in order to analyse whether clay can affect CAT activity, respiration and feeding. Using a single clone, we are able to attribute the differences to clay concentration. However, we cannot reject the possibility that the intensity of the response could vary in the two extremes of the lake gradient.

As an outcome of the different environmental features of both extremes of the lake, the highest abundances of *D. commutata* are reported in the turbid area (Fig. 3b) where it clearly dominates the zooplankton assemblage (Fig. 3c). In this area, with higher food quality (Laspoumaderes *et al.*, 2013), *Daphnia* faces stress imposed by glacial particles, increasing their CAT activity to reduce internal ROS, while GST remains low due to the low UVR in the water column. In the transparent area of the lake, with lower food quality, GST activities reach a maximum value and then decrease as a consequence of severe nutrient limitation imposed by high sestonic C:P values. Considering these favourable and unfavourable factors, the turbid area of the lake would be a better environment for a high-P demanding species like *Daphnia*. Despite the feeding interference imposed by glacial clay that causes an increase in CAT, the higher food quality (low C:P) seemed to compensate for the increased CAT activity and the P demand.

Glacier recession is a process that has accelerated in recent decades and this trend is expected to continue (IPCC, 2014). Large amounts of suspended sediment particles will continue to arrive in receiving lakes as long as glacier melting continues. In this sense, Lake Mascardi is a peculiar lake, as the inputs of glacial clay in one extreme creates a gradient that represents a spatial scale: different glacial lake ontogenetic stages (Sommaruga, 2015). This feature allows stronger projections about future scenarios of global change to be predicted. In the future, two possible scenarios may occur: (i) Higher rates of glacier recession due to global warming will increase the inputs of suspended material to receiving lakes. Filter feeding zooplankton will be exposed to the stress of feeding interference by increased glacial clay. In consequence, the benefits of suspended particles found in this study, might be overshadowed by an excess of suspended particles reaching a deleterious concentration. (ii) Glacier recession may continue with a similar trend, with no great changes in the contribution of glacier sediment in lakes. In this case, filter feeding zooplankton with high-P

requirements will continue to find a positive trade-off among the costs and benefits of inhabiting turbid environments. It remains an open question as to which of these scenarios will dominate the landscape in the future. However, in both cases, glacier recession will continue until the glaciers melt out, and lakes will become clear. At this point, the transparent end of the lake may represent the future of the entire system.

CONCLUSIONS

Glacier recession modifies the clay load to lakes, that in turn affects water transparency. This change in transparency has many effect in planktonic filter-feeders such as *Daphnia*. On the one hand, clay affects the feeding and respiration of *Daphnia* that increases CAT activity. However, clay also changes the underwater light, both UVR and PAR, so UVR oxidative stress increases as the lake becomes more transparent but at the same time, as the light:nutrient ratio increases, food becomes more P limiting. Consequently, high-P demanding species (such as *Daphnia*) may reach the level where the increase in oxidative stress is no longer followed by an increase in enzymatic response due to high C:P ratio of the food.

ACKNOWLEDGEMENTS

We thank Dr. Amanda Rugenski for her helpful comments. The Delegación Regional Patagonia of the Administración de Parques Nacionales for granting permission to collect material for this study.

FUNDING

This work was supported by Fondo Nacional de Ciencia y Técnica PICT2012-0929, PICT2014-1002, PICT2015-0418. The authors are CONICET researchers.

REFERENCES

- Aebi, H. (1984) Catalase in vitro. *Methods Enzymol.*, **105**, 121–126.
- APHA (2005) Standard Methods for the Examination of Water and Wastewater. American Public Health Association, AWWA, Washington, DC.
- Balseiro, E., Modenutti, B., Queimaliños, C. and Reissig, M. (2007) *Daphnia* distribution in Andean Patagonian lakes: effect of low food quality and fish predation. *Aquatic Ecol.*, **41**, 599–609.
- Balseiro, E., Souza, M. S., Modenutti, B. and Reissig, M. (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., Demeester, L. and Spaak, P. (1999) Environmental stress and local adaptation in *Daphnia magna*. *Limnol. Oceanogr.*, **44**, 393–402.
- Borgeraas, J. and Hessen, D. O. (2000) UV-B induced mortality and antioxidant enzyme activities in *Daphnia magna* at different oxygen concentrations and temperatures. *J. Plankton Res.*, **22**, 1167–1183.
- Borgeraas, J. and Hessen, D. O. (2002) Variations of antioxidant enzymes in *Daphnia* species and populations as related to ambient UV exposure. *Hydrobiologia*, **477**, 15–30.
- Cho, Y.-W., Park, E.-H. and Lim, C.-J. (2000) Catalase, glutathione S-transferase and thioltransferase respond differently to oxidative stress in *Schizosaccharomyces pombe*. *J. Biochem. Mol. Biol.*, **33**, 344–348.
- Cooper, W. J. and Lean, D. R. S. (1989) Hydrogen peroxide concentration in a northern lake: photochemical formation and diel variability. *Environ. Sci. Technol.*, **23**, 1425–1428.
- Dahms, H. U. and Lee, J. S. (2010) UV radiation in marine ectotherms: molecular effects and responses. *Aquat. Toxicol.*, **97**, 3–14.
- Demott, W. R. (1986) The role of taste in food selection by freshwater zooplankton. *Oecologia.*, **69**, 334–340.
- Giorgio, M., Trinei, M., Migliaccio, E. and Pelicci, P. G. (2007) Hydrogen peroxide: a metabolic by-product or a common mediator of ageing signals? *Nat. Rev. Mol. Cell. Biol.*, **8**, 722–728.
- Gliwicz, Z. M. (1990) Food thresholds and body size in cladocerans. *Nature*, **343**, 638–640.
- Gliwicz, Z. M. and Pijanowska, J. (1989) The role of predation in zooplankton succession. In Sommer, U. (ed.), *Plankton Ecology: Succession in Plankton Communities*. Springer, Heidelberg.
- Gonçalves, R. J., Villafañe, V. E. and Helbling, E. W. (2002) Photorepair activity and protective compounds in two freshwater zooplankton species (*Daphnia menucoensis* and *Metacyclops mendocinus*) from Patagonia, Argentina. *Photochem. Photobiol. Sci.*, **1**, 996–1000.
- Góth, L., Lenkey, Á. and Bigler, W. N. (2001) Blood catalase deficiency and diabetes in Hungary. *Diabetes. Care.*, **24**, 1839–1840.
- Habig, W. H., Pabst, M. J. and Jakoby, W. B. (1974) Glutathione S transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, **249**, 7130–7139.
- Hader, D. P., Williamson, C. E., Wangberg, S. A., Rautio, M., Rose, K. C., Gao, K., Helbling, E. W., Sinha, R. P. et al. (2015) Effects of UV radiation on aquatic ecosystems and interactions with other environmental factors. *Photochem. Photobiol. Sci.*, **14**, 108–126.
- Hansson, L.-A., Hylander, S. and Sommaruga, R. (2007) Escape from UV threats in zooplankton: a cocktail of behavior and protective pigmentation. *Ecology*, **88**, 1932–1939.
- Hayes, J. D., Flanagan, J. U. and Jowsey, I. R. (2005) Glutathione transferases. *Annu. Rev. Pharmacol. Toxicol.*, **45**, 51–88.
- Heinsbroek, L. T. N., Van Hooff, P. L. A., Swinkels, W., Tanck, M. W. T., Schrama, J. W. and Verreth, J. A. J. (2007) Effects of feed composition on life history developments in feed intake, metabolism, growth and body composition of European eel, *Anguilla anguilla*. *Aquaculture.*, **267**, 175–187.
- Helbling, W., Zagarese, H. and Williamson, C. (2003) UVR effects on aquatic ecosystems: a changing climate perspective. In Helbling, W. and Zagarese, H. (eds), *UV Effects in Aquatic Organisms and Ecosystems*, Royal Society of Chemistry, Cambridge, pp. 547–568.
- Hylander, S., Jephson, T., Lebret, K., Von Einem, J., Fagerberg, T., Balseiro, e.g., Modenutti, B. E., Souza, M. S. et al. (2011) Climate-induced input of turbid glacial meltwater affects vertical distribution

- and community composition of phyto- and zooplankton. *J. Plankton Res.*, **33**, 1239–1248.
- Hylander, S., Larsson, N. and Hansson, L. A. (2009) Zooplankton vertical migration and plasticity of pigmentation arising from simultaneous UV and predation threats. *Limnol. Oceanogr.*, **54**, 483–491.
- IPCC. (2014) *Cambio climático 2014: Informe de síntesis.*, Grupo Intergubernamental de Expertos sobre el Cambio Climático.
- Kessler, K., Lockwood, R. S., Williamson, C. E. and Saros, J. E. (2008) Vertical distribution of zooplankton in subalpine and alpine lakes: ultraviolet radiation, fish predation, and the transparency-gradient hypothesis. *Limnol. Oceanogr.*, **53**, 2374–2382.
- Kilham, S. S., Kreeger, D. A., Lynn, S. G., Goulden, C. E. and Herrera, L. (1998) COMBO—a defined freshwater culture medium for algae and zooplankton. *Hydrobiologia*, **377**, 147–159.
- Kim, J., Kim, S., An, K. W., Choi, C. Y., Lee, S. and Choi, K. (2010) Molecular cloning of *Daphnia magna* catalase and its biomarker potential against oxidative stresses. *Comp. Biochem. Physiol. C: Pharmacol. Toxicol. Endocrinol.*, **152**, 263–269.
- Kirk, K. L. (1991) Suspended clay reduces *Daphnia* feeding rate: behavioral mechanisms. *Freshw. Biol.*, **25**, 357–365.
- Kirk, K. L. (1992) Effects of suspended clay on *Daphnia* body growth and fitness. *Freshw. Biol.*, **28**, 103–109.
- Koenings, J. P., Burkett, R. D. and Edmundson, J. M. (1990) The exclusion of limnetic cladocera from turbid glacier-meltwater lakes. *Ecology*, **71**, 57–67.
- Laspoumaderes, C., Modenutti, B. E., Souza, M. S., Bastidas Navarro, M., Cuassolo, F. and Balseiro, e.g. (2013) Glacier melting and stoichiometric implications for lake community structure: zooplankton species distributions across a natural light gradient. *Global Change Biol.*, **19**, 316–326.
- Leech, D. M., Padeletti, A. and Williamson, C. E. (2005) Zooplankton behavioral responses to solar UV radiation vary within and among lakes. *J. Plankton Res.*, **27**, 461–471.
- Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
- Martyniuk, N., Modenutti, B. and Balseiro, e.g. (2014) Can increased glacial melting resulting from global change provide attached algae with transient protection against high irradiance? *Freshw. Biol.*, **59**, 2290–2302.
- Masiokas, M. H., Villalba, R., Luckman, B. H., Lascano, M. E., Delgado, S. and Stepanek, P. (2008) 20th-century glacier recession and regional hydroclimatic changes in northwestern Patagonia. *Global Planet. Change*, **60**, 85–100.
- Modenutti, B., Perez, G., Balseiro, E. and Queimaliños, C. (2000) Relationship between light availability, chlorophyll *a* and total suspended solid in a glacial lake of South Andes. *Verh. Internat. Ver. Limnol.*, **27**, 2648–2651.
- Modenutti, B. E., Balseiro, e.g., Bastidas Navarro, M., Laspoumaderes, C., Souza, M. S. and Cuassolo, F. (2013) Environmental changes affecting light climate in oligotrophic mountain lakes: the deep chlorophyll maxima as a sensitive variable. *Aquat. Sci.*, **75**, 361–371.
- Moeller, R. E., Gilroy, S., Williamson, C. E., Grad, G. and Sommaruga, R. (2005) Dietary acquisition of photoprotective compounds (mycosporine-like amino acids, carotenoids) and acclimation to ultraviolet radiation in a freshwater copepod. *Limnol. Oceanogr.*, **50**, 427–439.
- Rautio, M. and Tartarotti, B. (2010) UV radiation and freshwater zooplankton: damage, protection and recovery. *Freshw. Rev.*, **3**, 105–131.
- Rellstab, C. and Spaak, P. (2007) Starving with a full gut? Effect of suspended particles on the fitness of *Daphnia hyalina*. *Hydrobiologia*, **594**, 131–139.
- Rose, K. C., Hamilton, D. P., Williamson, C. E., McBride, C. G., Fischer, J. M., Olson, M. H., Saros, J. E., Allan, M. G. *et al.* (2014) Light attenuation characteristics of glacially-fed lakes. *J. Geophys. Res. Biogeosci.*, **119**, 1446–1457.
- Shindo, Y. and Hashimoto, T. (1995) Antioxidant defence mechanism of the skin against UV irradiation: study of the role of catalase using acatalasaemia fibroblasts. *Arch. Dermatol. Res.*, **287**, 747–753.
- Siebeck, O., Vail, T., Williamson, C. E., Vetter, R., Hessen, D., Zagarese, H. E., Little, E., Balseiro, e.g. *et al.* (1994) Impact of UV-B radiation on zooplankton and fish in pelagic freshwater ecosystems. *Arch. Hydrobiol. Beih. Ergebn. Limnol.*, **43**, 101–104.
- Sommaruga, R. (2015) When glaciers and ice sheets melt: consequences for planktonic organisms. *J. Plankton Res.*, 10.1093/plankt/fbv027.
- Souza, M. S., Balseiro, E., Laspoumaderes, C. and Modenutti, B. (2010) Effect of ultraviolet radiation on acetylcholinesterase activity in freshwater copepods. *Photochem. Photobiol.*, **86**, 367–373.
- Souza, M. S., Modenutti, B. E. and Balseiro, e.g. (2007) Antioxidant defences in planktonic crustaceans exposed to different underwater light irradiances in Andean lakes. *Water. Air. Soil. Pollut.*, **183**, 49–57.
- Sternner, R. W., Elser, J. J., Fee, E. J., Guildford, S. J. and Chrzanowski, T. H. (1997) The light: nutrient ratio in lakes: the balance of energy and materials affects ecosystem structure and process. *Am. Nat.*, **150**, 663–684.
- Suzuki-Ohno, Y., Kawata, M. and Urabe, J. (2012) Optimal feeding under stoichiometric constraints: a model of compensatory feeding with functional response. *Oikos*, **121**, 569–578.
- Thompson, D. W. J., Solomon, S., Kushner, P. J., England, M. H., Grise, K. M. and Karoly, D. J. (2011) Signatures of the Antarctic ozone hole in Southern Hemisphere surface climate change. *Nat. Geosci.*, **4**, 741–749.
- Wiedner, C. and Vareschi, E. (1995) Evaluation of a fluorescent microparticle technique for measuring filtering rates of *Daphnia*. *Hydrobiologia*, **302**, 89–96.
- Williamson, C. E. and Neale, P. J. (2009) Ultraviolet light. In Likens, G. (ed.), *Encyclopedia of Inland Waters*. Elsevier, Oxford, pp. 705–714.
- Wolinski, L., Laspoumaderes, C., Bastidas Navarro, M., Modenutti, B. E. and Balseiro, e.g. (2013) The susceptibility of cladocerans in North Andean Patagonian lakes to volcanic ashes. *Freshw. Biol.*, **58**, 1878–1888.
- Wolinski, L., Modenutti, B., Souza, M. S. and Balseiro, E. (2016) Interactive effects of temperature, ultraviolet radiation and food quality on zooplankton alkaline phosphatase activity. *Environ. Pollut.*, **213**, 135–142.
- Zemp, M., Hoelzle, M. and Haeblerli, W. (2009) Six decades of glacier mass-balance observations: a review of the worldwide monitoring network. *Ann. Glaciol.*, **50**, 101–111.