Glacier melting and stoichiometric implications for lake community structure: zooplankton species distributions across a natural light gradient

CECILIA LASPOUMADERES, BEATRIZ MODENUTTI, MARÍA SOL SOUZA, MARCELA BASTIDAS NAVARRO, FLORENCIA CUASSOLO and ESTEBAN BALSEIRO
Laboratorio de Limnología, INIBIOMA, CONICET-UNComahue, Quintral 1250, Bariloche 8400, Argentina

Abstract

Glaciers around the globe are melting rapidly, threatening the receiving environments of the world's fresh water reservoirs with significant changes. The meltwater, carried by rivers, contains large amounts of suspended sediment particles, producing longitudinal gradients in the receiving lakes. These gradients may result in changes in the light : nutrient ratio that affect grazer performance by altering elemental food quality. Thus, glacial melting may induce a shift in the phytoplankton carbon : nutrient ratio and hence influence the dominance of herbivorous zooplankton through stoichiometric mechanisms. To test this hypothesis, we combined field and experimental data, taking advantage of a natural light intensity gradient caused by glacial clay input in a deep oligotrophic Patagonian lake. Across this gradient, we evaluated the abundances of two consumer taxa with different phosphorus requirements, the copepod *Boeckella gracilipes* and the cladoceran *Daphnia commutata*, using a six-station transect along the lake. We found significant differences in light : nutrient ratio and stoichiometric food quality of the seston, together with a switch from dominance of P-rich *Daphnia* in low carbon : nutrient stations to dominance of low-P copepods in high carbon : nutrient stations. The laboratory experiments confirmed that the difference in the carbon : nutrient ratio across the gradient is sufficient to impair *Daphnia* growth. The overall patterns are consistent with our prediction that shifts in the environmental light : nutrient ratio as a result of glacial melting would contribute to shifts in the dominance of stoichiometrically contrasting taxa in consumer guilds.

Keywords: *Boeckella gracilipes*, *Daphnia commutata*, food quality, glacial clay, light : nutrient hypothesis, Phosphorus, receiving environments, turbidity

Introduction

Global warming will have consequences for all types of aquatic ecosystems (Liboriussen *et al.*, 2005; Acuna *et al.*, 2008; Van de Waal *et al.*, 2009). Increasing temperatures are causing glaciers around the globe to melt rapidly (Masiokas *et al.*, 2008), threatening the receiving environments of the world's fresh water reservoirs with significant changes. The meltwater, carried by rivers, contains large amounts of suspended sediment particles, producing turbid waters in the receiving lakes; thus, lacustrine environments have been recognized as important sentinels of climate change (Williamson *et al.*, 2008, 2009). Because heavy glacial clay inputs cause a turbidity-induced attenuation of photosynthetically active radiation (PAR), they can be expected to result in light limitation for primary producers (Huisman *et al.*, 2004). Zooplankton performance and abundance is known to be affected by numerous environmental factors, such as food supply rates (Smith & Cooper, 1982), food particle size (Sommer, 1989) and fish predation pressure (Brooks & Dodson, 1965). Recent works in ecological stoichiometry have suggested that food quality, notably the phosphorus (P) content relative to the carbon (C) content, also plays an important role in influencing zooplankton performance (Sterner & Elser, 2002; Urabe *et al.*, 2002). Sterner *et al.* (1997) proposed the 'light : nutrient hypothesis' (LNH hereafter) that explicitly links the supply of two key resources, light and P, with the elemental content of primary producers. This hypothesis predicts that under high PAR intensities and low levels of inorganic P, phytoplankton nutrient limitation becomes more severe, resulting in a biomass with a disproportionate accumulation of C relative to P. This increase in C : P ratio in the primary producers results in a decrease in food quality for grazers (Sterner & Elser, 2002). However, what environmental factors regulate the impact of such stoichiometric mechanisms on zooplankton and how might these interact with other factors? Because the nutrient composition of sestonic material influences the

Correspondence: Cecilia Laspoumaderes, tel. + 54 294 442 3374, fax + 54 294 442 2111, e-mails: claspoumaderes@comahue-conicet.gob.ar; chilideres@yahoo.com.ar

performance of herbivorous zooplankton (Elser *et al.*, 1996; Sterner *et al.*, 1997), shifts in the light : nutrient ratio can affect multiple aspects of pelagic ecosystem function, such as the efficiency of energy transfer through the food web (Hessen *et al.*, 2008).

One corollary of stoichiometric theory and the LNH in P-limited systems is that the impact of the light : nutrient ratio and thus food quality should vary for different zooplankton species according to their somatic P requirements. This is because organisms with elevated body nutrient content (low somatic C : P) should be more susceptible to poor food quality when available food develops high C : nutrient ratios (Sterner & Elser, 2002; Hall *et al.*, 2004). A variety of data indicate that the elemental composition of zooplankton varies significantly among grazer species and taxonomic groups. For example, copepods usually require high amounts of nitrogen (N) and low amounts of P, while the herbivore *Daphnia* typically has a relatively higher P and lower N content (Andersen & Hessen, 1991; Hall *et al.*, 2004). Consequently, when *Daphnia* consumes phosphorus-poor food, imbalances between the nutrient content of the food and its own body become important (Hall *et al.*, 2004). Indeed, *Daphnia* growth has been reported to be limited by P at molar seston C : P ratios above 300 (Hessen *et al.*, 2002; Urabe *et al.*, 2002). Relatively high C : P ratios are common in lakes; therefore, *Daphnia* often experiences P limitation in nature (Elser & Hassett, 1994; Balseiro *et al.*, 2007). These findings suggest that the relationship between light and nutrients could determine the relative dominance of taxa requiring different amounts of P in grazer assemblages and predict shifts in grazer composition across gradients in light and nutrient supplies (Elser *et al.*, 2000; Hall *et al.*, 2004). Surprisingly, no studies have examined how light-driven differences in phytoplankton P content affect the relative abundance of different taxa of herbivorous zooplankton in natural environments. It is an open question whether *Daphnia* and copepods respond to spatial variation in resource stoichiometry in nature as the ecological stoichiometry hypothesis predicts, affecting community structure and dynamics.

While the light climate in low-nutrient lakes is usually characterized by high transparency (Morris *et al.*, 1995; Callieri *et al.*, 2007), inputs of finely ground rock particles in glacier-influenced lakes strongly affect vertical light attenuation and may thereby influence lacustrine communities. Indeed, upper lakes in glacial basins often have a grey or whitish appearance, while those lower down in the basin are less turbid because all particles have settled out of the water column (Modenutti *et al.*, 2000). Such shifts in the effects of glacial clay on the light environment can also be observed within a single lake as glacial clay settles, moving away from the source input. Therefore, we predict that the glacier melting would impact the receiving environments via changes in the optical properties of the lakes, thus affecting the nutritional state by changes in the C : P ratio of producers along the turbidity gradient. This, in turn, would induce changes at the grazer level, leading to the dominance of P-rich species in the more turbid end of the gradient and the dominance of P-poor species in the more transparent end.

The Patagonian Andes is the largest glaciered area in South America, and north of 45°S, there are many relatively small glaciers that have been affected by past and present climate changes (Masiokas *et al.*, 2008). In this study, we take advantage of a natural light intensity gradient in Lake Mascardi (North Andean Patagonia, Argentina) caused by inputs of glacial clay from the glaciers of Tronador Mountain that have shown a continuous and maintained recession over the last 30 years (Rabassa *et al.*, 1978; Leiva, 1999; Masiokas *et al.*, 2008). In this study, we aim to test the hypothesis that glacial melting induces a shift in the carbon : nutrient ratio of phytoplankton and hence influences the dominance of two herbivorous zooplankton competitors through stoichiometric mechanisms. In lakes of the Patagonian Andes, the calanoid copepods of the genus *Boeckella* have relatively low P content (Balseiro *et al.*, 1997; Laspoumaderes *et al.*, 2010; Souza *et al.*, 2010), whereas *Daphnia commutata* has a relatively high P content (Balseiro *et al.*, 2008). In particular, we expect that the changes in food quality should affect *Daphnia* more directly due to its high P requirements. To test this, over 2 years, we assessed light and nutrient conditions, seston quality (C : P ratio), quantity and composition as well as the abundances of *D. commutata* and *B. gracilipes* along a six-station transect (9 km in length) across the light : nutrient gradient in Lake Mascardi. In addition, we carried out a *Daphnia* growth assay to assess if its growth is constrained by food quality across a C : P gradient similar to that found across the lake's light gradient.

Materials and methods

Study site and sampling procedures

Lake Mascardi (41°15′ – 41°25′S; 71°28′ – 71°39′W) is a V-shaped lake with a western arm (Tronador arm, Zmax = 118 m) and an eastern arm (Catedral arm, Zmax = 218 m) (Fig. 1). The lake is glacial in origin, with a deep basin and well-defined margins. The northernmost end of Tronador arm receives the Upper Manso River, which begins at the largest glacier (Manso Glacier) of Tronador Mountain (3554 m a.s.l.). Glacier fluctuations on Mountain Tronador have been observed since 1976 and show a continuous
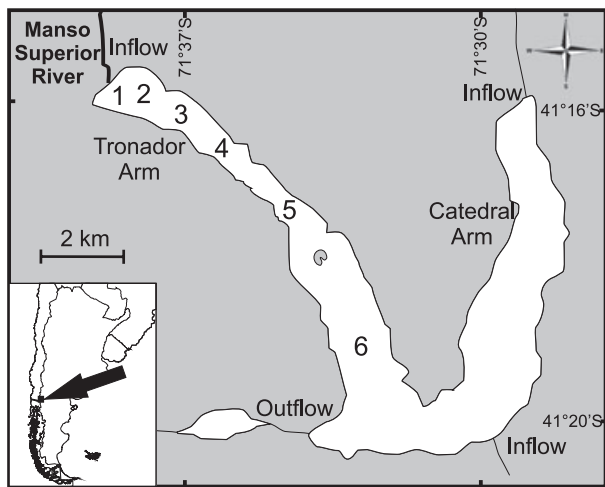
Fig. 1 Map of Lake Mascardi with sampling stations (1–6). The Manso Superior River is the glacial clay input.

and maintained recession (Masiokas *et al.*, 2008). During the warm months of the year, the glacier discharges large amounts of glacial clay (Iriondo, 1974), and the streams that drain this glacier are therefore classified as 'white-waters' (Chillrud *et al.*, 1994). The suspended sediment particles cause a sharp longitudinal gradient in light along the western arm of Lake Mascardi (Modenutti *et al.*, 2000; Balseiro *et al.*, 2007), leading to differences between the two arms of the lake. The Tronador arm has a high load of suspended glacial clay, while the Catedral arm has clear water streams and no glacial clay. The fact that the lake outlet is located at the confluence of the two arms maintains the differences between them.

In two successive austral summers (January 2009 and January 2010), we sampled a six-station transect across the Tronador arm at increasing distances from the meltwater input of the Manso River to include the entire light intensity gradient (Fig. 1). At each station, we measured the vertical profiles of temperature and downward irradiance with a submersible radiometer (PUV 500B; Biospherical Instruments, San Diego, CA, USA). Photosynthetically Active Radiation attenuation depths were used as proxies of water transparency at different stations. Replicated lake water samples were obtained with a Schindler-Patalas trap from the epilimnion (0, 5, and 15 m depth) and hypolimnion (30 and 45 m depth) for chlorophyll *a* concentration (Chl *a*) and sestonic carbon and nutrient analyses. The epilimnion was considered to be the mixed layer, which was determined 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. Epilimnion attenuation coefficients, temperature profiles and average nutrient concentrations were used to calculate the light : nutrient ratio at each station (see below). Zooplankton were sampled with closing plankton nets from the epilimnion (0–15 m) and the hypolimnion (15–45 m) to include migrating individuals. All samples were transferred to acid-washed propylene bottles and carried to the laboratory

in thermally insulated containers. For zooplankton quantification, one sample from each interval was stored in the freezer at −20 °C. Sampling was conducted in full sunshine on windless days, starting at station 1 (nearest to the clay inputs; see Fig. 1) at 12:00 hours noon and ending at approximately 14:00 hours at station 6 (farthest from the glacial inputs).

Laboratory procedures

Immediately after sampling, live zooplankton were manually separated under a stereomicroscope for elemental analyses. The zooplankton were rinsed in Milli-Q water and then identified and classified by species under a stereomicroscope in 5 mL Bogorov chambers. Calanoid copepods of similar size (~80 individuals, 0.7 mm in length) and *Daphnia* of similar size (~20 individuals, 1.8 mm in length) were separately placed onto precombusted (2 h at 450 °C) GF/F Whatman filters and analysed for C content using a Thermo Finnigan EA 1112 CN elemental analyser (Thermo Scientific, Milano, Italy). Phosphorus analyses were conducted using a similar number of freshly separated individuals of similar size as those used in the C analyses. Animals were placed in 45 mL of Milli-Q water and then digested with persulfate at 1.5 atm (121 °C) for 1 h, followed by colorimetric analysis via the molybdate reaction (APHA, 2005). Zooplankton species were quantified from the frozen samples in 5 mL Bogorov chambers under a stereomicroscope. *Daphnias*' adult sizes along the gradient were measured by taking lateral images and then processing the images using Image-Pro Plus (Media Cybernetics, Bethesda, MD, USA) software.

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 C using a Thermo Finnigan EA 1112 CN elemental analyser (Thermo Scientific). Particulate phosphorus (PP) was calculated for each sample as the difference between total phosphorus (TP) and total dissolved phosphorus (TDP). Total phosphorus (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). Epilimnetic and hypolimnetic sestonic C : P atomic ratios were calculated by averaging the depth-specific seston C : P ratios for the 0, 5, and 15 m depths and the 30 and 45 m depths respectively.

Total suspended solids (TSS) were quantified by filtering 1 L of lake water through preweighted GF/F glass fibre filters (Whatman™), which were dried for at least 48 h at 60 °C and then reweighed. Chlorophyll *a* was determined by filtering 100 mL of the water samples from each station and depth through GF/F glass fibre filters (Whatman™), extraction with 90% ethanol (Nusch, 1980) and quantification with a 10-AU fluorometer (Turner Designs, Sunnyvale, CA, USA) that had been previously calibrated against direct spectrophotometric measurements. Dissolved organic carbon (DOC) and a_{440} were measured on lake water that had been filtered through precombusted GF/F glass fibre filters (Whatman™). DOC was quantified in a Shimadzu TOC VCSH Carbon analyser, and a_{440} was quantified in a double beam spectrophotometer Shimadzu UV2450 in 10 cm cells with Milli-Q water as a blank.

To determine if phytoplankton species composition and size (cells <20 μm, considered as edible algae) changed across the gradient of glacial clay, two stations located near each extreme of the transect were compared (stations 2 and 6). Phytoplankton samples (200 mL) were fixed with Lugol's solution and then counted and measured with an inverted microscope using 50 mL Utermöhl chambers. Algal cells were measured with an image analysis system (Image ProPlus, Media Cybernetics).

Experimental conditions and experimental design

Growth rates of *D. commutata* neonates were measured in the laboratory under different C : P food ratios to test whether the difference in food C : P across the natural lake gradient (see Results) could affect their growth rate. To obtain different levels of food C : P, the green algae *Chlamydomonas reinhardtii* was grown at 15 °C on COMBO medium (Kilham *et al.*, 1998) with the phosphate concentration reduced (2.5 μmol P L^{-1}) in a chemostat at a dilution rate of 0.25 day^{-1}. Water in the growth chamber was mixed by bubbling with air filtered through a 0.2-μm filter. The cultures reached stable states before the start of the experiment. We added different amounts of dissolved inorganic P (K$_2$HPO$_4$) to batches of the chemostat P-depleted algae to spike them with P and lower their C : P ratio while keeping other food quality parameters constant (Plath & Boersma, 2001). The P-depleted algae were incubated with the dissolved inorganic P for 30 min before the algae were distributed into the new experimental containers. Approximately 1 h passed from when the inorganic P was added until the *D. commutata* were feeding on the spiked algae. The algae were diluted to 30 μmol C L^{-1} using P-free COMBO medium before being used in the experiments. The C concentration of the algae culture for daily food dilution was calculated from the *in vivo* fluorescence using a previously established calibration curve between the fluorescence and C concentration, measured in a Thermo Finnigan EA 1112 CN elemental analyser (Thermo Scientific).

For the two different treatments, actual concentrations of total particulate P and C were monitored by routine analysis (see data analyses). To obtain food for the experiments, a known volume of algal culture from the chemostat was centrifuged at 3000 g, and the supernatant was discharged. The pellet was resuspended in Milli-Q water and subjected to particulate C and P analyses. On the basis of these measurements and using the same procedure, we collected and added chemostat algae to reach a concentration of 30 μmol C L^{-1} (a C concentration similar to that of Lake Mascardi) in 50-mL experimental beakers. A clonal population of *D. commutata* was started from a single female isolated from the population of Lake Mascardi. The clone was maintained under laboratory conditions and fed with *C. reinhardtii* for at least 20 generations prior to starting the experiment. The experiment began by transferring one *Daphnia* neonate (less than 24 h old) to each flask with the corresponding food C : P level. The flasks were completely filled to decrease the risk of neonates getting caught in the surface tension. We ran each food treatment with 15

replicates for 10 days, and every 2 days, the COMBO from the 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 μmol photons m^{-2} s^{-1}) photoperiod. Prior to this and every 2 days, the body length and area of each animal were measured by taking lateral images and then processing the images using Image-Pro Plus (Media Cybernetics) software, following Acharya *et al.* (2004) and Balseiro *et al.* (2007).

Calculations

PAR attenuation coefficients ($K_{d\ PAR}$) were determined from the slope of the linear regression of the natural logarithm of downwelling PAR irradiance vs. depth. To assess the correlation between the sestonic C : P ratio and the light : nutrient ratio within the epilimnion, we calculated the mean light level of the mixed layer (I_m) (Sterner *et al.*, 1997; Balseiro *et al.*, 2007) according to the formula:

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

where Z_m indicates the depth of the mixed layer. The mixed layer was estimated as described above. We then calculated the light : phosphorus ratio (I_m : TP μmol L^{-1}) (Sterner *et al.*, 1997) for each station where TP is the average TP concentration for epilimnetic water samples.

To quantify the stoichiometric imbalance between seston and zooplankton across the light gradient, we calculated the difference between seston C : P atomic ratio and the C : P atomic ratio for each zooplankton species at each station as: Imbalance = log C : P$_{food}$ − log C : P$_{consumer}$. We applied this general formula following Sterner & Elser (2002) to analyse relative imbalances of *Daphnia* and copepods with their resources.

Growth rates of *Daphnia* neonate experiments were estimated as:

$$GR(day^{-1}) = \frac{\ln[A_{(f)}] - \ln[A_{(i)}]}{Time(days)}$$

where $A_{(f)}$ and $A_{(i)}$ are the final and initial lateral areas, expressed in μm^2. These GR were then combined with the GR obtained from natural lake water from Lake Mascardi and other lakes of the area, obtained from Balseiro *et al.* (2007).

Phosphorus concentration, light : nutrient ratio and seston C : P ratio along the transect were analysed by linear regression. Epilimnetic and hypolimnetic C : P ratios and the temperature among stations were compared with a Kruskal-Wallis (K-W) nonparametric analysis, and when necessary, the Tukey *post-hoc* test was applied. Differences in stoichiometric imbalance for the two zooplankton species were tested with an ANOVA. The comparison of species composition between station 2 and 6 was performed by applying a paired *t*-test, and the growth rates of the two experimental treatments

were compared using a *t*-test. Normality and homoscedasticity were confirmed before the analyses; when these conditions were not fulfilled, a K-W test was applied.

Results

We found substantial differences across the lake transect in total suspended solids, optical properties, nutrients, temperature and zooplankton species distribution. Total suspended solids decreased from station 1 (3.83–3.36 mg L^{-1}) to station 6 (0.59–0.76 mg L^{-1}), showing that glacial clay sinks with increasing distance from the input. As a result of the declining effects of glacial clay input from the Manso River, attenuation coefficients decreased steadily from station 1 ($K_d = 0.24$ m^{-1}) to station 6 ($K_d = 0.16$ m^{-1}) (Fig. 2a). TSS and K_d had a highly significant correlation ($r^2 = 0.616$, df = 10, $P = 0.002$), showing that the changes in the optical properties of the lake along the transect were due to the inorganic suspended particles. The DOC concentrations were extremely low (DOC 0.48–0.58 mg L^{-1}) with no increasing or decreasing trend along the gradient ($r^2 = 0.006$, df = 28, $P = 0.680$). The water colour (a_{440}) was also very low (0.033–0.066 m^{-1}) and without a trend along the transect ($r^2 = 0.09$, df = 28, $P = 0.095$). As a result of the decreased K_d, epilimnetic mean light intensity (I_m) increased with increasing distance from the river input. Total Phosphorus (TP) was constant along the transect (2009: $P = 0.169$; 2010: $P = 0.136$) in both the epilimnion and the hypolimnion (2009: $P = 0.069$; 2010: $P = 0.125$) (Fig. 2b). As predicted by

the light : nutrient hypothesis, with increasing light intensities and no or little change in TP, both I_m : TP ratio and sestonic C : P ratio increased (nutritional quality decreased) along the gradient (C : P 2009: $r^2 = 0.43$, df = 17, $P = 0.003$; 2010: $r^2 = 0.41$, df = 16, $P = 0.006$. I_m : TP 2009: $r^2 = 0.78$, df = 5, $P = 0.019$; 2010: $r^2 = 0.79$, df = 5, $P = 0.018$) (Figs 2c and d). Consistent with the LNH, we observed strong positive relationships between I_m : TP and sestonic C : P ratios (2009: $r^2 = 0.90$, df = 5, $P = 0.004$; 2010: $r^2 = 0.87$, df = 5, $P = 0.007$) (Fig. 3). In contrast and consistent with the lower light intensities, seston C : P ratios in the hypolimnion were generally lower than in the epilimnion (2009, K-W = 5.034, $P = 0.025$; 2010, K-W = 13.069, $P < 0.001$) with no difference between the stations (2009, K-W = 9.231, $P = 0.100$; 2010, K-W = 0.818, $P = 0.976$), ranging from 85 to 150, with the exception of an increase to 250 at station 6 in 2009. Water temperature was lower in 2010 than in 2009 (Fig. 4). Despite this difference, the temperature pattern in Lake Mascardi showed the same trend in both years; it increased with increasing distance from the glacial input in both sampling years (2009, K-W = 83.107, $P < 0.001$; 2010, K-W = 163.974, $P < 0.001$). The first three stations were the coldest (Tukey test, $P < 0.05$), stations 4 and 5 were intermediate (Tukey test, $P < 0.05$), and station 6 was the warmest (Tukey test, $P < 0.05$) for both sampling years (Fig. 4).

Chlorophyll *a* concentration was always less than 1.5 μg L^{-1}, and vertical distributions showed the presence of a deep chlorophyll maximum (DCM) at all
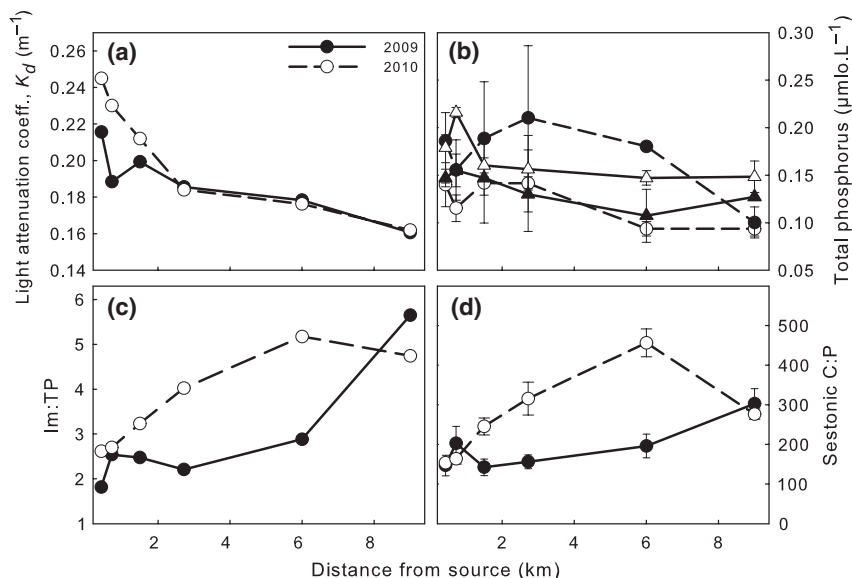

Fig. 2 (a) Attenuation coefficient (K_d), (b) total phosphorus (μmol L^{-1}), circles are the epilimnion and triangles are the hypolimnion, (c) light nutrient ratio (I_m : TP), and (d) sestonic C : P (atomic) (mean ± SE), at increasing distance (km) from the river input.
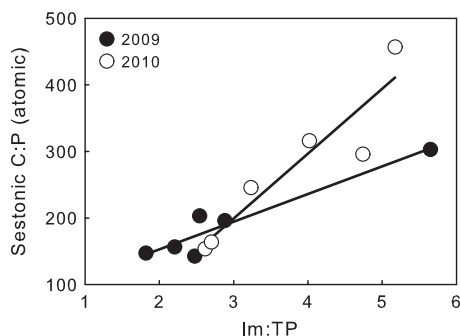
Fig. 3 Relationship between sestonic C : P ratio (atomic) and light : nutrient ratio (I_m : TP) in the lake. (2009: $r^2 = 0.90$, df = 5, $P = 0.004$; 2010: $r^2 = 0.87$, df = 5, $P = 0.007$).
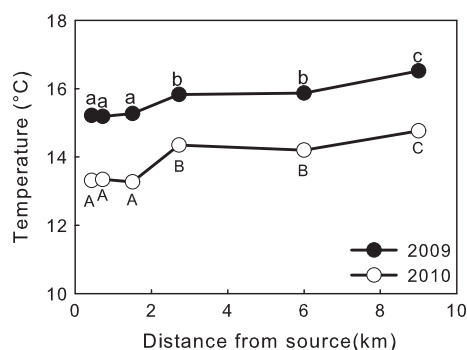

Fig. 4 Temperature across the transect (mean ± SE). Same letters indicate no significant differences between stations ($P > 0.05$). Capital letters are for 2010 and lowercase letters for 2009.

stations. The depth of the DCM increased towards station 6 (Fig. 5), and chlorophyll concentrations at depth were also generally higher moving towards station 6 (Fig. 5). The phytoplankton community was dominated by nanoflagellates and did not vary across the horizontal gradient (Table 1). The most abundant species were the Haptophyceae *Chrysochromulina parva*, followed by

the Cryptophyceae *Rhodomonas lacustris*, which together made up more than 85% of the total phytoplankton cell abundance. In all cases, we did not observe significant differences in phytoplankton species composition at the two ends of the gradient [$t(21) = 1.859$, $P = 0.08$] (Station 2 and 6, Table 1). Thus, the assemblage was dominated all along the gradient by edible nanoplanktonic cells (<20 μm), and the proportion of this size class did not change significantly (Table 1).

Zooplankton communities were dominated by the calanoid copepod *B. gracilipes* and the cladoceran *D. commutata*. These two species represent almost the entire zooplankton biomass in the lake. The remaining zooplankton were rotifers (such as *Polyarthra vulgaris*, *Keratella cochlearis* and *Synchaeta* spp.) whose abundances did not change across the gradient. In contrast, there was a clear variation in crustacean zooplankton species abundance across the gradient with the absolute and relative abundances of *D. commutata* declining while those of *B. gracilipes* increased from station 1 to station 6 (Fig. 6). At the stations closer to the Manso River input, *Daphnia* was most abundant (*Daphnia*: 1100 ind m^{-3}, *B. gracilipes*: 600 ind m^{-3}), but at the opposite end of the transect, *Boeckella* dominated (*Daphnia*: 70 ind m^{-3}, *B. gracilipes*: 1600 ind m^{-3}) (Figs 6c and d). The same pattern was observed in both study years. The species differed in their C : P somatic ratios. In *D. commutata*, C : P varied between 50 and 80 (Fig. 7), whereas in *B. gracilipes*, C : P ranged from 125 to 350 (Fig. 7). In general, C : P ratios of the two zooplankton species did not vary significantly along the transect for animals sampled from the hypolimnion or the epilimnion (all cases tested $P > 0.05$). However, in 2009, copepods' C : P ratio increased with increasing distance from the clay input ($r^2 = 0.21$, df = 21, $P = 0.032$).

Combination of the seston and zooplankton elemental data showed that, moving away from the source of clay input and towards clearer water, *Daphnia* faced an increasing stoichiometric imbalance with its food resources (2009, $r^2 = 0.49$, df = 17, $P < 0.001$; 2010,
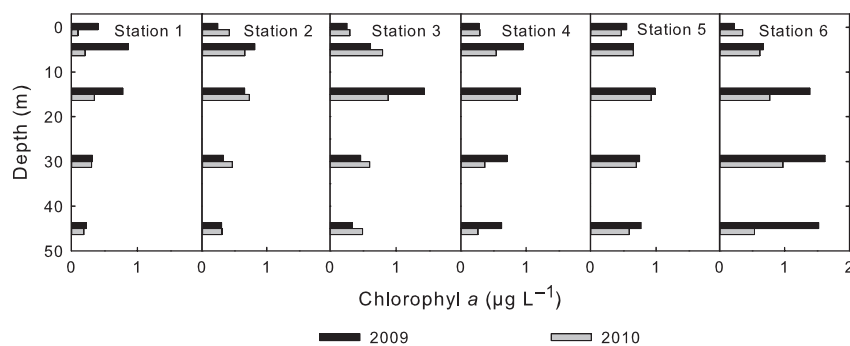

Fig. 5 Vertical profiles of Chlorophyll *a* (μg L^{-1}) along the Lake Mascardi gradient.

Table 1 Composition of phytoplankton and proportion of cells of size less than 20 μm (edible cells) at the two ends of the Lake Mascardi gradient (stations 2 and 6) in the two sampling years. Mean cell numbers are in cell mL^{-1}, SD, standard deviation; prop., proportion to total cell abundance
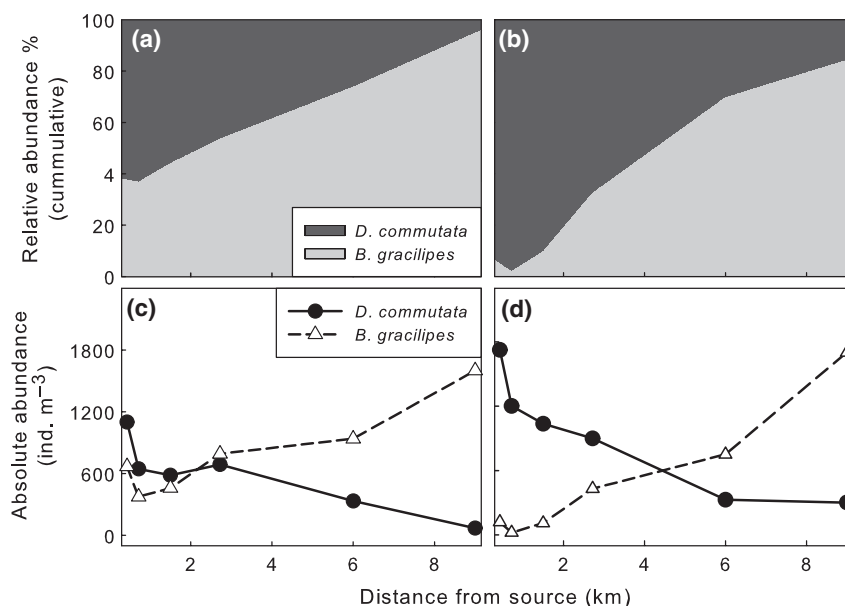
	Station 2 (2009)		Station 6 (2009)		Station 2 (2010)		Station 6 (2010)	
	Mean ± SD	Prop.	Mean ± SD	Prop.	Mean ± SD	Prop.	Mean ± SD	Prop.
Haptophyceae	255 ± 2.7	0.65	371 ± 125.2	0.67	208 ± 54.4	0.73	191 ± 61.4	0.64
Cryptophyceae	110 ± 26.0	0.28	120 ± 41.9	0.21	72 ± 27.5	0.25	77 ± 46.7	0.26
Dinophyceae	27 ± 15.7	0.07	59 ± 6.0	0.11	5 ± 4.7	0.02	23 ± 12.8	0.08
Chrysophyceae	20 ± 11.5	0.05	49 ± 27.9	0.09	7 ± 5.8	0.03	15 ± 5.2	0.05
Bacillaryophyceae	1 ± 1.9	0.00	0 ± 0.0	0.00	0 ± 0.0	0.00	4 ± 4.1	0.01
Chlorophyceae	2 ± 0.1	0.01	2 ± 0.9	0.00	1 ± 0.2	0.00	1 ± 1.0	0.00
Cyanophyceae			5 ± 0.0	0.01			3 ± 3.2	0.01
Proportion of cells <20 μm	0.99		0.98		0.99		0.98	

$r^2 = 0.50$, df = 19, $P < 0.001$) (Fig. 8). In contrast, the stoichiometric imbalance experienced by *B. gracilipes* was much lower than that of *Daphnia* (ANOVA, $F_{1,74} = 101.423$, $P < 0.001$) and did not increase along the gradient (Fig. 8) (2009, $P = 0.753$; 2010, $P = 0.107$), at least partially reflecting the plasticity of its somatic C : P ratio (Fig. 7). There was a clear negative relationship between the abundance of *Daphnia* with the sestonic C : P ratio (2009: $r^2 = 0.78$, df = 5, $P = 0.020$; 2010: $r^2 = 0.66$, df = 5, $P = 0.48$,) (Fig. 9a) and the I_m : TP (2009: $r^2 = 0.80$, df = 5, $P = 0.017$; 2010: $r^2 = 0.68$, df = 5, $P = 0.042$) (Fig. 9b). *Daphnia* adults varied from 2.3 to 2.5 mm in body length, and we did not find any trend or differences in size along the gradient ($r^2 = 3.7e^{-7}$, df = 202, $P = 0.99$). *Daphnia* abundance

decreased as the C : P ratio and the I_m : TP increased, resulting in an increase in copepod dominance at lower food qualities as *Daphnia* dominated areas with good quality food. Strikingly, combining data from both years and considering the temperature, a multiple regression analysis shows that C : P and temperature explain 85% of the variation in *Daphnia* abundance ($R^2 = 0.85$, df = 9, $P < 0.001$).

Growth experiment

The food concentration in the experiments was on average 30 μmol C L^{-1} (range: 26–35 μmol C L^{-1}). This C concentration was slightly higher than what is found in Lake Mascardi (~20 μmol C L^{-1}) to eliminate the

Fig. 6 Species abundance across the sampling gradient. (a) and (b) Relative abundances. (c) and (d) Absolute abundances. Left panels (a and c) are for 2009 and right panels (b and d) are for 2010.
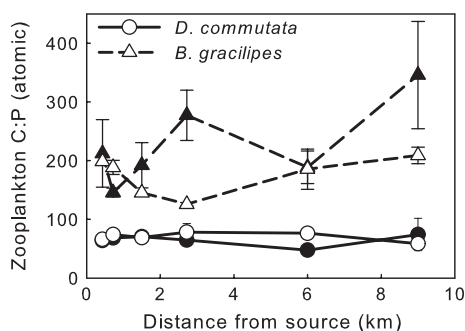
Fig. 7 Elemental atomic C : P ratios of zooplankton (mean ± SE). Open figures are for 2010 and closed figures are for 2009.
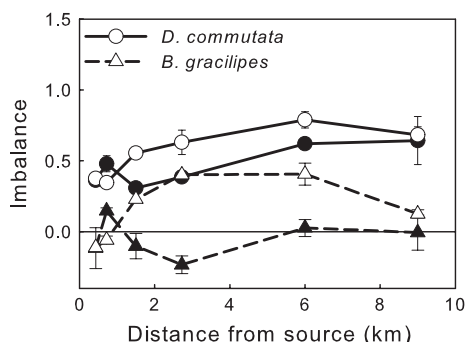

Fig. 8 Elemental imbalance between consumers and their food along the gradient (mean ± SE). Open figures are for 2010 and closed figures are for 2009.

potential interference of food quantity effects. The C : P atomic ratio for the high quality treatment was 200 (SE 15), and this value is equivalent to those of the first lake sampling stations (Fig. 2d). The low quality treatment had a C : P of 400 (SE 17), close to that of lake sampling stations 5 and 6 (Fig. 2d). *Daphnia commutata* growth rates varied from 0.09 to 0.18 day^{-1} and were

significantly affected by the food quality (t-test = 3.41, P = 0.003). The growth rates obtained in this experiment fitted well in the growth rate–C : P relationship obtained in previous experiments (Balseiro *et al.*, 2007) carried out with natural seston from Lake Mascardi and other lakes of the area (r^2 = 0.781, df = 38, P < 0.001) (Fig. 10).

Discussion

In Lake Mascardi, we observed a positive correlation between light : phosphorus supply ratios in the water column and the seston C : P ratio (Fig. 3). Concurrently, we observed shifts in zooplankton community structure (Fig. 6), as the abundances of P-rich *Daphnia* relative to low-P *Boeckella* declined as light penetration, light : nutrient ratio (Fig. 9a) and seston C : P (Fig. 9b) increased with increasing distance from the glacial input. These two herbivorous crustaceans compete for algal food because both feed on the same algal types (Balseiro *et al.*, 2001; Modenutti *et al.*, 2003). Across the gradient, our results showed that the total phytoplankton biomass, as revealed by Chl *a* concentration, increased slightly towards the clear end of the gradient (Fig. 5), without any change in species composition (Table 1). Thus, neither the *Daphnia* decline nor the shift in *Daphnia/Boeckella* relative abundances could have been caused by food quantity. In addition, the experiment showed that the difference in the C : P ratio in the gradient is sufficient to impair *Daphnia* growth and cause a decrease in its performance, resulting in lower competitive abilities in the transparent-low quality sector. Thus, these data provide the first evidence from direct field observations in support of the role of light : nutrient ratio in influencing zooplankton community structure and distributions via shifts in stoichiometric food quality, suggesting that the changes in glacial melting rates will affect lake community structure.
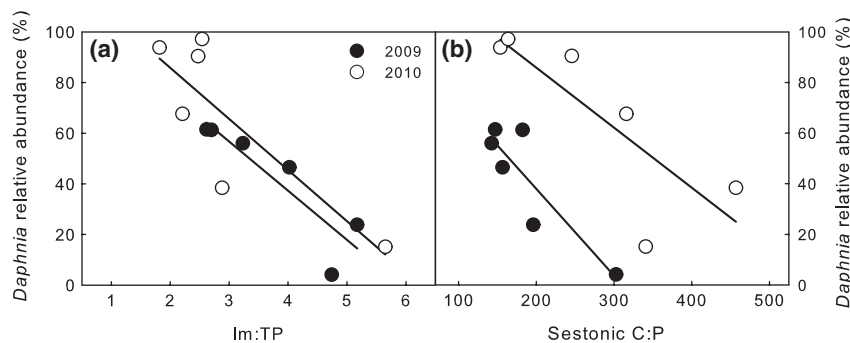

Fig. 9 *Daphnia commutata* relative abundance vs. (a) I_m : TP, 2009: r^2 = 0.80, df = 5, P = 0.017; 2010: r^2 = 0.68, df = 5, P = 0.042; and (b) sestonic C : P (atomic), 2009: r^2 = 0.78, df = 5, P = 0.020; 2010: r^2 = 0.66, df = 5, P = 0.048. 2009 (closed circles) and in 2010 (open circles).
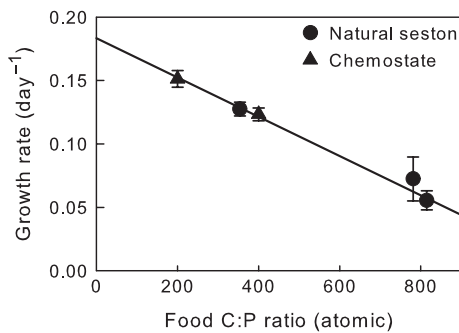
Fig. 10 Experimental growth rate of *Daphnia commutata* (mean ± SE) under different food C : P ratios. Circles indicate experiments with natural seston as food. Triangles indicate chemostat cultures as food ($r^2 = 0.781$, df = 38, $P < 0.001$).

An important factor associated with our study is the possible shifting role of visual predation by fish in affecting the absolute and relative abundances of *Daphnia* and *Boeckella* across the gradient. 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 in the more transparent end of the lake (Balseiro *et al.*, 2007). Therefore, the predation risk may act in the same direction as the LNH, impairing *Daphnia*'s performance due to the higher risk of visual predation and low food quality. Although predation could have contributed to the distribution pattern observed in Lake Mascardi, the growth experiments (natural lake water and chemostat algae) strongly support the stoichiometric hypothesis. If the *Daphnia* population is experiencing a stoichiometric constraint that lowers its growth rate (as shown by our experiments), this would imply that under predation pressure, the prey population along the gradient may not compensate for the losses due to the reduced growth as food quality decreases. In contrast, a population not affected by low quality food might have been able to grow sufficiently to compensate for predation losses. As a result, although predation might lower the population abundance, the stoichiometric constraints would be key to population success, affecting population growth and thus mortality compensation. In addition, the adult size analyses showed that there were no changes in size along the gradient, which would be expected under moderate or high visual fish predation due to size selective feeding (Gliwicz & Pijanowska, 1989); even a decrease in size induced by fish chemical cues might be expected under such conditions (Boersma *et al.*, 1999). Finally, some whole-lake food web manipulations in oligotrophic lakes have shown that in lakes where C : P is above the *Daphnia* threshold elemental ratio (C : P above 300), food quality may be insufficient to support increased populations of

Daphnia, despite reductions in levels of planktivory (Elser *et al.*, 1995, 1998; Mackay & Elser, 1998). This means that *Daphnia* abundance in lakes with C : P ratios above its threshold will be regulated by food quality mechanisms rather than by fish predation pressure.

Suspended inorganic particles affect the feeding efficiency of crustacean zooplankton. In this sense, *Daphnia* would be more strongly affected than calanoid copepods, as copepods have more selective feeding strategies (Richman & Dodson, 1983; Koenings *et al.*, 1990; Balseiro *et al.*, 2001). Thus, *Boeckella* would be capable of avoiding the ingestion of high amounts of glacial clay, while *Daphnia*, as a filter feeder, would not (Gliwicz, 1986). Despite the advantage that copepods would have over *Daphnia* in the more turbid area of the lake, *Daphnia* dominates the area, taking advantage of its ability to outcompete copepods when food quality is high.

Daphnia's body C : P ratio did not change significantly across the gradient. While changes in *Daphnia* C : P content due to changes in food quality have been obtained in laboratory experiments (Plath & Boersma, 2001; Demott, 2003; Balseiro *et al.*, 2008), the C : P ratio of *D. commutata* from Lake Mascardi did not change significantly until the food C : P ratio was increased above approximately 600 : 1 (Balseiro *et al.*, 2008). This suggests that, while the stoichiometric imbalance observed in Lake Mascardi did impair *D. commutata*'s growth (present growth rate experiment), it did not exceed its capacity to regulate body elemental composition. On the other hand, *B. gracilipes* showed less strict homeostatic regulation because its somatic C : P ratio varied almost threefold (125–350) across the transect (Fig. 7). This higher plasticity and higher C : P ratio resulted in a much lower imbalance with its resources (Fig. 8). Although *D. commutata*'s homeostatic capacity may not have been exceeded, our growth experiments showed that the increased imbalance provoked a decline in *Daphnia* growth (Fig. 10). Because *Boeckella* has a higher overall C : P ratio, it can take advantage of the decline of *Daphnia* along the gradient, as *Daphnia* would become a weaker competitor with declining food quality, which results in lower growth rates. Under these conditions, *Boeckella* can become dominant, and the plankton community structure can change from being dominated by *Daphnia* to being dominated by copepods.

In addition, the transparent end of the gradient is more transparent not only for PAR but also for ultraviolet radiation (UVR), and it has been demonstrated that stoichiometric constraints (high food C : P ratio) affect *Daphnia*'s capacity to respond to UVR-increased oxidative stress (Balseiro *et al.*, 2008). This means that in the more transparent end of the gradient with higher P

limitation, not only *Daphnia*'s growth rate is decreased but also its fitness is reduced by its inability to cope with the UVR indirect effect. The fact that P limitation increases across the gradient in the same direction as the increase in UVR stress adds additional support to the hypothesis that *Daphnia* becomes less abundant as the lake becomes more transparent mainly due to an increase in P limitation. Although the copepods could suffer a similar effect of food quality on cellular defence (Souza *et al.*, 2010), the copepods experienced a less severe imbalance than *Daphnia*. Thus, it could be expected that under similar conditions, the effect on cellular defence would also be lower. On the other hand, copepods at intermediate latitudes are less susceptible to UVR than cladocerans (Zagarese *et al.*, 1997; Leech & Williamson, 2000; Cooke & Williamson, 2006). Moreover, because DOC did not vary along the gradient, changes in UVR attenuation due to DOC increase could not be expected during deglaciation, as was reported for small lakes in Alaska (Williamson *et al.*, 2001). Nor could changes in algae composition, as observed by Parker *et al.* (2008), be expected. Indeed, we did not observe any change in algae composition along the gradient.

Gradients in food quality could have a combined effect with temperature, as there is an increased risk of P limitation at higher temperatures (Persson *et al.*, 2011). High temperatures facilitate high somatic growth rates, but pose a higher dietary demand for P to maximize growth than do lower temperatures (Persson *et al.*, 2011). In Lake Mascardi, areas with a higher C : P ratio also had higher temperatures, which would increase the P limitation for *Daphnia* growth. These results imply that the effect of low food quality might have been stronger than what we observed in our experiments, where we only manipulated food quality. Thus, the net effect of glacial melting as a consequence of global warming would result in changes in zooplankton community structure by combining nutrient limitation and increased temperature. Strikingly, lake water in 2010 was colder than in the previous year, and consistent with Persson *et al.* (2011), relative and absolute abundances of *Daphnia* were higher, which supports the hypothesis that a higher C : P ratio would have a less severe effect when temperature is lower. Consistent with this, the multiple regression analysis shows that the two variables together explain a high proportion of the variance in *Daphnia* abundance, supporting the effect of temperature on P limitation.

In conclusion, our results show that global warming, through glacial melting, will have important effects not only through losses of the worlds' water reservoirs but also by altering the community structure of the environments that are receiving these substantial amounts of glacial material. Glacial recession will affect water transparency, and it is likely that although glacial clay inputs to lakes will increase when glacial recession ends, the lessening or lack of clay input will allow lakes to become transparent again. This scenario would result a humped effect on food quality. First, the relative content of P will increase because of the lower light : nutrient ratio and therefore benefit species such as *Daphnia* with high P demand. However, as glaciers disappear, the C : P will increase due to an increase in transparency, and thus, species with lower P demand, such as calanoid copepods, will become dominant because of stoichiometric constraints.

References

Acharya K, Kyle M, Elser JJ (2004) Effects of stoichiometric dietary mixing on *Daphnia* growth and reproduction. *Oecologia*, **138**, 333–340.

Acuna V, Wolf A, Uehlinger U, Tockner K (2008) Temperature dependence of stream benthic respiration in an Alpine river network under global warming. *Freshwater Biology*, **53**, 2076–2088.

Andersen T, Hessen DO (1991) Carbon, nitrogen, and phosphorus content of freshwater zooplankton. *Limnology and Oceanography*, **36**, 807–814.

APHA (2005) *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, AWWA, Washington, DC.

Balseiro EG, Modenutti BE, Queimaliños CP (1997) Nutrient recycling and shifts in N:P ratio by different zooplankton structures in a South Andes Lake. *Journal of Plankton Research*, **19**, 805–817.

Balseiro EG, Modenutti BE, Queimaliños CP (2001) Feeding of *Boeckella gracilipes* (Copepoda, Calanoida) on ciliates and phytoflagellates in an ultraoligotrophic Andean lake. *Journal of Plankton Research*, **23**, 849–857.

Balseiro E, Modenutti B, Queimaliños C, Reissig M (2007) *Daphnia* distribution in Andean Patagonian lakes: effect of low food quality and fish predation. *Aquatic Ecology*, **41**, 599–609.

Balseiro E, Souza MS, Modenutti B, Reissig M (2008) Living in transparent lakes: low food P:C ratio decreases antioxidant response to ultraviolet radiation in *Daphnia*. *Limnology and Oceanography*, **53**, 2383–2390.

Boersma M, Demeester L, Spaak P (1999) Environmental stress and local adaptation in Daphnia magna. *Limnology and Oceanography*, **44**, 393–402.

Brooks JL, Dodson SL (1965) Predation, body size, and composition of plankton. *Science*, **150**, 28–35.

Callieri C, Modenutti B, Queimaliños C, Bertoni R, Balseiro E (2007) Production and biomass of picophytoplankton and larger autotrophs in Andean ultraoligotrophic lakes: differences in light harvesting efficiency in deep layers. *Aquatic Ecology*, **41**, 511–523.

Chillrud SN, Pedrozo FL, Temporetti PF, Planas FH, Froelich PN (1994) Chemical weathering of phosphate and germanium in glacial meltwater streams: effects of subglacial pyrite oxidation. *Limnology and Oceanography*, **39**, 1130–1140.

Cooke SL, Williamson CE (2006) Positive effects of UV radiation on a calanoid copepod in a transparent lake: do competition, predation or food availability play a role? *Journal of Plankton Research*, **28**, 171–179.

Demott W (2003) Implications of element deficits for zooplankton growth. *Hydrobiologia*, **491**, 177–184.

Elser JJ, Hassett RP (1994) A stoichiometric analysis of the zooplankton-phytoplankton interaction in marine and freshwater ecosystems. *Nature*, **370**, 211–213.

Elser JJ, Luecke C, Brett MT, Goldman CR (1995) Effects of food web compensation after manipulation of rainbow trout in an oligotrophic lake. *Ecology*, **76**, 52–69.

Elser JJ, Dobberfuhl DR, Mackay NA, Schampel JH (1996) Organism size, life history and N:P stoichiometry. *BioScience*, **46**, 674–685.

Elser JJ, Chrzanowski TH, Sterner RW, Mills KH (1998) Stoichiometric constraints on food-web dynamics: a whole-lake experiment on the Canadian Shield. *Ecosystems*, **1**, 120–136.

Elser JJ, Sterner RW, Gorokhova E *et al.* (2000) Biological stoichiometry from genes to ecosystems. *Ecology Letters*, **3**, 540–550.

Gliwicz ZM (1986) Suspended clay concentration controlled by filter-feeding zooplankton in a tropical reservoir. *Nature*, **323**, 330–332.

Gliwicz ZM, Pijanowska J (1989) The role of predation in zooplankton succession. In: *Plankton Ecology: Succession in Plankton Communities* (ed. Sommer U), pp. 253–295. Springer, Verlag, Berlin.

Hall SR, Leibold MA, Lytle DA, Smith VH (2004) Stoichiometry and planktonic grazer composition over gradients of light, nutrients, and predation risk. *Ecology*, **85**, 2291–2301.

Hessen DO, Faerovig PJ, Andersen T (2002) Light, nutrients, and P:C ratios in algae: grazer performance related to food quality and quantity. *Ecology*, **83**, 1886–1898.

Hessen DO, Leu E, Farovig P, Falkpetersen S (2008) Light and spectral properties as determinants of C:N:P-ratios in phytoplankton. *Deep Sea Research Part II: Topical Studies in Oceanography*, **55**, 2169–2175.

Huisman J, Sharples J, Stroom JM, Visser PM, Kardinaal WEA, Verspagen JMH, Sommeijer B (2004) Changes in turbulent mixing shift competition for light between phytoplankton species. *Ecology*, **85**, 2960–2970.

Iriondo M (1974) Modelo de sedimentación en el Lago Mascardi Provincia de Río Negro. *Revista Asociación Geológica Argentina*, **29**, 349–358.

Kessler K, Lockwood RS, Williamson CE, Saros JE (2008) Vertical distribution of zooplankton in subalpine and alpine lakes: ultraviolet radiation, fish predation, and the transparency-gradient hypothesis. *Limnology and Oceanography*, **53**, 2374–2382.

Kilham SS, Kreeger DA, Lynn SG, Goulden CE, Herrera L (1998) COMBO – a defined freshwater culture medium for algae and zooplankton. *Hydrobiologia*, **377**, 147–159.

Koenings JP, Burkett RD, Edmundson JM (1990) The exclusion of limnetic cladocera from turbid glacier-meltwater lakes. *Ecology*, **71**, 57–67.

Laspoumaderes C, Modenutti B, Balseiro E (2010) Herbivory versus omnivory: linking homeostasis and elemental imbalance in copepod development. *Journal of Plankton Research*, **32**, 1573–1582.

Leech DM, Williamson CE (2000) Is tolerance to UV radiation in zooplankton related to body size, taxon, or lake transparency? *Ecological Applications*, **10**, 1530–1540.

Leiva JC (1999) Recent fluctuations of the Argentinian glaciers. *Global and Planetary Change*, **22**, 169–177.

Liboriussen L, Landkildehus F, Meerhoff M *et al.* (2005) Global warming: design of a flow-through shallow lake mesocosm climate experiment. *Limnology and Oceanography: Methods*, **3**, 1–9.

Mackay NA, Elser JJ (1998) Factors potentially preventing trophic cascades: food quality, invertebrate predation, and their interaction. *Limnology and Oceanography*, **43**, 339–347.

Masiokas MH, Villalba R, Luckman BH, Lascano ME, Delgado S, Stepanek P (2008) 20th-century glacier recession and regional hydroclimatic changes in northwestern Patagonia. *Global and Planetary Change*, **60**, 85–100.

Modenutti B, Perez G, Balseiro E, Queimaliños C (2000) Relationship between light availability, chlorophyll *a* and total suspended solid in a glacial lake of South Andes. *Verhandlungen des Internationalen Verein Limnologie*, **27**, 2648–2651.

Modenutti B, Queimaliños C, Balseiro E, Reissig M (2003) Impact of different zooplankton structures on the microbial food web of a South Andean oligotrophic lake. *Acta Oecologica-International Journal of Ecology*, **24**, S289–S298.

Morris DP, Zagarese H, Williamson CE *et al.* (1995) The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. *Limnology and Oceanography*, **40**, 1381–1391.

Nusch EA (1980) Comparison of different methods for chlorophyll and phaeopigment determination. *Archiv fur Hydrobiologie, Ergebnisse der Limnologie*, **14**, 14–36.

Parker BR, Vinebrooke RD, Schindler DW (2008) Recent climate extremes alter alpine lake ecosystems. *Proceedings of the National Academy of Sciences*, **105**, 12927–12931.

Persson J, Wojewodzic MW, Hessen DO, Andersen T (2011) Increased risk of phosphorus limitation at higher temperatures for Daphnia magna. *Oecologia*, **165**, 123–129.

Plath K, Boersma M (2001) Mineral limitation of zooplankton: stoichiometric constraints and optimal foraging. *Ecology*, **82**, 1260–1269.

Rabassa J, Rubulis S, Suarez J (1978) Los glaciares del Monte Tronador, Parque Nacional Nahuel Huapi, Rio Negro, Argentina. *Anales de Parques Nacionales*, **14**, 259–318.

Richman S, Dodson SI (1983) The effect of food quality on feeding and respiration by Daphnia and Diaptomus. *Limnology and Oceanography*, **28**, 948–956.

Smith DW, Cooper SD (1982) Competition among Cladocera. *Ecology*, **63**, 1992.

Sommer U (1989) *Plankton Ecology. Succession in Plankton Communities*. Springer-Verlag, Berlin.

Souza MS, Modenutti B, Carrillo P, Villar-Argaiz M, Medina-Sánchez JM, Bullejos F, Balseiro E (2010) Stoichiometric dietary constraints influence the response of copepods to ultraviolet radiation-induced oxidative stress. *Limnology and Oceanography*, **55**, 1024–1032.

Sterner RW, Elser JJ (2002) *Ecological Stoichiometry. The Biology of Elements from Molecules to the Biosphere*. Princeton University Press, Princeton, New Jersey.

Sterner RW, Elser JJ, Fee EJ, Guildford SJ, Chrzanowski TH (1997) The light: nutrient ratio in Lakes: the balance of energy and materials affects ecosystem structure and process. *The American Naturalist*, **150**, 663–684.

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

Van de Waal DB, Verschoor AM, Verspagen JMH, Van Donk E, Huisman J (2009) Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. *Frontiers in Ecology and the Environment*, **8**, 145–152.

Williamson CE, Olson OG, Lott SE, Walker ND, Engstrom DR, Hargreaves BR (2001) Ultraviolet radiation and zooplankton community structure following deglaciation in Glacier Bay, Alaska. *Ecology*, **82**, 1748–1760.

Williamson CE, Dodds W, Kratz TK, Palmer MA (2008) Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. *Frontiers in Ecology and the Environment*, **6**, 247–254.

Williamson CE, Saros JE, Vincent WF, Smol JP (2009) Lakes and reservoirs as sentinels, integrators, and regulators of climate change. *Limnology and Oceanography*, **54**, 2273–2282.

Zagarese HE, Feldman M, Williamson CE (1997) UV-B-induced damage and photoreactivation in three species of *Boeckella* (Copepoda, Calanoida). *Journal of Plankton Research*, **19**, 357–367.