Advanc. Limnol. **65**, p. 257–271
Freshwater Phytoplankton of Argentina

Acidophilic phytoplankton in Argentina: the case study of Lake Caviahue (Patagonia)

Mónica M. Diaz* & Guadalupe Beamud

With 7 figures and 2 tables

Abstract: This article presents a review of the studies carried out over a 10-year period in Lake Caviahue, a natural acidic lake located in Patagonia. The main patterns of the phytoplankton populations are described.

Five extremophiles phytoplanktonic species were studied in relation to the zooplankton and nutrients bioavailability under the environmental conditions registered in Lake Caviahue: very low pH (~ 3), high concentrations of iron (18.4 mg L^{-1}) and sulphur (130 mg L^{-1}). During the 10-years studies, the biomass (0.2 and 1.4 mg fw L^{-1}) and the biodiversity were very low being *Keratococcus rhaphidioides* the dominant species (60 to 100% of the total biomass). *Philodina* sp. (Bdelloidea) was the only zooplankter responsible of the zooplankton biomass. From the lack of relationship between phyto-zooplankton biomass, as well as from the results obtained in the feeding experiments, we conclude that no control of algal abundance by the zooplankton occurs.

The microalgae were nitrogen limited in the lake and according to the results from experiments with nutrient addition, the phytoplankton showed nitrogen limitation on growth rate and yield. The species have also the capacity to use mixotrophically alternative sources of organic and inorganic carbon and organic nitrogen. The importance of the uptake and the expression of CO_2 concentrating mechanisms (CCMs) were demonstrated in *Euglena* and in the ellipsoidal form of *Watanabea* but not in the spherical form of *Watanabea* and in *Palmellopsis*. The two forms of *Watanabea* differed in their possession of a CCM.

Keywords: Acidophilic phytoplankton, natural acidic lake, Patagonia

Introduction

Only exceptional organisms among the planktonic communities can tolerate extreme environmental conditions such as those registered in Lake Caviahue: very low pH (~ 3), high aluminium (Al), iron (Fe), and sulphate ($SO_4^{=}$) contents (Pedrozo et al. 2001, 2008). These organisms growing at extreme environments have been named acidophilic extremophiles

Authors' address:
Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA), Universidad Nacional del Comahue, CONICET, Quintral 1250, 8400 Bariloche, Argentina.
*Corresponding author: monica.diaz@crub.uncoma.edu.ar

DOI: 10.1127/1612-166X/2014/0065-0045 1612-166X/2014/0065-0045 $ 0.00

(Pick 1999). From the point of view of their metabolism, the extremophiles photoautotrophs of Lake Caviahue have to thrive besides the high protons concentration with, high phosphorus (P) concentrations (0.45 mg P L^{-1}), very low nitrogen (N) concentrations (70 µg N L^{-1}), low N:P ratio (ranging between 0.1–0.2) and relative low concentrations of pure carbon (C) dioxide dissolved in the water (0.5–1.7 mg C L^{-1}). As a consequence of the low pH, the inorganic carbon is only present as dissolved CO_2 and HCO_3^- is unavailable in extremely acid waters. The 1% of the surface PAR irradiance is located at 16 m depth, including only the epilimnion, but there are no differences between the epi- and hypolimnetic phytoplankton biomass.

Microalgae satisfy their inorganic carbon requirements through photosynthesis, although it is well known that some autotrophic algae are able to take up particulate and/or dissolved carbon directly (Rivkin & Putt 1987). Mixotrophy is the capability of one organism to combine the two nutrition strategies at the same time: phototrophy and heterotrophy. The facultative heterotrophy has been reported for a number of photosynthetic algae able to switch in darkness from photosynthetic carbon fixation to nutrition by direct absorption (osmotrophy) or uptake of organic molecules (Vincent & Goldman 1980, Jones 1994, Glibert & Legrand 2006). The mixotrophic strategy could be advantageous in environments were light and inorganic carbon are low such as acidic lakes. The CO_2 concentration in Lake Caviahue is in the range of the half-saturation concentration (0.2 to 2.4 mg C L^{-1}) of the primary carboxylation enzyme Rubisco (ribulose bis-phosphate carboxylase-oxygenase) which fixes CO_2 into stable C_3 products but which will also competitively undergo an oxygenation reaction leading to a net loss of CO_2 (Falkowski & Raven 2007). This, coupled with the relatively low affinity of all eukaryotic Rubisco for CO_2, has led many species of photoautotrophs to operate a CO_2 concentrating mechanism (CCM) that elevates the concentration of CO_2 around Rubisco, suppressing the oxygenase reaction and promoting photosynthesis (Raven 1997, Giordano et al. 2005).

Most of the existing works on the ecology of phyto- and zooplankton living in acidic lakes are based on lakes that were acidified by the input of anthropogenic emissions (Kwiatkowski & Roff 1976, Olaveson & Nalewajko 1994, Geller et al. 1998, Nixdorf et al. 1998, Kamjunke et al. 2004). These acidic lakes were formed by acid rain deposition, acid mine drainage or the filling of large-scale excavations caused by mining exploitation. Their waters shown an orange-red colour due to the high concentrations of dissolved ferric iron (up to 500 mg L^{-1}), low P and high N concentrations (< 10 µg P L^{-1}; > 1 mg N L^{-1}), as well as low inorganic carbon content (IC = 0.3–0.6 mg C L^{-1}) (Tittel et al. 2005). Besides, evidence of phytoplankton nutrient limitation has been obtained, both from the N:P ratios as from added nutrients experiments, especially for inorganic carbon (Olaveson & Stokes 1989, Tittel et al. 2005). Because of these characteristics, mainly C and secondarily P were considered the nutrients controlling phytoplankton growth in acidic lakes (Olaveson & Nalewajko 1994, Beulker et al. 2003, Tittel et al. 2005). At the naturally acidic Lake Caviahue (the lake described in this article), the C and N are scarce, but the P is abundant, driving to a low N:P and C:P relationships. The consequent N limitation for algal growth was evidenced in the studies carried on both in the lake (Pedrozo et al. 2001, Beamud et al. 2007, 2010b) as well as in its acid tributary: River Agrio (Baffico et al. 2004).

The present article summarizes the results obtained over a 10-year period of work carried on at the naturally acidic Lake Caviahue. We present here the main patterns of the phytoplankton populations. After considering the biomass with relation to environmental and biotic factors, we also evaluate the importance of nitrogen and carbon on the growth rate and yield of phytoplankton, the capacity of several acidophilic microalgae to use alternative sources of carbon and assess the effect of CO_2 on the rate of photosynthesis in order to test whether or not there is any evidence for a CCM.

Study area

Lake Caviahue is a deep glacial and volcanic lake located in Andean Patagonia's north-western region, at 1600 m above sea level, within the Copahue Provincial Park. It has a pH ~ 3, similar to that of acidic mining lakes, although it is naturally acidified by the waters of the Upper Agrio River. Its acidity springs from the headwaters close to the crater of the Copahue Volcano and results in high aluminium (Al), Fe, and sulphate ($SO_4^{=}$) contents in the lake. A full description about study area was presented in Pedrozo et al. (2001, 2008) and Beamud et al. (2010b). The lake has a horseshoe shape, with a North and a South arm (Fig. 1). The morphometric features along with some chemical characteristics (metals and sulphate), chlorophyll *a* and dissolved oxygen contents are shown in Table 1. Water temperature in summer reaches 17.0°C (surface) and 6.5°C (bottom). In winter, the longest season (from early June to later October), the entire water column is mixed and the temperature ranges from 4 to 8°C.

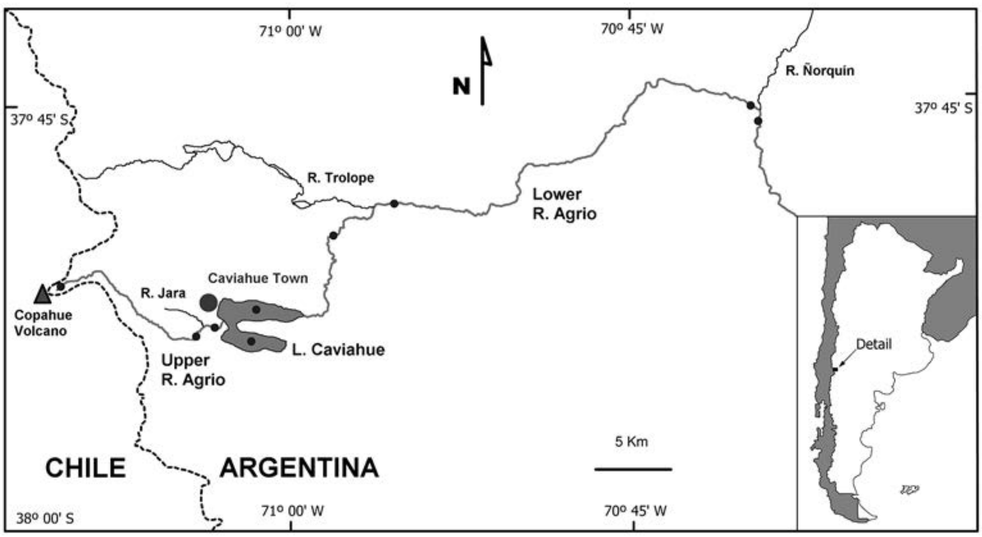

Fig. 1. Map of the study area of the Copahue – Rio Agrio – Lake Caviahue Basin.

Table 1. Morphometric and chemical characteristics, and chlorophyll *a* contents of Lake Caviahue. From Pedrozo et al. (2001, 2008), Beamud et al. (2010b), Rapacioli (1985).

		Lake	North arm	South arm
Max length	(km)	9.75		
Max width	(km)	4.72		
Z max	(m)	95		
Z mean	(m)	51.4		
Area	(km^2)	9.22		
Volume	(Hm^3)	0.474		
Tw	(years)	2.6		
Al	(mg L^{-1})		30.3	31.1
$SO_4^=$	(mg L^{-1})		402	362.3
Fe	(mg L^{-1})		22.2	21.7
Ca	(mg L^{-1})		23.6	21.7
K	(mg L^{-1})		6.7	5.9
Chl *a*	(mg m^{-3})		0.21	0.27
DO	(mg L^{-1})		10.2	10.8

Phytoplankton composition

Lake Caviahue is characterized by a very low diversity of phytoplankton species and the prevalence of green algae. Five planktonic species have been found since the first sampling in 1998 until nowadays, although two dominant populations were present over the years: the small green algae (37 μm^3 of biovolume) *Keratococcus rhaphidioides* (60–100% to the total biomass) and the two cell forms of *Watanabea* sp.: spherical cells: 170 μm^3 and ellipsoidal cells: 25 μm^3 of biovolume (0–22% of the total biomass). *K. rhaphidioides* and *Watanabea* sp. are non-motile species that colonized the lake in all depths during the period under evaluation. Other species present were less important quantitatively or rare: chlorophytes motiles *Chlamydomonas* sp. (80 μm^3; 0–5% total biomass) and *Palmellopsis* sp. (282 μm^3; 0–25% total biomass), and one euglenophyte: *Euglena mutabilis* (4890 μm^3; 0–40% total biomass).

Keratococcus rhaphidioides was the dominant species for almost the whole studied period (1998–2006), with the exception of March 2001 (sampling after the Copahue Volcano eruption) where *Watanabea* sp. (previously determined as *Viridiella* sp.) had the greater biomass (Pedrozo et al. 2001, Beamud et al. 2007, 2010b). It is important to point out that there is no replacement of species over the time, which means that no seasonal succession as is expected in the freshwater plankton of the lakes occurs in Lake Caviahue (Beamud et al. 2010a).

Phytoplankton biomass, nutrients variation through the years and zooplankton control

The Figures 2 and 3 show the epilimnetic (average of six samples taken between 0 to 30 m) and hypolimnetic nutrients (P, N and C) concentrations (average of six samples taken between

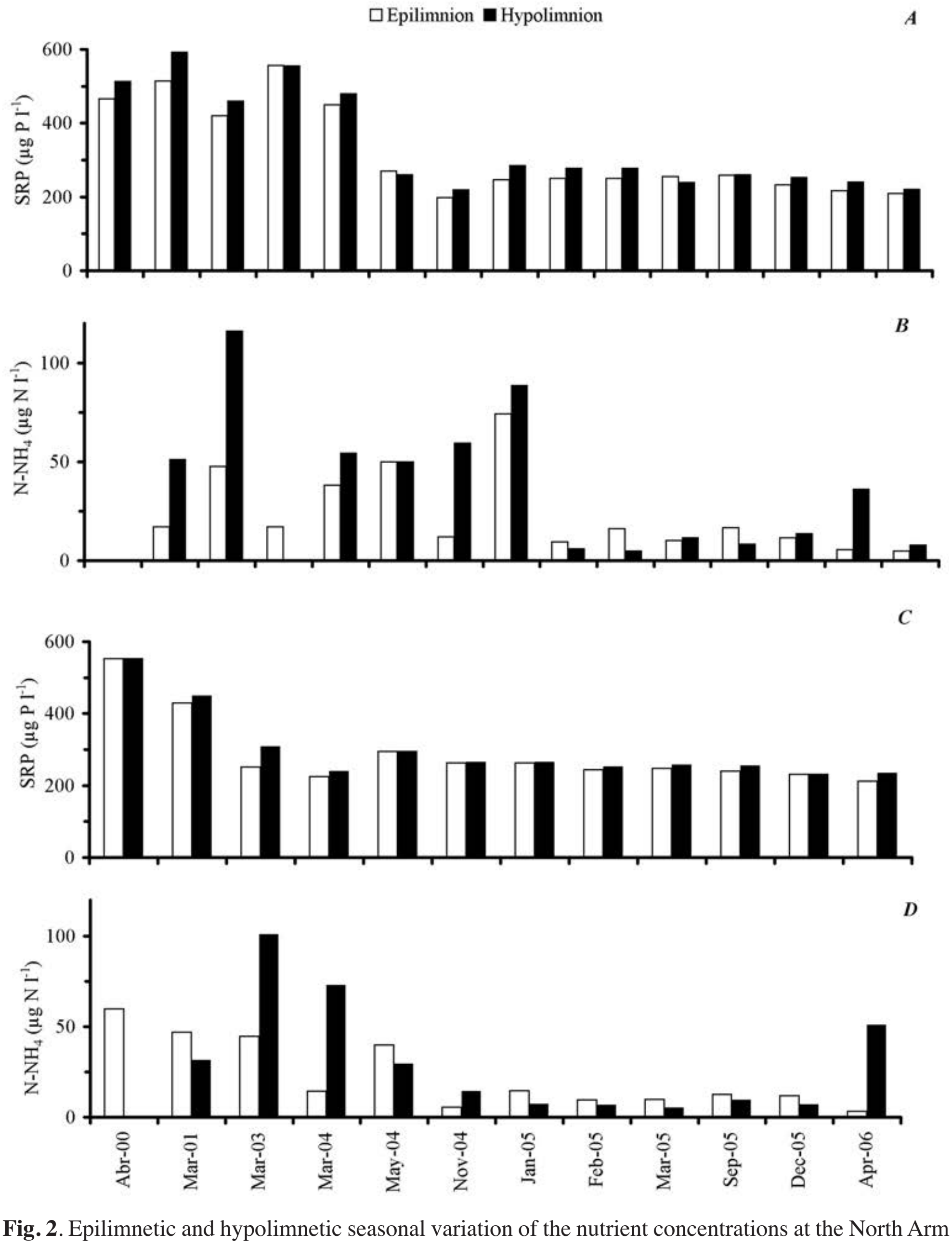

Fig. 2. Epilimnetic and hypolimnetic seasonal variation of the nutrient concentrations at the North Arm (*A, B*) and South Arm (*C, D*) of Lake Caviahue from 2000 to 2006: SRP in mg P L^{-1} (*A, C*) and N-NH_4 (*B, D*) in mg N L^{-1}.

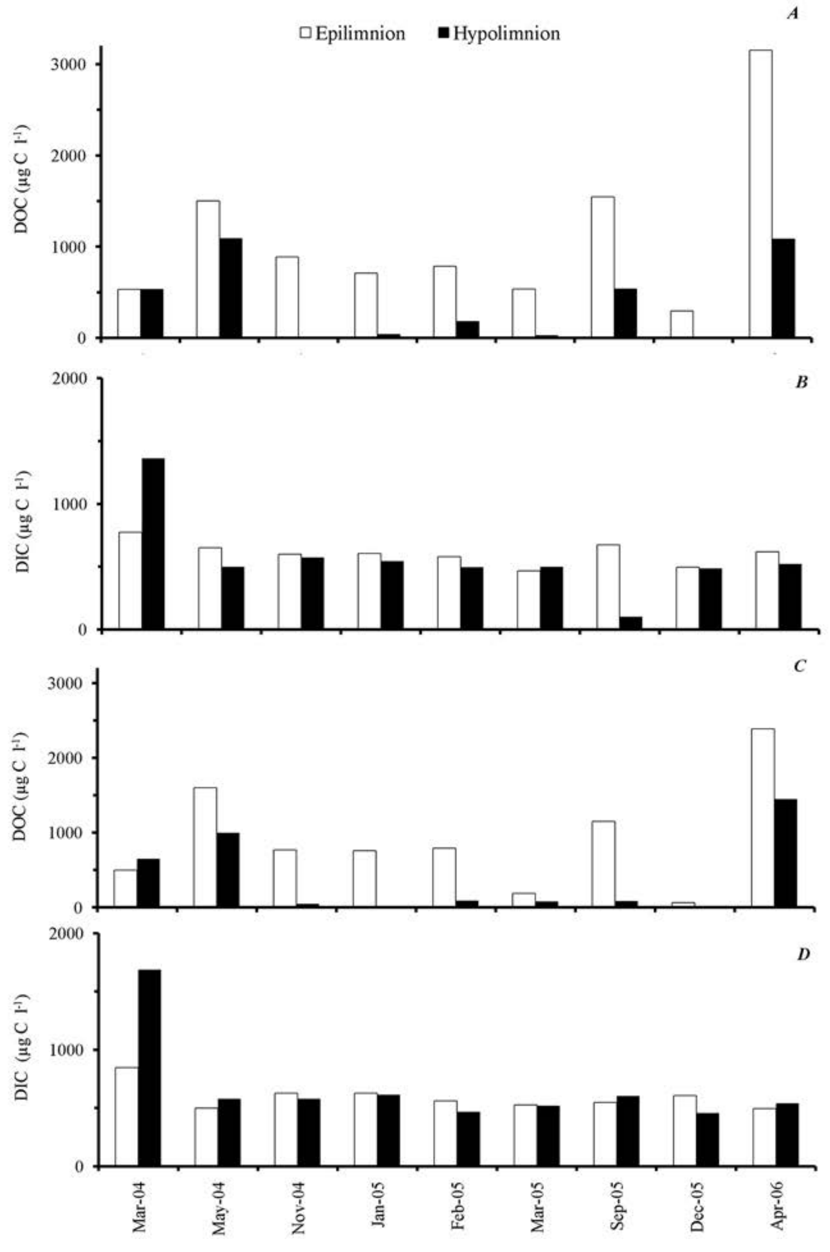

Fig. 3. Carbon seasonal variation at the epliminion and hypolimnion of the North Arm (*A, B*) and South Arm (*C, D*) of Lake Caviahue from 2004 to 2006: DOC in mg C L^{-1} (*A, C*) and DIC in mg C L^{-1} (*B, D*). Modified from Beamud et al. (2010a).

40 to 90 m depth) of the North and South arms of the lake. N and P concentrations showed similar values at the water column (Figs. 2 A to D). SRP (Soluble Reactive Phosphorus) was always the main fraction (50–90%) of the TP (Total Phosphorus) on account of acidity (Pedrozo et al. 2008, Beamud et al. 2010a). High concentrations of SRP were measured along the water column, both in the epilimnion as well as in the hypolimnion. Two periods can be split along the years: the first one (1998–2004) with highest SRP concentrations and a second one where the P contents decreased and remained constant through the sampling dates (Figs. 2A and 2C). N-NH_4 (ammonium) concentrations registered lower values than P ones, and the contents through the years were not constant (Figs. 2B and 2D). The highest values were mostly observed at early autumn and a severe decrease was observed after November 2004 in the concentrations in both arms. The decrease in soluble phosphorus content was related to changes in magmatic activity of the Copahue Volcano (Pedrozo et al. 2008), and possibly the drop of the ammonium concentrations could also be influenced by a decrease of the volcanic fluids. The average N:P ratio calculated from the measured concentrations in the 1998–2006 period ranged from 0.02–0.19, and these values led to a N deficiency for the phytoplankton of the lake (Beamud et al. 2007, 2010b).

Organic and inorganic carbon had different patterns over the time, but each form of carbon had the same pattern in the lake as a whole, independently of the arm (Figs. 3A to D). Dissolved organic carbon (DOC) had low values and showed a seasonal pattern with maximum values in autumn and winter, and minimum contents in summer (Figs. 3A and C). The values of dissolved inorganic carbon (DIC) were also low and the concentrations of the epi- and hypolimnion for the studied period were constant (Figs. 3B and D). In most of the sampling dates epilimnion contents for both DOC and DIC were greater than the hypolimnion ones. The dissolved carbon content in the lake was low, coinciding with those reported in other environments of low pH (Kamjunke et al. 2004). The major source of the dissolved CO_2 is the atmosphere, but CO_2 can also come from groundwater, photo-oxidation of organic compounds in the respiratory activity of microorganisms (Gross 2000) or volcanic gases (Satake & Saijo 1974). Photosynthesis under an acidic pH is strongly dependent on the DIC available. All the DIC is present as CO_2, rapidly depleted in the absence of bicarbonate and only counterbalanced by a high rate of exchange between CO_2 and atmospheric CO_2. In the case of the photic zone of Lake Caviahue, the measurements of inorganic carbon concentrations averaged 0.5 mg C L^{-1}. This is three times the calculated air-equilibrium concentration for a lake temperature of 15ºC, 630 mm Hg and an altitude of 1600 m. This suggests that in this acid lake, inorganic carbon is probably not limiting if other environmental factors such as light and other nutrients are sub-optimal (Diaz & Maberly 2009).

The total phytoplankton biomass in the water column of the lake was low: between 0.2 and 1.4 mg fw L^{-1} (Fig. 4), both at the epilimnion (showed as the average of six depths from 0 to 30 m; Fig. 4 A) as well as at the hypolimnion (showed as the average of six depths from 40 to 90 m; Fig. 4B). Although the magnitude in the variation of the biomass values is low, it could be observed certain seasonality, with maximum values in late spring-early summer and minimum in autumn. The lowest value was found in March 2001 (less than 0.2 mg fw L^{-1}) after the eruption of the Copahue Volcano, which took place in July 2000. The eruption brought an important change in underwater light penetration due to the great amount of volcanic ash coming from the crater distant 11 km, increasing the suspended material and decreasing the Secchi disc depth and the algal biomass.

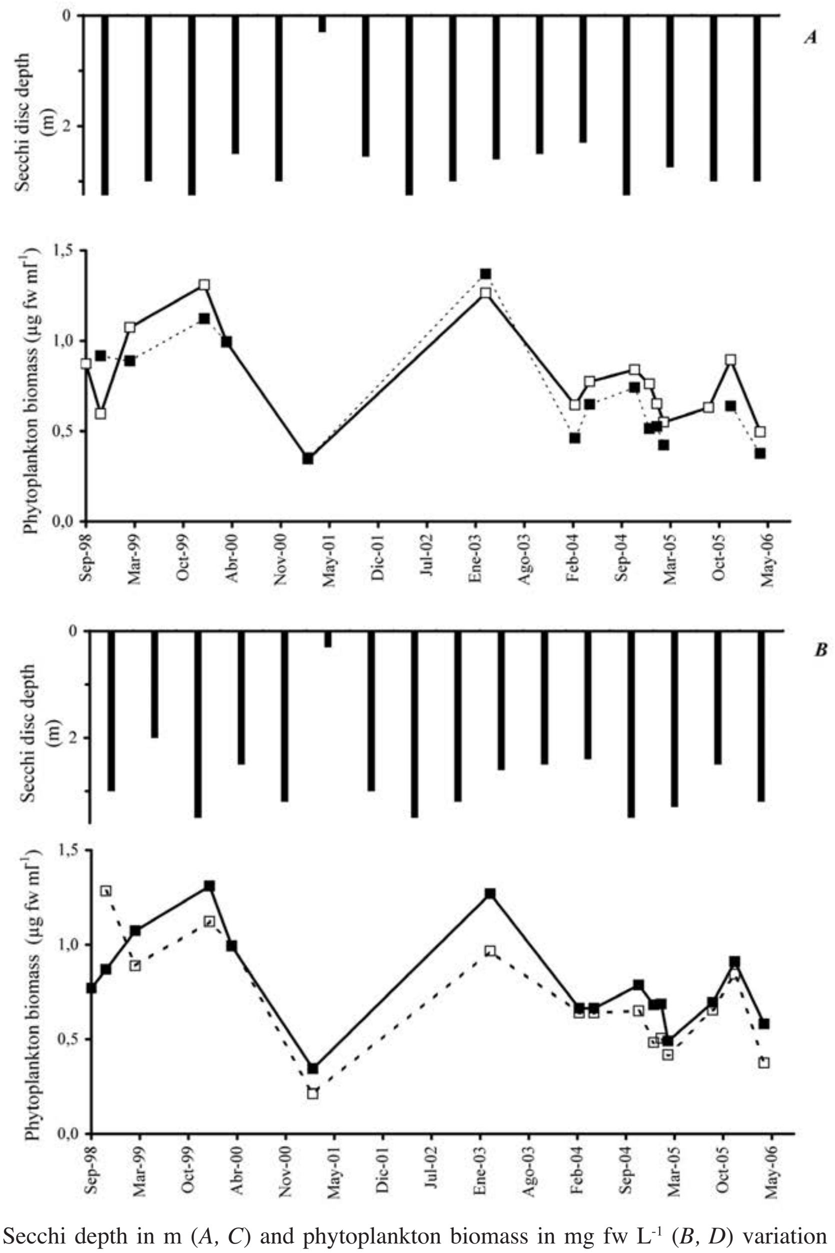

Fig. 4. Secchi depth in m (*A, C*) and phytoplankton biomass in mg fw L^{-1} (*B, D*) variation in Lake Caviahue from 1998 to 2006: (*A, B*) North arm and (*C, D*) South arm.

The vertical distribution of the phytoplankton biomass was distinctive with similar values at all depths, even in strata where light is no longer available for algae (Beamud et al. 2010b). Seasonal variation in the biomass values was lower than that observed in temperate (Wetzel, 2001) and acidic mining lakes (Beulker et al. 2003).

Philodina sp. (Bdelloidea) was the only rotifer recorded throughout 1998–2006 period and responsible for the zooplankton biomass measurement. Occasionally some ciliates were found (Pedrozo et al. 2001). Besides zooplankton quantification, laboratory experiments were performed with removal of radioactively labelled particles (algae) to check if zooplankton species feed on phytoplankton species. Radioactivity of zooplankton and suspended algal food source was determined. Filtration and ingestion rates were calculated according to Haney (1973). The Ingestion rate of the rotifers (I, in cells ml^{-1} h^{-1}) for the North arm was 0.61 (± 0.006, n = 3) and 0.58 (± 0.006, n = 3) for the South arm. When the biomass was used for the calculations, approximately the same values were found in both arms: 2.7 x 10^{-5} mg fw ml^{-1} h^{-1} (± 3 x 10^{-7}, n = 3) for the North and 2.9 x 10^{-5} mg fw ml^{-1} h^{-1} (± 3 x 10^{-7}, n = 3) for the South. None of the calculated rates was high enough to suggest that there is a substantial top-down control by zooplankton. Similar results emerge from the relationships between the biomass of phytoplankton and zooplankton obtained for 2004–2006 (Fig. 5). There is no clear relationship between both variables, which can be an indication of no control of algal abundance by the zooplankton. In addition, by comparing the ingestion rate of zooplankton and phytoplankton abundance, we may deduce that the rotifers consumed per day around 0.004% of the density and/or biomass of phytoplankton. This value would support the argument that there is no control on primary producers.

The biomass maxima of phytoplankton in relation to nutrients availability (N, P and C) and zooplankton content are consistent with those of other Patagonian lakes, but in Lake Caviahue are related to ammonium, organic and inorganic carbon, and not to phosphorus or zooplankton (Beamud 2009). A multifactorial analysis (MFA) was performed to identify interrelationships between different parameters or environmental variables (zooplankton biomass, chlorophyll *a*, SRP, TP, NH_4, dissolved oxygen, conductivity, pH, organic and inorganic carbon) on the vertical distribution of phytoplankton (Beamud et al. 2010a). The analysis revealed differences among superficial, intermediate and deep layers of the lake. Different variables (or values thereof) characterized each group of depths obtained in the MFA. It

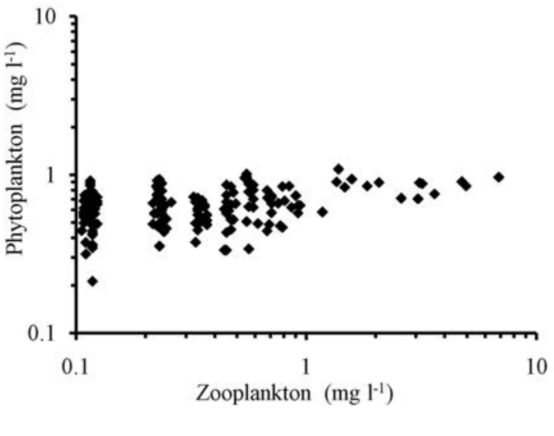

Fig. 5. Rotifer biomass (mg L^{-1}) versus phytoplankton biomass (mg L^{-1}) in Caviahue Lake (2004–2006). Both axes are in logarithmic scale.

was observed that the superficial depths were grouped by high values of phytoplankton and zooplankton biomass, organic and inorganic carbon, dissolved oxygen and pH for all seasons. Deeper strata were grouped by high values of chlorophyll *a*, NH_4 and P (dissolved and particulate) over the seasons. The joint analysis of the variables and depths confirmed that there was no clear marked seasonality based on physical, chemical or biological variables. The lake is very different to any other temperate lake in so many ways that, for instance, the PEG model (Sommer et al. 1986), which describes step by step the seasonal events which occur in phyto- and zooplankton of an idealized standard lake does not fit for Lake Caviahue.

Nutrient limitation: algal bioassays

Several bioassays were performed with unialgal non-axenic cultures of the dominant algal species isolated from Lake Caviahue: *Keratococcus rhaphidioides* (inorganic and organic nutrient experiments), *Watanabea* sp. (inorganic nutrient experiments) and *Euglena mutabilis* (inorganic nutrient experiments).

Inorganic nutrient (N, P and C) experiments: The N+P experiments consisted in the addition of ammonium (NH_4), nitrates (NO_3) and phosphates (P) separately or combined (P + NH_4 and P + NO_3) to algal cultures. In the N + IC experiments we added ammonium and/or bicarbonates to the cultures. The growth rate (μ) per day (d^{-1}) of each species was measured and the results are showed in Figure 6.

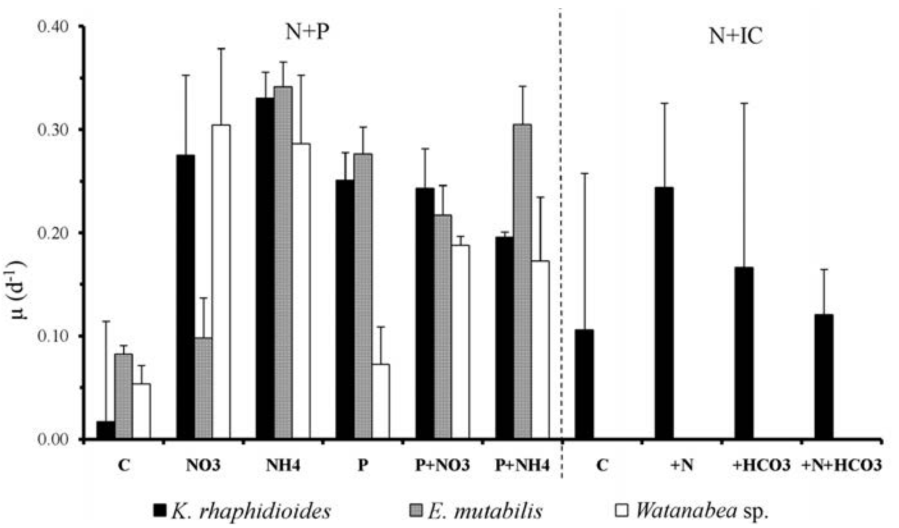

Fig. 6. Growth rates (d^{-1}) for *Euglena mutabilis*, *Watanabea* sp. and *Keratococcus rhaphidioides* for inorganic nutrient experiments (N + P and N + IC). Treatments: control (C), plus ammonium (+ NH_4), plus nitrates (+ NO_3), plus phosphorus (+ P); plus phosphorus and ammonium (+ P + NH_4), plus phosphorus and nitrates (+ P + NO_3). Each point represents three replicates average (± 1 standard deviation). Modified from Beamud et al. (2010b).

Keratococcus rhaphidioides: After the addition of N and P, the species showed the highest growth rates for NH_4 (0.33 d^{-1}), followed for the other two non-combined treatments (NO_3 = 0.28 and P = 0.25 d^{-1}). Again, when ammonium and bicarbonates were added, the first treatment had the highest μ (0.24 d^{-1}), followed by the treatment containing bicarbonates (μ = 0.17 d^{-1}).

Euglena mutabilis: The species better grow up when NH_4 was added as the only nutrient (0.34 d^{-1}), followed by treatments with P (0.28 d^{-1}) added alone or in combination with NH_4 (0.30 d^{-1}).

Watanabea sp.: The treatment NO_3 had the highest growth rate (0.30 d^{-1}), followed by NH_4 (0.28 d^{-1}) and P combined treatments (P+NO_3 = 0.19 d^{-1}, P + NH_4 = 0.17 d^{-1}).

The nitrogen addition produced the highest growth rates in the experiments suggesting that the CO_2 availability is sufficient to meet the carbon requirements of phytoplankton in the lake, and that the experiments and algae growth should not be limited by carbon. The response to the addition of NH_4, NO_3 and/or PO_3 was different for every species. *K. rhaphidioides* showed higher growth rate with NH_4 addition (in first place), alone or together with phosphates or inorganic carbon. For *E. mutabilis* the N and P jointly addition resulted important for the greatest growth rate. Finally, *Watanabea* sp. showed a different behaviour than the other species, since the highest growth was observed in the treatments containing NO_3 (Beamud et al. 2010b). One of the factors that could explain the prevalence of *K. rhaphidioides* and the quite-low *Watanabea* sp. biomass in Lake Caviahue was the availability of NH_4 and NO_3. Since nitrates are not available in the lake, *Watanabea* sp. can not develop high biomass or have a significant growth rate. In laboratory cultures, we observed that *Watanabea* sp. grew faster than *K. rhaphidioides* (μ = 0.63 d^{-1}, μ = 0.26 d^{-1}, respectively), when using acidic media enriched with NO_3.

Organic nutrient (N and C) experiments: For these bioassays, the dominant algal species were incubated with different radioactively labelled substrates: ^{3}H-leucine, ^{3}H-glucose, ^{3}H-thymidine, ^{14}C-aspartic acid, ^{14}C-acetic acid and bicarbonate. Assimilation rates of the organic substrates were determined after light and dark incubation of the cultures. The results showed that *Keratococcus rhaphidioides, Euglena mutabilis* and *Watanabea* sp. reached the 50%, 40% and 10% of total biomass, respectively. The total autotrophic biomass during the incubations was 0.31 μg fw ml^{-1} and 5.94 μg fw ml^{-1}, for light and dark, respectively (Beamud 2009).

Table 2 shows the assimilation of organic substrates according to the fraction of the cell sizes. Algae fraction (≥ 2 μm) was able to incorporate between 85 to 92% of available organic carbon (μg C L^{-1} h^{-1}) under light and dark conditions. Higher uptake rates were found in the light compared to dark incubations. Particularly, the assimilation of organic C sources in the dark was higher for leucine (0.5 μg C L^{-1} h^{-1}). This was also found in light incubations for leucine (0.39 μg C L^{-1} h^{-1}), but also high uptake rates for acetate (0.17 μg C L^{-1} h^{-1}) and aspartic acid (0.17 μg C L^{-1} h^{-1}) were encountered. Primary production rate (IC assimilation) of the cultures was high (8.44 μg C L^{-1} h^{-1}). The results from the experiments indicate that the three species studied were able to assimilate thymidine, acetate, aspartic acid and leucine under lab conditions. *Keratococcus rhaphidioides*, *Euglena mutabilis* and *Watanabea* sp. utilize different sources of organic carbon and nitrogen in the light and/or in the dark. In experiments carried out in situ at 0–10 m depth in Lake Caviahue, where the algae were incubated with radiolabelled leucine and bicarbonates, *K. rhaphidioides* and *Watanabea* sp. had an important

Table 2. Incorporation rate (µg C L^{-1} h^{-1}) ^{3}H-glucose (glu), ^{14}C-aspartic acid (asp), ^{14}C-acetic acid (ace), ^{3}H-thymidine (thy) and ^{3}H-leucine (leu) and photosynthetic incorporation of inorganic carbon $NaH_{14}CO_3$ (IC) during light and dark incorporation. Size fractions from Lake Caviahue cultures: 0.2 µm: total (bacteria and algae), > 2 µm and > 5 µm: algae. IC in the dark was not determined. Each value represents three replicates average with standard deviation.

	0.2 µm	> 2 µm	> 5 µm
Light (µg C l^{-1} h^{-1})			
glu	0.07 (± 0.00)	0.07 (± 0.00)	
asp	0.20 (± 0.01)	0.17 (± 0.02)	
ace	0.20 (± 0.01)	0.17 (± 0.02)	
thy	0.09 (± 0.02)	0.08 (± 0.02)	
leu	0.56 (± 0.01)	0.39 (± 0.05)	
IC			8.44 (± 0.00)
Dark (µg C l^{-1} h^{-1})			
glu	0.04 (± 0.03)	0.04 (± 0.03)	
asp	0.06 (± 0.01)	0.05 (± 0.01)	
ace	0.06 (± 0.01)	0.05 (± 0.01)	
thy	0.11 (± 0.01)	0.08 (± 0.01)	
leu	0.82 (± 0.03)	0.51 (± 0.01)	

assimilation of these substrates. The algal growth in the dark together with the in situ results, are indicative of a possible use and metabolisation of organic carbon and nitrogen at deeper strata.

The genus *Watanabea* sp. was established by Hanagata et al. (1998) to separate some species of *Chlorella*. Several species of *Chlorella* are able to grow using different forms of dissolved organic nitrogen and glucose (Droop 1974, Wheeler et al. 1974, Timperley et al. 1985, Vincent & Goldman 1980, Graneli et al. 1999), similarly to the *Watanabea* species found at Lake Caviahue. The euglenophyte *E. mutabilis* is able to enhance its growth under light incubations using different kind of aminoacids and glucose (Olaveson & Stokes 1989). No dark growth was determined for this species with different organic substrates. In the case of *K. rhaphidiodes*, it is the first time that its ability to assimilate organic substrates has been studied.

The limited availability of nitrogen and carbon at Lake Caviahue, leads to the phytoplankton to exploit different sources of organic carbon and nitrogen, according to the requirements of the species. Thus, it is clearly indicated that besides photosynthesis, algae can meet simultaneously their matter and energy requirements by mixo- and heterotrophic uptake of organic carbon and nitrogen. Nevertheless, the addition of N in lab experiments yielded maximum growth, which suggests that this nutrient limits productivity after Liebig's law of the minimum.

Carbon, though scarce, could be in sufficient concentrations to many species of algae isolated from the River Agrio – Lake Caviahue Basin. The ionic dissociation rates of CO_2 species and the exchange between atmospheric CO_2 and water CO_2 take place sufficiently fast, that is unlikely a severe C limitation in pelagic regions with good mix, even under low TIC (Total Inorganic Carbon) conditions (Wetzel 2001). Some species have a CCM while others

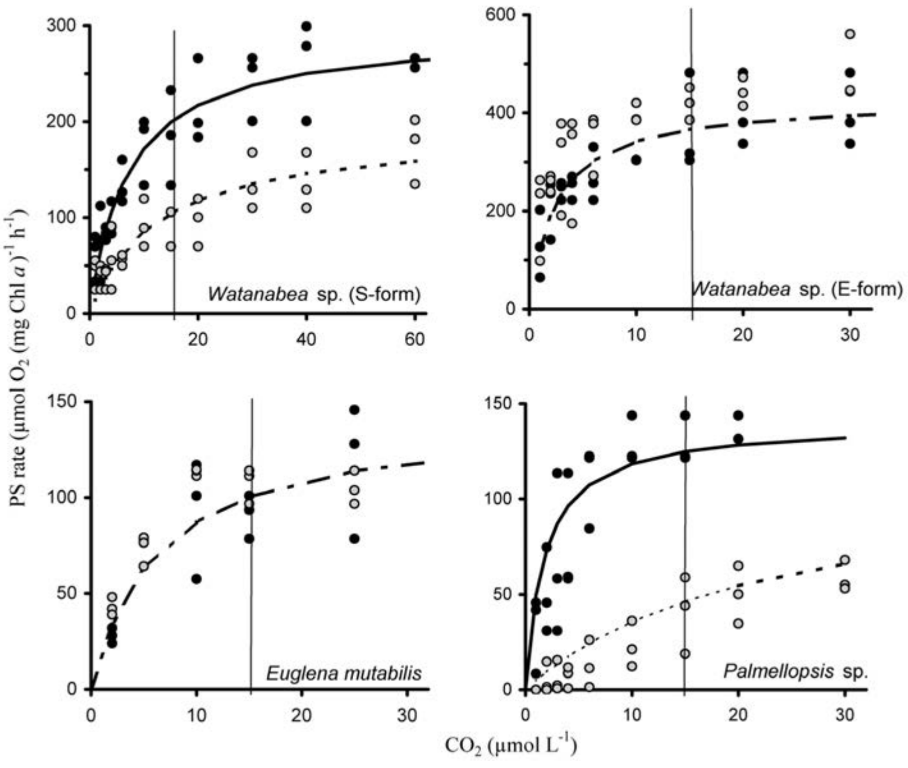

Fig. 7. Rate of photosynthesis vs. concentration of CO_2 at 100 mmol photon m^{-2} s^{-1} for three species or forms at low (●) and atmospheric (○) concentrations of oxygen: *Watanabea* sp. S-form (spheroidal) and E-form (ellipsoidal), *Euglena mutabilis* and *Palmellopsis* sp. Triplicate experimental results shown and modelled response using the Michaelis-Menten equation for low (- - - -) and atmospheric (—) levels of oxygen or for combined data (-·-·-·-) where responses do not vary significantly with oxygen. The vertical line at 15 CO_2 μmol represents the air equilibrium concentration. Modified from Diaz & Maberly (2009).

appear to lack one. CCMs are known to be present or absent depending on growth conditions, but air-levels of CO_2 have always been sufficient to activate the CCM (Giordano et al. 2005). In Lake Caviahue two species have been found with CCM (Fig. 7) and where a high affinity for CO_2 was demonstrated: *Euglena* and the ellipsoidal form of *Watanabea* (Diaz & Maberly 2009). This investigation on the two growth forms of *Watanabea* sp. suggests that the ellipsoidal form has a CCM while the spheroidal form does not. Interestingly, this appears to be the first time that different forms of the same species have been shown to have different abilities to use inorganic carbon even when grown under identical conditions. The chlorophyte *Palmellopsis* appears to lack a CCM. The presence or absence of CCMs in the other two species of the lake (*Keratoccocus rhaphidioides* and *Chlamydomonas acidophila*) has not been studied yet. The high affinity for CO_2 in all the species studied suggests that inorganic carbon does not strongly-limit photosynthesis even at air-equilibrium.

Acknowledgments

This research work was partially supported by grants from ANPCyT PICT 1105, Universidad Nacional del Comahue Program 04/B166 and CONICET PIP 10013.

References

Baffico, G., Diaz, M., Wenzel, M., Koschorreck, M., Schimmele, M., Neu, T. & Pedrozo, F. (2004): Community structure and photosynthetic activity of epilithon from a highly acidic (pH ≤ 2) mountain stream in Patagonia, Argentina. – Extremophiles **8**: 463–473.

Beamud, S. G., Diaz, M. & Pedrozo, F. (2007): Summer phytoplankton composition and nitrogen limitation of the deep, naturally-acidic (pH ~ 2.2) Lake Caviahue, Patagonia, Argentina. – Limnologica **37**: 37–48.

Beamud, S. G. (2009): Control del crecimiento y la distribución vertical del fitoplancton de un lago ácido natural: lago Caviahue. –Doctoral Thesis. Univ. Nacional del Comahue, Bariloche.

Beamud, S. G., Diaz, M. M., Baccalá, N. & Pedrozo, F. L. (2010a): Analysis of patterns of vertical and temporal distribution of phytoplankton using multifactorial analysis: Acidic Lake Caviahue, Patagonia, Argentina. – Limnologica **40**: 140–147.

Beamud, S. G., Diaz, M. M. & Pedrozo, F. L. (2010b): Nutrient limitation of phytoplankton in a naturally acidic lake (Lake Caviahue, Argentina). – Limnology **11**: 103–113.

Beulker, C., Lessmann, D. & Nixdorf, B. (2003): Aspects of phytoplankton succession and spatial distribution in an acidic mining lake (Plessa 117, Germany). – Acta Oecol. **24**: S25–S31.

Diaz, M. & Maberly, S. (2009): Carbon concentrating mechanisms in acidophilic algae. – Phycologia **48**:77–85.

Droop, M. R. (1974): Heterotrophy of carbon. – In: Stewart, W. D. (ed.): Algal physiology and biochemistry. – Univ. of California Press, California, pp. 530–559.

Falkowski, P. G. & Raven, J. A. (2007): Aquatic photosynthesis. – Princeton Univ. Press, Princeton.

Geller, W., Klapper, H. & Schultze, M. (1998): Natural and anthropogenic sulfuric acidification of lakes. – In: Geller, W., Klapper, H. & Salomons, W. (eds.): Acidic mining lakes: acid mine drainage, limnology and reclamation. – Springer, Berlin, pp. 3–14.

Giordano, M., Beardall, J. & Raven, J. A. (2005): CO_2 concentrating mechanisms, environmental modulation, and evolution. – Ann. Rev. Plant Biol. **56**: 99–131.

Glibert, P. M. & Legrand, C. (2006): The diverse nutrient strategies of harmful algae: focus on osmotrophy. – In: Graneli, E. & Turner, J. T. (eds.): Ecology of harmful algae. – Ecol. Stud. **189**: 163–175.

Granéli, E., Carlsson, P. & Legrand, C. (1999): The role of C, N and P in dissolved and particulate organic matter as a nutrient source for phytoplankton growth, including toxic species. – Aquat. Ecol. **33**: 17–27.

Gross, W. (2000): Ecophysiology of algae living in highly acidic environments. – Hydrobiologia **433**: 31–37.

Hanagata, N., Karube, I., Chihara, M. & Silva, P. C. (1998): Reconsideration of the taxonomy of ellipsoidal species of *Chlorella* (Trebouxiophyceae, Chlorophyta), with establishment of *Watanabea* gen. nov. – Phycol. Res. **46**: 221–229.

Haney, J. F. (1973): An in situ examination of the grazing activities of natural zooplancton communities. – Arch. Hydrobiol. **72**: 87–132.

Jones, R. I. (1994): Mixotrophy in planktonic protists as a spectrum of nutritional strategies. – Mar. Mic. Food Webs **8**: 87–96.

Kamjunke, N., Gaedke, U., Tittel, J., Weithoff, G. & Bell, E. M. (2004): Strong vertical differences in the plankton composition of an extremely acidic lake. – Arch. Hydrobiol. **161**: 289–306.

Kwiatkowski, R. E. & Roff, J. (1976): Effects of acidity on the phytoplankton and primary productivity of selected northern Ontario lakes. – Can. J. Bot. **54**: 2546–2561.

Nixdorf, B., Mischke, U. & Lessmann, D. (1998): Chrysophytes and Chlamydomonads: Pioneer colonists in extremely acidic mining lakes (pH < 3) in Lusatia (Germany). – Hydrobiologia **369/370**: 315–327.

Olaveson, M. M. & Stokes, P. M. (1989): Responses of the acidophilic alga *Euglena mutabilis* (Euglenophyceae) to carbon enrichment at pH 3. – J. Phycol. **25**: 529–539.

Olaveson, M. M. & Nalewajko, C. (1994): Acid rain and freshwater algae. – In: Rai, L. C., Gaur, J. P. & Soeder, C. J. (eds.): Algae and Water Pollution. – Adv. Limnol. **42**: 99–123.

Pedrozo, F. L., Kelly, L., Diaz, M. M., Temporetti, P., Baffico, G., Kringel, R., Friese, K., Mages, M., Geller, W. & Woelfl, S. (2001): First results on the water chemistry, algae and trophic status of Andean acidic lake system of volcanic origin in Patagonia (Lake Caviahue). – Hydrobiologia **452**: 129–137.

Pedrozo, F., Temporetti, P., Beamud, G. & Diaz, M. (2008): Volcanic nutrient inputs and trophic state of Lake Caviahue, Patagonia, Argentina. – J. Vol. Geo. Res. **178**: 205–212.

Pick, U. (1999): *Dunaliella acidophila* – a most extreme acidophilic alga. – In: Seckbach, J. (ed.): Enigmatic microorganisms and life in extreme environments. Kluwer Acad. Publ., Netherlands, pp. 465–478.

Rapacioli, R. A. (1985): El Lago Caviahue y su Cuenca. – EPAS Report, Provincia del Neuquén, Argentina.

Raven, J. A. (1997): Putting the C in phycology. – Eur. J. Phycol. **32**: 319–333.

Rivkin, R. B. & Putt, M. (1987): Heterotrophy and photoheterotrophy by Antartic microalgae: light-dependent incorporation of amino acids and glucose. – J. Phycol. **23**: 442–452.

Satake, K. & Saijo, Y. (1974): Carbon dioxide content and metabolic activity of microorganisms in some acid lakes in Japan. – Limnol. Oceanogr. **19**: 331–338.

Sommer, U., Gliwicz, Z. M., Lampert, W. & Duncan, A. (1986): The PEG–Model of seasonal succession of planktonic events in fresh waters. – Arch. Hydrobiol. **106**: 433–471.

Timperley, M. H., Vigor-Brown, R. J., Kawashima, M. & Ishigami, M. (1985): Organic nitrogen compounds in atmospheric precipitation: their chemistry and availability to phytoplankton. – Can. J. Fish. Aquat. Sci. **42**: 1171–1177

Tittel, J., Bissinger, V., Gaedke, U. & Kamjunke, N. (2005): Inorganic carbon limitation and mixotrophic growth in *Chlamydomonas* from and acidic mining lake. – Protist. **156**: 63–75.

Vincent, W. F. & Goldman, C. R. (1980): Evidence of algal heterotrophy in Lake Tahoe, California-Nevada. – Limnol. Oceanogr. **25**: 89–99.

Wetzel, R. G. (2001): Limnology. – Academic Press, California.

Wheeler, P. A., North, B. B. & Stephens, G. C. (1974): Amino acid uptake by marine phytoplankters. – Limnol. Oceanogr. **19**: 249–259.