

Mixotrophic ciliates in North-Patagonian Andean lakes: stoichiometric balances in nutrient limited environments

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ABSTRACT

Mixotrophic ciliates in North-Patagonian Andean lakes: stoichiometric balances in nutrient limited environments

Transparent ultraoligotrophic lakes in the North-Patagonian Andes have a particular microbial food web, with the presence of large mixotrophic ciliates. These organisms exhibit different features that allow them to colonize either the epilimnion (*Stentor araucanus*) or the metalimnion (*Ophrydium naumanni*). *S. araucanus* is a dark pigmented (stentorin) species resistant to ultraviolet radiation and needs high light supply to maintain endosymbiotic algal photosynthesis. In contrast, *O. naumanni* dominates the photosynthetic biomass in the deep chlorophyll maxima (metalimnion) of these lakes, being photosynthetically efficient at low light intensities but susceptible to photoinhibition at epilimnetic light irradiances. Analysis of food vacuoles revealed a weak niche overlap, however light climate, shaped by temporal or spatial variations in thermocline depth, resulted in a key factor modulating the relative success of these mixotrophic ciliate species. Overall, these species are stoichiometrically, carbon to nutrients, more balanced than the bulk seston, but the mechanisms by which each species regulates the elemental balance differ. *O. naumanni* increases bacterivory with light, thus increasing phosphorus uptake, while *S. araucanus* regulates carbon fixation. The low carbon:nutrient ratio of these organisms would represent a very good food source for zooplankton. Finally, we pointed out that different effects of local and global changes will affect negatively the particular ciliate assemblage of North-Patagonian Andean lakes.

Key words: mixotrophy, stoichiometric constraints, transparency

RESUMEN

Ciliados mixotróficos en lagos andinos norpatagónicos: balances estequiométricos en ambientes limitados por nutrientes

Los lagos andinos nord-patagónicos son ultraoligotróficos, muy transparentes y poseen una red trófica microbiana particular, con la presencia de grandes ciliados mixotróficos. Estos organismos exhiben diferentes características que les permiten colonizar el epilimnion (*Stentor araucanus*) o el metalimnion (*Ophrydium naumanni*). *S. araucanus* es una especie pigmentada oscura (stentorina) resistente a la radiación ultravioleta y que necesita un alto suministro de luz para mantener la fotosíntesis de sus algas endosimbióticas. En contraste, *O. naumanni* domina la biomasa fotosintética en los niveles de clorofila máxima profunda (metalimnion) de estos lagos, siendo fotosintéticamente eficiente a bajas intensidades de luz, pero susceptible a la fotoinhibición en el epilimnion. El análisis de las vacuolas alimentarias reveló una baja superposición de nichos, sin embargo, el clima óptico, modulado por variaciones temporales o espaciales en la profundidad de la termoclina, resultó un factor clave para la alternancia de estas dos especies. En general, estas especies son estequiométricas más balanceadas (carbono:nutrientes) que el seston, pero los mecanismos por los cuales cada especie regula el equilibrio elemental difieren. *O. naumanni* aumenta la bacterivoría con la luz, lo que aumenta la incorporación de fósforo, mientras que *S. araucanus* regula la fijación de carbono. La baja proporción de carbono: nutrientes de estos organismos representa una muy buena fuente de alimento para el zooplancton. Finalmente, señalamos que los diferentes efectos de cambios locales y globales afectarán negativamente esta particular biota de ciliados mixotróficos de los lagos andinos del norte de la Patagonia.

Palabras clave: mixotrofia, restricciones estequiométricas, transparencia

INTRODUCTION

Mixotrophy is a nutrition mode that combines both phototrophy and phagotrophy to support growth (Jones, 1997; Stoecker, 1998). The combination of photosynthetic and organic carbon ingestion is very common among protists; thus, this strategy is widely distributed in the eukaryotic tree of life (Stoecker *et al.*, 2009). Photosynthetic capability includes the maintenance of phototrophic structures (organelles or whole cells) inside their cytoplasm (Putt, 1990; Perriss *et al.*, 1994) and in particular, ciliates acquired phototrophy by a transient or permanent association between host and symbiont or preyed organelles (Stoecker *et al.*, 2009). Positive photosynthetic rates were observed both, when ciliates sequester chloroplasts from ingested prey or when photosynthetic cells act as endosymbionts (Reisser *et al.*, 1985; Stoecker & Silver, 1987; Perriss *et al.*, 1994). Genetic analyses of the endosymbionts of freshwater ciliates have revealed that these algae belong mainly to the chlorophyte lineage (mostly species of the genus *Chlorella*) (Summerer *et al.*, 2008). Independently of the strategy of photosynthesis acquisition,

mixotrophic ciliates may represent an important fraction of autotrophic plankton communities (Laybourn-Parry *et al.*, 1997; Modenutti *et al.*, 2000; Macek *et al.*, 2001; Woelfl & Geller, 2002).

In Andean-Patagonian transparent and oligotrophic lakes, the presence of an important assemblage of mixotrophic ciliates has been reported (Foissner & Woelfl, 1994; Modenutti, 1997; Modenutti *et al.*, 2000; Woelfl & Geller, 2002). In particular, a permanent mixotrophic association has been recorded in ciliates such as the peritrich *Ophrydium naumanni* and the heterotrich *Stentor araucanus* (Modenutti, 1997) (Fig.1). These organisms contain photosynthetic endosymbionts (*Chlorella* sp.) that are capable of replication within the host cell and, when ciliate reproduction takes place, they are distributed into the daughter cells so that successive protist generations are already paired with endosymbionts (Modenutti, 2014). Since mixotrophy requires investment in both photosynthetic and heterotrophic cellular apparatus, the benefits must outweigh these costs (Tittel *et al.*, 2003). Thus, the presence of these mixotrophic ciliates suggests that this is a successful strategy under conditions of abundant light and nutrient limita-

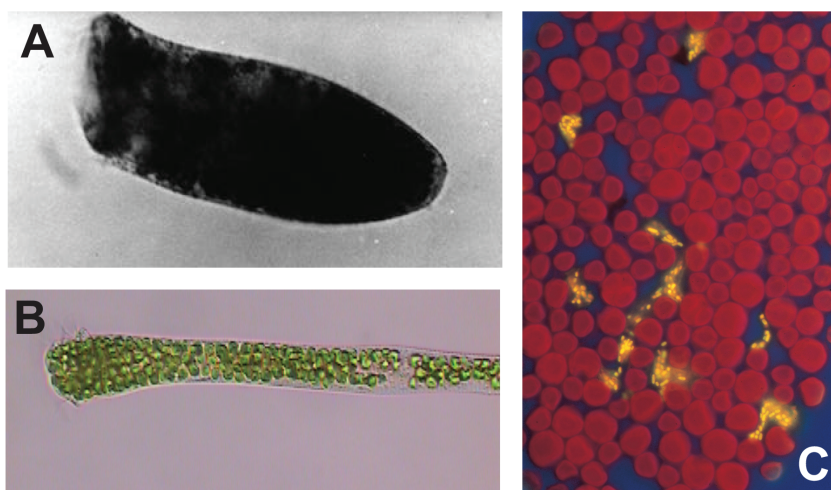


Figure 1. Microphotograph of the mixotrophic ciliates *Stentor araucanus* (A) and *Ophrydium naumanni* (B) under direct microscope and *O. naumanni* (C) under epifluorescent microscope (blue light) showing endosymbionts (*Chlorella* sp. in red) and prey (picocyanobacteria in yellow). *Microfotografía de los ciliados mixotróficos Stentor araucanus (A) y Ophrydium naumanni (B) ajo microscopio directo y O. naumanni (C) bajo microscopio de epifluorescencia (luz azul) mostrando células endosimbiontes (Chlorella sp. en rojo) y presas (picocianobacterias en amarillo).*

tion as are the features of North-Patagonian Andean lakes of Argentina (Modenutti *et al.*, 2013a). In lake ecosystems, high sestonic Carbon: Phosphorus (C:P) ratios are associated with high light: phosphorus ratios (Sterner *et al.*, 1997). Under high light 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 (Elser & Hassett, 1994; Hessen *et al.*, 2002). In this sense, “ecological stoichiometry” has been applied to describe the role of multiple chemical elements in controlling trophic processes (Sterner & Elser, 2002; Andersen *et al.*, 2004). Thus, herbivore consumers living in transparent lakes would be constrained by poor stoichiometric food quality. However, since mixotrophic ciliates combine phagotrophy and phototrophy their C:nutrient ratio can be balanced through these two nutritional modes (Flynn & Mitra, 2009; Mitra *et al.*, 2016). Here, we will analyze two alternative strategies to colonize the water column by two mixotrophic ciliate species (*O. naumanni* and *S. araucanus*) with the final outcome of a balanced stoichiometry. Finally, we will discuss the possible anthropogenic and global effect on these lakes that may affect these two mixotrophic species.

The lakes and the mixotrophic ciliates

Large and deep lakes in North-Patagonian Andes of Argentina are located in South America around 41° S (Fig. 2). They conform an extended lake district from glacial origin shared with Chile and named Araucanian lakes (Thomasson, 1963). Lakes of Argentina are very deep (up to 464 m in Lake Nahuel Huapi) and are located at 750 m a.s.l. The lakes are cold-temperate monomictic with a stratification during spring-summer (Baigún & Marinone, 1995).

These lakes are very transparent environments with extended euphotic zones (up to 50 m) that include the epilimnion, the metalimnion and part of the hypolimnion (Fig. 3). Nutrient concentration is low, less than 5 µg/L and 100 µg/L of TP and TN, respectively, and these concentrations are evenly distributed along the water column (Corno *et al.*, 2009). The same pattern was observed for

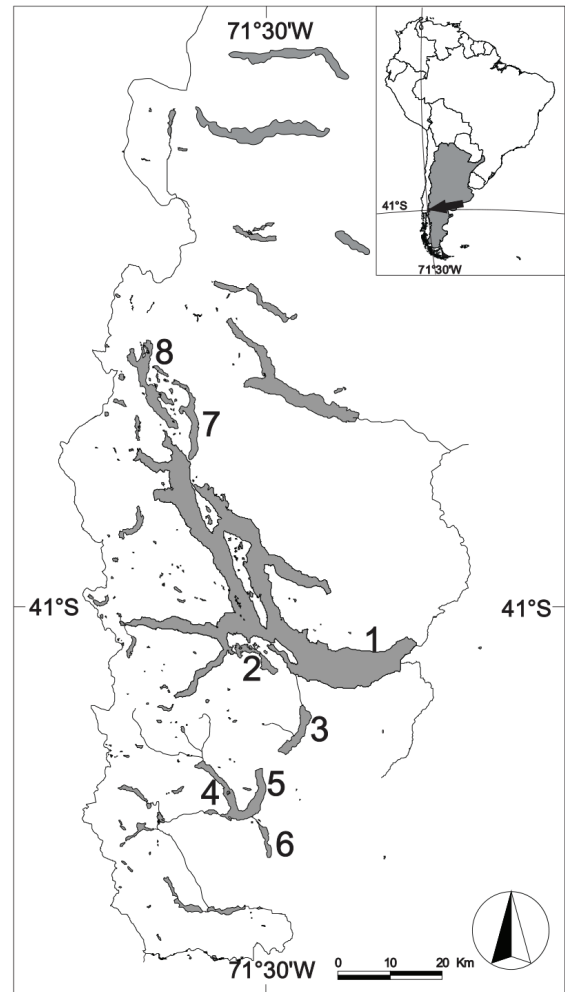


Figure 2. Map of the North Patagonian Andean lake area of Argentina. Numbers indicate most distinctive lakes studied: 1 Nahuel Huapi, 2 Moreno, 3 Gutiérrez, 4 Mascaridi Tronador arm, 5 Mascaridi Catedral arm, 6 Guillermo, 7 Correntoso and 8 Espejo. *Mapa de los lagos Andinos Norpatagónicos de Argentina. Los números indican los principales lagos estudiados. 1 Nahuel Huapi, 2 Moreno, 3 Gutiérrez, 4 Mascaridi Tronador arm, 5 Mascaridi Catedral arm, 6 Guillermo, 7 Correntoso and 8 Espejo.*

the dissolved organic carbon (DOC) (less than 0.5 mg/L) (Morris *et al.*, 1995; Modenutti *et al.*, 2013a). This condition causes that the potentially hazardous ultraviolet radiation (UVR) affects an extended portion of the euphotic zone because of the high penetration of the short wavelengths (UVR-A and UVR-B) (Fig. 3). In addition, and also due to the low DOC concentration (low

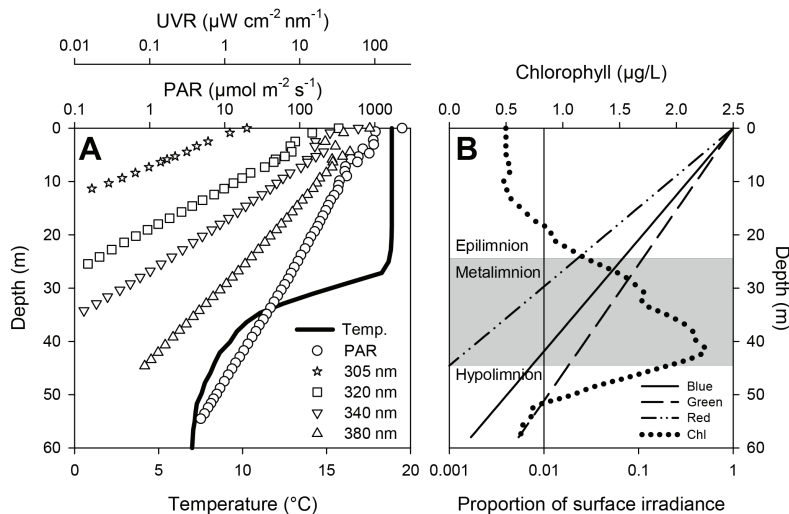


Figure 3. Light, temperature and Chlorophyll *a* vertical profile in deep north Patagonian lakes of Argentina. (A) Light (PAR, and four bands of UVR) and temperature vertical profiles in a typical north Patagonian deep lake of Argentina. (B) Vertical profiles of specific colours, showing the predominance of blue-green light in the metalimnion, and Chlorophyll *a* vertical profiles showing the presence of a deep chlorophyll maxima (DCM) at the metalimnion. *Perfiles verticales de luz, temperatura y clorofila a de lagos profundos norpatagónicos de Argentina. (A) Perfiles verticales de temperatura y de luz fotosintéticamente activa (PAR) y cuatro bandas de radiación ultravioleta (UVR). (B) Perfiles verticales de colores específicos mostrando la predominancia de azul y verde en el metalimnion, y un perfil vertical de clorofila a mostrando la existencia de un máximo profundo de clorofila (DCM) en el metalimnion.*

chromophoric dissolved organic matter), optical quality of the lakes is characterized by a relative rapid attenuation of red light while blue and green light penetrate deeper (Pérez *et al.*, 2002) (Fig. 3). Thus, these environments have been defined as high light/low nutrient environments (Balseiro *et al.*, 2004). Among the peculiarities of their biota, the peritrich *O. naumanni* and the heterotrich *S. araucanus*, both with stable association with endosymbiotic algae, have been recorded since the early studies of Thomasson (1963). Further studies have indicated that these ciliate species are restricted to the more transparent and deeper lakes of the area (Modenutti, 1997).

Under high light scenarios including UVR, a strong photoinhibition effect can occur (Lesser, 1996; Falkowski & Raven, 2007). As expected, in the upper 10 m layer of the water column (Fig. 3) there is a strong photoinhibition for autotrophs (Villafañe *et al.*, 2004) that causes low photosynthetic efficiency (Callieri *et al.*, 2007). In addition, due to the UVR effect, plankton avoid upper layers during daytime (Alonso *et al.*, 2004; Balseiro *et al.*, 2007; Modenutti *et al.*, 2018) with the

exception of the pigmented ciliate *S. araucanus* (Modenutti *et al.*, 1998; Modenutti *et al.*, 2005). However, as a counterpart, light is not limiting for autotrophs at least up to ~30–40 m of the water column, and implies the development of deep chlorophyll maxima (DCM) located mainly in the metalimnion (Modenutti *et al.*, 2013a). Accordingly, the highest photosynthetic efficiency was observed near the 1% of surface PAR (photosynthetically active radiation) (Callieri *et al.*, 2007). These DCM are constituted by endosymbiotic algae of *O. naumanni* (Queimaliños *et al.*, 1999), picocyanobacteria (Callieri *et al.*, 2007), dinoflagellates and nanoflagellates (Modenutti *et al.*, 2013a). Interestingly, predator and prey (*O. naumanni* and picocyanobacteria) coexist at this level (Modenutti & Balseiro, 2002).

Light protection or surface avoidance

To cope with scenarios of extremely high epilimnetic irradiances in these Andean lakes, mixotrophic ciliates exhibit two different strategies: a strong photoprotection (Modenutti *et al.*,

2005) or the avoidance of the upper levels of the water column (Modenutti *et al.*, 2004). The highly illuminated epilimnetic layers include both PAR and UVR wavelengths, and the net effect on the phytoplankton community at these upper levels is a strong photoinhibition with DNA damage and very low phytoplanktonic biomass (Villafañe *et al.*, 2004). Nevertheless, these upper levels of the water column are colonized by the mixotrophic ciliate *S. araucanus* (Woelfl & Geller, 2002) that presents an extremely high resistance to UVR (Modenutti *et al.*, 1998). This resistance was attributed to the presence of subcortical dark pigmented granules (with stentorin) (Modenutti *et al.*, 1998; Modenutti *et al.*, 2005) and mycosporin-like aminoacids (Tartarotti *et al.*, 2004). Thus, photoprotection seems to be a predominant strategy in this epilimnetic species.

However, since the metalimnion is included in the euphotic zone autotrophs can avoid the upper levels of the water column developing DCM. Metalimnetic DCM colonization by phototrophic organisms represents a trade-off between higher survival and lower cell-specific primary produc-

tion (Modenutti *et al.*, 2004). In particular, 40–80 % of Chl *a* concentration at the DCM was observed to be composed by endosymbiotic *Chlorella* of the ciliate *O. naumanni* (Queimaliños *et al.*, 1999). This important contribution can be also associated with the raise of Chl *b* in *Chlorella* that allow to profit from the prevailing green and blue wavelengths (< 500 nm) at the DCM (Pérez *et al.*, 2002; Pérez *et al.*, 2007) (Fig. 3).

Living at the edge and stoichiometric balances: high vs low irradiance

Purely phototrophic organisms tend to increase in C content and thus in C: nutrient ratio as light increases (Sterner *et al.*, 1997); however, mixotrophs compensate for increased C fixation by phagotrophy to obtain N and P (Jones, 2000; Modenutti & Balseiro, 2002). Mixotrophic ciliates combine phagotrophy and phototrophy in the same organism and the combination of these nutritional modes imply a more balanced stoichiometric composition (Flynn & Mitra, 2009). Also, this combined nutrition seems to confer important ecological advantages as it provides

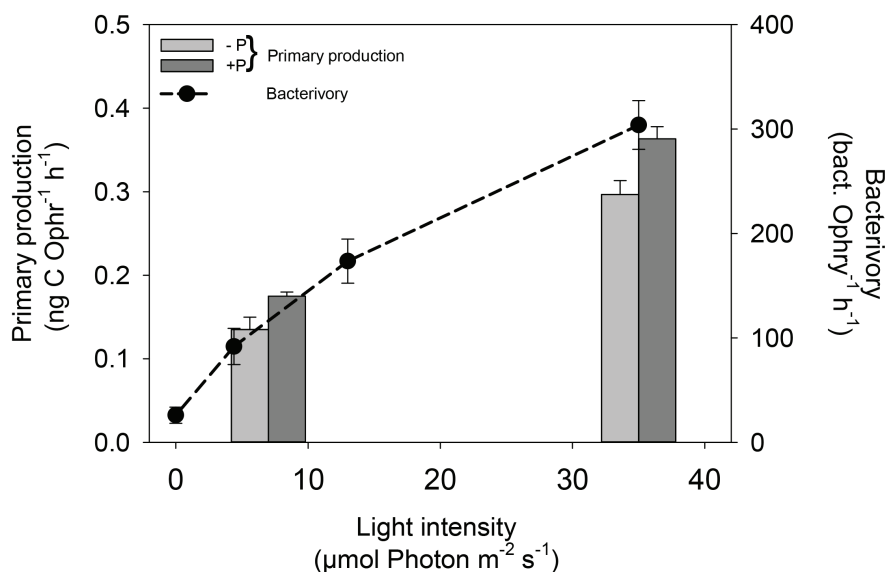


Figure 4. Bacterivory of *Ophrydium naumanni* in a light gradient (black dots and dashed lines) and Net Primary Production (NPP) of *Ophrydium naumanni* cultured with (dark bars) and without (light grey bars) P enriched bacteria. Error bars are 1 s.e. *Bacterivoría de Ophrydium naumanni en un gradiente de luz (puntos negros y línea de guiones) y Producción Primaria Neta (NPP) de Ophrydium naumanni cultivados con (barras oscuras) y sin (barras claras) bacterias enriquecidas en P. Las barras de error indican 1 error estándar.*

greater flexibility in the planktonic environment (Stoecker *et al.*, 2009).

While the sestonic C:P ratio (atomic) of deep North-Patagonian Andean lakes range from 340 to 1200 (Balseiro *et al.*, 2007), the C:P ratio of *S. araucanus* and *O. naumannii* is significantly lower (170 ± 27 , mean \pm S.E.) (Modenutti *et al.*, 2018). This balanced C:P ratio represents a noticeably increase in P content relative to the bulk lake seston. Thus, mixotrophic ciliates show a rather strict stoichiometric homeostasis, meaning that, from a stoichiometric perspective, these species would be a good food source in systems with low food quality (Modenutti *et al.*, 2018).

Mixotrophic ciliate stoichiometric balance may not be the pure result of light-nutrient ratio of the lake because their elemental ratio results of the interactions between photosynthesis and prey consumption. This outcome was clear in the case of *O. naumannii* conforming metalimnetic DCM at 1 % of PAR. Combining field and laboratory experiments (^{14}C fixation and bacterivory experiments) we determined that *O. naumannii* balances photosynthesis and prey consumption (Modenutti & Balseiro, 2002; Modenutti *et al.*, 2004). At low irradiance (1 % of surface PAR) C fixation and bacterivory are both related with light availability as *O. naumannii* ingests more bacteria as C fixation increases (Fig. 4). Furthermore, if *O. naumannii* is fed with P enriched bacteria (see dark grey bars in Fig. 4) C fixation increases when compared with non-enriched prey, at the same light intensity (Fig.4). Summarizing, *O. naumannii* at the DCM (metalimnion) balance C fixation (autotrophic nutrition) with the P uptake (phagotrophic nutrition).

However, the ^{14}C fixation experiments carried out with *S. araucanus* gave very different results. As expected for a resistant UVR organism, at 0.3 m depth the net primary production (NPP) did not decrease in the presence of UV-B wavelength (similar values in quartz tubes vs. quartz tubes wrapped with MylarTM) (Modenutti *et al.*, 2005). Strikingly, we observed no trend in NPP with light, since all conditions (static or moving incubations) with light $> 100 \mu\text{mol m}^{-2} \text{s}^{-1}$ had NPP $> 1 \text{ ng C ind}^{-1} \text{ h}^{-1}$ (Fig. 5). However, a significant drop in NPP was observed when light was $< 100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 5) and this limit is far from the

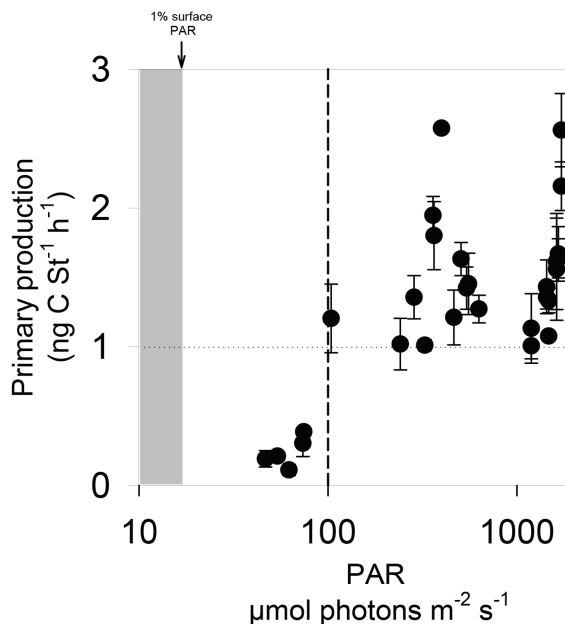


Figure 5. Net Primary Production of *Stentor araucanus* in fixed and moving incubations, protected or not from UVR. Vertical dashed line indicates $100 \mu\text{mol photon m}^{-2} \text{s}^{-1}$, and horizontal dotted lines a NPP of $1 \text{ ng C St}^{-1} \text{h}^{-1}$. Note the drop in NPP when light is below $100 \mu\text{mol photon m}^{-2} \text{s}^{-1}$. *Producción Primaria Neta (NPP) de Stentor araucanus en incubaciones fijas y móviles protegidas o no de la radiación UV. La línea de guiones vertical indica $100 \mu\text{mol fotones m}^{-2} \text{s}^{-1}$ y la línea de puntos horizontal muestra NPP de $1 \text{ ng C St}^{-1} \text{h}^{-1}$. Nótese la brusca caída en NPP cuando la luz es menor a $100 \mu\text{mol fotones m}^{-2} \text{s}^{-1}$.*

zone where *O. naumannii* inhabits ($\sim 15 \mu\text{mol m}^{-2} \text{s}^{-1}$). In addition, the analysis of food vacuoles did not show bacteria inside *S. araucanus*' cell meaning that at least for long summer periods photosynthesis of the endosymbiotic algae is the main nutrition mode of this ciliate (Modenutti *et al.*, 2008). The presence of these dark granules act as a light umbrella for the endosymbiotic algae, since stentorin absorbs PAR mainly between 400 and 600 nm (Moller, 1962; Modenutti *et al.*, 2005). In this sense, the irradiance below $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ appeared to be insufficient to allow endosymbiotic algae to produce (C fixation) under such a dark pigmented umbrella. Consequently, granules have a shading effect and black ciliates such as *S. araucanus* may be able to regulate internal light intensity for endosymbiotic algae and thus, elemental balance in this ciliate

would be achieved by regulating internal light and not by phagotrophy.

Independently of the stoichiometric strategy involved, the overall seston C:P ratio in a lake with strong influence of such mixotrophs would be lower than that of purely phototrophic organisms (Modenutti *et al.*, 2018). Given that zooplankton predators are constrained by the elemental content of their prey (Laspoumaderes *et al.*, 2015), the stoichiometric balance of mixotrophs may be a key factor affecting the stoichiometry of predator–prey relationships. This was the case of the cyclopoid copepod *Mesocyclops araucanus* that feeds upon *S. araucanus* (Kamjunke *et al.*, 2012) at the epilimnion during night (Modenutti *et al.*, 2018). In this relationship it is also important the presence of the pigment stentorin that cause an increase in the oxidative stress of the organisms who prey upon *S. araucanus*. Under these circumstances, *M. araucanus* would be exposed to an increase in visible light stress that drives a diel vertical migration with a deeper distribution (1.5 fold deeper than other microcrustaceans, *Daphnia* and *Boeckella*) during day (Modenutti *et al.*, 2018).

The future

Lakes act as early sentinels to environmental changes (Williamson *et al.*, 2008). In particular, the decline in biodiversity of freshwater biota is higher than for terrestrial or marine organisms (Jenkins & Boulton, 2003). Thus, it would be crucial to understand the causes and consequences of the loss of biodiversity caused by changes in aquatic ecosystems (Williamson *et al.*, 2008). In this sense, it would be necessary to develop applications of ecological and evolutionary knowledge for addressing environmental problems (Sutherland *et al.*, 2009). At present, this mixotrophic biota is threatened by local and global changes that may affect negatively the particular ciliate assemblage of North-Patagonian Andean lakes.

These glacial deep lakes can be considered as sensitive environments to global change. Patagonia is under the influence of the Antarctic ozone "hole" receiving occasionally enhanced levels of ultraviolet B radiation (UVR-B, 280–315 nm) (Villafañe *et al.*, 2001). This condition would

cause a number of detrimental effects in aquatic organisms (Zagarese & Williamson, 1994; Helbling & Zagarese, 2003). In this sense, pigmented members of the genus *Stentor* (i.e. *S. araucanus* and *S. amethystinus*) would be favored because of their high resistance to UVR (Modenutti *et al.*, 1998; Tartarotti *et al.*, 2004). Interannual variations in the thermocline depth due to wind will also cause changes in the mean light intensity of the epilimnion and would affect the coexistence of *S. araucanus* and *O. naumanni* (Modenutti *et al.*, 2008) shifting the ratio of these two species, towards *S. araucanus* if wind decreases or favoring *O. naumanni* if wind becomes stronger.

Glaciers are retreating as a consequence of climate change almost everywhere around the world — including in the Alps, Himalayas, Andes, Rockies, Alaska and Africa (Zemp *et al.*, 2015). In Patagonia, there are clear examples of glacial retraction in the Mount Tronador ice cap (Worni *et al.*, 2012; Ruiz *et al.*, 2015). This situation affects the lakes that receive water from the glaciers, i.e. Lake Mascaradi. This lake has two arms (Catedral and Tronador) that can be considered separate lakes since the two arms differ in their hydrology because of three headwater glaciers (Manso, Castaño Overo and Alerce glaciers) that drain only into Mascaradi Tronador arm (Modenutti *et al.*, 2013a). Planktonic organisms (i.e. picocyanobacteria) were observed to be sensitive to changes in glacial clay inputs that cause, in turn, a decrease in light (Hylander *et al.*, 2011; Bastidas Navarro *et al.*, 2018). Mixotrophic ciliates are absent in lake sectors that receives glacial clay (i.e. Lake Mascaradi Tronador Arm) probably because clay particles interfere with feeding, although populations remain in the areas without such input (i.e. Lake Mascaradi Catedral Arm). Another negative effect of suspended particles on this biota was due to volcanic eruptions that produced sudden catastrophic effects. Northern Patagonia is an active volcanic region with historically high eruption frequency (Inbar *et al.*, 1995). The Puyehue-Cordón Caulle Volcanic Complex (40.59° S - 72.11° W, 2200 m a.s.l.) erupted explosively on 4th June 2011. The volcano produced 1.46 km³ of rhyolitic volcanic material (Silva Parejas *et al.*, 2012), a similar amount

to that erupted by Mount St. Helens in 1980. The lakes in the area were covered by this material affecting nutrient inputs and light conditions (Modenutti *et al.*, 2013b). Under these conditions, mixotrophic ciliate disappeared and the microbial food web changed towards a more autotrophic one (diatoms and phytoflagellates), probably due to the interference by the volcanic ash and the improvement in light conditions (i.e. reduced photoinhibition) (Modenutti *et al.*, 2013b).

The main cause of the loss of biodiversity can be attributed to the local influence of human activities on different ecosystems. In fact, human activities have deeply altered lacustrine environments, through the modifications of landscapes, direct exploitation of species or by affecting biogeochemical cycles. Alteration and loss of the habitats is the transformation of the natural areas by different human activities. In the area there is an example of how this situation will affect ciliate biota. The small, closed basin of Lake Morenito (41°S, 71°W; 764 m a.s.l.; 0.82 km²; 12 m maximum depth) is located 20 km west from the city of Bariloche and until 1960 this lake was a bay of Lake Moreno West (Modenutti *et al.*, 2000). At that time a road was constructed, separating this bay and creating the new Lake Morenito. In the new lake a littoral zone where *Schoenoplectus californicus* increased its relative importance and the water exchange with the main basin was deeply reduced. As a consequence dissolved organic matter increased in the new lake in comparison with the original Lake Moreno (0.5 mg/L in Lake Moreno vs 3 mg/L in Lake Morenito) (Modenutti *et al.*, 2000). This situation caused a change in light climate of the new Lake Morenito shifting the blue-green under water climate of Lake Moreno (Fig. 2) to a red predominance (Pérez *et al.*, 2002). These changes would be the key factors for the absence of the two mixotrophic ciliate species (rich in Chl b) (Modenutti *et al.*, 2000). Interestingly, the studies of short sediment cores from Lake Morenito showed a change in the chironomid assemblage in response to an increase in trophic enrichment after the road was constructed (Massafferro *et al.*, 2005). Thus, biota diversity can be severely affected by this kind of change in landscape and basin.

Summarizing, these final considerations

imply that endemic elements, such as mixotrophic ciliates, can disappear being replaced by other species of more autotrophic metabolism. From an ecosystem perspective, lake functioning will result altered because of the replacement of the links in the microbial food web. Thus, the loss of this particular biota would drive changes in nutrient recycling, in top down and bottom up effects and in the major ecosystem services.

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REFERENCES

- ALONSO, C., V. ROCCO, J. P. BARRIGA, M. A. BATTINI & H. ZAGARESE. 2004. Surface avoidance by freshwater zooplankton: Field evidence on the role of ultraviolet radiation. *Limnology and Oceanography*, 49: 225-232.
- ANDERSEN, T., J. J. ELSER & D. O. HESSEN. 2004. Stoichiometry and population dynamics. *Ecology Letters*, 7: 884-900. DOI: 10.1111/j.1461-0248.2004.00646.x
- BALGÚN, C. & M. C. MARINONE. 1995. Cold-temperate lakes of South America: do they fit Northern hemisphere models? *Archiv für Hydrobiologie*, 135: 23-51.
- BALSEIRO, E. G., C. P. QUEIMALIÑOS & B. E. MODENUTTI. 2004. Grazing impact on autotrophic picoplankton in two south andean lakes (Patagonia, Argentina) with different light:nutrient ratios. *Revista Chilena de Historia Natural*, 77: 73-85.
- BALSEIRO, E. G., B. E. MODENUTTI, C. QUEIMALIÑOS & M. REISSIG. 2007. *Daphnia* distribution in Andean Patagonian lakes: effect of low food quality and fish predation. *Aquatic Ecology*, 41: 599-609.
- BASTIDAS NAVARRO, M., N. MARTYNIUK, E. BALSEIRO & B. MODENUTTI. 2018. Effect of glacial lake outburst floods on the

- light climate in an Andean Patagonian lake: implications for planktonic phototrophs. *Hydrobiologia*, 816: 39-48.
- CALLIERI, C., B. E. MODENUTTI, C. QUEIMALIÑOS, R. BERTONI & E. G. BALSEIRO. 2007. Production and biomass of picoplankton and larger autotrophs in Andean ultraoligotrophic lakes: Differences in light harvesting efficiency in deep layers. *Aquatic Ecology*, 41: 511-523.
- CORNO, G., B. E. MODENUTTI, C. CALLIERI, E. G. BALSEIRO, R. BERTONI & E. CARAVATI. 2009. Bacterial diversity and morphology in deep ultraoligotrophic Andean lakes: The role of UVR on vertical distribution. *Limnology and Oceanography*, 54: 1098-1112.
- ELSER, J. J. & R. P. HASSETT. 1994. Stoichiometric analysis of the zooplankton-phytoplankton interaction in marine and freshwater ecosystem. *Nature*, 370: 211-213.
- FALKOWSKI, P. G. & J. A. RAVEN 2007 *Aquatic photosynthesis*, 2nd edition ed. Blackwell Science, Malden, MA, USA.
- FLYNN, K. J. & A. MITRA. 2009. Building the "perfect beast": modelling mixotrophic plankton. *Journal of Plankton Research*, 31: 965-992. DOI: 10.1093/plankt/fbp044
- FOISSNER, W. & S. WOELFL. 1994. Revision of the genus *Stentor* Oken (Protozoa, Ciliophora) and description of *S. araucanus* nov. spec. from South American lakes. *Journal of Plankton Research*, 16: 255-289.
- HELBLING, W. & H. E. ZAGARESE. 2003. *UV effect on aquatic organisms and ecosystems*. Royal Society of Chemistry,
- HESSEN, D. O., P. J. FÆRØVIG & T. ANDERSEN. 2002. Light, nutrients, and P:C ratios in algae: grazer performance related to food quality and quantity. *Ecology*, 83: 1886-1898.
- HYLANDER, S., T. JEPHSON, K. LEBRET, J. VON EINEM, T. FAGERBERG, E. G. BALSEIRO, B. E. MODENUTTI, M. S. SOUZA, C. LASPOUMADERES, M. JÖNSSON, P. LJUNGBERG, A. NICOLLE, P. A. NILSSON, L. RANÅKER & L.-A. HANSSON. 2011. Climate-induced input of turbid glacial meltwater affects vertical distribution and community composition of phyto- and zooplankton. *Journal of Plankton Research*, 33: 1239-1248. DOI: 10.1093/plankt/fbr025
- INBAR, M., H. OSTERA, C. PARICA, M. REMESAL & F. SALANI. 1995. Environmental assessment of 1991 Hudson volcano eruption ashfall effects on southern Patagonia region, Argentina. *Environmental Geology*, 25: 119-125.
- JENKINS, K. M. & A. J. BOULTON. 2003. Connectivity in a dryland river: short-term aquatic microinvertebrate recruitment following floodplain inundation. *Ecology*, 84: 2708-2723.
- JONES, H. L. J. 1997. A classification of mixotrophic protists based on their behaviour. *Freshwater Biology*, 37: 35-43.
- JONES, R. I. 2000. Mixotrophy in planktonic protists: an overview. *Freshwater Biology*, 45: 219-226.
- KAMJUNKE, N., M. KRAMPS, S. CHAVEZ & S. WOELFL. 2012. Consumption of large, *Chlorella*-bearing ciliates (*Stentor*) by *Mesocyclops araucanus* in North Patagonian lakes. *Journal of Plankton Research*, 34: 922-927. DOI: 10.1093/plankt/fbs051
- LASPOUMADERES, C., B. MODENUTTI, J. ELSEER & E. BALSEIRO. 2015. Does the stoichiometric carbon:phosphorus knife edge apply for predaceous copepods? *Oecologia*, 178: 557-569. DOI: 10.1007/s00442-014-3155-8
- LAYBOURN-PARRY, J., S. J. PERRISS, G. G. R. SEATON & J. ROHOZINSKI. 1997. A mixotrophic ciliate as a major contributor to plankton photosynthesis in Australian lakes. *Limnology and Oceanography*, 42: 1463-1467.
- LESSER, M. P. 1996. Acclimation of phytoplankton to UV-B radiation: oxidative stress and photoinhibition of photosynthesis are not prevented by UV-absorbing compounds in the dinoflagellate *Prorocentrum micans*. *Marine Ecology Progress Series*, 132: 287-297.
- MACEK, M., K. ŠIMEK & T. BITTL. 2001. Conspicuous Peak of Oligotrichous Ciliates Following Winter Stratification in a Bog Lake. *Journal of Plankton Research*, 23: 353-363(311).
- MASSAFERRO, J., S. R. GUEVARA, A. RIZZO & M. ARRIBÈRE. 2005. Short-term

- environmental changes in Lake Morenito (41° S, 71° W, Patagonia, Argentina) from the analysis of sub-fossil chironomids. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 15: 23-30. DOI: 10.1002/aqc.640
- MITRA, A., K. J. FLYNN, U. TILLMANN, J. A. RAVEN, D. CARON, D. K. STOECKER, F. NOT, P. J. HANSEN, G. HALLEGRAEFF, R. SANDERS, S. WILKEN, G. MCMANUS, M. JOHNSON, P. PITTA, S. VAGE, T. BERGE, A. CALBET, F. THINGSTAD, H. J. JEONG, J. BURKHOLDER, P. M. GLIBERT, E. GRANIELI & V. LUNDGREN. 2016. Defining Planktonic Protist Functional Groups on Mechanisms for Energy and Nutrient Acquisition: Incorporation of Diverse Mixotrophic Strategies. *Protist*, 167: 106-120. DOI: 10.1016/j.protis.2016.01.003
- MODENUTTI, B. E. 1997. Distribución de los ciliados planctónicos *Ophrydium naumanni* y *Stentor araucanus* en lagos oligotróficos andinos. *Revista de la Sociedad Mexicana de Historia Natural*, 47: 79-83.
- MODENUTTI, B. E., E. G. BALSEIRO & R. MOELLER. 1998. Vertical distribution and resistance to ultraviolet radiation of a planktonic ciliate *Stentor araucanus*. *Verhandlungen Internationale Vereinigung Limnologie*, 26: 1636-1640.
- MODENUTTI, B. E., E. G. BALSEIRO & C. P. QUEIMALIÑOS. 2000. Ciliate community structure in two South Andean lakes: the effect of lake water on *Ophrydium naumanni* distribution. *Aquatic Microbial Ecology*, 21: 299-307.
- MODENUTTI, B. E. & E. G. BALSEIRO. 2002. Mixotrophic ciliates in an Andean lake: dependence on light and prey of an *Ophrydium naumanni* population. *Freshwater Biology*, 47: 121-128.
- MODENUTTI, B. E., E. G. BALSEIRO, C. CALLIERI, C. QUEIMALIÑOS & R. BERTONI. 2004. Increase in photosynthetic efficiency as a strategy of planktonic organisms exploiting deep lake layers. *Freshwater Biology*, 49: 160-169.
- MODENUTTI, B. E., E. G. BALSEIRO, C. CALLIERI, R. BERTONI & C. P. QUEIMALIÑOS. 2005. Effect of UV-B and different PAR intensities on the primary production of the mixotrophic planktonic ciliate *Stentor araucanus*. *Limnology and Oceanography*, 50: 864-871.
- MODENUTTI, B. E., E. G. BALSEIRO, C. CALLIERI & R. BERTONI. 2008. Light versus food supply as factors modulating niche partitioning in two pelagic mixotrophic ciliates. *Limnology and Oceanography*, 53: 446-455.
- MODENUTTI, B. E., E. G. BALSEIRO, M. BASTIDAS NAVARRO, C. LASPOUMADERES, M. S. SOUZA & F. CUASSOLO. 2013a. Environmental changes affecting light climate in oligotrophic mountain lakes: The deep chlorophyll maxima as a sensitive variable. *Aquatic Sciences*, 75: 361-371. DOI: 10.1007/s00027-012-0282-3
- MODENUTTI, B. E., E. G. BALSEIRO, J. J. ELSER, M. BASTIDAS NAVARRO, F. CUASSOLO, C. LASPOUMADERES, M. S. SOUZA & V. DÍAZ VILLANUEVA. 2013b. Effect of volcanic eruption on nutrients, light, and phytoplankton in oligotrophic lakes. *Limnology and Oceanography*, 58: 1165-1175. DOI: 10.4319/lo.2013.58.4.0000
- MODENUTTI, B. E. 2014. Mixotrophy in Argentina freshwaters. *Advances in limnology*, 65: 359-374.
- MODENUTTI, B. E., L. WOLINSKI, M. S. SOUZA & E. G. BALSEIRO. 2018. When eating a prey is risky: Implications for predator diel vertical migration. *Limnology and Oceanography*, 63: 939-950. DOI: 10.1002/lno.10681
- MOLLER, K. M. 1962. *On the nature of stentorine*. *Compte rendue travaux Carlsberg*, 32: 471-498.
- MORRIS, D. P., H. ZAGARESE, C. E. WILLIAMSON, E. G. BALSEIRO, B. R. HARGREAVES, B. E. MODENUTTI, R. MOELLER & C. QUEIMALIÑOS. 1995. The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. *Limnology and Oceanography*, 40: 1381-1391.
- PÉREZ, G. L., C. P. QUEIMALIÑOS & B. E. MODENUTTI. 2002. Light climate and plankton in the deep chlorophyll maxima in North Patagonian Andean lakes. *Journal of Plankton Research*, 24: 591-599.

- PÉREZ, G. L., C. QUEIMALIÑOS, E. G. BALSEIRO & B. E. MODENUTTI. 2007. Phytoplankton absorption spectra along the water column in deep North Patagonian Andean lakes (Argentina): Limnology of Temperate South America. *Limnologia*, 37: 3-16.
- PERRISS, S. J., J. LAYBOURN-PARRY & R. I. JONES. 1994. Chlorophyll contents and photosynthetic rates of the freshwater mixotrophic ciliate *Strombidium viride* (Ciliophora: Oligotrichida). *Archiv fur Hydrobiologie*, 130: 473-483.
- PUTT, M. 1990. Abundance, chlorophyll content and photosynthetic rates of ciliates in the Nordic Seas during summer. *Deep Sea Research Part A, Oceanographic Research Papers*, 37: 1713-1731.
- QUEIMALIÑOS, C. P., B. E. MODENUTTI & E. G. BALSEIRO. 1999. Symbiotic association of the ciliate *Ophrydium naumanni* with *Chlorella* causing a deep chlorophyll maximum in an oligotrophic South Andes lake. *Journal of Plankton Research*, 21: 167-178.
- REISSER, W., R. MEIER, H. D. GORTZ & K. W. JEON. 1985. Establishment, Maintenance, and integration mechanisms of endosymbionts in Protozoa. *Journal of Protozoology*, 32: 383-390.
- RUIZ, L., E. BERTHIER, H. M. MASIOKAS, P. PITTE & R. VILLALBA. 2015. First surface velocity maps for glaciers of Monte Tronador, North Patagonian Andes, derived from sequential Pléiades satellite images. *Journal of Glaciology*, 61: 908-922. DOI: 10.3189/2015JoG14J134
- SILVA PAREJAS, C., L. LARA, D. BERTIN, A. AMIGO & G. OROZCO. 2012. The 2011-2012 eruption of Cordón Caulle volcano (Southern Andes): Evolution, crisis management and current hazards, p. 9382. EGU General Assembly Conference Abstracts.
- STERNER, R. W., J. J. ELSER, E. J. FEE, S. J. GUILDFORD & T. H. CHRZANOWSKI. 1997. The light:nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. *The American Naturalist*, 150: 663-684.
- STERNER, R. W. & J. J. ELSER. 2002. *Ecological stoichiometry. The biology of elements from molecules to the biosphere*. Princeton University Press, Princeton, NJ USA.
- STOECKER, D. K. & M. W. SILVER. 1987. Chloroplast retention by marine planktonic ciliates. *Annals of the New York Academy of Sciences*, 503: 562-565.
- STOECKER, D. K. 1998. Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. *European Journal of Protistology*, 34: 281-290.
- STOECKER, D. K., M. D. JOHNSON, C. DE VARGAS & F. NOT. 2009. Acquired phototrophy in aquatic protists. *Aquatic Microbial Ecology*, 57: 279-310.
- SUMMERER, M., B. SONNTAG & R. SOMMARUGA. 2008. Ciliate-symbiont specificity of freshwater endosymbiotic *Chlorella* (Trebouxiophyceae, Chlorophyta). *Journal of Phycology*, 44: 77-84.
- SUTHERLAND, W. J., W. M. ADAMS, R. B. ARONSON, R. AVELING, T. M. BLACKBURN, S. BROAD, G. CEBALLOS, I. M. COTE, R. M. COWLING, G. A. DA FONSECA, E. DINERSTEIN, P. J. FERRARO, E. FLEISHMAN, C. GASCON, M. HUNTER, JR., J. HUTTON, P. KAREIVA, A. KURIA, D. W. MACDONALD, K. MACKINNON, F. J. MADGWICK, M. B. MASCIA, J. MCNEELY, E. J. MILNER-GULLAND, S. MOON, C. G. MORLEY, S. NELSON, D. OSBORN, M. PAI, E. C. PARSONS, L. S. PECK, H. POSSINGHAM, S. V. PRIOR, A. S. PULLIN, M. R. RANDS, J. RANGANATHAN, K. H. REDFORD, J. P. RODRIGUEZ, F. SEYMOUR, J. SOBEL, N. S. SODHI, A. STOTT, K. VANCE-BORLAND & A. R. WATKINSON. 2009. One hundred questions of importance to the conservation of global biological diversity. *Conservation Biology*, 23: 557-567. DOI: 10.1111/j.1523-1739.2009.01212.x
- TARTAROTTI, B., G. BAFFICO, P. TEMPORETTI & H. E. ZAGARESE. 2004. Mycosporine-like amino acids in planktonic organisms living under different UV exposure conditions in Patagonian lakes. *Journal of Plankton Research*, 26: 753-762.
- THOMASSON, K. 1963. Araucanian lakes. *Acta*

- Phytogeographica Sueca, 47: 1-139.
- TITTEL, J., V. BISSINGER, B. ZIPPEL, U. GAEDKE, E. BELL, A. LORKE & N. KAMJUNKE. 2003. Mixotrophs combine resource use to outcompete specialists: Implications for aquatic food webs. *Proceeding of the National Academy of Sciences*, 100: 12776-12781.
- VILLAFANE, V., E. W. HELBLING & H. E. ZAGARESE. 2001. Solar ultraviolet radiation and its impact on aquatic systems of Patagonia, South America. *Ambio*, 30: 112-117.
- VILLAFANE, V. E., A. G. J. BUMA, P. BOELEN & E. W. HELBLING. 2004. Solar UVR-induced DNA damage and inhibition of photosynthesis in phytoplankton from Andean lakes of Argentina. *Archiv fur Hydrobiologie*, 161: 245-266.
- WILLIAMSON, C. E., W. DODDS, T. K. KRATZ & M. A. PALMER. 2008. Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. *Frontiers in Ecology and the Environment*, 6: 247-254.
- WOELFL, S. & W. GELLER. 2002. Chlorella-bearing ciliates dominate in an oligotrophic North Patagonian lake (Lake Pirehueico, Chile): abundance, biomass and symbiotic photosynthesis. *Freshwater Biology*, 47: 231-242.
- WORN, R., M. STOFFEL, C. HUGGEL, C. VOLZ, A. CASTELLER & B. LUCKMAN. 2012. Analysis and dynamic modeling of a moraine failure and glacier lake outburst flood at Ventisquero Negro, Patagonian Andes (Argentina). *Journal of Hydrology*, 444-445: 134-145. DOI: 10.1016/j.jhydrol.2012.04.013
- ZAGARESE, H. E. & C. WILLIAMSON. 1994. Modeling the impact of UV-B radiation on ecological interactions in freshwater and marine ecosystems. In: *Stratospheric ozone depletion/UV-B radiation in the biosphere*. R. H. Biggs & M. E. B. Joyner (ed.):315-328. Springer Verlag, Berlin, Germany.
- ZEMP, M., H. FREY, I. GÄRTNER-ROER, S. U. NUSSBAUMER, M. HOELZLE, F. PAUL, W. HAEBERLI, F. DENZINGER, A. P. AHLSTRØM, B. ANDERSON, S. BAJRACHARYA, C. BARONI, L. N. BRAUN, B. E. CÀCERES, G. CASASSA, G. COBOS, L. R. DÀVILA, H. DELGADO GRANADOS, M. N. DEMUTH, L. ESPIZUA, A. FISCHER, K. FUJITA, B. GADEK, A. GHAZANFAR, J. O. HAGEN, P. HOLMLUND, N. KARIMI, Z. LI, M. PELTO, P. PITTE, V. V. POPOVNIN, C. A. PORTO-CARRERO, R. PRINZ, C. V. SANGEWAR, I. SEVERSKIY, O. SIGURDSSON, A. SORUCO, R. USUBALIEV & C. VINCENT. 2015. Historically unprecedented global glacier decline in the early 21st century. *Journal of Glaciology*, 61: 745-762. DOI: 10.3189/2015JoG15J017

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CUARTA DEL GOBIERNO
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PARA LA TRANSICIÓN ECOLÓGICA
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