

Drivers of phytoplankton diversity in Patagonian and Antarctic lakes across a latitudinal gradient (2150 km): the importance of spatial and environmental factors

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Abstract We investigated the phytoplankton structure in 60 lakes across a latitudinal gradient (2150 km), from Austral Patagonia to Antarctica, including environments ranging from oligotrophic to eutrophic. We analysed the latitudinal variation of species richness (local and regional) and evenness, as well as the similarity decay in phytoplankton composition in Patagonia and Antarctica. The following hypotheses were tested: (1) there is a decline of phytoplankton species richness with increasing latitude; (2) phytoplankton structure is influenced by both geographical and environmental factors. (3) The predominant algal

trophic strategy (autotrophic vs mixotrophic) is influenced by lake trophic status. Phytoplankton was analysed using a polyphasic approach (morphologically based species diversity, functional diversity, dominant molecular diversity). We found a significant decline in phytoplankton richness with increasing latitude. Multivariate analyses showed that phytoplankton is structured by the lake geographic position (mainly latitude) and variables related with trophic state (nutrients, conductivity and pH). The autotrophs/mixotrophs ratio increased towards higher trophic states. The initial similarity was higher in Antarctica than in Patagonia, whereas the halving distance was lower for Antarctic lakes. The three biodiversity approaches of this meta-analysis evidenced that both geographic and environmental factors influence phytoplankton diversity at large spatial scale, although the local effect was stronger.

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Introduction

Since the eighteen century, the naturalists have been interested in exploring the distribution of the species and their coexistence in different habitats, but the biogeography of the macroorganisms has received much more attention. Only in recent decades, studies

on microbial biogeography supported by molecular tools allowed a deeper knowledge of the microbial diversity, leading to great advances in this field of the science (see Martiny et al., 2006; Soininen, 2012). Although there is no universal definition of ‘microorganism’, it is accepted that the term encompasses from bacteria to microscopic eukaryotic members (such as microalgae, heterotrophic flagellates, ciliates). Their main ecological features (large population size, short generation time and high dispersion), determine that historical factors are less important than biological and physical factors in microbial biogeography (Dolan, 2005). The well-known hypothesis coined by Baas-Becking (1934)—‘everything is everywhere, but the environment selects’—was defended in a series of publications (e.g. Finlay, 1998; Finlay, 2002; Fenchel & Finlay, 2004), emphasizing the cosmopolitanism of the microorganisms (ubiquity model). Nevertheless, although current evidence seems to confirm that the environmental selection is in part responsible for spatial variation in microbial diversity, many recent studies dispute the idea that ‘everything is everywhere’ (Martiny et al., 2006). In this sense, another model proposed by Foissner (2006, 2008), usually known as ‘moderate endemism model’, postulates that approximately a third of free living protists would present a restricted geographic distribution.

Regarding the studies on microalgae biodiversity, some problems concerning species definition hamper the interpretation of biogeography in these organisms (Coesel & Krienitz, 2008). According to the morphologic species concept, groups of morphologically identical organisms are considered as species (Futuyama, 1998). However, there are many cryptic species morphologically similar but genetically different whose distribution may differ, which was confirmed by molecular analyses (e.g. Darling et al., 2004; Saez & Lozano, 2005). In addition, another problem that hinders reliable data on algal biogeography is under-sampling, thus rare species may be overlooked; moreover, many species can be in a dormant stage waiting for favourable conditions to become active (Foissner, 2008). Molecular methods based on 18S rRNA gene amplification and sequencing have revealed an astonishing diversity of microbial eukaryotes (Epstein & López-García, 2008 and cites therein). On the other hand, traditional community descriptors such as species richness may be supplemented by metrics based on functional attributes of the

communities (Petchey et al., 2004). Functional diversity has received a great interest since it provides key information linking ecosystem function and biodiversity (e.g. Schmera et al., 2009). The positive relationship between ecosystem functioning and species richness is often attributed to the greater number in functional groups (see Magurran, 2008). Functional classifications were used to analyse phytoplankton communities of large lake data sets. For example, Kruk et al. (2010) used a phytoplankton classification based on simple morphological traits and analysed more than 200 lakes situated in climate zones ranging from subpolar to tropical, and the results showed that this classification captured much of the variability in functional properties among the phytoplankton.

One aspect that has received a great attention in ecological biogeography is the latitudinal variation of biodiversity (e.g. Rosenzweig, 1995; Gaston, 2000; Willig et al., 2003). Although the most common pattern is the decrease of richness with increasing latitude (with some exceptions), it has been recognized that the patterns may depend on the spatial scale and taxonomic hierarchy—namely species, genera, families, etc. (Willig et al., 2003 and cites therein). Hillebrand (2004) performed a meta-analysis with more than 600 latitudinal gradients obtained from the literature, corroborating that the latitudinal decline in diversity is an ubiquitous phenomenon, but weaker and flatter gradients were observed in freshwater environments. This study also showed that gradients on regional scales were significantly stronger and steeper than on local scales.

Besides the interest in the gradients of local diversity (alpha diversity), in the last decades, there has been an increase of investigations focused on the species turnover of community composition (beta diversity) in space and time. Soininen (2010) examined recent findings in species turnover along abiotic and biotic gradients, and concluded that the latitudinal gradient in turnover is scale dependent both in space and time. At narrow study extents, turnover seems to be faster in the tropics, but the pattern is reversed at broad extents, accelerating the turnover towards the poles. Across the geographic distance, the decay of similarity in the communities can be associated to niche-based community processes under different environmental conditions, spatial configuration of the landscape, and to neutral theory due to the limited dispersal of the organisms (Soininen et al., 2007a and

cites therein). In a study of 100 small lakes from Finland, where all planktonic communities were examined (bacteria, phytoplankton and zooplankton), the distance decay of similarity was found to be related to study scale, environment and organism characteristics (Soininen et al., 2011).

The role of the spatial and environmental factors in shaping biodiversity of the aquatic communities was analysed in several studies. Beisner et al. (2006) compared the role of these factor in structuring different communities ranging from bacteria to fish and concluded that less motile species (zooplankton and fish) are better predicted by spatial factors than good dispersers (bacteria and phytoplankton). In line with these findings, Mazaris et al. (2010) proposed that biogeography of macroorganisms is affected by geographic distance, whereas that of microorganisms whose dispersion is passive, is mainly determined by local environmental conditions. The relative importance of spatial and environmental factors in shaping phytoplankton diversity is matter of current debate. Some previous studies found that local environmental factors were the main drivers (e.g. Vanormelingen et al., 2008; Mazaris et al., 2010; Lopes et al., 2011), whereas others showed that both spatial and environmental factors (Soininen et al., 2007a) were important in structuring community. Nonetheless, other authors reported that neither environment nor spatial factors explained a significant proportion of the total variation in phytoplankton structure (Beisner et al., 2006; Nabout et al., 2009). It has been postulated that the importance of the geographic factors would be stronger at larger spatial scales (e.g. Soininen, 2012).

The environmental conditions in the lakes, and particularly their trophic states seem to influence the trophic strategies of the phytoplankton species. Freshwater systems with high nutrient supply and without light limitation provide good scenarios for algae to develop at the expense of the photosynthesis (Irigoien et al., 2005). Under low nutrient conditions, the mixotrophic behaviour enables to exploit both light and prey sources, which was experimentally demonstrated by Tittel et al. (2003) and Katechakis & Stibor (2006), showing that mixotrophs overcome strictly autotroph and heterotroph protists only under poor nutrient conditions.

In this study, we analysed the phytoplankton communities of 60 lakes located across a latitudinal gradient of more than 2100 km from Austral

Patagonia (45°S) to Antarctica (63°S), to determine the main factors that influence the community structure. We also covered a gradient of trophic conditions, including environments ranging from oligotrophic to eutrophic, and we analysed the predominant algal trophic strategies (mostly autotrophic vs mixotrophic) in lakes with contrasting trophic states. The phytoplankton diversity was analysed using a polyphasic approach (morphologically based species composition, functional groups and dominant eukaryotic molecular diversity).

We postulated the following hypotheses: (1) the species richness of phytoplankton in lakes decreases with increasing latitude. (2) In large spatial scales (as that of the present study) phytoplankton structure is influenced by both geographic and environmental factors. 3) The autotrophs/mixotrophs ratio increases towards higher trophic states.

Study sites

Latitudinal gradient of lakes

We sampled 60 freshwater bodies located across a latitudinal transect of 2150 km over 19° of latitude, from Austral Patagonia (Argentina) to the Antarctic Peninsula: Along this gradient we selected lakes with different morphometries and trophic states in order to cover the variability within each region. According to their nutrient condition and chlorophyll *a* (Chl *a*), lakes varied from oligotrophic to eutrophic (Table 1).

From North to South lakes can be grouped in 7 different zones (Fig. 1), whose geographic coordinates (centroids) are indicated between brackets: (1) steppe lakes from Chubut Province (45°34'S; 69°3'W); (2) lakes from NW of Santa Cruz (47°22'S; 71°42'W); (3) lakes from the Patagonian Strobel plateau (48°37'S; 71°17'W); (4) lakes at the SW of Santa Cruz (49°29'S; 72°50'W); (5) Tierra del Fuego lakes (54°41'S; 67°47'W); (6) lakes from Potter Peninsula, King George Island (62°3'S; 58°31'W); (7) lakes from Hope Bay, Antarctic Peninsula (63°18'S; 57°12'W).

Patagonian lakes are included in two main limnoregions: Andean Patagonia and Patagonian Plateau (Quirós & Drago, 1999). In the Andean region lakes are deep, large and vary from ultraoligotrophic to oligotrophic; most of them were formed by glacial and tectonic processes. In the Patagonian Plateau, lakes are

Table 1 Trophic status of the studied lakes and their corresponding ranges for nutrients and chlorophyll *a*

Trophic state	DIN (mg l ⁻¹)	P-PO ₄ (mg l ⁻¹)	Chlorophyll <i>a</i> (µg l ⁻¹)	Freshwater environments
Oligotrophic to oligo-mesotrophic	0.04–0.31	0.02–1.39	≤2.9	PATAGONIA 15 Deep lakes 10 Shallow lakes ANTARCTICA 8 Shallow lakes
Mesotrophic	0.3–0.42	0.04–0.3	3–9.9	PATAGONIA 10 Shallow lakes ANTARCTICA 1 Shallow lake
Eutrophic	0.05–0.28	0.10–15.8	≥10	PATAGONIA 1 Large shallow lake 14 Shallow lakes ANTARCTICA 1 Shallow lake

DIN dissolved inorganic nitrogen (nitrates + nitrites + ammonium). Data gathered from several field campaigns; detailed information was published in Schiaffino et al. (2011) and Tell et al. (2011)

shallower and range from mesotrophic to eutrophic. In the insular southernmost part of Patagonia (Tierra del Fuego), some lakes are placed near the mountains and share the typical characteristics of the Andean lakes, others are located in the steppe region of the island, which are shallow and meso to eutrophic; finally, other group of environments are typically humic-stained lakes. The main geological and climatic characteristics of the Patagonian regions were described in previous papers (Schiaffino et al., 2011; Tell et al., 2011; Saad et al., 2013).

In Antarctica, we sampled lakes in two different zones: Potter Peninsula in King George Island and Hope Bay at the Antarctic Peninsula. In both regions, lakes are shallow and vary from oligotrophic to eutrophic (Izaguirre et al., 1998; Vinocur & Unrein, 2000; Izaguirre et al., 2003), depending on the influence of marine animals to which they are subjected.

Materials and methods

Samples were obtained from 2004 to 2012 in successive field campaigns. In Antarctica, lakes were sampled during austral summers (January–March), whereas in Patagonia in late spring (October–

November), when lakes are usually well mixed (Baigun & Marinone, 1995). Lakes were sampled once or twice, and in this case we integrated temporal samples in one single community per lake. In deep lakes, integrated samples were collected within the euphotic zone, from the surface down to 5 m, whereas in shallow lakes samples were obtained subsuperficially (upper 30 cm). Temperature, pH and conductivity were measured with a Horiba D-54 metre, and dissolved oxygen (DO) with HI 9146 Hanna or Hatch HQ30d portable metres. Underwater photosynthetically active radiation (PAR) was measured with a LI-COR radiometre equipped with a submersible spherical quantum sensor (Li-193 SA; Li-Cor PAR, NE, USA). The vertical PAR attenuation coefficient (K_d) was calculated as the slope of the relationship between \ln (irradiance) and depth (Kirk, 1994). Samples for nutrient and Chl *a* analyses were filtered through Whatman[®] GF/F filters. Nutrient analyses (ammonium, nitrate and phosphate) were performed using a Hach[™] DR/2800 spectrophotometre and their corresponding reagent kits. Dissolved inorganic nitrogen (DIN) was obtained as nitrate + ammonium. Chl *a* concentration (corrected for phaeopigments) was estimated by spectrophotometry and the equations given in Marker et al. (1980). Dissolved organic carbon (DOC) was determined using a high-

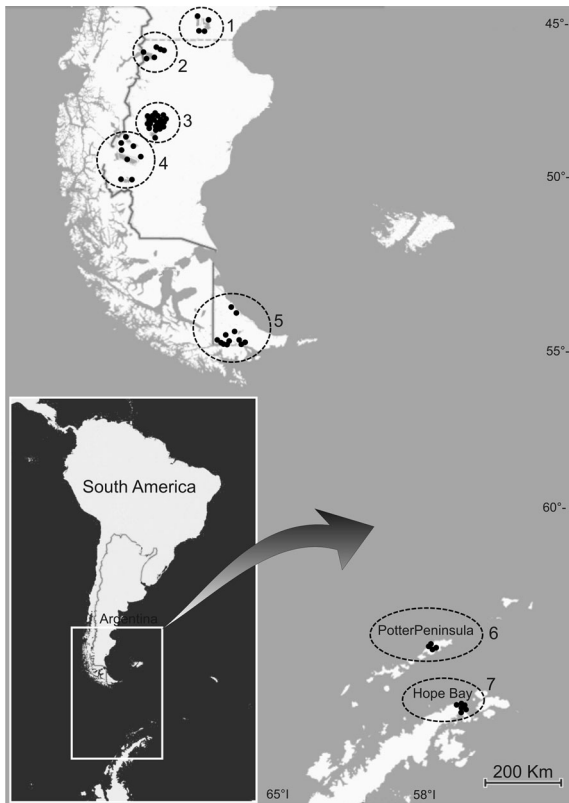


Fig. 1 Geographic location of the studied lakes across the Patagonian-Antarctic transect. 1 Steppe lakes from Chubut Province, 2 lakes from NW of Santa Cruz, 3 lakes from the Patagonian Strobel plateau, 4 lakes at the SW of Santa Cruz, 5 Tierra del Fuego lakes, 6 lakes from Potter Peninsula, King George Island, 7 lakes from Hope Bay, Antarctic Peninsula

temperature Pt catalyst oxidation method (Shimadzu analyser TOC-5000A), following the recommendations of Sharp et al. (1993).

Phytoplankton samples for quantitative analyses were fixed with 1% acidified Lugol's iodine solution. Phytoplankton counts were performed using a CKX41 Olympus inverted microscope, and the counting error was estimated according to Venrick (1978). Biovolumes were calculated using appropriate geometric formulae (Hillebrand et al., 1999; Sun & Liu, 2003). On the other hand, the species recorded in the samples were classified into functional groups using the classification of Reynolds et al. (2002), updated by Padisák et al. (2009). We also discriminated the phytoplankton species in relation to their main trophic strategies, between mostly autotrophic species (those that are not able to ingest preys) and mixotrophic species, including in this group only species that are potentially

phagotrophic, according to Flynn et al. (2013); for the classification of the taxa into these two groups we consulted several published papers (e.g. Jones, 2000; Urabe et al., 2000; Boëchat et al., 2007; Unrein et al., 2007; Raven et al., 2009; Unrein et al., 2010; Izaguirre et al., 2012; Unrein et al., 2014) on mixotrophy and also we performed ingestion experiments in different lakes (Saad et al., in preparation).

Molecular analyses

In each lake, we also collected water samples for molecular analyses of the plankton fractions. We aimed to compare the latitudinal patterns of richness corresponding to the morphologically based species determinations with that of the molecular diversity.

Depending on the trophic state of the lake, between 500 and 2000 ml were sequentially filtered through 20, 3 and 0.2 μm pore size polycarbonate filters, which were preserved in cryovials with lysis buffer at -80°C until DNA extraction. For the analysis of the nanoplankton fraction, we used the 3 μm filters. The extracted DNA for each lake was used as a template for PCR amplification of eukaryotic 18S rRNA genes. Then, with the product of the PCR, we applied the fingerprinting analysis DGGE (denaturing gradient gel electrophoresis). This method is usually used to retrieve the dominant organisms in each lake (e.g. Schiaffino et al., 2011). The analysis was carried out with a DGGE-2000 system (CBS Scientific Company). Digitised DGGE images were analysed using the GEL-PRO 4.0 software (USA). DGGE bands were excised from the gel and sequenced. The sequences obtained (around 500 bp) were screened for chimaeras, and then compared with public DNA databases using BLAST. The detailed procedures of DNA extraction, PCA amplification, DGGE analyses and band sequencing were described in previous papers (Unrein et al., 2005; Schiaffino et al., 2011).

Numerical analyses

For each individual lake, we obtained the local phytoplankton species richness (LSR) as a surrogate of diversity, and for the whole data set we analysed its latitudinal variation from Patagonia to Antarctica by means of simple regression. We also used the Fisher transformed correlation coefficient r_z (Rosenberg et al., 2000) to analyse the relationship between

species richness and latitude; the gradient strength is reflected by r_z , whereas the gradient steepness is the slope of the linear regression of diversity on latitude (Hillebrand, 2004).

We analysed the regional species richness (RSR) along the latitudinal gradient. The regional scale was defined for latitudinal grids of approximately $1^\circ \times 1^\circ$. Beta diversity, a measure of the change in species composition between samples along transects or across environmental gradients (Magurran, 2008), was obtained as the ratio between RSR and LSR (Soininen, 2010). On the other hand, we calculated the Shannon–Weaver diversity index (H) and the Pielou's evenness for each lake (Jost, 2010). We calculated initial similarity (similarity at one km distance) according to Soininen et al. (2007a). We analysed the similarity decay in phytoplankton composition with distance for the two main regions of the latitudinal gradient: Patagonia and Antarctica. Similarity was measured using Sorensen index; we calculated the halving distance, which is the distance that halves the similarity from its value at one km distance (Soininen et al., 2007a).

To analyse the prevailing trophic strategists of the phytoplankton species in the studied gradient, we performed simple regression of ratios between the abundances of strictly autotrophs and mixotrophs with Chl *a* (as an indicator of lake trophic status).

We applied different multivariate analyses such as canonical correspondence analysis (CCA) or redundancy analysis (RDA) to explore the controlling factors of phytoplankton composition in the lakes. The choice of CCA or RDA in each case was based in a previous detrended correspondence analysis (DCA): when the response of the biological data was unimodal we used CCA, and when it was lineal we used RDA (Ter Braak, 1994).

For the whole data set of lakes, we first performed a CCA based on the species abundances and the environmental variables. On the other hand, we applied RDA to estimate how much variation in the abundance of the phytoplankton functional groups (sensu Reynolds et al., 2002) was explained by the environmental variables. Calculations were performed with the software CANOCO (Ter Braak, 1991). The statistical significance of the first axis and of all the axes was tested by a Monte Carlo permutation test, and the importance of each variable was assessed by forward selection. For both multivariate analyses,

species whose contribution was less of 10% of the total phytoplankton abundance in all lakes were excluded.

On the other hand, to analyse the relative importance of environmental factors and spatial factors (geographic coordinates) we employed a canonical partitioning procedure (Borcard et al., 1992), both for species and functional groups approaches. The whole variation of the phytoplankton matrix was partitioned as: non-spatial environmental variation (environment alone), spatial variation that is not shared by the environmental variables (space alone), spatially structured environmental variation (environment + space) and unexplained variation and stochastic fluctuations. The significance of these components was evaluated with a Monte Carlo permutation test. We used partial CCA since the response was unimodal, and calculations were performed with the software CANOCO (Ter Braak, 1991).

Diversity analysis using molecular data

A matrix was constructed taking into account the presence or absence of individual bands of DGGE in all lanes of the gel, and the relative intensity of each band compared to the total band intensity in the lane. We analysed the latitudinal variation in the number of Operational Taxonomic Units (OTUs) along the gradient, which is assumed as the dominant eukaryotic nanoplankton richness. The correlation between band richness and latitude was also analysed using the Fisher transformed correlation coefficient (r_z).

This matrix of the DGGE bands was tested against a matrix of the environmental and spatial data using CCA, following the same procedure previously described.

Also for this approach, a canonical partitioning procedure was performed to disentangle the effects of the environment and space.

Results

Environmental variables

From West to East of Patagonia, lakes exhibit a gradient in their trophic states. Near the Andes, deep lakes are oligotrophic (mean values: Chl *a* $0.29 \mu\text{g l}^{-1}$; P-PO4 0.09 mg l^{-1} ; DIN 0.09 mg l^{-1}), and have low K_d (average 0.70 m^{-1}), and low conductivity (average

100.3 $\mu\text{S cm}^{-1}$). In this zone, we also sampled some shallow lakes that exhibit mesotrophic conditions (mean Chl *a* 2.67 $\mu\text{g l}^{-1}$ and mean K_d 1.48 m^{-1}). Towards the East, steppe lakes (Patagonian Plateau) represent the most eutrophic lakes of the dataset, with the highest mean Chl *a* values (12.41 $\mu\text{g l}^{-1}$), dissolved nutrients (P- PO_4 1.56 mg l^{-1} ; DIN 0.14 mg l^{-1}) and DOC (37.34 mg l^{-1}); some of them are also characterized by high conductivities. In insular Patagonia (Tierra del Fuego), besides deep oligotrophic lakes and eutrophic shallow lakes, we also sampled humic lakes and ponds that present oligotrophic conditions (mean Chl *a* 0.37 $\mu\text{g l}^{-1}$) but are characterized by brown waters due to the high DOC contents of allochthonous origin (4.71 mg l^{-1}). Finally, the studied Antarctic lakes are shallow and most of them range from oligotrophic to mesotrophic (mean Chl *a* 2.89 $\mu\text{g l}^{-1}$); however, some particular lakes exhibit a natural eutrophication due to the input of nutrients derived from penguin rookeries. Ranges of the physical and chemical variables and mean values for the different regions are summarised in Table 2.

Diversity analyses based on morphological determinations

Species diversity

A total of 321 phytoplankton species were identified from the microscopical examinations for the whole set of lakes from Austral Patagonia and Antarctica (see phytoplankton composition in Online Resource 1). The latitudinal analysis of the diversity (Fig. 2a, b) showed that both local and regional phytoplankton species richness showed a significant decreasing pattern with increasing latitude, but the regional decline was stronger than local gradient (RSR: $r^2 = 0.188$, slope = -0.611 , $P = 0.001$; LSR: $r^2 = 0.487$, slope = -0.152 , $P = 0.036$). Contrarily, no clear latitudinal trend was observed for evenness. The gradient strength reflected by the transformed correlation coefficient between local species richness and latitude was significant ($r_z = -0.54$; $P < 0.05$).

Comparing the two main regions of the studied gradient (Patagonia and Antarctica), we found that the initial similarity was higher in Antarctica (0.59) than in Patagonia (0.28). Oppositely, the halving distance was lower for Antarctic lakes (129.7 km) than for the Patagonian lakes (156.6 km). The similarity decay for

both regions is shown in Fig. 3a,b. The regression parameters are: $r^2 = 0.22$, slope = -0.0012 ; $P = 0.046$ (Patagonia); $r^2 = 0.91$, slope = 0.0023 , $P < 0.0001$ (Antarctica).

On the other hand, we found that the proportion of phytoplankton taxa with different trophic strategies was linked to lake trophic status. As we mentioned, our study covered a wide range of trophic states, and we found that the strictly autotrophs/mixotrophs ratio significantly increased towards higher lake trophic states ($r^2 = 0.322$; $P < 0.0001$) (Fig. 4).

The biplot (samples and environmental variables) corresponding to the CCA based on phytoplankton abundance with respect to the environmental variables is shown in Fig. 5a. The first two axes accounted for 49.8% of the total variance (axis 1: 31.7%; axis 2: 18.1%). The first axis was mainly correlated with temperature and pH (intra-set correlation coefficients: -0.90 and -0.87 , respectively). The high contribution of temperature in the analysis is directly associated to the latitudinal gradient, since both variables are strongly correlated. The second axis was mainly defined by conductivity (intra-set correlation coefficient: 0.83). Monte Carlo tests were significant for the first canonical axis ($P = 0.004$) and for all canonical axes ($P = 0.008$). This analysis showed an ordination of the lakes according to their geographic position and the trophic status. The Antarctic lakes (white circles) are ordinated at the right part of the figure, towards higher concentrations of DO and lower temperatures and pH values. Most of the oligotrophic Patagonian systems, including large deep lakes and humic lakes are placed at an intermediate position of this gradient (grey circles). On the other hand, most of the shallow mesotrophic and eutrophic lakes from the Patagonian steppe (black circles) are ordinated at the left part of the figure (higher temperature and pH values), or at the upper part of the graph (higher conductivity).

In addition, we performed the same analysis, but including the geographic coordinates, latitude and longitude, and excluding temperature because this is highly correlated with latitude (figure not shown). In this case, the two first axes explained 44.2% of the variance (axis 1: 28.2%; axis 2: 16.0%), and also Monte Carlo tests were significant for the first canonical axis ($P = 0.004$) and for all canonical axes ($P = 0.006$). This analysis evidenced that the first axis was mainly correlated with the geographic coordinates; the intra-set correlation coefficients were 0.85

Table 2 Mean values and ranges of the physical and chemical variables measured in the different groups of lakes for the period of the study

	Deep Andean lakes	Shallow Andean lakes	Patagonian Plateau/steppe lakes	Humic lakes and ponds Tierra del Fuego	Antarctic lakes
Temperature (°C)	7.68 4.50–10.0	11.38 6.9–15.6	15.42 10.0–27.0	8.64 6.1–13.4	2.07 0–8.40
pH	7.34 6.70–8.0	7.36 6.27–8.2	8.46 7.84–9.38	7.37 6.5–8.14	6.73 6.15–8.0
Conductivity ($\mu\text{S cm}^{-1}$)	100.28 20–210	212.4 50–610	2143.10 78.80–25800	343.94 54.90–1284	257.07 19.30–1860
Dissolved oxygen (mg l^{-1})	11.12 9.30–11.90	10.53 8.1–12.67	10.36 8.10–14.90	9.99 7.7–10.97	15.05 5.19 to >16
Chlorophyll <i>a</i> ($\mu\text{g l}^{-1}$)	0.29 0.10–1.02	2.67 0–11.32	12.41 0.11–56.60	0.372 0.29–0.51	2.89 0.22–17.49
DIN (mg l^{-1})	0.09 0.04–0.17	0.11 0.03–0.17	0.14 0.02–0.71	0.0784 0.041–0.13	1.75 0.015–16.80
P-PO ₄ (mg l^{-1})	0.09 0.02–0.25	0.30 0.02–0.96	1.56 0.05–15.80	0.058 0.03–0.08	1.56 0.01–15.00
DOC (mg l^{-1})	6.16 4.00–9.30	8.3 6.1–11	37.34 5.40–211.60	14.32 6.9–24.8	4.71 0.38–41.90
K _d (m^{-1})	0.70 0.09–1.61	1.48 0.49–3.06	3.53 0.00–28.52	2.20 0.79–3.29	0.59 0.13–1.24

DIN dissolved inorganic nitrogen (nitrates + nitrites + ammonium). *DOC* dissolved organic carbon, *K_d* vertical PAR attenuation coefficient

for latitude and -0.85 for longitude. The ordination of the lakes was very similar to that obtained with temperature and without geographic coordinates. It is clear that the temperature is the most important variable involved in the gradient of the latitude, whereas trophic status of the studied lakes are more related with longitude.

According to the results of the partial CCA, both spatial and environmental factors exerted influence on the phytoplankton composition. The overall explained variation in this analysis was 30.7% (environment alone: 23.4%; space alone: 4.5%; environment + space: 2.8%), thus a high percentage corresponded to unexplained variation and stochastic fluctuations. Monte Carlo test was significant for the first axis (environment alone: $P = 0.03$; space alone: $P = 0.012$).

Phytoplankton functional groups diversity

The phytoplankton functional groups observed in the different groups of lakes from Patagonia and Antarctica are listed in Online Resource 2. From the 40

functional groups described by Reynolds et al. (2002) and Padisák et al. (2009), 26 were registered in the studied lakes. Patagonian steppe lakes showed the highest richness in functional groups (25) and the Antarctic lakes the lowest one (15). The RDA based on the abundance of the phytoplankton functional groups and the environmental variables also showed an ordination of the lakes in relation to their geographic position along the gradient and their trophic status (Fig. 5b). In this analysis, the two first axes accounted for 82.6% of the variance (axis 1: 58.6%; axis 2: 24%). Monte Carlo tests were significant for the first canonical axis ($P = 0.002$) and for all the canonical axes ($P = 0.002$). The first axis was inversely correlated with temperature and pH, and directly with DO (intra-set correlation coefficients: -0.59 ; -0.57 ; 0.41 , respectively), whereas the second axis was mainly defined by DIN (intra-set correlation coefficient: 0.60). Antarctic lakes (white circles) are ordered towards the right side of the figure, at higher DO values, and lower temperatures and pH. At an intermediate position are placed most of the

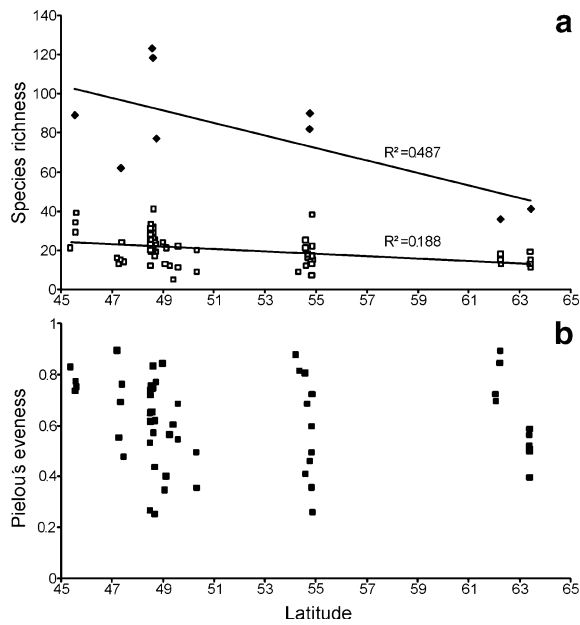


Fig. 2 **a** Latitudinal variation of regional species richness (RSR) and local species richness (LSR) along the studied gradient. LSR corresponds to individual lakes. See text for details about the regions considered in RSR. **b** Latitudinal variation in Pielou's evenness along the gradient

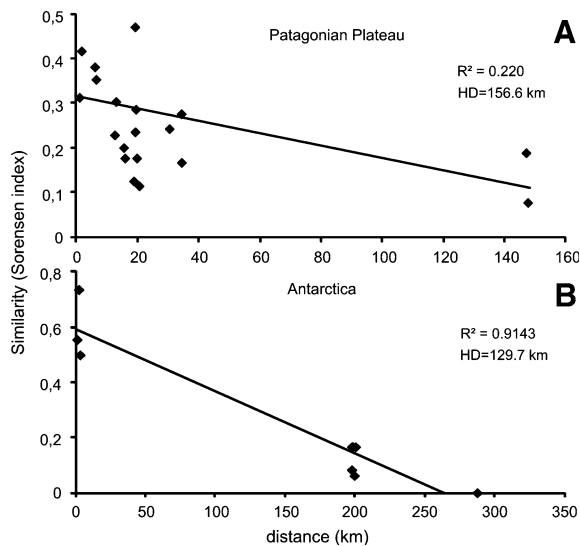


Fig. 3 Distance decay of similarity for the two main regions of the gradient of lakes: **a** Patagonian Plateau, **b** Antarctica. *HD* halving distance

Patagonian oligotrophic water bodies, including Andean deep lakes and humic environments from Tierra del Fuego (grey circles). At the opposite side are ordered most lakes of the Patagonian steppe (black

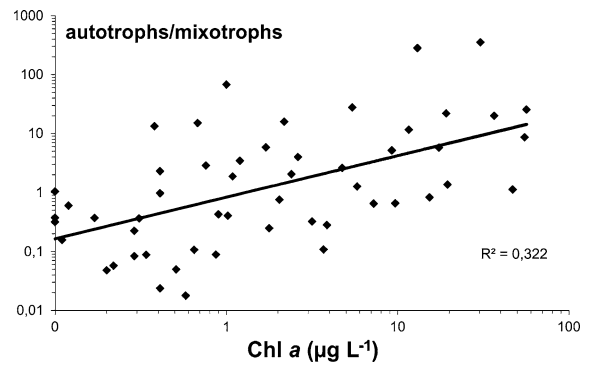


Fig. 4 Simple regression of the ratio between the species abundance classified according to their trophic strategies with chlorophyll *a*. *Autotrophs* phytoplankton taxa that are not capable of phagotrophic behaviour; *mixotrophs* phytoplankton taxa that are able to ingest preys

circles), towards higher temperatures and pH values; moreover, some particular steppe lakes are placed in the lower part of the figure at higher conductivities.

For the functional groups, the results of the partial CCA also evidenced that spatial and local factors are important in shaping the phytoplankton structure. The overall explained variation in this analysis was 46.7% (environment alone: 28.5%; space alone: 5.9%; environment + space: 12.3%). Monte Carlo test was significant for the first axis (environment alone: $P = 0.034$; space alone: $P = 0.022$).

Diversity analyses based on molecular approach

The number of DGGE bands per sample ranged from 4 to 40 for the whole set of lakes. Analysis of the gels gave a total of 1072 bands, with 82 different DGGE positions for the Patagonian lakes and 47 for the Antarctic lakes (Unrein et al., 2005). These OTUs would represent the total dominant eukaryotic plankton richness in the studied lakes. From the total bands obtained for both regions, 32 different positions were successfully sequenced for Patagonian lakes, and 18 for Antarctica (Unrein et al., 2005).

From these molecular analyses, we also found a significant decreasing pattern in the dominant eukaryotic plankton richness with increasing latitude, with $r^2 = 0.385$, slope = -0.92 , $P < 0.0001$ (Fig. 6). In this case, the standardized correlation coefficient between band richness and latitude was $r_z = -0.73$ ($P < 0.05$).

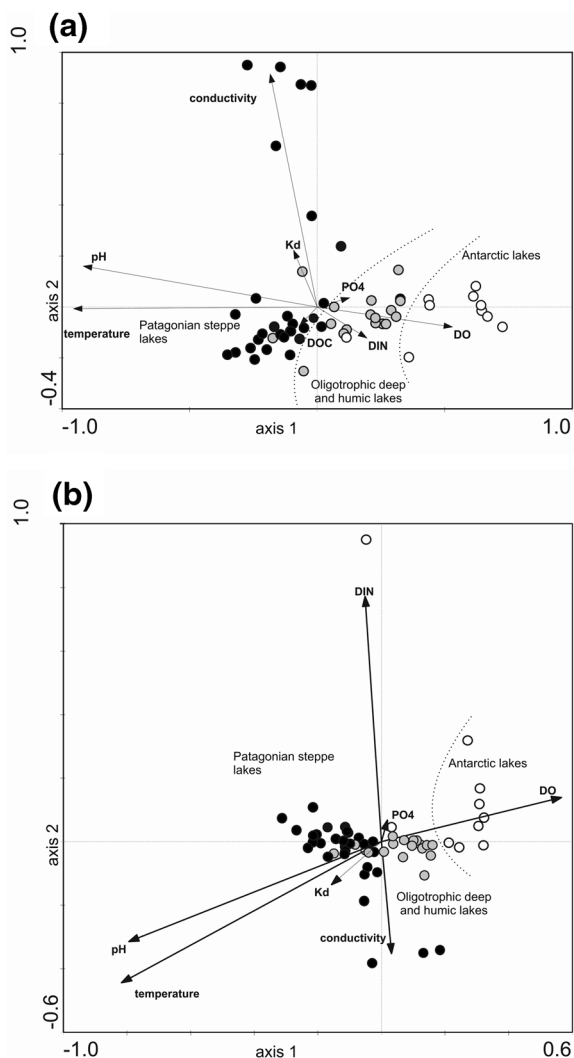


Fig. 5 **a** Biplot (samples and environmental variables) of the CCA based on the phytoplankton species abundance. **b** Biplot (samples and environmental variables) of the RDA based on the abundance of the phytoplankton functional groups. Patagonian steppe lakes (*black circles*); Oligotrophic deep and humic lakes (*grey circles*); Antarctic lakes (*white circles*). *DOC* dissolved organic carbon, *DIN* dissolved inorganic nitrogen, *DO* dissolved oxygen, *PO4* soluble reactive phosphorus, *Kd* PAR vertical light attenuation coefficient

The multivariate analysis CCA based on the molecular diversity gave similar results to those obtained with the biodiversity based on microscopic examinations (figure not shown). Also in this case, both environmental factors as well as geographic position of the lakes were important in shaping the eukaryotic molecular diversity. In the CCA using the DGGE band intensity profiles and both environmental

and spatial variables, the first two axes explained a 62.2% of the total variance (axis 1: 32.7%, axis 2: 29.5%). The environmental variables were significantly correlated with the first axis ($P = 0.046$), and the test of significance of all canonical axes was also significant ($P = 0.006$). The first axis is mainly defined by phosphate and latitude (intraset correlation coefficients: 0.91 and -0.40 , respectively); the second axis is mainly correlated with latitude and longitude (intraset correlation coefficients: -0.46 and -0.25 , respectively).

The canonical variation partitioning performed to discriminate the relative importance of environmental and spatial factors showed that both exerted influence on molecular biodiversity. The relative importance of the spatial components was 11.7%, while that for environmental factors, was 12.0% (Monte Carlo permutation test $P < 0.05$), and for spatial component of environmental influences, it was 0.6% (environment + space).

Discussion

The results of our study evidenced clear geographic patterns in the phytoplankton diversity across the studied gradient of lakes, as well as the influence of the environmental and biological factors on the community structure. As it was expected (Hypothesis 1), we found a decreasing trend of species richness with increasing latitude (from Patagonia to Antarctica), in accordance with the most generalized pattern described for different groups of organisms (e.g. Willig et al., 2003; Hillebrand, 2004). This tendency was also found for particular algal groups in previous analyses

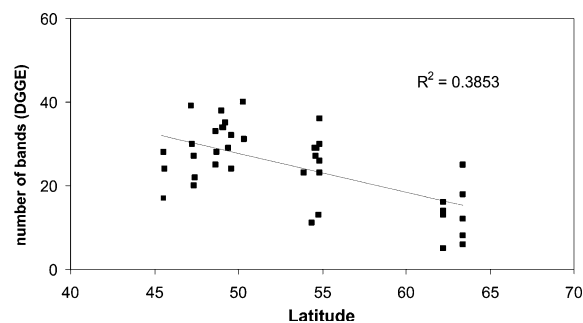


Fig. 6 Variation in the dominant eukaryotic nanoplankton OTUs (number of bands in the denaturing gradient gel electrophoresis: DGGE) across the latitudinal gradient of lakes

of the same region: a decline in diatom species richness from Patagonia to Antarctica was reported in the analysis of Maidana et al. (2005), and an inverse correlation between the species richness of chlorococcaleans and latitude was also observed in a gradient of Patagonian lakes (Tell et al., 2011).

Contrarily, our results did not evidence any clear geographic pattern in relation to evenness. In a meta-analysis of a large number of aquatic data sets, Soininen et al. (2012) observed that in a great number of cases species richness was not correlated with evenness. They argued that richness and evenness may respond to different environmental factors, or may respond in a different way to a given factor, thus reflecting independent components of biodiversity. As an example, Hillebrand et al. (2007) demonstrated that in many aquatic communities species evenness decreases with fertilization, while richness shows the opposite tendency. In our study, the heterogeneity in the trophic states of the selected lakes can be masking the latitudinal pattern in evenness. We also found that the number of bands per lake obtained by the molecular analysis DGGE (assumed as dominant planktonic eukaryotes), decreased with increasing latitude. Interestingly, the slope of this regression was higher than the slope of the regression of the species richness on latitude based on the microscopical analyses for the whole phytoplankton community. Although the molecular technique employed does not allow obtaining the real diversity, because only the most abundant taxa are generally retrieved by this analysis, the results suggest that the gradient steepness in richness may be higher than that obtained from studies based purely on morphology. Further analyses based on massive sequencing are necessary to confirm this hypothesis, and in this sense our recent molecular studies using Illumina are revealing a great number of eukaryotic taxa along this latitudinal gradient, and the presence of some sequences exclusive of some lakes of the region, which are probably endemic (Lara et al., in preparation). The importance of the molecular tools in biogeographic studies of protists was stressed by different authors (e.g. Coesel & Krienitz, 2008; Weisse, 2008). In addition, some more recent studies have supplemented the traditional community descriptor species richness by the functional diversity, which is regarded as a key in understanding the link between ecosystem function and biodiversity (Schmera et al., 2009), and different

measures of functional diversity have been proposed (e.g. Petchey et al., 2004; Petchey & Gaston, 2007). In this sense, we emphasise the importance of using polyphasic approaches to analyse biogeographic patterns in microorganisms.

Regarding the variation in RSR, our results evidenced that the gradient on regional scale was stronger and steeper than on local scales, in accordance with the pattern described by other authors (Hillebrand, 2004; Soininen, 2010). Examining separately two parts of the spatial gradient (Patagonia versus Antarctica), we found that the initial similarity was higher in Antarctica than in Patagonia, whereas the halving distance was lower for Antarctic lakes than for the Patagonian lakes. The separation of these two zones in this analysis is justified by the great geographic discontinuity between Patagonia and Antarctica due to the circumpolar waters. Our results fit in the trends found by Soininen et al. (2007a), since the initial similarity was higher at high latitudes, and on the other hand, the similarity decreased faster at higher latitude.

The multivariate analyses revealed that the phytoplankton structure is affected by both spatial and environmental factors, which confirms our second hypothesis. The CCA or RDA performed, based on morphological and molecular analyses of biodiversity (species composition, functional groups and band patterns obtained by DGGE), showed a high importance of the spatial factors (mainly latitude) and variables related with lake trophic status in the differences among lakes. In the studied gradient, temperature is inversely correlated with latitude, whereas variables associated with trophic status of the lakes (nutrients, pH, conductivity and K_d) are associated in some degree with longitude. As it was described, most Andean lakes are deep and oligotrophic, whereas steppe lakes are shallower and range from mesotrophic to eutrophic. Moreover, the partitioning variance analyses allowed the separation of the effects of space and environment, evidencing a higher influence of the latter on the phytoplankton structure, but also an effect of the geographic factors. Nevertheless, a great percentage corresponded to unexplained and stochastic variations. For the same set of lakes, previous studies that involved an analysis of the bacterioplankton diversity gave similar results (Schiaffino et al., 2011).

The relative importance of historical (past environmental conditions and dispersal limitation) versus

environmental factors (contemporary conditions) seems to be related to the sampling scale (Martiny et al., 2006). At a very wide spatial scale (e.g. ten of thousands of kilometres), the influence of the environmental factors would be relatively less important than the distance indeed. At small scales (few kilometres) in general, there is no effect of the distance but the environmental conditions are more relevant. At intermediate scales (up to 3000 km), as that of our analysis, both historical contingencies, as well as ecological contemporary factors may be important (Martiny et al., 2006 and cites therein). Studies on phytoplankton biodiversity conducted in other regions based on partial analysis also showed differing results depending on the scale. At small scales (<350 km) contemporary environmental conditions were found to be the only predictor of phytoplankton composition (e.g. Vanormelingen et al., 2008; Mazaris et al., 2010), whereas at larger scales was the combined effect of environment and space, although the local conditions were more relevant (e.g. Soininen et al., 2007b). Nevertheless, other studies (e.g. Beisner et al., 2006; Nabout et al., 2009) also reported that neither environment nor space were significant in shaping phytoplankton structure. Although the evidences are controversial, analyzing the background on the subject, it seems that microbial communities are more strongly spatial structured at larger study scales (Soininen, 2012 and cites therein).

For the trophic gradient of the studied lakes, we also found that the proportion of phytoplankton taxa potentially phagotrophic (namely, mixotrophic species) were more abundant in oligotrophic lakes than in mesotrophic and eutrophic ones. Thus, we observed an increasing trend of the strict autotrophs/mixotrophs ratio towards higher Chl *a* values, supporting the third hypothesis of our work. This tendency was also observed for a set of lakes at a narrower extent, which included only lakes from Tierra del Fuego (Saad et al., 2013).

In summary, our meta-analysis showed a latitudinal decline in phytoplankton biodiversity along the gradient of the analysed lakes, and the effect of the environmental factors in shaping the communities. The combination of approaches to analyse the phytoplankton biodiversity (species composition based on microscopy, dominant molecular OTUs and phytoplankton functional groups) gave more strength to the results obtained, since the three approaches showed

similar patterns. All the analyses evidenced that both geographic factors (mainly latitude) and environmental conditions in the individual lakes (mainly trophic status) influenced the phytoplankton diversity, although the local effect was stronger. Our results support the idea that at large spatial scales both effects are drivers of the community structure.

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