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# Microbial planktonic communities of freshwater environments from Tierra del Fuego: dominant trophic strategies in lakes with contrasting features

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We analysed the structure of the microbial plankton communities of different types of freshwater environments from the southernmost region of South America (Tierra del Fuego). Water bodies were grouped in four categories: humic lakes, clear oligotrophic lakes, beaver ponds and steppe shallow lakes, which differed in their nutrient and dissolved organic carbon (DOC) contents. We tested if microbial planktonic communities were different among lakes with dissimilar nutrient and DOC concentrations, analysing to what extent the known large-scale patterns of lake trophic structure applies to a diverse but localized set of lakes. We found that mixotrophs dominated over strict autotrophs in both humic and clear oligotrophic systems, whereas in eutrophic lakes autotrophy was a successful strategy. The functional phytoplankton approach also allowed the separation between oligotrophic (clear and humic) and eutrophic systems, with different functional groups. The lowest abundances of picoplankton were found in oligotrophic lakes, picoeukaryotes being more abundant than picocyanobacteria in beaver ponds and humic lakes. Our results show that in low nutrient environments, mixotrophic strategies thrive over strict autotrophs suggesting the paramount importance of the microbial loop when compared with high trophic status systems where the prevalence of autotrophy indicates that the energy flux depends on phytoplankton.

**KEYWORDS:** phytoplankton; microbial communities; lakes; trophic-DOC paradigm; Tierra del Fuego

## INTRODUCTION

The ratio of total heterotrophic to autotrophic biomass in freshwater plankton communities of temperate lakes has been found to decline along gradients of increasing phytoplankton biomass (e.g. [del Giorgio and Gasol, 1995](#)). The balance of phytoplankton production and community respiration is below unity in unproductive lakes, where heterotrophic biomass is high in relation to autotrophic biomass, suggesting that these planktonic food webs must be subsidized by allochthonous organic matter ([del Giorgio et al., 1999](#)). On the other hand, the possible interaction between the concentration of dissolved organic carbon (DOC) and phosphorus or chlorophyll as determinants of the main energy pathway along trophic gradients has been previously proposed by [del Giorgio and Peters \(del Giorgio and Peters, 1993\)](#).

[Jansson et al. \(Jansson et al., 2000\)](#) found differences between dark-coloured lakes where the food chain is based on bacterioplankton energy mobilization from allochthonous DOC, and moderately coloured lakes where total pelagic production is based mainly on phytoplankton photosynthesis. Further studies carried out by [Karlsson et al. \(Karlsson et al., 2002\)](#) in a wider range of systems indicated the similarity between humic and clear oligotrophic lakes, pointing to the fact that in these systems to a large extent carbon and energy sources other than phytoplankton production are utilized for secondary production within the pelagic systems. The importance of the DOC concentration in aquatic systems has been widely demonstrated (e.g. [Steinberg et al., 2006](#) and citations therein). A typical and useful way to classify lakes is by taking into account this parameter together with nutrient concentration (especially dissolved reactive phosphorus, DRP). Rodhe ([Rodhe, 1969](#)) was one of the first scientists who formalized this idea and proposed a classification by setting lakes among two gradients: a eutrophic one (nutrient concentration) and an allo-trophic one (DOC concentration). Later, [Williamson et al. \(Williamson et al., 1999\)](#) further contributed to this model, considering also DOC “quality” as a third axis in the analysis, in recognition of the strong variation of this property among lakes. Allochthonous DOC is generally composed of more recalcitrant and highly coloured humic and fulvic acids, while autochthonous DOC generated by phytoplankton or macrophytes is usually more labile and less coloured ([Jones, 1992](#); [Keskitalo and Eloranta, 1999](#); [Wetzel, 2001](#)).

Considering plankton trophic strategies, as well as theoretical ([Stoecker, 1998](#); [Jost et al., 2004](#); [Crane and Grover, 2010](#); [Ward et al., 2011](#)) and observational experimental studies ([Bergström et al., 2003](#); [Pålsson and Granéli, 2004](#); [Katchakis and Stibor, 2006](#); [Unrein et al., 2007](#)), strict autotrophy and heterotrophy seem to be suitable strategies in high nutrient environments, while mixotrophy would be a better one under low nutrient conditions. On the other hand, humic substances affect light penetration ([Kirk, 1994](#)), and consequently primary production and algal biomass will also be affected ([Jones, 1992](#); [Carpenter et al., 1998](#)).

As environmental conditions select groups of species that share similar adaptive characteristics, there is a broad consensus that communities are more reliable indicators of habitat conditions than are the presence or absence of component species ([Naselli-Flores and Barone, 2011](#) and citations therein). In this sense, the structure of communities may be analysed by determining their functional diversity (FD). In particular, [Weithoff \(Weithoff, 2003\)](#) proposed this concept for phytoplankton, where FD is related to the functional multiplicity within the community rather than the multiplicity of species; functional traits were defined by this author as a property of an organism that can be measured and that influence one or more essential processes such as growth, reproduction, etc. Different phytoplankton functional classifications were proposed which allow the comparison of phytoplankton assemblages from different types of lakes taking into account their sensibilities and tolerances to light and nutrient conditions among other variables (e.g. [Reynolds et al., 2002](#); [Salmaso and Padisák, 2007](#); [Kruk et al., 2010](#)). The dominant plankton trophic strategies above mentioned may be successfully analysed within this functional framework along environmental gradients at regional or local scales (e.g. [Tolotti et al., 2005](#); [Sarmento and Descy, 2008](#); [Izaguirre et al., 2012](#)).

The high diversity of water bodies present in Tierra del Fuego, which differ in their trophic status and concentration of humic substances, provides an excellent scenario for comparing the trophic strategies of the microbial communities prevailing under different nutrient and DOC conditions. Several floristic studies have been performed (e.g. [Tell and Mosto, 1982](#); [Tell et al., 2011](#)) where peat bogs have received comparatively more attention including both floristic and ecological studies (e.g. [Mataloni and Tell, 1996](#); [Mataloni, 1999](#); [González](#)

*Garraza et al.*, 2012). Regarding the overall limnological information of the lakes located in this region, Mariazzi *et al.* (Mariazzi *et al.*, 1987) carried out the first survey, and analysed 19 water bodies with different geological origin and characteristics. Recently, Conzonno and Ulibarrena (Conzonno and Ulibarrena, 2010) published a characterization based on the hydrochemical data obtained in that survey with further hydrochemistry analyses. According to these studies, the ponds located in the north Extra Andean region show high nutrient concentrations and a high salinity gradient. In the southern region near the Andes, the ponds are surrounded by peat material and are characterized by high concentration of humic substances, whereas large lakes are transparent, ultraoligotrophic and with soft waters. Thus, the available limnological data for this region indicate the occurrence of different water bodies with contrasting DOC concentrations and trophic status.

Our study constitutes the first characterization of the structure of microbial communities of the different freshwater environments on the island. We have surveyed water bodies with contrasting limnological characteristics in relation to their morphometry, DOC content and trophic status. We hypothesize that microbial planktonic communities will be different among geographically close lakes with dissimilar nutrient and DOC concentrations. We particularly analysed the differences in relation to the dominant phytoplankton functional groups and the proportion of protists with different trophic strategies: mixotrophs, strict autotrophs and heterotrophs. Finally, we hypothesize that the large-scale patterns of lake trophic structure found in temperate climate zones apply to a diverse but geographically close set of lakes in extreme Patagonia.

## METHOD

### Study area

Tierra del Fuego ( $52^{\circ}27' - 55^{\circ}03'S$ ;  $65^{\circ}06' - 70^{\circ}26'W$ ) is the southernmost region of the American continent. Geographically is considered an island as it is separated from the continent by the Magallanes Strait. Our study was focused in the Argentinean part of the island (Tierra del Fuego Province). Temperature in this region is lower than in Europe at the same latitude due to a colder oceanic current (in contrast with the warmer one in North Atlantic); as a result, vegetation resembles to that of boreal maritime places like the Scandinavian coast (Collantes and Faggi, 1999).

The north area of the island is characterized by a climax community of “coirón” (*Festuca gracillima*) steppe

that covers plateaus and low mountains, where the soil is mainly sandy-rocky; average annual temperature is about  $5^{\circ}\text{C}$  and precipitation between 100 and 270 mm. Small shallow lakes, most of them temporary, prevail in this area. Towards the south, there is an ecotonal area, where annual precipitation reaches about 2000 mm in the western portion, decreasing to 700–800 mm towards the east. The dominant trees, “ñire” (*Nothofagus antarctica*), “lenga” (*Nothofagus pumilio*) and a cypress (*Austrocedrus chilensis*), may form mixed or single species forests. In this zone, meadow wetlands and bogs are also present, and the climate is temperate and humid with an average annual temperature of about  $5.4^{\circ}\text{C}$ . In the South-eastern region of the island (Mitre Peninsula), the climate is much colder and drier, with a climax community dominated by “guindo” (*Nothofagus betuloides*). Bogs are common in this area with different plant associations. Finally, the south-western portion is a mountainous area, strongly influenced by glacial activity, with rocky or sandy immature soils and a characteristic mountain climate, with snowfall and hail at any moment of the year. Lakes with glacial or periglacial origin, and valleys lined with bogs where ponds are formed are encountered in this zone (Cabrera, 1971; Iturraspe and Urciuolo, 2000). In this survey, we have studied water bodies located in three of the cited regions, with the exception of the Mitre Peninsula since it is an inaccessible area by land.

### Sampling and physico-chemical analyses

We selected 12 water bodies of Tierra del Fuego with different limnological characteristics in order to cover the representative lentic freshwater ecosystems in the region: oligotrophic lakes (Escondido, Fagnano, Acigami, Yehuin), humic lakes (Laguna Negra, Laguna Victoria), steppe eutrophic shallow lakes (San Luis and Laguna de los Cisnes) and ponds affected by beavers (a, b, c, d; among which the last three are typically beaver ponds). The geographic position of the water bodies (Fig. 1) was recorded using a GPS (geographic positioning system; Garmin, KS, USA). Table I shows the available morphometric data of the lakes studied.

Samples were collected during three different sampling periods in most of the lakes: October 2008, January 2010 and April 2012 (spring, summer and autumn, respectively). These were taken from the euphotic zone at the first meter of the water column. Temperature, pH and conductivity were measured *in situ* with a Horiba D-54 meter (Horiba, Kyoto, Japan), and dissolved oxygen (DO) with HI 9146 Hanna portable meter (Hanna Instruments, 150 PD, Italy). Samples for nutrient, colour estimation and Chl *a* analyses were immediately filtered through Whatman® GF/F filters



**Fig. 1.** Location of the water bodies studied.

(Whatman, Maidstone, UK). Dissolved inorganic nitrogen (DIN = ammonium + nitrate + nitrite) and DRP concentrations were determined using a Hach<sup>TM</sup>DR/2800 spectrophotometer (Hach Company, CO, USA) and their corresponding reagent kits (detection limit for all nutrients  $0.01 \text{ mg L}^{-1}$ ) on GF/F filtrates. The absorption coefficient at 320 nm ( $a_{320}$ ), as a metric of coloured DOC in natural waters, was measured with a spectrophotometer. Concentrations of Chl *a*, corrected for phaeopigments, were determined by spectrophotometry before and after acidification (HCl 0.1 N). Extractions of pigments from the GF/F filters were carried out using hot ethanol (60–70°C) and overnight storage at 4°C. Calculation of Chl *a* was based on the equations published by Marker *et al.* (Marker *et al.*, 1980). Aliquots of filtered water of each lake were acidified and stored at 4°C for the analysis of DOC, which was determined with the high temperature Pt catalyst oxidation method (Shimadzu analyzer TOC-5000A, SM 5310B technique, detection limit:  $0.1 \text{ mg L}^{-1}$ ) following the recommendations of Sharp *et al.* (Sharp *et al.*, 1993). *In situ* underwater measurements of photosynthetically active radiation (PAR) were obtained using a LI-COR radiometer 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).

## Microbial community analyses

Quantitative phytoplankton samples were fixed with 1% acidified Lugol's iodine solution. Phytoplankton counts were performed using an Olympus CKX41 inverted microscope (Utermöhl, 1958) at  $\times 400$  magnification,

and the counting error was estimated according to Venrick (Venrick, 1978). In all cases, we considered the individual algae as the unit (unicell, colony, coenobium or filament), and we estimated cell numbers per colony or filament. Individual biovolumes were calculated using appropriate geometric formulae according to their shapes and the mean dimensions of the organisms in the samples (Hillebrand *et al.*, 1999; Sun and Liu, 2003). For colonial organisms, calculations were made for the whole colony including mucilage. Biomass was estimated from biovolume, assuming unit specific gravity. The phytoplankton species were classified into the functional groups proposed in the classifications of Reynolds *et al.* (Reynolds *et al.*, 2002), reviewed by Padisák *et al.* (Padisák *et al.*, 2009). Ciliates were also counted with the inverted microscope from samples fixed with 1% acidified Lugol's iodine solution, using 10 mL chambers.

Samples for epifluorescence microscopy were preserved by the addition of filtered (0.22 µm Millipore, MA, USA) cold glutaraldehyde 10% (1% final concentration). Fixed samples were filtered through a 0.2 µm pore-size polycarbonate black filter, and cells were stained with DAPI (4,6-diamidino-2-phenyl-indole;  $10 \mu\text{g mL}^{-1}$  final concentration), following the procedure outlined by Porter and Feig (Porter and Feig, 1980). The samples were stored at  $-20^{\circ}\text{C}$  until analysis in the laboratory. Total heterotrophic bacteria, picocyanobacteria (Pcy) and picoeukaryotes (Peuk) abundances were determined by epifluorescence using an Olympus microscope (Olympus BX40F4, Tokyo, Japan) at  $\times 1000$  magnification. Bacterioplankton counting was performed using UV excitation. Pcy and Peuk were counted using both blue and green-wavelength excitation. For the quantification of heterotrophic nanoflagellates (HNF), aliquots of the samples fixed with glutaraldehyde (1% final concentration) were filtered through polycarbonate filters of 0.8 µm pore size (Poretics), stained with DAPI and the counting was carried out using UV excitation.

## Statistical analyses

The similarity in phytoplankton structure among water bodies was first assessed by Cluster Analysis based on the species abundance, using the Bray–Curtis index and the unweighted pair-group mean average (UPGMA) algorithm with software SPSS 15.0.1 (StatSoft, Tulsa, OK, USA).

Redundancy analyses (RDA) were used to estimate how much variance of the abundance of the phytoplankton functional groups (Reynolds *et al.*, 2002, updated by Padisák *et al.*, 2009) was explained by the environmental variables. Previously, we performed a detrended correspondence analyses, and as the data showed a linear

**Table I:** Type, geographic position and main morphometric features ( $z_{mean}$ : mean depth,  $z_{max}$ : maximum depth) of the water bodies studied, and ranges with mean values (between brackets) of the physical and chemical variables measured in each system. DIN, dissolved inorganic nitrogen; DRP, dissolved reactive phosphorus; DOC, dissolved organic carbon;  $K_d$ : vertical PAR extinction coefficient (PAR),  $\diamond$  sampled in one occasion.

Type	Victoria Humic	Negra Humic	Escondido Oligo	Fagnano Oligo	Acigami (Roca) Oligo	Yehuin Oligo	Pond a Beaver	Pond b Beaver	Pond c Beaver	Pond d Beaver	San Luis Steppe	Cisnes Steppe
Latitude (°S)	54.78	54.84	54.68	54.59	54.83	54.36	54.85	54.87	54.60	54.85	53.79	53.92
Longitude (°W)	67.70	68.59	67.81	67.62	68.56	67.78	68.58	67.35	67.63	67.5	67.78	67.60
Altitude (m a.s.l.)	103	29	120	27	20	50	15	11	43	12	8	10
Area (km <sup>2</sup> )	0.09	0.14	6.05	646.69	19.20	42.48	0.001	0.14	0.0003	0.04	3.12	3.75
$Z_{mean}$ (m)	—	—	38.0	80.7	22.4	42.7	<1	<1	<1	<1	—	1.1
Temperature (°C)	4.9–13.5 (8.5)	6.1–15.9 (9.8)	4.5–10.7 (7.7)	6.7–9.7 (8.4)	4.5–13.3 (8.7)	8.0	13.4	8.2	8.3–14.8 (10.6)	6.9–14.4 (10.6)	10.7	12.7
pH	6.4–7.4 (6.8)	6.0–6.9 (6.3)	6.0–7.4 (6.7)	6.2–7.5 (6.9)	6.9–7.6 (7.3)	8.0	8.2	7.9	6.5–8.5 (7.3)	6.3–8.6 (7.5)	8.1	9.0
Conductivity (µS cm <sup>-1</sup> )	34.9–55.6 (48.5)	65.5–77.1 (69.5)	69.5–104.6 (92.4)	59.4–87.5 (77.2)	61.1–79.4 (72.7)	197.0	1284.0	110.3	138–205 (161.5)	181–272 (226.5)	313	25 800
Dissolved oxygen (mg L <sup>-1</sup> )	9.7–12.1 (10.9)	8.9–11.5 (10.17)	9.8–11.5 (10.8)	10.3–11.7 (11.2)	8.2–11.2 (10.0)	11.5	10.5	10.7	7.7–15.5 (12.0)	12.7–13.6 (13.1)	10.7	12.0
Chlorophyll <i>a</i> (µg L <sup>-1</sup> )	0.1–1.6 (0.7)	0.1–2.2 (0.9)	0.1–1.5 (0.6)	0.1–1.1 (0.6)	0.1–4.0 (2.0)	0.1	0.3	0.3	0.3–9.3 (4.8)	11.3–33.5 (22.4)	0.7	0.4
DIN (mM)	0–4.14 (2.09)	0.71–9.29 (4.05)	0.71–5.07 (2.40)	1.43–5.14 (2.90)	1.43–5.36 (3.39)	9.78	5.64	2.93	0–6 (2.24)	0.71–2.14 (1.43)	5.79	9.57
DRP (mM)	0–2.90 (1.29)	0.32–2.26 (1.29)	1.29–1.61 (1.40)	0.65–1.61 (1.07)	0.32–3.22 (1.77)	1.29	0.97	2.58	0.32–2.58 (1.51)	0.64–1.29 (0.97)	5.48	12.90
DOC (mg L <sup>-1</sup> )	6.4–10.8 (8.6)	8.1–12.0 (10.0)	2.3–7.1 (4.7)	2.6–5.6 (4.1)	2.1–5.1 (3.6)	9.3	6.9	17.1	11.3–24.8 (18.0)	9.1	17.8	50.2
$K_d$ (m <sup>-1</sup> )	1.8–2.8 (2.3)	0.8–1.0 (0.8)	0.6–0.7 (0.7)	0.7	1.5	9.4	2.2	3.0	3.3	0.7	2.1	2.7

response we applied RDA. Calculations were performed with the program CANOCO (ter Braak, 1988). The analysis was based on the abundance of the functional groups and the environmental variables corresponding to each lake and date. The statistical significance of the first axis and of all the axes was tested by a Monte Carlo permutation test. The importance of each variable was assessed using forward selection. In order to compare the multivariate analysis based on phytoplankton functional groups with an analysis done using the phytoplankton species data set, we performed another RDA analysis based on the species abundance matrix, excluding those species with a contribution of less than 10% of the total community abundance in any individual lake.

We also performed simple regressions of ratios between abundances of nano- and microplankton classified by their trophic strategies (mixotrophs, strict autotrophs, strict heterotrophs) with Chl *a* as an indicator of lake trophic status. In order to compare the trends found for the water bodies of Tierra del Fuego with those observed in lakes encompassing a larger geographical area, we included an additional analysis performed with data obtained in freshwater environments across a transect of more than 2100 km stretching from Argentinean Patagonia (45°S) to Maritime Antarctica (63°S) (Izaguirre *et al.*, unpublished data). The significance of the regressions was tested applying functional regression model II by means of variance analysis (Zar, 2010).

## RESULTS

The water bodies surveyed presented a wide range of variation in their physicochemical variables (Table 1). Water temperature was generally lower in large oligotrophic lakes, with lower variation among sampling dates. The maximum value was registered in Laguna Negra lake in January 2010 (15.9°C) and the minimum in Escondido and Acigami lakes in October 2008 (4.5°C). DO concentrations were relatively high in all water bodies, mean values varying from 10 to 13.1 mg L<sup>-1</sup>; extreme concentrations were registered in the beaver ponds due to their small size and shallowness.

Conductivity and pH values were lower in humic lakes (mean 48.5–69.5 µS cm<sup>-1</sup> and 6.3–6.8, respectively). The low pH values registered in humic lakes are related to the high influence of the moss *Sphagnum* sp. lining the bottom and littoral areas of these lakes. The highest values of conductivity and pH were recorded in the steppe shallow lakes, particularly at Laguna de los Cisnes, which showed extreme values (25 800 µScm<sup>-1</sup> and pH = 9) probably due to the high marine influence caused by its location relatively close to the sea-shore.

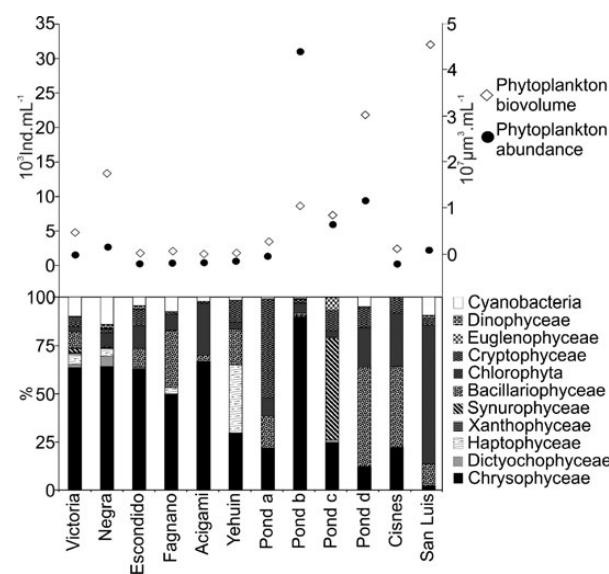
Nutrient concentrations were in general very low, and in some cases values were below the detection limit. The lowest values were found in deep oligotrophic lakes, with mean concentrations of DRP ranging from 0.97 to 1.29 mM, and DIN from 2.14 to 10 mM. Higher values were found in steppe lakes, with DRP from 5.48 to 12.9 mM and DIN from 5.71 to 9.28 mM.

Chlorophyll *a* (Chl *a*) concentrations showed the lowest values in oligotrophic and humic lakes (mean 0.83 and 0.82 µg L<sup>-1</sup>), whereas the highest ones were recorded in beaver ponds (mean 10.96 µL<sup>-1</sup>). DOC concentrations were higher in steppe lakes (mean 34 mg L<sup>-1</sup>), followed by beaver ponds (mean 15.6 mg L<sup>-1</sup>) and humic lakes (mean 9.3 mg L<sup>-1</sup>); oligotrophic lakes showed the lowest concentrations (mean 5.5 mg L<sup>-1</sup>). Large oligotrophic lakes were the least coloured (0.5–1.6 m<sup>-1</sup>), humic lakes showed high colour values (mean 7.4 m<sup>-1</sup>) and the ponds had a high range of variation (4.15–11.63 m<sup>-1</sup>).

## Microbial community composition

### Phytoplankton

The lowest phytoplankton abundances and biovolumes were registered in deep oligotrophic lakes (mean values: 392 ind. mL<sup>-1</sup> and 3.4 × 10<sup>5</sup> µm<sup>3</sup> mL<sup>-1</sup>, density and biovolume, respectively), whereas the highest mean values were found in beaver ponds (11 895 ind. mL<sup>-1</sup> and 1.3 × 10<sup>7</sup> µm<sup>3</sup> mL<sup>-1</sup>). In the humic lakes, phytoplankton abundance showed intermediate values (2086 ind. mL<sup>-1</sup> and 1.1 × 10<sup>7</sup> µm<sup>3</sup> mL<sup>-1</sup>) (Fig. 2). Steppe lakes exhibited contrasting phytoplankton



**Fig. 2.** Upper panel: Total phytoplankton biovolume (right *Y*-axis) and phytoplankton abundance (left *Y*-axis) in the lakes studied; lower panel: relative proportion of the abundance of the different algal groups.

**Table II:** Main phytoplankton functional groups with their ecological features, observed in the four types of freshwater environments

Functional group	Main ecological features	Most frequent and/or abundant taxa	Freshwater environments where the group was best represented
Lo	Deep and shallow lakes, tolerates low nutrient conditions	<i>Chroococcus</i> spp. <i>Coelosphaerium</i> spp.	Humic lakes
N	In the epilimnia of stratified lakes or in shallow lakes, tolerates nutrient deficiency	<i>Tabellaria tabulata</i>	Humic lakes
X3	Nanoplanktonic species from oligotrophic lakes, tolerates low base status, includes phagotrophic taxa	<i>Chromulina</i> sp. <i>Chrysocapsa planctonica</i> <i>Chrysochromulina</i> sp. <i>Monochrysis</i> sp. <i>Ochromonas</i> sp. <i>Pseudopedinella</i> sp.	Humic lakes—clear oligotrophic lakes
E	Oligotrophic environments, base poor lakes or heterotrophic ponds, includes phagotrophic taxa	<i>Dinobryon cylindricum</i> <i>Dinobryon</i> sp. <i>Mallomonas</i> spp.	Humic lakes—some beaver ponds
X2	Shallow meso-eutrophic environments, tolerates stratification	<i>Chlamydomonas minutissima</i> <i>Chlamydomonas</i> sp. <i>Ochromonas</i> sp. (some spp.) <i>Plagioselmis lacustris</i> <i>Plagioselmis nanoplanctica</i>	Beaver ponds
X1	Shallow eutrophic to hypertrophic lakes, Tolerates stratification	<i>Chlorella</i> sp. <i>Monoraphidium arcuatum</i> <i>Monoraphidium circinale</i> <i>Monoraphidium contortum</i> <i>Monoraphidium griffithii</i> <i>Cryptomonas</i> spp.	Beaver ponds—steppe lakes
Y	Includes large cryptomonads that tolerate low light conditions, enriched lakes		Beaver ponds—steppe lakes
F	Deeply mixed meso-eutrophic lakes, tolerates high turbidity	<i>Oocystis parva</i>	Steppe lakes
J	Shallow enriched lakes, ponds and rivers	<i>Willea</i> sp.	Steppe lakes
K	Nutrient-enriched waters, shallow lakes	<i>Aphanocapsa delicatissima</i>	Steppe lakes
D	Shallow turbid waters	<i>Synedra</i> sp.	Beaver ponds
P	Eutrophic epilimnia, with tolerance to mild light, sensitive to stratification	<i>Aulacoseira granulata</i>	Steppe lakes
MP	Littoral diatoms that are drifted to the plankton	<i>Epithemia longisetosa</i> <i>Fragilaria construens</i> <i>Navicula</i> spp.	Beaver ponds

For each functional group, the most frequent and/or abundant taxa are indicated.

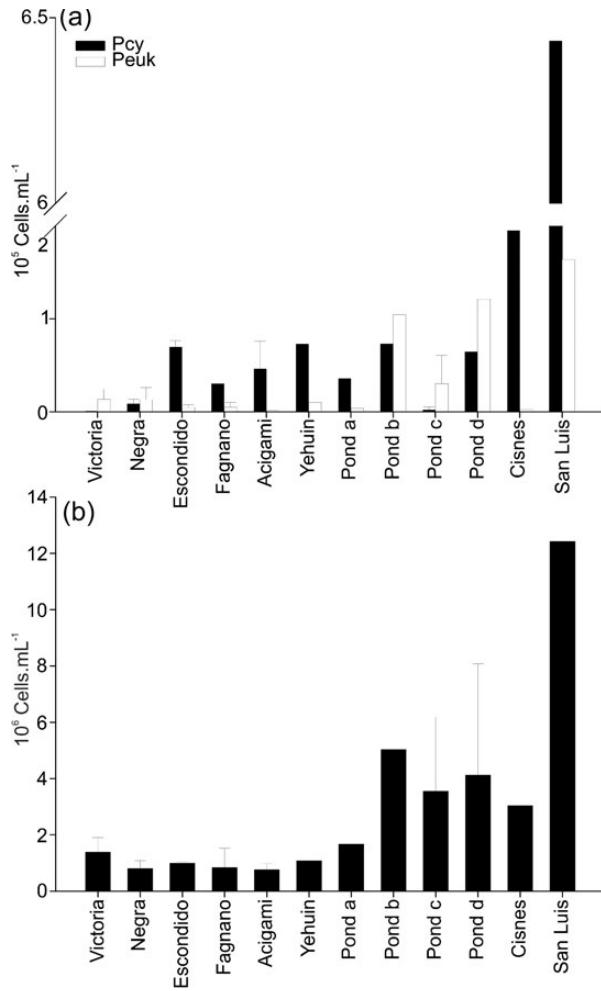
abundances; San Luis had the highest biovolumes among the lakes studied ( $4.5 \times 10^7 \mu\text{m}^3 \text{ mL}^{-1}$ ), whereas lower values were observed at Laguna de los Cisnes ( $1.2 \times 10^6 \mu\text{m}^3 \text{ mL}^{-1}$ ) because this shallow lake was in a “clear water phase” when we collected the samples due to a high abundance of large size copepods (personal observation) (Fig. 2).

Algal composition differed among the lakes studied. In general, heterokontophytes (particularly chrysophytes) were best represented in all water bodies, except steppe lakes. In low nutrient environments, humic lakes and great oligotrophic lakes, the phytoplankton was composed of more than 50% of Chrysophyceae, with *Ochromonas* spp. as one of the dominant taxa. Bacillariophyceae, Cryptophyceae and Chlorophyceae were also quite abundant, particularly in steppe lakes and beaver ponds (Fig. 2). A total of 181 phytoplankton species were recorded in all lakes. The more abundant and frequent taxa in each type of water body are indicated in Table II, which were classified in phytoplankton

functional groups according to Reynolds *et al.* (Reynolds *et al.*, 2002) and Padisák *et al.* (Padisák *et al.*, 2009). The main ecological features of the different functional groups are also indicated in this table. The more abundant taxa belong to 12 functional groups: Lo, N, X3, E (mainly registered in humic and clear oligotrophic lakes); X2, X1, Y, D (beaver ponds and steppe lakes); F, J, K, P (only in steppe lakes).

#### *Autotrophic picoplankton (APP) and bacterioplankton*

The picoplankton community was least abundant in humic and oligotrophic lakes:  $9.1 \times 10^5$ – $1.7 \times 10^6$  cells  $\text{mL}^{-1}$  for bacterioplankton and  $4 \times 10^4$ – $8.1 \times 10^4$  cells  $\text{mL}^{-1}$  for APP. The highest densities were recorded in Lake San Luis, with  $8.07 \times 10^5$  and  $1.2 \times 10^7$  cells  $\text{mL}^{-1}$  for APP and bacterioplankton, respectively. Picocyanobacteria (Pcy) were more abundant than picoeukaryotes (Peuk), except in humic lakes and beaver ponds. Phycoerythrin (PE)-rich Pcy were more abundant in the clear oligotrophic lakes, whereas phycocyanin

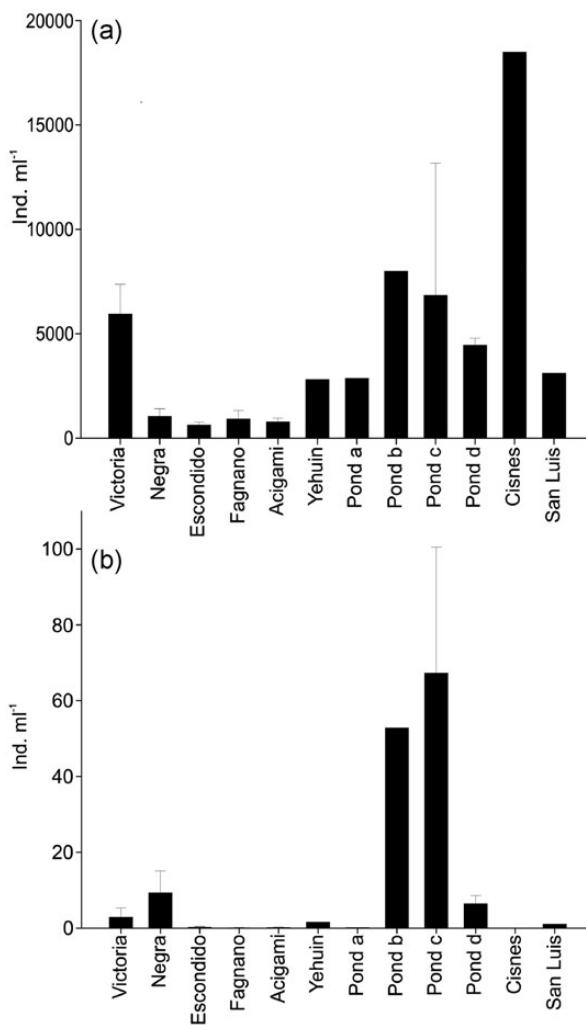


**Fig. 3.** Abundance of autotrophic picoplankton (a) and bacterioplankton (b) in the lakes studied. Pcy, picocyanobacteria; Peuk, picoeukaryotes.

(PC)-rich Pcy dominated in one of the humic lakes (Laguna Negra) and were also abundant in the shallow lake San Luis. Figure 3a and b shows the mean abundance of APP and bacterioplankton in each lake.

#### Heterotrophic nanoflagellates (HNF) and ciliates

The abundance of HNF and ciliates (Fig. 4a and b) also showed important differences between lakes. Ciliates were very abundant in beaver ponds b and c (53 and 67 ind.  $\text{mL}^{-1}$ ), while in the other water bodies densities were relatively low (less than 9 ind.  $\text{mL}^{-1}$ ). Regarding HNF, the steppe lake Laguna de los Cisnes had the highest abundance ( $1.8 \times 10^4$  ind.  $\text{mL}^{-1}$ ); intermediate values were observed in lake Victoria and in the beaver ponds ( $4.5 \times 10^3$ – $8 \times 10^3$  ind.  $\text{mL}^{-1}$ ), and the lowest abundances were found in Laguna Negra (humic lake) and in the oligotrophic lakes Escondido, Fagnano and Acigami, ranging from  $6.4 \times 10^2$  to  $1.0 \times 10^3$  ind.  $\text{mL}^{-1}$ .



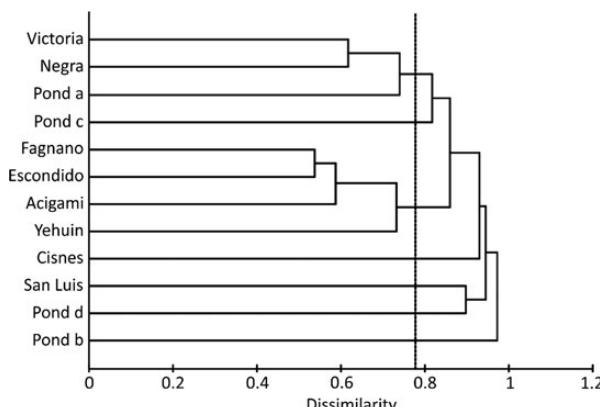
**Fig. 4.** Abundance of heterotrophic nanoflagellates (a) and ciliates (b) in the lakes studied.

Interestingly, for both ciliates and HNF, a high variability was observed in pond 27.

#### Multivariate analyses

The cluster analysis based on phytoplankton species abundance (Fig. 5) showed the differences among the lakes studied. Two main groups are clearly separated at a high dissimilarity level: one of them is formed by the clear oligotrophic lakes (Fagnano, Escondido, Acigami and Yehuin), and the other one includes the two humic lakes (Victoria, Negra) and pond a. On the other hand, the most eutrophic water bodies, beaver ponds and steppe lakes, did not form a distinct group, probably because their higher heterogeneity and variability.

The results of the RDA based on the phytoplankton functional groups abundance with respect to environmental variables are shown in Figure 6a and b.

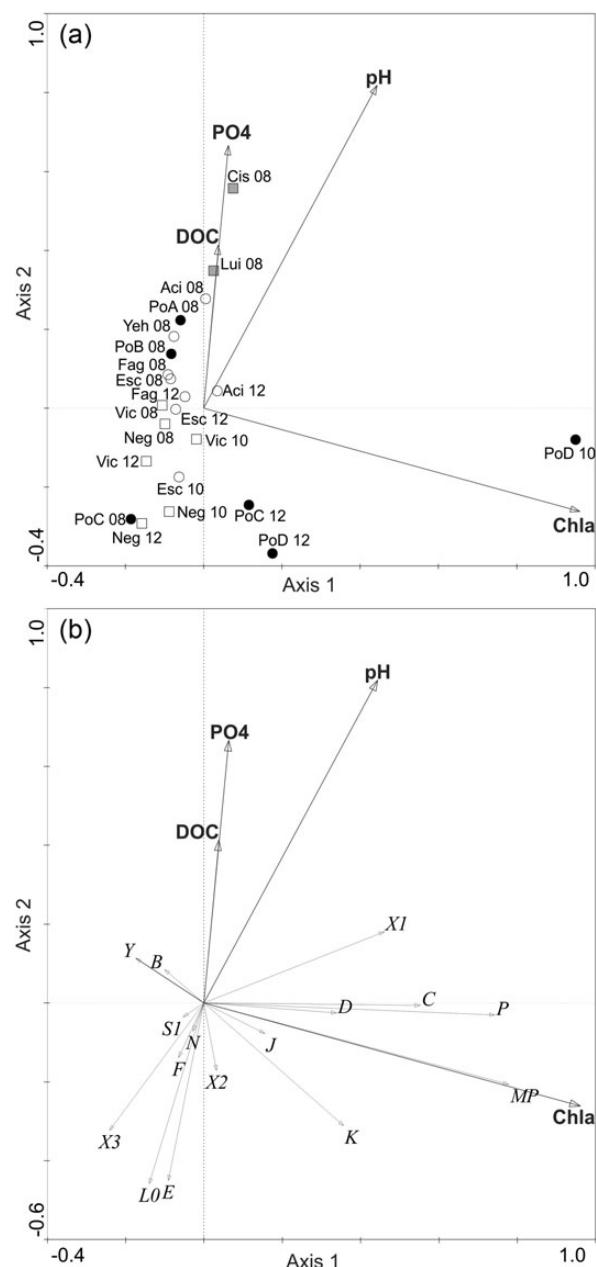


**Fig. 5.** Cluster analysis based on the phytoplankton species abundances in each water body, using the Bray–Curtis index and UPGMA algorithm.

Significant variables in terms of the variance explained in the distribution of functional groups are indicated by solid arrows. The first two axes accounted for 75.4% of the variance (axis 1: 52.6%; axis 2: 22.8%). The first axis was mainly defined by a combination of Chl *a* and pH (intra-set correlation coefficients: 0.78 and 0.36, respectively); axis 2 was mainly correlated with pH and DRP (intra-set correlation coefficients: 0.61 and 0.50, respectively). Monte Carlo tests were significant for the first canonical axes and for all canonical axes ( $P = 0.05$  and  $P = 0.02$ ). The RDA based on the species abundances showed similar results (Figure not presented), and also in this case the first axis was mainly defined by a combination of Chl *a* and pH and the second axis by pH and DRP.

These analyses showed an ordination of the lakes following a gradient of pH and a DRP concentration. Steppe lakes (San Luis and Laguna de los Cisnes) are placed in the upper part of the figure, positively associated to high DRP concentrations and pH values. The humic lakes (Laguna Negra and Victoria) ordinate at the lower side of the Figure, whereas most of the oligotrophic lakes ordinate in the middle of the gradient. On the other hand, beaver ponds tend to follow a positive Chl *a* gradient and are placed at the right of the Figure. In general terms, the spring samples of 2008 are closely located and related to higher DOC and DRP contents, whereas those of summer 2010 and early autumn 2012 are more dispersed in the plot and related to lower concentrations; the differences in 2008 samples are probably associated with the effect of the melting that occurred in spring some days before our sampling.

Taking into account the phytoplankton functional classification, groups X3, L0 and E were particularly associated with oligotrophy and low pH, being dominant in humic and in large oligotrophic lakes. According to



**Fig. 6.** Biplot of the redundancy analysis based on the abundance of the phytoplankton functional groups according to Reynolds *et al.* (2002). **(a)** Ordination of the samples in relation to the environmental variables. **(b)** Functional groups and environmental variables. Victoria (Vic), Negra (Neg), Escondido (Esc), Fagnano (Fag), Acigami (Aci), Yehuin (Yeh), Pond a (PoA), Pond b (PoB), Pond c (PoC), Pond d (PoD), San Luis (Lui), Cisnes (Cis). White circles: oligotrophic lakes; white squares: humic lakes; black circles: beaver ponds; grey squares: steppe lakes. Numbers in lake labels indicate the year of sampling.

Reynolds *et al.* (Reynolds *et al.*, 2002) and Padisák *et al.* (Padisák *et al.*, 2009), codon X3 is typical of oligotrophic environments and includes many small nanoplanktonic species mainly belonging to Chrysophyceae but also some Chlorophyceae. L0 includes some small colonial

Cyanobacteria and some thecate dinoflagellates that can tolerate low nutrient conditions. Group E is usually encountered in oligotrophic lakes, and is comprised of many colonial and scaled Chrysophyceae (i.e. *Dinobryon* spp.; *Mallomonas* spp.). Many of the species belonging to groups X3 and E are potentially mixotrophic.

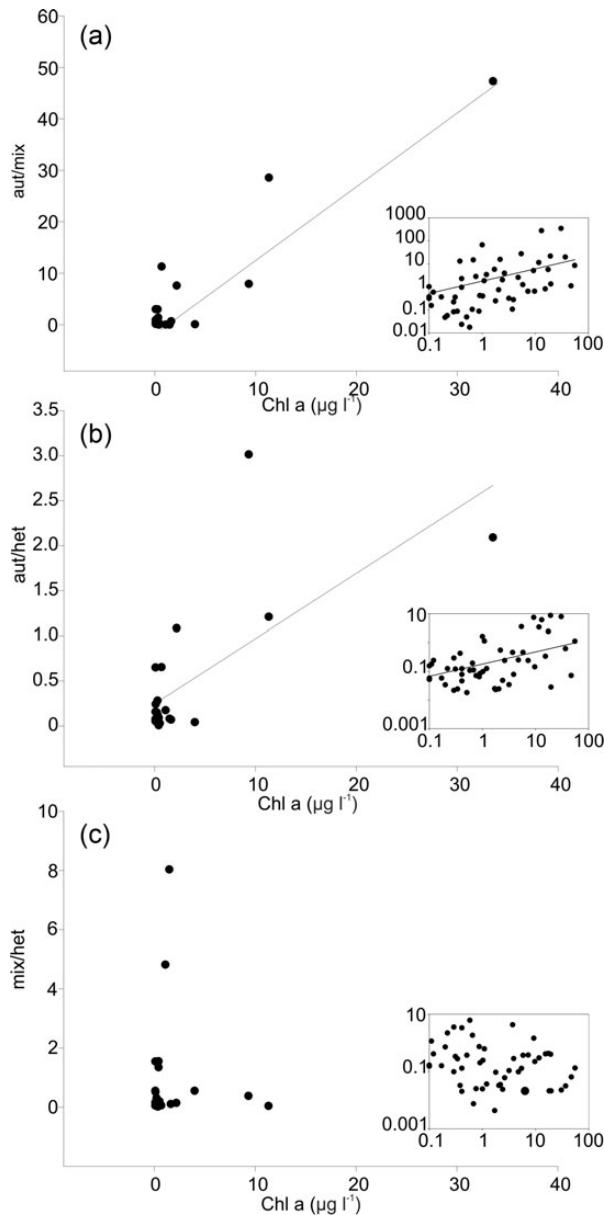
On the other hand, groups C, P, MP, X1 and J were associated with high Chl *a* (beaver ponds). Codon C includes several centric diatoms that live in eutrophic small and medium sized lakes with species sensitive to the onset of stratification; group P is also usually found in the eutrophic epilimnion and is formed of diatoms and desmids that are able to survive under mild light; MP is a codon defined for littoral diatoms that are drifted to the plankton. X1 is typical in shallow eu-hypertrophic environments and includes many small chlorococcaceans, whereas codon J comprises mainly coenobia chlorococcaceans living in shallow eutrophic waters.

#### *Nanoplankton and microplankton abundances in the trophic status gradient*

The results of the regression analyses of the trophic ratios (strict autotrophs/mixotrophs, strict autotrophs/heterotrophs, mixotrophs/heterotrophs) on Chl *a* are illustrated in Figure 7a–c. In this figure, we plotted together the data belonging to Tierra del Fuego lakes with data obtained across a latitudinal gradient of lakes from Patagonia to Antarctica (own unpublished data). A clear increasing trend of the ratio strict autotrophs/mixotrophs in relation to Chl *a* was observed ( $R^2 = 0.87$ ,  $n = 22$ ,  $P < 0.001$ ). On the other hand, the ratio strict autotrophs/heterotrophs also showed an increasing tendency towards higher Chl *a* values ( $R^2 = 0.49$ ;  $n = 22$ ;  $P < 0.001$ ). For the larger data set of Patagonian lakes, we found the same trends but fitting to a logarithmic model ( $R^2 = 0.30$ ,  $n = 53$ ,  $P < 0.001$ ;  $R^2 = 0.26$ ,  $n = 53$ ,  $P < 0.001$  for autotrophs/mixotrophs and autotrophs/heterotrophs, respectively) (see small plots in Fig. 7a and b). Contrarily, no clear trend was observed in both cases in relation to the rate mixotrophs/heterotrophs ( $R^2 = 0.05$ ;  $n = 22$  and  $R^2 = 0.0085$ ;  $n = 53$ , respectively).

## DISCUSSION

Considering the wide range of variation in nutrients and DOC concentrations observed among the lakes, the trophic-DOC paradigm (Rodhe, 1969), and then trophic-CDOC model (Williamson *et al.*, 1999) successfully describe the variety of aquatic environments present in Tierra del Fuego. These environmental variables influence planktonic food chains by altering the physical and



**Fig. 7.** Simple regressions of ratios between abundances of nano- and microplankton classified by their trophic strategies with chlorophyll *a*. Main plots correspond to data from Tierra del Fuego lakes; small plots (bottom right of the figure) correspond to a latitudinal gradient of lakes from Austral Patagonia to Antarctica (Izaguirre *et al.*, unpublished data). Autotrophs (Aut), Mixotrophs (Mix), Heterotrophs (Het). Regression adjustment: (a)  $R^2 = 0.87$ ,  $n = 22$ ,  $P < 0.001$ ;  $R^2 = 0.30$ ,  $n = 53$ ,  $P < 0.001$ . (b)  $R^2 = 0.49$ ,  $n = 22$ ,  $P < 0.001$ ;  $R^2 = 0.26$ ,  $n = 53$ ,  $P < 0.001$ . (c)  $R^2 = 0.87$ ,  $n = 22$ ,  $P < 0.001$ ;  $R^2 = 0.30$ ,  $n = 53$ ,  $P < 0.001$ .  $R^2 = 0.87$ ,  $n = 22$ ,  $P < 0.001$ ;  $R^2 = 0.30$ ,  $n = 53$ ,  $P < 0.001$ .  $0.05$ ,  $n = 22$ ;  $R^2 = 0.0085$ ,  $n = 53$ .

chemical environment. Our study shows important differences in the structure of the microbial communities among the water bodies surveyed with different physico-chemical properties, mainly phosphorus concentrations and DOC content. From clear oligotrophic and humic

lakes to beaver ponds and steppe lakes, following an increasing gradient in Chl *a*, increasing patterns in the ratios autotrophs/mixotrophs and autotrophs/heterotrophs were found. We observed this gradient in the geographically close lakes from Tierra del Fuego, which was also found across a long latitudinal gradient of lakes from Patagonia to Antarctica. In this sense, our results are consistent with the patterns described by del Giorgio and Gasol (del Giorgio and Gasol, 1995), who reported a decline in the ratio heterotrophic/autotrophic biomass in freshwater plankton communities of temperate lakes along gradients of increasing phytoplankton biomass.

From oligotrophic and humic lakes to beaver ponds and steppe lakes, following an increasing gradient in Chl *a*, increasing patterns in the ratios autotrophs/mixotrophs and autotrophs/heterotrophs were found.

Tierra del Fuego exhibits two types of low nutrient systems: the large oligotrophic lakes and the humic lakes, both characterized by low phytoplankton biomass (expressed as Chl *a*). Some authors have reported lower yields of chlorophyll per unit of total phosphorus in humic systems, which may result from lower availability of light due to increased water colour or to lower bioavailability of phosphorus (Phillips *et al.*, 2008 and citations therein). Despite the evident differences in morphometry and limnological features between deep oligotrophic and humic lakes, their microbial communities show a certain degree of similarity, both in terms of phytoplankton species and functional groups. The dominance of flagellated algae comprised in X3, E and L0 functional groups, and in particular of those species capable of ingesting prey (phagotrophic type of mixotrophy), is typical in these systems. Saad (Saad, 2011 and unpublished data) experimentally demonstrated the ingestion of prey by taxa in Victoria and Escondido Lakes. Their ecological success can be explained in different ways. On the one hand, mixotrophy tends to be a suitable strategy in environments with low nutrients and good light climate (i.e. oligotrophic clear lakes), thus mixotrophs tend to dominate over strictly autotrophic organisms due to their capability to incorporate nutrients by prey ingestion (Medina-Sánchez *et al.*, 2004; Pålsson *et al.*, 2005). On the other hand, in environments with humic coloured waters characterized by relatively low Kd values and high  $\alpha_{320}$  absorbances, the mixotrophic flagellates that are able to turn from autotrophy to heterotrophy to supplement their carbon budget under light limitation may thrive. As it was mentioned, the humic lakes of Tierra del Fuego are also characterized by low nutrient contents; thus, both conditions contribute to create a propitious scenario for mixotrophic algae. Similar humic boreal lakes show comparable phytoplankton structure (e.g. Isaksson *et al.*, 1999; Bergström *et al.*, 2003; Willén,

2003; Poniewozik *et al.*, 2011). On the other hand, the results of our study also showed that in oligotrophic lakes heterotrophic protists were proportionally more abundant than strict autotrophs.

Beaver ponds exhibit the highest trophic status among the freshwater environments of the south-west region of Tierra del Fuego as a consequence of the introduction of the North American beaver *Castor canadensis* (Anderson *et al.*, 2009). This is not unexpected as one of the processes by which invasive species may impact an entire ecosystem is via ecosystem engineering, creating, destroying or modifying habitat (Crooks, 2002). Planktonic microbial communities differed among ponds and sampling dates, probably due to their small size, and consequently a higher influence of the environmental conditions. Moreover, beaver ponds present different intensities of disturbance related to the stages of colonization of these rodents; this is demonstrated by the lack of a distinctive group in the cluster analysis based on species abundances. The functional groups representative of these water bodies are X1, X2, Y and P, typical of enriched waters.

In agreement with the data reported by Mariazzi *et al.* (Mariazzi *et al.*, 1987), the shallow lakes from the steppe also showed a relatively high trophic status and higher conductivity values than those located near the mountains. As it has been described by Quirós and Cuch (Quirós and Cuch, 1985) and Quirós and Drago (Quirós and Drago, 1999), those Patagonian lakes located in the ecotone steppe region are usually characterized by relatively high concentrations of total phosphorus and phytoplankton Chl *a*, ranging from mesotrophic to eutrophic. In addition, the extremely high conductivity observed in the shallow lake Cisnes could also be explained by the input of sea ingressions/regressions during quaternary age in that region (Conzonno and Ulibarrena, 2010). The shallow lakes of the steppe are typically affected by strong wind stress, which causes high turbidity due to resuspension promoting nutrient flux to the water column. These environmental conditions favour the development of high densities of phytoplankton, particularly chlorophytes and diatoms, represented by functional groups E, J, X1 and P. The dominance of these groups in the shallow lakes studied agrees with the phytoplankton structure reported for other Patagonian lakes from the steppe (e.g. Izaguirre, 1993; Díaz *et al.*, 2000; Gonçalves *et al.*, 2010).

The phytoplankton functional approach applied in this study (Reynolds *et al.*, 2002—updated by Padisák *et al.*, 2009) allowed a good discrimination of the different types of water bodies. The multivariate analysis based on this classification showed the separation of the lakes according to their phytoplankton structure.

The abundance of both bacterioplankton and phototrophic picoplankton also varied according to the trophic gradient among the lakes, showing higher values with increasing trophic status. These results agree with the patterns commonly described for other sets of lakes (e.g. Bird and Kalff, 1984; Cole *et al.*, 1988; Gasol and Duarte, 2000; Bell and Kalff, 2001; Schiaffino *et al.*, 2013). Oligotrophic lakes and humic water bodies exhibited the lowest abundances of heterotrophic and autotrophic picoplankton, and PE-rich Pcy dominated in all the oligotrophic systems. This pattern consistent with that described by Callieri (Callieri, 2008) based on data from a wide range of water bodies and trophic conditions (Vörös *et al.*, 1998), and on data obtained in North Patagonian lakes (Callieri *et al.*, 2007). Recently, a study based on flow cytometry, where 32 Patagonian water bodies located along a latitudinal and trophic gradient (Schiaffino *et al.*, 2013) were analysed, confirmed that PE-rich Pcy were best represented in oligotrophic lakes, whereas PC-rich Pcy in eutrophic ones. On the other hand, our results showed that Peuk were more abundant than Pcy in humic lakes and ponds, in agreement with the findings of Schiaffino *et al.* (Schiaffino *et al.*, 2013) who reported that Peuk prevail over Pcy with increasing light attenuation and/or trophic status. According to different studies, Peuk seems to be favoured under light limiting conditions (Stockner and Antia, 1986; Craig, 1987; Søndergaard, 1991; Vörös *et al.*, 2009). Nevertheless, in the relatively turbid steppe lakes, this tendency was not observed, indicating that probably another environmental factor may be involved in this case. These high conductivity environments had the maximum densities of Pcy; this group is very tolerant to a great variety of environments, particularly saline conditions where halotolerant strains prevail (Van Hove *et al.*, 2008).

Our studies showed that ciliates were only abundant in two beaver ponds, which is probably explained by their higher trophic status, since ciliates tend to increase in meso- to eutrophic environments (Šimek *et al.*, 1995 and citations therein). It is important to mention that studies conducted in some oligotrophic lakes from North Patagonia demonstrated the great importance of ciliates in trophic food webs (e.g. Queimaliños *et al.*, 1999; Modenutti and Balseiro, 2002; Balseiro *et al.*, 2004) as a high abundance of mixotrophic ciliates is usually associated with the deep chlorophyll maxima in the metalimnion. In our study, sampling was performed in the epilimnion, and thus we cannot dismiss the possibility of higher abundances of ciliates and other planktonic groups at greater depths. For example, Bird and Kalff (Bird and Kalff, 1989) found high concentrations of mixotrophic algae (i.e. *Dinobryon* and *Ochromonas*) in the metalimnion of an oligo-mesotrophic lake in Quebec.

We conclude that in the trophic gradient of lakes analyzed here there exists a change in the structure of the microbial community that is the main support for secondary production. At the oligotrophic end, energy mobilization is guided by bacteria and the microbial loop is important; in these low nutrient environments, different mixotrophic strategies (according to light conditions) would thrive over strict autotrophs, and the energy source would be mainly allochthonous organic matter. In agreement with that reported by other authors (e.g. Raven, 1997; Jones, 2000), when moving along the trophic gradient, towards a higher level, the energy mobilization starts to be guided by phytoplankton; as nutrients are not limiting, mixotrophy is no longer an effective strategy and strict autotrophy predominates, and the main source of energy for higher trophic levels would be organic matter from phytoplankton (autochthonous). Thus, the adaptive features of mixotrophic protists, which allow these organisms to prevail when nutrient concentrations, light conditions and/or prey abundances do not satisfy the demand of purely phototrophic or heterotrophic protists, scale-up to the community showing tendencies in the pelagic metabolism of lakes, both at small and large spatial scales.

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## REFERENCES

- Anderson, C. B., Pastur, G. M., Lencinas, M. V. *et al.* (2009) Do introduced North American beavers *Castor canadensis* engineer differently in southern South America? An overview with implications for restoration. *Mammal Rev.*, **39**, 33–52.
- Balseiro, E. G., Queimaliños, C. P. and Modenutti, B. E. (2004) Grazing impact on autotrophic picoplankton in two south andean lakes (Patagonia, Argentina) with different light:nutrient ratios. *Rev. Chil. Hist. Nat.*, **77**, 73–85.
- Bell, T. and Kalff, J. (2001) The contribution of picophytoplankton in marine and freshwater systems of different trophic status and depth. *Limnol. Oceanogr.*, **46**, 1243–1248.

- Bergström, A.-K., Jansson, M., Drakare, S. *et al.* (2003) Occurrence of mixotrophic flagellates in relation to bacterioplankton production, light regime and availability of inorganic nutrients in unproductive lakes with differing humic contents. *Freshwat. Biol.*, **48**, 868–877.
- Bird, D. F. and Kalf, J. (1984) Empirical relationship between bacterial abundance and chlorophyll concentration in fresh and marine waters. *Can. J. Fish. Aquat. Sci.*, **41**, 1015–1023.
- Bird, D. F. and Kalf, J. (1989) Phagotrophic sustenance of a metalimnetic phytoplankton peak. *Limnol. Oceanogr.*, **34**, 155–162.
- Cabrera, A. L. (1971) Fitogeografía de la República Argentina. *Bol. Soc. Argent. Bot.*, **14**, 1–2.
- Callieri, C. (2008) Picophytoplankton in freshwater ecosystems: the importance of small-sized phototrophs. *Freshwat. Rev.*, **1**, 1–28.
- Callieri, C., Modenutti, B., Queimaliños, C. *et al.* (2007) Production and biomass of picophyto-plankton and larger autotrophs in Andean ultraoligotrophic lakes: differences in light harvesting efficiency in deep layers. *Aquat. Ecol.*, **41**, 511–523.
- Carpenter, S. R., Cole, J. J. and Kitchell, J. F. (1998) Impact of dissolved organic carbon, phosphorus, and grazing on phytoplankton biomass and production in experimental lakes. *Limnol. Oceanogr.*, **43**, 73–80.
- Cole, J. J., Findlay, S. and Pace, M. L. (1988) Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar. Ecol. Prog. Ser.*, **43**, 1–10.
- Collantes, M. and Faggi, A. M. (1999) Los humedales del sur de Sudamérica. In Malvárez, A. I. (eds), *Tópicos sobre humedales subtropicales y templados de Sudamérica*. UNESCO, Montevideo.
- Conzonno, V. H. and Ulibarrena, J. (2010) Hydrochemistry of Lakes of the Patagonian Province of Tierra del Fuego (Argentina). *Environ. Earth Sci.*, **59**, 1431–1436.
- Craig, S. R. (1987) The distribution and contribution of picoplankton to deep photosynthetic layers in some meromictic lakes. *Acta Acad. Aboensis*, **47**, 55–81.
- Crane, K. W. and Grover, J. P. (2010) Coexistence of mixotrophs, autotrophs, and heterotrophs in planktonic microbial communities. *J. Theor. Biol.*, **262**, 517–527.
- Crooks, J. A. (2002) Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos*, **97**, 153–166.
- del Giorgio, P. A., Cole, J. J., Caraco, N. F. *et al.* (1999) Linking planktonic biomass and metabolism to net gas fluxes in northern temperate lakes. *Ecology*, **80**, 1422–1431.
- del Giorgio, P. A. and Gasol, J. M. (1995) Biomass distribution in freshwater plankton communities. *Am. Nat.*, **146**, 135–152.
- del Giorgio, P. A. and Peters, R. H. (1993) The influence of DOC on the bacteria-chlorophyll relationship in lakes. *Verh. Internat. Verein. Limnol.*, **25**, 359–362.
- Díaz, M. M., Pedrozo, F. and Baccala, N. (2000) Summer classification of Southern Hemisphere temperate lakes. *Lake. Reserv. Res. Manag.*, **5**, 213–229.
- Gasol, J. M. and Duarte, C. M. (2000) Comparative analyses in aquatic microbial ecology: how far do they go? *FEMS Microbiol. Ecol.*, **31**, 99–106.
- Gonçalves, R. J., Souza, M. S., Aigo, J. *et al.* (2010) Responses of plankton and fish from temperate zones to UVR and temperature in a context of global change. *Ecol. Austral.*, **20**, 129–153.
- González Garraza, G., Mataloni, G., Iturraspe, R. *et al.* (2012) The limnological character of bog pools in relation to meteorological and hydrological features. *Mires Peat*, **10**, 1–14.
- Hillebrand, H., Claus-Dieter, D., Kirschtel, D. *et al.* (1999) Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.*, **35**, 403–424.
- Isaksson, A., Bergström, A.-K., Blomqvist, P. *et al.* (1999) Bacterial grazing by phagotrophic phytoflagellates in a deep humic lake in northern Sweden. *J. Plankton Res.*, **21**, 247–268.
- Iturraspe, R. and Urciuolo, A. (2000) *Clasificación y caracterización de las cuencas hidrálicas de Tierra del Fuego. Actas XVIII Congreso Nacional del Agua. Río Hondo*, Santiago del Estero, June/2000.
- Izaguirre, I. (1993) Comparative analysis of the phytoplankton of six lentic environments from the Province of Chubut (Argentina). *Physis*, **48**, 7–23.
- Izaguirre, I., Sinistro, R., Schiaffino, M. R. *et al.* (2012) Grazing rates of protists in wetlands under contrasting light conditions due to floating plants. *Aquat. Microb. Ecol.*, **65**, 221–232.
- Jansson, M., Bergström, A. K., Blomqvist, P. *et al.* (2000) Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. *Ecology*, **81**, 3250–3255.
- Jones, R. I. (1992) The influence of humic substances on lacustrine planktonic food chains. *Hydrobiologia*, **229**, 73–91.
- Jones, R. I. (2000) Mixotrophy in planktonic protists: an overview. *Freshwat. Biol.*, **45**, 219–226.
- Jost, C., Lawrence, C. A., Campolongo, F. *et al.* (2004) The effects of mixotrophy on the stability and dynamics of a simple planktonic food web model. *Theor. Popul. Biol.*, **66**, 37–51.
- Karlsson, J., Jansson, M. and Jonsson, A. (2002) Similar relationships between pelagic primary and bacterial production in clearwater and humic lakes. *Ecology*, **83**, 2902–2910.
- Katechakis, A. and Stibor, H. (2006) The mixotroph Ochromonas tuberculata may invade and suppress specialist phago- and phototroph plankton communities depending on nutrient conditions. *Oecologia*, **146**, 692–701.
- Keskitalo, J. and Eloranta, P. (1999) *Limnology of Humic Water*. Backhuys Publishers, Leiden, Netherlands, 284 pp.
- Kirk, J. T. O. (1994) *Light and Photosynthesis in Aquatic Ecosystems*. 2nd edn. Cambridge University Press, Cambridge, 509 pp.
- Kruk, C., Huszar, V. L. M., Peeters, E. *et al.* (2010) A morphological classification capturing functional variation in phytoplankton. *Freshwat. Biol.*, **55**, 614–627.
- Mariazzi, A., Conzonno, V., Ulibarrena, J. *et al.* (1987) Limnological investigation in Tierra del Fuego, Argentina. *Biol. Aquat.*, **10**, 1–74.
- Marker, A. F. H., Nusch, A., Rai, H. *et al.* (1980) The measurement of photosynthetic pigments in freshwater and standardization of methods: conclusions and recommendations. *Arch. Hydrobiol. Beih. Ergeb. Limnol.*, **14**, 91–106.
- Mataloni, G. (1999) Ecological studies on algal communities from Tierra del Fuego peat bogs. *Hydrobiologia*, **391**, 157–170.
- Mataloni, G. and Tell, G. (1996) Comparative analysis of the phytoplankton communities of a peat bog from Tierra del Fuego (Argentina). *Hydrobiologia*, **325**, 101–112.
- Medina-Sánchez, J. M., Villar-argaz, M. and Carrillo, P. (2004) Neither with nor without you: a complex algal control on bacterioplankton in a high mountain lake. *Limnol. Oceanogr.*, **49**, 1722–1733.
- Modenutti, B. E. and Balseiro, E. G. (2002) Mixotrophic ciliates in an Andean lake: dependence on light and prey of an Ophrydium naufragium population. *Freshwat. Biol.*, **47**, 121–128.
- Naselli-Flores, L. and Barone, R. (2011) Fight on plankton! Or, phytoplankton shape and size as adaptive tools to get ahead in the struggle for life. *Cryptogamie Algol.*, **32**, 157–204.

- Padisák, J., Crossetti, L. O. and Naselli-Flores, L. (2009) Use and misuse in the application of the phytoplankton functional classification: a critical review with updates. *Hydrobiologia*, **621**, 1–19.
- Pålsson, C. and Granéli, W. (2004) Nutrient limitation of autotrophic and mixotrophic phytoplankton in a temperate and tropical humic lake gradient. *J. Plankton Res.*, **26**, 1005–1014.
- Pålsson, C., Kritzberg, E. S., Schristoffersen, K. et al. (2005) Net heterotrophy in faroe islands clear-water lakes: causes and consequences for bacterioplankton and phytoplankton. *Freshwater Biol.*, **50**, 2011–2020.
- Phillips, G., Pietiläinen, O.-P., Carvalho, L. et al. (2008) Chlorophyll–nutrient relationships of different lake types using a large European dataset. *Aquat. Ecol.*, **42**, 213–226.
- Poniewozik, M., Wojciechowska, W. and Solis, M. (2011) Dystrophy or eutrophy: phytoplankton and physicochemical parameters in the functioning of humic lakes. *Oceanol. Hydrobiol. St.*, **40**, 22–29.
- Porter, K. G. and Feig, Y. S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.*, **25**, 943–948.
- Queimaliños, C. P., Modenutti, B. E. and Balseiro, E. G. (1999) Symbiotic association of the ciliate *Ophyridium naumanni* with *Chlorella* causing a deep chlorophyll a maximum in an oligotrophic south Andes lake. *J. Plankton Res.*, **21**, 167–178.
- Quirós, R. and Cuch, S. (1985) Relaciones entre visibilidad, fósforo total y concentración de clorofila en 32 lagos patagónicos, Argentina. In *En. Actas XII Congreso Nac. del Agua*. Mendoza, Tomo IC, 27 pp.
- Quirós, R. and Drago, E. (1999) The environmental state of Argentinean lakes: an overview. *Lakes Reserv. Res. Manage.*, **4**, 55–64.
- Raven, J. A. (1997) Phagotrophy in phototrophs. *Limnol. Oceanogr.*, **42**, 198–205.
- Reynolds, C. S., Huszar, V., Kruk, C. et al. (2002) Towards a functional classification of the freshwater phytoplankton. *J. Plankton Res.*, **24**, 417–428.
- Rodhe, W. (1969) Crystallization of eutrophication concepts in Northern Europe. In *Eutrophication: Causes, Consequences, Correctives*. Natl. Acad. Sci. Publ. 1700, pp. 50–64.
- Saad, J. F. (2011) Impacto de depredación de protistas fagotróficos y su relación con la producción primaria y bacteriana en un lago oligotrófico y uno distrófico de Tierra del Fuego (Argentina). Tesis Doctoral. Universidad de Buenos Aires.
- Salmaso, N. and Padisák, J. (2007) Morpho-functional groups and phytoplankton development in two deep lakes (Lake Garda, Italy and Lake Stechlin, Germany). *Hydrobiologia*, **578**, 97–112.
- Sarmento, H. and Descy, J.-P. (2008) Use of marker pigments and functional groups for assessing the status of phytoplankton assemblages in lakes. *J. Appl. Phycol.*, **20**, 1001–1011.
- Schiaffino, M. R., Gasol, J. M., Izquierdo, I. et al. (2013) Picoplankton abundance and cytometric group diversity along a trophic and latitudinal lake gradient. *Aquat. Microbial. Ecol.*, **68**, 231–250.
- Sharp, J. H., Peltzer, E. T., Alperin, M. J. et al. (1993) Procedures subgroup report. *Mar. Chem.*, **41**, 37–49.
- Simek, K., Bodkova, J., Macek, M. et al. (1995) Ciliate grazing on picoplankton in a eutrophic reservoir during the summer phytoplankton maximum: a study at the species and community level. *Limnol. Oceanogr.*, **40**, 1077–1090.
- Søndergaard, M. (1991) Phototrophic picoplankton in temperate lakes: seasonal abundance and importance along a trophic gradient. *Int. Rev. Gesamt. Hydrobiol.*, **76**, 505–522.
- Steinberg, C. E. W., Kamara, S., Prokhotskaya, V. Y. et al. (2006) Dissolved humic substances—ecological driving forces from the individual to the ecosystem level?. *Freshwat. Biol.*, **51**, 1189–1210.
- Stockner, J. G. and Antia, N. J. (1986) Algal picoplankton from ecosystems: a multidisciplinary perspective. *Can. J. Fish. Aquat. Sci.*, **43**, 2472–2503.
- Stoecker, D. K. (1998) Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. *Eur. J. Protistol.*, **34**, 281–290.
- Sun, J. and Liu, D. (2003) Geometric models for calculating cell biovolume and surface area for phytoplankton. *J. Plankton Res.*, **25**, 1331–1346.
- Tell, G., Izquierdo, I. and Allende, L. (2011) Diversity and geographical distribution of *Chlorococcales* (*Chlorophyceae*) in contrasting lakes along a latitudinal transect in Argentinean Patagonia. *Biodivers. Conserv.*, **20**, 703–727.
- Tell, G. and Mosto, O. (1982) Orden Chlorococcales. In Guarera, S., Gamundi, I. and Halperin, D. (eds), *Flora Criptogámica de Tierra del Fuego*. CONICET, Buenos Aires, 147 pp.
- ter Braak, C. J. F. (1988) CANOCO-aFORTRAN program for canonical community ordination by [partial] [detrended] [canonical] correspondence analysis, principal components analysis and redundancy analysis (version 2.1). Report LWA-88-02. Agricultural Mathematics Group, Wageningen, 95 pp.
- Tolotti, M., Rott, E., Thies, H. et al. (2005) Functional species groups of summer phytoplankton in relation to lake restoration: a long-term study of Piburger See, Austria. *Verh. Internat. Verein. Limnol.*, **29**, 891–894.
- Unrein, E., Massana, R., Alonso-sáez, L. et al. (2007) Significant year-round effect of small mixotrophic flagellates on bacterioplankton in an oligotrophic coastal system. *Limnol. Oceanogr.*, **52**, 456–469.
- Utermöhl, H. (1958) Zur Vervollkommenung der quantitativen Phytoplankton- methodik. *Mitt. Int. Vér. Theor. Angewan. Limnol.*, **9**, 1–38.
- Van Hove, P., Vincent, W. F., Galand, P. E. et al. (2008) Abundance and diversity of picocyanobacteria in High Arctic lakes and fjords. *Algol. Stud.*, **126**, 209–227.
- Venrick, E. L. (1978) How many cells to count? In Sournia, A. (ed), *Phytoplankton Manual*. UNESCO, Paris, France.
- Vörös, I., Callieri, C., V-Balogh, K. et al. (1998) Freshwater picocyanobacteria along a trophic gradient and light quality range. *Hydrobiologia*, **369/370**, 117–125.
- Vörös, I., Mózes, A. and Somogyi, B. (2009) A five-year study of auto-trophic winter picoplankton in Lake Balaton, Hungary. *Aquat. Ecol.*, **43**, 722–734.
- Ward, B. A., Dutkiewicz, S., Barton, A. D. et al. (2011) Biophysical aspects of resource acquisition and competition in algal mixotrophs. *Amer. Nat.*, **178**, 98–112.
- Weithoff, G. (2003) The concepts of “plant functional types” and “functional diversity”. In lake phytoplankton, a new understanding of phytoplankton ecology? *Freshwat. Biol.*, **48**, 1669–1675.
- Wetzel, R. G. (ed) (2001) *Limnology*, 3rd edn. Academic, San Diego.
- Willén, E. (2003) Dominance patterns of planktonic algae in Swedish forest lakes. *Hydrobiologia*, **502**, 315–324.
- Williamson, C. E., Morris, D. P., Pace, M. L. et al. (1999) Dissolved organic carbon and nutrients as regulators of lake ecosystems: resurrection of a more integrated paradigm. *Limnol. Oceanogr.*, **44**, 795–803.
- Zar, J. H. (2010) *Biostatistical Analysis*. Prentice Hall, Englewood Cliffs.