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Distribution patterns of the abundance of major bacterial and archaeal groups in Patagonian lakes

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We explored the distribution patterns of bacterial and archaeal abundances at the phylum and class level using catalyzed reporter deposition-fluorescence *in situ* hybridization (CARD-FISH), examining the among (across 35 water bodies) and within-lake (intra-annual seasonality) patterns in Patagonia (Argentina). *Alphaproteobacteria*, *Betaproteobacteria* and *Actinobacteria* globally dominated the bacterioplankton, whereas *Gammaproteobacteria* and *Archaea* never exceeded 3 and 6% of the community, respectively. The different groups showed seasonality, with simultaneous peaks of all bacterial group absolute abundances during late winter or spring, and with peaks of *Archaea* during winter, late spring and summer. The bacterial groups presented roughly similar relative abundances in all seasons, whereas *Archaea* varied in their relative contribution to community structure. Multivariate analyses showed that dissolved organic carbon was an important variable structuring the community at the studied taxonomic resolution (using absolute and relative abundances), in both among and within-lake patterns. The absolute abundance of most bacterial groups was significantly higher in mesotrophic and eutrophic systems than in oligotrophic ones (except *Actinobacteria*), whereas their relative abundances did not change among trophic states (except *Bacteroidetes*). The lake grouping obtained from CARD-FISH was consistent with previous work using polymerase chain reaction-denaturing gradient gel electrophoresis data: deep oligotrophic lakes clustered together, whereas small and shallow water bodies grouped separately.

KEYWORDS: *Bacteria*; *Archaea*; patagonian water bodies; prokaryotic group distribution patterns; CARD-FISH

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INTRODUCTION

Inland waters harbor a high number of diverse planktonic microorganisms that are involved in biogeochemical cycling, aquatic food webs and key ecological processes (Logue and Lindström, 2008). During the last decade, the analysis of the prokaryotic diversity and distribution patterns has received growing attention (Martiny *et al.*, 2006; Van der Gucht *et al.*, 2007). Nevertheless, although there is increasing information, the great variability in lake properties (physical, chemical and biological), as well as the climate conditions of the region where lakes are located, requires more insight into their microbial structure. Variability in environmental conditions across space and time represents habitat heterogeneity, which shapes biological communities (Shade *et al.*, 2008; Stocker and Seymour, 2012). Ecological niche separation of coexisting microbial taxa might be triggered by resource availability (bottom-up control) and/or mortality factors (top-down control), leading to distinct within-lake spatial patterns (longitudinal and vertical) and temporal patterns of distribution of the different microbes (Salcher, 2014). These spatial patterns can range from micro-scale (μm–mm) to larger scales (m–km) patchiness (e.g. Green and Bohannan, 2006; Pinel-Alloul and Ghadouani, 2007; Van der Gucht *et al.*, 2007; Salcher *et al.*, 2011).

Molecular techniques have resulted in significant advances in knowledge of prokaryotic diversity, allowing the evaluation of patterns in the spatial and temporal distribution of *Bacteria* (Logue and Lindström, 2008). On the other hand, though *Archaea* have been found to be ubiquitous in a variety of freshwater habitats (Schleper *et al.*, 2005; Chaban *et al.*, 2006; Casamayor and Borrego, 2009), less is known about their seasonal variations and their spatial patterns among lakes (see Keough *et al.*, 2003; Auguet and Casamayor, 2008, 2012; Auguet *et al.*, 2010), as well as their relative contribution to the microbial communities in different types of lakes.

Many studies on prokaryotic plankton communities, both in marine and freshwater ecosystems, have highlighted the importance of different factors shaping their community structure (e.g. Lindström *et al.*, 2005; Šimek *et al.*, 2005; Logue *et al.*, 2008; Corno and Jürgens, 2008; Barberán and Casamayor, 2010). These studies suggest that both intrinsic (e.g. dispersal rate, trophic factors) and extrinsic (such as latitude, ecosystem size, habitat isolation) factors are involved in the turnover of community composition in space and time. The relevance of each factor varies across large geographical gradients (Soininen, 2010; Schiaffino *et al.*, 2011). Among the factors that regulate prokaryotic assemblages, some of the most important are temperature, ultraviolet radiation, quality and quantity of dissolved organic matter, nutrient concentrations and grazing pressure (e.g. Lindström *et al.*, 2005; Pernthaler, 2005; Glaeser *et al.*, 2010; Newton and McMahon, 2011; Logue *et al.*, 2015), which can vary in relation to the geographic position, watershed and surrounding landscape of the lakes. In addition, the seasonal changes in environmental conditions, such as temperature or nutrient concentrations, affect prokaryotic community composition (Donner *et al.*, 1996; Yannarell *et al.*, 2003; Rösel *et al.*, 2012). Recent reports based on high-frequency multiyear datasets of site-specific studies have shown that seasonal patterns in bacterial community structure recur in freshwater ecosystems and these seasonal patterns indicate that some microbial communities change directionally according to environmental conditions (Rösel *et al.*, 2012; Kara *et al.*, 2013; Tammert *et al.*, 2015).

The domains *Bacteria* and *Archaea* (Woese and Fox, 1977) encompass virtually all possible genetic diversity and lifestyles. Although there are unifying traits for freshwater bacteria in each phylum, there are also ecological divergences at this higher taxonomic rank (Newton *et al.*, 2011). *Actinobacteria*, a cosmopolitan freshwater phylum, is often a numerically dominant group in lakes, and their small cell sizes constitute a key strategic characteristic against predators and UV damage (Pernthaler *et al.*, 2001; Warnecke *et al.*, 2005). A large group of bacteria, the phylum *Cytophaga–Flavobacterium–Bacteroidetes* (hereafter referred to as *Bacteroidetes*) has also been reported from freshwater systems, and are known to have an important role in biopolymer degradation (Kirchman, 2002). Within the phylum *Proteobacteria*, Class *Alphaproteobacteria* are numerically dominant in marine ecosystems (Morris *et al.*, 2002), but also abundant and ubiquitous in freshwater (Newton *et al.*, 2011; Salcher *et al.*, 2011; Gerea *et al.*, 2013). Members of this group were found to be good competitors in freshwater ecosystems at low nutrient concentrations, being able to degrade a variety of organic compounds (Salcher *et al.*, 2013; Salka *et al.*, 2014). *Betaproteobacteria* are abundant in freshwater systems (Glöckner *et al.*, 1999) and some subgroups within it are favored by enriched nutrient conditions (Newton *et al.*, 2011), whereas *Gammaproteobacteria* are more abundant in oceans (Rusch *et al.*, 2007) or saline lakes (Wu *et al.*, 2006) than in freshwater environments. Finally, the domain *Archaea* contains kingdoms *Proteoarchaeota* (including *Crenarchaeota*, *Thaumarchaeota*, *Aigarchaeota*, *Korarchaeota*) and *Euryarchaeota* (Petitjean *et al.*, 2015). *Archaea* were initially thought to thrive in extreme environments as described by temperature, pH and salinity (Karlin *et al.*, 2005), but recent observations suggest that they are also present in more moderate habitats (Massana *et al.*, 2000; Auguet *et al.*, 2010).

We studied the distribution patterns of the abundance of major bacterial and archaeal groups in 35 Patagonian

water bodies located along 1500 km of distance, analyzing the among-lakes pattern, and the intra-annual within-lake pattern in two selected lakes with contrasting DOC. We also studied the relationship between these patterns and the environmental variables. Assuming that the lake characteristics influence the microbial composition, we hypothesized that the large differences among the lakes studied (location and environmental variables) are clearly reflected in the distribution patterns of the prokaryotic group abundances. To maximize the effort, we used seven probes in the catalyzed reporter deposition-fluorescence *in situ* hybridization (CARD-FISH) methodology and compared the data with a diversity analysis previously obtained in the same set of lakes using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) (Schiaffino *et al.*, 2011).

METHOD

Study sites

We sampled 35 freshwater bodies located in the Andean Patagonia and Patagonian Plateau, from 72° 55′ to 67° 21′ W of longitude, and along a gradient of 1500 km of distance, from 41° 03′ to 54° 52′ S of latitude (from Río Negro to Tierra del Fuego Provinces, Argentinean Patagonia). Different types of water bodies ranging in area from 0.0003 to 1419 km^2 (mean: 152 km^2) were sampled: deep lakes (L), shallow lakes (SL) and ponds (P). Descriptions of the regions studied, geographic positions of the lakes, as well as their main limnological characteristics were detailed in Schiaffino *et al.* (Schiaffino *et al.*, 2013) and Gerea (Gerea, 2013). The trophic states of the water bodies was characterized according to levels of phytoplankton chlorophyll *a* (chl *a*) and nutrient concentrations, using the reference values proposed by Wetzel (Wetzel, 2001).

Sampling design, environmental and biotic parameters

The 35 water bodies were sampled once in the euphotic zone during spring (2007, 2008 and 2009), whereas for studying the intra-annual seasonality, samples were collected monthly between January 2010 and January 2011 (except in May, August and December) from the euphotic zone of 2 of the 35 water bodies (Lakes Morenito and Escondido, Andean Patagonia). In deep lakes, integrated samples comprising the first 5 m were collected within the epilimnion, whereas in shallow lakes and ponds, samples were obtained from about 30 cm below the surface. Temperature, pH, conductivity and dissolved oxygen (DO) were measured *in situ* with portable meters (Horiba D-54 meter, Kyoto, Japan and Hanna HI 9146, Villafranca, Italy). Nutrients (ammonium N-NH_4, nitrate N-NO_3, nitrite N-NO_2 and phosphate P-PO_4), chl *a*, dissolved organic carbon (DOC) and diffuse attenuation coefficient (K_{dPAR}) were analyzed following the methods described in Schiaffino *et al.* (Schiaffino *et al.*, 2011) and Gerea (Gerea, 2013). Dissolved inorganic nitrogen (DIN) was defined as the sum of nitrate, nitrite and ammonium. Heterotrophic flagellates (HF) were enumerated using epifluorescence microscopy (Olympus BX40F4, Japan) with blue and UV wavelength excitations on samples filtered through 0.6-μm pore-size polycarbonate filters. Samples were previously fixed with cold glutaraldehyde 10% (1% final concentration) and stained with 4,6-diamino-2-phenylindole (DAPI, 10 μg mL^{-1}, final concentration).

Catalyzed reporter deposition-fluorescence *in situ* hybridization

Major bacterial and archaeal groups (archaeal kingdoms, bacterial phyla and proteobacterial classes) were detected by CARD-FISH using group-specific phylogenetic probes. Water samples were fixed with formaldehyde (2% final concentration) and aliquots between 0.1 and 5 mL were concentrated on 0.2-μm pore-size white polycarbonate filters, which were dried and kept frozen until processed. Whole-cell *in situ* hybridizations of sections from the polycarbonate filters were performed as described by Pernthaler *et al.* (Pernthaler *et al.*, 2002) and Sekar *et al.* (Sekar *et al.*, 2003) using the following oligonucleotide probes: EUB338-II-III, to target most *Bacteria* including *Verrucomicrobia* and *Planctomycetes* (Amann *et al.*, 1990; Daims *et al.*, 1999); ALF968, specific for *Alphaproteobacteria* (Neef, 1997); BET42a, specific for *Betaproteobacteria* (Manz *et al.*, 1992); GAM42a, to target *Gammaproteobacteria* (Manz *et al.*, 1992); CF319a, to target the *Cytophaga–Flavobacterium–Bacteroidetes* group (hereinafter *Bacteroidetes*) (Manz *et al.*, 1996); HGC69a, specific for *Actinobacteria* (Amann *et al.*, 1995); CREN554, to target *Crenarchaeota* (Massana *et al.*, 1997); and EURY806, specific for *Euryarchaeota* (Teira *et al.*, 2004). The probes were supplied by Thermo Electron Corporation (Waltham, MA, USA) with an aminolink (C6) at the 5′ end, ligated with a horseradish peroxidase enzyme (Urdea *et al.*, 1988). After hybridization, the signal was amplified with Alexa 488-labeled tyramide and counter-stained with DAPI. Filter pieces were mounted on a slide and observed by epifluorescence microscopy (Olympus BX40F4, Japan) under blue light and UV excitation. We used two different abundances: the relative abundance, defined as the contribution of each bacterial and archaeal group to the

prokaryotic community, which was always calculated as a percentage of DAPI counts (% of DAPI); and the absolute cell abundances, obtained from each probe counts (cells mL^{-1}). Hybridization efficiency was defined as the fraction of *Bacteria* + *Archaea* (DAPI-stained cells) hybridized with probes EUB338-II-III + EURY806 + CREN554.

Polymerase chain reaction-denaturing gradient gel electrophoresis

The bacterial PCR-DGGE raw data of Patagonian lakes presented in another context in Schiaffino *et al.* (Schiaffino *et al.*, 2011) was used to perform a cluster analysis that was compared with that obtained from CARD-FISH data (absolute abundances of bacterial groups).

Statistical analysis

Correlations between all variables were carried out using Spearman's ρ tests. Using the bacterial PCR-DGGE band intensity pattern obtained from Schiaffino *et al.* (Schiaffino *et al.*, 2011) and the CARD-FISH absolute abundances of all bacterial groups, we constructed dissimilarity matrices among lakes. Cluster analyses (hierarchical clustering) were performed using Bray Curtis index and the Ward agglomeration method. Correlation between the CARD-FISH and PCR-DGGE dissimilarity matrices was performed with Mantel test (Spearman's correlation) using the same set of lakes. Variability in the abundances of each bacterial and archaeal group among water bodies (oligotrophic, mesotrophic and eutrophic) was identified using one-way analysis of variance (ANOVA) and Tukey-Kramer means *post hoc* comparison tests ($\alpha = 0.05$). Linear regression analyses between each log-transformed prokaryotic group and log-transformed independent environmental variables (chl *a*, temperature and DOC) were also performed. Equations of the regressions are presented as $\log(Y) = a + b \log(X)$, with $Y =$ cells mL^{-1}, $a =$ intercept, $b =$ slope, $X =$ independent variable (chl *a* in $\mu g\ L^{-1}$, temperature in °C, DOC in $mg\ L^{-1}$). Finally, to compare linear regressions and test for heterogeneity of slopes, analysis of covariance (ANCOVA) tests were conducted. The majority of variables for statistical tests were log10-transformed to correct deviations from normality and homoscedasticity, which were controlled respectively with Kolmogorov–Smirnov and Levene's tests ($\alpha = 0.01$). All these tests were performed with software XLSTAT (Addinsoft SARL, NY, USA) and SPSS 15.0.1 (StatSoft).

To identify the environmental factors controlling the among and within-lake planktonic prokaryote distribution patterns, we performed redundancy analyses (RDA) with a quantitative community matrix constructed with the absolute and relative abundances of the bacterial and archaeal groups and a second matrix obtained from environmental data corresponding to the same samples. RDA was used because a Detrended Correspondence Analysis performed with the quantitative community matrix determined that the gradient length along the first ordination axis was <3 standard deviation (SD), indicating that prokaryotic abundances showed a linear response (ter Braak and Smilauer, 2002). Multivariate analyses were performed with software CANOCO (ter Braak, 1991). Forward selection was used for adding environmental variables to the models and variables strongly correlated among them were eliminated from the analyses as they provided redundant information. Significance of the canonical axes was assessed using Monte Carlo permutation tests ($\alpha = 0.05$). The community absolute abundance matrix was Hellinger-transformed prior to applying multivariate methods (Legendre and Gallagher, 2001; Ramette, 2007).

RESULTS

Among-lake variation of bacterial and archaeal communities: 35 water bodies sampled once

The average cell detection with EUB338-II-III + EURY806 + CREN554 probes (hybridization efficiency) was 70% (SD = 20.0%, $n = 35$) of all DAPI-stained cells. Average relative abundance (% of DAPI counts) and average absolute abundance of each bacterial and archaeal group in the 35 water bodies are shown in Table I. *Alphaproteobacteria* was the best represented group followed by *Betaproteobacteria* and *Actinobacteria*, while *Gammaproteobacteria* and both archaeal groups were the least represented ones (Table I). In general, *Crenarchaeota* (0.5%, 1.4×10^4 cell mL^{-1}) was better represented than *Euryarchaeota* (0.1%, 4.0×10^3 cell mL^{-1}) along the gradient of lakes. Particularly, the eutrophic Lake Colhué Huapi (Patagonian Plateau) showed the highest relative and absolute abundances of *Crenarchaeota* (5.2%, 1.1×10^5 cell mL^{-1}) and *Euryarchaeota* (0.6%, 2.8×10^4 cell mL^{-1}). We also observed that all bacterial groups (except *Actinobacteria*) showed significantly higher absolute abundances in mesotrophic and eutrophic water bodies than in oligotrophic ones, whereas *Actinobacteria* and the archaeal groups did not change among lake trophic states (Table 1b). *Bacteroidetes* made a significantly higher relative contribution to community structure (relative abundance) in mesotrophic water bodies, when compared with oligotrophic ones (Table 1a).

Results of the RDA analyses using prokaryotic absolute and relative abundances versus environmental variables (both Monte Carlo's test for significance of first canonical

Table I: Relative contributions to community composition as percentages of total DAPI counts (a) and absolute abundances in cells mL^{-1} (b) of each studied bacterial and archaeal group

	Alphaproteobacteria	*Betaproteobacteria*	*Gammaproteobacteria*	*Actinobacteria*	*Bacteroidetes*	*Crenarchaeota*	*Euryarchaeota*
a							
O ($n = 16$)	28.3 a	15.5 a	0.7 a	9.7 a	6.4 b	0.6 a	0.2 a
M ($n = 14$)	32.8 a	25.4 a	0.7 a	15.3 a	12.8 a	0.1 a	0.1 a
E ($n = 5$)	27.2 a	15.6 a	0.8 a	7.5 a	6.6 ab	1.1 a	0.1 a
F-value	0.5	2.4	0.07	1.3	3.6	2.4	3.0
P-value	0.620	0.107	0.937	0.300	0.040	0.104	0.066
All lakes ($n = 35$)	30.0 (4.8–60.7)	19.5 (2.6–51.8)	0.7 (0.04–2.5)	11.7 (0.1–43.0)	9.0 (1.2–39.6)	0.5 (0.002–5.2)	0.1 (b.d.–0.6)
b							
O ($n = 16$)	6.2×10^5 b	3.1×10^5 b	1.2×10^4 b	2.3×10^5 a	1.1×10^5 b	7.4×10^3 a	3.0×10^3 a
M ($n = 14$)	3.1×10^6 a	2.2×10^6 a	4.9×10^4 a	1.2×10^6 a	6.9×10^5 a	1.6×10^4 a	3.1×10^3 a
E ($n = 5$)	9.3×10^6 a	4.5×10^6 a	1.0×10^5 a	1.4×10^6 a	1.6×10^6 a	3.1×10^4 a	9.6×10^3 a
F-value	14.5	9.7	10.1	2.7	17.4	2.7	2.3
P-value	0.0001	0.001	0.0001	0.079	0.0001	0.080	0.115
All lakes ($n = 35$)	2.9×10^6 (5.0×10^4–3.1×10^7)	1.7×10^6 (2.5×10^4–1.2×10^7)	4.0×10^4 (1.9×10^3–3.1×10^5)	7.7×10^5 (3.7×10^3–4.9×10^6)	5.5×10^5 (2.2×10^4–4.4×10^6)	1.4×10^4 (4.0×10^1–1.8×10^5)	4.0×10^3 (b.d.–2.8×10^4)

Ranges are given in parentheses and represent maximum and minimum values. One-way ANOVA test and *post hoc* Tukey-Kramer's test for unequal *n* were used to identify significant differences. Means in the same column followed by different letters (a or b) are significantly different at $P < 0.05$; b.d., below detection; O, oligotrophic; M, mesotrophic; E, eutrophic.

axis $P < 0.020$ and for all canonical axes $P < 0.004$) from the 35 studied water bodies showed differences (Fig. 1a and b). Only the RDA performed with absolute abundances showed the ordination of samples by lake trophic states and temperature (Fig. 1a), whereas this separation of water bodies was not clear in the RDA performed with prokaryotic relative abundances (Fig. 1b). The former analysis showed that samples located at the southern part of the spatial gradient (Tierra del Fuego Island) and many oligotrophic and large water bodies are situated in the left side of the graph with higher values of DO, whereas the majority of the eutrophic and mesotrophic shallow lakes and ponds are plotted together in the right side of the graph with higher values of DOC, temperature, conductivity, DIN and chl *a*, together with higher absolute abundances of all prokaryotic groups studied (Fig. 1a). However, the RDA performed with relative abundances (Fig. 1b) showed a clear separation of archaeal and *Gammaproteobacteria* groups (the less dominant studied prokaryotic groups) from the other bacterial groups (whose relative abundances also increased with higher values of DOC and temperature). Interestingly, in both RDA analyses (Fig. 1a and b), the most important and significant variable was DOC ($P < 0.044$), whereas temperature was also significant when using absolute abundances ($P = 0.002$, Fig. 1a), and conductivity when using relative abundances ($P = 0.044$, Fig. 1b).

Consistent with the analyses above, the absolute abundances of all bacterial groups correlated positively with temperature, DOC and HF, whereas only the relative abundance of *Bacteroidetes* increased with these variables (Supplementary data, Table SI). All the bacterial groups, except *Gammaproteobacteria* and *Actinobacteria*, showed decreased absolute and relative abundances with larger lake areas. Lake water temperature was not only correlated negatively to latitude but also to lake area ($r = -0.61$ and $r = -0.46$, both $P < 0.007$, respectively). *Archaea* did not show any significant correlation. The ratios between *Bacteria*:*Archaea* absolute abundances were positively related to temperature, DOC and HF, and negatively related to lake area. Only the highest correlations obtained with the absolute abundances were also significant correlations with the relative abundances (values highlighted in gray in Supplementary data, Table SI a and b, respectively).

In order to further study the responses of each prokaryotic group as a function of some environmental variables, the absolute abundances of each bacterial and archaeal group were regressed against chl *a* as a proxy of lake productivity level, temperature and DOC. All bacterial equations were significant (ANOVA, $P < 0.016$) except *Actinobacteria* abundance versus DOC, (Table II) and showed positive slopes, whereas not a single archaeal

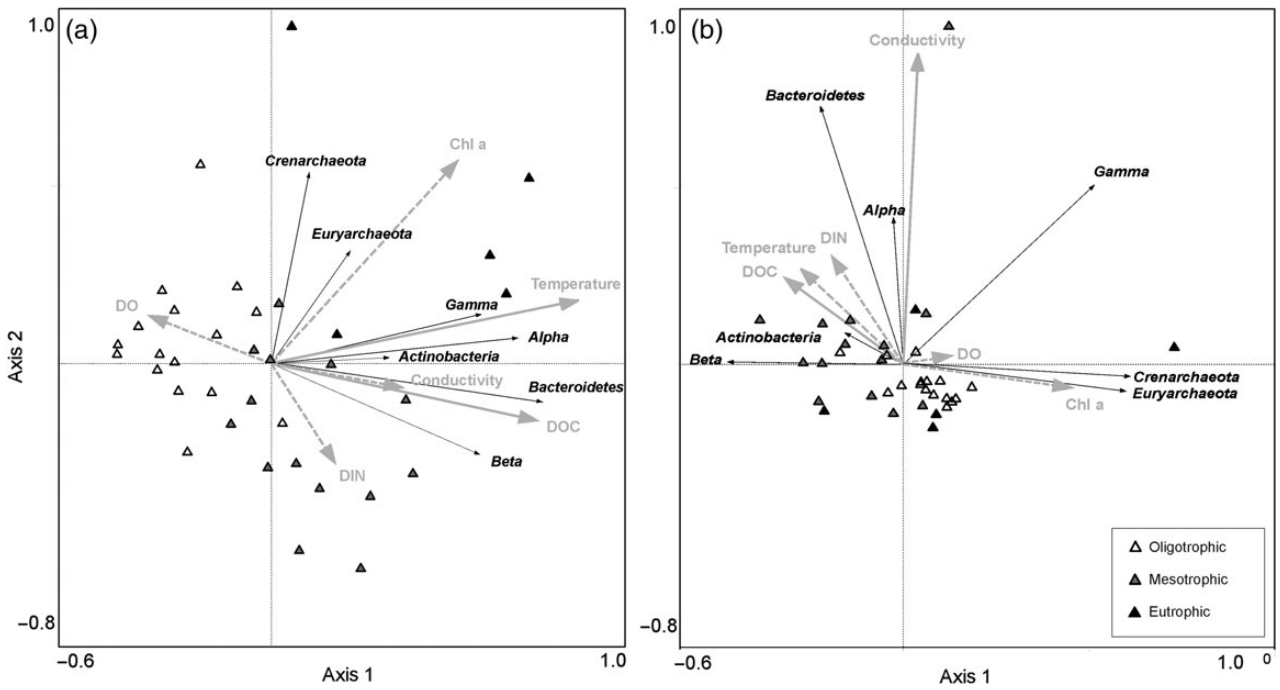

Fig. 1. Redundancy analysis (RDA) using the among-lake bacterial and archaeal group absolute (**a**) and relative (**b**) abundances and environmental data ($n = 35$). Significant environmental variables ($P < 0.05$) are indicated with gray solid arrows, whereas gray dashed arrows are not significant. Thin black arrows show the different bacterial and archaeal groups studied. DOC: dissolved organic carbon, DO: dissolved oxygen, Chl *a*: chlorophyll *a* content, DIN: dissolved inorganic nitrogen, Gamma: Gammaproteobacteria, Alpha: Alphaproteobacteria, Beta: Betaproteobacteria.

Table II: Regression analyses of log-transformed prokaryotic absolute abundance versus log-transformed environmental variables. Chl a: chlorophyll a; DOC, dissolved organic carbon; temperature

	Chl *a*				Temperature				DOC			
	R^2 corrected	Intercept ± SE	Slope ± SE	*P*-value	R^2 corrected	Intercept ± SE	Slope ± SE	*P*-value	R^2 corrected	Intercept ± SE	Slope ± SE	*P*-value
DAPI	0.40	6.55 ± 0.06	0.40 ± 0.08	**0.0001**	0.32	4.81 ± 0.43	1.72 ± 0.42	**0.0001**	0.46	5.53 ± 0.20	0.99 ± 0.18	**0.0001**
Alphaproteobacteria	0.25	6.02 ± 0.09	0.41 ± 0.11	**0.001**	0.36	3.74 ± 0.52	2.24 ± 0.50	**0.0001**	0.35	4.90 ± 0.27	1.08 ± 0.24	**0.0001**
Betaproteobacteria	0.21	5.71 ± 0.10	0.39 ± 0.13	**0.004**	0.16	4.01 ± 0.64	1.68 ± 0.61	**0.0100**	0.34	4.54 ± 0.29	1.13 ± 0.26	**0.0001**
Gammaproteobacteria	0.20	4.25 ± 0.08	0.33 ± 0.10	**0.004**	0.18	2.74 ± 0.52	1.48 ± 0.50	**0.006**	0.26	3.39 ± 0.26	0.83 ± 0.23	**0.0010**
Actinobacteria	0.17	5.31 ± 0.13	0.46 ± 0.17	**0.009**	0.14	3.26 ± 0.83	2.02 ± 0.79	**0.016**	0.08	4.50 ± 0.44	0.79 ± 0.39	0.054
Bacteroidetes	0.31	5.26 ± 0.09	0.47 ± 0.12	**0.0001**	0.38	2.79 ± 0.55	2.43 ± 0.52	**0.0001**	0.47	3.91 ± 0.26	1.30 ± 0.23	**0.0001**
Crenarchaeota	0.08	3.53 ± 0.12	0.30 ± 0.15	0.056	0.01	2.60 ± 0.78	0.92 ± 0.75	0.23	−0.01	3.84 ± 0.41	0.27 ± 0.37	0.467
Euryarchaeota	0.08	3.23 ± 0.11	0.27 ± 0.14	0.064	−0.01	2.65 ± 0.76	0.58 ± 0.72	0.42	−0.03	3.29 ± 0.38	0.02 ± 0.34	0.943

Parameters details of the different regressions follow the next form: log prokaryotic abundance (cell mL^{-1}) = intercept (± SE) + slope (± SE) × log environmental variable. Bold values are significant ($P < 0.05$), $n = 35$. The significant slopes are highlighted in gray. SE, standard error.

equation was significant (ANOVA, $P > 0.056$, Table II). We tested for the significance of the homogeneity of slopes, performing an ANCOVA that included interaction of covariates. The homogeneity of slopes assumption was only rejected for DOC (ANCOVA, $P = 0.001$, $n = 35$), indicating that only the slopes between prokaryotic absolute abundances and DOC differed among groups, whereas the slopes of prokaryotic absolute abundances versus chl *a* (ANCOVA, $P = 0.944$, $n = 35$), and temperature (ANCOVA, $P = 0.424$, $n = 35$) did not differ among groups (each prokaryotic group abundance responded similarly to chl *a* or temperature). The highest slope between absolute bacterial abundance and DOC was found for *Bacteroidetes* and the lowest for the archaeal groups (Table II). The slopes of all bacterial groups (except *Actinobacteria*) significantly differed when compared with the slopes of archaeal groups (Tukey tests, $P < 0.05$). The slope of *Actinobacteria* did not differ with any other prokaryote groups studied (Tukey tests, $P > 0.05$).

The correlation between the dissimilarity matrices obtained with the CARD-FISH absolute abundances of bacterial groups and the bacterial PCR-DGGE band intensity pattern for the same set of Patagonian lakes was positive and significant (Mantel test, $r = 0.25$, $P < 0.0001$, $n = 29$). With these approaches (CARD-FISH and PCR-DGGE) two main groups were observed and in general the deep, large and oligotrophic water bodies clustered together (Group II, Fig. 2), but the small, shallow and meso-eutrophic water bodies grouped separately and formed two subgroups (Group I, Fig. 2).

Within-lake variation of bacterial and archaeal communities: 2 lakes sampled during 10 months

As mentioned, from the multivariate analyses, we observed that DOC was one of the most important variables explaining the prokaryotic among-lake pattern. Taking into account these results, we selected two lakes with contrasting DOC concentrations (lakes Morenito and Escondido) in order to analyze the within-lake pattern. These lakes, located near the Andes, are cold polymictic and are separated about 3.7 km each other. Both are shallow (maximum depth < 10 m) and have a glacial origin. In general, both lakes showed similar temporal patterns of physical and chemical parameters during the study period (Fig. 3). As expected, the lowest temperatures were observed during winter (Fig. 3a). On all sampling dates, Lake Escondido (a humic-acid-rich lake) had higher DOC concentrations than Lake Morenito, but both showed a peak in March (late summer) (Fig. 3b). In general, Lake Morenito showed higher biotic values (chl *a* and total prokaryotes) than

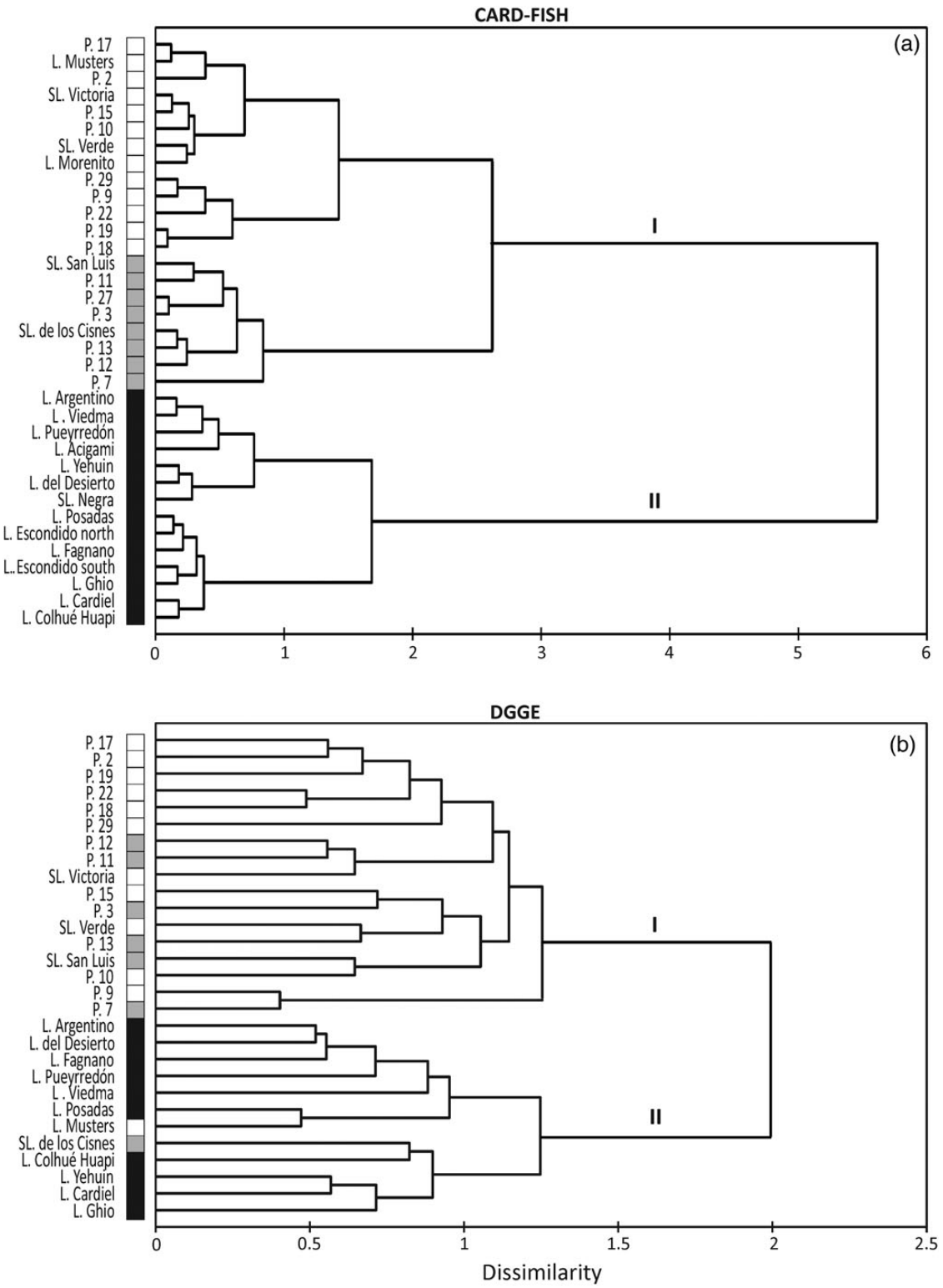

Fig. 2. Cluster analysis using Bray Curtis index performed with data obtained from CARD-FISH (**a**) ($n = 35$ water bodies) and PCR-DGGE (**b**) ($n = 29$) bacterial band intensity pattern using the same set of water bodies. SL: shallow lake, P: pond, L: lake. Group I, squares highlighted in gray and white (showing two subgroups) and Group II squares highlighted in black.

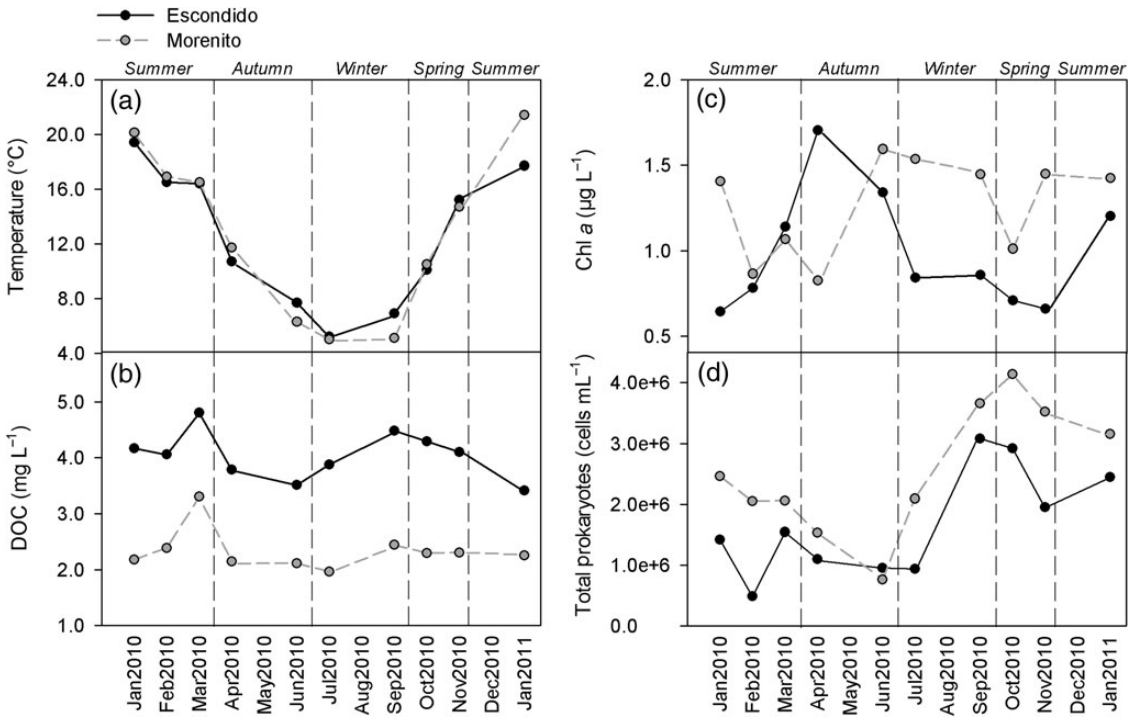

Fig. 3. Monthly variation in Lake Morenito (gray circles) and Escondido (black circles) of abiotic (physical and chemical) and biotic parameters: temperature (**a**), dissolved organic carbon (DOC) (**b**), chlorophyll *a* (Chl *a*) (**c**), total heterotrophic prokaryotes (sum of all studied probes) (**d**).

Lake Escondido (Fig. 3c and d). In autumn, phytoplankton biomass (measured as chl *a*, Fig. 3c) showed a peak, whereas total abundances of heterotrophic prokaryotes were lower (Fig. 3d). Chl *a* was positively correlated with total nitrogen ($r = 0.66$, $P = 0.002$, $n = 20$) and negatively with DOC ($r = -0.55$, $P = 0.011$, $n = 20$).

Average hybridization efficiency, defined as the portion of *Bacteria* + *Archaea* hybridized with probes EUB338-II-III + EURY806 + CREN554, was 77% (SD = 12.5%, $n = 20$). The prokaryotic groups studied varied seasonally in their absolute abundances over the year (Fig. 4). All the bacterial groups showed simultaneous peaks during late winter or spring (Fig. 4a and c), whereas their relative abundances were roughly similar in all seasons (Fig. 5). *Actinobacteria* and *Alphaproteobacteria* were the most abundant groups followed by *Betaproteobacteria*. *Gammaproteobacteria* was the least abundant bacterial group (<1.2% and $<2.0 \times 10^4$ cell mL^{-1}). All bacterial groups, and particularly *Bacteroidetes*, were much more abundant in Lake Morenito than in the humic Lake Escondido on all sampling dates (Fig. 4a and c, Fig. 5). *Archaea* never exceeded 6% of total DAPI counts, but their absolute and relative abundances varied during the study period (Fig. 4b and d, Fig. 5). On average, Lake Morenito showed higher relative and absolute abundances of *Euryarchaeota* than *Crenarchaeota*, with maximum archaeal values in late spring and summer 2011, whereas in Lake Escondido, *Crenarchaeota* were in general more abundant and showed peaks during summer 2010 and 2011, and winter (Figs 4b and d, and 5).

Results of the RDA using prokaryotic absolute and relative abundances versus environmental variables (Monte Carlo's test for significance of first canonical axis, $P < 0.040$, and for all canonical axes, $P < 0.010$) from each sampling date of both lakes are shown in Fig. 6a and b. In both graphs, most of the samples from the humic-acid-rich Lake Escondido are plotted together in the left side of the graph with higher levels of DOC together with higher absolute abundances of *Gammaproteobacteria*. Most samples from Lake Morenito are placed together in the right side of the graph with higher values of conductivity, simultaneous to higher abundances of *Alphaproteobacteria* and *Bacteroidetes*. Furthermore, in both graphs *Crenarchaeota* and *Euryarchaeota* groups are ordinated together with higher temperatures and pH values. However, *Actinobacteria* and *Betaproteobacteria* showed different ordination when using absolute (Fig. 6a) or relative (Fig. 6b) abundances. The absolute abundances of both groups were higher together with higher values of DOC (Fig. 6a), but when considering their contribution to total community structure (Fig. 6b), their relative abundances decreased together with higher values of DOC. In addition, these analyses did not show a marked ordination of samples by season. Even though all samples from

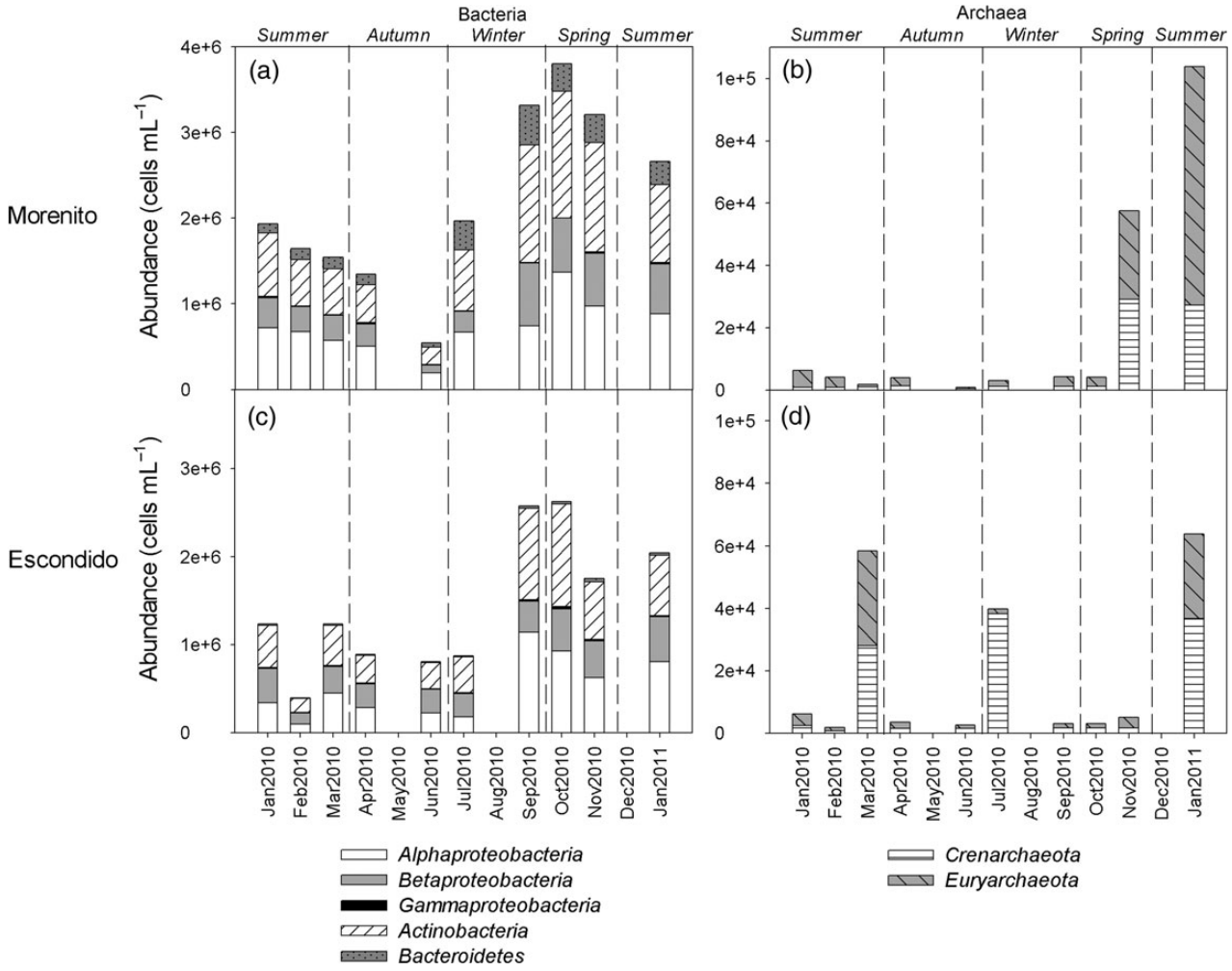

Fig. 4. Monthly absolute abundances of the different bacterial groups in Lakes Morenito (**a**) and Escondido (**c**) and different archaeal groups in Lakes Morenito (**b**) and Escondido (**d**).

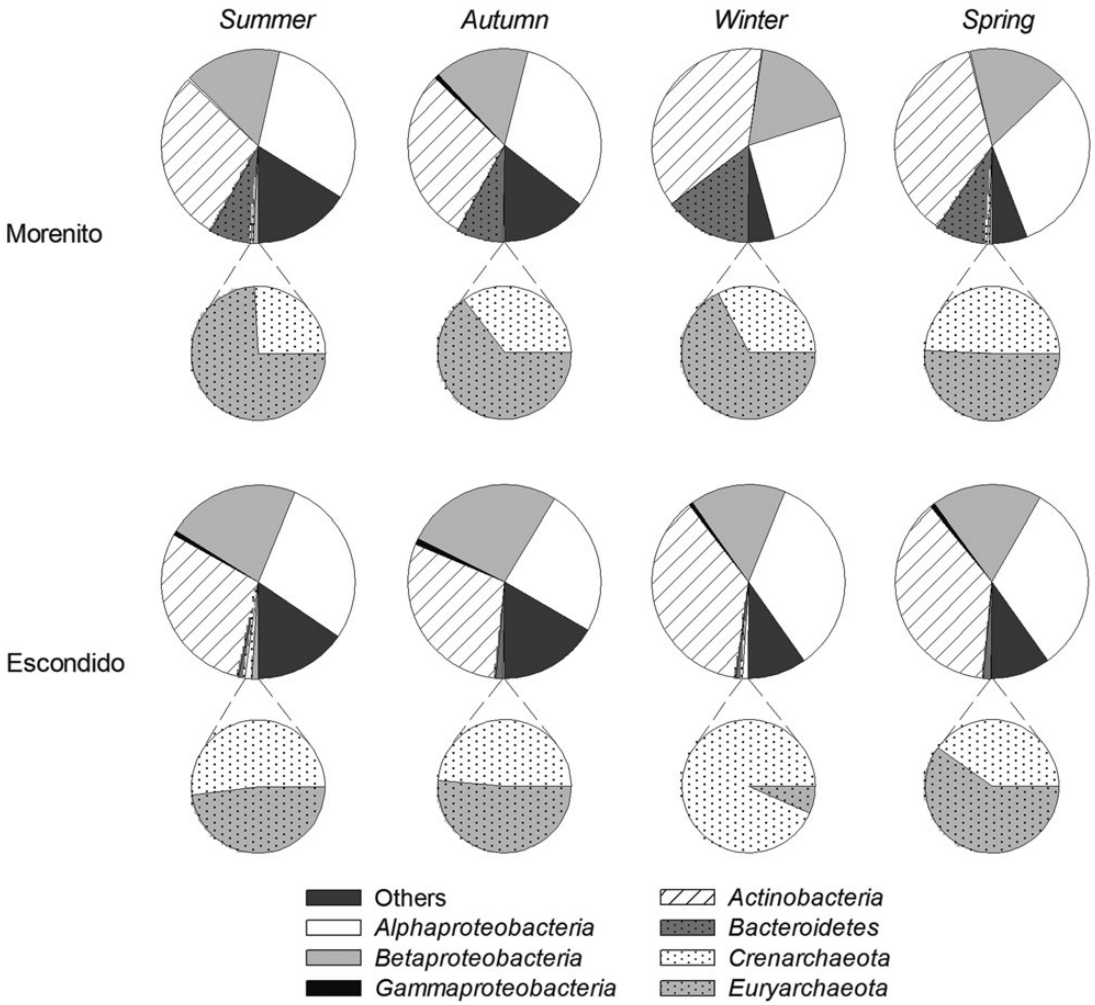

Fig. 5. Average seasonal relative abundances (hybridization percentage of total DAPI counts) of bacterial and archaeal groups in Lakes Morenito and Escondido. Larger pie charts correspond to the total composition (including all bacterial and archaeal groups), the below smaller pie charts show a detail for the archaeal groups. "Others" stand for cells detected by DAPI but not hybridized by the used bacterial and archeal probes.

winter are plotted together at lower temperatures, not all samples from summer are ordinated with higher temperatures in the upper side of the graph (Fig. 6a and b). The RDA analysis showed that the most important and significant variable shaping the absolute abundances was DOC ($P = 0.002$); temperature and conductivity showed lower significances ($P = 0.052$ and $P = 0.054$, respectively) (Fig. 6a). The relative abundances were affected by DOC and pH ($P = 0.024$ and $P = 0.028$, respectively), while conductivity showed a lower significance ($P = 0.080$). The other variables included in the analyses were not significant.

Correlation analyses (Spearman's ρ test, $n = 20$) between the abundance (both absolute and relative) of each group and environmental variables are consistent with the previous analysis, showing that *Euryarchaeota* absolute abundances increased with higher temperatures ($r = 0.59$, $P = 0.007$), and not only the absolute but also their relative abundances increased with increasing levels of pH ($r = 0.53$ and $r = 0.49$, both $P < 0.03$). *Crenarchaeota* absolute abundances also correlated with pH ($r = 0.46$, $P = 0.04$), and their absolute and relative abundances increased with increasing levels of DOC ($r = 0.46$ and $r = 0.56$, both $P < 0.04$). The absolute and relative abundances of *Bacteroidetes* increased with decreasing DOC values ($r = -0.67$ and $r = -0.64$, both $P < 0.003$) and with increasing values of conductivity ($r = 0.62$ and $r = 0.60$, both $P < 0.006$). The ratios *Bacteria*:*Archaea* of absolute abundances were negatively related to pH values ($r = -0.54$, $P = 0.014$) and to temperature ($r = -0.48$, $P = 0.033$).

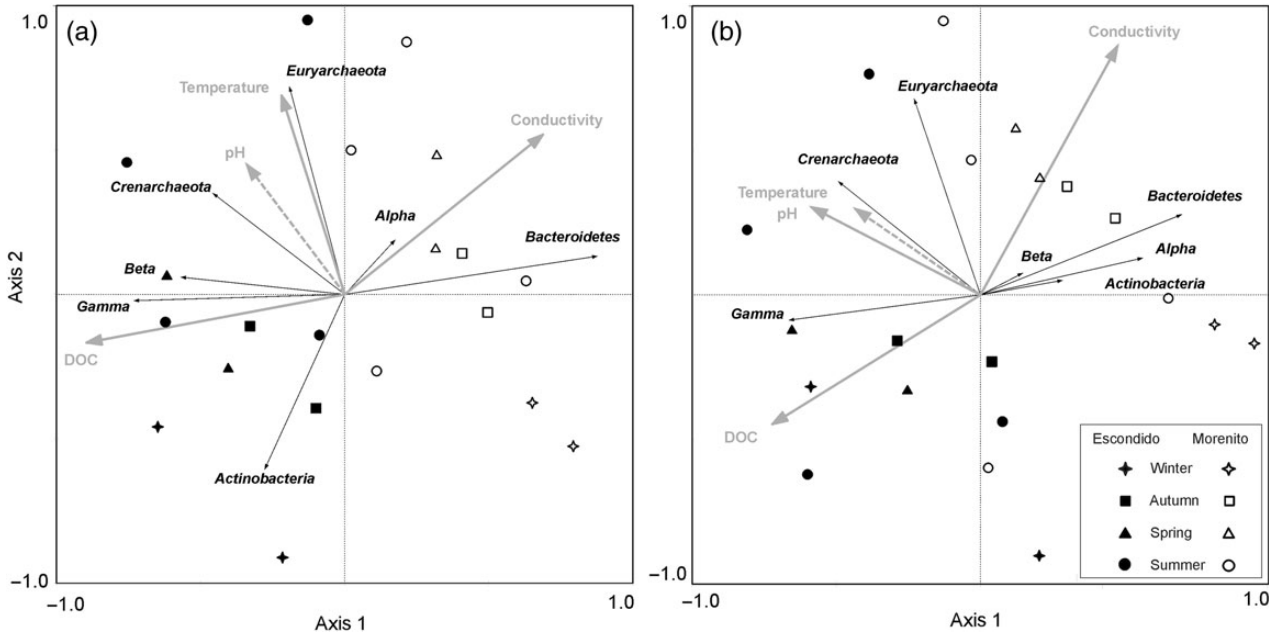

Fig. 6. Redundancy analysis (RDA) based on the within-lake (intra-annual seasonality) bacterial and archaeal group absolute (**a**) and relative (**b**) abundances and environmental data ($n = 20$) from Lakes Morenito and Escondido. Significant ($P < 0.05$) and almost significant ($P < 0.08$) environmental variables are indicated with gray solid arrows, whereas gray dashed arrows indicate not significant variables ($P > 0.08$). Thin black arrows show the different bacterial and archaeal studied groups. DOC: dissolved organic carbon, Gamma: Gammaproteobacteria, Alpha: Alphaproteobacteria, Beta: Betaproteobacteria.

DISCUSSION

We studied the among-lake and within-lake distribution patterns of heterotrophic prokaryote community structure at the kingdom, phylum and class level of taxonomic resolution, and observed that local environmental variables strongly shaped both bacterial and archaeal group abundances. Linkages of prokaryotic distribution (in absolute and relative abundances) with environmental parameters were examined by RDA analyses (Figs. 1 and 6). This approach, like any multivariate analysis, provides insights into complex correlations and interdependencies, and revealed relations of community structure to environmental parameters with some distinct variations depending on whether the analysis was done using absolute (DOC and temperature, Figs. 1a and 6a) or relative (DOC, pH, conductivity, Figs. 1b and 6b) prokaryotic abundances. In the among-lake and within-lake patterns, the multivariate analyses showed that DOC was one of the most important variables structuring the community at the taxonomic resolution used, for both absolute and relative abundances, whereas temperature was an important driver shaping their absolute, but not relative abundances. Absolute and relative abundances give different information about the community, the former provide an idea of the population sizes, biomass and turnover, while the latter indicates the dominance of a specific group over the others. It has been postulated that planktonic prokaryotes are mainly determined by substrate availability and grazing (Jürgens and Güde, 1994) together with environmental conditions (Allgaier and Grossart, 2006). Some of the most important physical and chemical factors that regulate bacterial assemblages are temperature, organic matter and nutrient concentrations (Logue *et al.*, 2008). Particularly, DOC is a relevant energy source for bacterioplankton communities (e.g. Eiler *et al.*, 2003; Kritzberg *et al.*, 2006), and it is the main driver dominating matter and energy flux in freshwater systems (Wetzel, 1995). The availability of DOC for bacterioplankton is mainly related to the lability of the molecules comprising it, its origin and age (Farjalla *et al.*, 2002). Bastidas Navarro *et al.* (Bastidas Navarro *et al.*, 2014) found that different bacterial communities in Patagonian Andean lakes seem to have similar metabolic pathways in order to be able to exploit the available DOC molecules. In addition, Lindström *et al.* (Lindström *et al.*, 2005) found that other local environmental factors, such as temperature and pH are also important in the distribution of typical freshwater bacterial groups. Although, it is well known that the major phylogenetic groups encompass members with quite different ecological strategies (Alonso and Pernthaler, 2006) and taxa within a phylum are not necessarily functionally or metabolically similar (Peura *et al.*, 2012), it has been suggested that the two phylogenetically independent domains of life (i.e. *Archaea* and *Bacteria*) share similar broad trends, suggesting a commonality in the types of factors that are important for prokaryotic distribution (Auguet *et al.*, 2010).

In contrast with the apparent similarity in community structure (at the high-level group) between lakes, the among-lake and within-lake patterns did not show the same trends for many of the studied prokaryotic groups (Figs. 1 and 6). When comparing these two patterns taking into account the absolute abundances (Figs. 1a and 6a), *Alphaproteobacteria* and *Bacteroidetes* showed an opposite ordination than the other prokaryotic groups. At the among-lake scale, all the prokaryotic groups ordered together with higher DOC and temperature values whereas, in the within-lake scale, *Alphaproteobacteria* and *Bacteroidetes* ordered together with lower values of DOC and temperature. However, when taking into account the relative abundances (which translate into community structure, Figs. 1b and 6b), the archaeal and all the bacterial groups differed in their ordination comparing the among-lake and within-lake patterns. In the among-lake scale, *Alphaproteobacteria*, *Betaproteobacteria*, *Actinobacteria* and *Bacteroidetes* relative abundances ordered together with higher values of DOC and temperature (Fig. 1b) whereas, in the within-lake pattern, they ordered toward lower values of these environmental variables (Fig. 6b). The relative abundances of *Gammaproteobacteria* and archaeal groups, the less dominant groups studied, showed an opposite trend compared with the above-mentioned groups (Figs 1b and 6b). Therefore, we observed more similar trends comparing the within and among-lake patterns when considering absolute abundances (*Betaproteobacteria*, *Gammaproteobacteria*, *Actinobacteria* and archaeal groups showed similar trends) than when taking into account the relative abundances. This variation among the within and among-lake patterns could reflect the existence of different dominant ecotypes or tribes (genetically closely related but physiologically distinct populations; e.g. Salcher, 2014) in the water bodies studied.

In addition, the among-lake and within-lake patterns studied showed the dominance of the same three bacterial groups (*Alphaproteobacteria*, *Betaproteobacteria* and *Actinobacteria*). *Betaproteobacteria* and *Actinobacteria* have been described as typical and dominant members of freshwater systems (Glöckner *et al.*, 1999; Newton *et al.*, 2011). A few years ago, *Alphaproteobacteria* were thought to be only dominant in marine environments and rare in lacustrine systems (Salcher *et al.*, 2011), but, more recently, they have been found to be abundant also in freshwaters (Salcher *et al.*, 2011; Heinrich *et al.*, 2013). Our results are consistent with these findings and show the dominance of

the typical bacterial members of freshwaters. On the other hand, we found that *Gammaproteobacteria* always accounted for less than 3% of total DAPI counts. Similarly, this group accounted for less than 2% of total cell density in a high mountain lake (Pernthaler *et al.*, 1998). *Gammaproteobacteria* are generally more abundant in saltwater environment, oceans (Biers *et al.*, 2009) or saline lakes (Wu *et al.*, 2006), than in freshwaters. Coincidently, we found that this group had a higher contribution to community structure in a high conductivity (25 800 μm cm^{-1}) shallow lake (De Los Cisnes, Tierra del Fuego Province; Schiaffino *et al.*, 2013). Furthermore, the abundances of *Archaea* never exceeded 6% of total DAPI counts. Glockner *et al.* (Glockner *et al.*, 1999) and Pernthaler *et al.* (Pernthaler *et al.*, 1998) found that estimates of the abundance of *Archaea* in some small freshwater lakes (as detected by FISH) were similar to our estimates insofar as they rarely exceed 10%. Vila-Costa *et al.* (Vila-Costa *et al.*, 2013) found that *Archaea* were one to two orders of magnitude less abundant than *Bacteria*, but more evenly distributed (as detected by 454 pyrosequencing) in high mountain lakes.

Among-lake pattern in bacterial and archaeal community structure

On average, *Crenarchaeota* were better represented than *Euryarchaeota* in the 35 lakes studied. Massana *et al.* (Massana *et al.*, 2000) observed *Crenarchaeota* to be widespread in a variety of aquatic environments that included both coastal and open oceans. Non-thermophilic *Crenarchaeota* are also predominant in great lakes from North America, Africa and Eurasia (Keough *et al.*, 2003) and in high mountain lakes from the Central Pyrenees (Auguet and Casamayor, 2008).

In the among-lake pattern, most prokaryotic groups showed higher absolute abundances in mesotrophic and eutrophic water bodies than in oligotrophic ones, whereas *Actinobacteria* and the archaeal groups did not change significantly (Table Ib). Consistent with this, the distribution patterns of the absolute abundance of most bacterial groups increased with higher values of chl *a,* temperature and DOC (Table II, Supplementary data, Table SIa, Fig. 1a), except for *Actinobacteria*, whose absolute abundances did not regress significantly against DOC values and showed lower R^2 (Table II) and r (Supplementary data, Table SIa) values and thus an expected smaller arrow in the RDA analysis (Fig. 1a) compared with the other groups, suggesting a different and more complex distribution pattern across lakes. Bulk bacterial abundance and biomass have been seen to co-vary with the trophic states of a variety of aquatic environments (Bird and Kalff, 1984; Cole *et al.*, 1988; Gasol and Duarte, 2000), but less is known about the relationship at more specific bacterial phylogenetic levels in freshwaters. Temperature influenced the spatial distribution of prokaryotic group abundances, as has already been reported by other authors for *Bacteria* in lakes and oceans (e.g. Coveney and Wetzel, 1995; Li, 1998; Lefort and Gasol, 2013). De Figueiredo *et al.* (de Figueiredo *et al.*, 2010) found that some bacterial groups (*Alphaproteobacteria*, *Betaproteobacteria* and *Actinobacteria)* were also associated with high temperature and conductivity values in a eutrophic lake.

As mentioned, absolute abundances of most bacterial groups significantly regressed against chl *a*, temperature and DOC values (Table II). In line with our results, *Bacteria* and chl *a* are generally related with a positive relationship and a log–log slope < 1 between heterotrophic and autotrophic biomass (Simon *et al.*, 1992; Gasol and Duarte, 2000), indicating that bacterial biomass varies proportionally less than chl *a*. This coupling between autotrophs (phytoplankton biomass estimated as chl *a* concentration) and heterotrophs suggests that bacteria prefer to use the dissolved organic matter produced by phytoplankton to support growth through bacterial production (Nagata, 2000; Moran *et al.*, 2002), suggesting commensal interactions between phytoplankton and bacteria. Interestingly, each prokaryotic group studied increased their absolute abundances at the same rate with temperature and chl *a* values, whereas their absolute abundances increased at different rates with increasing DOC contents (results from ANCOVA and Table II). In contrast to our results, significantly different log–log regression slopes were found between bacterial group absolute abundances and chl *a* concentration in marine samples (Lefort and Gasol, 2013). Particularly, the slopes of bacterial groups (except *Actinobacteria*) differed significantly only when compared with the slopes of archaeal groups. Among bacterial groups, *Bacteroidetes* showed the highest slope value (1.30 ± 0.23) when regressed against DOC (although did not differ significantly from the other bacterial groups) and was the only group whose relative contribution to total prokaryotic community significantly changed with lake trophic state (Table Ia and Supplementary data, Table SIb). As mentioned, this group seems to play a particularly important role in the degradation of complex biopolymers (Kirchman, 2002). Accordingly, an increase in the likelihood of *Bacteroidetes* occurring during periods or sites with high external DOC loading or alga-derived DOC inputs has been documented (Newton *et al.*, 2011 and cites therein).

Although CARD-FISH and PCR-based DGGE approaches explore different taxonomic levels, it is noteworthy that when comparing the lake grouping obtained with these two different approaches targeting bacterial communities, we found comparable results (Fig. 2): deep,

large and oligotrophic water bodies clustered together, whereas small and shallow ones grouped separately. CARD-FISH is useful to enumerate specific cell groups, while the PCR-based technique DGGE handles relative intensities of bands (i.e. the dominant members of the community) and targets a higher level of taxonomic resolution.

Within-lake pattern in bacterial and archaeal community structure

From the multivariate analyses of the among-lake study, we observed that DOC was one of the most important variables explaining the prokaryotic abundance patterns, so we selected two lakes with different and contrasting DOC concentration to study the intra-annual seasonality, and thus to better understand and complete the snapshot vision (water bodies sampled once) resulting from the across lake study.

The five bacterial groups studied varied synchronously in their absolute abundances through the year (Fig. 4a and c), showing similar seasonal trends. As mentioned, *Actinobacteria*, *Alphaproteobacteria* and *Betaproteobacteria* were the best represented groups in all seasons (Fig. 5), showing the highest absolute abundances in spring and late winter (Fig. 4a and c). Peura *et al.* (Peura *et al.*, 2012) also found peaks of bacterioplankton abundance during spring in boreal lakes, with dominance of *Betaproteobacteria* and *Actinobacteria*. We also observed that, while *Bacteria* were abundant in spring and late winter, phytoplankton biomass (measured as chl *a* concentration) showed a peak during autumn (Fig. 3c). The same results were observed by Peura *et al.* (Peura *et al.*, 2012) in boreal lakes. The lack of a relationship between bacterial abundance and chl *a* in the two North Patagonian lakes could be related to the dominance of mixotrophic algae in these lakes. Despite marked differences in absolute abundances, all the bacterial groups showed roughly similar relative abundances during the four seasons (Fig. 5), which suggests that all the groups are regulated seasonally in a similar way. Archaeal groups not only varied markedly in their absolute abundances (Fig. 4b and d) but also their relative contribution to community structure (Fig. 5) during the study period. In Lake Morenito, *Euryarchaeota* had maximum absolute abundances values in late spring and in summer (2011), whereas in the humic-acid-rich Lake Escondido, *Crenarchaeota* showed peaks during both summer (2010 and 2011) and winter. Interestingly, Murray *et al.* (Murray *et al.*, 1998) reported strong seasonality of archaeal rRNA concentrations in Antarctic nearshore marine waters, with the highest values occurring during the austral winter. This same pattern was also observed by Galand *et al.* (Galand *et al.*, 2010) in the coastal Mediterranean Sea. In addition, Auguet *et al.* (Auguet *et al.*, 2011) observed a marked seasonal increase in archaeal group abundances from spring to summer in oligotrophic alpine lakes.

We observed a strong within-lake variation in the absolute and relative abundances of bacterial and archaeal groups mostly shaped by DOC, temperature, pH and conductivity (Fig. 6), suggesting the influence of lake local environmental factors on community structure. *Actinobacteria* and *Betaproteobacteria* were the only groups that showed different ordination when comparing absolute (Fig. 6a) or relative (Fig. 6b) abundances. This could be explained because as mentioned, the absolute abundances are more linked to the population sizes, biomass and turnover of a community, whereas the relative abundances are related to the dominance of a specific group over the other. On the other hand, both absolute and relative abundances of *Crenarchaeota* and *Euryarchaeota* ordinated together with higher values of pH and were also positively correlated with this environmental variable (Spearman's ρ test). Coincidently, the most important variable shaping the *Bacteria:Archaea* ratio, was pH, suggesting proportionally higher abundances of *Archaea* when compared with those of *Bacteria* with increasing pH. The pH of an ecosystem is often a master driver of bacterial community composition (Fierer and Jackson, 2006), but it also seems to be important driving the intra-annual seasonality of archaeal abundances. Auguet and Casamayor (Auguet and Casamayor, 2012) found well-defined archaeal community patterns with pH as the main potential driving environmental factor. In addition, *Euryarchaeota* absolute abundance was positively correlated with temperature and *Crenarchaeota* absolute and relative abundances were positively correlated with DOC. Consistent with our results, Auguet *et al.* (Auguet *et al.*, 2011) found that temperature was the environmental variable that better explained spring, summer and winter archaeal assemblage structure in alpine lakes.

Although we found that the same factors could be important for bacterial and archaeal distribution, the observed within-lake shifts in bacterial and archaeal abundance could reflect the dependence of these prokaryotic populations on seasonally variable biogeochemical processes. The different annual peaks in *Bacteria* and *Archaea* suggest that these two picoplankton domains could have different growth and loss mechanisms, possibly playing different roles in freshwater ecosystems, as already reported from the oceans (Curch *et al.*, 2003).

In summary, we found across lake and intra-annual variation patterns in the abundance of various heterotrophic prokaryotic groups (five major groups of *Bacteria* and two of *Archaea*) in the water bodies studied. The environmental variables were related to both relative and absolute abundances, indicating a complex interaction of

abiotic factors behind the structuring of prokaryotic communities. In both among-lake and within-lake patterns, multivariate analyses showed that DOC was an important variable shaping the community at the taxonomic resolution studied, using both absolute and relative abundances, while temperature was an important driver shaping their absolute, but not relative abundances. Our results confirm that archaeal and *Gammaproteobacteria* groups are small contributors to community structure in these lakes, which are dominated by *Alphaproteobacteria*, *Betaproteobacteria* and *Actinobacteria*. The distribution pattern over time showed that all the bacterial groups showed simultaneous absolute abundance peaks in late winter or spring, whereas abundance of *Archaea* changed markedly with peaks in summer, spring and winter. In contrast to *Archaea*, each bacterial group had roughly similar relative contribution among seasons, suggesting that these two prokaryotic domains could have different temporal ecological strategies.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

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