Diet and feeding selectivity of the Andean Flamingo *Phoenicoparrus andinus* and Chilean Flamingo *Phoenicopterus chilensis* in lowland wintering areas

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Abstract

Flamingos *Phoenicopteridae* sp. are gregarious birds that travel long distances between breeding and feeding sites. Here we describe the diet and feeding selectivity of two flamingo species, the Andean Flamingo *Phoenicoparrus andinus* and Chilean Flamingo *Phoenicopterus chilensis*, which coexist in a lowland area of Argentina. Environmental characteristics and available food resources were assessed at twelve lakes where feeding flocks of both species of flamingos occurred. Food items found in faeces (16S rRNA for bacteria and archaea) and microscopic analyses (for Cyanobacteria, microalgae and microinvertebrates) were analysed, and the birds' feeding selectivity and niche overlap were estimated. Results showed that the lakes were of eutrophic to hypereutrophic status, and with hypohaline to mesohaline salinity levels. Predominant microorganisms belonged to the Planctomycetes, Verrucomicrobia, Chloroflexi, Euryarchaeota, Cyanobacteria, Bacillariophyta and Copepoda phyla. Euryarchaeota and Firmicutes were the main phyla found in the faeces, with Chloroflexi and Planctomycetes also present in smaller quantities. Proteobacteria were well represented in Andean Flamingo faeces, but Verrucomicrobia were scarce in both species. Cyanobacteria, Bacillariophyta,

Copepoda, Cladocera, and Rotifera were abundant in Chilean Flamingo faeces, and larger organisms belonging to Ostracoda, Nematoda, and Diptera were also found. The most consumed taxa were in the intermediate to large size range (10^4 to 2×10^5 µm^3, and 10^8 to 2×10^8 µm^3). Andean Flamingo faeces were composed mainly of microalgae, especially diatoms. Cladocera and Copepoda species were found to a lesser extent, showing the flamingos' preference for intermediate prey sizes (10^4 to 2×10^5 µm^3). Food selection was probably dependent on the spatial variability in prey availability, as both positive selectivity (for Bacillariophyceae) and avoidance (for Copepoda) were observed in Chilean Flamingos. In contrast, Andean Flamingos showed a high positive selection for diatoms, and strong negative selection for microinvertebrates. Both flamingo species can apparently coexist whilst feeding on a wide spectrum of microorganisms, but trophic niches differed in the amounts of Cyanobacteria, microalgae and microinvertebrates taken. Such a low niche overlap probably contributes to the coexistence of both sympatric species in similar waters.

Key words: bacteria, microalgae, microinvertebrates, microorganisms, niche overlap, 16S rRNA, trophic selectivity.

Flamingos *Phoenicopteridae* sp. are long-lived, gregarious birds that live in shallow saline lakes from sea level to 4,500 m above sea level (a.s.l.) (Ogilvie & Ogilvie 1986; Caziani *et al*. 2007), and travel long distances between breeding and feeding sites (McCulloch *et al*. 2003; Caziani *et al*. 2007). Flamingos are filter feeders with a specialised bill, adapted to feed on small organisms found in shallow waters (Gray 1869; Jenkin 1957; Mascitti & Kravetz 2002). Factors such as predation, disturbances, and environmental conditions are known to affect their abundance and distribution (Arengo & Baldassarre 1995; Barisón *et al*. 2014; Henriksen *et al*. 2015), but several studies indicate that their distribution is influenced mainly by food abundance and quality (Vareschi 1978; Hurlbert *et al*. 1986; Arengo & Baldassarre 1995; Tuite 2000; Arengo & Baldassarre 2002; Krienitz & Kotut 2010; Kaggwa *et al*. 2013; Kumssa &

Bekele 2014; Henriksen *et al*. 2015; Krienitz *et al*. 2016). An accurate knowledge of flamingo diets and their trophic preferences therefore is needed to understand habitat selection.

Three flamingo species inhabit the southern region of South America (Caziani *et al*. 2007): the Chilean Flamingo *Phoenicopterus chilensis*, the Andean Flamingo *Phoenicoparrus andinus* and the James' Flamingo *P. jamesi*. The Chilean Flamingo occurs in a wide variety of wetlands, including salt and freshwater lakes, estuaries, and marine coasts from Peru to Tierra del Fuego in Argentina, and from Chile to southern Brazil and Uruguay (Canevari 1983; Bucher 2006). The Andean and James' Flamingos have a narrower distribution, with both species using the High Andean saline lakes of Argentina, Bolivia, Chile, and Peru as breeding and feeding areas during summer (Caziani *et al*. 2007; Marconi *et al*.

2007). In winter, when these lakes freeze over, most populations migrate to lowland lakes in Argentina (Romano *et al*. 2002; Caziani *et al*. 2007; Cruz *et al*. 2013). The southernmost wintering area is Pampa de las Lagunas, a large wetland system that constitutes a sympatric area for Andean and Chilean Flamingos, where some lakes support thousands of individuals of both species, while others are occupied by only one of the species (Romano *et al*. 2009).

Regarding trophic preferences, several reports show that the Andean Flamingo is able to feed on diatoms, whereas the Chilean Flamingo is omnivorous and can consume cyanobacteria, insect larvae and microcrustacea (Aravena 1928; Zotta 1932; Mascitti & Kravetz 2002; Tobar *et al*. 2014; De los Ríos Escalante 2015). On the basis of exclusion experiments, Hurlbert & Chang (1983) linked the abundance of Andean Flamingos to diatom dominance, and the abundance of Chilean Flamingos to cyanobacteria and microcrustacea dominance. All of the inferences were indirect, however, and insufficient to assess the trophic preferences of the two species.

Filter feeders ingest mud when feeding (Jenkin 1957), and Mascitti (1998) suggested that sediments represent about 75% of gizzard volume in Andean Flamingos. This feeding strategy could contribute significantly to bird nutrition because organic matter and microscopic organisms can constitute about 20% of the mud dry weight, from which bacteria represent a substantial part (del Hoyo *et al*. 1992). Yet neither bacteria nor archaea have been studied as potential food sources for flamingos, despite the fact that techniques

such as high-throughput sequencing can now identify such organisms, and thus provide insights into the microorganisms that constitute the flamingos' diet (Rinke *et al*. 2013).

Knowledge of available food sources, together with items consumed, is needed for a better understanding of filter feeders' preferences and possible selection of their prey (Cody 1985). The gut content of flamingos is difficult to assess non-invasively because of their neck anatomy, but analysis of faecal content provides an alternative method for investigating their diet. The aim of this study was to describe the diet and feeding selectivity of Andean and Chilean Flamingos coexisting in a lowland wintering area, and to identify possible differences in their trophic profiles. We assessed the environmental characteristics and the availability of trophic resources at twelve lakes by observing feeding flocks of both species at these sites. Trophic items found in faeces were analysed via molecular and microscopic observation, in order to estimate the feeding selectivity and niche overlap of these two flamingo species.

Methods

Study area

Pampa de las Lagunas is a wetland system (33.70º–34.30ºS, 61.42º–62.53ºW) located in a highly agricultural region of Argentina's central-east plain in South America (Pasotti *et al*. 1984; Romano *et al*. 2005). It encompasses an area of *c*. 800,000 ha dotted with lakes and different permanent and temporary water bodies (Fig. 1). The climate is temperate with mean annual rainfall
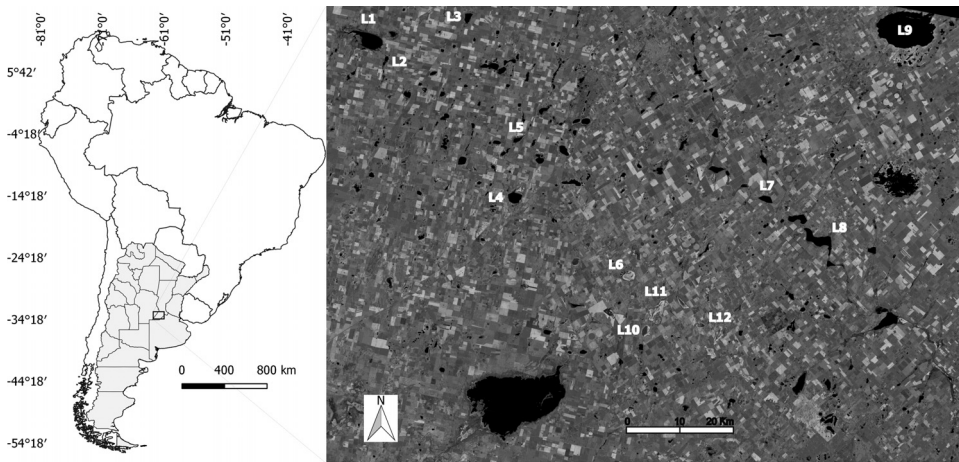
Figure 1. Map of South America showing the location of Pampa de las Lagunas region (left), and a detail of the sampled lakes (right). L1 = Las Tunas, L2 = La Dulce, L3 = La Badenia, L4 = Sancti Spiritu, L5 = M1, L6 = MT3, L7 = Encadenadas 3, L8 = Encadenadas 5, L9 = Melincué, L10 = Bella Vista, L11 = Martín García, L12 = Morgan. Landsat-5 TM image, Path/Row: 227/084, April 2011.

of 800–1,000 mm, concentrated during summer and autumn (Biasatti *et al.* 1999). Altitude ranges from 80–130 m a.s.l., and the low gradient (slope= 0.1%) prevents water runoff (Pasotti *et al.* 1984). The lakes included in the study (Fig. 1) were shallow (< 3 m depth) and with an area of < 20 km², except for Melincué Lake (L9) that has a maximum depth of 5–7 m and an area of 40–200 km² depending on hydroclimatic cycles (Sosnovsky & Quirós 2006; Battauz *et al.* 2013). The flamingos prefer to feed in lake areas < 0.40 m deep (Canevari 1983).

Field sampling

A total of twelve lakes were sampled over three southern hemisphere winters (in August 2011, 2012, 2013), which corresponded to the flamingos' feeding season in this region. All flamingos were counted at each lake and

identified to species level, using 10× binoculars or 15/45× spotting scopes and manual counters. Habitat sampling was undertaken at the littoral area of each lake, where the flamingos were feeding. Water temperature, dissolved oxygen, conductivity, and pH were determined *in situ* using a multiparametric probe (Lutron YK 2001, Coopersburg, PA, USA). Transparency of water was measured as Secchi disc depth, water depth was measured with a ruler, and altitude and geographical position were recorded with a portable geographical positioning device (GARMIN® Map78). Water samples (2 l) were preserved by acidification to pH 2 with sulphuric acid, and transported refrigerated to the laboratory for nutrient determinations.

    Samples for archaea and bacteria analyses by DNA sequencing were collected from

the bottom of the lakes at the sediment surface (at sites L3, L5, L9 and L10). Three subsamples of 1 cm³ were extracted from a core of 10 cm² of surface and 3 cm depth; they were homogenised, pooled together and immediately placed on ice and stored at –20°C until they were processed in the laboratory.

Water for Cyanobacteria and microalgae quantification was sampled with a bottle and fixed *in situ* with 1% of Lugol's acidified solution. Additional qualitative samples were collected with a 10 m pore mesh for taxonomical determinations and fixed with 4% formalin. Microinvertebrates were collected by filtering between 10–30 litres of water with a conical network of 50 m pore mesh, and fixed with 10% formalin.

Faeces of both Andean and Chilean Flamingos were collected when a bird was observed *in situ* feeding and defecating. Faecal samples on the shore, which had not come into contact with water, were collected using a sterile 10 ml pipette. The pipette was introduced inside the faeces, taking care that the portion extracted was not contaminated with lake sediment. Two to three faeces per sample were pooled, dissolved up to 100 ml with distilled water, and fixed with 10% formalin. Twenty-five samples were taken for microscopic microorganism quantifications, and four samples were preserved for molecular analyses of microorganisms without the addition of distilled water.

Chemical analyses

Total phosphorus was estimated following the ascorbic acid method, and total nitrogen was measured by the Kjeldahl method (American Public Health Association 1999). Turbidity was estimated using a nephelometric method (HACH 2100 N). Salinity (g l⁻¹) was calculated using temperature (°C) and conductivity data, adjusting the formula proposed by Fofonoff & Millard (1983) with field data.

Archaea and bacteria pyrosequencing

DNA was isolated using the Power Biofilm DNA Isolation Kit (MO BIO Laboratories, Inc.) according to the supplied protocol. The V3–V5 hypervariable region of the 16S rRNA gene was amplified using the universal primers F357 and R926 (Supporting Materials: Appendix S1).

All DNA samples were sequenced in INDEAR (Rosario, Santa Fe, Argentina) using tag-pyrosequencing (see online Supporting Materials) with 454 GS FLX (Roche-454 Life Sciences, Branford, CT, USA). Raw sequences for each sample were stored in FASTQ format in the NCBI Sequence Read Archive (SRA) under the accession number SRP029444. The QIIME software package v.1.7.0 (Caporaso *et al.* 2010) was used to process the sequencing data. Sequences were clustered into Operational Taxonomic Units (OTUs) using UCLUST at the 97% similarity level using the most abundant sequence as the representative for each OTU. Each representative OTU sequence was taxonomically classified using the Ribosomal Database Project (RDP) with a bootstrap confidence of 80%. A maximum likelihood reference tree was constructed using RaxML, as implemented in ARB software package (Ludwig *et al.* 2004) using reference 16S rRNA gene sequences with near full

length (> 1,300 nt) from cultured isolates. Later, partial 16S rRNA gene sequences from each sample and closely related environmental uncultured 16S rRNA gene sequences were inserted into the reference tree without altering tree topology applying a maximum parsimony criterion and a 50% base frequency filter.

OTU tables were subsampled using ten replicates for each sampling effort at increasing intervals of 100 sequences, so alpha diversity indexes were calculated on each subsample of the rarefaction curve and on the complete OTU table (including all sequences). The diversity metrics calculated were CHAO1 (which estimates the species richness), and the Shannon-Wiener index (H') (Shannon & Weaver 1949) for lakes and flamingo faeces samples.

Cyanobacteria, microalgae and microinvertebrate microscopic analyses

Cyanobacteria and microalgae were counted using an inverted microscope and sedimentation chambers until reaching at least 100 individuals of the most frequent species (Utermöhl 1958). Individuals were considered as the unit present in the samples (unicell, colony, coenobium, or filament). Cell dimensions were measured to calculate biovolume (mm^3 l^{-1}) based on geometric equations (Hillebrand et al. 1999). Species identifications were also performed with a direct microscope at 400× and 1,000× magnification. Bacillariophyta frustules were treated with hydrogen peroxide (100 volumes) and hydrochloric acid at a temperature of 80°C for 2 h and washed with distilled water before permanent mounting with Naphrax® (refractive index = 1.74) (Battarbee 1986). Taxonomic identification for Cyanobacteria was based on Komárek & Anagnostidis (1999, 2005) and Komárek (2013); for Bacillariophyta on Krammer & Lange-Bertalot (1986, 1988, 1991 a,b) and Round et al. (1990); and, for the rest of the taxonomic group, on Prescott (1978), Komárek & Fott (1983), Tell & Conforti (1986), Menezes (1994) and González (1996).

Microinvertebrates were analysed qualitatively and quantitatively with optical and stereoscopic microscopes. Rotifers and nauplii larvae were counted using 1 ml Kolkwitz chambers, and cladocerans, copepods, and other larger organisms using 5 and 10 ml Bogorov chambers in at least 100 individuals of the numerically dominant organism. Density (individuals ml^{-1}) was calculated, and biovolume (mm^3 l^{-1}) was estimated in 10 individuals of each species for every sample, using body measurements and relating these dimensions to geometric shapes (Ruttner-Kolisko 1977; Dumont et al. 1975). Body measurements and certain taxonomic characters helped to identify to specific level the remains of specimens found in faeces. Taxonomic classifications were based on different authors for Rotifera (Ruttner-Kolisko 1974; Koste 1978; José de Paggi 1978, 1995), Cladocera (Pennak 1989; Paggi 1995, 1996, 1997), Copepoda (Reid 1985, Dussart & Defaye 2001; Domínguez & Fernández 2009) and on Dominguez & Fernández (2009) for other groups.

Microorganism diversity considering the assemblage composed of Cyanobacteria, microalgae, and microinvertebrate was

calculated with Shannon-Wiener index (H') for lakes and faecal content of both Andean and Chilean Flamingos. The relation of microorganism taxa to environmental characteristics and flamingo species was assessed with a multivariate ordination method (software CANOCO version 5). A redundancy analysis (RDA) was performed because the detrended correspondence analysis suggested that a linear method was appropriate, as the species gradient length was shorter than three standard deviations (after Braak & Smilauer 2012). The data set of the response variables were based on the biovolume of microorganism taxa present in more than three samples or in a percentage higher than 30% of total contribution. Response data were square root transformed and Hellinger standardised. The measured environmental variables and the abundance of each flamingo species were considered as explanatory variables. Automatic stepwise model building was used for selecting the subset of the most significant explanatory variables, with the forward selection method, which consists of adding explanatory variables one by one. The significance of all axes performed by the RDA ordination method was analysed using a Holm test, under an unrestricted model of 999 permutations ($P < 0.01$).

Similarities in the main microorganism taxa and taxonomic group biovolume present in the faecal content of Chilean Flamingos ($n = 19$) and Andean Flamingos ($n = 6$) were evaluated with SIMPER. Statistical differences according to microorganisms (for taxa and for taxonomic group) between both flamingo faecal content diets were determined with PERMANOVA on a Bray-Curtis triangular matrix with 9,999 permutations, which supports unbalanced data sets. For the analysis of flamingo diet, microorganisms were classified according to their size in five categories based on biovolume: I ($< 2 \times 10^3 \, \mu m^3$), II ($2 \times 10^3$ to $10^4 \, \mu m^3$), III (10^4 to $2 \times 10^5 \, \mu m^3$), IV (6×10^5 to $8 \times 10^7 \, \mu m^3$), and V (10^8 to $2 \times 10^8 \, \mu m^3$). A non-parametric Mann-Whitney U t-test analysed differences between the diet of Andean and Chilean Flamingos in the abundance of microorganism size category. Analyses were run with Past 3 software version 3.14 (Hammer et $al.$ 2001).

Trophic selectivity and niche overlap

Food selectivity was estimated with Strauss's index (Strauss 1979) based on the formula $Li = r_i - p_i$, where r_i is the relative abundance of a food item i in the diet (flamingo faeces), and p_i is the relative abundance of that item in food source (environmental samples). This index varies between –1 and 1. A zero value indicates no trophic selectivity (random feeding); negative values indicate that the food selection is negative because of inaccessibility or rejection of the item; positive values indicate a positive selection due to preference for some food items. A Mann-Whitney U t-test was used to test for differences between the trophic selectivity of Chilean and Andean Flamingos. Niche overlap between both species was quantified using Pianka's (1973) index whose values range from 0 (no overlap) to 1 (complete overlap), based on the formula $O_{jk} = \Sigma P_{ij}*P_{ik} / (\Sigma P_{ij}^2 + P_{ik}^2)^{-(1/2)}$, where p_{ij} is the proportion item i in the j sample. The niche overlap index was run with 'spaa' package for R software (R Core Team 2015).

Results

Lake characteristics, flamingo abundance and feeding sources

The water temperature of the lakes ranged between 9.2°C and 21.1°C during the winters of 2011–2013 inclusive. Salt concentration varied widely among lakes, and among years for each lake (from 3–32 g l⁻¹). Conductivity followed the same variation and, in all cases, pH values were high (above 8.5). Dissolved oxygen presented supersaturated values, and total phosphorus (< 0.9 mg l⁻¹) and nitrogen (< 26 mg l⁻¹) concentrations were also high (Table 1).

The abundance of flamingo species was highly variable among years and lakes (Table 1). In 2011, the abundance of Chilean Flamingos in the study area was substantially higher than that of Andean Flamingos (30,497 *vs.* 698 individuals, respectively); in 2013 the abundance of Chilean Flamingos was slightly higher (7,938 *vs.* 5,082 individuals), whilst in 2012 the opposite pattern was recorded with fewer Chilean than Andean Flamingos (4,495 *vs.* 8,738 individuals, respectively). There were also marked differences in numbers recorded across the lakes. Lake L9 had the highest abundance of Andean Flamingos during 2011 and 2012, and L6 during 2013, whereas L4 had the highest number of Chilean Flamingos during 2011, L10 during 2012, and L6 during 2013.

The sequencing of 16S rRNA showed, on average, a relative abundance of 12% of

Table 1. Limnological variables and flamingo species density in lakes of the Pampa de las Lagunas region ($n = 12$) during the winters of 2011 to 2013. CV% = coefficient of variation percentage.

Variable	Mean	Range	CV%
Temperature (°C)	15.12	9.20–21.10	22
Dissolved oxygen (mg l⁻¹)	12.19	5.10–19.20	40
Depth (m)	0.16	0.08–0.32	44
Secchi disc (m)	1.75	0.005–12.0	229
pH	9.38	8.50–10.20	6.12
Conductivity (ms cm⁻¹)	14.30	3.30–32.30	64
Salinity (g l⁻¹)	14.06	3.04–32.24	66
Total Phosphorus (mg l⁻¹)	3.54	0.90–10.63	79
Total Nitrogen (mg l⁻¹)	14.00	4–26	70
Flamingo species (individuals per lake)			
Phoenicoparrus andinus	950.50	0–6117	170
Phoenicopterus chilensis	2537.00	36–17,076	160

Table 2. Microorganisms, main taxonomic group and diversity indexes found in lakes of Pampa de las Lagunas region (*n* = 12 lakes) during the winters of 2011 to 2013. CV% = coefficient of variation percentage.

	Mean	Min.	Max.	CV%
Archaea+Bacteria (16S rRNA sequencing):				
Number of reads	2370.75	1,670	2,766	21
Chao1	471	220	656	43
OTUs	292	148	395	41
H' diversity index (bits ind.−1)	1.50	0.46	2.30	38
Evenness	0.46	0.26	0.83	42
Archaea (reads)	513.57	4	1,121	86
Euryarchaeota	513.57	4	1,121	86
Bacteria (reads)	1,439	8	3,883	35
Planctomycetes	448.57	1	1,078	90
Verrucomicrobia	189	1	511	94
Chloroflexi	210	0	444	76
Firmicutes	257	3	929	124
Deinococcus-Thermus	54	0	212	136
Bacteroidetes	42	2	90	84
Proteobacteria	72	2	250	113
SR1	89	0	27	147
Acidobacteria	33.29	0	149	174
NKB19	20	0	103	183
WS5	24.14	0	90	133
Cyanobacteria+Microalgae+Microinvertebrates (microscopic analyses):				
Number of taxa	14.46	8	28	35
Diversity index H' (bits ind.−1)	1.190	0.08	2.506	51
Evenness	0.20	0.10	0.37	35
Total biovolume (mm³ l−1)	1,269.53	47.62	7,705.85	172
Cyanobacteria (mm³ l−1)	669.42	0	5,593.26	239
Chroococcales	255.44	0	2,575.46	291
Oscillatoriales	112.20	0	899.02	238
Nostocales	301.78	0	2,945.37	282
Microalgae (mm³ l−1)	405.88	2.76	2,031.27	157
Chlorophyta	24.87	0	246.83	285
Bacillariophyta	302.32	2.20	1,784.44	165
Coscinodiscophyceae	4.50	0	35.48	229
Bacillariophyceae+Fragilariophyceae	297.81	0	1,748.96	165
Dinophyta	0.72	0	8.51	337
Euglenophyta	77.95	0	831.51	306
Microinvertebrates (mm³ l−1)	194.22	1.10	1,565.56	229
Rotifera	8.04	0	51.70	188
Cladocera	0.57	0	4.66	236
Copepoda	185.60	0	1,561.62	238
Nematoda	0.001	0	0.01	346
Ciliophora	0.003	0	0.03	346

Archaea and 88% of bacteria in lakes. The number of OTUs, as well as the microbial diversity, varied among the different lakes (Table 2). L3 and L5 had the richest and the most diverse microbial assemblage, with the greatest evenness. This was reflected in the rarefaction curve with a raised slope, while in the rest of the samples the slopes were close to zero (Supporting Materials: Fig. S1). Planctomycetes (bacteria), Euryarchaeota (archaea), Verrucomicrobia (bacteria), and Chloroflexi (bacteria) were the most abundant phyla in all lakes (50%–80%) (Fig. 2). Planctomycetes were especially abundant in lake L9, this sample having the lowest relative abundance of sequences affiliated to the Archaea domain. On the other hand, Archaea was well represented in lakes L10 and L5, also having relatively high sequence abundance in L3. Sequences associated with candidate phyla such as SR1, NK19, WS5 were highly represented in L3, as well as sequences that could not be associated with any known phylum in the bacteria domain. Sequences assigned as Bacteroidetes and Proteobacteria were scarcely represented, but they were present in all the lakes.

The analyses of Cyanobacteria, microalgae, and microinvertebrate biovolume showed that the former group was most abundant in the lakes, with a mean value of 669 mm^3 l^{-1} and a maximum of 5,593 mm^3 ml^{-1}. Nostocales prevailed over Oscillatoriales and Chroococcales. Potentially bloom species such as *Arthrospira* sp., *Oscillatoria* sp., *Planktolyngbya limnetica*, *Anabaenopsis elenkinii, A. milleri*, *Aphanocapsa* sp., and *Raphidiopsis curvata* were present. During 2011, Cyanobacteria blooms were observed in L2 and L5 lakes, with massive development of *Arthrospira* sp., *A. elenkinii*, and *A. milleri*, accompanied by other species.

Microalgae biovolume varied from 2.76–2,031 mm^3 l^{-1}, and microinvertebrates ranged from 1.1–1,566 mm^3 l^{-1} (Table 2). Bacillariophyta and Copepoda were the groups that alternatively sub-dominated the microorganism assemblage in the different lakes (Fig. 2). Of the Bacillariophyta, benthic species belonging to the Bacillariophyceae and Fragilariophyceae were the most abundant, whereas Coscinodiscophyceae contributed poorly to total microalgae biovolume (< 8%). Copepoda varied between 0–1,562 mm^3 l^{-1}, with Harpacticoida as the group with the highest biovolume. Cladocera and Rotifera were next in abundance, with *Daphnia spinulata* and species of the genus *Brachionus* being the main contributors, respectively, for these two groups.

Species richness was high and mainly due to Bacillariophyta (34 species), Chlorophyta (21), Cyanobacteria (19), Cladocera (5), Rotifera (7), and Copepoda (8). H' diversity index in lakes was low and never exceed 2.506 bits ind.$^{-1}$, and the same was true for measures of evenness (maximum = 0.37). Organisms in biovolume categories III and V were mainly from the plankton community.

The first two axes of the RDA significantly explained 11% of total microorganism biovolume variation (Monte Carlo permutation test for the first two axes: $P < 0.001$) (Fig. 3). The pH was the only significantly associated variable that explained taxa ordination ($P = 0.04$). Chilean Flamingo abundance was positively associated with lakes with higher pH and
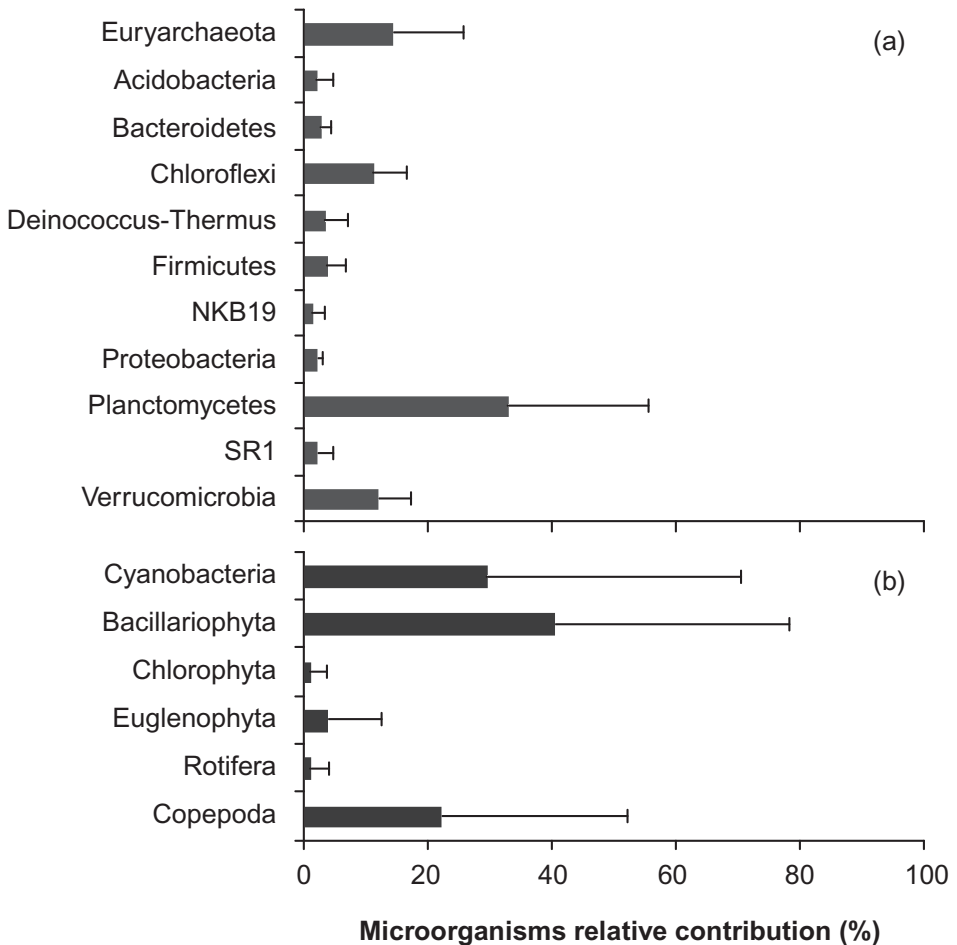
Figure 2. Main taxonomic groups of microorganisms found in lakes of Pampa de las Lagunas region. a) Relative contribution of different phyla to total sequenced reads for archaea and bacteria, and b) relative contribution of biovolume (mm³ l⁻¹) for Cyanobacteria, microalgae, and microinvertebrates, averaged through the different sampled lakes. Lines represent standard deviation.

phosphorus concentration, and was also positively associated with green algae: *Arthrospira* sp. and *Brachionus* sp. (*B. dimidiatus*, *B. plicatilis*, *B. pterodinoides*). The abundance of Andean Flamingos was closely associated with pennate diatoms (*Craticula cuspidata*, *C. ambigua*), filamentous Cyanobacteria (*Nodularia spumigera*, *Lyngbya*

sp., *Oscillatoria* sp.), green algae species (*Tetrastrum* sp., *Monoraphidium* sp.) and microinvertebrates (*Cephalodella* sp., copepodite and nauplii larvae).

Flamingo faeces composition

The 16S rRNA analyses provided evidence of similar food composition in Andean
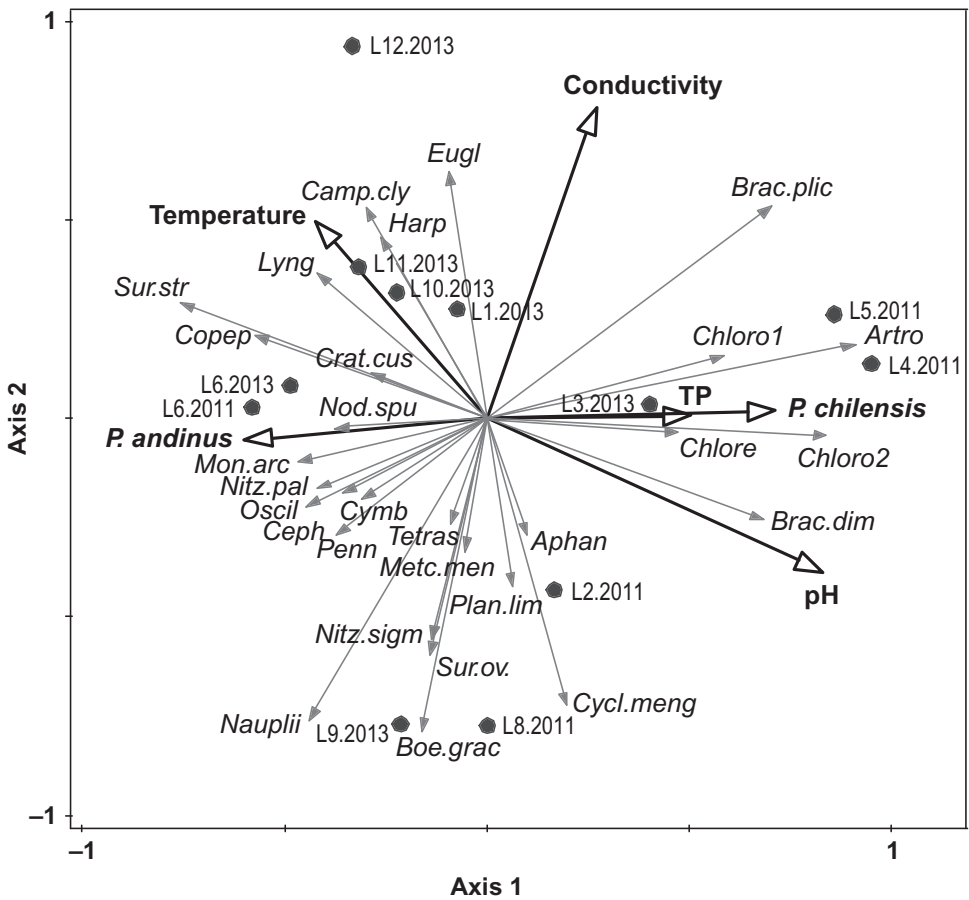
Figure 3. First two axes of the RDA showing the ordination of taxa biovolume of Cyanobacteria, microalgae, and microinvertebrate taxa biovolume (as response variables (grey arrows)), the environmental variables, and the abundance of flamingo species as explanatory variables (black arrows), and lake samples (circles).

Abbreviations: Eugl: *Euglena* sp.; *Camp.cly*: *Campylodiscus clypeus*; *Artro*: *Artrosphira* sp.; *Brac.plic*: *Brachionus plicatilis*; Harp: Harpacticoida; *Lyng*: *Lyngbya* sp.; Copep: Copepodites; *Nitz.sigm*: *Nitzschia sigma*; *Nitz.pal*: *Nitzschia palea*; *Oscil*: *Oscillatoria* sp.; *Boe.grac*: *Boeckella gracilis*; *Sur.ov*: *Surirella ovalis*; *Sur.str*: *Surirella striatula*; *Brac.dim*: *Brachionus dimmidiatus*; Chloro1, Chloro2: non-identified chlorococcal species; *Chlore*: *Chlorella* sp.; Penn: non-identified pennate diatom; *Met.men*: *Metacyclops mendocinus*; Nauplii: nauplii larvae; *Cycl.meng*: *Cyclotella meneghiniana*; *Ceph*: *Cephalodella* sp.; *Plan.lim*: *Planktolyngbya limnetica*; *Tetras*: *Tetrastrum* sp.; *Cymb*: *Cymbella*; *Aphan*: *Aphanocapsa* sp.; *Artro*: *Artrosphira* sp.; *Crat.cus*: *Craticulata cuspidate*; *Nod.spu*: *Nodularia spumigera*; *Mon.arc*: *Monoraphidium arcuatum*; TP: total phosphorus.

and Chilean Flamingo faeces, both having Euryarchaeota and Firmicutes as the predominant phyla and, in smaller amounts, Chloroflexi and Planctomycetes. Proteobacteria was well represented in the faeces of the Andean Flamingo, but Verrucomicrobia were hardly present in faeces of both flamingo species. With respect to diversity, both Chilean and Andean Flamingos showed similar diversity of CHAO and H' diversity index (Table 2).

Cyanobacteria accounted for the highest proportion of microorganisms in Chilean Flamingo faeces (49% on average biovolume), followed by microinvertebrates (31.05%), and microalgae (20.5%) (Fig. 4). In contrast, the average contribution of microalgae (97%) in Andean Flamingo faeces was greater than that of microinvertebrates (2.72%), and Cyanobacteria (0.3%). Oscillatoriales was the dominant Cyanobacteria group in Chilean Flamingo
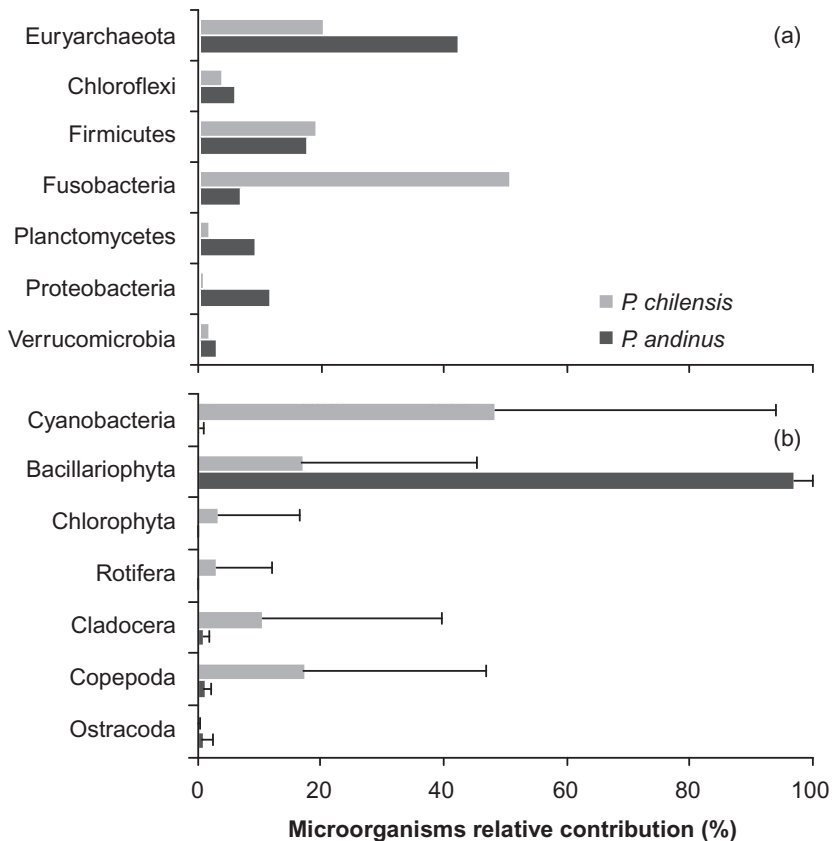

Figure 4. Main taxonomic groups of microorganisms present in the diet of *Phoenicopterus chilensis* and *Phoenicoparrus andinus*. a) Relative contribution of different phyla to total sequenced reads for archaea and bacteria, and b) relative contribution of biovolume (mm^3 l^{-1}) for Cyanobacteria, microalgae, and microinvertebrates, averaged through the different sampled faeces. Lines represent standard deviation.

Table 3. Trophic items found in Chilean Flamingo (*Chil.*) and Andean Flamingo (*And.*) faeces identified by 16S rRNA sequencing (*n* = 4 faeces samples), and microscopic analyses (*n* = 25 faeces samples), sorted in size-categories: I ($< 2 \times 10^3$ μm³), II (2×10^3 to 10^4 μm³), III (10^4 to 2×10^5 μm³), IV (6×10^5 to 8×10^7 μm³), and V (10^8 to 2×10^8 μm³). The taxonomic group to which they belonged is indicated: ARCH (Archaea), BACT (Bacteria); CYAN (Cyanobacteria); CHLO (Chlorophyta); BACI (Bacillariophyta); ZYGN (Zynematophyceae); EUGL (Euglenophyta); ROT (Rotifera); NEM (Nematoda); CLAD (Cladocera); COP (Copepoda); OSTR (Ostracoda); CILIO (Ciliophora); DIPT (Diptera).

ID	Group	Phyla/taxa	Chil.	And.
16S	ARCH	Euryarchaeota	+	+
rRNA	ARCH	Crenarchaeota		+
	BACT	Fusobacteria	+	+
	BACT	Firmicutes	+	+
	BACT	Chloroflexi	+	+
	BACT	Planctomycetes	+	+
	BACT	Proteobacteria	+	+
	BACT	Verrucomicrobia	+	+
	BACT	Bacteroidetes	+	+
	BACT	Deinococcus-Thermus	+	+
	BACT	WS5	+	+
	BACT	Actinobacteria	+	+
	CYAN	Cyanobacteria	+	+
	BACT	WPS-2	+	+
	BACT	Other	+	+
	BACT	SR1	+	+
	BACT	Spirochaetes	+	+
	BACT	Armatimonadetes	+	+
	BACT	WS4	+	+
	BACT	Gemmatimonadetes	+	
	BACT	WS1	+	+
	BACT	NKB19	+	+
	BACT	OD1	+	
	BACT	Acidobacteria		+
	BACT	BRC1	+	
	BACT	OP3	+	
I	CYAN	*Chroococcus limneticus* LEMMERMANN	+	+
	CYAN	*Chroococcus minutus* (KÜTZING) NÄGELI	+	
	CYAN	*Planktothrix* sp.	+	
	CYAN	Oscillatorial	+	
	CYAN	*Phormidium* sp.	+	
	CHLO	*Chlorella* sp.	+	
	CHLO	Chlorococcal	+	
	CHLO	*Monoraphidium minutum* (NÄGELI) KOMÁRKOVÁ-LEGNEROVÁ	+	
	BACI	*Cymbella* sp.	+	+
	BACI	*Fallacia pygmaea* (KÜTZING) STICKLE & D.G. MANN		+
	BACI	*Navicula* sp.	+	+
	BACI	*Gomphonema parvulum* (KÜTZING) KÜTZING	+	+
	BACI	*Nitzschia palea* (KÜTZING) W. SMITH	+	+
	BACI	*Nitzschia sigma* (KÜTZING) W. SMITH	+	+
	BACI	Other pennate diatoms	+	+

ID	Group	Phyla/taxa	*Chil.*	*And.*
II	CYAN	*Oscillatoria* sp.	+	+
	CYAN	*Planktolyngbya limnetica* (LEMM.) KOMÁRKOVÁ-LEGNEROVÁ & CRONBERG	+	
	CYAN	*Spirulina* sp.	+	
	CYAN	*Anabaenopsis elenkinii* V.V. MILLER	+	
	CHLO	Chlorococcal	+	
	CHLO	*Scenedesmus dimorphus* (TURPIN) KÜTZING	+	
	CHLO	*Tetrastrum triangulare* (CHODAT) KOMÁREK	+	
	ZYGN	*Closterium* sp.	+	
	BACI	*Cyclotella meneghiniana* KÜTZING	+	+
	BACI	*Craticula ambigua* (EHRENBERG) D. G. MANN	+	
	BACI	Other pennate diatoms	+	+
	EUGL	*Euglena* sp.	+	+
	EUGL	*Phacus acuminatus* STOKES	+	
III	CYAN	*Arthrospira* sp.	+	
	CYAN	*Lyngbya* sp.	+	
	CYAN	*Dolichospermum* sp.	+	
	CYAN	*Anabaenopsis milleri* WORONICHIN	+	
	CYAN	*Nodularia spumigena* MERTENS EX BORNET & FLAHAULT	+	+
	CHLO	*Pediastrum boryanum* (TURPIN) MENEGHINI	+	
	BACI	*Amphora* sp.	+	+
	BACI	*Anomoeoneis sphaerophora* PFITZER	+	+
	BACI	*Campylodiscus clypeus* (EHRENBERG) EHRENBERG EX KÜTZING		+
	BACI	*Craticula cuspidata* (KÜTZING) D. G. MANN	+	+
	BACI	*Surirella ovalis* BRÉBISSON	+	+
	BACI	*Surirella striatula* TURPIN	+	+
	BACI	*Tryblionella apiculata* W. GREGORY	+	+
	BACI	*Tryblionella levidensis* W. SMITH	+	+
	BACI	Other pennate diatoms	+	+
	NEM	Unidentified nematodes	+	+
IV	ROT	Eggs	+	
	ROT	*Brachionus dimidiatus* BRYCE	+	
	ROT	*Brachionus plicatilis* O. F. MULLER	+	
	ROT	*Brachionus pterodinoides* ROUSSELT	+	
	ROT	*Filinia* sp	+	
	ROT	*Cephalodella* sp.	+	
	ROT	*Keratella tropica* APSTEIN	+	
	CLAD	Ephippia	+	+
	CLAD	*Alona* sp.	+	
	CLAD	*Bosmina* sp.		+
	CLAD	*Daphnia* sp.	+	+
	CLAD	*Diaphanosoma* sp.	+	
	CLAD	*Liederbosmina* sp.		+
	CLAD	*Leydigia* sp.		+
	COP	Unidentified nauplii	+	+
	COP	Unidentified copepodites	+	
	COP	*Boeckella poopoensis* MARSH	+	
	COP	*Boeckella* sp.	+	
	OSTR	Ostracoda	+	+
	CILIO	Ciliophora		+
	DIPT	Diptera	+	
V	CLAD	*Daphnia spinulata* BIRABÉN	+	
	CLAD	*Moina* cf. *micrura* KURZ	+	+
	CLAD	*Moina wierzejski* RICHARD	+	+
	COP	*Boeckella gracilis* DADAY	+	
	COP	*Metacyclops mendocinus* WIERZEJSKI	+	
	COP	Unidentified harpacticoid copepods	+	+

faeces, and Nostocales in Andean Flamingo faeces. Regarding microalgae, Bacillariophyta was the main group in Andean Flamingo faeces (96.9%), whereas in Chilean Flamingo faeces diatoms were found in lower proportions (16.92%) while Copepoda and Cladocera were the most abundant microinvertebrates.

Significant differences in the faeces content of Andean and Chilean Flamingos were found for Cyanobacteria ($U = 18.5, P = 0.011$), total microalgae ($U = 6, P = 0.0004$), and Bacillariophyta ($U = 6, P = 0.0003$). A PERMANOVA on the biovolume of taxonomic groups in the faeces showed significant differences between flamingo species ($F = 6.57; P = 0.0003$), and the SIMPER analysis assessed a 91% of dissimilitude. Of the 16 taxonomic groups considered, pennate diatoms, Oscillatoriales, Copepoda, Cladocera and Nostocales accounted for almost all of the dissimilarity (Fig. 3), with other groups contributing less than 1% of the dissimilarity.

A total of 75 taxa was found in *P. chilensis* faeces and 34 taxa in those of *P. andinus*, with only 26 in common (Table 3). PERMANOVA comparing the biovolume of taxa revealed significant differences between Chilean and Andean Flamingo faeces ($F = 2.726, P = 0.0049$), with 95% dissimilarity shown by the SIMPER analysis. The taxa contributing most to these differences were *Surirella striatula*, *S. ovalis* and *Craticula cuspidata* with higher biovolume in Andean Flamingo faeces; and *Arthrospira* sp., *Anabaenopsis milleri*, *Lyngbya* sp., Harpacticoida, *Metacyclops mendocinus*, *Daphnia spinulata* and *Brachionus pterodinoides* with higher biovolume in Chilean Flamingo faeces. The H' diversity index and evenness values were low, with no significant differences between both flamingo species (Table 2).

Prey size varied between 84.78 and 1.5×10^8 μm³ for Andean Flamingos and 12.36 and 2×10^8 μm³ for Chilean Flamingos. The Andean Flamingo consumed a higher biovolume of organisms grouped into category III (10,293–159,821 μm³) than Chilean Flamingos, and significant differences were found between both ($U = 22, P = 0.033$). In contrast, category V was consumed in significantly greater quantities by Chilean than by Andean Flamingos ($U = 14, P = 0.028$).

Flamingos' feeding selectivity and trophic niche overlap

Both flamingo species showed positive selection (Strauss's index) for Fusobacteria and Firmicutes, although this was stronger among Chilean Flamingos (Fig. 5). Andean Flamingos showed negative selection for Planctomycetes, Chloroflexi, Verrucomicrobia and other groups, but less proportionately, and interspecific differences were not significant.

On considering the Cyanobacteria, microalgae and microinvertebrates, there was evidence for differences in feeding selectivity between the flamingos (Fig. 5). Chilean Flamingos positively selected Cladocera and Rotifera, and for the groups Chlorophyta, Bacillariophyta and Copepoda there was positive selection in some lakes, and negative selection in other lakes. Within the Cyanobacteria, Oscillatoriales were positively selected, whereas Nostocales and Chroococcales were negatively selected. At
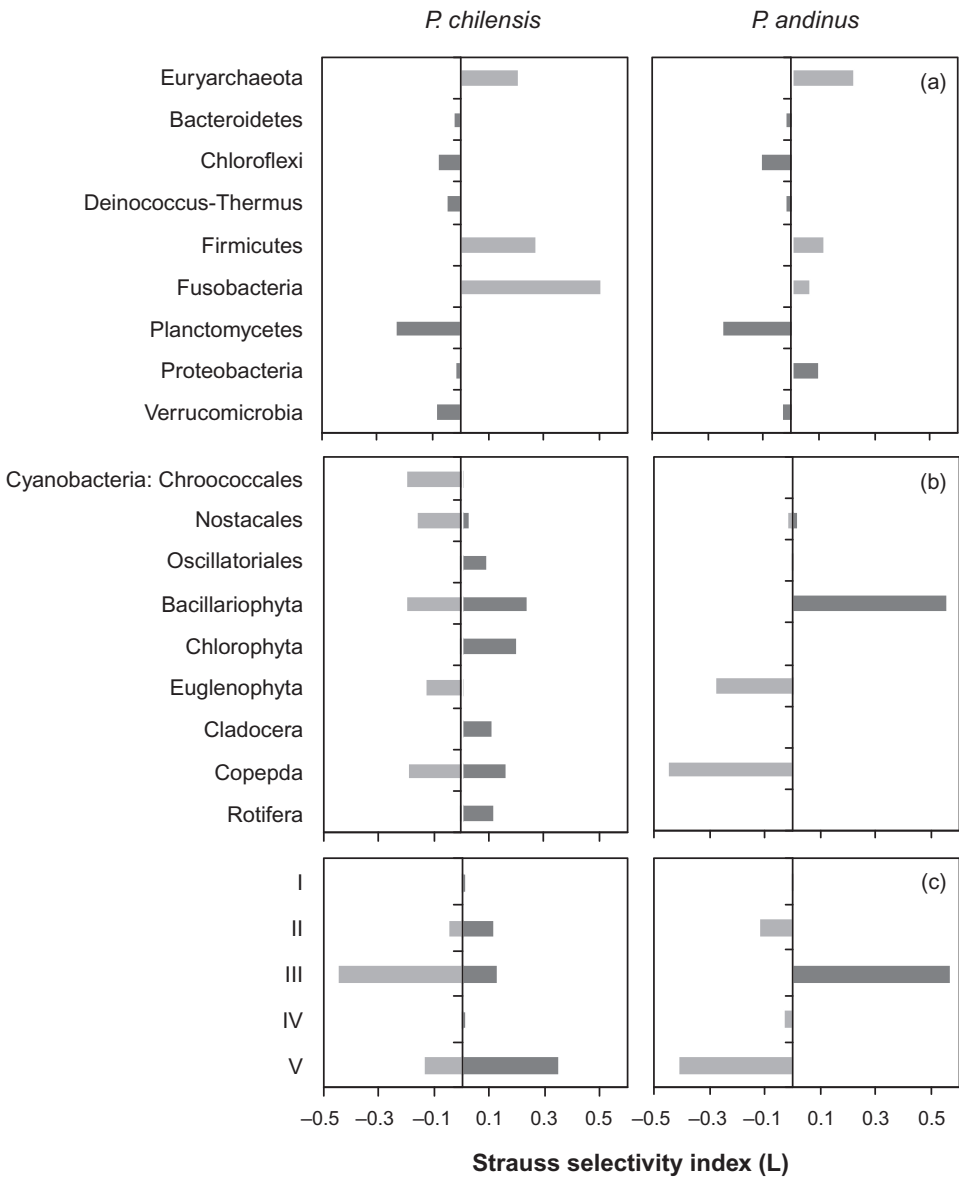
Figure 5. Trophic selectivity (Strauss index: L) for *Phoenicopterus chilensis* and *Phoenicoparrus andinus* applied to the abundance of a) archaea and bacteria, b) Cyanobacteria, microalgae and microinvertebrates, and c) their size categories: I ($< 2 \times 10^3 \ \mu m^3$), II ($2 \times 10^3$ to $10^4 \ \mu m^3$), III (10^4 to $2 \times 10^5 \ \mu m^3$), IV (6×10^5 to $8 \times 10^7 \ \mu m^3$), and V (10^8 to $2 \times 10^8 \ \mu m^3$). Strauss index indicates positive selection (L > 0), negative selection (L < 0), and absence of selectivity (L = 0).

species level, preferences were observed for *Daphnia spinulata*, *Metacyclops mendocinus* (Cladocera), *Boeckella* sp. (Copepoda), *Pediastrum boryanum* (Chlorophyta) and *Lyngbya* sp. (Cyanobacteria). The species negatively selected were *Surirella ovalis* and harpacticoid copepods. *S. striatula* was both positively and negatively selected. Andean Flamingos had a strong positive selectivity for Bacillariophyta and a negative selectivity for Copepoda and Euglenophyta (Fig. 4). *S. ovalis* and *S. striatula* were positively selected at species level and *Boeckella gracilis* and harpacticoids were negatively selected. Significant differences in the selectivity index of both flamingo species were found for Bacillariophyta ($U = 3$, $P = 0.0144$), Chlorophyta ($U = 5$, $P = 0.0258$) and Copepoda ($U = 5$, $P = 0.031$).

With regard to food size preferences, Chilean Flamingos showed a predominantly positive selection for category V and a negative selection for category III organisms. Conversely, Andean Flamingos positively selected category III and negatively selected category V (Fig. 5). Significant differences were found between both flamingo species for category III ($U = 5$, $P = 0.0217$) and V ($U = 5$, $P = 0.0319$). Niche overlap for Andean and Chilean Flamingos (Supporting Materials: Table S1) was high for bacteria and archaea (Pianka's index = 0.93) and for organism size categories (Pianka's index = 0.98), but it was low for Cyanobacteria, microalgae and microinvertebrate taxa (Pianka's index = 0.25).

Discussion

The abundance of Andean and Chilean Flamingos during the study years

demonstrated that the Pampa de las Lagunas wetland system is an important feeding area for the birds. Our results showed that populations of both species live in sympatry, sharing the same area but differing in their diets and trophic selectivity. The coexistence may be possible due to trophic niche differentiation.

Measures of nutrient concentrations at the lakes inhabited by flamingos indicated that they were eutrophic to hypereutrophic in condition (Nürnberg 1996), with water pH higher than 8.5, and hypohaline to mesohaline salinity levels (Hammer 1986). These characteristics are within the range of other lakes inhabited by flamingo (Caziani & Derlindati 2000; Blukacz *et al.* 2009; Kaggwa *et al.* 2013; Krienitz *et al.* 2016), but it is known that they could exploit a broader range of environmental conditions (Caziani *et al.* 2007; Derlindati 2008; Esté *et al.* 2012).

The molecular analysis showed a high microbial diversity in lake sediments available for feeding flamingos. This, the first bacteria and archaea inventory made in these lakes using high-throughput sequencing technology, found that the predominant phyla were Planctomycetes, Verrucomicrobia and Chloroflexi belonging to the bacteria domain, and Euryarchaeota belonging to the archaea domain. Similar phyla have been described in other extreme environments with flamingo flocks (Sorokin *et al.* 2014; Tazi *et al.* 2014) and in the alkaline and saline lakes of the Atacama Desert (Rasuk *et al.* 2016). Planctomycetes are widely distributed in saline environments of differing trophic status (Fuerst 1995), and Verrucomicrobia dominance is associated

with eutrophic conditions, especially with high phosphorus concentrations (Arnds *et al.* 2010). Euryarchaeota microorganisms are mainly aerobic heterotrophs that can also obtain energy from light due to bacteriorhodopsin. They are characteristically halophiles, and some of them are also resistant to hyperhaline conditions and provide the red pigmentation in the plumage of flamingos (Yim *et al.* 2015).

The lakes were characterised by high Cyanobacteria abundance favoured by the high nutrients, high conductivity and high pH (Reynolds 2006) found in the lakes. Bloom episodes are usually evident during summer due to the high temperatures (Paerl & Huisman 2008), but here blooms also occurred during winter, as observed in other environments (Kruk *et al.* 2015). Bacillariophyta and Copepoda followed the Cyanobacteria in biovolume levels. Bacillariophyta were widely distributed and had the highest species richness. *Cyclotella meneghiniana* was the only typically planktonic species. All the others were benthic or tychoplanktonic, and their appearance in the plankton was attributable to wind action and bird activity causing suspension of sediment in the shallow water column of the lakes (Wolin & Stone 2010). Cyanobacteria and diatoms have also been found to be dominant in other lakes inhabited by Chilean and Andean Flamingos (Hurlbert 1982; Hurlbert & Chang 1983; Salusso *et al.* 1997), and in African and Asian lakes inhabited by other flamingo species (Dadheech *et al.* 2013; Kaggwa *et al.* 2013).

Many studies support the view that differences in flamingo distribution are related to the availability and quality of food resources (*e.g.* Vareschi 1978; Hurlbert *et al.* 1986; Arengo & Baldassarre 2002; Krienitz & Kotut 2010; Kaggwa *et al.* 2013; Henriksen *et al.* 2015). We found that the highest abundances of Chilean Flamingos were closely associated with lakes characterised by the Cyanobacteria *Arthrospira* and species of the rotifer *Brachionus*. Previous work also found an association between the prevalence of Chilean Flamingo populations and environments with Cyanobacteria and microcrustaceans (Hurlbert 1982; Hurlbert *et al.* 1986; Mascitti 1998; Tobar *et al.* 2012). In contrast, the highest abundances of Andean Flamingos were recorded in lakes with Bacillariophyta and Copepoda dominance. Other studies pointed to the affinity of Andean Flamingos for diatoms, especially *Surirella* sp. (Hurlbert 1982; Hurlbert & Chang 1983). We found that an assemblage mainly composed by *Surirella striatula, Craticula cuspidata, Nitzschia palea, Campylodiscus cyclopeus* and *Cymbella* sp. was closely related to the abundance of Andean Flamingos at a site.

Nevertheless, the correlations between flamingo species abundance and microorganisms found in lakes give indirect knowledge on feeding preferences. This information was complemented with dietary studies that consider environmental supplies to show flamingos' feeding selectivity. One of the most significant findings of the present study was to show, for the first time, the archaeal and bacterial diversity of the flamingos' faeces. The importance of bacteria for flamingo diets is generally taken for granted, but has awaited the development and application of molecular

techniques to identify them. According to the classification of avian diet types proposed by Lópes *et al.* (2016), Andean and Chilean Flamingos could be considered iliovores as both ingest considerable amounts of mud with microbes when feeding.

High-throughput sequencing revealed that Planctomycetes (bacteria), Euryarchaeota (archaea), Verrucomicrobia (bacteria) and Chloroflexi (bacteria) were the most abundant phyla. The similar faecal content in Andean and Chilean Flamingos and the high niche overlap index of both species indicated no niche differentiation in relation to microbial assemblage. Antibiotic-resistant bacterial strains belonging to Proteobacteria, Actinobacteria and Firmicutes were detected, as was previously found in flamingo faeces from the Andean Puna analysed by isolation techniques (Dib *et al.* 2009; Fernández-Zenoff *et al.* 2015). Therefore, we suggest that birds could be acting as bacterial dispersers among regions, as stated in other studies (Palmagren *et al.* 1997; Shawkey *et al.* 2005; Dib *et al.* 2009).

Andean and Chilean Flamingo faeces differed mainly in their Cyanobacteria, microalgae and microinvertebrate content. The diet of Chilean Flamingos was composed primarily of Cyanobacteria, Bacillariophyta, Copepoda, Cladocera and Rotifera. They also consumed larger organisms belonging to Ostracoda, Nematoda and Diptera. The most consumed taxa were in the intermediate to large size range (categories III: 10^4 to 2×10^5 μm^3 and V: 10^8 to 2×10^8 μm^3). In some lakes where the Chilean Flamingo was abundant, *Arthrospira* sp. dominated in both food sources and

faeces. When *Arthrospira* sp. was absent, the birds fed mainly on *Planktolyngbya limnetica*, *Anabaenopsis elenkinii*, *Anabaenopsis milleri*, *Spirulina* sp. and diatoms (*Nitzschia palea*, *Cyclotella meneghiniana* and *Craticula cuspidata*). Diet studies for other flamingo species showed that, when Cyanobacteria markedly decreased, the birds change their diets and feed on diatoms, green algae, *Cryptomonas* and small insects (Ramesh & Ramachandran 2005, Krienitz & Kotut 2010, Tebbs *et al.* 2015). On the other hand, the most important microinvertebrates in the diet of Chilean Flamingos in our lakes were *Brachionus pterodinoides* (Rotifera), *Daphnia spinulata* (Cladocera), *Metacyclops mendocinus* and *Harpacticoida* sp. (Copepoda). Similarly, Hulbert (1982) proposed that *P. chilensis* feeds in an opportunistic way on the zooplankton present in High Andes salt lakes.

The diet of the Andean Flamingos was mainly composed of microalgae. The diatoms *Surirella striatula*, *S. ovalis Craticula cuspidata*, *Anomoneis sphaerophora*, *Campylodiscus clypeus* and *Cyclotella meneghiniana* were the most important food sources. To a lesser extent, they fed on Cladocera and Copepoda species. Hence, although they are able to consume large organisms, they preferred intermediate prey sizes (categories III: 10^4 to 2×10^5 μm^3). Likewise, Hulbert & Chang (1983) found that diatoms larger than 80 μm were also important in their diet, whereas smaller diatoms were not likely to be retained on filtering water and sediment through their beaks.

Although Chilean and Andean Flamingos differed in their food size preferences, a clear niche differentiation was not evident

at this level (there being high niche overlap according to organism size classification), as found for bacteria and archaea. Niche differentiation became evident on analysing food items at the taxa level. In addition, the number of different food items consumed was higher in Chilean Flamingos than in Andean Flamingos (75 and 34 taxa, respectively), suggesting a broader trophic niche.

Some of the mechanisms involved in the selection of prey during filter-feeding by flamingos are: (a) manipulation of the openness of the gape to adjust the mesh size for the inflow and outflow of water, (b) manipulation of the amplitudes of lingual motions to adjust pumping capacity, and (c) manipulation of the timing of lingual protraction-retraction relative to lingual elevation-depression, which directs a portion of the water outflow along proximal rather than distal lamellae, to match mesh size to filtering for smaller food sizes (Zweers *et al*. 1995). In this way, the specialization and the degree of functional versatility of the mesh size of the Chilean Flamingo corresponds to the exploitation of a wider variety of food sources than that of the Andean Flamingo (Tobar *et al*. 2017).

The Chilean Flamingo has been considered a generalist due to the broad variety of organisms in its diet (Mascitti & Kravetz 2002). On the other hand, the Andean Flamingo has been considered a specialist, because it mainly consumes phytoplankton and benthonic diatoms (Jenkin 1957; Zweers *et al*. 1995; Mascitti & Kravetz 2002). Consideration of the prey consumed indicates that the Chilean Flamingo is truly an omnivore, feeding on

Cyanobacteria (phytoplanktivore), insect larvae and microcrustacea (zooplanktivore) (Rodríguez 2005), although at some sites it has shown exclusively carnivore behaviour, with a trophic spectrum that encompassed only invertebrate prey (Hulbert 1982; Hurlbert *et al*. 1984; Tobar *et al*. 2014). We therefore conclude that the Chilean Flamingo is highly versatile in its diet and food selection, and that this varies in relation to the food available in its environment.

The Andean Flamingo showed a high positive selection for diatoms and strong negative selection of microinvertebrates such as copepods. Thus it preferred diatoms although also consumed some microinvertebrates. Lópes *et al*. (2016) similarly classified the Andean Flamingo as phytoplanktivore, but Martínez & González (2004) considered that it is omnivorous, as it may also feed on microcrustacea.

Acknowledgments

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