

Environmental changes affect picoplanktonic composition in Antarctic Peninsula ponds

Micaela DÍAZ^{1,2}, Leonardo LAGOMARSINO¹, Gabriela MATALONI³,
Marianela BELTRÁN^{4,5}, Marcela LIBERTELLI⁴ & Paulina FERMANI^{1,5*}

¹ Laboratorio de Ecología Acuática, INTECH-UNSAM-CONICET, Chascomús 7130, Argentina;

² Facultad de Ciencias Naturales y de la Salud, UNPSJB, Comodoro Rivadavia 9000, Argentina;

³ Laboratorio de Biodiversidad, Limnología y Biología de la Conservación, 3IA-UNSAM-CONICET, Buenos Aires 1650, Argentina;

⁴ Departamento de Biología de Predadores Tope, Instituto Antártico Argentino, Buenos Aires 1650, Argentina;

⁵ Laboratorio de Microbiología Ambiental, IBIOMAR/CESIMAR-CENPAT-CONICET, Puerto Madryn 9120, Argentina

Received 31 August 2023; accepted 9 November 2023; published online 30 March 2024

Abstract Antarctic Peninsula is experiencing one of the largest global warming events worldwide. Shallow water bodies generated by the melting of snow in summer are numerous, and they might act as sentinels of climate change due to their rapid response and ability to integrate catchment information. Shifts in climate can influence the structure of microbial communities which dominate these freshwaters ecosystems. Here, we characterize three ponds at Cierva Point (Antarctic Peninsula) by examining their physico-chemical and morphological characteristics and we explored how different factors modify the structure of the microbial community. We studied the abundance and biomass of heterotrophic bacteria, picocyanobacteria and picoeukaryote algae during January and February of two consecutive summers (2017 and 2018). We found that ponds had different limnological characteristics, due to their location, geomorphological features and presence of the surrounding flora and fauna. Physico-chemical parameters as well as microbial community differed between ponds, months and years. In 2017, most ponds were oligo to mesotrophic states. The larger accumulated rainfall (as a result of environmental changes on the Antarctic Peninsula) during 2018, particularly in February, causes nutrient runoff into water bodies. This affects those ponds with the highest seabird circulation, such as gentoo penguin, increasing eutrophication. As a result, picoplanktonic abundances were higher, and the community structure shifts to a largely heterotrophic bacteria dominated one. These results suggest that these communities could act as sentinels to environmental changes, anticipating a future with mostly hypertrophic ponds.

Keywords microorganisms, freshwater environments, climate change, Cierva Point

Citation: Diaz M, Lagomarsino L, Mataloni G, et al. Environmental changes affect picoplanktonic composition in Antarctic Peninsula ponds. *Adv Polar Sci*, 2024, 35(1): 108-122, doi: 10.12429/j.advps.2023.0018

1 Introduction

The Earth's temperature has increased on average 1.2 °C over the last 50 years, however not all regions

undergo the same warming (IPCC, 2021). Global climate change is magnified in polar sites and Antarctic Peninsula was one of the most rapidly warming regions globally (Lee et al., 2022; Siegert et al., 2019). Likewise, general warming with more intense rainfall is also expected towards the end of this century on the Antarctic continent (Vignon et al., 2021). Changes in precipitation will also be uneven (Walther et al., 2002), shifting from snow to rain at some

* Corresponding author, ORCID: 0000-0002-8036-9347, E-mail: pfermani@cenpat-conicet.gov.ar

time in the summer (Siegert et al., 2023).

Antarctic Peninsula warming and the effects of climate change on the continent are linked (King and Comiso, 2003). The strong interaction of terrestrial environments driven by fluctuations in the availability of liquid water has been observed (Hawes et al., 2023; Mataloni and Quintana, 2017). Liquid water plays a crucial role in supporting life and its regulatory role in the interactions between water and surrounding environments has been recognized, acknowledging the wetlands of Antarctica (Camacho et al., 2012; Mataloni and Quintana, 2017; Rochera et al., 2010). Wetlands are one of the world's most valuable ecosystems, playing a role in the hydrological cycle, and hosting a high biodiversity (Benzaquén et al., 2017). However, 35% of the world's wetlands have disappeared since 1970, mainly due to climate change (Davidson, 2018; Mieczan et al., 2019). Antarctic wetlands mainly consist of streams, ponds and lakes formed during the thawing process, representing the most variable terrestrial ecosystems. Despite their importance, freshwater environments in Antarctica are still understudied (Hawes et al., 2023).

Usually, shallow lakes and ponds can be sentinels for climate change due to their rapid response to changes and ability to integrate catchment information (Adrian et al., 2009). These systems experience fluctuations in temperature, sunlight regimes and chemical gradients, significantly influencing the abundances and life strategies of microorganisms (Fermani et al., 2022; Williamson et al., 2008). Polar lakes are particularly vulnerable to disturbances, characterized by low resilience and extended recovery periods (Mataloni and Quintana, 2017; Woodward et al., 2010). They are susceptible to impacts of climate warming, in which external factors outweigh the relative importance of in-lake processes, making them ideal sentinels of climate change (Siegert et al., 2023). Another consequence of climate change is the upward mobilization of allochthonous organic matter (OM) and nutrients that can rapidly alter the composition and function of microbial communities (Rofner et al., 2017). So, these fragile environments have served as natural laboratories for studying the effects of climate variations, allowing us to assess the expected ecological consequences of such changes (Grimm et al., 2013; Williamson et al., 2009).

Microorganisms have key roles in carbon and nutrient cycling, supporting the life of biosphere (Cavicchioli et al., 2019). Particularly, heterotrophic bacteria (HB) and phototrophic picophytoplankton (PPP) (picocyanobacteria–Pcy and picoeukaryote–Peuk) are among the world's most abundant (i.e., Fermani et al., 2013, 2015; Roland et al., 2010), distributed worldwide in all types of water bodies and trophic states (Callieri and Stockner, 2002). In Antarctic freshwaters ecosystems, planktonic food webs are characterized by short trophic levels (Ellis-Evans, 1996) where microorganisms dominate (Camacho et al., 2012). Shifts in climate can influence the structure and diversity of microbial communities (Cavicchioli et al., 2019), with the

potential modification of the lacustrine water body's metabolism.

Cierva Point (Antarctic Peninsula) is a landscape dominated by wetlands, where numerous ponds are located (Mataloni and Quintana, 2017). The area is characterized by its high environmental heterogeneity. Extensive studies of microalgae communities have revealed diverse taxonomic compositions influenced by surrounding environments (Allende and Mataloni, 2013; González Garraza et al., 2011; Mataloni et al., 2010). Furthermore, Cierva Point is inhabited by numerous species of birds, flora, fauna and invertebrates, providing it with an exceptional landscape value (Convey, 2011; González-Zevallos et al., 2013; Quintana et al., 2000). Due to their biological diversity, the Scientific Committee on Antarctic Research has designated Cierva Point and offshore islands as an Antarctic Specially Protected Area (ASPA) No.134 (Williamson et al., 2009). ASPAs are fundamental to reducing human impacts on Antarctic ecosystems (Wauchope et al., 2019). Among the 16 eco-regions, the northern area of the Antarctic Peninsula is one of the most protected; however, environmental changes seem to reach these areas as well.

Due to its importance, the objective of this study is to characterize three ponds located in Cierva Point, by examining their physical, chemical and morphological characteristics, as well as determine the impact of different stress factors on microbial communities. Specifically, we assess the abundance and biomass of HB, Pcy and Peuk during two consecutive Antarctic summers (2017 and 2018) in the months of greatest thawing (January and February). We hypothesize that the three ponds, although close in terms of distance, differ in trophic state, limnological variables, and/or picoplankton communities, based on pond morphology, environmental changes and the potential influence of Antarctic flora and/or fauna in their peripheral zones. Furthermore, we anticipate that water bodies will undergo temporary modifications as a result of climatic variations, which will impact on the microbial community structure. We expect that ponds with high nutrient loads, trophic states and temperatures will harbor greater microorganism abundance and biomass.

2 Materials and methods

2.1 Study sites and sample collection

The study area was situated at Cierva Point (64°09'S, 60°57'W) on the Danco Coast, in the northwestern part of the Antarctic Peninsula (Figure 1a), characterized by a complex mosaic of diverse environmental units (EUs) (Agraz et al., 1994). These EUs are featured by different reliefs and vegetation cover, which determine micro-watersheds and a high environmental heterogeneity with an important offer of habitats and microhabitats (Rau et al., 2000). Within this wetland system, the ASPA No. 134 consisting of Antarctic pools, ponds, streams, is dominated

by microbial mats and mostly covered by mosses like *Polytrichum* spp. (Allende and Mataloni, 2013; Hawes et al., 1999) (Figure 1b). The area has a cold marine climate

(mean annual air temperature ca. -3.2 °C) with an annual precipitation ranging between 400 and 1100 mm (Wilhelm et al., 2016).

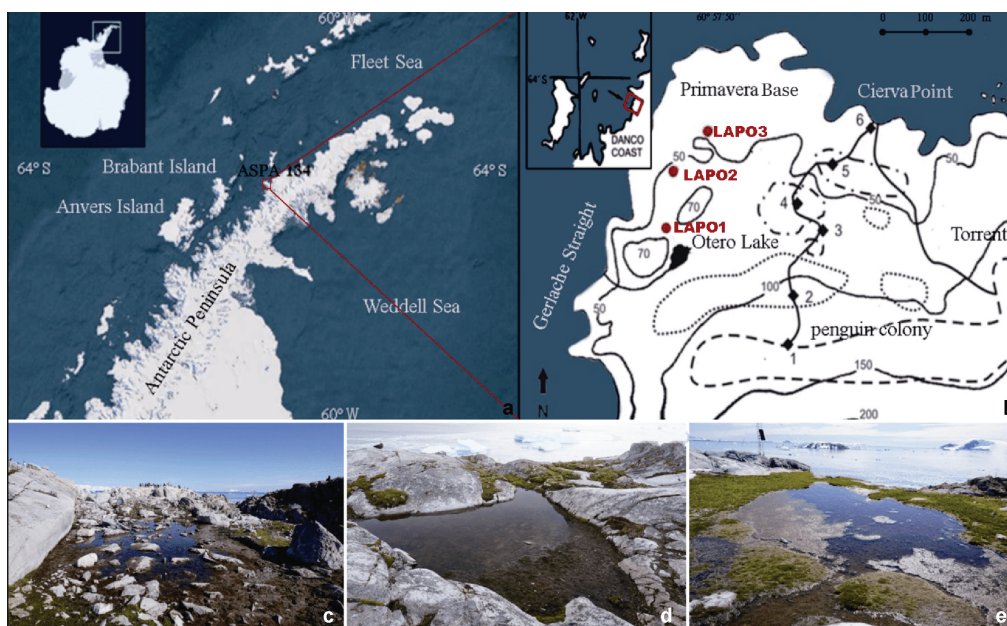


Figure 1 Map of the Antarctic Peninsula and studied ponds. **a**, general location of the Antarctic Specially Protected Area (ASP) No. 134, Cierva Point and offshore islands; **b**, map of Primavera Base (Argentina), with the studied water bodies (large pools = LAPO); **c**, LAPO1; **d**, LAPO2; **e**, LAPO3, adapted from Allende and Mataloni (2013).

During the Antarctic summer of 2017 and 2018, three ponds (large pools = LAPO) were sampled in this area. LAPO1 ($64^{\circ}9'22.44''S$, $60^{\circ}57'21.62''W$) located in a depression, was surrounded by clasts > 10 cm in size and adjacent to a gentoo penguin (*Pygoscelis papua*) rookery (Figure 1c). LAPO2 ($64^{\circ}9'26.63''S$, $60^{\circ}57'25.42''W$) situated on a terrace, was surrounded by clasts > 20 cm and mosses, and inhabited by a few skuas (*Catharacta maccormicki*) (Figure 1d). LAPO3 ($64^{\circ}9'28.35''S$, $60^{\circ}57'27.6''W$), closer to Primavera Base, had a flat microrelief. This pond was completely surrounded by mosses, and occasionally used by penguins on their transit to the sea (Figure 1e).

Sampling took place in each pond in January and February. The geographic position was established using Global Positioning Systems equipment (GPS eTrex, Garmin International, Olathe, KS, USA) and morphometric measures were taken in the field. The determination of the EU for each pond involved a careful examination of the Cierva Point maps, considering their specific locations. Furthermore, the width, length and maximum depth (Z) of each pond were measured using a tape measure (Figure 2a). The slope of each sampling site was calculated with a field laser clinometer (Scout DX 1000 ARC, Bushnell, Overland Park, KS, USA) (Figure 2b). Each pond was characterized based on relief type: depression (0) or flat (1). Additionally, the degree of slope was categorized as null (0) or gentle with a 5-degree inclination (1). The degree of disturbance in the surrounding area was assessed through on-site observations,

taking into account the presence of birds, mammals, rocks and mosses. Subsequently, we established an impact index of abundance and permanence of gentoo penguins or signs thereof (e.g., feathers or feces) in the area (Penguin Index: PI). The PI scale varies from 0 to 5, where 0 indicate absence of penguins or signs, 1 (occasional abundance), 2 (medium transit), 3 (medium abundance), 4 (high abundance), and 5 (nesting areas with extremely high abundance of penguins). Likewise, the Cover Index (CI) was used to indicate the relationship between the clast and moss (Figure 2c). The CI varies between 1 (pond covered by debris > 10 cm), 2 (pond covered by debris > 20 cm plus mosses) and 3 (pond covered only by mosses).

In situ measurements were conducted to determine the water temperature, conductivity and pH, using the multiparametric probe (Sension 156, Hach, Loveland, CO, USA) and a pH meter (HI98108, Hanna Instruments, Woonsocket, RI, USA), respectively. The transparency was measured using a Secchi disk. Then, subsurface water was collected in several polypropylene containers and transported to the Laboratory of Primavera Base in order to pre-analyze the samples for chemical and biological variables.

2.2 Analysis of chemical and biological variables

For the analysis of inorganic dissolved nutrients (nitrates– $N-NO_3^-$, nitrites– $N-NO_2^-$ and soluble reactive



Figure 2 Morphometric and coverage measures of studied ponds during sampling period. **a**, water depth (Z); **b**, slope; **c**, Cover Index (CI).

phosphorus–SRP), 100 mL of water from each sample was filtered through Whatman GF/F filters. Nitrates were reduced to nitrites using a cadmium reduction column; then nitrites were determined by diazotization (APHA, 1992). To determine organic nitrogen concentrations, the semi-micro-Kjeldahl method described by APHA (1992) was employed using unfiltered water. This method involved acid digestion using H_2SO_4 at high temperatures ($\sim 350^\circ C$), followed by alkaline distillation and subsequent volumetric titration with 0.1N HCl. Total nitrogen (TN) was considered as the sum of nitrates, nitrites and organic nitrogen. SRP was estimated using the molybdate-ascorbic acid technique (APHA, 1992). Total phosphorus (TP) concentrations were measured from unfiltered water through acid digestion using a combination of sulfuric acid and potassium persulfate. The molybdate-ascorbic acid method, following the analytical procedures was used for TP analysis (APHA, 1992). Chl-*a* concentrations were determined using a spectrophotometric method after methanol extraction, following Lopretto and Tell (1995). Trophic State Index (TSI) was calculated for each sampling date using Chl-*a* and TP concentrations (Carlson, 1977). The ponds were classified using a scale from 0 to 100, where TSI values < 20 indicate ultraoligotrophic conditions (1); between 30 and 40, oligotrophic conditions (2); values between 40 and 50, mesotrophic (3); between 50 and 70, eutrophic (4); and > 70, hypereutrophic conditions (5).

For picoplankton analysis (HB, Pcy and Peuk), 45 mL of water from each pond was fixed in 5 mL of 10% glutaraldehyde (final concentration 1%). In the field laboratory, two subsamples were taken and stained with 50 μL of DAPI (4',6-diamidino-2-phenylindole). Between 0.5 and 1 mL of replicates were filtered through 0.2 μm black polycarbonate filters for the quantification. Slides were mounted with filters and the samples were frozen ($-20^\circ C$) until analysis (Porter and Feig, 1980). Back in the laboratory of Chascomús (INTECH), filters were examined under Nikon E600 epifluorescence microscope equipped with UV-2E/C ultraviolet light excitation filter (excitation filter 340–380 nm BP, dichroic mirror 400 nm LP, barrier filter 435–485 nm BP), G-2A green light excitation filter (excitation filter 510–560 nm BP, dichroic mirror 565 nm

LP, barrier filter 590 nm LP) and a blue light excitation filter B-2A (excitation filter 450–490 nm BP, dichroic mirror 500 nm LP, barrier filter 515 nm LP). For HB count, UV light was used. HB were classified according to their size and state of cellular aggregation, being distinguished into unicellular (HB), aggregated (HB-ag) and filamentous (HB-fil); while Pcy and Peuk were observed from the fluorescence given by photosynthetic pigments, under green and blue excitation light, respectively (Callieri and Pinolim, 1995). At least 400 individuals and 20 fields of view were observed on each filter in order to account for an error less than 15 %.

To estimate unicellular HB biomass, an average biovolume (V) of $0.076 \mu m^3 \cdot cell^{-1}$ was used for LAPO1, and $0.059 \mu m^3 \cdot cell^{-1}$ was used for LAPO2 and LAPO3, which were previously determined for individuals from nearby maritime Antarctic water bodies and taking into account different trophic states (Unrein, unpublished data). For this purpose, photographs of bacteria were taken and the images were analyzed with Image-Pro Plus 4.5 software. Each image was processed according to the protocol proposed by Massana et al. (1997). To estimate the V of HB-fil, cell width, length and filament length were measured and approximated to simple geometric shapes. The averages V of filaments were $0.46 \mu m^3 \cdot cell^{-1}$. The HB biomass (CHB) for a single cell was estimated by applying the conversion factor proposed by Simon and Azam (1989): $C_{CHB, single} (pg C \cdot cell^{-1}) = 92 \times V^{0.598}$. The biovolume used for Pcy and Peuk were 2.3 and 1.7 $\mu m^3 \cdot cell^{-1}$, respectively, previously determined for individuals from Antarctic water bodies, kindly shared with us by Dr. Unrein and published by Allende and Mataloni (2013). The biomass of Pcy (C_{Pcy}) was calculated assuming a conversion factor of $230 fg C \cdot \mu m^{-3}$ (Worden et al., 2004); while the carbon content per cell of Peuk (C_{Peuk}) was estimated following the C : V ratio proposed by Menden-Deuer and Lessard (2000) as: $C_{C_{Peuk, single}} (pg C \cdot cell^{-1}) = 0.216 \times V^{0.939}$.

2.3 Statistical analysis

Similarity between ponds was performed through the Bray-Curtis (dis)similarity index, using morphometric and geological variables standardized. The statistical analyses of

simple agglomerative hierarchical clustering method (UPGMA) were performed through PAST 4.03 software (Hammer et al., 2001), using Euclidean distance, and the results were represented in a dendrogram.

To evaluate possible relationships between abiotic and biotic parameters, the nonparametric Spearman rank correlation was applied for all data set using Sigma Plot 12.0 software (Systat Software Inc., San Jose, CA, USA).

The multivariate ordination technique (Principal Component Analysis–PCA) was used in order to explore the ordination of ponds, according to abiotic and morphometric conditions, as well as to determine the variation of the community explained by the physical, chemical and/or environmental variables. Some highly correlated variables were excluded from the analysis (i.e., Secchi disk and TN). These analyses were made using the CANOCO 5.0 software (ter Braak and Šmilauer, 2012).

Air temperature (in °C) was calculated from monthly AIRS data, while the precipitation ($\text{mm}\cdot\text{d}^{-1}$) was calculated from daily AIRS-SSMI data, both obtained from Giovanni's website (<https://giovanni.gsfc.nasa.gov/giovanni/>, accessed on 7 August 2023). Data was performed for the period 2003 to 2018. The annual averages were compared for the years 2006, 2017, and 2018. Furthermore, air temperatures of San

Martín Base were provided by Servicio Meteorológico Nacional de Argentina and the years were compared using *t*-Student test.

All statistical analysis was accepted as significant at a probability level of $P < 0.05$.

3 Results

Geographical, morphometric and trophic state characterization of water bodies are summarized in Table 1. LAPO1 and LAPO2 were located in the same EU. However, the length, width, Z, CI and TSI, differed among the three ponds during January and February 2017 and 2018. Greatest depth was recorded in LAPO1 in 2018, while the largest variations in width and length occurred in LAPO2 and LAPO3, respectively. Most ponds displayed a Secchi disk equal to Z, with the exception of LAPO3 in February 2017 (Secchi disk = 10 cm). On the other hand, the three ponds had different TSI indices, according to the year and month. In 2017, LAPO1 showed meso-eutrophic characteristic, while LAPO2 and LAPO3 had oligotrophic to eutrophic conditions. In 2018, all ponds showed eutrophic to hypertrophic features, during both months.

Table 1 Geographical, morphometric and trophic state variables of ponds during sampling period

	January 2017			February 2017			January 2018			February 2018		
	LAPO1	LAPO2	LAPO3	LAPO1	LAPO2	LAPO3	LAPO1	LAPO2	LAPO3	LAPO1	LAPO2	LAPO3
EU	7	7	11		7	11	7	7	11	7	7	
Microrelief	1	0	1		0	1	1	0	1	1	0	
Slope	0	0	1		0	1	0	0	1	0	0	
Elevation/m	68	66	37		66	37	68	66	37	68	66	
Length/m	10.85	6	13.17		6	13.17	10.55	5.65	17.8	12.3	3.2	
Width/m	5.7	6.1	8.13	NS	6.1	8.13	5.2	2.45	5.8	6.2	6.1	NS
Z/cm	21	11	40		16	40	90	13	14	10	10.5	
PI	4	0	0		1	4	5	1	3	5	1	
CI	1	2	3		2	3	1	2	3	1	2	
TSI	3	2	2		4	5	5	5	5	5	5	

Notes: EU–environmental unit; Z–maximum depth; PI–Penguin Index; CI–Coverage Index; TSI–Trophic State Index; NS–not sampling

It is important to underline that LAPO1 was frozen in February 2017, and LAPO3 harbored a high number of penguins inside the water body in February 2018. Therefore, during the periods mentioned, these ponds were not sampled (NS).

Cluster analyses revealed that ponds constituted a separate cluster by year (Figure 3). Also, LAPO1 and LAPO2 had the shortest distance, making them more similar morphologically and geographically, compared to LAPO3, which formed a separate cluster, being more dissimilar.

Physico-chemical parameters of Antarctic ponds analyzed in this study differed between waters bodies, months and years (Figure 4 and Table 2). Water temperatures ranged from 2.4 to 18 °C, with the minimum value registered in February 2017 in LAPO2 (Figure 4a and Table 2). The pH in LAPO1 and LAPO2 were around neutral values, while LAPO3 had slightly more acidic pH numbers (Figure 4b). This parameter was positively correlated with N-NO_2^- ($r=0.88$, $P<0.0001$) and TP ($r=0.66$, $P<0.05$). Moreover, a wide range of conductivity values was observed with LAPO1 being up to two orders of

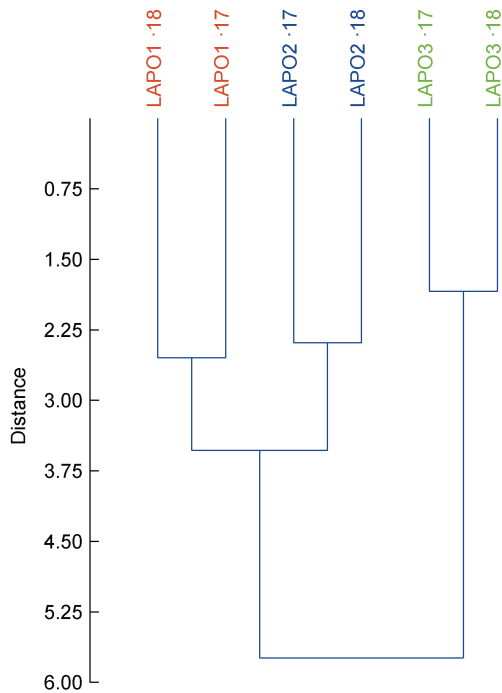
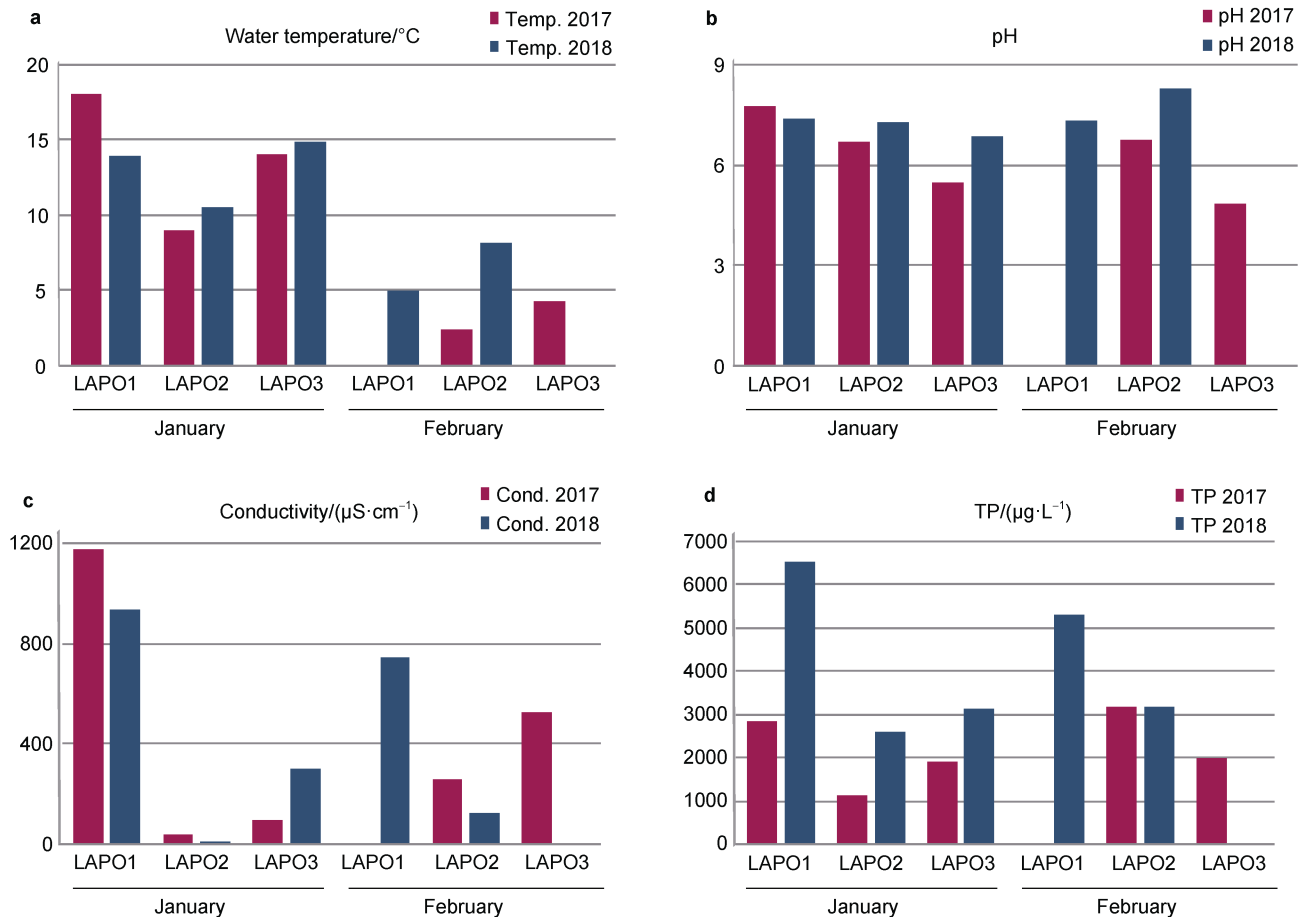


Figure 3 Cluster analysis dendrogram showing the dissimilarity among studied ponds during sampling period (large pools = LAPO; 17 indicates 2017; 18 indicates 2018).

magnitude higher than the other ponds (Figure 4c). Conductivity was positively correlated with SRP ($r=0.71$, $P<0.05$) and PI ($r=0.70$, $P<0.05$); in turn, PI was also correlated to N-NO_3^- ($r=0.69$, $P<0.05$). Nutrients and Chl-*a* concentrations were also highly variable (Table 2). In general, TP and N-NO_3^- were lower in 2017, reaching extremely high values in LAPO1 in both months of 2018 (Figures 4d and 4e). On the other hand, during January 2017, low Chl-*a* values were observed in all water bodies. This situation was reversed in February, where LAPO2 and LAPO3 doubled the concentrations. In contrast, in 2018 high Chl-*a* concentrations were overall recorded for both months (Figure 4f). This parameter was negatively correlated with water temperature ($r=-0.6$, $P<0.05$).

The abundance and biomass of PPP organisms also differed between waters bodies, months and years (Figure 5 and Table 3). Generally, numbers were greater in 2018, especially during February, reaching between one and two orders of magnitude higher (Figures 5a–5d). HB abundance ranged between $6.0 \times 10^5 \text{ ind}\cdot\text{mL}^{-1}$ in LAPO1 during January 2017 and $2.0 \times 10^7 \text{ ind}\cdot\text{mL}^{-1}$ in LAPO3 in February 2018. HB was negatively correlated with water temperature ($r=-0.84$, $P<0.001$) and positively correlated with Peuk density ($r=0.65$, $P<0.05$) and Chl-*a* concentration ($r=0.56$,



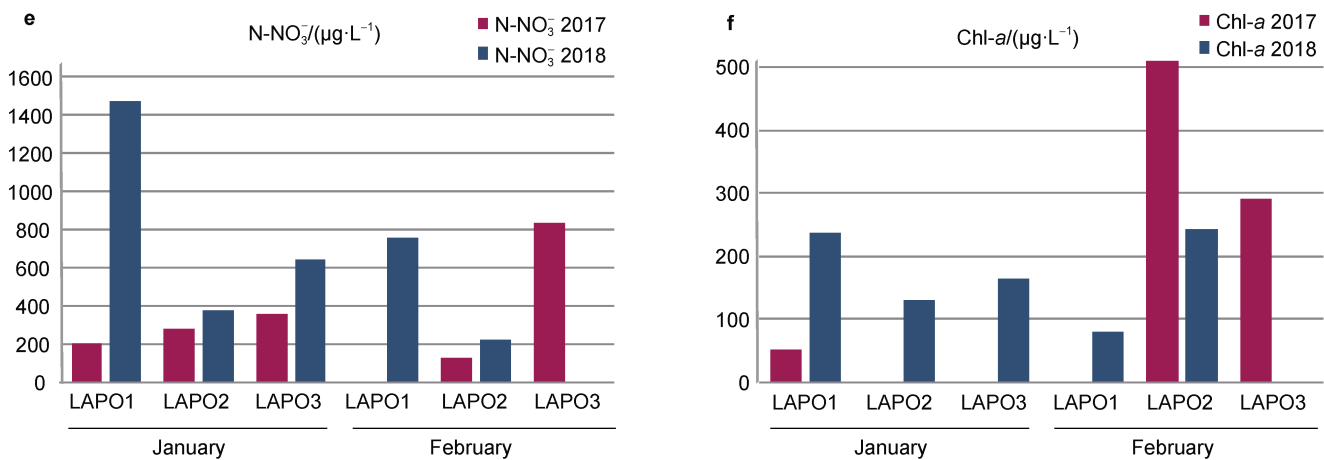


Figure 4 Physico-chemical parameters of studied ponds during sampling period. **a**, water temperature (Temp.); **b**, pH; **c**, conductivity (Cond.); **d**, total phosphorous (TP); **e**, nitrate (N-NO₃⁻); **f**, chlorophyll-*a* (Chl-*a*). LAPO indicates large pools.

Table 2 Average values and ranges (minimum and maximum) for months of physico-chemical variables

	2017		2018			
	LAPO1	LAPO2	LAPO1	LAPO2	LAPO1	LAPO2
Temp/°C	18.0 (18.0; 18.0)	5.7 (2.4; 9.0)	9.2 (4.3; 14.0)	9.45 (5.0; 13.9)	9.3 (8.1; 10.5)	14.8 (14.8; 14.8)
pH	7.7 (7.7; 7.7)	6.7 (6.6; 6.7)	5.1 (4.8; 5.5)	7.3 (7.3; 7.4)	7.8 (7.3; 8.3)	6.9 (6.9; 6.9)
Cond/(µS·cm ⁻¹)	1170.0 (1170.0; 1170.0)	145.5 (35.0; 256.0)	308.5 (92.0; 525.0)	837.0 (740.0; 934.0)	66.45 (10.5; 122.4)	298.0 (298.0; 298.0)
SRP/(µg·L ⁻¹)	2536.0 (2536.0; 2536.0)	544.4 (370.9; 718.0)	1094.0 (770.3; 1418.9)	2550.0 (2478.9; 2621.1)	1227.4 (1053.1; 1401.7)	1484.6 (1484.6; 1484.6)
TP/(µg·L ⁻¹)	2820.7 (2820.7; 2820.7)	2157.1 (1127.0; 3187.3)	1927.0 (1890.3; 1963.7)	5934.0 (5314.0; 6554.0)	2890.6 (2594.0; 3187.3)	3134.0 (3134.0; 3134.0)
N-NO ₃ ⁻ /(µg·L ⁻¹)	201.0 (201.0; 201.0)	203.1 (124.0; 282.0)	593.1 (353.1; 833.3)	1117.0 (755.0; 1479.0)	297.5 (218.0; 377.0)	640.0 (640.0; 640.0)
N-NO ₂ ⁻ /(µg·L ⁻¹)	15.0 (15.0; 15.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	145.5 (35.0; 256.0)	58.5 (3.0; 11.0)	1.0 (1.0; 1.0)
TN/(µg·L ⁻¹)	10349.0 (10349.0; 10349.0)	17381.1 (14480.1; 20283.0)	13697.5 (7728.0; 19667.0)	4469.2 (2126.1; 6776.0)	17180.0 (16888.0; 17472.0)	13888.2 (13888.2; 13888.2)
Chl- <i>a</i> /(µg·L ⁻¹)	51.0 (51.0; 51.0)	255.1 (2.0; 508.2)	146.2 (2.3; 291.0)	158.0 (79.1; 237.2)	185.5 (129.0; 242.1)	165.0 (165.0; 165.0)

Notes: Temp indicates temperature; Cond indicates conductivity; SRP indicates soluble reactive phosphorus; TP indicates total phosphorus; N-NO₃⁻ indicates nitrates; N-NO₂⁻ indicates nitrites; TN indicates total nitrogen; Chl-*a* indicates chlorophyll-*a*.

$P < 0.05$). Among the different bacterial morphotypes found (HB-ag and HB-fil), abundances were also higher in 2018. LAPO2 displayed a more stable community between the two months, however in LAPO1 and LAPO3 a clearly different composition was observed, increasing mainly in February (Figures 5c and 5d). HB-ag was negatively correlated with water depth ($r = -0.62$, $P < 0.05$), while HB-fil had a weak, but inverse relationship with water temperature ($r = -0.59$, $P < 0.05$). On the other hand, Pcy abundance fluctuated between 2.5×10^4 ind·mL⁻¹ (LAPO1 during January 2017) and 9.1×10^5 ind·mL⁻¹ (LAPO3 in February 2017). Pcy numbers did not show a clear pattern and were not significantly correlated to any studied parameters. Peuk cells were about one or two order of magnitude lower

than Pcy. These were relatively uncommon in the three ponds. The minimum and maximum values of Peuk were observed in LAPO3 in January 2017 and 2018 (3.6×10^3 ind·mL⁻¹, 1.0×10^5 ind·mL⁻¹, respectively), demonstrating the great variability of this ecosystem. Peuk abundances were positively correlated with Chl-*a* ($r = 0.65$, $P < 0.05$) and PI ($r = 0.5$, $P < 0.05$).

Excluding LAPO3, in terms of biomass, HB (100.5 ± 124.3 µg C·L⁻¹), Pcy (21.8 ± 9.5 µg C·L⁻¹) and Peuk (3.7 ± 2.8 µg C·L⁻¹) represented on average 79.7%, 17.3% and 2.9% of total picoplanktonic biomass, respectively, in 2017 (Table 3, Figures 5e and 5f). In 2018, the mean annual bacterial biomass was higher than previous year (541.85 ± 644.1 µg C·L⁻¹), reaching 86.1% of total picoplankton. This

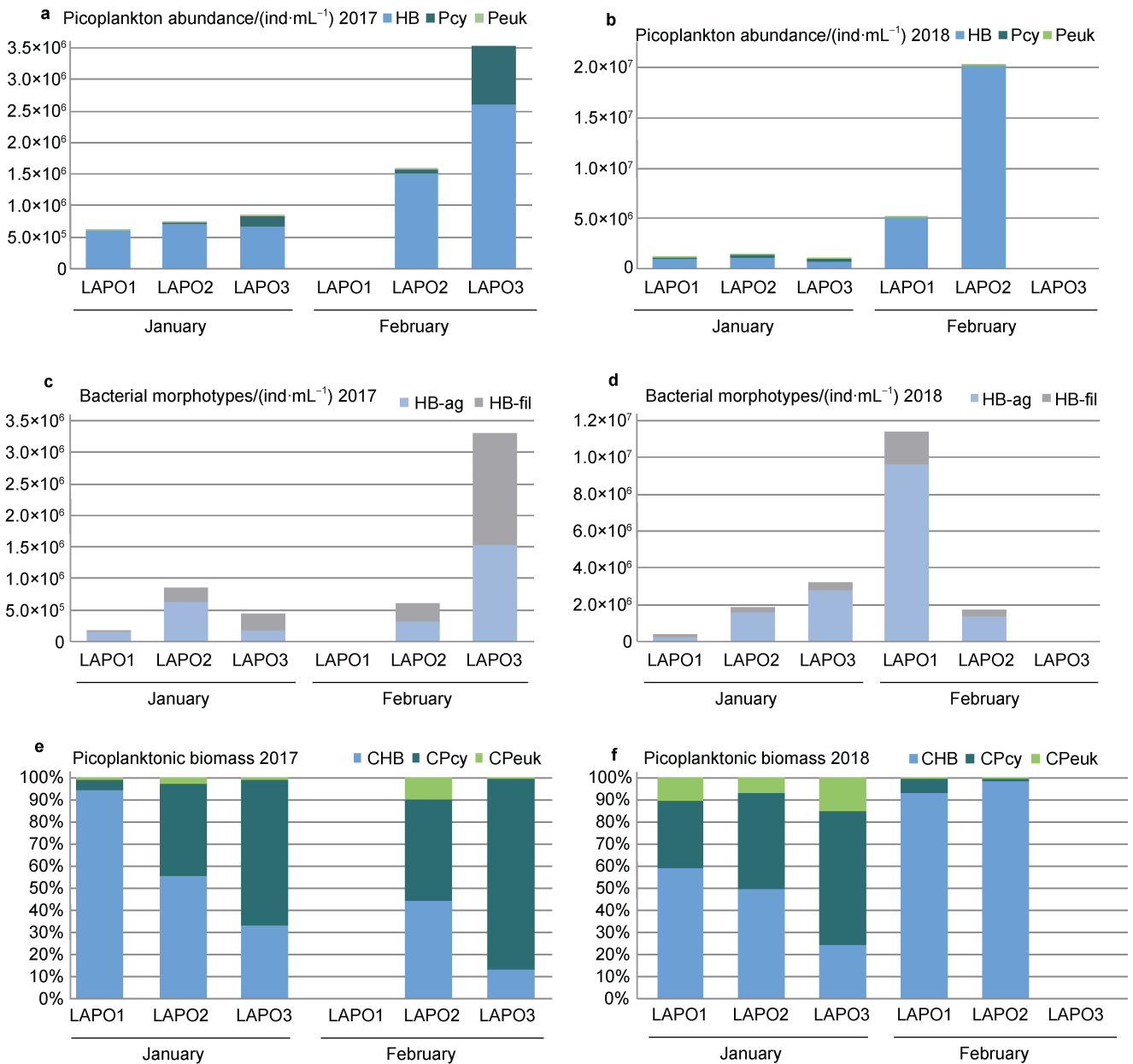


Figure 5 Abundance and biomass of picoplanktonic organisms of studied ponds during sampling period. **a**, picoplankton abundances in 2017; **b**, picoplankton abundances in 2018; **c**, bacterial morphotypes in 2017; **d**, bacterial morphotypes in 2018; **e**, picoplanktonic biomass in 2017; **f**, picoplanktonic biomass in 2018. LAPO indicates large pools.

ratio varies between month as shown in Figures 5e and 5f. The average relative biomass of bacterioplankton in LAPO1 is much higher than that in LAPO2 and LAPO3. Likewise, in LAPO3, Pcy's carbon content reached 86% in February 2017. Overall, in January of both years, a more stable picoplanktonic proportion was observed in each water body.

The principal components analysis (PCA) performed on the physico-chemical and morphometric variables for both years (2017 triangle, 2018 circle) is represented in Figure 6a. The first two components of the PCA explained 79.9% of cumulative variability of data. Ponds were sorted into three well-defined groups. LAPO1 was placed

according to the highest nutrient concentrations, conductivity, PI and morphological variables; while LAPO2 appeared on the opposite side of the plot. On the other hand, LAPO3 was mostly associated with higher slope, CI values and lower pH values. A second PCA was performed (Figure 6b) only based on biological variables. Axis 1 explained 30.8% of the total cumulative variance, while axis 2 explained 23.6% variance. In general, the abundances of all bacterial groups were higher in 2018 and they were mostly associated to LAPO1. Peuk numbers were also more abundant in ponds during 2018, whereas Pcy showed the opposite pattern and it was related to LAPO3.

Table 3 Average and ranges (minimum and maximum) values for months of biological variables

	2017			2018		
	LAPO1	LAPO2	LAPO3	LAPO1	LAPO2	LAPO3
HB/(ind·mL ⁻¹)	6.1×10 ⁵ (6.1×10 ⁵ ; 6.1×10 ⁵)	1.1×10 ⁶ (7.1×10 ⁵ ; 1.5×10 ⁶)	1.6×10 ⁶ (6.7×10 ⁵ ; 2.6×10 ⁶)	3.0×10 ⁶ (9.0×10 ⁵ ; 5.1×10 ⁶)	1.0×10 ⁷ (9.8×10 ⁵ ; 2.0×10 ⁷)	6.0×10 ⁵ (6.0×10 ⁵ ; 6.0×10 ⁵)
HB-ag/(ind·mL ⁻¹)	5.8×10 ⁴ (5.8×10 ⁴ ; 5.8×10 ⁴)	4.9×10 ⁵ (4.2×10 ⁵ ; 5.6×10 ⁵)	1.4×10 ⁶ (5.1×10 ⁴ ; 2.8×10 ⁶)	3.4×10 ⁵ (6.9×10 ⁴ ; 6.2×10 ⁵)	1.7×10 ⁶ (6.9×10 ⁵ ; 2.8×10 ⁶)	5.6×10 ⁶ (5.6×10 ⁶ ; 5.6×10 ⁶)
HB-fil/(ind·mL ⁻¹)	1.6×10 ⁴ (1.6×10 ⁴ ; 1.6×10 ⁴)	2.6×10 ⁵ (2.4×10 ⁵ ; 2.9×10 ⁵)	1.0×10 ⁶ (2.5×10 ⁵ ; 1.7×10 ⁶)	9.3×10 ⁵ (1.1×10 ⁵ ; 1.7×10 ⁶)	2.8×10 ⁵ (2.5×10 ⁵ ; 3.2×10 ⁵)	4.3×10 ⁵ (4.3×10 ⁵ ; 4.3×10 ⁵)
Pcy/(ind·mL ⁻¹)	2.5×10 ⁴ (2.5×10 ⁴ ; 2.5×10 ⁴)	4.9×10 ⁴ (3.8×10 ⁴ ; 6.1×10 ⁴)	5.4×10 ⁵ (1.7×10 ⁵ ; 9.1×10 ⁵)	9.7×10 ⁴ (4.2×10 ⁴ ; 1.5×10 ⁵)	1.8×10 ⁵ (4.2×10 ⁴ ; 3.1×10 ⁵)	2.8×10 ⁵ (2.8×10 ⁵ ; 2.8×10 ⁵)
Peuk/(ind·mL ⁻¹)	8.0×10 ³ (8.0×10 ³ ; 8×10 ³)	1.2×10 ⁴ (4.3×10 ³ ; 1.9×10 ⁴)	6.9×10 ³ (3.6×10 ³ ; 1.0×10 ⁴)	4.2×10 ⁴ (6.1×10 ³ ; 7.8×10 ⁴)	4.1×10 ⁴ (8.6×10 ³ ; 7.4×10 ⁴)	1.0×10 ⁵ (1.0×10 ⁵ ; 1.0×10 ⁵)
CHB/(μg C·L ⁻¹)	244.0 (244.0; 244.0)	28.8 (26.5; 31.1)	57.8 (45.4; 70.2)	238.5 (154.6; 322.3)	845.2 (188.4; 1502.0)	58.3 (58.3; 58.3)
CPcy/(μg C·L ⁻¹)	13.1 (13.1; 13.1)	26.2 (20.8; 32.4)	278.8 (90.2; 467.5)	51.2 (22.0; 80.4)	92.8 (20.1; 165.5)	147.7 (147.7; 147.7)
CPeuk/(μg C·L ⁻¹)	2.8 (2.8; 2.8)	4.2 (1.5; 6.8)	2.5 (1.3; 3.6)	14.9 (2.2; 27.8)	14.6 (3.0; 26.2)	36.5 (36.5; 36.5)

Notes: HB–unicellular heterotrophic bacteria; HB-ag–aggregated heterotrophic bacteria; HB-fil–filamentous heterotrophic bacteria; Pcy–picocyanobacteria; Peuk–picoeukaryote; CHB–total unicellular heterotrophic bacteria biomass; CPcy–picocyanobacteria biomass; CPeuk–picoeukaryote biomass.

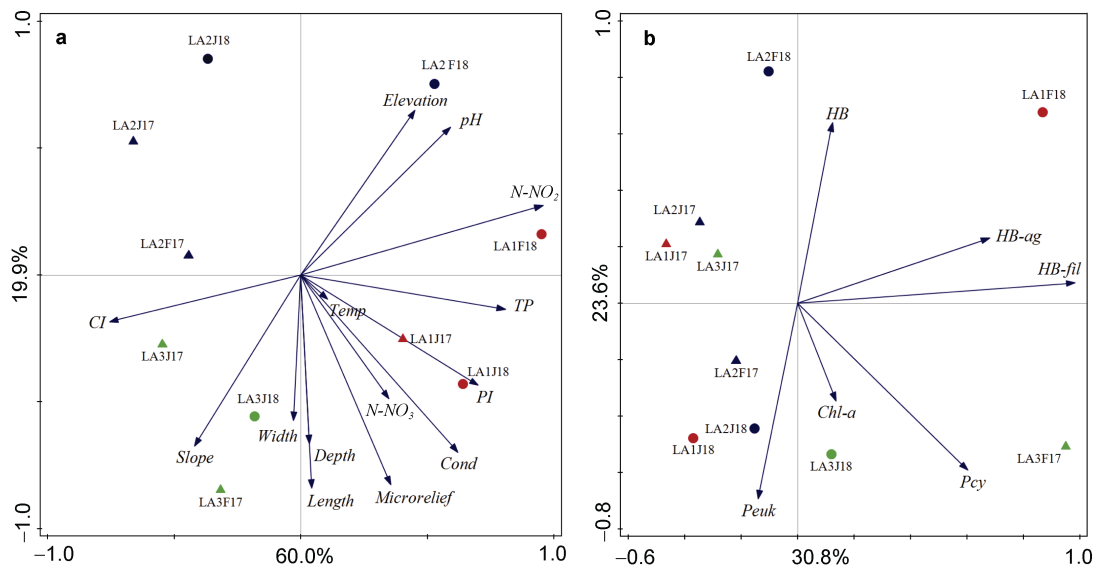


Figure 6 Principal components analysis (PCA) biplots showing the distribution of ponds during the sampled period based on morphometrical and physico-chemical variables. **a**, physico-chemical and morphometric variables vs ponds; **b**, ponds vs picoplanktonic abundances. LA1–3 indicates large pools 1–3; J = January; F = February; 17 = 2017; 18 = 2018. Data of 2017 and 2018 were presented in triangle and circle, respectively.

4 Discussion

Regional scale changes in temperature and precipitation are known to occur on the Antarctic Peninsula, threatening the stability of Antarctic ecosystems by altering nutrient dynamics that affect the abundance and distribution of microorganisms (Andersen et al., 2013; Hawes et al., 2023; Neale et al., 2021). Here, we found that ponds at

Cierva Point were susceptible to impacts of climate warming, in which external factors outweigh the relative importance of in-pond processes. Due to their small size, temporary physico-chemical fluctuations have greatly affected these water bodies, as well as the abundances and structure of microorganisms, which have the ability to adapt quickly. This study demonstrates that microorganism in Antarctic ponds can be effective indicators for monitoring climate change in the Antarctic Peninsula.

4.1 Climate variations

Since 1850, polar temperatures have risen twice as fast as the global average, being the Antarctic Peninsula one of the most affected regions (Siegert et al., 2019). Air temperatures at Cierva Point were within the reported ranges (Quintana et al., 2000). Averages temperatures for summer months of both years (2017–2018) ranged between -7.0 to 1.2 °C. Although no significant differences were recorded, the annual mean air temperature in 2018 was slightly higher than that in the previous year (Figure 7a) with a cloudier weather (Fermani personal observation). In addition, data from San Martín Base, an area further south on Cierva Point, revealed that minimum air temperatures were significantly higher in 2018 (t -Student, $P < 0.001$) (Figure 7b). Extreme temperature events have been recorded in 1982 at South Orkney Islands (60.78°S , 45.68°W) with a maximum of 19.8 °C, and at Esperanza Base (18.3 °C) (63.48°S , 57.08°E) in 2020 (Turner et al., 2021). Projections from climate models reveal that Antarctic coasts will experience a warming along with more frequent rainfall by the end of the century (Vignon et al., 2021). In Cierva Point, the mean annual precipitation between 2003 and 2007 was 850 mm (Figure 7c). However, over the next decade (2008–2018), our data generally supports an increase in annual precipitation (with the exception of 2012 and 2013), resulting in further accumulation in 2018 (960 mm). These gradual increases in temperatures and rainfall result in an enhancement in the thawing areas, leaving more ice-free environments and allowing for an extension in the development of Antarctic flora and fauna, that may affect the growth of microorganisms (Convey and Peck, 2019, Vignon et al., 2021). Observations of the landscape during the Antarctic summer campaigns carried out by this working group in the ASPA No. 134 (Fermani personal observation), show the loss of the territory covered by mosses to one more frequented and expanded by nesting birds such as skuas and gentoo penguins. In 2006, penguins made their nests higher up and on rocks, while today they have moved down and make their nests not only on rocks but also on mosses, leading to further area deterioration (Figure 8). After 1990, an increase in Pygoscelid penguins and nesting diversity was observed, possibly due to the low human impact in the area (González-Zevallos et al., 2013; McClintock et al., 2010; Quintana et al., 2000).

4.2 Antarctic ponds

Cierva Point hosts ponds with the usual morphometric characteristics (Ellis-Evans, 1996; Hawes et al., 1999). Due to their small size, they are susceptible to seasonal physico-chemical and/or climatic fluctuations. In winter, ponds are covered with ice as occurred in LAPO1. The thaw of snow will amplify the effects of climate change and the amplifying effects will eventually show in summer (Allende and Mataloni, 2013; Grimm et al., 2013). Thaw allows the

circulation of nutrients, favoring the development of planktonic microbes (Camacho, 2012). As predicted, Antarctic ponds studies showed differences in physical and chemical factors, affected by the surrounding area, and exhibiting high temporal variability (Allende and Mataloni, 2013; Hawes et al., 1999). LAPO1 and LAPO2 had similar morphological and abiotic characteristics, however, although those ponds are located in the same EU, the influence of morphometric, internal factors or surrounding flora and/or fauna were more relevant for the determination

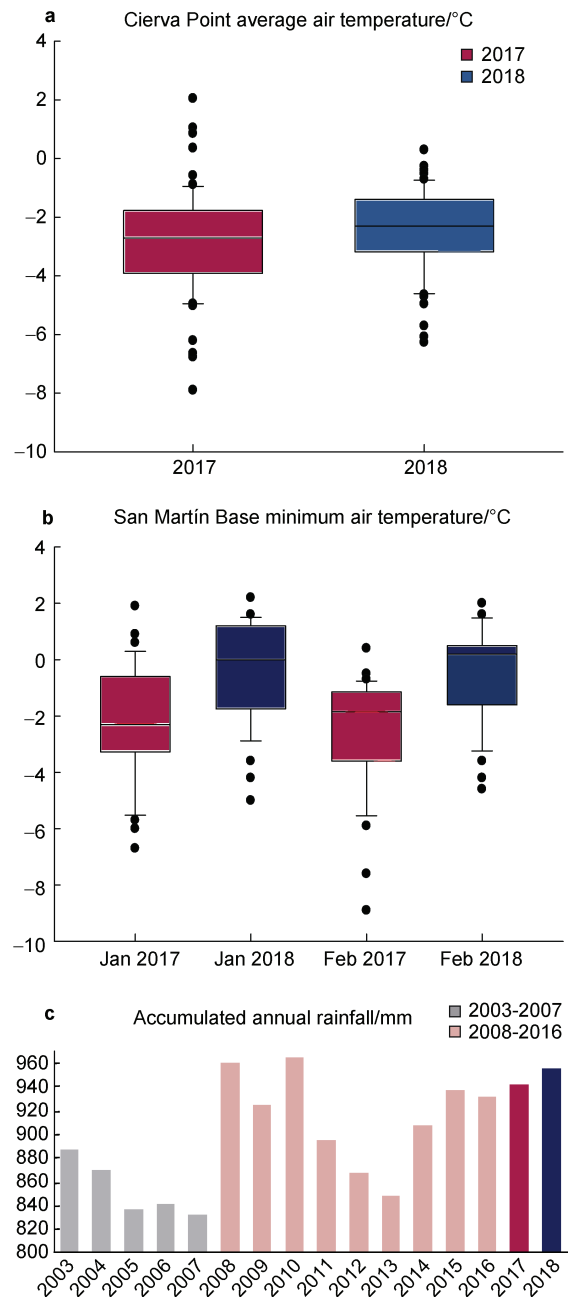


Figure 7 Meteorological variables. **a**, annual mean air temperature from Cierva Point; **b**, minimum air temperature from San Martín Base (Argentina); **c**, accumulated annual rainfall from Cierva Point.



Figure 8 Photos of ASPA No.134. Photos were taken in 2006 (a and c), 2017 (b) and 2018 (d).

of the microbial structure composition. Particularly, LAPO1 had the highest morphometric variability. This type of basin shape, together with their versatility and the presence of a gentoo penguin rookery in nearby areas (mostly in February 2018), make this pond change from mesoeutrophic to hypereutrophic conditions at the end of this study, and similar changes were found in other areas of the Antarctic Peninsula (Allende and Pizarro, 2006; Izaguirre et al., 2003). LAPO2 had mostly eutrophic waters, with a brief occurrence of sea bird. On the other hand, LAPO3 was more stable between the two years, featuring characteristics of dystrophic environments (Mieczan et al., 2019) with mosses in its surroundings and a slightly acidic pH value. Those differences were reflected in the abiotic factors, as well as in the microbial communities. It is known that water body shape the water runoff speed, and together with climate and soil type, is one of the most important determinants of biological productivity (Quirós, 2004; Rochera et al., 2013).

4.3 Environmental factors and their influence on the microbial community

Compared to other areas of the Antarctic Peninsula (Izaguirre et al., 2003; Rochera et al., 2010), water temperatures at Cierva Point were higher, on average, during the two studied years. The inverse relationship with prokaryotic densities would indicate that there is an optimal growth with lower irradiance. The high temperatures itself could not have a direct effect on HB abundance, but are likely to be influenced by indirect effects associated with global warming, like increased nutrient loading (Christoffersen et al., 2006).

Antarctic soils are characterized by poor development

(Fermani et al., 2007; González Garraza et al., 2011) and consequently, many Antarctic waters bodies are oligotrophic with very low dissolved nutrient concentrations. However, in Maritime Antarctic, fauna activity is considered to be a very important factor supplying nutrients to both terrestrial and freshwater ecosystems (Izaguirre et al., 2021; Mataloni et al., 2000). As viewed previously, areas with nesting penguins accumulate significant amounts of guano, which may ultimately enter nearby pools by runoff (Allende and Mataloni, 2013; Izaguirre et al., 2021). This could be observed in LAPO1, particularly in 2018. High phosphorus and nitrogenous concentrations were found in this pond, being the highest for the whole study period. This was reflected in an enhancement of conductivity values, which are similar to those observed in other hypertrophic ponds of Maritime Antarctic (Allende and Pizarro, 2006; Izaguirre et al., 2021). In contrast, lower nutrient concentrations were recorded in ponds with vegetation and low penguin activity, although within mesoeutrophic ranges.

Nutrient enrichment, supported by climate change and increases in rainfall, permitted a very active autotrophic community, as revealed by the high Chl-*a* concentrations (as proxy of phytoplankton biomass, especially in February when the penguin broods grow and circulate more actively throughout the area. Chl-*a* in LAPO2 was much higher than that recorded by Allende and Mataloni (2013) two decades ago. This could be due to an enhancement of primary productivity of larger autotrophic organisms. Phytoplankton is responsible for all primary production and at higher latitudes, the availability of sunlight in the ponds increases (Weiskopf et al., 2020). This phenomenon coupled with the increase in nutrients in February, triggers the phytoplankton

growing season, resulting in an enhanced release of OM available for HB (Azam et al., 1983). Also, allochthonous OM from the surrounding area, especially in February, would make HB grow (Rofner et al., 2017). Understanding how these changes impact the food web is crucial for maintaining high trophic levels. In this study, HB peaked in LAPO2 during February 2018. The positive correlations between Chl-*a*, Peuk and HB abundances could indicate that bacteria would be bottom-up regulated, and small environmental changes would modify their abundances. Also, the difference of bacterial morphotypes could be observed. LAPO2 had a more stable community, possibly due to a slight influence from the surroundings basin. While for LAPO1 (with a higher presence of penguins) and LAPO3 (neighboring mosses), the composition of morphotypes differs. The negative correlation between HB-ag and depth would indicate that the morphology of the basin influences the aggregation of microorganisms. Cell aggregation may provide additional nutrients, substrates or enzymes (Blom et al., 2010), and the matrix may possibly act as a physical barrier to protect HB from adverse factors (Pernthaler, 2005). Aggregates or filaments might also provide protection against predation (Fermani et al., 2013). Usually, filaments are mostly found in hypereutrophic systems (Sommaruga, 1995; Thelaus et al., 2008) as what we found particularly in February. Furthermore, in environments with high UV radiation, such as Antarctic Peninsula (Neale et al., 2021), filament formation has been observed (Corno et al., 2009).

On the other hand, it is well known that picophytoplankton would be of great importance in terms of biomass all along the trophic spectrum of Antarctic lakes (Ellis-Evans, 1996). Here, Pcy and Peuk abundances were also within the range observed for other studies in the area (Allende and Mataloni, 2013; Izaguirre et al., 2003). Pcy dominate over Peuk in all ponds with generally an order of magnitude higher (Callieri and Stockner, 2002). Pcy populations tend to be predominant in large, deep oligo-mesotrophic lakes (Callieri, 2010). However, in different shallow and eutrophic water bodies, their abundances were difficult to predict (Fermani et al., 2022; Silvano et al., 2011). Pcy are now known to inhabit a wide range of freshwater and brackish environments, likely due to their high adaptability and phenotypic plasticity (Berman et al., 2020; Huber et al., 2017). Here, the highest abundances and contribution to biomass were found in LAPO3, in agreement with some studies reported for dystrophic environments (Callieri, 2008). Moreover, despite their lower abundance, it is acknowledged that Peuk are major contributors to phytoplankton biomass (Mózes et al., 2006). In 2017, abundances and biomasses of these algae were within those reported records for Antarctic systems (Allende and Pizarro, 2006), but also differed between years, particularly in February. Also, high contribution to the biomass was observed in LAPO3 in 2018, which were

prevailing low-light and temperatures conditions, as well as dystrophic characteristics. Dystrophic lakes are characterized by the presence of humus substances migrating from the surrounding area. Humus dissolved in waters acidifies them and forms complexes of phosphorus compounds, ammonia and metal cations (Górnjak et al., 1999). With increasing dissolved organic carbon (DOC), the colour of water changes to brown, affecting the thickness of the photic zone and thus the visibility of the Secchi disk (Gray et al., 2022). Although, unfortunately, we do not have DOC estimates for all dates, the most extensive study of the wetlands of Cierva Point (unpublished data) revealed a DOC of $29.1 \text{ mg}\cdot\text{L}^{-1}$ for LAPO3 as well as a higher hardness ($124.8 \text{ mg}\cdot\text{L}^{-1}$). Our data are consistent with these additional results where a large number of dead mosses were observed together with an acidic pH, a mostly brown lacustrine colour (Fermani personal observation) and decreased of Secchi disk depth. In this regard, it has been noted that such environments with high concentrations of dissolved humic acid which are difficult to assimilate, can promote the growth of picophytoplankton (Callieri, 2008; Tamm et al., 2022).

Overall, total and relative abundances and biomasses of picoplanktonic community differed among water bodies (according to trophic state), months and years, as predicted by our hypothesis. The influences of the surroundings modify the structure of microorganisms which may be indicative of environmental changes. The increase in rainfall and temperature may result in the ponds remaining unfrozen for longer periods, allowing for more time for microbial biomass development within the ponds.

5 Conclusions

The studied ponds at Cierva Point had different limnological characteristics, due to their location, geomorphological features and presence of the surrounding flora and fauna. Waters bodies were affected by different stress factors, impacting the abundances and composition of microbial components. Seasonal physico-chemical and climatic fluctuations together with rainfall, bring increased nutrient runoff into water bodies, leading to eutrophication and modification of the microbial structure. These communities are not only able to integrate information on changes occurring in the basin, but also respond very quickly to variations in atmospheric and terrestrial inputs. Therefore, these ecosystems can serve as sentinels of climate change, as they are not directly affected by anthropogenic interventions (Camacho et al., 2012).

We could furthermore highlight that possibly the effects of climate change could leave a future landscape full of hypertrophic water bodies with high densities of HB, which could be susceptible to degradation by viruses (Weinbauer, 2004). We might also expect a decrease in the abundance and diversity of the picophytoplankton community, which could have consequences on higher

trophic levels such as flagellates and ciliates, their main predators (Fermani et al., 2022). In view of wetlands protections, particularly the ASPAs in Antarctic Peninsula, this study highlights the need to maintain basics monitoring studies in this area, as proposed by Hawes et al (2023).

Acknowledgments This work was supported by ANPCyT (Grant PICT-2016-2517) directed by Dr. G. Mataloni and the National Scientific and Technical Research Council-Argentina (CONICET). The authors are grateful to Ejército Argentino members during summer campaigns 2017 and 2018, for the logistic support at Primavera Base and the Instituto Antártico Argentino–Dirección Nacional del Antártico. We would also like to thank to F. Bertone from Servicio Meteorológico Nacional of Argentina for providing weather parameters for the Antarctic Peninsula. Also thank to Y. Sica, L. Burdman, D. González and V. Casa, for their assistance in the field; as well as to R. Escaray, for his help with the determination of organic nitrogen and F. Unrein for shared data of picophytoplankton biovolume. We thank two anonymous reviewers for their valuable comments on the manuscript.

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