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## Original Research Article

# Influence of fish introduction and water level decrease on lakes of the arid Patagonian plateaus with importance for biodiversity conservation

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## ABSTRACT

Biodiversity loss in shallow lakes is associated to several factors among which water level decrease and fish introduction are indicated as potential stressors. The Patagonian basaltic plateaus, located in one of the most arid regions of South America, hold thousands of shallow lakes and ponds and some large lakes, where vegetated lakes are the prime habitat for waterbirds, including endemic threatened species. We studied 31 different lakes at the two main Patagonian plateaus (Strobel and Buenos Aires) during successive spring-summer field trips from 2007 to 2016. We focused on the differences between fishless and stocked lakes under contrasting hydrologic regimes (relatively stable waters and decreasing water level). Our survey evidenced the effect of fish introduction and water level decrease on the limnological features of the lakes and their phyto and zooplankton communities. Arheic shallow lakes showed a decreased water level accompanied by an increased conductivity, and several shifted from a clear-vegetated state to a more turbid condition, with increased algal biomass and demise in submerged macrophytes. Fishless lakes presented higher total zooplankton and macrozooplankton biomass and lower phytoplankton biomass than fish stocked lakes. Our findings provide evidence that under a scenario of climate change, the effect of the decrease in water level may enhance the adverse effects of fish introduction.

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## 1. Introduction

At a global scale, continental aquatic environments are being subjected to a severe loss of biodiversity, which seems to be occurring even faster than in terrestrial ecosystems; this loss seems to be mainly associated with changes in land use, biotic exchanges and climate change (Sala et al. 2000; Abell 2002; Saunders et al. 2002).

Particularly, fish introductions are among the main causes of alterations in lakes. Most of the studies have been focused on top-down effects on pelagic and benthic communities (e.g. Carpenter and Kitchell 1996; Jeppesen et al. 1997) and regime shifts (Scheffer 1998 and references therein). The introduction of strictly planktivorous fish can alter plankton communities via cascading interactions in food webs: the increase in fish predation on zooplankton leads to a reduced grazing pressure on phytoplankton, and thus to an increase in algal biomass (Carpenter et al. 1985; Pace et al. 1999). There is less information about the effect of generalist fish that can feed both on pelagic and benthic preys, although it is known that they can create a strong coupling between habitats (e.g. Schindler and Scheuerell 2002; Vander Zanden and Vadeboncoeur 2002). In the case of generalist fish, the effects seem to depend on the introduced species and on the particular trophic structure of the lakes. Schaus and Vanni (2000) found that the omnivorous fish Gizzard shad (*Dorosoma cepedianum*) transports nutrients from sediments into the water column, impacting on phytoplankton through an interaction of top-down and bottom-up effects. Strock et al. (2013) found that after the introduction of the White perch (*Morone Americana*), which switches from strict planktivorous to a more generalist diet during ontogeny, the size of cladoceran ephippia increased up to 50%, suggesting that this fish has a predominant piscivorous role in this ecosystem. Fish introduction was also recognized to be a major threat to alpine lake biota (e.g. Knapp et al., 2001; Schabetsberger et al. 2009); one of the most utilized alien species in high altitude alpine lakes was the Brook trout (*Salvelinus fontinalis*), which has been introduced for sport fishing in several naturally fishless water bodies. Its introduction dramatically affected the assemblages of benthic macroinvertebrates, as well as the size spectrum and composition of the pelagic zooplankton due to the selective predation of the more visible taxa (Tiberti et al., 2014a; 2014b).

On the other hand, the sensitivity of lakes to climate change and their great potential as effective sentinels in this sense have been widely recognized (Williamson et al. 2008; Adrian et al. 2009; Schindler 2009). Lakes are sensitive not only to climate, but they also respond rapidly to changes and integrate information about modifications in the catchment (Adrian et al. 2009). Shallow lakes are particularly affected by changes in precipitation; the drawdown due to drought are more severe than in steep-sided systems, provoking important habitat loss for benthic taxa and animals that forage in these areas; the abiotic effects typically include increases in conductivity as well as changes in pH, turbidity and in the concentration of ions, nutrients and DOC (Lake, 2011 and cites therein). Moreover, changes in the water level of the shallow lakes may trigger shifts in the steady states of the lakes; for example, field studies comprising several years have shown the switch of a shallow lake from a state with profuse aquatic vegetation, either with free floating plants (O'Farrell et al., 2011), or with submerged vegetation (Sanchez et al., 2015) to a turbid regime with high phytoplankton biomass.

In general, the freshwater systems located in arid regions are considered among the most threatened habitats in the world (Williams 1999). Our study was focused on lakes located at the Patagonian steppe, one of the largest arid regions of South America, with less than 300 mm of annual rainfall (Cabrera, 1994), where two of the main threats would be the fish introduction and the climate change. Geologically, the Patagonian steppe is a complex landscape, mainly characterized by basaltic plateaus and tectonically uplifted pebble fans (Iriondo 1989). As in other arid regions of the world, water availability entails significant constraints on wildlife, whereby lakes have an important ecological role (Cuello et al. 2006; Lancelotti et al. 2009a). Particularly, the shallow lakes of the Patagonian steppe constitute the main feeding and breeding habitat for several waterbird species (Lancelotti et al. 2009b), including endemic and threatened species such as the hooded grebe (*Podiceps gallardoi*), which is listed as globally critically endangered (Bird Life International 2016) and whose populations have been estimated under 400 breeding pairs (Roesler et al. 2012). Thus, these environments have been listed as priority areas for waterbird conservation (Scott and Carbonell 1986; Canevari et al. 1998; Di Giacomo 2005). Besides, the lakes of this region constitute the prime habitat for other endemic species of vertebrates, invertebrates and also microalgae (e.g. Menu Marque et al. 2000; Perotti et al. 2005; Ortubay et al. 2006; Coesel et al. 2017).

Most lakes of the basaltic plateaus of Patagonia are naturally fishless (e.g. Lancelotti et al. 2009a; Lancelotti et al. 2016). However, during the last decade, many lakes located on public and private lands have been stocked with rainbow trout (*Oncorhynchus mykiss*), and there has been a considerable growth of the aquaculture industry in this region (Lancelotti et al. 2009a). Several studies have demonstrated that fish introductions cause alterations in the biodiversity and structure of aquatic communities in lakes of the Argentine Patagonia. In Laguna Blanca, a shallow lake of Northern Patagonia, the introduction of the native perch (*Percichthys* sp.) has triggered a drastic shift in macrophyte biomass and loss of bird and amphibian diversity (Cuello et al. 2006; Ortubay et al. 2006). The impact of both native and exotic fish on the planktonic food webs was also analyzed for other lakes of the Patagonian steppe by Reissig et al. (2006). The main results evidenced that the fish introduction changed the zooplankton structure leading to a narrower size spectrum; fish stocked lakes lacked *Daphnia* and large centropagid copepods, and a trophic cascade effect on phytoplankton was also evidenced from the higher abundances of cyanobacteria. In line with these observations, our first survey in some shallow lakes of the Strobel Plateau (one of the areas included in the present study) showed highest phytoplankton biomass in fish stocked lakes, and the dominance of cyanobacteria in one of them (Izaguirre and Saad 2014). Lakes of the Strobel Plateau also showed significant differences in zooplankton size and community structure between fishless lakes and those stocked with trout, with a significant reduction in the size of *Daphnia* spp. and copepods (*Boeckella* spp.), and absence of pelagic amphipods in stocked lakes, which are

abundant in fishless lakes (Lancelotti et al. 2016). All these mentioned organisms represent the primary prey of trout in this region (Lancelotti et al. 2015).

Argentina shows evidences of climate change, with contrasting patterns for different parts of its territory. In Patagonia reduced rainfall and increased temperatures over the Andes Ridge have led to the retreat of glaciers and a decrease in river flows (Barros et al. 2014). Climate projections indicate a probable decrease of snow in the mountains and a rise in temperature during the current century, which will cause the complete drought of several basins (Barros et al. 2014 and references therein). In agreement with this documented climate change in Patagonia, our studies in the lakes of the highland plateaus of Santa Cruz have shown a decrease in the water level in many shallow lakes and the complete drought of some others (Roesler et al. 2012).

The concomitant effects of fish introductions and climate change have been proposed as primary triggers of environmental degradation and habitat loss for waterbirds in this region (Roesler 2016). However, to date, these combined effects have not been evaluated. Thus, in the present study, we particularly focused on the differences between fishless and fish stocked lakes through a period with contrasting hydrologic regimes. The study was conducted in the two most important basaltic plateaus of Patagonia in terms of lake abundance and diversity of aquatic habitats: the Strobel and the Buenos Aires Plateaus. The Strobel Plateau hosts some lakes stocked with trout, whereas the Buenos Aires Plateau hosts only fishless lakes. We also analyzed the effect of the reduction of precipitation that is taking place in the region on the characteristics of the lakes and their communities, comparing the behavior of arheic lakes (i.e. isolated water bodies fed by precipitation) with that of chained lakes. The aim of this study is to produce a synthesis of the field data available since 2007 on the lakes of the two main Patagonian plateaus to assess the joint influence of fish introductions and climate change on the water bodies. The status and evolution of these lakes have paramount importance concerning the conservation of the main habitats of threatened waterbirds.

## 2. Materials and methods

### 2.1. Study area

The studied lakes are located in two basaltic Patagonian plateaus (Strobel and Buenos Aires Plateaus, Fig. 1), in Santa Cruz Province, Argentina (46°53'24"S; 71°20'03"W to 48°43'51"S; 70°59'29"W). These basaltic plateaus were formed during tectonic episodes in the Miocene-Pliocene periods (Panza and Franchi 2002) and hold thousands of shallow lakes and temporary ponds, as well as a few large lakes (>30 km<sup>2</sup>) (Roesler 2016).

The Strobel Plateau has an extension of 2500 km<sup>2</sup> and holds more than 1000 shallow lakes in wet years; its topography is relatively uniform, with a gentle slope towards the northwest, varying in altitude from 1200 m a.s.l. (west) to 700 m a.s.l. (east) (Lancelotti et al. 2010a). The Buenos Aires Plateau covers a total area of 3650 km<sup>2</sup>, has an altitude of about 1800 m a.s.l. in the west (with the Monte Zeballos Peak of 2700 m a.s.l.), decreasing towards the east, where it reaches near 700 m a.s.l. and holds more than 150 endorheic shallow lakes (Roesler 2016).

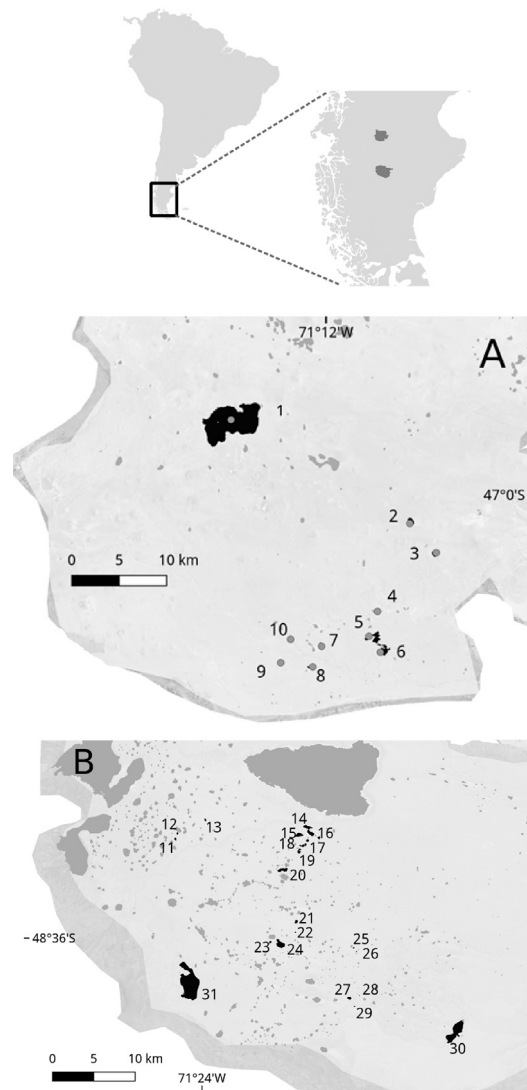
The climate of the region is characterized by its extreme conditions, with temperatures below –19 °C in winter and a great variation in the photoperiod over the year. The water regime is mostly determined by the balance between precipitation and evaporation (Pruel et al. 1998), favored by the high irradiance and strong winds. Lakes smaller than 30 km<sup>2</sup> remain frozen in the surface from early autumn throughout late spring. Due to the frequent wind storms (predominantly from the west), lakes are continuously mixed, thus preventing the formation of stable thermoclines (Lancelotti 2009).

The lakes of the Strobel Plateau have been classified in four general categories (Lancelotti et al. 2009a): turbid lakes, large vegetated lakes, large unvegetated lakes, and ponds. In large vegetated lakes, the aquatic macrophytes (dominated by *Myriophyllum* sp.) cover from 30 to 80% of the lake area, whereas in large unvegetated lakes, macrophytes cover less than 5% of lake area. Small ponds (<9 ha) are usually completely covered by plants (~90%).

As previously mentioned, most lakes of the basaltic plateaus of Patagonia are naturally fishless (e.g. Fjeldsø 1986; Lancelotti et al. 2010b; Lancelotti et al. 2016), but during the last two decades, more than 40 lakes of the Strobel Plateau have been stocked with rainbow trout (personal communication from farm owners). Conversely, the lakes of the Buenos Aires Plateau are still fishless; there was a single failed attempt at fish stocking in lake El Sello (Buria and Roesler unpublished information). Most fishless lakes of both plateaus are suitable habitat for several species of waterbirds. Particularly, Lake El Sello hosts the highest abundances of hooded grebe, with more than 350 individuals, which constitutes nearly 50% of its estimated population (Roesler et al. 2012). Recently, part of the Buenos Aires Plateau has been included within the Patagonia National Park (Parque Nacional Patagonia), which will favor the conservation of the aquatic environments and their wildlife.

### 2.2. Studied lakes, samplings and environmental variables

We analyzed samples collected in successive field trips, from 2007 to 2016. Lakes were visited one to six times (mostly three) during the warmer period (late spring to late summer), including two contrasting hydrological periods (higher water levels: 2007–2011; lower water levels: 2013–2016). The study included 21 lakes in the Strobel Plateau and 10 lakes in the Buenos Aires Plateau, which all together encompass the environmental diversity of the region according to the ecological characterization given by Lancelotti (2009) and Roesler (2016). The main morphometric features, regime, type of drainage and current state (2016) of the lakes are summarized in Table 1. The depths of the water bodies were estimated using a



**Figure 1.** Geographic location of the surveyed lakes. From north to south, the map indicates the position of the Buenos Aires (A) and Strobel (B) plateaus respectively. 1: El Sello; 2: 9 de Julio; 3: La Honda; 4: La Pedregosa; 5: E25; 6: Gaviotero; 7: La Soñada; 8: El Chapu; 9: El Huevo; 10: El Cervecero; 11: Rod 18; 12: Pif; 13: Rod 9; 14: Potrerillo; 15: Verde; 16: Temp; 17: La 9; 18: La 8; 19: La Herradura; 20: Campamento; 21: S94; 22: Nieve; 23: La Justita; 24: Pond X; 25: S64; 26: Pond 13; 27: S44; 28: Pond 11; 29: Pond 12; 30: Las Coloradas; 31: El Islote.

portable depth sounder (Speedtech). Stocked lakes and conservation priority based on their value as habitat for hooded grebe are also indicated in Table 1 (Lancelotti et al. 2009a, 2010b; Roesler et al. 2012). All analyzed lakes with fish were stocked with rainbow trout (*Oncorhynchus mykiss*); only Lake Temp was also stocked with brown trout (*Salmo trutta*). Trout do not reproduce in the studied lakes and their abundance is controlled by farmers by means of stocking and removal, to sustain a fish density of approximately 6000 individuals/km<sup>2</sup>.

Water samples from each lake were obtained from the pelagic zone at the upper water layer using a plastic cylindrical device (10 L), integrating waters from the surface to 50 cm. Due to the shallowness of most water bodies and the absence of stable thermoclines even in the few deeper lakes of the region, we assume that the upper layer represents adequately the pelagic habitat. We preserved 2 L for chemical and chlorophyll a (Chl<sub>a</sub>) analyses in plastic bottles previously rinsed with HCl 2% and MilliQ water, which were transported in darkness and cooled to the field laboratory. In each lake, we measured physical and chemical variables *in situ*: temperature, pH, and conductivity with a Horiba D-54 portable sensor; dissolved oxygen with a Hach<sub>TM</sub> HQ30 d m; underwater photosynthetically active radiation (PAR) with a LICOR radiometer, equipped with a submersible spherical quantum sensor (Li-193 SA; Li-Cor PAR, NE, USA), and transparency with a Secchi disk. The vertical PAR attenuation coefficient ( $K_d$ ) was calculated according to Kirk (1994). The sampling hour was in general around noon. In the field laboratory, a set of samples were filtered immediately through Whatman<sup>®</sup> GF/F filters for analyses of

**Table 1**

Main characteristics of the studied lakes located at the two Patagonian plateaus. Shallow lakes: maximum depth <10 m; deep: maximum depth >10 m ; very shallow lakes: maximum depth <1.5 m. Approximate values of depths at the pelagic zone are indicated.

	LAKE	surface area (Km <sup>2</sup> )	Depth	drainage	fish introduction	submerged plants	regime /shift	current state 2016	aquatic birds: importance for biodiversity conservation
<b>Strobel Plateau</b>	<b>Pond 11</b>	0.015	very shallow ~1 m	arheic basin	NO	YES	clear vegetated	Dry	NO
	<b>Pond 12</b>	0.092	shallow <5 m	arheic basin	NO	YES	clear vegetated	Dry	NO
	<b>Pond 13</b>	0.005	very shallow ~1 m	arheic basin	NO	NO	organic turbid	Dry	NO
	<b>Las Coloradas</b>	2.35	shallow <5 m	arheic basin	NO	YES	clear vegetated	almost dry	large and constant hooded grebe breeding colonies in the past.
	<b>S44</b>	0.15	shallow <5 m	arheic basin	NO	NO	inorganic turbid	Dry	use as stopover and feeding lake in the past by hooded grebe
	<b>S64</b>	0.03	shallow <5 m	arheic basin	NO	YES	clear vegetated	Dry	hooded grebe presence in the past
	<b>S94</b>	0.1	deep ~12 m	arheic basin	NO	YES	clear vegetated to organic turbid <sup>a</sup>	lower water level	breeding colonies of hooded grebe in the past
	<b>Pond X</b>	0.69	shallow <5 m	arheic basin	NO	NO	inorganic turbid	no significant variation in water level	NO
	<b>Justita</b>	0.07	shallow <5 m	arheic basin	NO	YES	clear vegetated to organic turbid <sup>a</sup>	lower water level	NO
	<b>Nieve</b>	0.02	shallow <5 m	arheic basin	NO	YES	clear vegetated	lower water level	NO
	<b>La Herradura</b>	0.14	deep ~18 m	endorheic chained lake	YES first stocking 2001	YES (in patches)	clear vegetated	higher water level	breeding colonies of hooded grebe in the past.
	<b>La 8</b>	0.15	shallow ~8 m	endorheic chained lake	YES first stocking 2001	YES (in patches)	clear vegetated	higher water level	breeding colonies of hooded grebe in the past
	<b>La 9</b>	0.09	shallow ~7 m	endorheic chained lake	YES first stocking 2004	YES (in patches)	clear vegetated	higher water level	breeding colonies of hooded grebe in the past
	<b>Potreriillo</b>	0.67	deep ~12 m	endorheic chained lake	YES first stocking 2002	NO	organic turbid	lower water level	NO
	<b>Temp</b>	0.08	shallow ~3 m	endorheic chained lake	YES first stocking 2005	YES (few patches)	clear vegetated	almost dry	breeding colonies of hooded grebe in the past
	<b>Pif</b>	0.03	shallow <5 m	arheic basin	NO	scarce	clear vegetated	dry	NO
	<b>Laguna verde</b>	0.4	deep ~15 m	endorheic modified	YES first stocking 2001	NO	organic turbid	no significant variation in water level	scattered presence of hooded grebe (stopover lake during spring migration)
	<b>Campamento</b>	0.4	deep ~10 m	endorheic chained lake	YES first stocking 2004	NO	organic turbid	no significant variation in water level	NO
	<b>Rod 18</b>	0.06	shallow ~6 m	arheic basin	NO	very scarce	Clear vegetated to organic turbid <sup>a</sup>	lower water level	NO
<b>Rod 9</b>	0.04	shallow <5 m	arheic basin	NO	YES	clear vegetated	lower water level	YES breeding colonies of hooded grebe	
<b>El islote</b>	6	deep ~57 m	endorheic	YES first stocking 2003	YES (few partches)	Clear	lower water level	use to hold one of the biggest known breeding colonies of hooded grebe	

(continued on next page)

Table 1 (continued)

	LAKE	surface area (Km <sup>2</sup> )	Depth	drainage	fish introduction	submerged plants	regime /shift	current state 2016	aquatic birds: importance for biodiversity conservation
<b>Buenos Aires Plateau</b>	<b>El Chapu</b>	0.178	shallow ~2 m	arheic basin	NO	YES	clear vegetated	lower water level	YES big concentration of waterbirds (including flamingoes)
	<b>El Cevercero</b>	0.121	shallow ~2 m	arheic basin	NO	YES	clear vegetated	lower water level	YES with hooded grebe
	<b>El Sello</b>	17.235	deep	endorheic	NO	YES	clear vegetated	no significant variation in water level	YES the most important lake in the present for hooded grebe with 25% of the global population.
	<b>9 de julio</b>	0.373	shallow ~3 m	arheic basin	NO	YES	clear vegetated	lower water level	YES
	<b>La Honda</b>	0.401	deep	arheic basin	NO	NO	clear mesohaline	lower water level	NO
	<b>E25</b>	0.87	shallow <5 m	endorheic	NO	NO	clear vegetated	higher water level	NO
	<b>Pedregosa</b>	0.07	shallow <5 m	endorheic	NO	NO	turbid	lower water level	NO
	<b>Gaviotero</b>	0.64	shallow <5 m	endorheic	NO	NO	clear vegetated	higher water level	NO
	<b>La soñada</b>	0.19	shallow <5 m	endorheic	NO	YES (in patches)	clear vegetated	higher water level	NO
	<b>El huevo</b>	0.03	shallow <5 m	endorheic	NO	YES (few partches)	clear vegetated	higher water level	NO

<sup>a</sup> Reduction in submerged vegetation in shallow lakes sampled over a three or more year period.



dissolved nutrients (ammonium, nitrate, phosphate, dissolved organic carbon (DOC)), and for Chla determinations. Another set of unfiltered samples were preserved frozen ( $-20^{\circ}\text{C}$ ) for analyses of total phosphorous (TP) and total nitrogen (TN), which were conducted after digestion (Valderrama 1981). TP and TN were only measured in samples collected in the 2014–2016 field trips. Dissolved nutrients were measured in the field laboratory, using a Hach<sub>TM</sub> DR/2800 spectrophotometer and the corresponding reagent kits. Filters for Chla determinations were transported frozen, and processed in the laboratory of the University of Buenos Aires (Buenos Aires, Argentina) by spectrophotometry, using acetone as solvent. The Chla concentrations (corrected for phaeopigments) were obtained using the equations given in Marker et al. (1980). Samples for DOC determinations were acidified (pH 2) and analyzed with a high-temperature Pt catalyst oxidation method (Shimadzu analyzer TOC-5000A), following the recommendations of Sharp et al. (1993).

### 2.3. Biological analyses

In each lake, phytoplankton samples for qualitative analyses were collected with a plankton net of  $15\ \mu\text{m}$  pore-size and then fixed with formaldehyde 2%. Unfiltered samples for quantitative phytoplankton analyses were obtained at the upper water layer of the lakes, using a plastic cylindrical device, integrating waters from the surface to 50 cm. Samples of 250 ml fixed with 1% acidified Lugol's iodine solution were stored in darkness and cooled until their counting. Phytoplankton counts were performed using a CKX41 Olympus inverted microscope, and the counting error was estimated according to Venrick (1978). Biovolumes were calculated using appropriate geometric formulae (Hillebrand et al. 1999; Sun and Liu 2003). Algal biomass was estimated from biovolume, assuming unit specific density.

We also analyzed the zooplankton composition and biomass in lakes of the Strobel Plateau for the 2013 field trip. Two 20-cm diameter mouth opening plankton nets of different mesh size ( $55\ \mu\text{m}$  and  $115\ \mu\text{m}$ , for micro- and macrozooplankton respectively) were towed horizontally. To avoid net clogging, in lakes with high seston amount, we obtained 5-m tows, whereas in clear lakes, we obtained 10-m tows. Samples were immediately fixed with 4% formalin. Microzooplankters present in three 1-ml subsamples from each sample were taken with a Hensen-Stempel pipette and counted in a Sedgewick-Rafter cell, using a binocular microscope. Macrozooplankters in three 5-ml subsamples taken with a Russell subsampler were counted in a Bogorov chamber using a stereoscopic microscope. Zooplankters were identified to species level, also discriminating males, females, and immatures. Up to twenty specimens of each taxon from Patagonian shallow lakes were measured using a binocular microscope to obtain mean body length. Mean biomass of each species was estimated by applying biovolumetric formulae for rotifers (Ruttner-Kolisko 1977), and then transformed to dry weight (Dumont et al. 1975) or body length-dry weight relationships (Bottrell et al. 1976; Pilati and Martínez 2003). Total biomass of each water body was calculated by multiplying the abundance of each species by its individual biomass, then adding up all values.

The abundance of submerged macrophytes was estimated qualitatively for each lake, considering three categories: profuse vegetation (approximately more than 50% of coverage), patchy vegetation, and lack of vegetation.

### 2.4. Climate variables

We analyzed precipitation data from 1960 to 2015 provided by the National Meteorological Service of Argentina (Servicio Meteorológico Nacional) from Gobernador Gregores and Perito Moreno towns, the nearest localities to the Strobel and Buenos Aires plateaus respectively. We calculated the annual accumulated precipitation and analyzed the occurrence of trends in this variable using a Mann-Kendall test. Data processing and analysis were conducted with R Software (R core Team 2016), and the package Kendall (McLeod 2011).

### 2.5. Statistical analyses

A stepwise discriminant analysis and one way multivariate ANOVA (MANOVA) followed by Tukey pos-hoc contrasts were performed for each plateau separately, in order to evaluate the differences among the lakes in relation to the fish introduction (presence, absence), and the changes in the water level (increase, relatively stable water level). The following variables were included in the analyses: Chla, soluble reactive phosphorus (P-PO<sub>4</sub>), dissolved inorganic nitrogen (DIN), pH, DOC and conductivity. Data were transformed (natural logarithm) to achieve the assumptions of multivariate normality and the homogeneity of variance-covariance matrices (Box's M test of equality of covariance). The analyses were performed with SPSS Software.

A Redundancy Analysis (RDA) was applied to explore the controlling factors of biological data in lakes of the Strobel Plateau; for this analysis we used data of field trips 2013 and 2015 for which there are zooplankton data. The biological data introduced in the analysis were: total phytoplankton biomass, total micro- and macrozooplankton biomass, zooplankton biomass/Chla ratio and abundance of aquatic macrophytes (considering the three categories of abundance previously mentioned). The choice of RDA was based on the linear response obtained in a previous Detrended Correspondence Analysis. The statistical significance of the first axis and of all axes was tested by the Monte Carlo permutation test, and the significance of each variable was obtained by forward selection. These analyses were performed with the software CANOCO (ter Braak and Smilauer 2002).

The differences in the zooplankton structure between fishless and fish stocked lakes were checked by one-way ANOVA, using SPSS Software. To fulfill the normality and homogeneity assumptions, data were log-transformed.

**Table 2**

Mean values of the main physical, chemical and biological variables registered in the different types of lakes

Plateau	Regime /shift	Fish introduction	Lake		Conductivity ( $\mu\text{S cm}^{-1}$ )	Chl a ( $\mu\text{g L}^{-1}$ )	DIN ( $\text{mg L}^{-1}$ )	P-PO4 ( $\text{mg L}^{-1}$ )	DOC ( $\text{mg L}^{-1}$ )	Kd ( $\text{m}^{-1}$ )	Phytoplankton biomass ( $\text{mg L}^{-1}$ )	Zooplankton biomass ( $\text{Mg DW L}^{-1}$ )	
STROBEL	Clear vegetated	YES	La Herradura	mean	434	10.24	0.08	0.21	35.84	2.04	13.54	522.11	
			La 8	sd	292	13.93	0.07	0.27	26.67	1.82	18.96	69.65	
		NO	La 9										
			Temp El islote										
	Organic turbid	YES	Las Coloradas	mean	383	5.50	0.10	0.32	30.68	1.98	10.94	748.98	
			S64	sd	433	6.75	0.18	0.42	15.15	1.30	24.74	658.78	
		NO	S94 (2011–2013)										
			Nieve Pif Rod 9 Rod 18 (2013) Justita (2011–2013)										
	Inorganic turbid	NO	Potrerrillo	mean	570	7.95	0.06	0.17	23.38	2.29	13.16	178.54	
			Laguna verde Campamento	sd	354	4.62	0.03	0.06	9.59	1.23	12.02	185.64	
		NO	Rod 18 (2014–2015)	mean	637	18.52	0.06	0.21	33.14	2.95	51.30	1253.63	
			Justita (2014) S94 (2014–2015)	sd	315	12.66	0.06	0.16	20.80	-	62.54	1309.42	
BUENOS AIRES	Clear vegetated	NO	S44	mean	5670	43.55	0.11	3.06	136.80	10.99	5.94	-	
			Pond X	sd	14	18.47	0.01	3.82	105.78	14.13	4.15	-	
	Inorganic turbid	NO	El Chapu	mean	150	8.93	0.06	0.12	19.15	2.45	23.17	-	
			El Cevercero	sd	100	10.85	0.06	0.22	19.21	2.57	35.09	-	
			El Sello 9 de julio E25 Gaviotero La soñada El huevo										
			Pedregosa	one data	1419	126,31	undetec.	0.48	118.6	28.11	29.51	-	
	Clear mesohaline	NO	La Honda	mean	14715	2.44	0.02	0.08	1438	0.92	6.01	-	
				sd	35	0.57	0.00	0.08	53.74	-	5.00	-	



### 3. Results

#### 3.1. Limnological variables

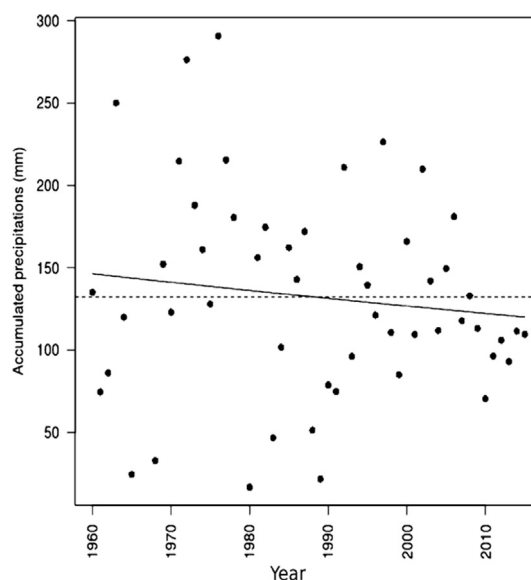
Mean values of the main physical, chemical and biological variables registered in the different types of lakes are shown in Table 2, whereas the detailed information of all the variables measured in each particular lake is presented in Supplementary material 1.

Values of the vertical attenuation coefficient ( $K_d$ ) reflected the different regimes of the lakes (clear vegetated, organic turbid, and inorganic turbid). The highest mean  $K_d$  values ( $10.99\text{--}28.11\text{ m}^{-1}$ ) were recorded in the inorganic turbid lakes of both plateaus, and the lowest ones in clear vegetated fishless lakes of the Strobel Plateau ( $1.98\text{ m}^{-1}$ ) and in one clear mesohaline lake of the Buenos Aires plateau ( $0.92\text{ m}^{-1}$ ).

Nutrients showed differences between both plateaus. In general, lakes of the Strobel plateau showed higher concentrations of P- $\text{PO}_4$ , varying mean values from  $0.17\text{ mg L}^{-1}$  (organic turbid lakes) to  $3.06\text{ mg L}^{-1}$  (inorganic turbid lakes); in the lakes of the Buenos Aires Plateau, average concentrations varied from  $0.08\text{ mg L}^{-1}$  (clear mesohaline lake) to  $0.48\text{ mg L}^{-1}$  (inorganic turbid lakes). DIN (nitrate + nitrite + ammonium) was relatively low in most lakes, with highest values in shallow lakes of the Strobel Plateau (average concentrations for the different lake types  $0.06\text{--}0.11\text{ mg L}^{-1}$ ), and values ranging from undetectable to  $0.06\text{ mg L}^{-1}$  in lakes of the Buenos Aires Plateau. In both plateaus, DOC values were relatively high in vegetated shallow lakes and very high in the most turbid lakes; extreme values were measured in the mesohaline lake (La Honda), where the precipitation of carbonates was observed in the shore.

#### 3.2. Environmental variables

Field observations during the last decade (2006–2016) have revealed a decreasing water level in most arheic basins. In the summer of 2016 a 71% of the studied lakes of Strobel Plateau showed a marked water level decrease (26% was dry or almost dry), whereas at the Buenos Aires Plateau a 50% of the studied lakes exhibited such condition. Satellite image analyses carried out by Lancelotti (unpublished data) confirmed the shrinking of the surface area of the lakes located at the Strobel Plateau. Nevertheless, some endorheic chained lakes receiving inputs from temporary streams presented higher water levels at the beginning of 2016. Meanwhile, several lakes (mainly large water bodies) showed no evident changes in water level. The decrease in the water level in the arheic basins seems to be associated with a decreasing trend in precipitation in this region and the noticeable dry period of the last decade, when, almost every year, the accumulated precipitations were lower than the historical mean (1960–2015), which is  $132.4\text{ mm}$  (Fig. 2). Spring-summer precipitations decreased significantly for this period (Mann-Kendall test  $p = 0.034$ ). However, no evident trend was observed at Perito Moreno, the nearest locality to the Buenos Aires Plateau (Mann-Kendall test  $p = 0.261$ ); data not shown). On the other hand, series of temperature for the Strobel Plateau indicates an increase in the average of daily maximum temperature from 1960 to 2015 (data not shown), both for cold and warm seasons, being more pronounced in spring-summer (Mann-Kendall test  $p = 0.003$ ).



**Fig. 2.** Historical series of accumulated precipitation from 1960 to 2015 for Gobernador Gregores (Santa Cruz Province), the nearest locality to the Strobel Plateau. The dotted horizontal line represents the historical mean value for the mentioned period ( $132.4\text{ mm}$ ). Data provided by the National Meteorological Service of Argentina.

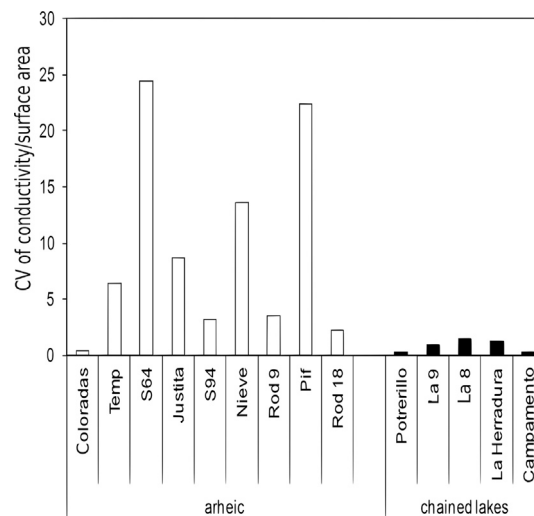
The decrease in water level in arheic shallow lakes was accompanied by increases in conductivity, whereas, in chained shallow lakes, conductivity showed seasonal variations, influenced by local thawing cycles. This contrasting behavior is evidenced by coefficient of variation of conductivity relativized by the surface lake area (Fig. 3), which showed higher values for the arheic shallow lakes than for the chained water bodies. In the Strobel Plateau, the highest conductivity values (around  $5600 \mu\text{S cm}^{-1}$ ) were measured in the most turbid lakes that have a high concentration of inorganic particulate material. The lakes surveyed in the Buenos Aires Plateau showed wide conductivity range, with a maximum of  $14740 \mu\text{S cm}^{-1}$  in an arheic basin located in a deep depression (lake Honda) and a minimum value of  $64.9 \mu\text{S cm}^{-1}$  in a small clear vegetated shallow lake (La Soñada). The decrease in the water level triggered a reduction in aquatic macrophytes in some shallow lakes; this pattern was particularly evident in three water bodies where we observed a simultaneous increase in conductivity (a proxy of water level) and phytoplankton Chla, leading to a turbid state (Fig. 4) that was evidenced by higher  $K_d$  values (2–6 fold increase over two years).

### 3.3. Biological analyses

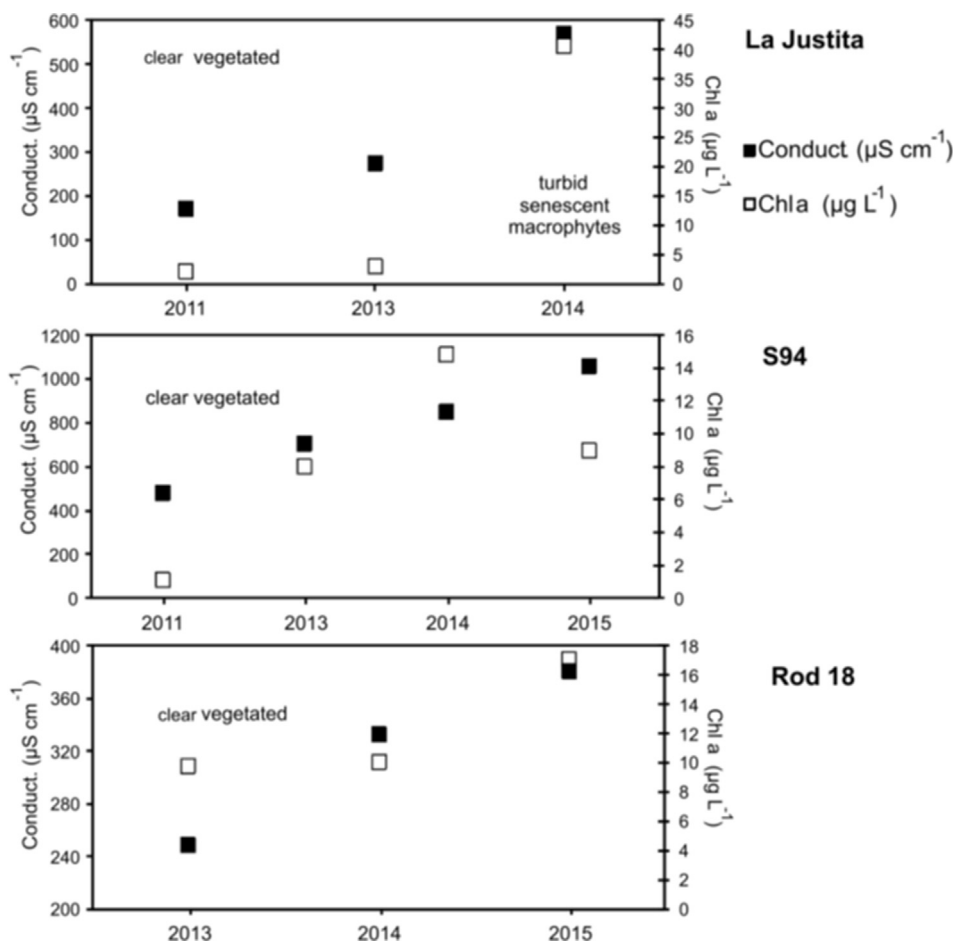
The lakes surveyed displayed a great range of variation in phytoplankton biomass, which was evaluated by Chla and algal biovolume (Table 2). Figure 5a illustrates the mean Chla for the lakes of the Strobel Plateau. The highest concentrations were observed in the most turbid lakes, including the two water bodies with high amount of inorganic suspended material ( $36.7$ – $56.6 \mu\text{g L}^{-1}$ ). In general, fish stocked lakes, as well as those lacking aquatic vegetation, presented intermediate to relatively high Chla values (mean  $6.6$ – $21.3 \mu\text{g L}^{-1}$ ), whereas fishless lakes completely colonized by submerged plants showed lower concentrations (mean  $3$ – $5.4 \mu\text{g L}^{-1}$ ); the minimum value was measured in the deep lake El Islote (fish stocked although with patchy vegetation in the littoral zone), which was sampled only in one occasion. In the Buenos Aires Plateau (Fig. 5b), the highest value of Chla ( $126.3 \mu\text{g L}^{-1}$ ) was measured in the most turbid shallow lake (La Pedregosa), which also had high amount of inorganic suspended material, whereas, in the other lakes surveyed (all of them clear vegetated), the values of Chla ranged between  $0.77$  and  $22.6 \mu\text{g L}^{-1}$ , corresponding the latter value to Lake El Chapu, where we observed a pronounced reduction of aquatic vegetation the last year sampled.

Values of the mean phytoplankton biomass obtained from algal biovolumes (fraction  $> 2 \mu\text{m}$ ) are shown in Table 2. In the Strobel Plateau, values ranged from  $1.11 \text{ mg L}^{-1}$  to  $101.18 \text{ mg L}^{-1}$ , with the lowest biomass in fishless clear vegetated shallow lakes, and also relatively low values in two fish stocked lakes with aquatic macrophytes (lakes La 8 and La 9). The highest algal biomass was found in a very small arheic shallow lake (Pond 12); inorganic turbid lakes exhibited rather low values compared with the very high Chla concentrations measured in these lakes, which probably was due to a high abundance of autotrophic picoplankton, not included in the biovolume calculations, but detected by epifluorescence and flow cytometry (Saad unpublished data). The lakes of the Buenos Aires Plateau exhibited comparatively lower phytoplankton biomass, with the highest mean value in El Chapu ( $54.26 \text{ mg L}^{-1}$ ) and the lowest one in La Soñada ( $1.18 \text{ mg L}^{-1}$ ).

The floristic list corresponding to the qualitative phytoplankton analyses is shown in Supplementary material 2. In general, the dominant algal group in terms of biomass in lakes of the Strobel Plateau was Chlorophyceae, with mean contributions  $>70\%$  in half of the lakes surveyed; some of the best represented taxa were *Chlamydomonas* spp., *Pediastrum boryanum*, *Scenedesmus acuminatus* and several species of *Monoraphidium*. Cyanobacterial biomass achieved higher values ( $15$ – $56\%$ ) in three fish stocked lakes (La Herradura, Potrerillo, Temp) and in a small pond (Pond 13), being *Aphanocapsa delicatissima*,



**Fig. 3.** Variation coefficient (CV) of conductivity relativized by lake surface area in arheic lakes and chained lakes from the Strobel Plateau. Higher values of this ratio indicate strong variations in conductivity due to changes in water level.

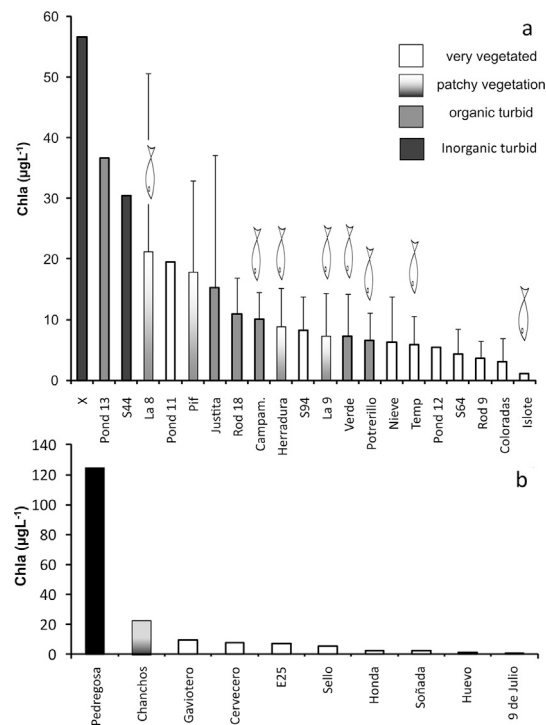


**Fig. 4.** Inter-annual variation in conductivity (as a proxy of water level) and Chl a in three shallow lakes of the Strobel Plateau that switched from a clear-vegetated state to a more turbid condition.

*Aphanocapsa elachista*, *Microcystis firma*, and *Aphanothece* sp. the most frequent species. Bacillariophyceae were also well represented in many lakes, whereas Chrysophyceae reached the highest biomass in a clear fishless lake. In the Buenos Aires Plateau, Chlorophyceae dominated in biomass in five water bodies (El Cervecero, 9 de Julio, La Honda, La Pedregosa and Gaviotero) and co-dominated in El Sello; this group was also well represented by *Chlamydomonas* spp. and *Monoraphidium* spp. in most of the lakes. Cyanobacteria showed the highest contribution to the biomass in lakes El Chapu and E25, and Bacillariophyceae in lakes La Soñada and El Huevo; among the cyanobacteria the most abundant species were *Pseudoanabaena catenata*, *A. delicatissima*, *Dolichospermum* sp. and *Planktothrix* sp., whereas diatoms were best represented by *Fragilaria* spp., *Nitzschia* spp., *Cocconeis placentula* and *Gomphonema* spp.

The zooplankton analyses of the Strobel Plateau (field trips 2013 and 2015) revealed differences in composition between fishless and fish stocked lakes; interestingly, in both types of lakes we found a remarkable degree of endemism among the species of all of the zooplankton groups (see zooplankton list – [Supplementary material 3](#)). The medium-sized calanoid copepod *Boeckella brasiliensis* (1.2–2.2 mm) dominated in biomass in three fish stocked lakes (La Herradura, Verde, La 8), whereas in lakes Potrerillo and Campamento the small *Boeckella gracilipes* (0.7–1.3 mm) was dominant, and in lake La 9 prevailed the medium-sized cladoceran *Daphnia dadayana* (1.1–2.5 mm). Among cladocerans, *Daphnia commutata* (1.0–2.7 mm) dominated in half of the fish stocked lakes; rotifers were best represented in these lakes by *Conochilus unicornis*, *Hexarthra fennica*, *Polyarthra dolichoptera*, *Keratella kostei* and *Brachionus kultrum*. In fishless lakes *B. brasiliensis*, together with the large species *Boeckella poppei* (1.9–3.3 mm) and *Boeckella longicauda* (2.3–2.8 mm), were dominant in biomass in most of the lakes; the large predatory calanoid copepod *Parabrotheas sarsi* (5.2–6.5 mm) was only present in two lakes (S94 and Pif); among cladocerans, dominance was shared by *D. dadayana*, *D. commutata*, *P. scopuliferus* and *Chydorus patagonicus*, whereas dominant rotifers in fishless lakes were *Euchlanis dilatata*, *K. kostei*, *B. kultrum*, *Notholca walterkosteii*, and undetermined Bdelloids.

The mean biomass of total zooplankton was significantly higher (ANOVA  $p = 0.042$ ) in fishless lakes ( $840.74 \mu\text{g L}^{-1}$ ) than in stocked lakes ( $334.71 \mu\text{g L}^{-1}$ ), and macrozooplankton was also significantly higher (ANOVA  $p = 0.033$ ) in fishless



**Fig. 5.** Mean Chla concentrations in lakes of the Strobel Plateau (a) and Buenos Aires Plateau (b), sorted according to decreasing concentrations; the colors of the bars represent the regimes of the water bodies, and fish stocked lakes are indicated with a symbol. Whiskers represent the standard deviations.

(822.21 µg L<sup>-1</sup>) than in fish stocked lakes (308.33 µg L<sup>-1</sup>). Microzooplankton biomass was lower in fishless (18.52 µg L<sup>-1</sup>) than in fish stocked lakes (26.38 µg L<sup>-1</sup>), although differences were not statistically significant. Among Crustacea, all groups considered in the analysis (small and large Copepoda; small and large Cladocera) had higher biomass in fishless lakes. The average values of the zooplankton biomass/Chla ratio were higher in fishless (965.53) than in fish stocked lakes (88.19), but differences between both types of lakes were not statistically significant.

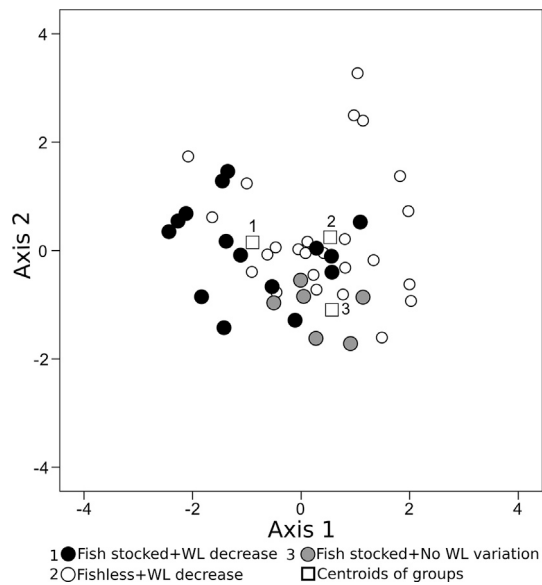
#### 3.4. Multivariate analyses

In the stepwise discriminant analysis performed for the lakes of the Strobel Plateau, the selected variables (greater weight in the analysis) were pH and P-PO<sub>4</sub>. MANOVA analysis evidenced differences among the group of lakes ( $\lambda_{\text{wilks}} = 0.606$ ;  $F_{4,84} = 5.970$ ;  $p < 0.0001$ ), where fish stocked lakes that presented relatively stable water level differed from lakes that showed a decrease in water level, both fishless (Tukey:  $F_{2,42} = 9.95$ ;  $p < 0.001$ ) and fish stocked (Tukey:  $F_{2,42} = 5.00$ ;  $p = 0.11$ ); however, no differences were achieved among fish stocked and fishless lakes affected by water level drop (Fig. 6). In the analysis corresponding to the lakes of the Buenos Aires Plateau, the selected variables were Chla and P-PO<sub>4</sub>. Also in this case, differences among lakes were related to the hydrological variations ( $\lambda_{\text{wilks}} = 0.526$ ;  $F_{1,15} = 13.52$ ;  $p = 0.02$ ), where lakes with decreased water level differed from those with stable water level (Tukey:  $F_{1,15} = 13.52$ ;  $p = 0.002$ ).

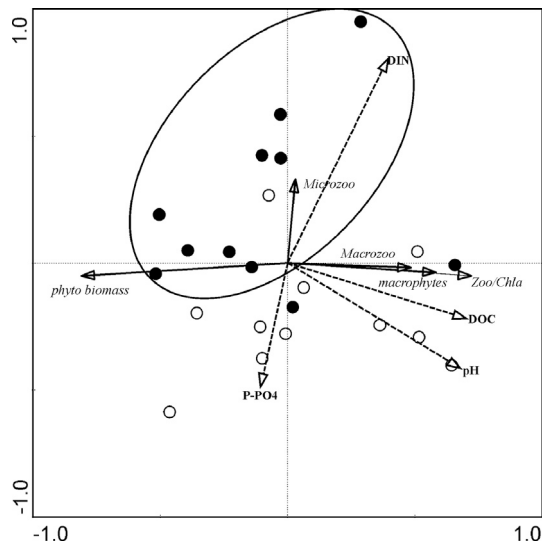
The RDA carried out with data of the Strobel Plateau of the 2013 and 2015 field trips reflected differences among lakes, mainly in relation to fish introduction and lake regime (Fig. 7). The two first axes accounted for 98% of the variance (axis 1: 94.8%; axis 2: 3.2%). Monte Carlo tests were significant for the first canonical axis ( $p = 0.004$ ) and for all axes ( $p = 0.01$ ). The first axis was positively correlated with DOC and pH (intra-set correlation coefficients 0.57 and 0.56 respectively) and the second axis was directly correlated with DIN (intra-set correlation coefficient 0.30). The triplot shows that most fish stocked shallow lakes are positioned on the left and upper quadrants of the graph, towards higher values of DIN, phytoplankton biomass and microzooplankton biomass. On the other hand, most fishless shallow lakes were located on the right and lower parts of the figure, showing opposite trends in relation to the mentioned environmental variables, towards increasing macrophyte abundance, together with higher macrozooplankton and zooplankton biomass/Chla ratio and lower microzooplankton biomass.

## 4. Discussion

The results of our investigations in the lakes of the plateaus of Austral Patagonia evidenced the effect of two main stressors, namely the fish introductions and the water level decrease (as a consequence of a reduction in precipitation), which are



**Fig. 6.** Biplot of the discriminant analysis based on physical and chemical data from lakes of the Strobel Plateau. Lakes were classified according to the presence/absence of fish introduction (fish stocked/fishless) and water level (WL) variation (decrease and no water level variation).



**Fig. 7.** Triplot corresponding to the Redundancy Analysis (RDA) based on environmental and biological data of lakes of the Strobel Plateau. The analysis was performed including only data of the 2013 and 2015 field trips.

affecting macrophyte and plankton communities. Particularly, in some lakes we observed inter-annual changes associated with water level reduction: decline in macrophyte cover and increase in phytoplanktonic chlorophyll a, depicting the strong influence of vegetation in the lake regime (clear vs. turbid). We also found differences in phytoplankton and zooplankton structure between fishless and fish stocked lakes, thus confirming with a large data set trends previously observed for a few number of lakes surveyed only at the Strobel Plateau (Izaguirre and Saad 2014; Lancelotti et al. 2016). In this study we further included the inter-annual variability that allowed analyzing the joint effect of fish stocking and water level reduction.

Regardless of the alternation of periods with different climatic conditions, climate projections in Patagonia (Barros et al. 2014) indicate reduction in precipitation and increasing temperatures, with consequences in freshwater water bodies (droughts in basins and reduced river flows). In this context, previous studies in the Strobel Plateau have pointed out that the abundance of lakes in this region is strongly related to the annual precipitation (Lancelotti et al. 2010a). The increased maximum temperatures over the last 55 years, the decreasing tendency in precipitation in the closest locality to the Strobel Plateau, and the fact that about 60% of the studied shallow lakes were dry or with lower water level in summer 2016, seem to

confirm this trend. Arheic lakes, which depend on the deposition of snow in the surrounding area, seem to be more affected by alterations in climate regimes than chained shallow lakes, which are fed by temporary streams and depend on a more extensive catchment. In this sense, the review published by [Adrian et al. \(2009\)](#) reports examples of non-regulated lakes, where the water level is a good indicator of climate change because it reflects the dynamic balance between water input (precipitation and runoff) and water loss (evaporation). Response variables are conductivity (or salinity) and pH. In our study we detected clear increases in conductivity in lakes with decreased water level. In some vegetated shallow lakes of the plateaus, the volume reduction was accompanied by a decrease in aquatic submerged plants (lower biomass and wilting) and an increase in phytoplankton biomass, which was noticeably evidenced by the Chla values. The reduction in water level is associated with a decrease in the surface area of lakes, detected by satellite images by Lancelotti (unpublished data). The positive feedback between submerged vegetation and water clarity and their associated mechanisms (physical, chemical and biological) have been largely discussed in relation to the alternative state theory in shallow lakes (e.g. [Jeppesen et al. 1990](#); [Scheffer et al. 1993](#); [Scheffer 2009](#)): vegetation enhances water clarity because it provides refuge for zooplankton, prevents resuspension, promotes sedimentation of suspended matter, competes (together with periphyton) for nutrients and light with phytoplankton and may excrete allelopathic substances. The effect of submerged vegetation on water clarity was evaluated across a wide climatic gradient in South America ([Kosten et al. 2009](#)), revealing that grazing on algae by zooplankton is generally higher in lakes with abundant submerged vegetation, which is enhanced in environments with low densities or absence of omnivorous fish, characteristics that are frequent in lakes of cold regions of South America.

Most lakes of the basaltic plateaus of Patagonia are naturally fishless, but stocking with rainbow trout started in the 1940s, and has been more intense in recent years ([Lancelotti et al. 2009a](#)). There is abundant worldwide literature with examples of fish stocking in historically fishless lakes. In the western national parks of Canada, about 95% of the lakes were fishless, and during the 20th century, 20% of them were altered by the introduction of non-native fish ([Donald 1987](#)). In a mountain region of western USA, less than 45% of the 95% naturally fishless lakes remain un-stocked ([Bahls 1992](#)), and in mountain lakes of southeastern Norway also a great proportion of lakes devoid of fish were stocked ([Hesthagen and Sandlund 2004](#)). It has been demonstrated that fish introductions in naturally fishless lakes have impacts on the planktonic food webs, generating trophic cascades, and may cause regime shifts (e.g. [Carpenter and Kitchell 1993](#); [Scheffer 1998](#); [Schindler et al. 2001](#); [Eilers et al. 2007](#)). On the other hand, fish stocking may generate changes in biodiversity in other aquatic communities (like pleustonic or benthic). Fishless lakes provide habitat for invertebrates and amphibians that have evolved in the absence of fish predation, and thus are unable to coexist with fish ([Schilling et al. 2008](#)). Significant effects of fish stocking on the macroinvertebrate community structure, with reduced species richness and abundance of taxa characteristic of fishless lakes, have been reported for kettle lakes and headwater lakes from Maine (USA) ([Schilling et al. 2009](#)).

Ecological alterations in aquatic ecosystems by introduction or translocation of native and exotic fish have also been reported for Patagonia. These alterations involved loss in biodiversity (e.g. [Ortubay et al. 2006](#)) and changes in the plankton structure, mainly lower zooplankton size spectrum and higher abundances of cyanobacteria ([Reissig et al. 2006](#)). The introduction of rainbow trout in lakes of the Strobel Plateau has triggered changes in the communities at several scales and organization levels. Comparison of fishless and fish stocked lakes in this region has revealed incipient changes in the relative proportion of different groups of pleustonic macroinvertebrates ([López, 2015](#)), higher phytoplankton biomass and greater cyanobacteria abundances in some fish stocked lakes ([Izaguirre and Saad 2014](#)), and differences in the size spectrum, with larger *Daphnia* spp. and *Boeckella* spp. in fishless lakes, the absence of pelagic amphipods (one of the largest prey for trout) in stoked lakes and high abundance of this item in fishless lakes ([Lancelotti et al. 2016](#)).

The results of the present investigation are in line with the previous evidences obtained in the lakes of the Strobel Plateau, but also showed the combined effect of fish introduction and the hydrological variability, as well as the role of the aquatic vegetation in the limnological characteristics of lakes and the structure of their planktonic communities. We also found differences in the zooplankton structure between fishless and fish stocked lakes, with a higher abundance of total zooplankton biomass and macrozooplankton biomass in fishless ones. Although differences were not significant due to the inter-annual variability, in general, the lowest Chla values were recorded in fishless water bodies profusely colonized by macrophytes. The multivariate analysis (RDA) performed with the data of the field trips 2013 and 2015 also showed a separation of the lakes in relation to the fish introduction and the presence of aquatic vegetation: samples of the fish stocked lakes were associated to higher phytoplankton biomass and microzooplankton, whereas clear vegetated fishless to higher abundances of macrozooplankton, evidencing the important role of both macrophytes and fish on plankton structure. To disentangle the effect of fish introduction from other stressors it would be necessary to perform *in situ* long-term experiments, either following the changes in a lake after fish stocking, or by removing the stocked fish; in this sense, a very recent experiment of trout removal was performed in lake El Islote at the Strobel Plateau.

On the other hand, the importance of the water level changes was also evident regarding the results of the MANOVA. Fishless and fish stocked lakes that experienced a reduction in their water level did not differ significantly in their limnological features, suggesting that water level drop is an overriding stressor, which at some point could be masking the effect of fish introduction on physical and chemical processes in lakes.

The clear shifts in the lake regimes observed from 2011 to 2015 in three shallow lakes of the Strobel Plateau (La Justita, S94 and R18) reinforce the relevance of water level fluctuation in the community structure. In these water bodies, we detected a switch from a clear vegetated state to a more turbid condition with higher Chla values and a clear decline in the submerged aquatic vegetation. These changes are consistent with the transition from a clear state to a turbid one described in the alternative equilibrium model ([Scheffer et al. 1993](#)). A similar behavior was reported for a shallow lake located in another



region of Argentina (Pampa plain), where the senescence of submerged aquatic plants, triggered by changes in the hydro-metric level, was accompanied by a drastic increase in phytoplankton biomass (Sanchez et al. 2015).

The aquatic communities of lakes of the Patagonian plateaus seem to be threatened by two combined forces: the fish introduction and the reduction in their volumes as a consequence of the climate change. We hypothesize that, under a scenario of climate change, the effect of the decreased water level may intensify the adverse effects of the fish introduction. The lakes of the region profusely colonized by macrophytes (>50%) are more transparent, and in general, the effect of fish seems to be less pronounced than in non-vegetated lakes, but the aquatic plants are severely affected by the hydrometric fluctuations. On the other hand, waterbirds depend on the aquatic vegetation and associated macroinvertebrates on which they feed. In this sense, information obtained in a study on the waterbird community in 83 similar lakes at Strobel Plateau showed that the abundance of waterbirds was lower in fish stocked lakes (Roesler 2016). Comparing past (1980's) and present (2012) data, the same author found that the Hooded Grebe suffered a population retraction of 98% in the stocked lakes. Although direct causation has not been evaluated yet, the population of this species declined drastically from 1320 individuals to only 25 individuals in those stocked lakes (Roesler 2016). As it was previously discussed, the rainbow trout has direct effects shaping the community of pelagic crustaceans, which represents the main food items of the Hooded Grebe (Lancelotti et al. 2016).

Therefore, maintaining the shallow lakes of this region of Patagonia in the natural conditions is crucial for the conservation of biodiversity. In this sense, the recent creation of a National Park at the Buenos Aires Plateau (Parque Nacional Patagonia) constitutes an excellent starting point.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.gecco.2018.e00391>.

## References

- Abell, R., 2002. Conservation biology for the biodiversity crisis: a freshwater follow-up. *Conserv Biol* 16, 1435–1437.
- Adrian, R., Reilly, C.M.O., Zagarese, H., Baines, S.B., Hessen, D.O., Keller, W., Livingstone, D.M., Sommaruga, R., Straile, D., Van Donk, E., 2009. Lakes as sentinels of climate change. *Limnol Oceanogr* 54, 2283–2297.
- Bahls, P., 1992. The status of fish populations and management of high mountain lakes in the western United States. *Northwest Sci* 66, 183–193.
- Barros, V.R., Boninsegna, J.A., Camilloni, I.A., Chidiak, M., Magrín, G.O., Rusticucci, M., 2014. Climate change in Argentina: trends, projections, impacts and adaptation. *WIREs Clim Change* 6, 151–169.
- Bird Life International, 2016. Species factsheet: *Podiceps gallardoi*. Accessed on August 2016. <http://www.birdlife.org/news/tag/podiceps-gallardoi>.
- Bottrell, H.H., Duncan, A., Gliwicz, Z.M., Grygierek, E., Herzig, A., Hillbricht-Ilkowska, A., Kurosawa, H., Larsson, P., Weglenska, T., 1976. A review of some problems in zooplankton production studies. *Norwegian J Zool* 24, 419–456.
- Cabrera, A.L., 1994. Regiones fitogeográficas argentinas. *Enciclopedia argentina de agricultura y jardinería*. Editorial Acme, Buenos Aires.
- Canevari, P., Blanco, D.E., Bucher, E.H., Castro, G., Davidson, I. (Eds.), 1998. *Los Humedales de la Argentina: Clasificación, Situación Actual, Conservación y Legislación*. Wetlands International Publication 46, Buenos Aires, Argentina.
- Carpenter, S.R., Kitchell, J.F., Hodgson, J.R., 1985. Cascading trophic interactions and lake productivity. *BioScience* 35, 634–639.
- Carpenter, S.R., Kitchell, J.F., 1993. *The Trophic Cascade in Lakes*. Cambridge Univ. Press, Cambridge, England.
- Carpenter, S.R., Kitchell, J.F., 1996. *The Trophic Cascade in Lakes*. Cambridge University Press, New York.
- Coesel, P.F.M., Porcel, S., van Geest, A., Izaguirre, I., 2017. Remarkable desmid species from the southern Patagonian highlands. *Fottea*. <https://doi.org/10.5507/fot.2016.019>.
- Cuello, M.E., Bello, M.T., Kun, M., Úbeda, C.A., 2006. Feeding habits and their implications for the conservation of the endangered semiaquatic frog *Ategnathus patagonicus* (Anura, Neobatrachia) in a northwestern Patagonian pond. *Phyllomedusa* 5, 67–76.
- Di Giacomo, A.S., 2005. Áreas Importantes para la Conservación de las Aves: Santa Cruz. In: Di Giacomo, A.S. (Ed.), *Áreas importantes para la conservación de las aves en Argentina. Aves Argentinas/Asociación Ornitológica del Plata*, Buenos Aires, pp. 404–407.
- Donald, D.B., 1987. Assessment of the outcome of eight decades of trout stocking in the mountain national parks, Canada. *North Am J Fish Manage* 7, 545–553.
- Dumont, H.J., van de Velde, I., Dumont, S., 1975. The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. *Oecologia* 19, 75–97.
- Eilers, J.M., Loomis, D., St Amand, A., Vogel, A., Jackson, L., Kann, J., Eilers, B., Truemper, H., Cornett, J., Sweets, R., 2007. Effects of repeated fish introductions in a formerly fishless lake: Diamond Lake, Oregon, USA. *Fund Appl Limnol/Arch Hydrobiol* 169, 265–277.
- Fjeldså, J., 1986. Feeding ecology and possible life history tactics of the hooded grebe *Podiceps Gallardoi*. *Ardea* 74, 40–58.
- Hesthagen, T., Sandlund, O.T., 2004. Fish distribution in a mountain area in south-eastern Norway: human introductions overrule natural immigration. *Hydrobiologia* 521, 49–59.
- Hillebrand, H., Claus-Dieter, D., Kirschtel, D., Pollinger, U., Zohary, T., 1999. Biovolume calculation for pelagic and benthic microalgae. *J Phycol* 35, 403–424.
- Iriondo, M., 1989. Quaternary lakes of Argentina. *Palaeogeogr Palaeoclimatol Palaeoecol* 70, 81–88.
- Izaguirre, I., Saad, J.F., 2014. Phytoplankton from natural water bodies of the Patagonian Plateau. *Adv Limnol* 65, 309–319.
- Jeppesen, E., Jensen, J.P., Kristensen, P., Søndergaard, M., Mortensen, E., Sortkjaer, O., Olrik, K., 1990. Fish manipulation as a lake restoration tool in shallow, eutrophic, temperate lakes 2: threshold levels, longterm stability and conclusions. *Hydrobiologia* 200/201, 219–227.

- Jeppesen, E., Jensen, J.P., Søndergaard, M., Lauridsen, T., Pedersen, L.J., Jensen, L., 1997. Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. *Hydrobiologia* 342/343, 151–164.
- Kirk, J.T.O., 1994. Light and Photosynthesis in Aquatic Ecosystems. Cambridge University Press, Cambridge.
- Knapp, R.A., Matthews, K.R., Sarnelle, O., 2001. Resistance and resilience of alpine lake fauna to fish introductions. *Ecological Monographs* 71, 401–421.
- Kosten, S., Kamarainen, A.M.Y., Jeppesen, E., van Nes, E.H., Peeters, E.T., Mazzeo, N., Sass, L., Hauxwell, J., Hansel-Welch, N., Lauridsen, T.L., Søndergaard, M., Bachmann, R.G., Lacerot, G., Scheffer, M., 2009. Climate-related differences in the dominance of submerged macrophytes in shallow lakes. *Glob Chang Biol* 15, 2503–2517.
- Lake, P.S., 2011. Drought and Aquatic Ecosystems, Effects and Responses. Wiley-Blackwell, Oxford.
- Lancelotti, J.L., 2009. Caracterización limnológica de lagunas de la Provincia de Santa Cruz y efectos de la introducción de Trucha Arco iris (*Oncorhynchus mykiss*) sobre las comunidades receptoras. PhD Thesis. Universidad Nacional del Comahue, Río Negro.
- Lancelotti, J.L., Pozzi, L.M., Yorio, P.M., Diéguez, M.C., Pascual, M.A., 2009a. Fishless shallow lakes of Southern Patagonia as habitat for waterbirds at the onset of trout aquaculture. *Aquat Conserv* 19, 497–505.
- Lancelotti, J.L., Pozzi, L.M., Márquez, F., Yorio, P.M., Pascual, M.A., 2009b. Waterbird occurrence and abundance in the Strobel plateau, Patagonia, Argentina. *Hornero* 24, 13–20.
- Lancelotti, J.L., Pascual, M.A., Gagliardini, A., 2010a. A dynamic perspective of shallow lakes of arid Patagonia as habitat for waterbirds. In: Meyer, P.L. (Ed.), *Ponds: Formation Characteristics and Uses*. Nova Science Publishers Incorporated, New York, pp. 208–231.
- Lancelotti, J.L., Pozzi, L.M., Yorio, P.M., Diéguez, M.C., Pascual, M.A., 2010b. Precautionary rules for exotic trout aquaculture in fishless shallow lakes of Patagonia: minimizing impacts on the threatened hooded grebe (*Podiceps gallardoi*). *Aquat Conserv* 8, 1–8.
- Lancelotti, J.L., Bandieri, L.M.B., Pascual, M.A., 2015. Diet of the exotic rainbow trout in the critical habitat of the threatened hooded grebe. *Knowl Manag Aquat Ec* 416, 1–11.
- Lancelotti, J.L., Marinone, M.C., Roesler, I., 2016. Rainbow Trout effects on zooplankton in the reproductive area of the critically endangered Hooded Grebe. *Aquat Conserv*. <https://doi.org/10.1002/aqc.2629>.
- Lopez, M.E., 2015. Estructura de las comunidades pleustónicas de macroinvertebrados en lagunas de la Patagonia extra-andina (Provincia de Santa Cruz) con y sin introducción de peces. Graduate Thesis. Universidad de Buenos Aires, Buenos Aires.
- Marker, A.F.H., Nusch, A., Rai, H., Riemann, B., 1980. The measurement of photosynthetic pigments in freshwater and standardization of methods: conclusions and recommendations. *Arch Hydrobiol Beih Ergebn Limnol* 14, 91–106.
- McLeod, A.I., 2011. Kendall rank correlation and Mann-Kendall trend test. R package version 2.2. <https://CRAN.R-project.org/package=Kendall>.
- Menu Marque, S., Morrone, J.J., Locascio, C., 2000. Distributional patterns of the south American species of *Boeckella* (Copepoda: Centropagidae): a track analysis. *J Crustacean Biol* 20, 262–272.
- O'Farrell, I., Izaguirre, I., Chaparro, G., Unrein, F., Sinistro, R., Pizarro, H., de Tezanos Pinto, P., Lombardo, R., Tell, G., 2011. Water level as the main driver of the alternation between a free-floating plant and a phytoplankton dominated state: a long term study in a floodplain lake. *Aquatic Sciences* 73 (2), 275–287.
- Ortubay, S., Cussac, V.E., Battini, M., Barriga, J., Aigo, J., Alonso, M.F., Macchi, P., Reissig, M., Yoshioka, J., Fox, S., 2006. Is the decline of birds and amphibians in a steppe lake of northern Patagonia a consequence of limnological changes following fish introduction? *Aquat Conserv* 16, 93–105.
- Pace, M.L., Cole, J.J., Carpenter, S.R., Kitchell, J.F., 1999. Trophic cascades revealed in diverse ecosystems. *Trends Ecol Evol* 14, 483–488.
- Panza, J.L., Franchi, M.R., 2002. Magmatismo Basáltico Cenozoico Extrandino. In: Haller, M.J. (Ed.), *Geología y Recursos Naturales de Santa Cruz*. Relatorio del XV Congreso Geológico Argentino, El Calafate, pp. 201–236.
- Paruelo, J.M., Beltran, A., Jobbagy, E., Sala, O.E., Golluscio, R.A., 1998. The climate of Patagonia: general patterns and controls on biotic. *Ecologia Austral* 8, 85–101.
- Perotti, M.G., Dieguez, M.C., Jara, F.G., 2005. Estado del conocimiento de humedales del norte patagónico (Argentina): aspectos relevantes e importancia para la conservación de la biodiversidad regional. *Rev Chil Hist Nat* 78, 723–737.
- Pilati, A., Martínez, J.J., 2003. Relación longitud- peso de siete especies de *Boeckella* (Copepoda: Calanoida) de la República Argentina. *Neotrópica* 49, 55–61.
- R Core Team, 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Reissig, M., Trochine, C., Queimaliños, C., Balseiro, E., Modenutti, B., 2006. Impacts of fish introduction on planktonic food webs in lakes of the Patagonian Plateau. *Biol Conserv* 132, 437–447.
- Roesler, I., 2016. Conservación del Macá Tobiano (*Podiceps gallardoi*): factores que afectan la viabilidad de sus poblaciones. PhD Thesis. Universidad de Buenos Aires, Buenos Aires.
- Roesler, I., Imberti, S., Casañas, H., Mahler, B., Reboreda, J.C., 2012. Hooded Grebe *Podiceps gallardoi* population decreased by eighty per cent in the last twenty-five years. *Bird Conserv Int* 22, 371–382.
- Ruttner-Kolisko, A., 1977. Suggestions for biomass calculation of plankton rotifers. *Arch Hydrobiol Beih Ergebn Limnol* 8, 71–76.
- Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., 2000. Global biodiversity scenarios for the year 2100. *Science* 287, 1770–1774.
- Sanchez, M.L., Lagomarsino, L., Allende, L., Izaguirre, I., 2015. Changes in the phytoplankton structure in a Pampean shallow lake in the transition from a clear to a turbid regime. *Hydrobiologia* 752, 65–76.
- Saunders, D.L., Meeuwig, J.J., Vincent, A.C.J., 2002. Freshwater protected areas: strategies for conservation. *Conserv Biol* 16, 30–41.
- Schabetsberger, R., Luger, M.S., Drozdowski, G., Jagsch, A., 2009. Only the small survive: monitoring long-term changes in the zooplankton community of an Alpine lake after fish introduction. *Biological Invasions* 11, 1335–1345.
- Schaus, M.H., Vanni, M.J., 2000. Effects of gizzard shad on phytoplankton and nutrient dynamics: role of sediment feeding and fish size. *Ecology* 81, 1701–1719.
- Scheffer, M., 1998. *Ecology of Shallow Lakes*. Chapman & Hall, New York.
- Scheffer, M., 2009. *Critical Transitions in Nature and Society*. Princeton University Press Oxford.
- Scheffer, M., Hopper, S.H., Meijer, M.-L., Moss, B., Jeppesen, E., 1993. Alternative equilibria in shallow lakes. *Trends Ecol Evol* 8, 275–279.
- Schilling, E.G., Loftin, C.S., Degoosh, K.E., Huryn, A.D., Webster, K.E., 2008. Predicting the locations of naturally fishless lakes. *Freshwat Biol* 53, 1021–1035.
- Schilling, E.G., Loftin, C.S., Huryn, A.D., 2009. Effects of the introduced fish on macroinvertebrate communities in historically fishless headwater and kettle lakes. *Biol Conserv* 142, 3030–3038.
- Schindler, D.W., 2009. Lakes as sentinels and integrators for the effects of climate change on watersheds, airsheds, and landscapes. *Limnol Oceanogr* 54, 2349–2358.
- Schindler, D.E., Scheuerell, M.D., 2002. Habitat coupling in lake ecosystems. *Oikos* 98, 177–189.
- Schindler, D.E., Knapp, R.A., Leavitt, P.R., 2001. Alteration of nutrient cycles and algal production resulting from fish introductions into mountain lakes. *Ecosystems* 4, 308–321.
- Scott, D.A., Carbonell, M.D.L., 1986. *A Directory of Neotropical Wetlands*. Cambridge.
- Sharp, J.H., Peltzer, E.T., Alperin, M.J., Cauwet, G., Farrington, J.W., Fry, B., Karl, D.M., Martin, J.H., Spitz, A., Tugrul, S., Carlson, C.A., 1993. Procedures subgroup report. *Marine Chemistry* 41, 37–49.
- Strock, K.E., Saros, J.E., Simon, K.S., McGowan, S., Kinnison, M.T., 2013. Cascading effects of generalist fish introduction in oligotrophic lakes. *Hydrobiologia* 711, 99–113.
- Sun, J., Liu, D., 2003. Geometric models for calculating cell biovolume and surface area for phytoplankton. *J Plankton Res* 25, 1331–1346.
- Ter Braak, C., Smilauer, P., 2002. *CANOCO Reference Manual and CanocoDraw for Windows User's Guide: Software for Canonical Community Ordination (Version 4.5)*. Microcomputer Power, Ithaca.

- Tiberti, R., Brighenti, S., Iacobuzio, R., Pasquini, G., Rolla, M., 2014a. Behind the impact of introduced trout in high altitude lakes: adult, not juvenile fish are responsible of the selective predation on crustacean zooplankton. *Journal of Limnology* 73, 593–597.
- Tiberti, R., von Hardenberg, A., Bogliani, G., 2014b. Ecological impact of introduced fish in high altitude lakes: a case of study from the European Alps. *Hydrobiologia* 724, 1–19.
- Valderrama, J.C., 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Mar chem* 10, 109–122.
- Vander Zanden, M.J., Vadeboncoeur, Y., 2002. Fishes as integrators of benthic and pelagic food webs in lakes. *Ecology* 83, 2152–2161.
- Venrick, E.L., 1978. How many cells to count? In: Sournia, A. (Ed.), *Phytoplankton Manual*. UNESCO, Paris, France.
- Williams, W.D., 1999. Conservation of wetlands in drylands: a key global issue. *Aquat Conserv* 9, 517–522.
- Williamson, C.E., Dodds, W., Kratz, T.K., Palmer, M.A., 2008. Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. *Front Ecol Environ* 6, 247–254.