Can top-down and bottom-up forces explain phytoplankton structure in a subtropical and shallow groundwater-connected lake?

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Abstract. Bottom-up and top-down control of phytoplankton is one of the most important hypothesis that explains and predicts the structure of aquatic community. Our aim was to elucidate whether predation and resource limitation can control phytoplankton composition and abundance in a subtropical shallow lake with groundwater connection to the river system. During 12 months, the lake was sampled at three points. Physico-chemical parameters, phytoplankton and zooplankton were sampled fortnightly, whereas fish were sampled every 3 months. The results showed that Euglenophyta dominated the total biovolume, followed by Dinophyta and Cryptophyta. As for the species composition, Chlorophyta was the dominant group (80 species recorded), followed by phylum Cyanobacteria (26 species recorded). Redundancy analysis (RDA) indicated that temperature and nitrate + nitrite concentration mainly explained biovolume changes, with zooplankton predation not having any measurable effect on phytoplankton during the high-water (HW) period. During low-water (LW) period top-down by fish was more important. At higher taxonomic resolution (species biovolume), phosphorus was another controlling factor. We concluded that phytoplankton in this lake is mainly regulated by hydrological changes as a macrofactor that affects nutrient availability and other environmental conditions. Even though bottom-up top-down forces do not have a central effect, we found evidence of positive nutrient influences at the HW period and fish effect at the LW period.

Additional keywords: control factors, floodplain lakes, hydrological connectivity, plankton.

Introduction

One of the most relevant topics in ecology has been the study of the biotic and abiotic factors that control the dynamics and the structure of communities (Carpenter *et al.* 1987; McQueen *et al.* 1989). Most ecologists agree that predation and resource limitation can alternatively regulate food-web interactions (Chase 2003; Borer *et al.* 2005). The hydrological fluctuation is another factor of great influence in floodplain systems (Amoros and Bornette 2002), where the spatial and temporal heterogeneity of water bodies and the transport of material and organisms is mediated by hydrological connectivity (Thomaz *et al.* 2007).

Amoros and Bornette (2002) discriminated among the three main kinds of hydrological connectivity. First, there is a direct connectivity when the main river or some tributaries are permanently or temporarily connected with its floodplain. The second type of connectivity is by river-water infiltration, and the third is by groundwater infiltration from hillslope aquifers; a combination of the three aforementioned types is also possible. Regarding this, ecologists have highlighted the importance of the direct connectivity in floodplain water ecosystems, which influences the transportation of suspended sediments, nutrients and organisms between the river and its floodplain (Junk *et al.* 1989; Tockner *et al.* 1999).

The distribution of phytoplankton biomass in the floodplain system is mainly determined by direct connectivity (Izaguirre *et al.* 2001). During high-water (HW) periods, flooding events have a dilution effect, changing the structure and composition of phytoplankton (García de Emiliani 1997; Huszar and Reynolds 1997). Conversely, during low-water (LW) periods, the enhancement of water transparency and an increase in habitat

heterogeneity have a considerable positive influence on phytoplankton density (Zalocar de Domitrovic 1992; Loverde-Oliveira *et al.* 2012).

Other factors such as nutrient enrichment (bottom-up) and predation (top-down) can have an effect on phytoplankton. Some studies have pointed out the importance of nutrient resource as one of the factors that influence phytoplankton density and species composition. Carignan and Neiff (1992) and Mayora *et al.* (2013) have shown that during HW periods, dissolved inorganic nitrogen penetrates into the floodplain, and is rapidly used by macrophytes, periphyton and phytoplankton.

Other authors have emphasised the importance of predation by zooplankton as a relevant factor that controls size distribution and abundance of phytoplankton, suggesting that the effects of predation is less important than resource availability in water bodies connected with the Paraná system (Sinistro *et al.* 2007; Sinistro 2010). It is necessary to consider that differences in size, morphology and palatability of phytoplankton can affect the strength of zooplankton predation (Lazzaro *et al.* 2003). Furthermore, evidence suggests that in those environments where direct connectivity is less pronounced, the structure of fish community changes (Rodríguez and Lewis 1997; Scarabotti *et al.* 2011). As a result, predatory fish significantly decrease in number, favouring planktivorous fish, and leading to a reduction in the predatory pressure of microcrustaceans (Cladocera and Copepoda) on phytoplankton (Byrnes *et al.* 2006; Gripenberg and Roslin 2007). In contrast, other studies have indicated that in those systems where microcrustaceans are more abundant, and direct hydrological connectivity allows the exchange of species between environments, zooplankton can exert an important effect on microalgae (Walks and Cyr 2004).

To sum up, both resource limitation and zooplankton predation can control phytoplankton; however, we do not have conclusive evidence about the strength of this process in shallow lakes of the Paraná River System, where the exchange of material and energy with the river system is reduced because direct water connection has been lost. Accordingly, the aim of the present work was to evaluate whether predation and resource limitation can control phytoplankton composition and abundance in a subtropical shallow lake with groundwater connection to the river system.

Materials and methods

Study area

'El Mirador Lake' is a small subtropical shallow lake in the Middle Paraná River system (31°37′S, 60°41′W, Argentina), with a surface area of ~3.76 ha and a maximum depth of 3.3 m. The lake water is mainly supplied with groundwater infiltration and rainfall, not being directly connected on the surface with the fluvial system. During the study, the perimeter of the lake was lined with a belt of emerging vegetation, with predominance of *Panicum elephantipes* (Nees ex Trin.) and *Ludwigia peploides* (Raven), without floating or submerged macrophytes (Fig. 1). With reference to the rain regime in the area, it had a mean (±s.d.) of 584 mm ± 187 during the HW period and 163 mm ± 20 during the LW period.

Samplings

Between December 2009 and November 2010, the lake was sampled every 15 days in three sampling points (one in the pelagic zone and two in the littoral zone), considering, for the
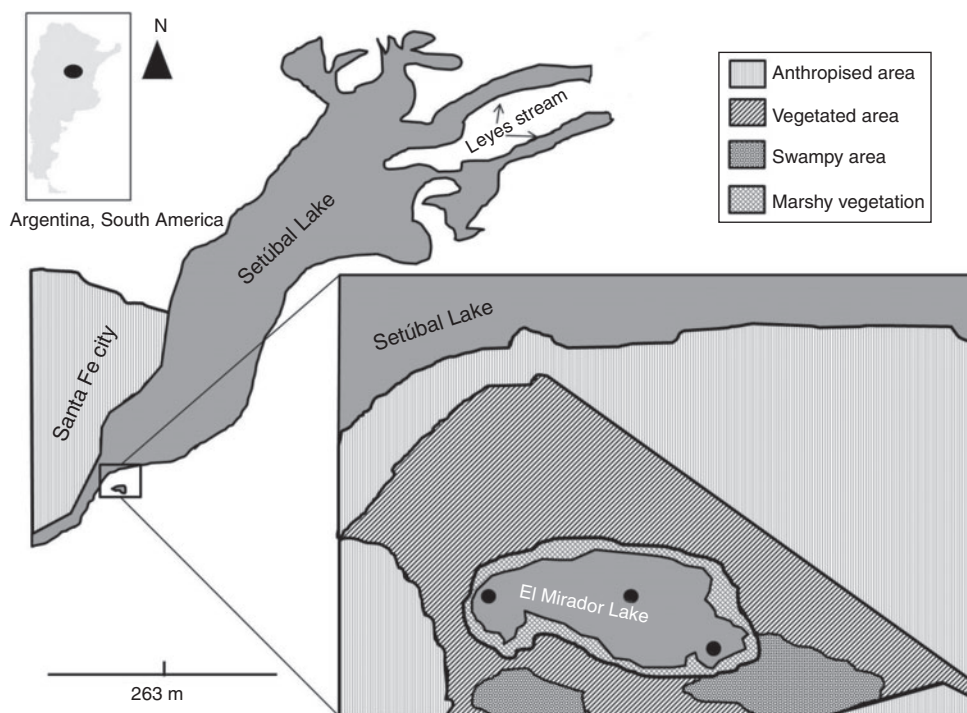

Fig. 1.   Localisation of 'El Mirador Lake' and the three sampling points used during the whole study.

present study, only the months of LW (less than 1.5 m) and HW (more than 2.3 m with respect to a point of reference) periods. Seven sampling dates were considered for both water periods (HW and LW). In each sampling point, HANNA portable meters were used to measure temperature (°C), pH, conductivity (μS cm^{-1}) and dissolved oxygen (mg L^{-1}). Transparency of water was measured with Secchi disc (cm) and depth (m) with an ultrasonic sensor. The euphotic zone was estimated using the formula proposed by Koenings and Edmundson (1991) (Z_{eu} = Secchi disc depth $\times$ 3.5). Phytoplankton, zooplankton and nutrient samples (for nitrate + nitrite and soluble reactive phosphorus) were collected. Fish sampling was performed on four occasions (January, May, September and December) in two microhabitats (pelagic and a littoral zones). This trimestral sampling is considered as an adequate frequency to describe fish control on plankton in isolated lakes of the region (Iglesias *et al.* 2008); even more so when one reproduction event (September in this study) is registered.

Samples for nutrient analysis were taken in 2-L bottles and filtered through Whatman GF/F glass-fibre filters. Soluble reactive phosphorous (SRP) was measured by the ascorbic acid method, and nitrate + nitrite (N–NO$_3^-$) was quantified by N–NO$_3^-$ reduction with metallic cadmium, and subsequent colourimetric determination of $^-$N–NO$_2^-$. Both analyses were performed using chemical sets from HACH Co.

Samples of phytoplankton were collected from the subsurface water by using 100-mL bottles, and were immediately fixed with 1% acidified Lugol solution. The quantitative analysis was conducted following the Utermöhl (1958) method, and the density obtained was expressed as individuals per millilitre. The biovolume of algae was measured following the method proposed by Hillebrand *et al.* (1999), and at least 10 individuals in each single taxon were measured. Biovolume data were expressed as square millimetres per litre, but only those species that represented more than 5% of the biovolume per sample were considered. Taxonomic identification was performed using keys and specific bibliography for each group.

As for the estimation of zooplankton density, 30 L of water was filtered using a Schindler-Patalas plankton trap with a conical conventional network (50 μm). The material collected was fixed *in situ* with 10% formalin and stained with erythrosine. The counts of the micro-zooplankton (Rotifera and nauplii) were carried out with a conventional optical microscope in chambers of the Kolkwitz type (1 mL). The counts of the macro-zooplankton (Cladocera and Copepoda) were performed in a Bogorov chamber (5 mL). A minimum of 100 individuals were counted in each sample.

Fish were sampled using a seine net of 25-m width and 5-m height, which tapered to 2.50 m at the ends and had a central bag of 5-mm knotless mesh. In each sampling point, the net was laid by encircling an area of 50 m^2 with the boat until both ends met. The net was quickly hauled for $\sim$10 m until it collapsed and fish got trapped in the bag. The sampling of littoral zones was conducted by encircling small areas ($<$10 m^2) according to the method proposed by Petry *et al.* (2003). The effort that each catch implied was the same in the different areas of the lake, with one haul being conducted in every sampling point. Fish were preserved *in situ* in 10% formalin, and in the laboratory they were identified, counted, measured and weighed. The sampling effort was standardised referring to the area covered by each haul.

Data analyses

The three sampling points were considered as replicates, after having previously confirmed that the samplings did not differ among them in phytoplankton total biovolume throughout the period of study ($P > 0.05$). The analyses were performed using Mann–Whitney comparison for independent samples and a significance level of 95% ($\alpha = 0.05$), with seven sampling dates for each water period (HW and LW).

Spearman correlations among the different environmental variables and phytoplankton richness *v.* hydrometric level of the lake were performed comparing HW and LW periods. Harrison diversity index was calculated so as to evaluate species turnover between periods. Redundancy analysis (RDA) was applied to find the variables with greater influence on phytoplankton because detrended correspondence analysis (DCA) showed that the gradient length of the response data was $<$3 (Lepš and Šmilauer 1999). In addition, those variables that were highly correlated (variance inflation factor $>$20) were removed from the analyses (Lepš and Šmilauer 1999). Phytoplankton biovolume was used as a response variable, whereas physical and chemical variables, density of Rotifera, microcrustaceans (Cladocera + Copepoda) and fish density were considered as explanatory variables. Quarterly fish data were used as an explanatory variable for phytoplankton data 1.5 months before and after fish sampling (two samples in the HW period and two in the LW period). This decision was taken for the following two reasons: first, only one reproduction event was observed during spring, and second immigration or emigration processes were negligible because the environment has only groundwater connection with the river system. All data were log-transformed to stabilise variance and to reduce the influence of dominant taxa on the arrangement, using error variance as standardisation procedure. In addition, the significance of every single variable and the combination of all canonical axes were determined using Monte Carlo permutation testing (499 permutations).

The influence of hydrological changes on phytoplankton assemblage was examined using the similarity percentage analysis (SIMPER) comparing species biovolume between HW and LW periods, and using the Bray–Curtis method. This analysis was accompanied by a non-parametric multivariate analysis of variance (NPMANOVA) to elucidate whether the differences found in the SIMPER analysis were statistically significant. RDA was used again (DCA gradient length of the response data was $<$3), but this time considering the relative biovolume of those species that contributed to 70% of the differences between HW and LW periods in SIMPER. This analysis was undertaken to evaluate the influence of the environmental variables on species composition. Biovolume values of species were log-transformed, and the significance of every single variable and the combination of canonical axes was determined using the Monte Carlo permutation testing (499 permutations). PAST software (Hammer *et al.* 2001) and CANOCO for windows 4.5 (ter Braak and Šmilauer 1998) were used to perform the aforementioned analyses.

Table 1.   Mean value and standard deviation (±s.d.) of environmental variables obtained for high-water (HW) and low-water (LW) periods
Mann–Whitney comparison test between both periods, and Spearman correlation between hydrometric level and environmental variables. Bold format indicates statistical significance at $P < 0.05$. SRP, soluble reactive phosphorus

Variable	HW period	LW period	Mann–Whitney test	Hydrometric level vs environmental variables
Temperature (°C)	27 ± 1.48	16 ± 4.31	$Z = -5.31, P = \mathbf{1.07 \times 10^{-7}}$	**Rho = 0.523, P = 0.000**
pH	7.29 ± 0.24	8.03 ± 0.55	$Z = -3.57, P = \mathbf{0.0003}$	**Rho = −0.541, P = 0.001**
Conductivity ($\mu S\ cm^{-1}$)	376 ± 71	1114 ± 251	$Z = -5.31, P = \mathbf{1.08 \times 10^{-7}}$	**Rho = −0.642, P = 0.000**
Dissolved oxygen ($mg\ L^{-1}$)	4 ± 1.96	7.91 ± 1.72	$Z = -4.41, P = \mathbf{0.003 \times 10^{-5}}$	**Rho = −0.431, P = 0.006**
SRP ($mg\ L^{-1}$)	0.13 ± 0.04	0.28 ± 0.15	$Z = -3.04, P = \mathbf{0.0023}$	**Rho = −0.410, P = 0.010**
$N–NO_3^-$ ($mg\ L^{-1}$)	1.37 ± 0.18	0.17 ± 0.098	$Z = -5.11, P = \mathbf{3.16 \times 10^{-7}}$	**Rho = 0.540, P = 0.000**
Z_{eu} (m)	1.66 ± 0.60	1.71 ± 0.65	$Z = -0.640, P = 0.522$	**Rho = 0.555, P = 0.000**
Microcrustaceans (individuals L^{-1})	49 ± 85	14 ± 27	$Z = -3.026, P = \mathbf{0.0025}$	Rho = 0.060, P = 0.717
Rotifera (individuals L^{-1})	503 ± 365	331 ± 338	$Z = -1.28, P = 0.200$	Rho = 0.264, P = 0.105
Fish density (individuals m^{-2})	14 ± 4	36 ± 8	$Z = -4.96, P = \mathbf{6.73 \times 10^{-7}}$	**Rho = −0.573, P = 0.000**

Results

Environmental and biological variables at hydrological phases

Temperature, $N–NO_3^-$ concentration and microcrustacean density had higher values during the HW period. In contrast, conductivity, dissolved oxygen, SRP, fish density and pH showed high values during the LW period. Statistical differences between phases were observed for every single variable except for Rotifera and Z_{eu}. Spearman correlation showed that all these variables were significantly correlated with the hydrometric level of the lake except, for Rotifera and microcrustacean density (Table 1).

Phytoplankton characterisation in HW and LW periods

In total, 150 phytoplankton species were recorded; the assemblage was dominated by Chlorophyta (80 taxa), followed by Cyanobacteria (26 taxa), and Ochrophyta, which was represented by Chrysophyceae (only 2 species and just 3.42% of Ochrophyta biovolume) and Bacillariophyceae with 24 species and representing the 96.58% of total Ochrophyta biovolume. Euglenophyta (17 taxa), Cryptophyta and Dinophyta (3 taxa each one) followed in number. Chlorophyta was the dominant group all throughout the year, representing 40% of the species richness in HW and LW periods. This group was followed by Cyanobacteria (20% of the species richness throughout the study), and Euglenophyta, with 10%. The other groups of phytoplankton had a low representation, and did not show changes in the number of species between the water phases (Fig. 2a). Harrison diversity index showed a small replacement of species between HW and LW periods (7.46%). A positive and statistically significant correlation was found between species richness and the hydrometric level of the lake (Rho = 0.846, $P < 0.001$, $n = 14$).

The highest values for biovolume were obtained for Euglenophyta in both HW and LW periods (respectively 25.8 ± 25.2 and $9.9 \pm 9.0\ mm^3\ L^{-1}$). It was followed by Dinophyta, which also showed high values during the HW period and Cryptophyta (respectively 12.3 ± 16.1 and $3.9 \pm 1.1\ mm^3\ L^{-1}$). The greater variability observed in Euglenophyta was attributed to anomalous high values during January (95.2, 67.5, 57.0 $mm^3\ L^{-1}$) in the HW phase, and in August (23.3, 14.9 and 16.4 $mm^3\ L^{-1}$) during the LW phase. Dinophyta presented extremely high values, mainly during February and March (49.6, 48.7, 27.9 $mm^3\ L^{-1}$).

The remaining groups of phytoplankton presented low biovolume values during the HW period, with values being $1.8 \pm 1.6\ mm^3\ L^{-1}$ for Cyanobacteria, $1.0 \pm 0.8\ mm^3\ L^{-1}$ for Chlorophyta and $0.2 \pm 0.54\ mm^3\ L^{-1}$ for L^{-1} Ochrophyta (Fig. 2b). During the LW period, Cyanobacteria was the dominant group ($2.8 \pm 4.5\ mm^3\ L^{-1}$), and was followed by Ochrophyta ($2.4 \pm 2.7\ mm^3\ L^{-1}$), Chlorophyta ($1.6 \pm 0.9\ mm^3\ L^{-1}$), Cryptophyta ($0.5 \pm 0.4\ mm^3\ L^{-1}$) and Dinophyta ($0.5 \pm 0.3\ mm^3\ L^{-1}$). Significant statistical differences were found for Euglenophyta, Ochrophyta, and Dinophyta between the HW and the LW periods, but not for the other groups of phytoplankton studied (Fig. 2b, Table 2).

Factors controlling phytoplankton abundance and species composition

The RDA (Fig. 3) demonstrated that phytoplankton biovolume was arranged following hydrological phases ($F = 2.317$, $P = 0.002$). The first axis represented a temporary gradient separating the HW from the LW period, which accounted for 54.4% of phytoplankton variability. The second axis accounted for 21%. Concerning the different variables used, it was found that biovolume was better described by several 4 of 10 variables (Table 3). In addition, all the environmental variables as well as all canonical axes accounted for 38.2% of total variability explanation.

During the HW period, Euglenophyta, Dinophyta and Cryptophyta were the prevailing groups, showing a positive correlation with $N–NO_3^-$ and temperature, but a negative association with conductivity and fish density (Fig. 3). Conversely, LW was characterised by a biovolume decrease in the majority phytoplankton groups except Chorophyta. This mostly coincided with the months of July and August when fish density and conductivity presented the highest concentration (Fig. 3).

Microcrustaceans (Cladocera + Copepoda) were better represented during the HW period, without showing a significant percentage of explanation for phytoplankton, but showing a negative correlation with fish, especially during July and August. Rotifera abundance was also better represented during the HW period, and exhibited a prominent negative correlation with fish density (Table 3). Fish assemblage showed an overdominance of small-sized fish (25–35 mm) largely belonging to
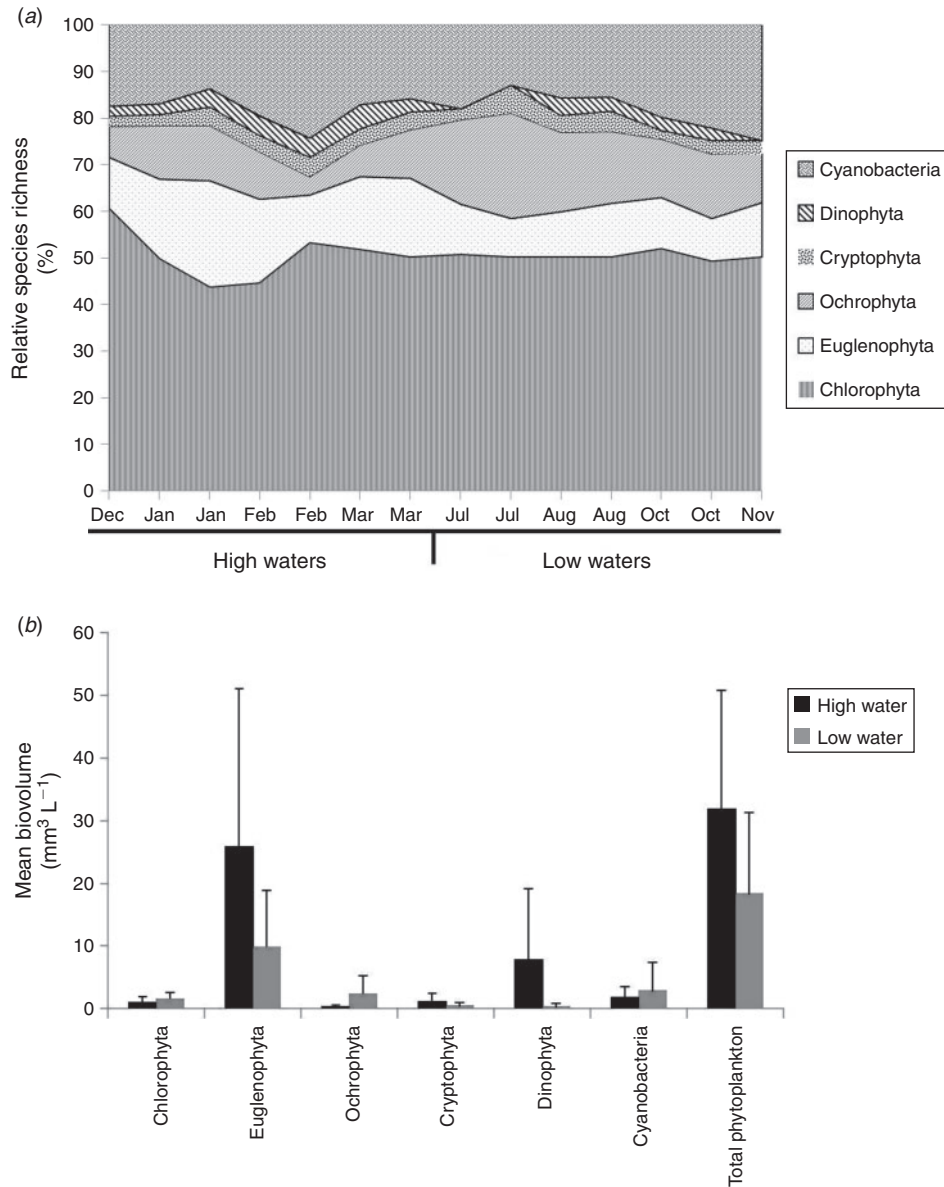
Fig. 2. (*a*) Relative species richness (%) of the different phytoplankton groups, and (*b*) mean biovolume values of phytoplankton groups registered during high- and low-water periods. Vertical bars indicate standard deviation during hydrological phases.

Table 2. Mann–Whitney comparison test between high- and low-water periods for the different phytoplankton groups considered
Bold format indicates statistical significance at $P < 0.05$

	Biovolume	
	Z	P-value
Total phytoplankton	−2.719	**0.0065**
Chlorophyta	−1.84	0.065
Euglenophyta	−2.662	**0.007**
Ochrophyta	−4.043	**5.28×10^{-5}**
Cryptophyta	−0.126	0.89
Dinophyta	−0.126	**0.015**
Cyanobacteria	−0.154	0.876

the species *Cheirodon interruptus* (Jenyns), which accounted for the 95–99% of the abundance and 55% of the biomass. Potentially piscivorous species were rare and were represented by the species *Rhamdia quelen* (Quoy and Gaimard) (8% in biomass) and *Hoplias malabaricus* (Bloch) (3% in biomass).

SIMPER analysis demonstrated a dissimilarity between the HW and LW periods that amounted to 58.57%, as was statistically significant (NPMANOVA: $F = 6.184$, $P = 0.0001$). In the same way, RDA using those species that contributed to 70% of dissimilarity, showed that phytoplankton was distributed according to a temporal gradient ($F = 1.927$, $P = 0.002$, 33.9% of explanation of the whole variance). The first axis described 34.8% of the variance separating the HW from the LW period, and the second axis accounted for 23.9% of the total variance.

The HW phase was mainly characterised by Dinophyta (*Durinskia baltica* and *Protoperidinium* sp.), Cryptophyta (*Cryptomonas ovata*), Euglenophyta (*Phacus* spp.) and Cyanobacteria (*Microcystis aeroginosa*). All these phytoplankton taxa were positively associated with temperature and $N-NO_3^-$, but negatively linked with SRP, conductivity and fish (Fig. 4). By contrast, July and August (LW period) were mostly characterised by centric diatoms (*Cyclotella meneghiniana* and a non-identified centric diatom). Chlorophyta species (*Coelastrum microporum, Pediastrum boryanum, Tetrahedron trigonum*),

Fig. 3. Redundancy analysis (RDA) plots for biovolume of the different phytoplankton groups represented during high-water (HW) and low-water (LW) periods. Response variables are Chlorophyta (Chloro), Ochrophyta (Ochro), Cryptophyta (Cryp), Euglenophyta (Eugl), Dinophyta (Dino) and Cyanobacteria (Cyan). Explanatory variables are nitrate + nitrite ($N-NO_3^-$), temperature (Temp), euphotic zone (Z_{eu}), Rotifera density (Rot), pH (pH), Cladocera + Copepoda density (Mcruc), dissolved oxygen (DO), fish density (Fish), conductivity (Cond) and soluble reactive phosphorus (SRP). Statistically significant variables are indicated by an asterisk.

Euglenophyta (*Trachelomonas volvocina*, *T. sidneyensis, Euglena rostrifera*) and Cyanobacteria (*Coelomoron* sp.) were also present in this water phase. This entire group of species demonstrated positive relations with conductivity (especially diatoms), SRP and fish density (Fig. 4).

Discussion

In the present study, we attempted to determine whether predation and resource limitation can control phytoplankton composition and abundance in a subtropical shallow lake with groundwater connection to the river system. We demonstrated that the hydrological fluctuation is the macrofactor that controls phytoplankton. It also affects resource availability, especially nutrients and other environmental variables, such as conductivity, which became secondary control factors affecting phytoplankton structure. A predominance of bottom-up or top-down effect, as the main determining mechanism of phytoplankton, was not observed in this shallow lake. Nevertheless, our results suggest that one of both forces became more important at the different hydrological phases. At the HW period, a bottom-up effect influenced biovolume positively, whereas the top-down effect was more important during the LW period. A nutrient threshold was high enough to exert a limitation effect on phytoplankton, but the enhancement of nitrates could be improving algal growth biomass. Fish predation pressure on zooplankton clearly reduced the effect of top-down control on phytoplankton. Our results suggested that *Cheirodon interruptus*, as an omnivorous predator, could also have a negative effect on phytoplankton through direct predation and a positive effect through nutrient remineralisation.

The periodic river–floodplain connection and disconnection transports water into the floodplain and depending on the residence time, water chemistry and previous hydrological conditions, a relevant effect on the community organisation of both the river and its landscape can be observed (Ahearn *et al.* 2006). In this shallow groundwater-connected lake, the increase in water level was accompanied by a rise in phytoplankton biovolume, with the opposite effect being displayed during the LW period. This is in contrast with directly connected floodplain lakes of the Paraná River system (Zalocar de Domitrovic 1990; Izaguirre *et al.* 2001) and other large river systems in the world

Table 3. Significance level and explanation percentage of the variables used in the redundancy analysis (RDA), based on phytoplankton biovolume and the most contributing species biovolume

Bold format indicates statistical significance at $P < 0.05$. SRP, soluble reactive phosphorous; %Expl., percentage explanation of the variable

Variable	RDA for biovolume			RDA for species		
	F-value	*P*-value	%Expl.	*F*-value	*P*-value	%Expl.
Temperature (°C)	7.29	**0.002**	**16.5**	4.646	**0.002**	**11.1**
Conductivity (μS cm^{-1})	5.87	**0.003**	**13.7**	3.100	**0.002**	**7.0**
SRP (mg L^{-1})	1.493	0.200	3.9	2.166	**0.014**	**7.7**
$N-NO_3^-$ (mg L^{-1})	7.048	**0.001**	**16.0**	3.685	**0.001**	**9.1**
Z_{eu} (m)	1.685	0.134	4.4	0.688	0.774	1.8
Microcrustaceans (individuals L^{-1})	1.685	0.138	4.3	1.243	0.206	3.3
Rotifera (individuals L^{-1})	1.044	0.362	2.7	1.110	0.348	2.9
Fish density (individuals m^{-2})	3.982	**0.006**	**9.7**	2.451	**0.006**	**6.2**

(e.g. Schemel *et al.* 2004; Mihaljević *et al.* 2009) where biovolume tends to increase during the isolation phase.

Euglenophyta and Dinophyta presented higher biovolume during the HW period, without displaying a predictable pattern because these groups tend to be better represented during isolation periods (García de Emiliani 1997; Zalocar de Domitrovic 2003; Mihaljević *et al.* 2009; Stević *et al.* 2013). Chlorophyta and Ochrophyta were better represented during the LW period, whereas Cryptophyta was better represented during the HW period. Chlorophyta and Cryptophyta are, in general, predominant at the beginning of the HW period, because they can tolerate a lower water-residence time through a higher reproduction rate, and can also remain in the subsurface layers resisting the turbidity increases. This pattern has already been observed in surface-connected shallow lakes of the Paraná River system and in other floodplain rivers (García de Emiliani 1997; Izaguirre *et al.* 2001; Reynolds *et al.* 2002; Descy *et al.* 2011).

The RDA split samples into two different water periods. In this context, N–NO$_3^-$ (16% of explanation in the RDA) and temperature (16.5%) were positively associated with higher abundance of Dinophyta, Cryptophyta and Euglenophyta during the HW period. It was during this period that the highest rainfall values were observed (584 ± 187 mm). Greater rainfall intensity could contribute to production of organic matter from surrounding vegetated areas (see Fig. 1) flow into the water body, which, in combination with higher temperatures, could favour the decomposition of organic matter, making nitrogen compounds more available for phytoplankton. Maberly *et al.* (2002) pointed out that nitrogen is a primary or a co-limiting nutrient for

phytoplankton in several inland water bodies, including subtropical shallow lakes. In this particular case, evidence did not suggest that nitrogen was a limiting nutrient, but a larger biovolume necessarily implied more resources (Chase 1999), which could partially explain the development of these phytoplankton groups during the HW period when they showed the highest biovolume.

The classical approach of the top-down effect describes that fish feed on zooplankton to generate a cascade effect. In the present study, Rotifera and microcrustaceans showed a negative relationship with fish density. This is consistent with the finding of Sinistro (2010) who reported a predominant predation effect on larger-bodied zooplankton by a planktivorous fish (*Jenynsia* sp.), and the subsequent reduced effect on phytoplankton abundance; this is also in accordance with other studies (Sommer *et al.* 2001). The high density of planktivorous–omnivorous fish registered during the whole study, which is consistent with the pattern observed in isolated lakes (Scarabotti *et al.* 2011), reduced the top-down control of zooplankton on phytoplankton (Byrnes *et al.* 2006; Gripenberg and Roslin 2007).

RDA showed that Dinophyta, Euglenophyta and particularly Cryptophyta were negatively correlated with fish abundance, which explained 9.7% of total variance. *C. interruptus* is regarded as a planktivorous-omnivorous fish that actually feed on both zooplankton and phytoplankton organisms (López Cazorla *et al.* 2003; Fernández *et al.* 2012). *C. interruptus* is a visual predator, and these phytoplankton taxa, previously mentioned, presented sizes similar to Rotifera (50–150 μm), which has been demonstrated to be strongly predated by this fish during

Fig. 4. Redundancy analysis (RDA) plots, showing (*a*) biovolume of phytoplankton species as response variables and (*b*) sampling dates and explanatory variables during high-water (HW) and low-water (LW) periods. Explanatory variables have the same codes as in Fig. 3. Phytoplankton species include *Oocystis parva* (*O. par*), *Cyclotella meneghiniana* (*Cy. meneg*), *Euglena clavata* (*E. clav*), *Tetraëdron trigonum* (*T. trigon*), *Coelastrum microporum* (*C. micro*), *Trachelomonas sidneyensis* (*T. sidne*), *Peridinium* sp. (*Peridi*), *Trachelomonas volvocina* (*T. volvo*), *Microcystis aeruginosa* (*M. aero*), *Cryptomona ovata* (*C. ova*), *Phacus longicaudus* (*P. long*), *Protoperidinium* sp. (*Protop.*), *Phacus brevicaudus* (*P. brevi*), *Monoraphidium arcuatum* (*M. arc.*), *Coelomoron* sp. (*Coelo*), *Durinskia baltica* (*D. balti*) and *Euglena rostrifera* (*E. rostr*). Statistically significant variables are indicated by an asterisk.

the same period (Senn, 2014). Our results suggested that this species could be feeding also on phytoplankton, and consequently, enhancing the top-down control during the LW period when fish density increases.

Moreover, RDA showed that, in the LW period, fish density and conductivity were the main controlling factors for phytoplankton groups (Chlorophyta and Ochrophyta). If we consider that phytoplankton could be controlled by fish predation, we also could assume that Chlorophyta was favoured during this phase, constituting a steady-state assemblage that was fully adapted to strong top-down conditions (Benndorf *et al.* 2002). This would explain the dominance of Chlorophyta during the LW period, because they are less susceptible to predation by fish because they have a smaller size and are able to reproduce faster than Euglenophyta and Dinophyta.

Ochrophyta and especially diatoms are well adapted to turbulence and high turbidity, being characteristics that allow them to survive during HW periods (Reynolds 1994). In the present study, their high abundance during the LW period could be explained, to a large extent, by an increase in the re-suspension process, which is mediated by the effect of wind on a shorter water column that enables them to survive in subsurface layers, a pattern that has already been reported in other shallow lakes (Divina de Oliveira and Calheiros 2000; Granado and Henry 2014). The high correlation found in the RDA between Ochrophyta and conductivity (which explained a 13.7%) during the LW period could also explain diatom co-dominance during the LW period. We attributed, in the present study, the greatest conductivity during the LW period to the effect of water volume reduction. It is likely that centric diatoms became dominant because they are better adapted to high concentrations of ions, a condition that also favours silica uptake for frustules production (Saros and Fritz 2000).

In regard to species richness, our results contrast with those found in other shallow lakes linked with larger river systems, in which an improvement of phytoplankton richness is recognised during the LW period (Zalocar de Domitrovic 1992; Descy *et al.* 2003; Granado and Henry 2014). In this isolated surface lake, the transportation of organisms between environments is restricted during floods, explaining the low value of Harrison diversity (7.46 %) obtained. Our results indicated that those changes observed in species richness were highly linked with the hydrometric level of the lake (Rho = 0.846 $P < 0.001$) which, in fact, presented a positive statistically significant correlation with other environmental variables such as temperature, light penetration (Z_{eu}) and N–NO_3^- concentration. These might favour some new species recorded in the HW period (42 ± 5 species registered) in comparison with the LW period, where a lower number of species was recorded (28 ± 5).

When a higher taxonomic resolution was used (species composition), RDA confirmed the pattern observed when phytoplankton groups were used, but SRP concentration also became important. The negative correlation between species of greater biovolume, such as *Cryptomonas ovata*, *Phacus* spp. and *Protoperidinium* sp., and the fish *C. interruptus*, enforce our argument of predation effects of this fish on phytoplankton (see Fig. 4).

In contrast, a higher representation of Chlorophyta (*Coelastrum microporum*, *Monoraphidium contortum*, *Oocystis parva*, *Tetraëdron trigonum*), one Cyanobacteria (*Coelomoron* sp.) and

three Euglenophyta (*Euglena rostrifera*, *E. clavata*, *Trachelomona curta*) species when SRP concentration was higher is consistent with previous findings where a high concentration of nutrients and a higher concentration of organic matter improve the development of these kind of algae in eutrophic systems (Jones 2000). During the LW period, it is expected that phosphorus liberation from sediment would be low because a higher oxygenation of the water column was observed; however, fish and SRP showed to be positively correlated, indicating a possible remineralisation effect mediated by fish. This phenomenon has been described as positive feedback by means of which fish can have a more pronounced effect on phytoplankton structure through nutrient re-mineralisation rather than as a result of predation on zooplankton (Rejas *et al.* 2005; Sarnelle and Knapp 2005). Future experimental studies will allow us to verify our hypothesis with respect to the multiple effects (predation and nutrient remineralisation) that this fish could have on phytoplankton biovolume. Our results are consistent with Hansson *et al.* (2004) who argued that in some phytoplankton taxa, physiological constraints and different competitive availability (in this study SRP concentration) may be more important for phytoplankton species than are top-down and bottom-up effects.

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