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Why predation is not a controlling factor of phytoplankton in a Neotropical shallow lake: a morpho-functional perspective

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Abstract This work explores the reasons why predation plays a minor role in structuring phytoplankton composition in a Neotropical shallow lake. Phytoplankton, zooplankton, and fish were sampled from a shallow lake over the course of a year, and the stomach contents of 80 individuals of the dominant omnivorous–planktivorous fish species were analyzed. The field study was complemented with a 5-day microcosm experiment in which the predation effects of micro, meso, and macrozooplankton were measured. Stomach content analysis revealed that fish predation was high, and primarily comprised by Cladocera (Ivlev's index >0.75). Both meso and macrozooplankton fractions are able to feed on colonial cyanobacteria, silica cell-wall organisms (<35 μm), and mixotrophic flagellate (>35 μm). Macrozooplankton, however, can feed on single cells, mucilaginous colonies, non-mucilaginous colonies, and silica cell-wall organisms (>35 μm) ($P < 0.05$ in all cases). Our results support the idea that absence of predation on phytoplankton is mainly mediated by fish predation on zooplankton and morpho-functional characteristics of algae which prevent zooplankton predation; showing fish a lack of direct predation effect on phytoplankton.

Keywords Top-down control · Phytoplankton features · Omnivorous fish effects · Zooplankton size

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Introduction

The two major paradigms that predict the effects of aquatic trophic interactions were introduced in the 80s by Carpenter et al. (1987), describing the trophic cascade hypothesis, and by McQueen et al. (1989), the top-down/bottom-up hypothesis. The first hypothesis predicts that changes in top predators affect the lower trophic levels, whereas the competing hypothesis predicts that top-down effects are stronger at the top of the food web and weaker toward the bottom. Nowadays, most ecologists agree that food web interactions can be highly variable through space and time (Chase, 2003), and some revisions made indicate that phytoplankton biomass would be better controlled by resources (bottom-up) than by grazing (top-down) (Benndorf et al., 2002).

A great amount of evidence has been published in the last decades supporting the idea that top-down plays a minor role as controlling factor of

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phytoplankton in Neotropical lakes (e.g., Fernando, 1994; Sarma et al., 2005; Von Rückert & Giani, 2008). Nevertheless, we still lack conclusive evidence of the main reasons which operate at the phytoplankton level. Four main hypotheses have been suggested. The first hypothesis was expressed by Lazzaro et al. (2003) who proposed that differences in size, morphology, and palatability of phytoplankton can affect the degree of zooplankton predation in the Brazilian lakes. Palatability has been historically considered, but referring especially to phytoplankton size into palatable (<35 μm) and unpalatable phytoplankton (>35 μm) (Lehman, 1988; Salmaso, 2002; Salmaso & Padisak, 2007), but without considering other morpho-physiological aspects such as, algae morphology, or cell-wall characteristics. The second hypothesis suggested that zooplankton of the Neotropical region play a minor grazing effect on phytoplankton due to their smaller size spectra, in comparison with zooplankton of temperate lakes (<20°C) where larger and more effective predators are dominant in the zooplankton assemblages (Hamza et al., 1995; Levine et al., 1999; Gillooly & Dodson, 2000; Sommer et al., 2003; Havens et al., 2007; Lacerot et al., 2013). The third hypothesis emphasized the role of small planktivorous fish which can exert a strong structuring effect on plankton communities both through predation of large zooplankton, and/or nutrient re-mineralization, which could support nutrient demand of primary producers (Drenner et al., 1986; Scasso et al., 2001; Boverí & Quirós, 2002; Vanni, 2002; Iglesias et al., 2008; Sinistro, 2010; Iglesias et al., 2011). Finally, the fourth hypothesis suggested that omnivorous–planktivorous fish—very common in subtropical lakes—can exploit mixed resources in several trophic levels including phytoplankton (DeVries & Stein, 1992; Beveridge & Baird, 2000; Zhang et al., 2006; Ke et al., 2007; Okun et al., 2008). In this study, we analyzed phytoplankton features, zooplankton predation ability, and the effect of omnivorous fish on phytoplankton and zooplankton through a field study and a short-term microcosm experiment. With this approach, we aimed to assess the four hypotheses and tried to shed light on the mechanisms explaining why predation plays a minor role as a control factor of phytoplankton in Neotropical shallow lakes.

Materials and methods

Sampling methods and plankton analyses in the shallow lake

From December 2009 to November 2010, we sampled 'El Mirador Lake' (31°37′S, 60°41′W, Argentina), a small (3.76 ha) subtropical shallow lake of the Middle Paraná River system. Samplings were obtained every 15 days in three points (two littoral and one limnetic) for phytoplankton, zooplankton, physical and chemical variables. Fish sampling was carried out in two microhabitats (one limnetic and one littoral zone) on four occasions (January, May, August, and November).

Phytoplankton samples were collected from subsurface water using 100 ml bottles, and were immediately fixed with 1% acidified lugol solution. The quantitative analysis was carried out following the Utermöhl (1958) method, and the density obtained was expressed as ind ml^{-1}. The counting error was estimated according to Venrick (1978), accepting a maximum error of 15%. Algae biovolume was measured following Hillebrand et al. (1999), and maximum linear dimension (MLD) (Lewis, 1976) was estimated by measuring at least 10 individuals for each taxa. Biovolume was expressed as $mm^3\ l^{-1}$ and MLD as μm.

To analyse palatability, phytoplankton was categorized into 10 different morpho-functional groups according to the criteria outlined by Weithoff (2003), which considers trophic habits (autotrophs, mixotrophs, and nitrogen fixing ability), mobility (with flagellum/s, lack of mobility, or buoyancy abilities), cell-wall or cell-protection (with mucilage, silica, or cellulose-proteins), and cellular organization as a measure of organisms shape (single-cell or cenobial/colonies algae) (Table 1). All classifications were calculated on biovolume basis, and only those groups that contributed more than 1% to total phytoplankton biovolume were considered in the analyses. The morpho-functional groups were also classified according to the size (<35 and >35 μm) as a complementary approach to access palatability.

For the estimation of zooplankton density, 30 l of water was filtered using a Schindler–Patalas trap with conical conventional plankton net (55 μm). The material collected was fixed in situ with formalin 10% and stained with erythrosine. Rotifera and Copepoda nauplii counts were carried out with an optical microscope in Kolkwitz type chamber (1 ml). Cladocera and

Table 1 Morpho-functional groups identified according to Weithoff (2003) and their characteristics

Metabolism	Motility	Cell protection	Cell organization	Code	Some examples for this lake
				a1Ab (Mucig Col.)	*Dictyosphaerum, Oocystis, Nephrocytium.*
				a1Ca (Single cell)	*Monoraphidium, Schoederia.*
	No mobile (1)	Mucilaginous (A)	Single cell (a)	**a1Cb (No mucig col.)**	*Ankistrodesmus, Actinastrum.*
(a) Autotrophic	Flagellum/s (2)	Silica (B)	Cenobial/ Colonies (b)	**a1Ba (Si cell wall)**	*Navicula, Nitzschia.*
	Aerotops (3)	Other (cellulose, proteins) (C)	Filaments (c)	**a3Ab (Cyan col.)**	*Microcystis*, *Aphanocapsa*, *Coelomoron.*
				a2Ca (Single flag.)	*Chlamydomonas*, *Chlorogonium*, *Phacotus.*
				a2Cb (Multi flag. col)	*Pandorina, Gonium.*
				a1Cc (Filaments)	*Oscillatoria, Phormidium.*
	No mobile (1)	Mucilaginous (A)	Single cell (a)	**m2Ca (Mix flag.)**	*Euglena*, *Lepocinclis*, *Cryptomonas*, *Peridinium.*
(m) Mixotrophic	Flagellum (2)	Silica (B)	Cenobial/ Colonies (b)	m2Bb (Mix flag. col.)	*Synura*
	Aerotops (3)	Other (cellulose, proteins) (C)	Filaments (c)		
	No mobile (1)	Mucilaginous (A)	Single cell (a)	n3Cb (N fix.)	*Dolichospermum*
(n) Nitrogen fixation	Flagellum (2)	Silica (B)	Cenobial/ Colonies (b)		
	Aerotops (3)	Other (cellulose, proteins) (C)	Filaments (c)		

Groups that represented more than 1% of the total phytoplankton biovolume in the lake are indicated in bold

Copepoda counts were carried out in a Bogorov chamber (5 ml). A minimum of 100 individuals were counted in each sample. Fish where classified according to their trophic guild using specific bibliography and personal observations. Fish biomass data were considered for the different analyses performed.

The relationship among the three biological matrixes considered phytoplankton morpho-functional groups, zooplankton groups, and fish trophic guilds was analyzed throughout the fish sampling period ($n = 4$ periods $\times$ 2 sites, for each variable) with a Spearman correlation test ($\alpha = 0.05$).

Stomach fish content analysis

We dissected eighty fish (20 per sampling date, including February, May, August, and November) of the dominant planktivorous fishes from the lake (those with >80% of total planktivorous abundance). Their stomach contents were individually analyzed. For this study, we only considered phytoplankton and zooplankton items of the diet, and excluded Diptera larvae, phytoperiphyton, or detritus parts (these were only considered qualitatively: presence or absence). Zooplankton was analyzed using an optical NIKON microscope at 200×, and biovolume was estimated by approximation to regular geometric shapes (Dumont et al., 1975; Ruttner-Kolisko, 1977). Phytoplankton was examined at 400×, and biovolume estimation was done following the same method explained above. Feeding selectivity was calculated using the formula proposed by Ivlev (1961): $E_i = (r_i - p_i)/(r_i + p_i)$ (where $E_i =$ feeding selectivity index; $r_i =$ relative biovolume of the food item i in the diet, and $p_i =$ relative biovolume

of item i in the environment. This selectivity index ranges from −1 to +1. When $E_i = 0$ selective feeding does not occur, when $E_i < 0$ food item i occurs less in the diet than would be expected by random feeding, indicating negative selection (avoidance or inaccessibility). When $E_i > 0$, food item i occurs more frequently in the diet than would be expected by chance, indicating positive selection (preference). We considered four selectivity categories (in absolute values) as follows: from 0 to 0.25 (absence of selectivity), from 0.26 to 0.50 (low selectivity), from 0.51 to 0.70 (moderate selectivity), and from 0.71 to 1 (high selectivity). The mean E_i obtained for each food item considered was compared to zero (null hypothesis), using a two-tailed, Student's one sample t test.

Microcosm zooplankton predation experiment

We performed a laboratory scale microcosm experiment to evaluate the effects of predation by different zooplankton fractions on phytoplankton composition. The experiment lasted 5 days and was run at 21°C, under constant photoperiod of 16:8 light–darkness hours. We took phytoplankton samples every 24 h (sample times: 0, 24, 48, 72, 96 h). Zooplankton density was estimated at the beginning (0 h) and at the end of the experiment (96 h).

Four treatments were developed in sixteen transparent cylindrical glass containers (13 cm in diameter by 22 cm in height) with a capacity of 3 l. Each treatment was repeated in 4 replicates ($n = 16$). The water used as culture medium in all vessels (18 l in total) was collected from El Mirador Lake, was filtered twice with 10 μm mesh before using, and had mean nutrient concentrations of 0.22 and 0.20 mg l^{-1} of soluble reactive phosphorus and nitrite–nitrate, respectively, at the beginning of the experiment. For this reason, nutrients were not considered as limiting factors for phytoplankton development (Reynolds, 2006) and were not added during the experiment. Immediately after filling the vessels with the culture medium, 100 l of water from El Mirador Lake was filtered through 55 μm mesh to remove large zooplankton. Next, the volume was filtered again through a 10 μm mesh to concentrate phytoplankton, which was then homogeneously distributed through their respective replicates (6 l of concentrated phytoplankton from lake was added to each vessel).

Finally, the different fractions of zooplankton were added to each treatment. The first treatment had only phytoplankton (phytoplankton treatment) and was used as a control to corroborate reproductive events in absence of predation. The second treatment contained phytoplankton plus the natural lake zooplankton composition (mainly Rotifera and Copepoda nauplii) (microzooplankton treatment). The third treatment contained phytoplankton plus zooplankton within a size range from 200 to 700 μm in size (mesozooplankton treatment). They were collected from a small pond located 200 meters from the lake in which insect larvae were the predominant potential predators of large zooplankton, mainly Notonectidae and Pleidae (both of the Order Hemiptera). These larvae insects were present in the pond, but were not used in the experiment. The fourth treatment used was composed by phytoplankton + zooplankton larger than 700 μm in size (macrozooplankton treatment); this zooplankton fraction was obtained from a small pond very close to the lake (<20 m), but without fish or other potential predators (Fig. 1a).

For the zooplankton assemblages used in microzooplankton, mesozooplankton, and macrozooplankton treatments, we filtered 60 l of water through a 55 μm mesh each (180 l in total). The concentrate was then distributed homogeneously throughout their respective replicates (concentrate from 12 l per replicate, per treatment). All treatments were gently aerated for 15 min every hour to ensure that the oxygenation of the water column was appropriate for zooplankton, and also to avoid the sedimentation effect on individual algae.

We measured water temperature (°C), conductivity (μS cm^{-1}), pH, and dissolved oxygen (mg l^{-1}) using HANNA portable meters. Phytoplankton samples were taken in 70 ml bottles (sub-superficial samples) and fixed with 1% acidified lugol solution. Biovolume estimation was performed following the methods and criteria explained in the previous section. Zooplankton density estimates were made at the beginning (using extra concentrated zooplankton that was only used for density estimation) and at the end of the experiment; the entire volume in the vessels were filtered through 55 μm mesh. Samples were stained with erythrosine and fixed with 10% formalin solution. The quali–quantitative analyses were made following the methods explained in the previous section.

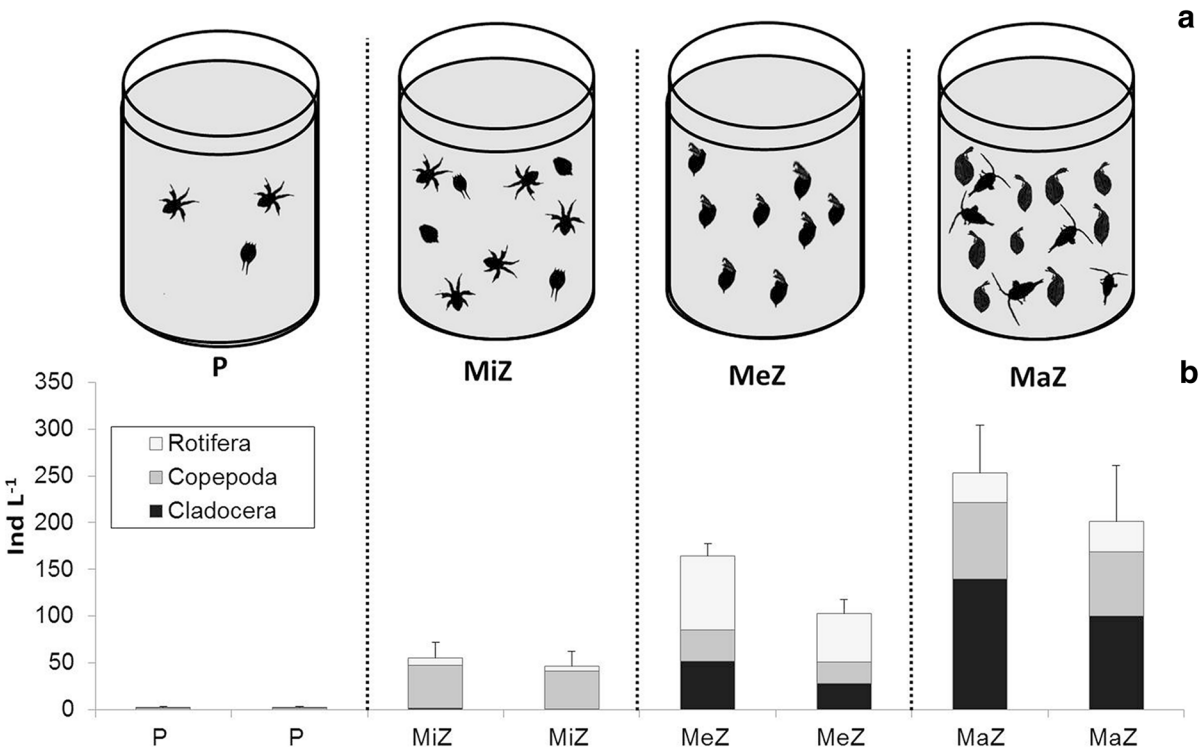

Fig. 1 Schematic illustration of the microcosms experiment used to test zooplankton predation on phytoplankton (**a**). The zooplankton composition and density used in each treatment at the beginning and at the end of the experiment are indicated for every treatment: phytoplankton (P), microzooplankton (MiZ), mesozooplankton (MeZ), and macrozooplankton (MaZ) (**b**)

Composition and relative biovolume of phytoplankton morpho-functional groups among treatments (phytoplankton, microzooplankton, mesozooplankton, macrozooplankton) at the onset of the experiment were compared with a similarity percentage analysis (SIMPER) using the Bray–Curtis distance. This analysis was accompanied by a non-parametric multivariate analysis (NPMANOVA) to verify the statistical significance of SIMPER. Statistical differences among treatments and sampling dates were tested using two-way repeated measures (RM) ANOVA for each component of the different morpho-functional groups. When ANOVA results were significant, Tukey's test was performed to analyse the effect of the treatment.

Zooplankton composition used in the different treatments at the beginning of the experiment

Species richness in the microzooplankton treatment was dominated by Rotifera with 12 species mainly represented by the genera *Lecane*, *Brachionus,* and *Lepadella*, followed by Cladocera (3 species), mainly *Symocephalus* sp. and copepod nauplii. The total mean zooplankton concentration in each replica was 55 ± 17 ind l^{-1}, and the assemblage was dominated by copepod nauplii (83% of total density) followed by Rotifera (13% of total density). In the mesozooplankton treatment, the assemblage was composed by 15 Rotifera species, three Copepoda species, and three Cladocera. Total zooplankton abundance in each replica was 164 ± 13 ind l^{-1}. The assemblage was co-dominated by Rotifera, which accounted for 48% of the total density (mainly *Lecane bulla* Gosse and *L. curvicornis* Murray), followed by Cladocera (32% of total density), principally *Moina reticulata* (Daday). Finally, the macrozooplankton treatment was composed of 11 Rotifera species, 1 Copepoda species, and 4 Cladocera. The total density of zooplankton in each replica was 253 ± 41 ind l^{-1}. The assemblage was dominated by Cladocera (63% of total density), primarily *Daphnia obtusa* (Kurz), followed by Copepoda (27% of total density), represented by the calanoid copepod *Argyrodiaptomus* sp. (Fig. 1b). The phytoplankton treatment had a mean total value

of 2 ± 1 ind l^{-1} (mainly Rotifera). The zooplankton densities used in each treatment (microzooplankton, mesozooplankton, macrozooplankton) correspond to natural values registered in similar isolated lakes associated with the Paraná River system (between 20 and 573 ind l^{-1} with maximum values registered of 1,200 ind l^{-1} (Bonetto & Martinez de Ferrato, 1966; Gagneten et al., 2000), making the densities used in this experiment comparable to the zooplankton densities registered in natural isolated lakes that lack planktivorous fish.

Results

Plankton characterization in the lake

A total of 150 phytoplankton species were recorded in the lake, being the assemblage dominated by mixotrophic flagellates taxa, which accounted for 15–94% of the total biovolume throughout the year. The biovolume of this group peaked in January and was dominated by algae >35 μm in MLD (98% of biovolume) (Fig. 2a). The second most abundant group was colonial cyanobacteria, which represented 2–77% of the total biovolume, and peaked in December, January, July, and August. This group was dominated by colonies <35 μm in MLD (100% of biovolume). The group of non-mucilaginous colonies reached little biovolume throughout the year, accounting for 0.17–13.53% of the total biovolume and being characterized by organisms <35 μm (100% of biovolume). Silica cell-wall group was found between July and November, representing between 0.25 and 28.31% of the total biovolume during this period, and was dominated by algae <35 μm in MLD (70% of year sampling). Single-cell flagellates (<35 μm), single-cell algae, and mucilaginous colonies (both >35 μm in MLD, 90% of individuals) were poorly represented in terms of biovolume throughout the study period (1–8% of total biovolume). Single-cell flagellates and single-cell algae had small peaks in biovolume between August and October, while mucilaginous colonies had similar biovolume values throughout the year with a peak in December (Fig. 2).

With respect to zooplankton, a total of 46 species were identified, and the assemblage was dominated by Rotifera (31 species), followed by Cladocera (10 species) and Copepoda (5 species). In terms of density, Rotifera was dominated by the genera *Brachionus* and *Lecane*, Cladocera by *Diaphanosoma* and *Chydorus*, and Copepoda by *Eucyclops* and *Mesocyclops*. Rotifera represented more than 80% of total density throughout the year, with peaks in March and October. Copepoda represented only between 0.05 and 10% of total density with peaks in January, March, and October. Cladocera was poorly represented throughout the year, with values lower than 4.5%, though there was a small peak in November (Fig. 3a).

The fish assemblage comprised 19 species, 8 of which were omnivorous, 5 detritivorous, 2 insectivorous, 2 planktivorous, and 2 piscivorous. More than 95% of the total fish density was dominated by the planktivorous species *Cheirodon interruptus* Jenyns. It represented 38–82% of the total biomass throughout the study period and had a mean standard length of 28.86 ± 3.69 mm. The second most abundant fish species, in terms of number and biomass, was *Prochilodus lineatus* Valenciennes, a detritivorous species (Fig. 3b).

The Spearman correlation test among the three biological matrixes showed lack of statistical correlation for zooplankton groups fish biomass ($P > 0.05$, for all groups), zooplankton versus phytoplankton morpho-functional groups ($P > 0.05$), or fish versus phytoplankton ($P > 0.05$), except for silica cell-wall algae (<35 μm) versus fish (Rho = 0.78 $P = 0.03$), when they were compared among the four fish sampling periods ($n = 4$ periods × 2 sites, for each variable).

Fish stomach content analysis

Analyzing the fish stomach content, we found 72 phytoplankton species. These mainly corresponded to the morpho-functional groups of non-mucilaginous colonies, mixotrophic flagellates, and silica cell-wall algae (accounting for more than 75% of total phytoplankton biovolume). Nevertheless, phytoplankton only represented 4% of total plankton biovolume, while zooplankton accounted for 96%. The latter was mainly represented by Cladocera—genera *Diaphanosoma* and *Moina* (between 18 and 82% of total zooplankton biovolume)—followed by Rotifera (between 10 and 52%)—genus *Brachionus*, *Lecane*, and a Bdelloidea (undetermined) organism—and Copepoda with Calanoids copepodites (between 13 and 30%). Other items found in the stomach, but not

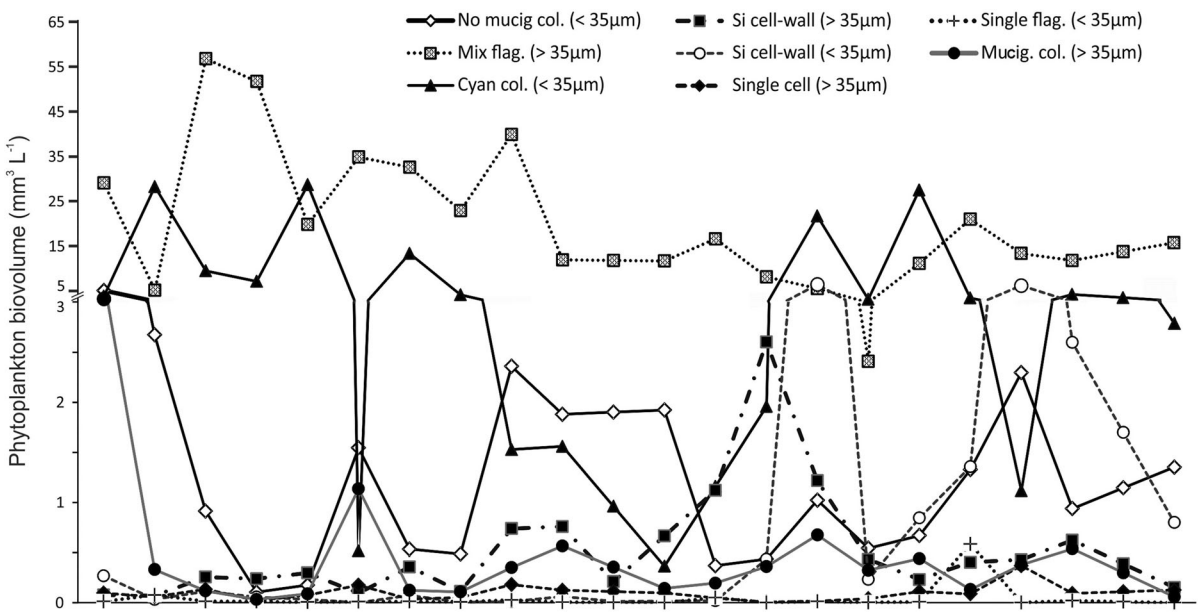

Fig. 2 Biovolume of the different phytoplankton morpho-functional groups registered in the lake throughout the year. Key: cyanobacteria colonies (cyan col.), mixotrophic flagellates (mix flag.), non-mucilaginous colonies (no mucig col.), single-cell algae (single-cell), single-cell flagellates (single flag.), mucilaginous colonies (mucig col.), silica cell-wall algae (Si cell-wall)

quantified were Diptera larvae insects (mainly during September and November), phytoperiphyton organisms (throughout the year), Ostracoda (only during November), and some vegetable fragments (throughout the year).

The Ivlev's index reveled that *C. interruptus* has a variable use of plankton items. Rotifera was negatively selected during the whole year with values between absence of selectivity ($E_i = -0.06$ during August) and moderate negative selectivity ($E_i = -0.61$); these values were statistically significant in all months ($P < 0.05$). Copepoda presented absence of selectivity during February ($P = 0.98$), low negative selectivity during November ($P < 0.05$), and low positive selectivity during May and August ($E_i = 0.34$ for both, but just statistically significant for May). Cladocera was highly positively preferred during February and August ($E_i > 0.71$, both $P < 0.05$) but showed absence of selectivity during May and November ($E_i < 0.25$, both $P < 0.05$) (Fig. 4). Regarding phytoplankton items, the Ivlev's index demonstrated that most groups were negatively preferred by *C. interruptus*, especially mixotrophic flagellates, mucilaginous colonies, single-cell flagellates, and colonial cyanobacteria [$E_i > (-0.71)$, $P < 0.05$ for almost all of them]. Non-mucilaginous colonies, however, were highly preferred during August while single-cell algae group during August and November ($E_i > 0.71$, $P < 0.05$ for both groups) (Fig. 4).

Impact of zooplankton feeding in the microcosms experiment

Environmental variables maintained similar values throughout the experiment: water temperature 21.28 ± 2.33°C, dissolved oxygen 8.42 ± 2.25 mg l^{-1}, pH 7.52 ± 0.31, and conductivity 1,220 ± 32.54 µS cm^{-1}. At the onset of the experiment, the SIMPER analysis revealed similar compositions (24.4% of dissimilarity, NPMANOVA, F = 1.57 $P = 0.13$) among treatments (phytoplankton, microzooplankton, mesozooplankton, macrozooplankton), when the biovolume of the different phytoplankton morpho-functional groups were considered.

Despite that a drop in phytoplankton biovolume was observed in all treatments throughout the experiment, the phytoplankton treatment showed higher values than the other treatments. This allowed us to infer the real effect of predation by zooplankton on phytoplankton morpho-functional groups (Fig. 5a). Moreover, in the phytoplankton treatment, several groups increased in biovolume (non-mucilaginous colonies, silica cell-wall

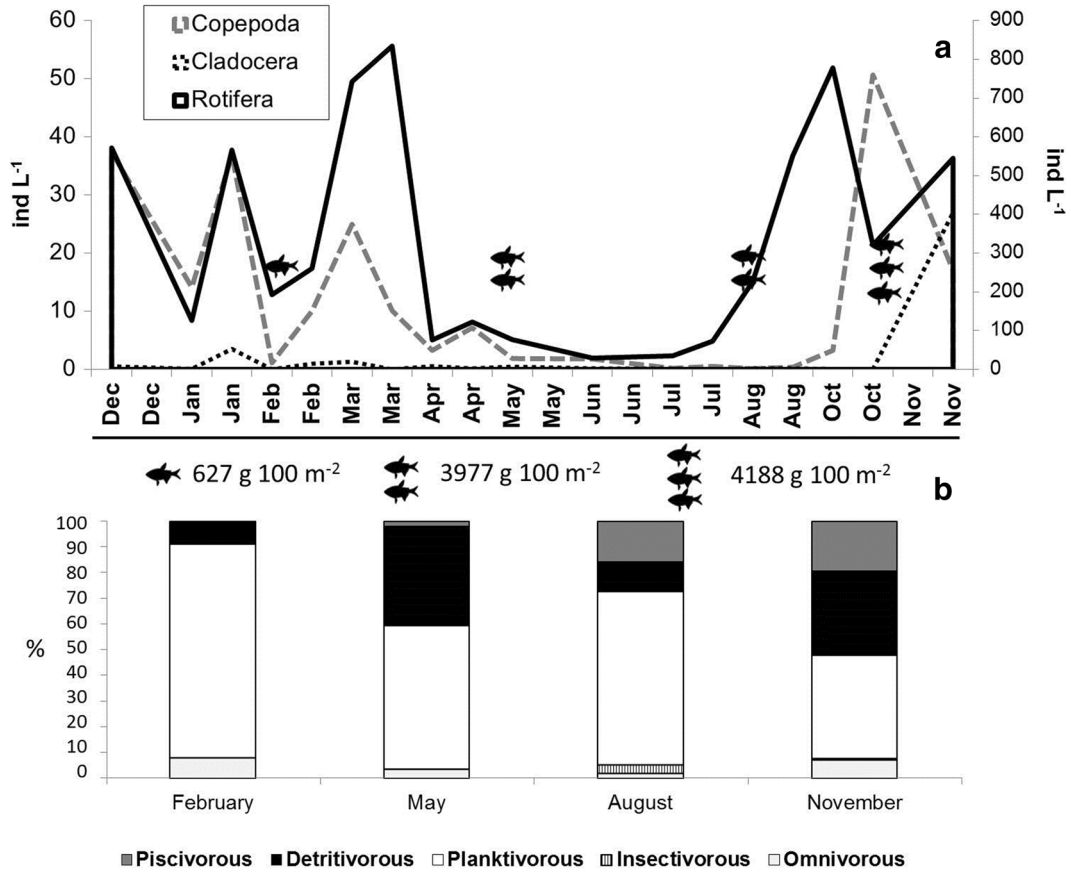

Fig. 3 Zooplankton structure throughout the year. Rotifera density is expressed on the right axis due to its high density relative to the other groups (**a**). Percentage contribution to the total biomass of each fish trophic guild found during the study (**b**)

algae, single-cell flagellates, and mixotrophic flagellates). This was particularly marked at 24 h for: mixotrophic flagellates (4% of increment), 128% for single-cell flagellates, 195% for silica cell-wall algae, and at 72 h with respect to 48 h for non-mucilaginous colonies (209%) (Fig. 5c, d, f, h).

In the microzooplankton treatment, drops in biovolume with respect to the phytoplankton treatment were observed at 24 h, especially in the groups: colonial cyanobacteria (>98% decrease), mixotrophic flagellates (47% decrease), and non-mucilaginous colonies (76% decrease). Silica cell-wall algae biovolume, however, increased (106%) after 24 h. Biovolume in the other morpho-functional groups (single-cell algae; single-cell flagellates, and mucilaginous colonies) was similar to those in the control (Fig. 5e–g). The (RM) ANOVA showed lack of statistical significance for any combination of phytoplankton versus microzooplankton treatment (Table 2).

In the mesozooplankton and macrozooplankton treatments, almost all of the morpho-functional phytoplankton groups presented low and constant values of biovolume (<0.1 $mm^3\ l^{-1}$) throughout the experiment. The exceptions were the silica cell-wall algae group and single-cell flagellates group. Silica cell-wall algae presented an irregular behavior throughout the study period in the different treatments used, but drops were evident at 24 and 48 h in mesozooplankton and macrozooplankton treatments (Fig. 5h). This group showed differences in treatments (for both <35 and >35 μm), time (only for Si cell-wall <35 μm), but not for their interaction (Table 2). Single-cell flagellates biovolume increased until 24 and 72 h in macrozooplankton treatment and underwent a marked decrease in all treatments at 48 h (Fig. 5f). The (RM) ANOVA showed lack of statistical significant differences for this group when phytoplankton treatment and the rest of the treatments were compared (Table 2).

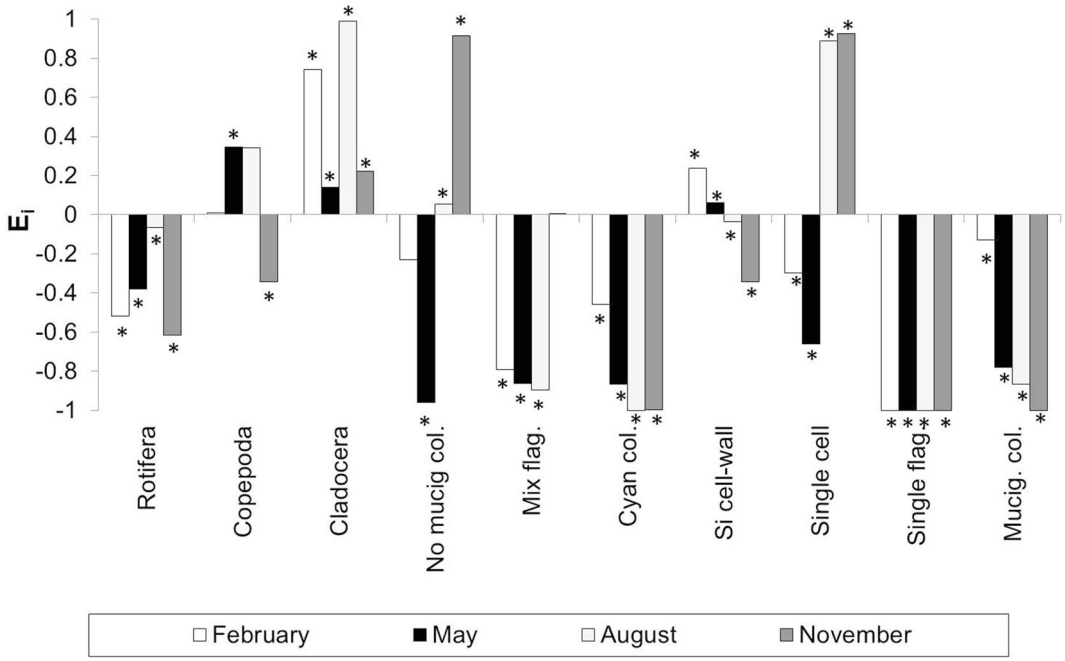

Fig. 4 Mean Ivlev's selectivity index (E_i) obtained for each zooplankton (Rotifera, Copepoda, and Cladocera) and phytoplankton groups considered (abbreviations for phytoplankton are the same than Fig. 2). Statistically significant values are indicated with (*asterisk*)

The biovolume of colonial cyanobacteria, mixotrophic flagellates, and non-mucilaginous colonies decreased until 24 h (>85%) in presence of meso and macrozooplankton. These values were maintained throughout the rest of the experiment (Fig. 5b–d). The single-cell group strongly decreased in macrozooplankton treatment (98% with respect to the onset of the experiment). This drop was also evident in the mesozooplankton treatment until 72 h. Mucilaginous colonies biovolume significantly dropped in the mesozooplankton and macrozooplankton treatments compared to the phytoplankton and microzooplankton treatments, where biovolume was rather similar throughout the experiment (Table 2). Differences with respect to phytoplankton and microzooplankton treatments were statistically significant for all of them through time and by the combination of both factors (time × treatment) (Table 2).

Discussion

In our intent of contribution to the discussion about why predation is a minor controlling factor of phytoplankton in shallow Neotropical lakes, we found in this lake that phytoplankton biovolume is mainly influenced by fish predation on zooplankton and morpho-physiological characteristics of algae which prevent zooplankton predation.

The first hypothesis: phytoplankton morpho-functional characteristics have an effect on zooplankton predation ability

In the microcosms experiment, we were able to demonstrate that phytoplankton MLD, biovolume and cell-wall characteristics have a role in the ability of phytoplankton to hinder zooplankton predation. Single cell, mucilaginous colonies, no-mucilaginous colonies, and silica cell-wall algae (>35 μm) were only effectively consumed by large zooplankton (macrozooplankton treatment), while single-cell flagellates remained unaffected by zooplankton (micro, meso, or macrozooplankton) feeding in all treatments.

The single-cell group was dominated by *Monoraphidium* spp. (principally *M. arcuatum* Korshicov and *M. griffithii* Berkeley). Both species are described as solitary cells, narrow fusiform, straight or slightly bent, and gradually tapering into the pointed apices between 45 and 75 μm of MLD. It is likely that the

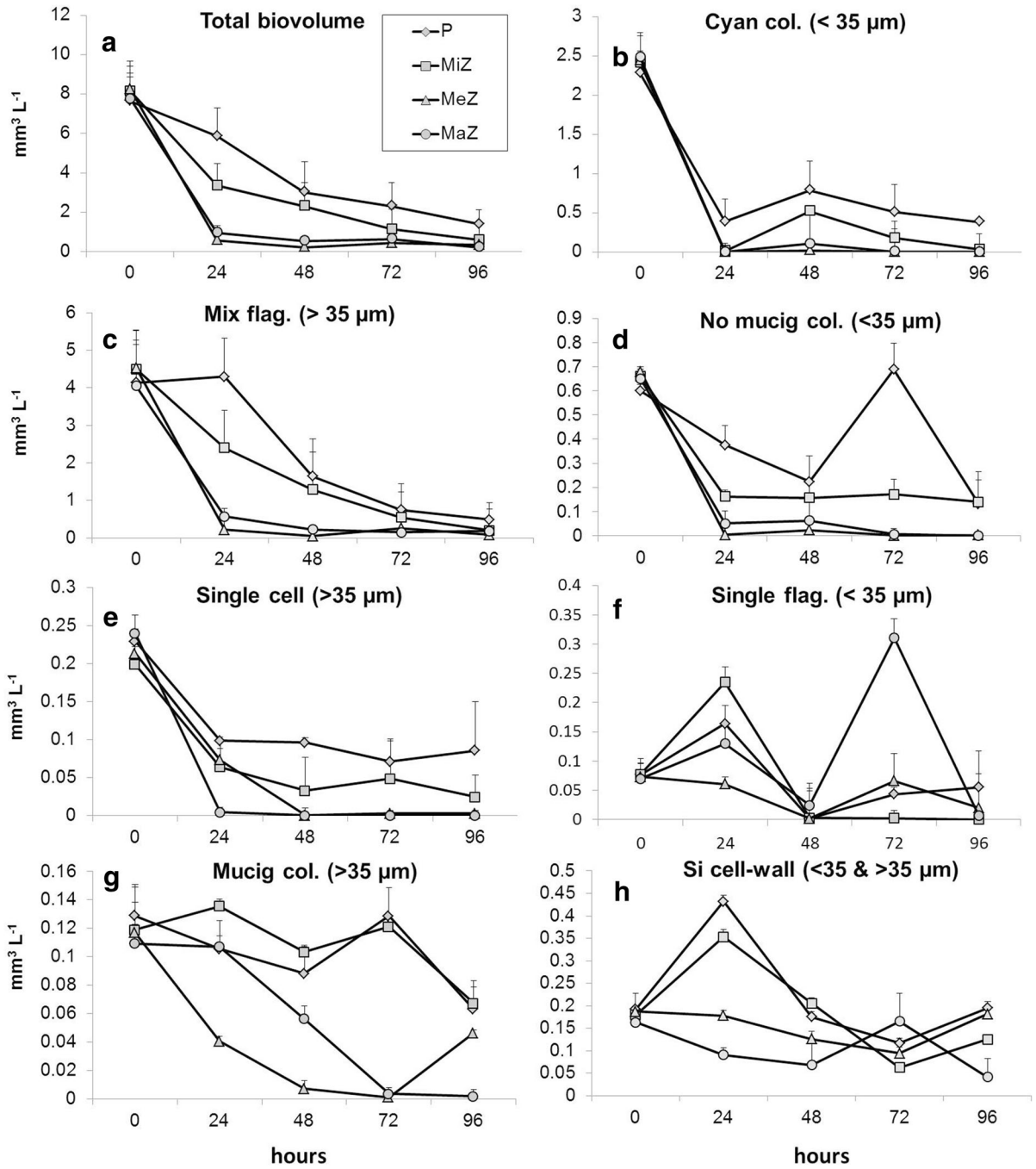

Fig. 5 Mean values and standard deviation (*vertical bars*) of total biovolume (**a**) and of each morpho-functional phytoplankton group (abbreviations are the same than Fig. 2) (**b**–**h**) in the microcosms experiment by time and per treatment: P (phytoplankton), MiZ (microzooplankton), MeZ (mesozooplankton), and MaZ (macrozooplankton)

shape, rather than the size, determined that only the largest feeders, like *Argyrodiaptomus* sp. and *D. obtusa*, can feed on single-cell algae (>35 µm). Mucilaginous colonies were dominated by the genera *Dictyosphaerium* and *Oocystis.* Both algae groups could be voluminous organisms which can represent a problem for small or less effective filter-feeders like *Moina reticulata* in this study. Particles cleared from

Table 2 Repeated measure ANOVA results for the biovolume of the different phytoplankton morpho-functional groups: cyanobacteria colonies (Cyan col.), non-mucilaginous colonies (no mucig col.), mixotrophic flagellates (mix flag.), silica cell-wall algae (Si cell-wall), single-cell algae (single-cell), single-cell flagellates (single flag.), and mucilaginous colonies (mucig col.) for every treatment: phytoplankton (P), microzooplankton (MiZ), mesozooplankton (MeZ), and macrozooplankton (MaZ)

	Cyan col. (<35 μm)	Single flag. (<35 μm)	Si cell-wall (<35 μm)	Si cell-wall (>35 μm)
Time	F = 4.46 P = **0.04**	F = 19.12 ***P*** = **0.003**	F = 4.102 ***P*** = **0.037**	F = 0.629 P = 0.664
Treatment	F = 10.75 P = **0.001**	F = 4.89 ***P*** = **0.019**	F = 3.46 ***P*** = **0.007**	F = 5.89 ***P*** = **0.010**
Time × treatment	F = 3.05 P = **0.03**	F = 2.49 ***P*** = **0.04**	F = 1.29 P = 0.269	F = 3.46 P = 0.052
Tukey				
P vs. MiZ	P = 0.573	P = 0.053	P = 0.432	P = 0.180
P vs. MeZ	***P*** = **0.03**	P = 0.63	***P*** = **0.008**	P = 0.206
P vs. MaZ	***P*** = **0.03**	P = 0.924	***P*** = **0.046**	***P*** = **0.006**
MeZ vs. MaZ	P = 0.364	***P*** = **0.018**	P = 0.738	P = 0.205
	Mix flag. (>35 μm)	No mucig col. (<35 μm)	Single cell (>35 μm)	Mucig col. (>35 μm)
Time	F = 72.39 ***P*** < **0.001**	F = 101.06 ***P*** < **0.001**	F = 40.86 ***P*** < **0.001**	F = 21.77 ***P*** < **0.001**
Treatment	F = 26.09 ***P*** < **0.001**	F = 36.03 ***P*** < **0.001**	F = 9.32 ***P*** = **0.002**	F = 30.76 ***P*** < **0.001**
Time × treatment	F = 5.66 ***P*** < **0.001**	F = 128.65 ***P*** < **0.001**	F = 2.07 ***P*** = **0.001**	F = 3.93 ***P*** = **0.00002**
Tukey				
P vs. MiZ	P = 0.405	P = 0.425	P = 0.993	P = 0.138
P vs. MeZ	***P*** < **0.001**	***P*** < **0.001**	P = 0.612	P = 0.468
P vs. MaZ	***P*** = **0.01**	***P*** < **0.001**	***P*** = **0.004**	***P*** = **0.00029**
MeZ vs. MaZ	P = 0.139	***P*** < **0.001**	***P*** = **0.034**	***P*** < **0.001**

In the case of statistical significance, Tukey's post hoc analyses were performed. Bold format indicates statistical significance at $P < 0.05$

water in Cladocera depend on the setae morphology and size on the moving appendages (Lehman, 1988), so Cladocera size seems to be an important factor to be considered when phytoplankton predation is analyzed.

Non-mucilaginous colonies were another group effectively removed only by macrozooplankton. This group was mostly represented by cenobial green algae such as *Coelastrum* spp. and *Ankistrodesmus* spp. Both algae are voluminous, non-motile, and without evident mucilaginous envelop, so size seems to be the most important factor. Calanoid copepods such as *Argyrodiaptomus* sp. (one of the dominant species in macrozooplankton treatment) are known to be capable of select live preys, distinguishing chemical properties, and showing preference for phytoplankton. In Cladocera, the only mechanism of food selection is size, ranging around 1 μm upper 30 μm for *Daphnia* (Sommer & Stibor, 2002; Tackx et al., 2003). As we said, copepod food selection does not only depend on food particle size, but minimal sizes for food particles are clearly larger than for cladocerans (Sommer & Stibor, 2002), so it is expected that in this experiment, *Argyrodiaptomus* (macrozooplankton treatment) was a more effective predators of phytoplankton.

Silica cell-wall (>35 μm) organisms were another group which only showed statistical difference in the macrozooplankton treatment and this is consistent with previous studies (Sommer et al. 2001, 2003). It is assumed that these algae cross the gut and remains photo-synthetically active, a state that is favored by their silica frustule, which prevents degradation by digestive enzymes (Porter, 1975). The resistance of this group to be digested has been observed also for other crustaceans (Devercelli & Williner, 2006) and could explain those changes observed over the course of the experiment (see Fig. 5h).

There was lack of evidence that single-cell flagellates biovolume was affected during the experiment by any zooplankton fraction (micro, meso or macrozooplankton). Single-cell flagellates were represented mainly by Volvocales, such as *Chlamydomonas*, *Chlorogonium*, and *Phacotus*. These genera are characterized by their high reproductive rate and small size in comparison with other algae— characteristics

which allow them to use nutrients more effectively than other groups (Litchman et al., 2010) and reproduce faster, explaining the pattern observed for this group during the experiment. The same pattern has been observed in other experiments of filter-feeding phytoplankton predators (Frau et al., 2013, 2016), reenforcing our hypothesis about the behavior of this group.

Finally, we found absence of statistically significance ($P > 0.05$) when microzooplankton (Rotifera and Copepoda nauplii) predation effect was tested. This is in contrast, with those studies made in temperate lakes by Cyr (1998) and Levine et al. (1999) who concluded that microzooplankton can exert an important predation effect on phytoplankton—comparable with the effect induced by macrozooplankton—when they are in similar densities. In this lake, Copepoda nauplii are never dominant and microphagous Rotifera (*Lecane* and *Brachionus* in this lake), which feed on detritus and Bacteria (Obertegger et al., 2011), do not exert a direct predation effect on phytoplankton. This pattern of microphagous Rotifera dominance has been found in other water systems of the Neotropical region (e.g., José de Paggi & Paggi, 2008) so a microzooplankton effect on phytoplankton should not be expected in these kind of systems.

The second hypothesis: zooplankton of Neotropical lakes are less effective grazer than zooplankton of temperate lakes

Results indicate that those more suitable groups of phytoplankton to be predated by meso and macrozooplankton are those of small size (<35 μm) such as cyanobacteria colonies and small silica cell-wall algae, or groups with cell-wall protection which would be flexible enough to be handled by predators in spite of their relatively large size (mixotrophic flagellates). With respect to Cyanobacteria colonies, recent studies suggest that small cyanobacteria—such as those found in this microcosm experiment—without toxic strains, can be effectively removed from the water column by phytoplanktophagous zooplankton (Kozlowsky-Suzuki et al., 2003; Panosso et al., 2003) supporting our findings.

Changes observed in silica cell-wall algae (<35 μm) biovolume in presence of meso and macrozooplankton are consistent with previous studies (González, 1998, 2000; Sommer et al., 2001, 2003) which have demonstrated that diatoms are positively ingested by zooplankton. Nonetheless, as we explained above, this group could survive to digestive enzymes which would be determining a more erratic behavior in time.

The mixotrophic flagellates group was largely represented by euglenoids, such as *Euglena* spp., *Lepocinclis* spp., and *Phacus* spp., whose size was >35 μm. Their cell-wall (the pellicle) is composed of proteinaceous strips underneath the cell membrane. This pellicle is supported by dorsal and ventral microtubules that give the possibility of being highly flexible, and may explain why these voluminous algae can be eaten even by mesozooplankton in spite their size (>100 μm of MLD at least in this study). Our results are consistent with those found by Eskinazi-Sant'Anna et al. (2002) who found that Cladocera and Copepoda can effectively feed on euglenoids despite their high motility and size.

The third hypothesis: a high fish predation over zooplankton prevents its predation effect on phytoplankton

Cheirodon interruptus is a fish widely distributed in streams and shallow lakes throughout the Neotropical region (Ferriz et al., 2011), and in the El Mirador Lake, it was the most abundant species (>95% of total density and >40% of total biomass). Several authors have already proved that planktivorous fish of the Neotropical region such as, *Odontesthes bonariensis* Valenciennes and *Jenynsia multidentata* Günther, can exert an important predation effect on zooplankton (Boverí & Quirós, 2002; Iglesias et al., 2008; Sinistro, 2010). Our results with *C. interruptus* also demonstrated a high selectivity on Cladocera and youngsters of Copepoda (considered as two important predators of phytoplankton). Compared to the cladocerans, copepods have more swimming appendages, a more developed sensory system, and better neuromuscular coordination (Caparroy et al., 2000; Dussart & Defaye, 2001). These characteristics allow them the ability to perform rapid evasive movements when they detect deformations in the flow field as hydrodynamic signals generated by predator activities, and would explain the lower positive selectivity index values obtained for this group in the stomach content analyses. In addition, these results also explain the low density obtained for Copepoda in the lake analysis. Considering that *C. interruptus* predated on

youth Copepoda, it is likely to found a low density of Copepoda adults in the lake.

High predation pressure on Cladocera and Copepoda promotes the development of Rotifera, since they can reproduce faster and are smaller. As zooplankton assemblage was mainly dominated by microphagous Rotifera during the lake surveys, measurable effects of zooplankton on phytoplankton could not be expected ($P > 0.05$ in the Spearman correlation) when planktivorous fish are present in high densities.

The fourth hypothesis: planktivorous–omnivorous fish can effectively feed on phytoplankton

Cheirodon interruptus was classified by Ringuelet (1975) as a visual, omnivorous plant–benthivorous filter feeder species, and Fernández et al. (2012) demonstrated that they can feed on insect larvae, epiphytic algae, zooplankton, and phytoplankton. This last author, however, lacked a quantification of the relative contribution of these items on diet as we did in the present study. Our results suggest the absence of statistical significance in the effect of *C. interruptus* on phytoplankton being included several reasons, which are mainly related with phytoplankton features (principally size and evasive strategies) and fish abilities to predate on it.

Mixotrophic flagellates and single-cell flagellates were two groups highly and negatively selected by *C. interruptus.* Both phytoplankton groups encompass single-cell algae with flagellum, which provide them with mobility across the water column under stable water conditions. Previous studies have demonstrated that phyto-flagellates (mixotrophic and single-cell flagellates in this study) can avoid predation by metazoan filter feeders such as fish by attaching to large particles, migrating to anoxic areas, surviving periods of strong predation with high reproduction rate, or even by producing cysts that are stocked in the sediments (Leadbeater & Green, 2003). Furthermore, Jakobsen (2001) demonstrated that some dinoflagellates (included in this study in the mixotrophic flagellates group) can avoid predators by responding to hydromechanical signals that are generated at a distance from the predators. Considering that detailed auto-ecological studies are only available for a few species, many questions still require intensive study, since several defense mechanisms in phyto-flagellates have not been interpreted (Leadbeater & Green, 2003).

Non-mucilaginous colonies (<35 μm) and single-cell algae (>35–150 μm in MLD) were positively preferred by *C. interruptus* (Ivlev's index > 0.7), mostly during August and November. Considering that *C. interruptus* is a visual predator, it is highly probable that these algae could be passively selected (phytoplankton represented 4% of total plankton biovolume in the stomach content) and ingested due to their small size in comparison with the smallest well-represented item found in the stomach content (Rotifera between 150 and 350 μm of MLD, above 50% of stomach content). A similar explanation could be given for mucilaginous colonies and colonial cyanobacteria, since both groups were negatively selected by the fish.

Previous studies made in temperate areas, with *Dorosoma* spp. (gizzard shads), *Oreochromis* spp. (tilapias), and *Hypophthalmichthys molitrix* Valenciennes (silver carp), have shown an effective predation effect on phytoplankton, as equal as, zooplankton (DeVries & Stein, 1992; Beveridge & Baird, 2000; Zhang et al., 2006; Ke et al., 2007). Nonetheless, these species have a greater size (from 1,300 to 140,000 mm in standard length, except *Dorosoma* species (284–399 mm), and they are all filter-feeding species. In comparison, omnivorous species studied in the Neotropical region (e.g., *O. bonariensis*, *J. multidentata* and *C. interruptus* in this study) have a maximum length <500 mm and are visual predators, so a direct predation effect on phytoplankton should not be expected.

Conclusions

In this study we aimed to test four main hypotheses which could explain the absence of top-down effects on phytoplankton in subtropical shallow lakes. We demonstrated that morpho-functional characteristics of phytoplankton has an effect on zooplankton predation and that zooplankton can feed effectively on phytoplankton depending on its size and phytoplankton cell-wall characteristics (first and second hypothesis). We also demonstrated that the intense predation of fish on meso and macrozooplankton (third hypothesis) prevents predation effects of zooplankton on

phytoplankton, without fish exerting a direct predation effect on phytoplankton (fourth hypothesis).

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