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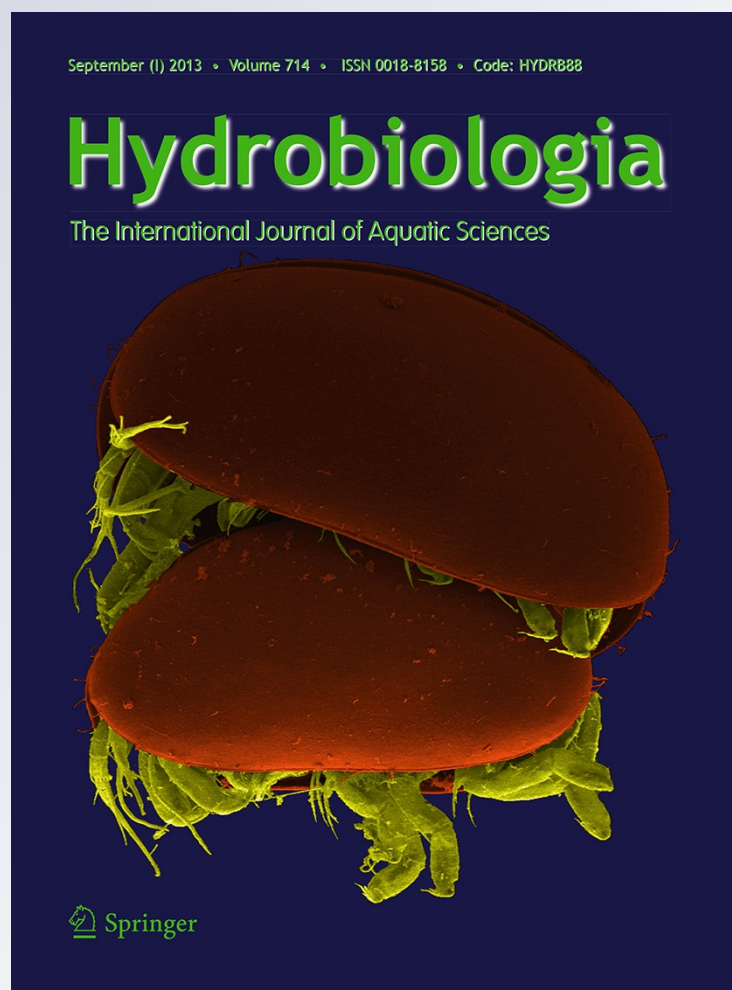
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The microbial food web structure of a hypertrophic warm-temperate shallow lake, as affected by contrasting zooplankton assemblages

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Abstract The composition of zooplankton is known to affect the structure of the microbial trophic web. The zooplankton of the hypertrophic Laguna Chascomús (Argentina) is generally dominated by rotifers and cyclopoids copepods. An unusual dominance by small-cladocerans was observed after a massive winter fish kill in 2007. We hypothesized that small-cladocerans would increase the grazing pressure on heterotrophic flagellates (HF), reducing the degree of coupling between HF and picoplankton. The aim of this study was to investigate the microbial food web structure under two contrasting zooplankton assemblages. The lake was sampled every other week between 2007 and 2009. The abundances of heterotrophic bacteria (HB) and picocyanobacteria (Pcy) laid among the highest values reported for aquatic

systems ($>10^8$ and 10^7 cells ml^{-1} , respectively). Pcy averaged 53% of total picoplanktonic biomass. When small-cladocerans dominated zooplankton HF reached the higher abundance ($>10^5$ cells ml^{-1}) and picoplankton showed the opposite pattern, while the proportion of grazing resistant morphologies (i.e. microaggregates of Pcy) was higher. In contrast, when rotifers dominated, HF abundance decreased and picoplankton increased. Our data suggest that the degree of HF–HB coupling was affected by changes in zooplankton dominance. In contrast to our initial hypothesis, the present results suggest that large numbers of rotifers ($>5,000$ ind. l^{-1}) are more efficient than small-cladocerans at controlling HF populations.

Keywords Picoplankton · Heterotrophic flagellates · Trophic cascade · Trophic coupling

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Introduction

The abundance and composition of aquatic organisms might be modulated either by resource availability (“bottom-up”) or predation (“top-down”). The heterotrophic flagellates (HF) are considered a key group within aquatic microbial food webs, since they are efficient predators of bacteria, and also constitute an important food source for ciliates and crustacean zooplankton. Thus, HF play a pivotal role in aquatic microbial communities by channelling picoplankton

production to higher trophic levels (Ducklow et al., 1986; Sherr & Sherr, 1994).

HF not only prey upon heterotrophic bacteria (HB) (Boenigk & Arndt, 2002), but also upon autotrophic picoplankton (Weisse et al., 1990; Šimek et al., 1997; Sherr & Sherr, 2002). In fact, under certain circumstances, picocyanobacteria (Pcy) may be the preferred prey (Christoffersen, 1994; Pernthaler et al., 1996; Callieri et al., 2002), as well as the main source of carbon (Tarbe et al., 2011). It is known that HF may control the abundance, production and size structure of bacterial communities (Chrzanowski & Šimek, 1990; Pernthaler et al., 1996; Jürgens & Jeppesen, 2000). Moreover, HF predation may induce the development of resistance morphologies, such as filaments and aggregates (Jürgens & Matz, 2002; Blom et al., 2010). Comparatively, the effect of HF on autotrophic picoplankton has received less attention.

HF populations may in turn be affected by predation by larger grazers (Arndt, 1993; Sanders & Wickham, 1993; Arndt et al., 2000). It is well known that virtually all planktonic metazoan groups (hereafter zooplankton) compete with, and prey upon, HF (Sanders et al., 1994; Jürgens et al., 1996), but the intensity of predation varies widely with the type of predator (Arndt, 1993; Jürgens & Jeppesen, 2000; Jürgens & Matz, 2002; Sommer & Sommer, 2006). For example, large filter-feeder cladocerans (i.e. *Daphnia* spp.) use a filter to remove microbial food from suspension (Finlay & Esteban, 1998), thereby can feed over a wide range of particle size. These large filter-feeders are more efficient at controlling HF than selective raptorial-feeders (i.e. copepods), which prefer larger preys (Sommer & Sommer, 2006). As a consequence, changes in zooplankton composition may impact on the HF abundance, and also indirectly affect the abundance and size structure of preys (Jürgens & Matz, 2002), and the degree of HF-preys coupling (Gasol, 1994; Tadonlécé et al., 2004). Even though the contrasting impact of large cladocerans versus copepods on the microbial food web has been well studied (see reviews by Jürgens & Matz, 2002; and Sommer & Sommer, 2006), the effect of small cladocerans and rotifers is comparative less known.

Although the structure of the microbial trophic web has been investigated over a wide range of trophic conditions, there is still a paucity of studies dealing with highly eutrophic environments (but see Sommaruga, 1995; Šimek et al., 1997; Jürgens & Jeppesen,

2000; Wieltchnig et al., 2001; Chen et al., 2010). Laguna Chascomús (Pampa region, Argentina) is a highly productive (Torremorell et al., 2009), hypertrophic, shallow lake. The zooplankton composition of the lake is strongly shaped by fish planktivory and is typically dominated by small rotifers and cyclopoid copepods; while other zooplankton groups are comparatively less abundant (calanoid copepods) or virtually absent (cladocerans) (Diovisalvi et al., 2010, unpublished data).

In many aspects, Laguna Chascomús is a rather predictable environment that, in spite of the marked inter-annual variability in hydrological and weather variables that characterizes the region (Sierra et al., 1994; Rennella & Quirós, 2006), displays recurring seasonal patterns of several important variables like total phosphorus (TP), phytoplankton biomass, suspended particulate matter and water transparency (Torremorell et al., 2007, 2009; Llames et al., 2009; Lagomarsino et al., 2011; Pérez et al., 2011). However, singular (i.e. catastrophic) events may affect the lake sporadically. Quite relevant for the context of this study was a massive fish kill that occurred in winter 2007, after an unusually long period of cold weather. Inspection of plankton samples, which are part of our routine sampling of Laguna Chascomús, revealed a remarkable shift in the zooplankton towards the dominance of small cladocerans (*Moina* spp., *Bosmina* spp.) during spring 2007–summer 2008. However, during the next spring-summer period, zooplankton reverted to the characteristic assemblage of Laguna Chascomús dominated by rotifers.

Here, we report the results of a 2-year study, aimed at investigating the microbial community structure (particularly, the relationship between HF and HB) in an highly eutrophic lake, under two contrasting zooplankton communities (small cladocerans vs. rotifers). We hypothesized that the increase in the abundance of cladocerans would increase the grazing pressure on HF, thus reducing the degree of coupling between HF and picoplankton.

Materials and methods

Study area

Laguna Chascomús (35°36'S 58°W) is located in north-eastern Buenos Aires Province within the

Pampa region of Argentina. The region has a warm-temperate climate, with a mean annual temperature and precipitation of about 15.3°C and 935 mm, respectively (Iriondo & Drago, 2004), but with a recurrent alternation of wet and dry periods, resulting in large inter-annual variability (Sierra et al., 1994).

Laguna Chascomús is a large (area 30.1 km²), shallow (mean depth 1.9 m) and highly turbid lake (mean Secchi disk 0.11 m) belonging to the River Salado watershed (Dangavs, 1976). Nutrient concentrations are very high and typical of hypertrophic systems, mean total phosphorous concentration fluctuate around 610 µg l⁻¹ (Lagomarsino et al., 2011) and total nitrogen around 1,000 µg l⁻¹ (Torremorell et al., 2009). The mixing regime is polymictic due to the persistence and strength of winds. Primary productivity and the concentration of suspended particulate matter are directly related to the incident solar radiation (Torremorell et al., 2007, 2009).

Sampling

The lake was sampled every other week from May 2007 to May 2009. Measurements of temperature, pH (Orion pH-meter), conductivity (Cond) (Hach conductimeter), nephelometric turbidity (Hach), dissolved oxygen concentration (DO) (YSI 5000 Meter) and Secchi disk readings were made in situ. Water depth was measured at a gaging station. Subsurface water samples were collected at a central point of the lake with 10 l polypropylene containers and transported to the laboratory for analyses of chemical parameters and biological communities. It is worth mentioning that due to the shallowness and the large area of the lake, vertical as well as horizontal homogeneity of the water column is commonly observed for most parameters (Torremorell et al., 2007).

Chemical analyses

Lake water was filtered through Whatman GF/F filters. Nitrates (N-NO₃⁻) and nitrites (N-NO₂⁻) were measured by Cd reduction followed by diazotization and ammonia (N-NH₄⁺) by the indophenol blue method (APHA, 1998). TP from unfiltered water samples was converted to soluble reactive phosphorus (SRP) after an acid digestion with potassium persulfate and measured by ascorbic acid method. Chlorophyll

a concentration (Chl-*a*) was assessed after extraction with methanol (Lopretto & Tell, 1995). Total suspended solid (TSS, also referred to as seston) concentration was determined by weighing the dried residue (60°C) resulting from the filtration of a water sample through prerinsed and precombusted (530°C, 2 h) GF/F filters. Non-volatile particulate matter (also referred to as ash content) was estimated by reweighing the GF/F filters after combustion at 530°C for 3 h (APHA, 1998). The ash-free dry weight (AFDW) was estimated as the difference between TSS and ash. Dissolved organic carbon (DOC) was measured on filtered (GF/F 0.7 µm) water samples using the high temperature Pt catalyst oxidation method (Shimadzu TOC-5000) following Sharp (1993). The instrument has a detection limit of 4 ppb of TOC and is routinely calibrated against a sodium phthalate standard. DOC analysis were performed by Don Morris at Lehigh University.

Biological communities

Picoplankton and HF abundance

Within the picoplankton fraction (size range: 0.2–2 µm, Sieburth et al., 1978), we distinguished HB, Pcy and picoeukaryotic (Peuk) algae. Although heterotrophic picoflagellates (<2 µm) overlap the upper end of the picoplankton size range, they were processed slightly differently and their abundances were pooled with larger-sized heterotrophic nanoflagellates. HF < 20 µm are collectively referred to as HF. Only non-pigmented flagellates were included in this group. Picoplankton and HF samples were preserved in 45 ml falcon flask with ice-cold filtered glutaraldehyde 10% (final concentration 1%).

Due to the high density of organisms and high amount of suspended particulate matter, the samples had to be diluted with distillate water prior to counting. First, 1 ml of lake water sample was brought to 30 ml (1:30 dilution). For picoplankton enumeration (HB, Pcy and Peuk), 1.2 ml of the 1:30 dilution (i.e. 40 µl of the original lake water sample) were brought to 5 ml with distillate water, stained with 50 µl of DAPI (0.5 mg ml⁻¹) for 10 min (Porter & Feig, 1980), and filtered through a 0.22-µm pore-size black polycarbonate filter (MSI). For HF counting, 3 ml of the same 1:30 dilution (i.e. 100 µl of the original lake water sample) were filtered through a 0.8-µm pore-size black

polycarbonate filter (MSI). All filters were mounted onto a microscope slide with a drop of immersion oil for fluorescence (Immersol 518 F) and stored at -20°C .

Samples were inspected at $1,000\times$ magnification using Nikon Eclipse 600 microscope equipped with HBO 50 W lamp, and a filter set for blue light excitation (BP 450–490 nm, FT 500 nm, LP 515 nm), green light excitation (BP 510–560 nm, FT 565 nm, LP 590 nm) and UV excitation (BP 340–380 nm, FT 400 nm, BP 435–485). Single-cell HB were counted under UV light excitation. Pcy and Peuk were clearly recognizable under blue and green light excitation, due to of their characteristic photosynthetic pigments fluorescence (Callieri & Pinolini, 1995). Pcy micro-aggregates (i.e. 4–40 cells loosely attached within inconspicuous mucilage) were recurrently observed in our samples and recorded separately. Conspicuous aggregates of cyanobacteria (i.e. *Aphanocapsa* sp. (Nägeli) were not considered. Filamentous HB ($>4\ \mu\text{m}$ length) were assumed to be inedible for HF and were measured and counted on the $0.8\text{-}\mu\text{m}$ pore-size filter. HF were counted under blue and UV light excitation and sorted into three size categories: <5 , $5\text{--}10$ and $>10\ \mu\text{m}$. A minimum of 25 fields was inspected for HB and Pcy, and 200 for HF.

Ciliates abundance

Unfiltered water samples were preserved in 100 ml PVC flasks with 1% acidified Lugol's iodine solution. Although ciliates are customarily counted using the Utermöhl method, the high density of particulate matter present in lake water made this procedure unfeasible. To circumvent this problem, 6 ml of samples were bleached with a few drops of thiosulphate (Macek et al., 2008) and subsequently fixed with 2% formalin; 500 μl of this preparation were stained with 50 μl of DAPI and gently filtered through a $2\text{-}\mu\text{m}$ pore-size black polycarbonate filter (Sherr & Sherr, 1993). Ciliates were enumerated by epifluorescence microscopy under UV excitation, and sorted into three size categories: <10 , $10\text{--}20$ and $>20\ \mu\text{m}$. A minimum of 200 fields was counted at $1,000\times$ magnification.

Zooplankton abundance

The zooplankton was collected by pouring 50 l of lake water into a $45\text{-}\mu\text{m}$ plankton net. Samples were preserved in 4% formalin. Rotifer and nauplii enumeration was

performed under a direct scope at $100\times$ magnification, on 1 ml Sedgwick-Rafter counting cell. Crustaceans were counted at $20\times$ magnification under a dissecting scope using a 5-ml Bogorov counting chamber (Gannon, 1971). For all groups of zooplankton, the number of subsamples counted was adjusted in order to admit a maximum counting error of 20%. Zooplankton species were identified following Ruttner-Kolisko (1974), Pontin (1978) and Koste (1978) for rotifers, and José de Paggi (1994), Pennak (1989) and Reid (1985) for crustaceans.

Estimation of picoplankton biomass

The average HB, Pcy and Peuk biovolume (V) in Laguna Chascomús had been previously estimated in 0.053, 0.351 and $1.097\ \mu\text{m}^3$, respectively (Kranewitter, 2010). Using these estimates, the individual bacterial cell carbon content (C_{bact}) was estimated according to Simon & Azam (1989) as: $C_{\text{bact}}\ (\text{fgC cell}^{-1}) = 92 \times V^{0.598}$. Individual cell carbon content for Pcy was calculated assuming a conversion factor of 230 $\text{fgC}\ \mu\text{m}^{-3}$ (Worden, 2004). Whereas, Peuk cell carbon content (C_{peuk}) was estimated following the C:V relationship proposed by Menden-Deuer & Lessard (2000) as: $C_{\text{peuk}}\ (\text{pgC cell}^{-1}) = 0.216 \times V^{0.939}$.

Empirical models

The degree of uncoupling (D) between HF and HB abundances was estimated according to Gasol (1994), as the vertical distance from the actual HF abundance to the maximum attainable abundance (MAA), predicted by the model (Eq. 6), for the observed HB abundance:

$$D = -2.55 + 1.04 \times \text{Log}(\text{HB}) - \text{Log}(\text{HF}). \quad (1)$$

where HB and HF (cells ml^{-1}) are the abundance of HB and HF, respectively. The model assumes that HB are the only food source for HF, and that HF responds in a proportional way to HB abundance regardless bacterial size.

Bacterial production (BP) was estimated using the empirical models proposed by White et al. (1991) for freshwater systems and assuming a steady state between population growth and mortality (Eq. 5):

$$\text{Log}(\text{BP}) = 0.43 + \text{Log}(\text{HB}) + 0.031 \times T, \quad (2)$$

where BP ($\mu\text{gC l}^{-1}\ \text{day}^{-1}$) is the bacterial production, HB (cells ml^{-1}) is the abundance of HB and T ($^{\circ}\text{C}$) is water temperature.

Bacterial grazing by HF (G_{HF}) was calculated following Vaqué et al. (1994, Eq. 1):

$$\text{Log}(G_{\text{HF}}) = -3.21 + 0.99 \times \text{Log}(\text{HF}) + 0.028 \times T + 0.55 \times \text{Log}(\text{HB}), \quad (3)$$

where HB and HF (cells ml⁻¹) are the abundance of HB and HF, respectively; G_{HF} (HB ml⁻¹ h⁻¹) is the grazing rate of HF on HB and T (°C) is water temperature. This empirical model assumes that HF grazing are the major contribution to HB mortality.

Data analysis

The nonparametric Spearman rank correlation was applied for all data set. All statistical analysis was accepted as significant at a probability level of $P < 0.05$. After graphical inspection of the data, we noticed that the collected data tended to form two distinct groups. Following Hessen (2006), we used recursive partitioning analysis to identify potential variables and their corresponding threshold values that better explained the segregation of the data points into the two groups. Recursive partitioning (Zhang & Singer, 1999) is a tool for choosing among alternative explanatory parameters without any strong a priori assumptions about causality.

Results

Average values of physical and chemical parameters are summarized in Table 1. Mean depth remained fairly constant (1.9 m) during the best part of the study, but after September 2008 it displayed a decreasing trend. By the end of the study mean depth reached 1.1 m (Fig. 1a). This parameter was negatively correlated with conductivity ($r = -0.80$, $P < 0.05$), nephelometric turbidity ($r = -0.74$, $P < 0.05$) and TP concentration ($r = -0.55$, $P < 0.05$). The concentrations of nutrients and Chl-*a* were similar to values measured previously and well within the hypertrophic range (Table 1). Chl-*a* showed a temporal pattern, with the highest values occurring during the warm seasons and a general increasing trend towards the end of the study (Fig. 1b). Chl-*a* concentration was negatively correlated with lake depth ($r = -0.60$, $P < 0.05$) and positively correlated with nephelometric turbidity ($r = 0.92$, $P < 0.001$), TSS ($r = 0.75$, $P < 0.001$),

AFDW concentration ($r = 0.79$, $P < 0.001$) and TP ($r = 0.75$, $P < 0.001$). In turn, nephelometric turbidity was positively correlated with the TSS ($r = 0.96$, $P < 0.001$), AFDW ($r = 0.87$, $P < 0.01$) and negatively with depth Secchi disk ($r = -0.82$, $P < 0.01$).

The pattern of water temperature was similar among years (Fig. 1a). A remarkable exception corresponds to winter 2007 (data not shown) that displayed an unusually long (>1 month) period of low temperatures (<10°C). Dissolved oxygen during this period fluctuated around 10–11 mg l⁻¹. During this low temperature period, a massive winter fish kill event took place. As an attempt to assess the dimension of this fish kill, our colleagues from the Ecology and Fish Production Laboratory (IIB-INTECH) performed a visual inventory of the dead fish that crowded the lakeshore. On a single day, they counted nearly 17,000 dead fish, mainly planktivorous and microphagous *Cyphocharax voga* (Günther), *Parapimelodus valenciennesi* (Kröyer), along with several other

Table 1 Average values (AVG), standard deviation (SD) and range (maximum and minimum values) of main physical, chemical and biological parameters, measured during the study period

	AVG	SD	Maximum	Minimum
Temp. (°C)	17.1	5.2	25	6
pH	9.1	0.2	9.5	8.7
Cond. (mS cm ⁻¹)	2.5	0.5	3.7	1.8
DO (mg l ⁻¹)	9.2	1.6	12.1	6.0
Secchi (cm)	8.8	2.9	16	4
Z (m)	1.7	0.3	2.0	1.1
N-NO ₃ ⁻ (µg l ⁻¹)	15	16	86	0
N-NO ₂ ⁻ (µg l ⁻¹)	9	17	109	0
N-NH ₄ ⁺ (µg l ⁻¹)	13	16	66	0
TP (µg l ⁻¹)	788	243	1,251	263
SRP (µg l ⁻¹)	15	10	41	3
Chl- <i>a</i> (µg l ⁻¹)	365	186	860	43
AFDW (mg l ⁻¹)	86.4	35.6	45.0	193.4
TSS (mg l ⁻¹)	245.9	120.9	591.7	87.0
DOC (mg l ⁻¹)	28.8	7.9	47.4	10.7

In all cases $n = 46$

Temp temperature, Cond conductivity, DO dissolved oxygen, Secchi Secchi depth, Z mean depth, TP total phosphorous, SRP soluble reactive phosphorus, AFDW ash-free dry weight, TSS total suspended solid, DOC dissolved organic carbon, Chl-*a* chlorophyll *a*

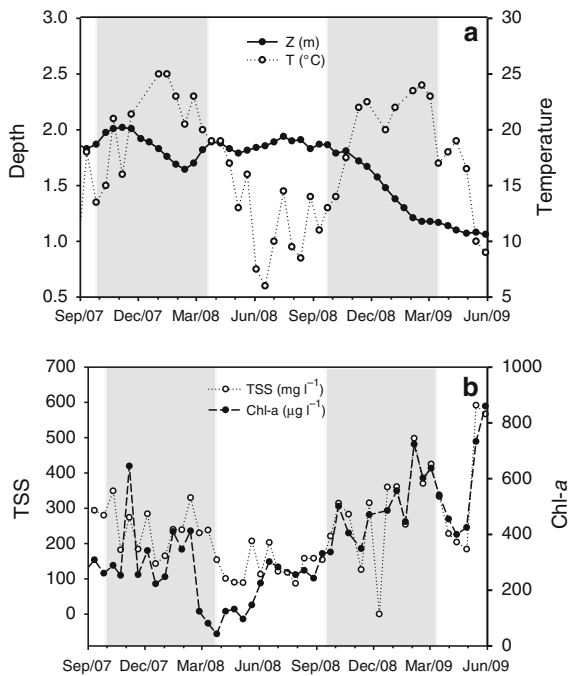


Fig. 1 Temporal changes of **a** lake depth (Z) and water temperature (T), and **b** chlorophyll a ($Chl-a$) and total suspended solid (TSS) concentrations in Laguna Chascomús during the study period. *Shaded areas* correspond to warm (spring and summer) seasons

benthonic fish species, such as *Hypostomus commersoni* (Valenciennes) (Solimano, pers. commun.).

The abundance and biomass of picoplanktonic organisms were very high (Table 2). HB (single-cell) abundance was always higher than 2.3×10^7 cells ml⁻¹, and displayed an increasing trend with time (Fig. 2a), with higher abundances recorded towards the end of the study period. Filamentous HB (mean filament length: 22 µm) followed the same temporal pattern, but they never exceeded 1.2×10^6 filaments ml⁻¹ (Table 2). HB abundance was positive correlated with Chl- a , AFDW, TP and DOC concentrations ($r = 0.71$, $r = 0.71$, $r = 0.55$, $r = 0.55$ respectively; $P < 0.05$). Single-cell, phycocyanine-rich Pcy abundance fluctuated between 3.2×10^6 and 2.5×10^7 cells ml⁻¹. They increased along the study and showed the lowest annual values in March, at the end of each summer seasons (Fig. 2a). Pcy microaggregates of on average 12 cells per aggregate, ranged between 1.6×10^6 and 8.9×10^6 cells ml⁻¹, without showing a clear seasonal pattern. Peuk were about an order of magnitude lower than Pcy, and represented always less than 6% of the total abundance

of autotrophic picoplankton (Pcy + Peuk). Peuk behaved seasonally with an annual peak during summer time (Fig. 2a).

In terms of biomass, single-cell HB (953 ± 408 µgC l⁻¹), Pcy ($1,044 \pm 418$ µgC l⁻¹) and Peuk (43 ± 38 µgC l⁻¹) represented on average 44, 53 and 3% of total picoplanktonic biomass, respectively. However, these proportions varied seasonally: the percentage of HB biomass reached the highest values (up to 54% of the total picoplanktonic biomass) always in summer, but it only amounted $\sim 35\%$ in winter (Fig. 3a). Filamentous HB (341 ± 180 µgC l⁻¹) represented on average 26% of the total biomass of HB and displayed no evident temporal pattern (Fig. 3b). The percentage of Pcy cells forming microaggregates (349 ± 164 µgC l⁻¹) averaged 22% of the total Pcy biomass. This proportion was relatively constant all year round except during the first summer (January–March 2008) when it reached 32–65% (Fig. 3c).

HF abundance ranged from 7.4×10^3 to 1.1×10^5 cells ml⁻¹ (Table 2). Their density neither displayed a defined temporal trend nor a repeatable seasonal trend (Fig. 2b). On average, small cells (<5 µm) contributed 69.1%, medium-size cells (5–10 µm) 26.9%, and large cells (>10 µm) 4% of total HF abundance. The density of ciliates varied haphazardly over time, with abundances ranging from zero to 1,207 cells ml⁻¹ (Table 2; Fig. 2b). Small (<10 µm), medium size (10–20 µm) and large (>20 µm) represented 27, 44 and 29%, respectively.

The zooplankton was represented by the cyclopoid copepod, *Acanthocyclops robustus* (Sars), the calanoid copepod, *Notodiaptomus incompositus* (Brian), small cladocerans (mostly *Bosmina huaronensis* (Delachaux) and *Moina micrura* (Kurz)) and rotifers (predominantly, *Brachionus caudatus* (Barrois & Daday), *B. havanaënsis* (Rousselet) and *Keratella tropica* (Apstein)). As a whole the abundance of zooplankton displayed marked seasonality, with obvious maxima occurring during spring and summer, and much lower densities of all groups during fall and winter. During the spring 2007–summer 2008, cladocerans were particularly abundant (Fig. 2c), while the abundance of rotifers was relatively low. An early spring peak of *B. huaronensis* (up to 611 ind. l⁻¹) was followed by a mid-summer peak of *M. micrura* (up to 240 ind. l⁻¹). The dominance of small cladocerans was preceded by the massive mortality of planktivorous and microphages fish occurring during the winter of 2007 (June–July). In contrast, the

Table 2 Average abundance (AVG), standard deviation (SD) and range (maximum and minimum) for the different planktonic components measured during the study period

Abundance	AVG	SD	Maximum	Minimum
HB single-cells (cells ml ⁻¹)	6.0×10^7	2.6×10^7	1.1×10^8	2.3×10^7
HB filaments (filaments ml ⁻¹)	4.9×10^5	2.9×10^5	1.2×10^6	5.0×10^4
Pcy single-cells (cells ml ⁻¹)	1.4×10^7	5.5×10^6	2.5×10^7	3.2×10^6
Pcy microaggregates (cells ml ⁻¹)	4.3×10^6	2.0×10^6	8.9×10^6	1.6×10^6
Peuk (cells ml ⁻¹)	2.2×10^5	2.0×10^5	1.0×10^6	1.4×10^4
HF total (cells ml ⁻¹)	3.5×10^4	2.1×10^4	1.1×10^5	7.4×10^3
HF < 5 μm (cells ml ⁻¹)	2.5×10^4	1.6×10^4	6.8×10^4	4.4×10^3
HF 5–10 μm (cells ml ⁻¹)	9.2×10^3	6.7×10^3	3.5×10^4	7.4×10^2
HF > 10 μm (cells ml ⁻¹)	1.3×10^3	2.0×10^3	9.2×10^3	0
Ciliates total (cells ml ⁻¹)	427	278	1,207	0
Ciliates < 10 μm (cells ml ⁻¹)	105	93	426	0
Ciliates 10–20 μm (cells ml ⁻¹)	205	155	568	0
Ciliates > 20 μm (cells ml ⁻¹)	117	166	1,009	0
Rotifers (ind. l ⁻¹)	936	1,273	5,103	44
Nauplii cyclopoids (ind. l ⁻¹)	272	276	1,238	40
Nauplii calanoids (ind. l ⁻¹)	50	63	306	3
Copepods (ind. l ⁻¹)	107	95	514	19
Cladocerans (ind. l ⁻¹)	78.0	139.2	611.4	0.2

In all cases $n = 46$

HB heterotrophic bacteria, Pcy picocyanobacteria, Peuk picoeukaryote, HF heterotrophic flagellates

next year (from spring 2008 to summer 2009), the zooplankton assemblage was dominated by rotifers, which reached densities $>5,000$ ind. l⁻¹, while the abundance of cladocerans remained low. The pattern of copepods did not differ so markedly between the two growing seasons, although they tended to display higher abundances on 2007–2008 than on 2008–2009.

Two distinct clusters of data are apparent in Fig. 4a. Samples collected before July 16th 2008 tend to plot to the left of samples collected after that date. The distinction between these two periods was based on a recursive partitioning analysis performed on the degree of decoupling (D) (Fig. 5). We have noticed that D tended to be higher during the second half of the study. Among the several variables investigated, the recursive partitioning analysis identified time to be the variable that better explained the splitting of the data. Samples collected before July 16th 2008 displayed lower uncoupling (D) than samples collected after that date (Fig. 5), which explains the segregation of data points (circles and diamonds) in Fig. 4a. The average HB:HF ratio before July 16th 2008 was lower ($1,099 \pm 687$) than the average ratio after that date ($3,476 \pm 2,171$). The estimates of HB production

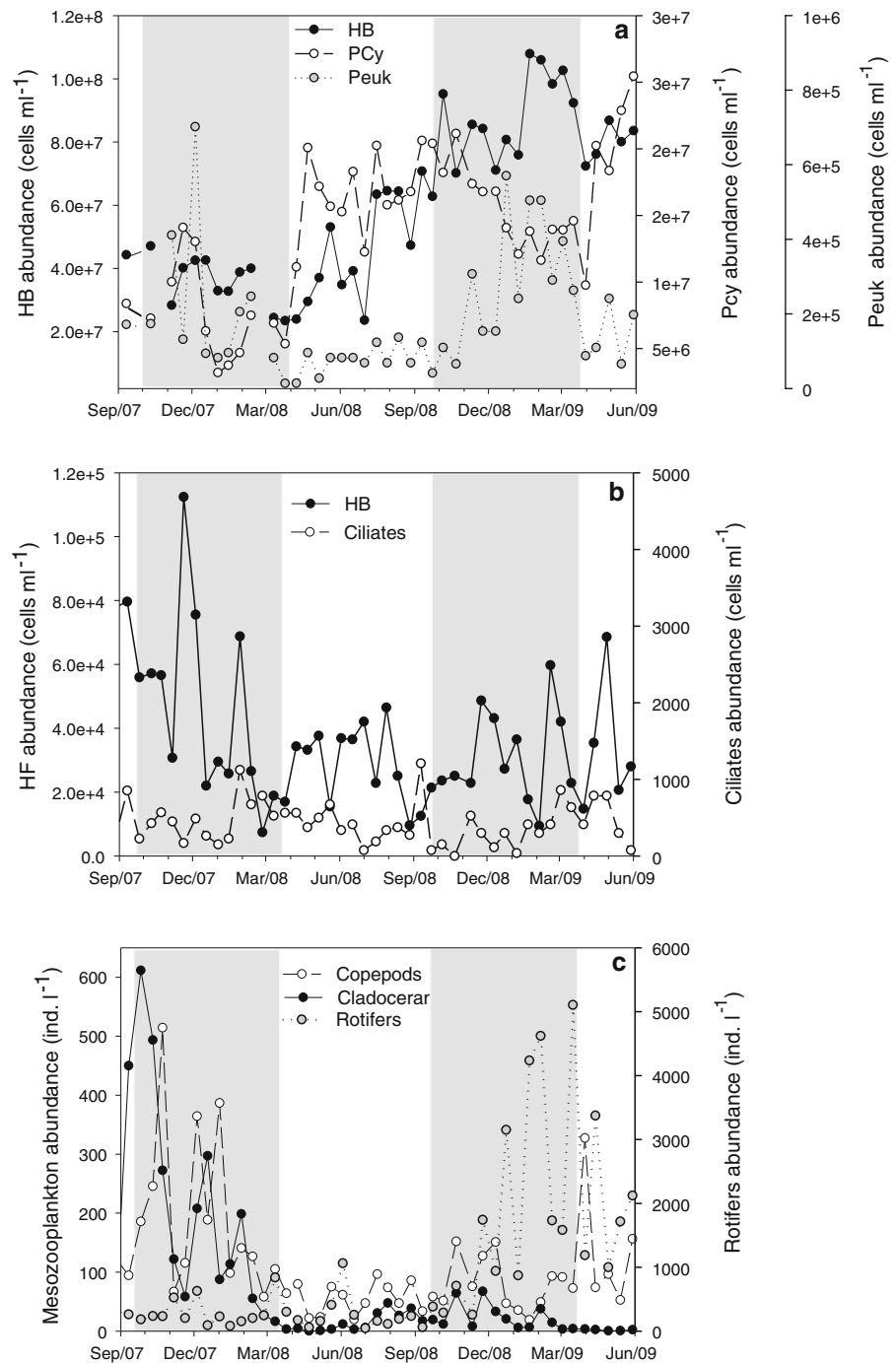
(BP) averaged $318 (\pm 178) \mu\text{C l}^{-1} \text{ day}^{-1}$ and $540 (\pm 264) \mu\text{C l}^{-1} \text{ day}^{-1}$ for 2007–2008 and 2008–2009, respectively. On the other hand, HB grazing by HF (G_{HF}) averaged $399 (\pm 363) \mu\text{C l}^{-1} \text{ day}^{-1}$ during 2007–2008 and $366 (\pm 274) \mu\text{C l}^{-1} \text{ day}^{-1}$ during 2008–2009.

Data also tended to cluster in two groups when HF abundance was plotted against Pcy abundance (Fig. 4b). However, the segregation of data by period was less marked than in the HF–HB plot. The average Pcy:HF ratio before July 16th 2008 was lower (286 ± 206) than after that date (687 ± 380).

Discussion

The first, and perhaps most salient, characteristic of the picoplankton community in Laguna Chascomús is its great abundance. HB abundances (up to 1.1×10^8 cells ml⁻¹) are higher than those reported in most hypertrophic lakes worldwide (e.g. Sommaruga, 1995; Jürgens & Jeppesen, 2000; Eiler & Bertilsson, 2004; Tijdens et al., 2008; Chen et al., 2010) and close to the

Fig. 2 Temporal changes of organisms in Laguna Chascomús during the study period, **a** abundances of single-cells heterotrophic bacteria (*HB*), picocyanobacteria (*PCy*) and picoeukaryote (*Peuk*); **b** heterotrophic flagellates (*HF*) and ciliates and rotifers. *Shaded areas* correspond to warm (spring and summer) seasons



highest values ever reported for pelagic systems (3.6×10^8 cells ml⁻¹ in an alkaline saline lake, Kilham, 1981). The abundances of phycocyanine-rich Pcy (up to 2.7×10^7 cells ml⁻¹), which make the bulk of the autotrophic picoplankton, are among the highest values

ever reported in natural aquatic systems (Hirose et al., 2003; Callieri, 2007).

Two other distinctive features of the picoplankton in this lake are the relative proportion of the autotrophic components and the weak seasonality displayed by

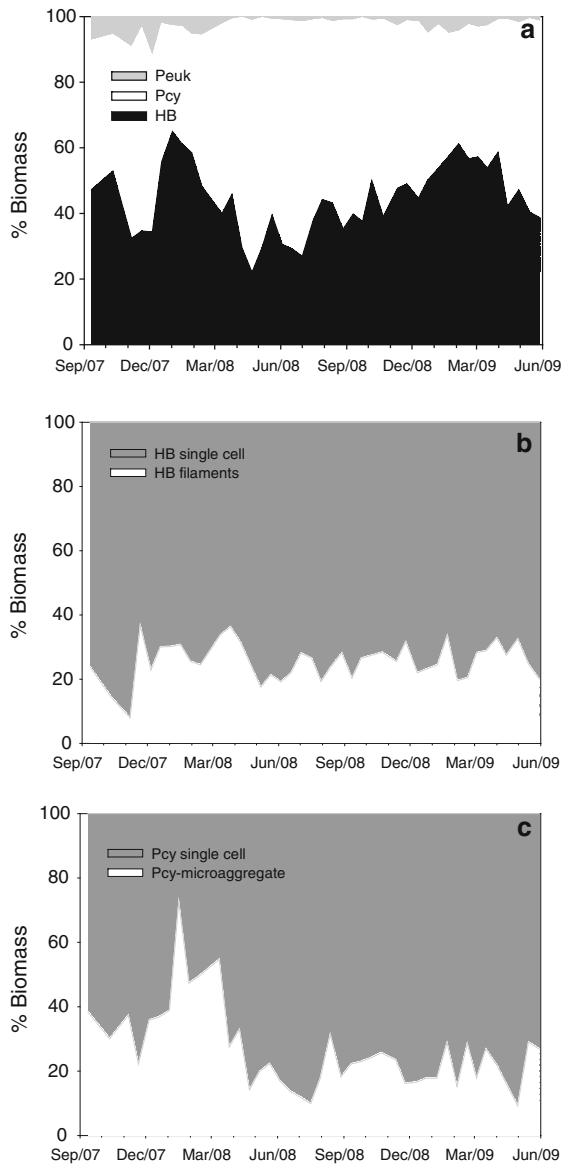


Fig. 3 **a** Relative biomass of single-cell *HB*, *Pcy* and *Peuk*, **b** single-cell and filaments of *HB* and **c** *Pcy* single-cell and microaggregates of *Pcy* along the study period

them. On a biomass basis, the *Pcy*:*HB* ratio in Laguna Chascomús resulted very high (minimum: 35%, mean: 53%) and the abundance of *Pcy* never dropped below 3.2×10^6 cells ml^{-1} . Similar relative compositions of picoplankton and seasonal patterns of *Pcy* abundance (e.g. constrained within a narrow range year round) have been reported for some shallow subtropical lakes (Carrick & Schelske, 1997) and deep tropical lakes (Sarmiento et al., 2008; Stenuite et al., 2009). In most temperate systems, the abundance of *Pcy*

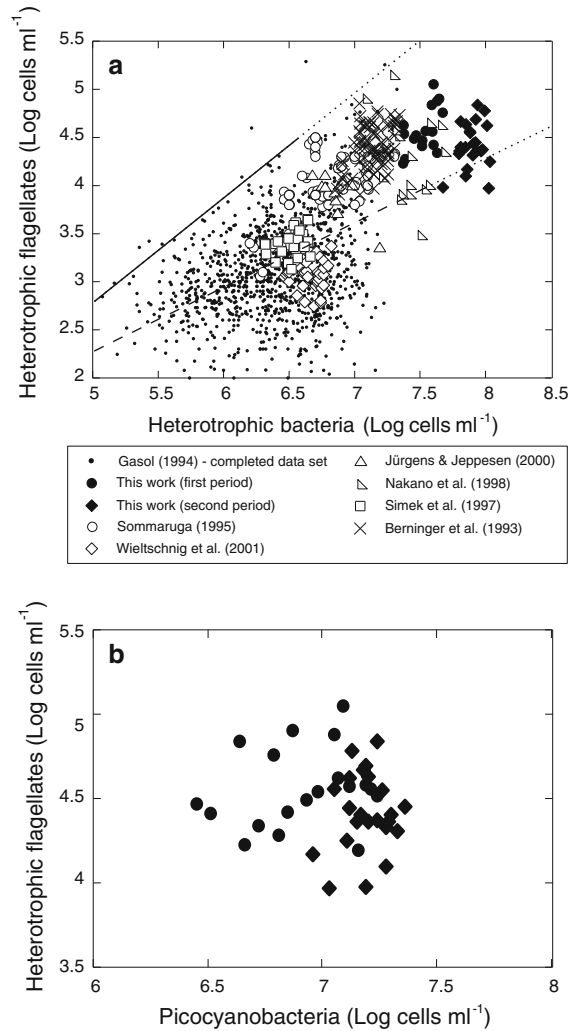


Fig. 4 **a** Scatter plot of HF versus HB abundances (log–log) of our own observations, together with the data set from Gasol (1994) and other simultaneous estimations of HB and HF abundances collected from the literature. Only eutrophic and hypertrophic shallow lakes were considered. We excluded papers that presented only single points or means. A total of six articles were found to be included in this plot. Data were taken from tables or read from graphs with an image analyzer. The *upper boundary line* is the empirically derived MAA for HF, and the *dashed line* is the mean realized abundance (MRA), according to Gasol (1994). The vertical distance between MAA and an individual observation is a measure of the degree of uncoupling (*D*) between predators (HF) and resources (HB). **b** Plot of HF versus *Pcy* single-cells (log–log) of our own observations. *Solid circles* and *diamonds* in both panels represent samples collected before and after July 16th 2008, respectively (refer to the text for further details)

typically remains low during the best part of the year, except for one or two outbursts in the warmer season (Callieri, 2010). The similarities between Laguna

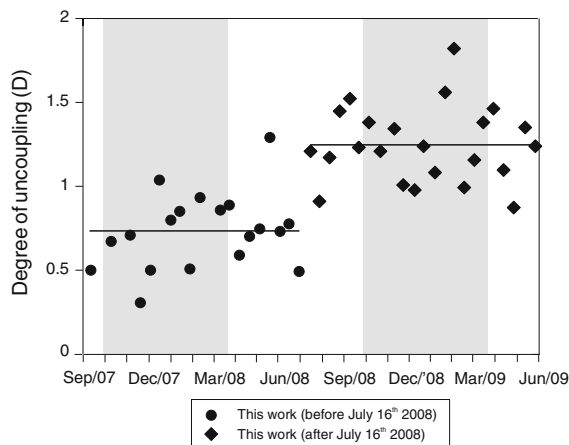


Fig. 5 Degree of uncoupling (D) between heterotrophic flagellates and heterotrophic bacteria throughout the study. Solid circles and diamonds represent samples collected before and after July 16th 2008, respectively. Horizontal lines indicate the mean value for each period. Shading areas correspond to the spring–summer periods

Chascomús and, tropical and subtropical lakes may be a reflection of its intermediate climatic position or may be related to the fact that the light climate in tropical (Sarmiento, 2012), as well as in polymictic hypertrophic turbid lakes, such as Laguna Chascomús (Pérez et al., 2011), is less variable than in deep, stratifying temperate lakes.

Picoplanktonic organisms may develop resistant morphologies, such as filamentous and microaggregates, in response to HF predation (Pernthaler, 2005 and references therein). Filamentous HB are particularly common in hypertrophic lakes (e.g. Sommaruga, 1995; Jürgens & Jeppesen, 2000; Hahn & Höfle, 2001; Thelaus et al., 2008). In Laguna Chascomús filamentous HB make up a significant proportion of the HB biomass (>20%). However, no relationship was found between the abundance, biomass or proportion of filamentous HB and the abundance of HF. It is known that some bacterial strains have permanently a filamentous morphotype independent of the presence of a predator (Hahn et al., 1999), this might explain the constancy of filamentous bacteria recorded in Chascomús regardless the abundance of HF. On the other hand, the presence of Pcy microaggregates has been reported in several freshwater environments (Callieri, 2007 and references therein). The origin of Pcy microaggregates is not fully understood. It is possible that some microaggregates may represent detachments

from larger colonies (i.e. *Aphanocapsa* sp.). However, there is also evidence suggesting that they might derive from aggregation of single-cell Pcy (Passoni & Callieri, 2001; Crosbie et al., 2003). In particular, Jezberová & Komárková (2007) demonstrated that the grazing activities of a nanoflagellate induced the formation of microaggregates in a culture of Pcy single-cell. In our study, higher proportion of Pcy microaggregates were recorded during the period in which small cladocerans dominated zooplankton and HF abundance and HF:prey ratio reached the highest values.

HF abundances were also among the highest values (>10⁵ cells ml⁻¹) reported for natural pelagic environments (Sanders et al., 1992; Sommaruga, 1995; Auer & Arndt, 2001; Hirose et al., 2003; Chen et al., 2010). The diet of HF typically includes picoplanktonic preys. Several studies have shown that protists can ingest both HB and Pcy (e.g. Weisse et al., 1990; Callieri et al., 2002; Tsai et al., 2007), but only a handful studies have simultaneously analyzed the grazing rates on both types of preys (e.g. Šimek et al., 1997; Sakka et al., 2000; Christaki et al., 2001; Tarbe et al., 2011; Izaguirre et al., 2012). All these studies reported higher clearance rates on Pcy than on HB. Along the same line, several authors (Jürgens & Matz, 2002 and cites herein) demonstrated that ~0.5 μm diameter particles (i.e. close to average HB size) would experience 4–6 times lower mortality due to HF grazing than ~1 μm diameter particles (i.e. close to average Pcy cell size). Published clearance rates (CR) of HF on Pcy estimates range between 0.3 and 58 nL HF⁻¹ h⁻¹ (Šimek et al., 1997; Callieri et al., 2002; Tarbe et al., 2011; Izaguirre et al., 2012). The importance of HF as major grazers of autotrophic prokaryotes becomes apparent when one considers that, even assuming the lowest CR value, HF could still remove as much as 25% of the Pcy biomass per day. These results are in line with the idea that Pcy microaggregates would be formed as a result of high grazing pressure on Pcy single-cell.

In Laguna Chascomús, the structure of the zooplankton community is strongly affected by intense fish planktivory (Diovisalvi et al., 2010). During the first year of this study, and after the massive winter fish kill, we recorded unusually high abundances of small cladocerans (*Moina* spp., *Bosmina* spp.) and low rotifer abundances. This atypical zooplankton composition reverted to the characteristic zooplankton assemblage of Laguna Chascomús, one dominated by

rotifers and cycloid copepods, within about a year. The role of fish in shaping the zooplankton community in Laguna Chascomús has also been observed experimentally. In a mesocosm experiment from which fish had been excluded, Llamas et al. (2009) observed the development of small-sized cladocerans populations within a few weeks. Similar results were observed in other tropical and subtropical shallow lakes (Rennella & Quirós, 2006; Boveri & Quirós, 2007; Havens et al., 2009; Teixeira de Mello et al., 2009; Iglesias et al., 2011).

The relationship between HF and their preys (HB and Pcy) seems to have been differentially affected by the two distinct zooplankton assemblages that dominated in the two consecutive years: dominance of small cladocerans versus dominance of rotifers. Even though the proportion of grazing resistant morphologies (i.e. filaments) did not change between years, the absolute abundance of HB steadily increased and the HF:HB ratio decreased. This effect has been better investigated for the relationship between HF and HB. Gasol (1994) proposed an empirical model to relate the abundances of HF and HB. He noticed that the joint distribution of Log HF abundance and Log HB abundance was bounded by straight line, which he called “maximum attainable abundance” (MAA). Gasol (1994) further used the MAA as a reference to define the “degree of uncoupling”, i.e. the vertical distance (D) between the MAA line and the actual HF abundance. In theory, low D values indicate low levels of predation on HF, and vice versa. HB abundances in Laguna Chascomús are nearly an order of magnitude higher than the upper limit of Gasol’s (1994) dataset, and also higher than more recently published reports for highly eutrophic shallow lakes (Berninger et al., 1993; Sommaruga, 1995; Šimek et al., 1997; Nakano et al., 1998; Jürgens & Jeppesen, 2000; Wieltchnig et al., 2001; Hirose et al., 2003; Chen et al., 2010). Nevertheless, the validity of Gasol’s original model seems unaffected, as neither the values reported more recently, nor our own from Laguna Chascomús, exceeds the original MAA line. During the period when the zooplankton community was dominated by small cladocerans, the degree of uncoupling between HF and HB (D) (sensu Gasol, 1994) was relatively low. On the other hand, during the period when the zooplankton was dominated by rotifers (i.e. the “typical” condition in Laguna Chascomús), the values of D were significantly higher. Moreover, our estimates of BP ($318 \pm 178 \mu\text{C l}^{-1} \text{ day}^{-1}$) and bacterial grazing by HF ($399 \pm 363 \mu\text{C l}^{-1} \text{ day}^{-1}$) were roughly balanced

during the first part of the study, whereas during the second part of the study, BP ($540 \pm 264 \mu\text{C l}^{-1} \text{ day}^{-1}$) exceeded the estimated bacterial grazing ($G_{\text{HF}} = 366 \pm 274 \mu\text{C l}^{-1} \text{ day}^{-1}$). One of the major changes observed during the study was the gradual increase in HB abundance, which affected the estimates of D , G_{HF} and BP. This increasing trend in HB was also observed even if the abundance was standardized by lake depth. The different approaches are therefore consistent in suggesting that HB and HF were more coupled during the first half of the study.

By analogy with the analysis performed for HB, we plotted the Log HF abundance versus Log Pcy abundance. Similarly to the results observed for HF–HB, the HF–Pcy data tended to cluster in two groups. As mentioned above the relationship between HF and Pcy, has been less intensively studied, and unfortunately an analogue estimate of the degree of uncoupling (D) has not yet been developed. Therefore the choice of July 16th 2008 to separate the two periods is somehow arbitrary, which may explain why the segregation of data by period is less marked than in the HF–HB plot. Moreover, the proportion of micro-aggregates of Pcy (i.e. potentially grazing resistant morphologies) was higher during the first summer period (January–March 2008) when small cladocerans dominated.

The evidence presented in this study suggests that the relationship between the abundances of HF and their potential preys is affected by the composition of the zooplankton assemblage. The composition of zooplankton is known to affect the structure of the microbial trophic web (see reviews by Jürgens & Matz, 2002; Sommer & Sommer, 2006). But most studies have focused on comparisons between *Daphnia* dominated and copepod dominated zooplankton communities. In general, the degree of decoupling is higher in *Daphnia* dominated environments than in copepod-dominated lakes (Gasol et al., 1995). Both *Daphnia* dominated and copepod dominated zooplankton assemblages are more representative of temperate, stratifying lakes. On the other hand, rotifer, which are often the dominant group in polymictic shallow lakes (Berninger et al., 1993; Sommaruga, 1995; Jürgens & Jeppesen, 2000; Chen et al., 2010) appear to have an intermediate effect on the HF (Gasol et al., 1995). We used published clearance rates of rotifers on HF to calculate proportion of the HF community that could have been consumed by rotifers

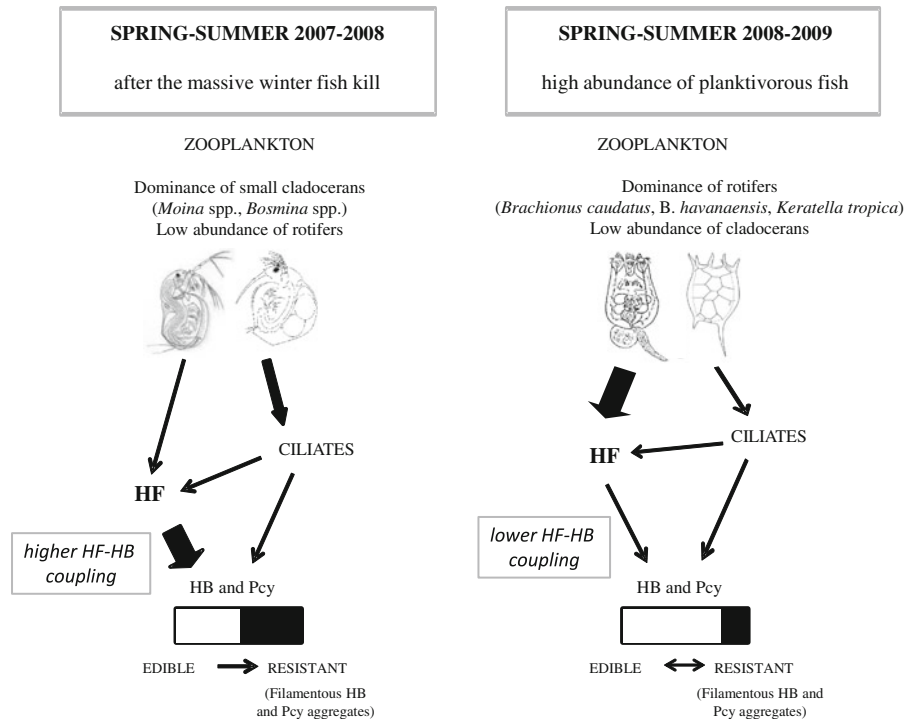


Fig. 6 Simplified scheme showing the trophic interactions between zooplankton and the components of the microbial food web in Lake Chascomús during two consecutive years, with contrasting zooplankton communities (modified from Jürgens &

Matz, 2002). The size of the *arrows* is indicative only, and thicker indicates greater predation pressure to that community. *Bars* *HB* and *Pcy* were built randomly

during our study. Clearance rates estimates range from 0.38 to 1.23 ml ind.⁻¹ day⁻¹ (Dolan & Gallegos, 1991; Sanders et al., 1994; Jürgens et al., 1996), but in order to be conservative, we used the lowest value. During the first part of the study, the rotifer abundance averaged 350 ind. l⁻¹ and could have potentially consumed about 10% of the HF biomass per day. While during the second part of the study, rotifers often exceeded 1,500 ind. l⁻¹ and theoretically, they might have grazed ~90% of the HF biomass daily. Dolan & Gallegos (1991) observed a strong top-down control of HF by rotifers in the Rhone River Estuary when rotifers achieved densities comparable with our study (1,000–5,000 ind. l⁻¹), which cascaded down as an indirect positive effect on HB abundance. Studies from other lakes also revealed that rotifers can have a strong structuring effect on microbial plankton communities (Burian et al., 2012), being responsible for a high mortality of HF (Arndt, 1993; Pernthaler et al., 1996) and affecting the HF–HB relationship (Tadonléké et al., 2004). Finally, zooplankton assemblages dominated by small cladocerans (e.g. *Bosmina* spp.,

Moina spp.), which are known to have much less effective filtration capacity than daphnids (Köthe & Benndorf, 1994; Jürgens & Stolpe, 1995; Zölner et al., 2003), can be presumed to have a lesser effect in structuring the microbial community than *Daphnia*. Contrarily to our hypothesis, the present results suggest that the effect of small cladocerans dominated zooplankton may be even smaller than that of produced by high densities of rotifers, which may explain the differences in the microbial food web structure observed between years.

Our study showed that changes in the composition of the zooplankton community might affect the relationship between HF and their preys (HB and Pcy), and possibly, although less evident, the structure of the potential preys (i.e. Pcy morphology). The resulting heterotrophic cascade for the two contrasting zooplankton communities is outlined in Fig. 6. The observed changes in zooplankton community could in turn be linked to processes affecting the top of the trophic web (winter fish kill) and ultimately to an infrequent weather event (a prolonged period of low

winter water temperature). The data reported in this work are consistent with empirical models describing the relationship between HF and HB, but extend the range of published HB and Pcy abundances. Even after including additional data published after Gasol's (1994) revision, it is evident that hypertrophic environments are still underrepresented. Considering the high number of shallow lakes, many of which are naturally eutrophic or have undergone eutrophication in recent decades, the paucity of data at the high productivity end of the gradient seems most likely a consequence of scientific bias rather than the statistical outcome of the world's distribution of lakes.

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