Available online at www.sciencedirect.com
ScienceDirect
Limnologica 37 (2007) 88–99
LIMNOLOGICA
www.elsevier.de/limno

Experimental study of the zooplankton impact on the trophic structure of phytoplankton and the microbial assemblages in a temperate wetland (Argentina)

Rodrigo Sinistro[a,*], María Laura Sánchez[a], María Cristina Marinone[b], Irina Izaguirre[a,c]

[a]*Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C1428EHA Buenos Aires, Argentina*
[b]*Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C1428EHA Buenos Aires, Argentina*
[c]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*

Received 7 July 2006; received in revised form 23 August 2006; accepted 1 September 2006

Abstract

An experimental study using mesocosms was conducted in the main shallow lake of a temperate wetland (Otamendi Natural Reserve, Argentina) to analyse the impact of zooplankton on phytoplankton and the microbial assemblages. The lake is characterised by the presence of a fluctuating cover of floating macrophytes, whose shading effects shape the phytoplakton community and the ecosystem functioning, which was absent during the study period. The experiment was run in situ using polyethylene bags, comparing treatments with and without zooplankton. The cascade effect of zooplankton on phytoplankton and the lower levels of the microbial food web (ciliates, heterotrophic nanoflagellates (HNF) and picoplankton) were analysed.

A significant zooplankton grazing on the nano-phytoplankton fraction (3–30 μm) was observed. Conversely, large algae (filamentous cyanobacteria, colonial chlorophytes and large diatoms) increased in all mesocosms until day 10, suggesting that they were not actively grazed by zooplankton during this period. However, from day 10 until day 17 this fraction decreased in the enclosures with mesozooplankton, probably due to an increase in the abundance of large herbivores.

The results of the experiment would also indicate a trophic cascade effect on the lower levels of the microbial community. In the treatment where zooplankton was removed, the abundance of ciliates followed the same increasing pattern as the abundance of HNF, but with a time lag in its response. In the enclosures without zooplankton, HNF remained relatively constant throughout the experiment, whereas ciliates strongly decrease during the last week. Total picoplankton abundance increased in the enclosures with mesozooplankton, thus supporting the existence of a four-link trophic cascade (copepods–microzooplankton–HNF–picoplankton). Zooplankton composition changed significantly from the beginning until the end of the experiment; cyclopoid nauplii and rotifers were notoriously dominant at t_0, whereas 10 days later the community showed a more equitable proportion of cyclopoids, calanoids, nauplii, cladocerans and rotifers.

Keywords: Zooplankton; Phytoplankton; Microbial communities; Top–down control; Wetlands; Trophic cascade

*Corresponding author.
E-mail address: rs@ege.fcen.uba.ar (R. Sinistro).

0075-9511/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.limno.2006.09.001

Introduction

The effects of zooplankton grazing on the structure of prey assemblages, and its impact at the lower levels via trophic cascades were discussed in many papers (Carpenter & Kitchell, 1988; Carpenter, Kitchell, & Hodgson, 1985; Gliwicz, 2002; Pace, Cole, & Carpenter, 1998; Schnetzer & Caron, 2005; Sommer et al., 2001).

Zooplankton grazing usually provokes a decrease in phytoplankton biomass; however, because of the effect of the selective feeding, some inedible algae may increase their abundances in a lake during active grazing phases, since they can take advantage of the availability of nutrients when the competition pressure with other algae diminishes (Queimaliños, Modenutti, & Balseiro, 1998). Zooplankton composition, in turn, also determines the responses of the grazing pressure on phytoplankton. Particularly, microphagous and macrophagous zooplankton may exert a different top–down impact on the phytoplankton community (Sommer et al., 2003).

On the other hand, the impact of zooplankton on the lower levels of the aquatic food web is mainly mediated by the removal of protozooplankton (mainly ciliates) and heterotrophic nanoflagellates (HNF). An increase of HNF, in turn, leads to a higher grazing pressure on picoplankton. Trophic cascade at the microbial level have been both described and analysed for marine and freshwater ecosystems (Adrian, Wickham, & Butler, 2001; Jürgens & Jeppesen, 2000; Katechakis, Stibor, Sommer, & Hansen, 2002; Schnetzer & Caron, 2005). Although ciliates can also contribute to the grazing on picoplankton, HNF are often the main grazers on this fraction (Hahn & Hofle, 2001; Sanders, Porter, Bennett, & DeBiase, 1989). Another important effect of lake zooplankton is the recycling of nutrients, particularly when they are limiting for phytoplankton (Attayde & Hansson, 1999; Balseiro, Modenutti, & Queimaliños, 1997; Carrillo, Reche, Sánchez Castillo, & Cruz-Pizarro, 1995; Queimaliños et al., 1998). Nevertheless, the importance of the zooplankton regenerating effect depends on the trophic status of the system. In oligotrophic lakes, which are more dependent on internal recycling, zooplankton regulates the availability of nutrients via excretion (Urabe, 1993).

This study was conducted in a temperate wetland of the Lower Paraná River (Otamendi Natural Reserve, Argentina), where investigations on the phytoplankton community have been carried out during the last 8 years. The characteristics of the water bodies of this area are thoroughly described in previous papers (Izaguirre et al., 2004; Izaguirre, Sinistro, O'Farrell, Unrein, & Tell, 2001; O'Farrell, Sinistro, Izaguirre, & Unrein, 2003). These works show that the macrophyte cover is one of the main factors in the selection of the phytoplankton species in this wetland, because it strongly modifies the light climate conditions in the water column. More recently, different experimental studies using enclosures have been carried out in the main shallow lake of this reserve. In particular, one recent paper was focused on the influence of the underwater light climate on the morphometric characteristics of the phytoplankton, at both the population and community levels (O'Farrell, de Tezanos Pinto, & Izaguirre, in press). Subsequent experimental studies in microcosms highlighted the presence of a rich microbial community, with high abundances of autotrophic and heterotrophic picoplankton (HPP), and a great variety of HNF, ciliates and mixotrophic algae. In these experiments, we analysed the influence of the light deficiency due to floating macrophytes on the microbial communities, and some of the main interactions within the microbial food web, such as the mixotrophic behaviour of some algae that can prey on bacteria (Sinistro, Izaguirre, & Asikian, 2006). Experimental manipulations are very useful to analyse the interactions occurring within a trophic cascade (Carpenter & Kitchell, 1988; Carpenter et al., 1985), and particularly, the recent experimental approaches applied in this wetland are revealing interesting interactions among the microbial assemblages.

The present study was mainly aimed at analysing the predation impact of zooplankton on phytoplankton in this wetland by evaluating its effect in terms of abundance, size structure and species composition of the phytoplankton community. On the other hand, we also analysed the responses of the different components of the microbial community: HNF, ciliates and picoplankton fractions, by comparing their temporal evolution in the presence or absence of zooplankton. The following hypotheses were tested in the studied shallow lake:

(1) Zooplankton will effectively control phytoplankton abundance by grazing.
(2) Grazing will be more important on the nanoplanktonic algae, and thus the inedible species will profit from the nutrients released by zooplankton, increasing their abundances when the competitive pressure with other algae decreases.
(3) The absence of zooplankton will favour the increase of HNF and ciliates, thus promoting a negative cascade effect on the picoplankton.

Methods

Study site

The experiment was carried out in the main shallow lake (Laguna Grande) of the Otamendi Natural Reserve, Buenos Aires Province, Argentina (34°10′–34°17′S; 58°48′–58°53′W) (Fig. 1). This shallow

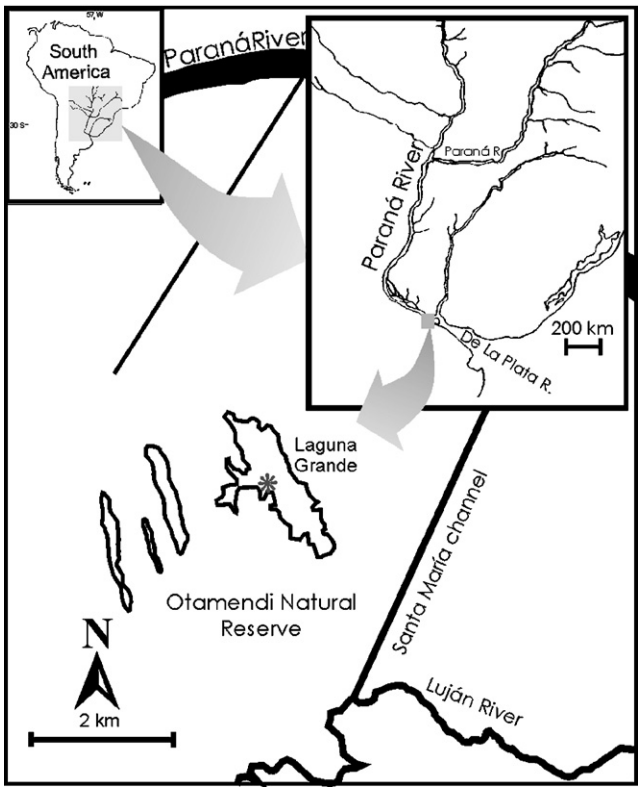

Fig. 1. Map of the Paraná River Basin in Argentina, showing the location of the lake and the site where the experiment was conducted.

lake has a surface area of approximately 28 ha, and its littoral zone exhibits profuse aquatic vegetation, mainly composed by rooted and floating species, whose spatial distribution and biomass experience marked fluctuations during the year and among years. In particular, during the present study period, the lake showed sparse vegetation cover. Full descriptions of the region, climate and vegetation are provided in our previous works (Izaguirre et al., 2004; Sinistro et al., 2006). Those papers also report limnological information of the main shallow lake, obtained during a 2-year study. Water temperature fluctuates between 9.4 and 30.3 °C, having annual mean values around 20 °C. The concentrations of phosphates are rather high and typical of eutrophic systems, but nitrogen can be occasionally limiting for phytoplankton under conditions of active algal growth (Sinistro et al., 2006). According to the descriptions given by Williamson, Morris, Pace, and Olson (1999), the aquatic systems of this wetland can be defined as typical 'mixotrophic lake ecosystems', with high DOC and total P contents.

Experimental design

The experiment was carried out in six mesocosms (50-L high-density polyethylene bags supported by floating devices) placed 100 m offshore in "Laguna Grande". The temporal evolution of the mesocosms was analysed from March 31 to April 17, 2006. Samples and measurements were obtained at t_0 (initial time), t_1 (3 days), t_2 (6 days), t_3 (10 days) and t_4 (17 days). Owing to the sparse vegetation cover present during the study period, the shading effect on the mesocosms was negligible.

Two treatments by triplicate were compared: (1) mesocosms with all planktonic components (zooplankton, phytoplankton, HNF, ciliates and picoplankton) and (2) mesocosms without mesozooplankton but with the other planktonic fractions. The six enclosures were first filled with water filtered through a 55-μm-mesh net to exclude zooplankton. Then, a concentrate sample of zooplankton was obtained from the same lake, by filtering 230 L of water through a 55-μm-mesh net, and divided into four aliquots of equal volume. Three of them were added to each one of the three bags including zooplankton, and the fourth one was fixed with 5% formaldehyde to quantify the initial concentration of zooplankton. The initial abundance of zooplankton added to the bags was similar to that registered in the natural environment during periods of high zooplankton densities, according to previous samples obtained in this lake.

Sampling and laboratory procedures

Total chlorophyll *a* was estimated in each one of the mesocosms and sampling date, using a Cyclops-7 fluorometer (Turner Designs, USA). The abundance of the different plankton fractions, except zooplankton, was estimated for all sampling dates. Two 50-mL samples were taken from each one of the mesocosms. One of these samples was fixed with acidified lugol 1% and used to quantify micro-phytoplankton, nano-phytoplankton and ciliates following the Utermöhl (1958) method. Chambers of 5 and 10 mL (depending on the plankton abundance) were left to sediment for 24 h, and the counting error was estimated according to Venrick (1978), accepting a maximum error of 15%. In the phytoplankton counting, algae were separated according to their size fractions. Two main groups of phytoplanktonic algae were recognised: nano-phytoplankton (algae with GALD $<30\,\mu m$) and large algae ($>30\,\mu m$). In the case of algae $>30\,\mu m$, we discriminated among filamentous species, colonial species and large diatoms, in order to analyse possible differences due to the presence of inedible species. Moreover, the abundances of eukaryotic algae and cyanobacteria were also discriminated.

A second sample from each mesocosm was preserved with ice-cold filtered glutaraldehyde 2% and used for picoplankton and HNF counts. Two subsamples of this

sample were filtered through 0.2 and 0.6 μm pore-size black polycarbonate filters Isopore GTPB and DTTP, Millipore, for picoplankton and nanoflagellates, respectively. A volume of 2 mL was filtered for picoplankton enumeration and of 3 mL for HNF. The material was stained with DAPI (Porter & Feig, 1980). Filters were mounted on a microscope slide with a drop of immersion oil for fluorescence (Immersol 518 F). Using epifluorescence microscopy, autotrophic eukaryote picoplankton and autotrophic prokaryote picoplankton was counted from the fluorescence given off by photosynthetic pigments, under blue and green light excitation (Callieri & Pinolini, 1995). HPP and HNF were counted under UV excitation. We used a Zeiss Axioplan microscope equipped with HBO 50 W lamp, a plan-apochromat 100× objective and a filter set for blue light excitation (BP 450–490 nm, FT 510 nm, LP 520 nm), green light excitation (BP 546 nm, FT 580 nm, LP 590 nm) and UV excitation (BP 365 nm, FT 395 nm, LP 397).

Zooplankton abundance was estimated only at t_0 and t_4, since sampling for zooplankton requires a large volume of water. At the end of the experiment the whole content of the enclosures was filtered through a 55-μm net. Micro- and protozooplankton samples were analysed in a 1-mL Sedgwick-Rafter counting cell under a binocular microscope, and subsamples of dense samples were taken with a Hensen-Stempel pipette. Macrozooplankton samples were examined and enumerated in a 5-mL Bogorov chamber under a stereomicroscope, and large samples were subsampled with a Russell device. Naupliar stages were discriminated. The number of aliquots to be counted (at least three) was calculated in order to keep the estimation error below 10%. Zooplankton abundance was expressed as individuals per litre of water.

Physical and chemical data

The following physical and chemical variables were measured in all of the enclosures on every sampling date: dissolved oxygen, temperature, pH and conductivity, with portable electronic meters Hanna HI 9143, HI991301 (Hanna Instruments, USA). A sample for nutrient analyses was also collected from each mesocosms and sampling date. Soluble reactive P (SRP), nitrates (N-NO_3) and ammonia (N-NH_4) were measured with a Hach DR/2010 spectrophotometer, using the corresponding kits of Hach reagents.

Data analyses

To analyse the statistical differences between treatments (with and without mesozooplankton), and among sampling dates, two-way repeated measures (RM) ANOVA were performed for each one of the components of the microbial assemblages, with treatment as the main factor and time as the RM. To test for the significance of the differences between treatments, post hoc comparisons were made by Student–Newman–Keuls test (SNK) (Underwood, 1997).

In the case of the zooplankton, a Wilcoxon test (Zar, 1996) was performed in order to analyse if the change in the community structure from t_0 to t_4 was significant ($p<0.05$). Correlations between pairs of biotic and abiotic variables were estimated using the Spearman non-parametric correlation coefficient (Conover, 1980).

Results

Physical and chemical properties of the water

Variations of the physical and chemical variables analysed in the enclosures throughout the experiment are shown in Fig. 2(a–f). Water temperature in the mesocosms followed the environmental variations of this parameter, varying from 23.8 °C (at the beginning of the experiment) to 16.6 °C (at the end). Differences between treatments were not significant with a significance level of 95%.

The mean values of dissolved oxygen varied between 6.4 and 8.6 mg L^{-1}. From t_3 to t_4, lower values of dissolved oxygen were observed in the treatment with mesozooplankton. This fact was corroborated by the inverse Spearman correlations between this parameter and the densities of cladocerans and copepods ($r=-0.76$ and $r=-0.78$, respectively; $p<0.05$). Nevertheless, taking into account all of the sampling dates, the RM ANOVA did not show significant differences in the oxygen values between treatments, whereas differences among dates were significant ($p<0.00001$).

The pH values ranged between 8.23 and 8.54, on average. The RM ANOVA evidenced significant differences between treatments and among times ($p=0.04$; $p=0.00005$ respectively). Temporal variations of the conductivity throughout the experiment were negligible (mean values ranged from 0.98 to 1.03 mS cm^{-1}).

Dissolved inorganic nitrogen (DIN = N-NH_4^+ + N-NO_3^-) increased significantly ($p=0.005$ for RM ANOVA) from t_3 to t_4 in both treatments. Higher mean values of DIN were registered at the end of the experiment in the mesocosms containing zooplankton, and in spite of the positive correlations between DIN and calanoids ($r=0.91$; $p<0.05$) and cladocerans ($r=0.90$; $p<0.05$), the differences between treatments were not statistically significant. The reduced form of nitrogen (ammonia) always prevailed over its oxidised form (nitrate) as usual in this wetland because of the high redox conditions.

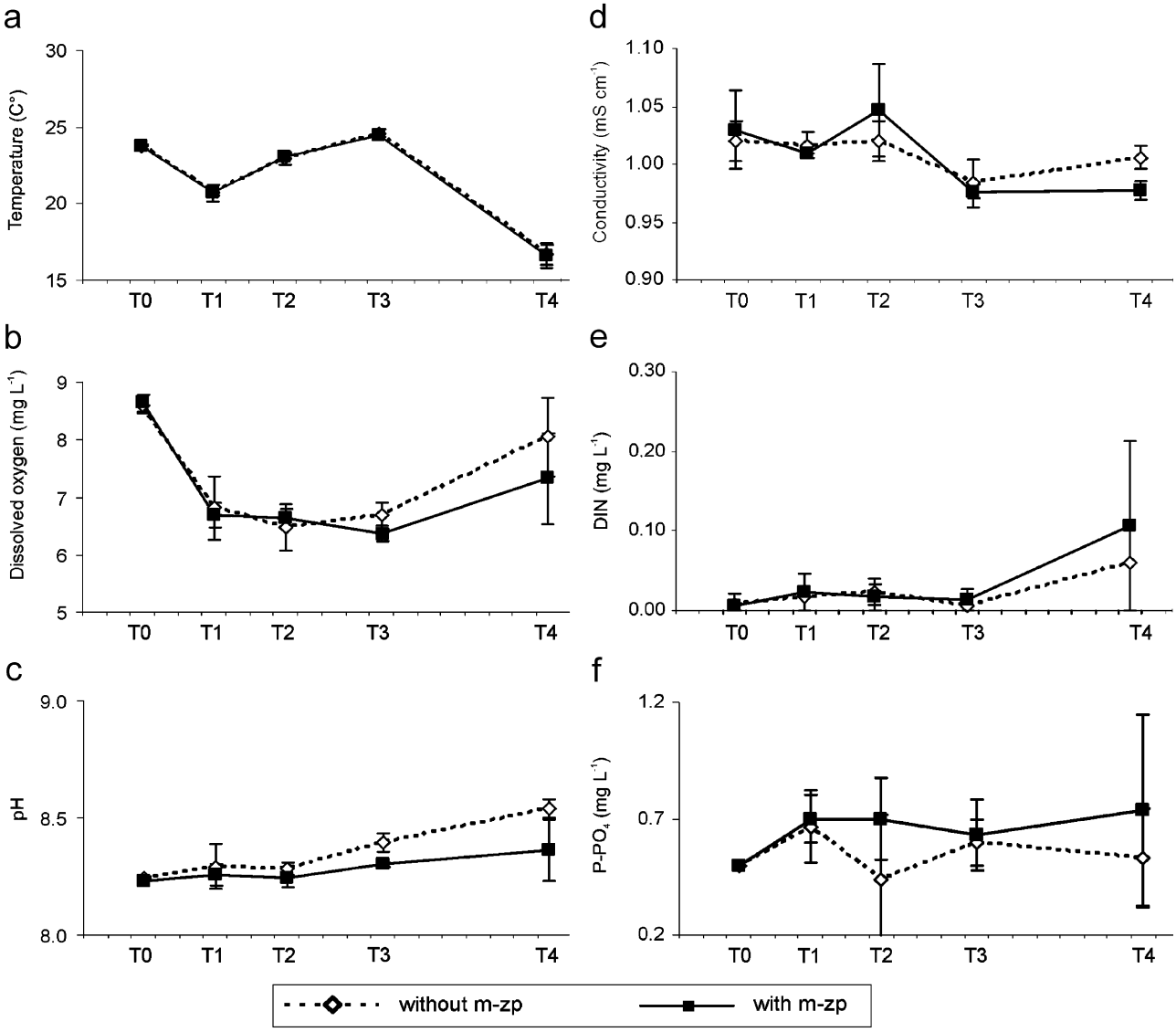

Fig. 2. Variation of the physical and chemical variables measured inside the enclosures without and with mesozooplankton (m-zp), during the experimental period. (a) Water temperature; (b) dissolved oxygen; (c) pH; (d) conductivity; (e) dissolved inorganic nitrogen (DIN); (f) phosphates.

Phosphate ($P\text{-}PO_4$) concentration showed different temporal patterns depending on the treatment. In the enclosures with mesozooplankton, mean concentrations increased from t_0 ($0.50\,mg\,L^{-1}$) to t_4 ($0.73\,mg\,L^{-1}$). On the contrary, in the enclosures without mesozooplankton values remained more constant and did not show any temporal pattern. In spite of this, the RM ANOVA did not evidence significant differences between treatments. Phosphate values were always rather high, and can be considered as non-limiting for phytoplankton.

Chlorophyll *a*

Mean chlorophyll *a* values registered were typical of eutrophic systems, ranging between 24.0 and $42.7\,\mu g\,L^{-1}$ (Fig. 3). Its concentration decreased in all of the enclosures at the very beginning of the experiment, from t_0 to t_1. From t_3 (day 10) until the end of the experiment, the differences between both treatments were notorious, presenting higher values in the mesocosms without mesozooplankton. The RM ANOVA showed significant differences between treatments ($p = 0.002$), and among dates ($p = 0.0004$).

Nano-phytoplankton (mostly algae 3–30 μm)

This size fraction (Fig. 4a) was dominated by Chlorophyceae and Cryptophyceae, such as *Monoraphidium contortum*, *Monoraphidium circinale*, *Monoraphidium minutum*, *Monoraphidium griffithii*, *Cryptomonas*

marssonii, *Cryptomonas erosa*, *Plagioselmis* sp., and several species of *Scenedesmus*, *Chlamydomonas*, *Chlorella*, and *Crucigenia*. Among the cyanobacteria, the dominant taxa were *Merismopedia tenuissima*, *Woronichinia elorantae*, *Romeria leopoliensis*, and *Aphanocapsa delicatissima*. Mean densities varied from 33,089 to 97,997 ind. mL^{-1}.

The abundance of this algal fraction decreased in the enclosures with mesozooplankton respect to those that excluded this component, and differences between treatments were significant according to the RM ANOVA ($p<0.054$). This analysis also revealed significant differences in the density of this algal fraction among dates ($p=0.000006$). Contrasts showed that differences in the density were significant between t_0 and t_4 in the treatment with mesozooplankton, and non-significant in the treatment with phytoplankton only ($p<0.05$).

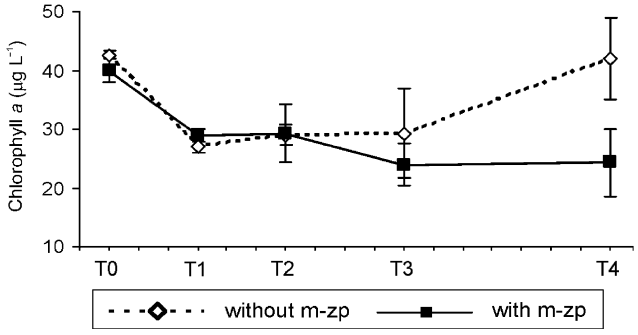

Fig. 3. Variation of total chlorophyll a during the experimental period in the enclosures without and with mesozooplankton (m-zp). Bars represent standard deviations.

Micro-phytoplankton fraction (algae >30 µm)

The micro-phytoplankton fraction (Fig. 4b) was dominated by filamentous cyanobacteria, with *Planktolyngbya limnetica*, *Raphidiopsis mediterranea, Anabaena* sp. and *Planktothrix aghardii*, as the more frequent species. Mean densities varied from 1345 to 24,966 ind. mL^{-1}.

Among chlorophytes and diatoms, the more frequent species were *Dictyosphaerium pulchellum*, *Closterium acutum* var. *variabile*, *Closterium aciculare*, *Staurastrum* sp., *Pediastrum tetras*, *Actinastrum hantzschii*, *Nitzschia acicularis* and *Aulacoseira granulata* var. *granulata*.

Although the RM ANOVA showed significant differences in the abundance of this algal fraction between treatments ($p<0.05$), in Fig. 4b it is evident that algae >30 µm increased in all the mesocosms from t_0 to t_3, suggesting that no grazing impact occurred on this fraction until day 10. Contrasts clearly showed that differences between treatments were only evident from t_3 to t_4 ($p<0.05$).

Heterotrophic nanoflagellates (HNF) and ciliates

The mean densities of HNF varied from 4571 to 9031 ind. mL^{-1}, while ciliates varied between 70 and

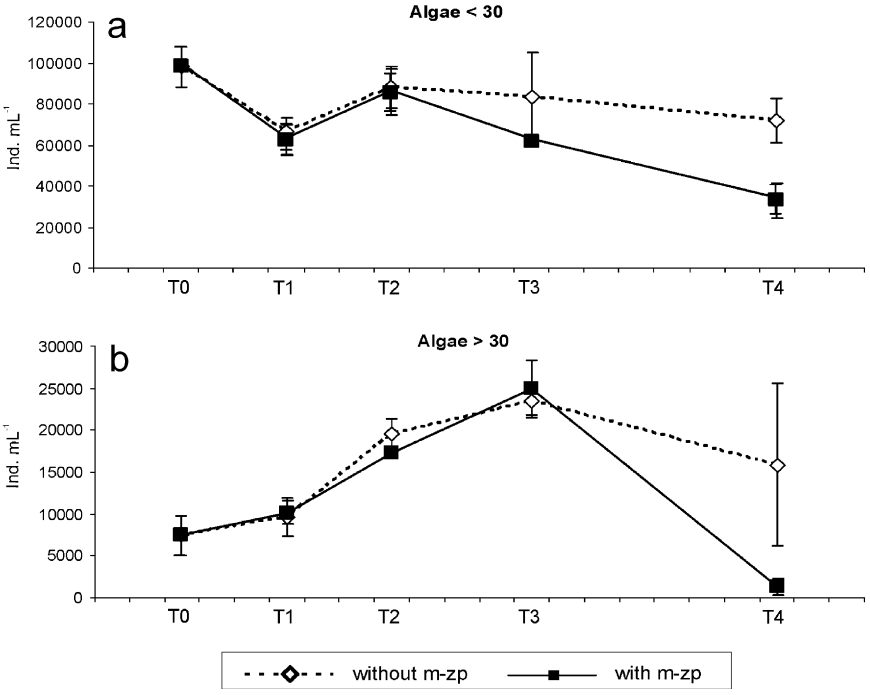

Fig. 4. Temporal variation of the abundance of the phytoplankton in the enclosures without and with mesozooplankton (m-zp). (a) Nano-planktonic algae (mainly 3–30 µm); (b) large algae (>30 µm). Bars represent standard deviations.

579 ind. mL^{-1} (Fig. 5). The assemblage of ciliates was mainly composed of Oligotrichida. On day 6, the abundance of HNF showed a peak in the enclosures without mesozooplankton. The temporal evolution of ciliates followed the same increasing pattern as HNF, but with a time lag in its peak abundance. On the contrary, in the enclosures with mesozooplankton, ciliates did not reach peaks of abundance, and their densities remained relatively constant until they strongly decreased towards the end of the experiment. Nevertheless, the RM ANOVA did not evidence significant differences between treatments for ciliates or HNF. In the case of HNF differences among dates were significant ($p = 0.014$).

Zooplankton

The zooplankton community comprised rotifers, cyclopoid copepods, calanoid copepods and cladocerans (Anomopoda and Ctenopoda). Among the rotifers, *Brachionus calyciflorus*, *Brachionus havanaensis*, *Brachionus austrogenitus*, *Polyarthra vulgaris*, *Filinia longiseta* and *Hexarthra mira* were the dominant taxa. Calanoids were mainly represented by *Notodiaptomus incompositus* and *Notodiaptomus spiniger*, whereas *Metacyclops mendocinus* was the most frequent cyclopoid. The dominant cladoceran was *Diaphanosoma brevireme*.

Fig. 6 illustrates the zooplankton composition at t_0 and t_4. At the beginning of the experiment, the mean densities of the different groups in the treatment with mesozooplankton were 12 calanoids L^{-1} (all stages included), 608 cyclopoids L^{-1} (all stages included) and 561 rotifers L^{-1}. On the final date, the assemblages exhibited a more equitable composition: 197 calanoids L^{-1}, 262 cyclopoids L^{-1}, 258 cladocerans L^{-1} and 111 rotifers L^{-1}. Differences in zooplankton composition between t_0 and t_4 were significant ($p < 0.0001$).

It is worthy to point out that, even when the water used to fill the mesocosms without mesozooplankton was filtered through a 55-μm-mesh net, some microzooplanktonic stages or smaller organisms, like eggs and early naupliar stages of cyclopoid, and small rotifers could have passed the net. Thus, at the end of the experiment, we found some small zooplankters in the treatment without mesozooplankton, whose densities were significantly lower than those in the mesocosms with mesozooplankton ($p = 0.019$).

Inverse pair correlations between the abundance of the nano-phytoplankton fraction and most of the zooplankton components were observed: adults and early stages of calanoids ($r = -0.89$; $p < 0.05$), calanoid nauplii ($r = -0.89$; $p < 0.05$), adult cladocerans ($r = -0.77$; $p < 0.05$) and early stages of cladocerans ($r = -0.90$; $p < 0.05$).

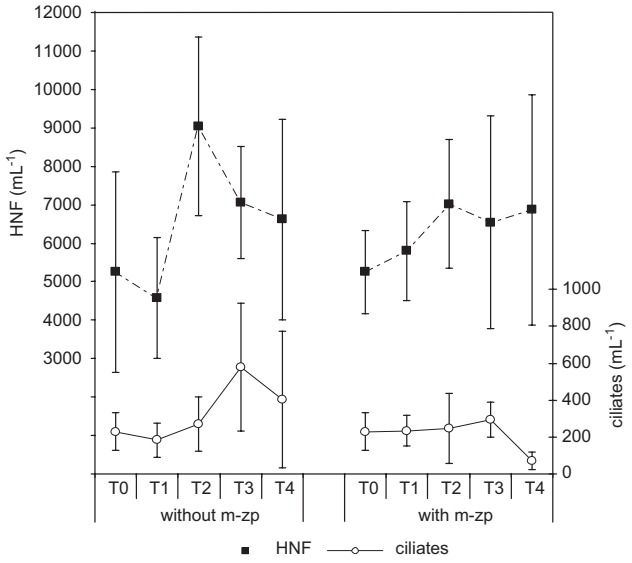

Fig. 5. Temporal variation of the abundance of the heterotrophic nanoflagellates (HNF) and ciliates in the enclosures without and with mesozooplankton (m-zp). Bars represent standard deviations.

Picoplankton fraction

The temporal evolution of the picoplankton fractions is shown in Fig. 7. Pico-eukaryotes (P-eu) exhibited lower abundances, ranging from 1.26×10^5 to 3.80×10^5 cells mL^{-1}, and were essentially represented by *Chlorella*-like cells. According to the RM ANOVA, the abundance of P-eu were significantly different between treatments ($p < 0.04$) and among dates ($p = 0.00002$). The abundances of ciliates and P-eu were inversely and significantly correlated ($r = -0.37$; $p < 0.05$).

Pico-cyanobacteria (Pcy) algae (*Synechococcus* and *Synechocystis*-like cells), varied from 4.61×10^5 to 6.30×10^5 cells mL^{-1}. The RM ANOVA did not evidenced significant differences between treatments or among dates. The total abundance of picoplankton varied between 1.4×10^6 and 1.76×10^6 cells mL^{-1}. In the enclosures with mesozooplankton, we observed an increase in the abundance of this fraction (Fig. 7).

Heterotrophic bacteria varied between 0.64×10^6 and 1.04×10^6 bact. mL^{-1}, increasing from the beginning until the end of the experiment in both treatments. Although the RM ANOVA did not reveal significant differences between treatments, we found significant ($p = 0.03$) differences among dates.

Discussion

Most of the studies conducted in the aquatic systems of the Otamendi Reserve were focused on the biodiversity and ecology of the phytoplankton community

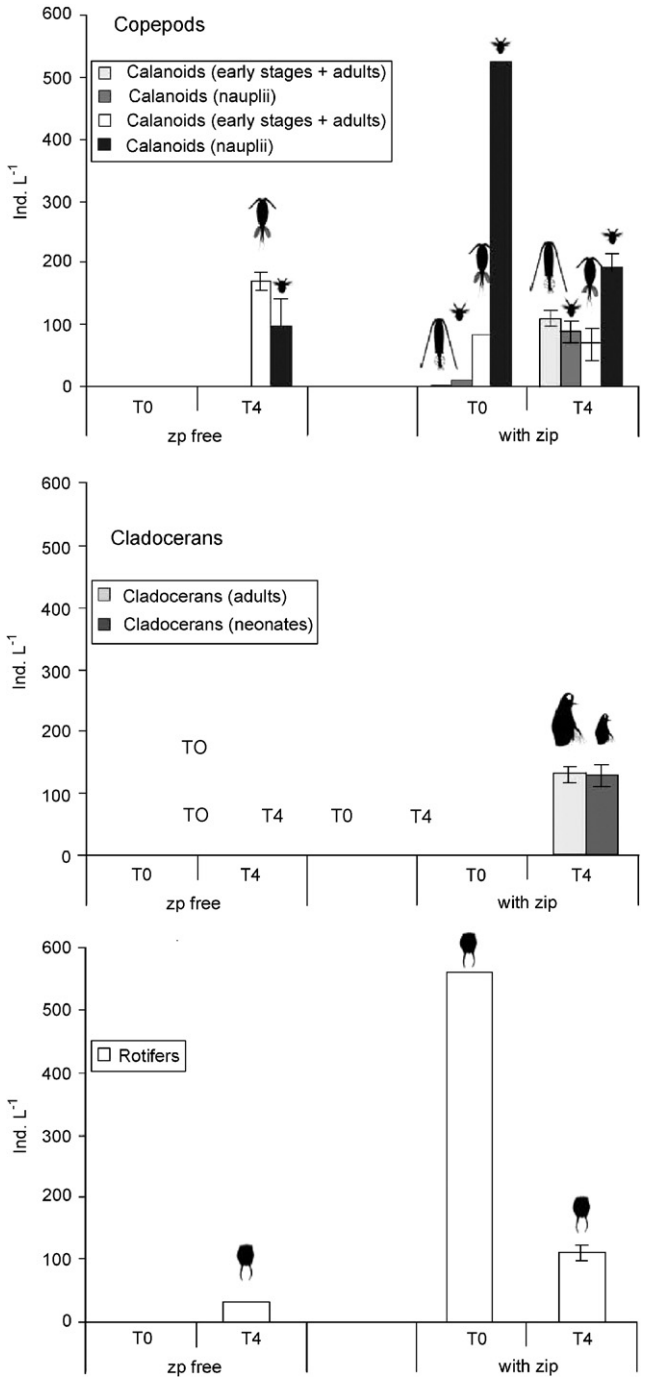

Fig. 6. Zooplankton composition in the enclosures without and with mesozooplankton (m-zp) at t_0 and t_4.

(Izaguirre et al., 2004, 2001; O'Farrell et al., 2003). These studies revealed that one of the most distinctive features of the water bodies of this wetland is the presence of profuse aquatic vegetation, whose spatial distribution and biomass vary throughout the year. Macrophytes exert a great influence on the structure and dynamics of planktonic communities, also playing an important role in the complex interactions that take place in the lakes of this wetland. Recent experimental studies carried out in the main shallow lake revealed the existence of some interactions among the microbial assemblages, and the influence of the floating macrophytes that determines a strong light attenuation in the water column, which affects the structure of the microbial communities (Sinistro et al., 2006).

The phytoplanktonic communities of this wetland are characterised by their high biodiversity. Many taxa among more than 300 phytoplankton species identified are mixotrophic. The microbial assemblages comprise a great variety of HNF and ciliates, heterotrophic bacteria and autotrophic picoplankton (including eukaryote and prokaryote species).

The rich zooplankton community is represented by many species of rotifers, cyclopoids, calanoids, and cladocerans, which are reported for the first time for this wetland. During some periods of the year, when zooplankton abundance is very high (total density $>800\,\text{ind.}\,\text{L}^{-1}$), clear water phases are evident in the lake (Sinistro, unpublished data). Differing from the zooplankton communities of shallow lakes in the Northern Hemisphere, which are frequently dominated by *Daphnia* spp. (e.g., Jeppesen et al., 2005), the studied lake usually exhibits a high abundance of copepods and *Diaphanosoma*.

Regarding the temporal evolution of the nano-phytoplankton fraction during the experiment, a decrease in its concentration was observed from t_0 to t_1 in both treatments, probably because of an enclosure effect. Chlorophyll *a* concentration showed the same pattern. Thereinafter, the results of our experiment showed that zooplankters exerted a significant grazing pressure on the nano-phytoplankton fraction. Conversely, the increase in the abundance of the large algae in both treatments until day 10 suggests that this fraction has been negatively selected by the zooplankton during this period. At the beginning of the experiment, the zooplankton assemblages comprised a great number of rotifers and copepod nauplii. In this sense, our results support the concept of Hulot's model (Hulot, Lacroix, Lescher-Moutoué, & Loreau, 2000), which divides the herbivorous zooplankton in two categories (small and large), which prey on two phytoplankton categories (unprotected and protected ones). Large algae (usually $>35\,\mu\text{m}$) and gelatinous algae are considered protected species. Small herbivores (mainly rotifers and copepod nauplii) feed only on unprotected phytoplankton, whereas large herbivores (cladocerans plus adult and pre-adult copepods) can also prey on protected algae. Thus, the decrease of large algae (mainly represented by thin filamentous cyanobacteria) from t_3 to t_4 in the mesocosms with mesozooplankton may be explained by an increase in the abundance of large herbivores in the enclosures. In spite of their difficulty of ingesting long filaments, certain cladocerans are able to arrange them into spaghetti-like bundles (Nadin-Hurley & Duncan,

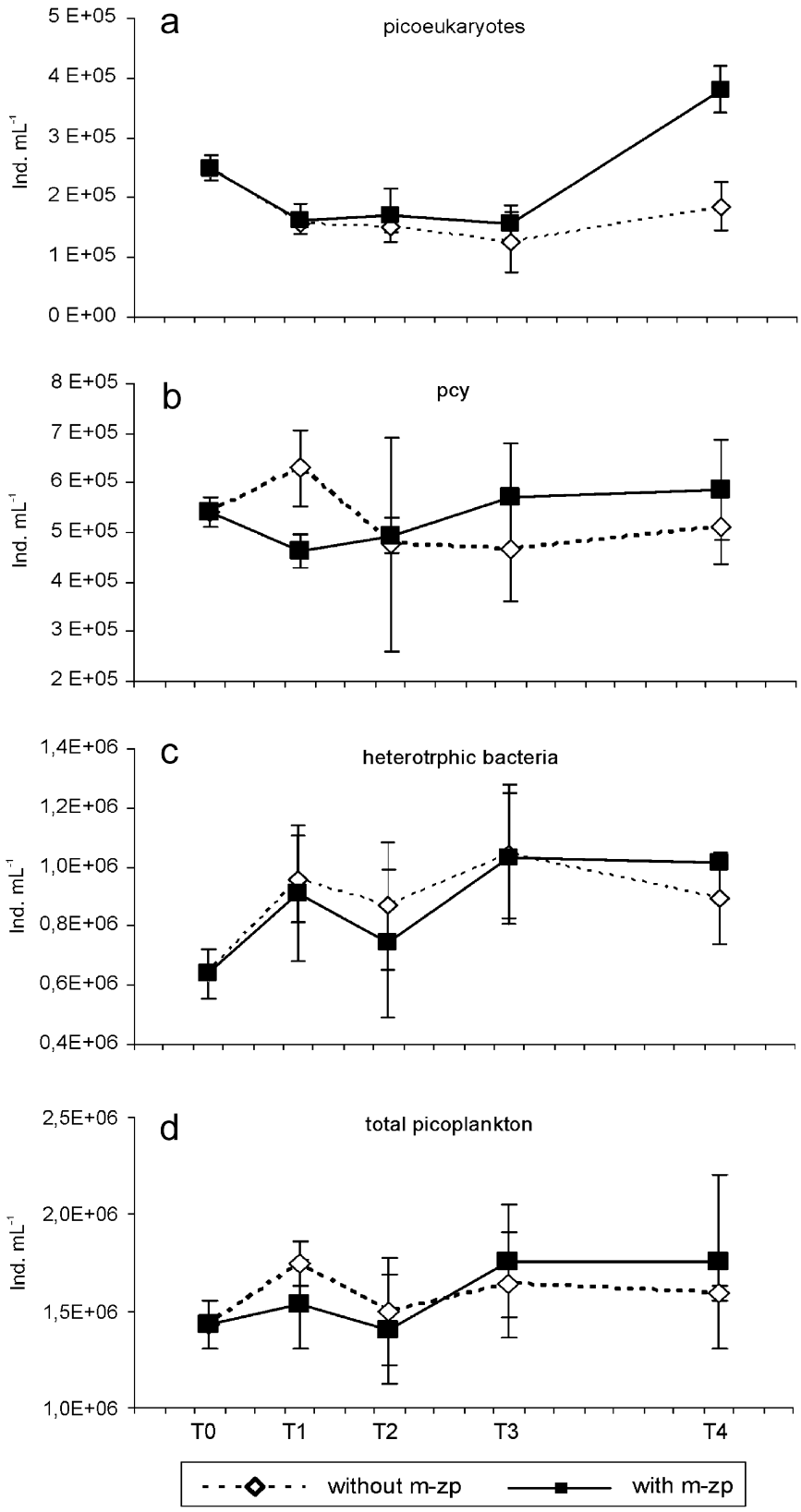

Fig. 7. Variation of the autotrophic and heterotrophic picoplankton fraction during the experiment in the enclosures without and with mesozooplankton (m-zp). (a) Picoplanktonic eukaryotes; (b) picoplanktonic cyanobacteria (Pcy); (c) heterotrophic bacteria; (d) total picoplankton. Bars represent standard deviations.

1976). Adults of copepods can feed on large algae and ciliates, which are actively captured and manipulated with their maxillae and maxillipeds (Reynolds, 2006). The final decline of ciliates (Fig. 5) in both treatments (consider that some smaller stages and eggs remained in the treatment without mesozooplankton) can be explained by the direct effects (predation, interference) and indirect effects (resource competition) produced by copepods, cladocerans and even rotifers on these protozoans (Adrian & Schneider-Olt, 1999; Arndt, 1993; Gilbert, 1993; Jack & Gilbert, 1994).

Sommer et al. (2003) have proposed that a higher functional diversity of the zooplankton community would be more effective in reducing phytoplankton biomass. The results of our experiment seem to support this hypothesis, because the control of large algae would have only started when the zooplankton in the enclosures included abundant adults of copepods and cladocerans.

Regarding the third hypothesis of this work, the results of our experiment seem to support the concept of a four-link trophic cascade described in other works (Schnetzer & Caron, 2005; Sommer et al., 2003), since a trophic cascade effect on the lower levels apparently occurred. As already mentioned, in the enclosures lacking zooplankton, ciliate abundance followed the same increasing pattern as HNF, but with a time lag in its response. Lags in the response are due to the different generation times among trophic levels, which were well described by Carpenter et al. (1985). On the other hand, total picoplankton abundance increased in the enclosures with mesozooplankton, reaching its peak only after a marked decrease of small ciliates. Although HNF are the main grazers of picoplankton, small ciliates can also exert a grazing pressure on this fraction (Callieri, Karjalainen, & Passoni, 2002; Pernthaler, 2005; Pernthaler et al., 1996). From the analysis of the temporal evolution of the heterotrophic bacteria, it is evident that their abundance increased in all of the enclosures from t_0 to t_4. A possible explanation for this increase is that HNF and small ciliates usually prefer to consume autotrophic picoplankton rather than heterotrophic bacteria (Pernthaler et al., 1996).

Nevertheless, the zooplankton impact on the microbial assemblages involves confounding interactions, and there is some controversy about the trophic cascades at these levels. For example, Pace and Funke (1991) predicted that the cascading trophic interactions can be truncated at the level of protozoa, and explained the possible causes of this fact. In another study, Schnetzer and Caron (2005) also have reported that the responses of the pico-prokaryotes to copepod abundances did not follow the expected pattern assuming the four-link cascade copepods–microzooplankton–HNF–picoplankton. In particular, copepod grazing activity increases the substrate available for bacterial growth, thus compensating for their decrease due to predation. In another study, Adrian et al. (2001) found a strong response of the ciliates to mesozooplankton manipulations, weak responses by the HNF and no response by the bacteria, thus supporting the idea of a diluted cascading effect at the lower levels of the microbial food web.

In this work we did not analyse the bottom–up effects, but according to the studies carried out in this wetland during the last 7 years, there is evidence that P is not limiting for phytoplankton growth, but N can be limiting under certain conditions (Unrein, 2001). Due to the high redox conditions, the prevalent form of nitrogen in this wetland is its reduced form (ammonia). Although there is a continuous release of nitrogen from the sediments of the lake to the water column, its uptake by algae would be very fast because the concentration of DIN is usually very low. In our experiment we found relatively high phosphate concentrations and rather low levels of DIN. The last increase registered in all of the mesocosms, on day 14, would be explained by a rain that occurred the day before sampling. The role of nitrogen in the regulation of the food webs in this wetland deserves further studies that combine the effects of top–down and bottom–up.

Our investigation is the first one to deal with trophic cascades in a typical mixotrophic shallow lake, with high content of humic substances, belonging to the Lower Paraná River floodplain. This paper provides evidence of the zooplankton impact on phytoplankton and the microbial assemblages for this wetland, also showing the complex cascade interactions occurring within the microbial food web.

Acknowledgements

We thank the staff of the Otamendi Natural Reserve (Administración de Parques Nacionales) for their assistance in the field work. This research was supported by UBA (University of Buenos Aires), CONICET and a grant of ANCYPT (Argentina) (PICT 01-12332). We thank the valuable suggestions made by two anonymous reviewers.

References

Adrian, R., & Schneider-Olt, B. (1999). Top–down effects of crustacean zooplankton on pelagic microorganisms in a mesotrophic lake. *Journal of Plankton Research*, *21*, 2175–2190.

Adrian, R., Wickham, S. A., & Butler, N. M. (2001). Trophic interactions between zooplankton and the microbial community in contrasting food webs: The epilimnion and deep

chlorophyll maximum of a mesotrophic lake. *Aquatic Microbial Ecology*, *24*, 83–97.

Arndt, H. (1993). Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates) – a review. *Hydrobiologia*, *255*, 231–246.

Attayde, J. L., & Hansson, L.-A. (1999). Effects of nutrient recycling by zooplankton and fish on phytoplankton communities. *Oecologia*, *121*, 47–54.

Balseiro, E. G., Modenutti, B. E., & Queimaliños, C. P. (1997). Nutrient recycling and shifts in N:P ratio by different zooplankton structures in a south Andes lake. *Journal of Plankton Research*, *19*, 805–817.

Callieri, C., Karjalainen, S. M., & Passoni, S. (2002). Grazing by ciliates and heterotrophic nanoflagellates on picocyanobacteria in Lago Maggiore, Italy. *Journal of Plankton Research*, *24*, 785–796.

Callieri, C., & Pinolini, M. L. (1995). Picoplankton in Lake Maggiore, Italy. *Internationale Revue der Gesamten Hydrobiologie*, *80*, 491–501.

Carpenter, S. R., & Kitchell, J. F. (1988). Consumer control of lake productivity. Large-scale experimental manipulations reveal complex interactions among lake organisms. *BioScience*, *38*, 764–769.

Carpenter, S. R., Kitchell, J. F., & Hodgson, J. R. (1985). Cascading trophic interactions and lake productivity: Fish predation and herbivory can regulate lake ecosystems. *BioScience*, *35*, 634–639.

Carrillo, P., Reche, I., Sánchez Castillo, P., & Cruz-Pizarro, L. (1995). Direct and indirect effects of grazing on the phytoplankton seasonal succesion in an oligotrophic lake. *Journal of Plankton Research*, *17*, 1363–1379.

Conover, W. J. (1980). *Practical nonparametric statistics*. New York: Wiley.

Gilbert, J. J. (1993). Rotifers as predators on small ciliates. *Hydrobiologia*, *255/256*, 247–253.

Gliwicz, Z. M. (2002). On the different nature of top–down and bottom–up effects in pelagic food webs. *Freshwater Biology*, *47*, 2296–2312.

Hahn, M. W., & Hofle, M. G. (2001). Grazing of protozoa and its effect on populations of aquatic bacteria. *FEMS Microbiology Ecology*, *35*, 113–121.

Hulot, F. D., Lacroix, G., Lescher-Moutoué, F., & Loreau, M. (2000). Functional diversity governs ecosystem response to nutrient enrichment. *Nature*, *405*, 340–344.

Izaguirre, I., O'Farrell, I., Unrein, F., Sinistro, R., dos Santos Afonso, M., & Tell, G. (2004). Algal assemblages across a wetland, from a shallow lake to relictual oxbow lakes (Lower Paraná River, South America). *Hydrobiologia*, *511*, 25–36.

Izaguirre, I., Sinistro, R., O'Farrell, I., Unrein, F., & Tell, G. (2001). Algal assemblages in anoxic relictual oxbow lakes from the Lower Paraná floodplain (Argentina). *Nova Hedwigia*, *123*, 95–106.

Jack, J. D., & Gilbert, J. J. (1994). Effects of *Daphnia* on microzooplankton communities. *Journal of Plankton Research*, *16*, 1499–1512.

Jeppesen, E., Søndergaard, M., Jensen, J. P., Havens, K. E., Anneville, O., Carvalho, L., et al. (2005). Lake responses to reduced nutrient loading – an analysis of contemporary long-term data from 35 case studies. *Freshwater Biology*, *50*, 1747–1771.

Jürgens, K., & Jeppesen, E. (2000). The impact of metazooplankton on the structure of the microbial food web in a shallow, hypertrophic lake. *Journal of Plankton Research*, *22*, 1047–1070.

Katechakis, A., Stibor, H., Sommer, U., & Hansen, T. (2002). Changes in the phytoplankton community and microbial food web of Blanes Bay (Catalan Sea, NW Mediterranean) under prolonged grazing pressure by doliolids (Tunicata), cladocerans and copepods (Crustacea). *Marine Ecology Progress Series*, *234*, 55–69.

Nadin-Hurley, C. M., & Duncan, A. (1976). A comparison of daphnid gut particles with the sestonic particles in two Thames Valley reservoirs throughout 1970 and 1971. *Freshwater Biology*, *6*, 109–123.

O'Farrell, I., de Tezanos Pinto, P., Izaguirre, I. (in press). A pattern of morphological variability in phytoplankton in response to different light conditions. *Hydrobiologia*.

O'Farrell, I., Sinistro, R., Izaguirre, I., & Unrein, F. (2003). Do steady state assemblages occur in shallow lentic environments from wetlands? *Hydrobiologia*, *502*, 197–209.

Pace, M. L., Cole, J. J., & Carpenter, E. J. (1998). Trophic cascades and compensation: Differential responses of microzooplankton in whole-lake experiments. *Ecology*, *79*, 138–152.

Pace, M. L., & Funke, E. (1991). Regulation of planktonic microbial communities by nutrients and herbivores. *Ecology*, *73*, 904–914.

Pernthaler, J. (2005). Predation on prokaryotes in the water column and its ecological implications. *Nature Reviews Microbiology*, 1–10.

Pernthaler, J., Šimek, K., Sattler, B., Schwarzenbacher, A., Bobkova, J., & Psenner, R. (1996). Short-term changes of protozoan control on autotrophic picoplankton in an oligo-mesotrophic lake. *Journal of Plankton Research*, *18*, 443–462.

Porter, K. G., & Feig, Y. S. (1980). The use of DAPI for identifying and counting aquatic microflora. *Limnology and Oceanography*, *25*, 943–948.

Queimaliños, C. P., Modenutti, B. E., & Balseiro, E. G. (1998). Phytoplankton responses to experimental enhancement of grazing pressure and nutrient recycling in a small Andean lake. *Freshwater Biology*, *40*, 41–49.

Reynolds, C. S. (2006). *The ecology of phytoplankton*. Cambridge: Cambridge University Press.

Sanders, R. W., Porter, K. G., Bennett, S. J., & DeBiase, A. E. (1989). Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. *Limnology and Oceanography*, *34*, 673–687.

Schnetzer, A., & Caron, D. A. (2005). Copepod grazing impact on the trophic structure of the microbial assemblage of the San Pedro Channel, California. *Journal of Plankton Research*, *27*, 959–971.

Sinistro, R., Izaguirre, I., Asikian, V. (2006). Experimental study on the microbial plankton community in a South American wetland (Lower Paraná River Basin) and the effect of the light deficiency due to the floating macrophytes. *Journal of Plankton Research*. 〈http://plankt.oxfordjournals.org/cgi/content/abstract/fbl008v1〉 (advance access published online 5 December 2006).

Sommer, U., Sommer, F., Santer, B., Jamieson, C., Boersma, M., Becker, C., et al. (2001). Complementary impact of copepods and cladocerans on phytoplankton. *Ecology Letters*, *4*, 545–550.

Sommer, U., Sommer, F., Santer, B., Zöllner, E., Jürgens, K., Jamieson, C., et al. (2003). *Daphnia* versus copepod impact on summer phytoplankton: Functional compensation at both trophic levels. *Oecologia*, *135*, 639–647.

Underwood, A. J. (1997). *Experiments in ecology: Their logical design and interpretation using analysis of variance*. London: Cambridge University Press.

Unrein, F. (2001). *Efecto de los nutrientes y el pH sobre el crecimiento y la estructura del fitoplancton en ambientes de la llanura aluvial del Paraná Inferior*. Ph.D. thesis, Facultad de Ciencias Exactas y Naturales, University of Buenos Aires, Argentina.

Urabe, J. (1993). N and P cycling coupled by grazers' activities: Food quality and nutrient release by zooplankton. *Ecology*, *74*, 2337–2350.

Utermöhl, H. (1958). Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitteilungen Internationale Vereinigung für theoretische und angewandte Limnologie*, *9*, 1–38.

Venrick, E. L. (1978). How many cells to count? In A. Sournia (Ed.), *Phytoplankton manual* (pp. 167–180). Paris: Unesco.

Williamson, C. E., Morris, D. P., Pace, M. L., & Olson, O. G. (1999). Dissolved organic carbon and nutrients as regulators of lake ecosystems: Resurrection of a more integrated paradigm. *Limnology and Oceanography*, *44*, 795–803.

Zar, J. H. (1996). *Biostatiscal analysis* (3rd ed.). Upper Saddle River, NJ: Prentice Hall.