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Top-down and bottom-up regulation of planktonic communities in a warm temperate wetland

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This field experimental study simultaneously analysed the effects of predation (top-down) and nutrients (bottom-up) on planktonic communities (phytoplankton, zooplankton, heterotrophic nanoflagellates and ciliates) in a warm temperate wetland in South America. The top-down and bottom-up controls were investigated by assessing the impact of omnivorous–planktivorous fish (*Jenynsia* sp.) and the effects of nutrient input from natural lake sediments, respectively. Three treatments and a control were run in triplicate in mesocosms and samples were taken at Days 0, 3, 7 and 15. The control contained all the planktonic components while treatments included all planktonic components plus the addition of either planktivorous fish (F), natural wetland sediments in dialysis bags (S) or both of them (SF). A bottom-up effect due to nutrient release from sediment (mainly total phosphorus) was noticed in treatments S and SF. Phytoplankton abundance increased in all treatments compared with the control. Thus, phytoplankton appeared to be bottom-up controlled while fish exerted a strong predation pressure on zooplankton (top-down), because treatments F and SF showed a marked decrease in mesozooplankton abundance. The results obtained in this study agree with the hypothesis that phytoplankton regulation by zooplankton might be weaker in warm temperate systems than in temperate ones.

INTRODUCTION

Whether the control of the food webs is exerted by upper trophic levels on lower ones or vice versa has long been the subject of scientific debate (Hairston *et al.*, 1960; Carpenter *et al.*, 1985). Although both forces are known to occur in nature, they may differ in magnitude. Shapiro (Shapiro *et al.*, 1975) has provided evidence indicating that community biomass and productivity are regulated by the next higher trophic level. Also, Carpenter and Kitchell (Carpenter and Kitchell, 1988) postulated that in aquatic ecosystems, the complex trophic relationships are interconnected in a cascade or by a network of links, so that a change in any component will have an effect on the other ones. On the other hand, several authors found that nutrient loading explained a great amount of variation in phytoplankton biomass and production (Schindler, 1978; Smith, 1982; McCauley *et al.*, 1989).

Until some decades ago, fish were not included in studies concerning trophic interactions because they were thought to play a minor role in the regulation of aquatic communities. Currently, fish are known to interact with all trophic levels either directly or indirectly through trophic cascades, nutrient transport and nutrient re-suspension (Matthews, 1998). The strength of the top-down control critically depends on the

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prey/predator size ratio at all the trophic levels. Gliwicz and Pijanowska (Gliwicz and Pijanowska, 1989) postulated that the composition of a zooplankton community may become dominated by small-bodied species because planktivorous fish, which are visual predators, consume selectively large-bodied zooplankters. Gliwicz (Gliwicz, 2003) observed that the composition and distribution of zooplankton size differ markedly between systems with and without zooplanktivorous fish. In this sense, Brooks and Dodson (Brooks and Dodson, 1965) state that large-bodied zooplankters are more efficient at grazing on phytoplankton than their smaller competitors, which are restricted to consume small particles. Thus, the strength of the top-down control critically depends on the prey/predator size ratio at all trophic levels.

Another important effect of zooplankton is related to nutrient recycling, especially when this is limiting for phytoplankton (Carrillo *et al.*, 1995; Balseiro *et al.*, 1997; Queimaliños *et al.*, 1998; Attayde and Hansson, 1999). In oligotrophic lakes, which are highly dependent on internal recycling, zooplankton may play a major role in nutrient availability; however, their importance depends on the trophic state of the system.

In general, grazing by zooplankton leads to a decrease in phytoplankton biomass. Nonetheless, phytoplankton regulation by zooplankton might be weaker in tropical systems than is generally found in temperate regions (Von Rückert and Giani, 2008). In addition, zooplankton may affect community structure as some non-edible algae may become more abundant during the period of active grazing due to selective feeding. Under such conditions, larger sized algae are subjected to a lower interspecific competitive pressure and can make use of an abundant supply of nutrients from zooplankton excretion (Queimaliños *et al.*, 1998).

Recent studies suggest that zooplankton biomass is lower in subtropical than in temperate lakes, particularly when considering biomass of phytoplankton (Havens *et al.*, 2009). One possible explanation is that in subtropical lakes the effect of predation by planktivorous fish is the main factor controlling the biomass of large zooplankton (Jeppesen *et al.*, 2005, 2007, Meerhoff *et al.*, 2007; Iglesias *et al.*, 2008). Moreover, the low biomass and scarcity of large effective grazers (Hamza *et al.*, 1995; Havens *et al.*, 1996) do not produce changes either in biomass or in phytoplankton composition in subtropical lakes (Havens *et al.*, 2009).

In South America, there have been several studies assessing the effect of planktivorous fish on zooplankton (Northcote *et al.*, 1990; Boveri and Quirós, 2007; Meerhoff *et al.*, 2007; Iglesias *et al.*, 2007; Sinistro *et al.*, 2007; Iglesias *et al.*, 2008). Few experimental studies were carried out the combined effect with the addition of nutrients (Rejas *et al.*, 2005; Acuña *et al.*, 2008). Moreover, Rejas *et al.* (Rejas *et al.*, 2005) showed deviations from trophic cascade-based expectations, suggesting that trophic cascades may be weak in tropical lakes.

Here we experimentally examine the simultaneous effects of predation (top-down) and nutrients (bottom-up) on planktonic communities in a warm temperate wetland. The top-down control was investigated by assessing the impact of planktivorous fish predation on the abundance, size structure and species composition of zooplankton, and its cascading effect on some microbial components [phytoplankton, ciliates and heterotrophic flagellates (HNF)]. The bottom-up control was investigated by determining the effects of nutrient release from the natural wetland sediments on phytoplankton composition and abundance.

METHOD

Study site

The experiment was carried out in the main shallow lake (Laguna Grande) of the Otamendi Natural Reserve, a warm temperate floodplain wetland in Argentina (34°10′ to 34°17′S; 58°48′ to 58°53′W) (Fig. 1). The water body has a surface area of ~156 ha, and the littoral exhibits aquatic vegetation. The climate of the region is temperate with rainfall throughout the year; the

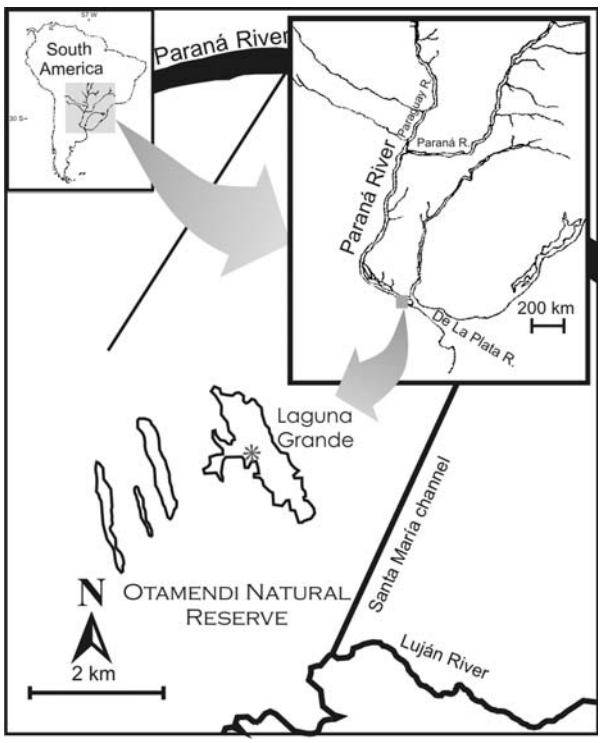

Fig. 1. Map of the study area showing the site where mesocosms were installed.

environmental temperature of the warmest month is above 22°C. Moreover, the wetland system of the region modifies the main climatic variables (i.e. extreme temperature, hydrological deficiency) thus generating conditions more similar to the humid subtropical climate than to the temperate sub-humid characteristic from the surrounding area (Malvares, 1999). The concentrations of phosphates in the water are high and typical of eutrophic systems but dissolved inorganic nitrogen (DIN) may become limiting for phytoplankton under conditions of active algal growth (Sinistro *et al.*, 2006). Following Williamson *et al.* (Williamson *et al.*, 1999) we classify the aquatic systems of this wetland as "mixotrophic lake ecosystems", with high dissolved organic carbon and total phosphorus (TP) contents.

Experimental design

The experiment was performed in the pelagic area of "Laguna Grande" (100 m offshore) in an area without submerged, emergent or floating plants, using a mesocosm approach (50 L high-density polyethylene bags equipped with floating devices). Bags were not open to the sediments. The experimental design consisted of three treatments and a control, in triplicate. The different treatments assessed either the separate or the combined effects of "top down" and "bottom up" as follows:

- Control (C): planktonic components (zooplankton, phytoplankton, HNF and ciliates) without sediments and fishes.
- Sediments (S): planktonic components and lake sediments in a dialysis bag, without fishes.
- Fish (F): planktonic components plus planktivorous fish (*Jenynsia* sp.) and without sediments.
- Sediments + Fish (SF): planktonic components, lake sediments in a dialysis bag and planktivorous fish (*Jenynsia* sp.).

In S and SF, we used lake sediments as nutrient sources in an attempt to reproduce the usual way in which nutrients are released from the sediments into the water column. The dialysis tubing cellulose membrane with a pore size excluding molecules larger than 12 400 MW, Sigma-Aldrich (dialysis bags) was filled with sediment from the Natural Reserve Otamendi. The dialysis bags were placed inside the mesocosms which allowed the interchange of gases and dissolved nutrients but prevented the entrance of organisms into the water column, because the sediment layer may be a reservoir of resting stages of planktonic organisms (Ortega-Mayagoitia *et al.*, 2003). The surface of the dialysis bags was equivalent to the surface exposed to the water column if the mesocosms were open at the bottom (0.11 m^2).

The planktivorous fish (*Jenynsia* sp.) used in the experiment were collected from the environment. The number of specimens added in each mesocosm (79 fish m^{-2}) was based on literature concerning the abundance of the planktivorous fish in wetlands at similar latitudes (Mazzeo *et al.*, 2003; Iglesias *et al.*, 2008). The maximum total length of fishes ranged on average between 1.5 and 2 cm.

The experiment started on 25 September and finished in 10 October 2006; samples and measurements were obtained at Days 0, 3, 7 and 15.

Sampling and laboratory procedures

The abundance of the different planktonic fractions was estimated on all sampling dates, except for zooplankton. Samples of 50 mL were taken from each mesocosm and preserved in 1% acidified Lugol's iodine solution for microphytoplankton and nanophytoplankton quantification, following Utermöhl (Utermöhl, 1958). Counting error was estimated according to Venrick (Venrick, 1978), accepting a maximum error of 15%. Algae were sorted by size during phytoplankton counting based on the size-selective predation by the different zooplankters: small and edible algae with greatest axial linear dimension (GALD) less than 30 μm and large and usually un-edible algae (GALD > 30 μm). In turn, the latter fractions were separated into eukaryotes, cyanobacteria, filamentous species, colonial species and large diatoms to detect possible differences in non-edible species. Ciliates and HNF abundance was counted simultaneously to phytoplankton, counting at least 100 individuals of each group.

Zooplankton abundance was estimated at the beginning and at the end of the experiment (Days 0 and 15) because of the large water volume required for zooplankton counting. At the end of the experiment, the content of the enclosures was filtered through a 55 μm pore mesh. Micro- and protozooplankton samples were analysed in 1 mL Sedgwick–Rafter counting cells under a binocular microscope, and subsamples were obtained using a Hensen-Stempel pipette. Macrozooplankton was counted in 5 mL Bogorov chambers under a stereoscope microscope, and subsamples were taken with a Russell device. The larval stages were recorded and the number of counted aliquots (at least three) was calculated with a maximum error of 10%.

Physicochemical data

Dissolved oxygen, temperature, pH and conductivity were measured *in situ* in all enclosures and sampling dates using portable electronic meters Hanna HI 9143

and HI991301 (Hanna Instruments, USA). Likewise, samples were collected for nutrient analyses in the laboratory. Dissolved phosphorus ($P\text{-}PO_4$), nitrate and nitrite ($N\text{-}NO_3 + N\text{-}NO_2$) and ammonia ($N\text{-}NH_4$) were measured with a Hach DR/2010 spectrophotometer, using the corresponding kits of HACH reagents. Nitrogen forms were analysed as DIN ($DIN = N\text{-}NH_4 + N\text{-}NO_3 + N\text{-}NO_2$). Total fractions were assessed at the beginning and at the end of the experiment. TP and total nitrogen (TN) were determined as $P\text{-}PO_4$ and $N\text{-}NO_3 + N\text{-}NO_2$with a Hach DR/2010 spectrophotometer after their simultaneous digestion with the persulfate method (American Public Health Association, 2005).

Data analyses

Statistical differences among treatments and sampling dates were tested using two-way repeated measures (RM) ANOVA for each component of the microbial assemblages, using fish and sediment as the mains factor and time as the RM (Zar, 1996). Later on, Duncan's a posteriori multiple comparisons were carried out to identify the treatment(s) that showed significant differences; this test has rules for computing a minimum average risk least sgnificant difference (Bliss, 1967). TN and TP concentrations at the beginning and the end of the experiment were compared by means of a one-way ANOVA. The treatments with fish (F and SF) were excluded from this analysis, because the digestion of the samples did not include fishes. Thus, part of the biomass product of the predation of fish on the zooplankton was lost from the analysis.

RESULTS

Physicochemical variables

Water temperature ranged between 15.5 and 22.8°C (Fig. 2A) with no significant differences among treatments. Dissolved oxygen decreased significantly throughout the experiment in all treatments, always remaining above 4.9 mg L^{-1} (Fig. 2B). Treatments with (S and SF) and without sediments (C and F) exhibited differences over time, where the oxygen concentration was higher in S and SF at Day 3; whereas on Day 15 it increased in treatments C and F (Table I). No significant differences were found among treatments, but at the end of the experiment there was a trend towards a lower dissolved oxygen concentration in the treatments with sediments (S, SF). Mean pH values decreased from 9.03 to 8.16 (Fig. 2C) throughout the experiment with no significant differences among treatments at Days 0, 3, 7, and significant differences with lower pH values occurred in treatments with sediments at the end of the experiment (15 days). Mean conductivity ranged between 2.12 and 2.38 mS cm^{-1} with significant differences during the experiment but no significant differences among treatments (Fig. 2D).

Dissolved phosphorus ($P\text{-}PO_4$) (Fig. 2E) decreased significantly from Day 0 (2.9 and 3.2 μM) to Day 15 (undetectable) in all the enclosures: differences among treatments were not significant. Final values (15 days) were below concentrations potentially limiting for phytoplankton growth [0.1 μM P, Reynolds (Reynolds, 2006)]. Likewise, DIN (Fig. 2F) significantly decreased towards the end of the experiment in all treatments with significant differences among treatments with and without sediments on Day 3, where DIN concentrations were higher in treatments without sediments. On Day 0 mean values ranged between 87 and 103 μM and on Day 15 between 15 and 21 μM: the lowest values occurred at Day 7. Ammonia contributed more to DIN than nitrate, and both nitrogenous forms followed the same temporal trend. Final concentrations were above values potentially limiting for phytoplankton growth [7 μM N, Reynolds (Reynolds, 2006)]. Results suggest that at the onset and Day 3 phytoplankton growth was not limited by the availability of nutrients, but on Day 7 phytoplankton growth was limited by nitrogen but not by phosphorous and by the end the opposite scenario occurred.

At the onset of the experiment, TN ranged between 131 and 153 μM and TP between 16 and 29 μM; no significant differences [$F(1,4)$ 2.2, $P > 0.2$] were encountered among treatments with and without sediments. At the end of the experiment, TN was similar under both conditions (range: 209–306 μM). Conversely, TP was significantly higher [$F(1,4)$ 13.9, $P < 0.02$] in the treatments with sediments (49–67 μM) than in treatments without them (39–49 μM). This suggests that more P than N was released from the sediments, as no changes in TN were observed.

Zooplankton

At the beginning of the experiment, mean total zooplankton density was 2.3×10^2 ind. L^{-1} and the community was composed of similar proportions of rotifers, adult and nauplii cyclopoid copepods and cladocerans (Fig. 3). Abundances of adult and nauplii of calanoid copepods were scarce (Fig. 3). Among rotifers, the dominant species were *Brachionus calyciflorus*, *B. havanaensis*, *B. austrogenitus*, *B. quadridentatus*, *Polyarthra vulgaris*, *Testudinella patina*, *Filinia* cf. *longiseta* and *Keratella morenoi*.

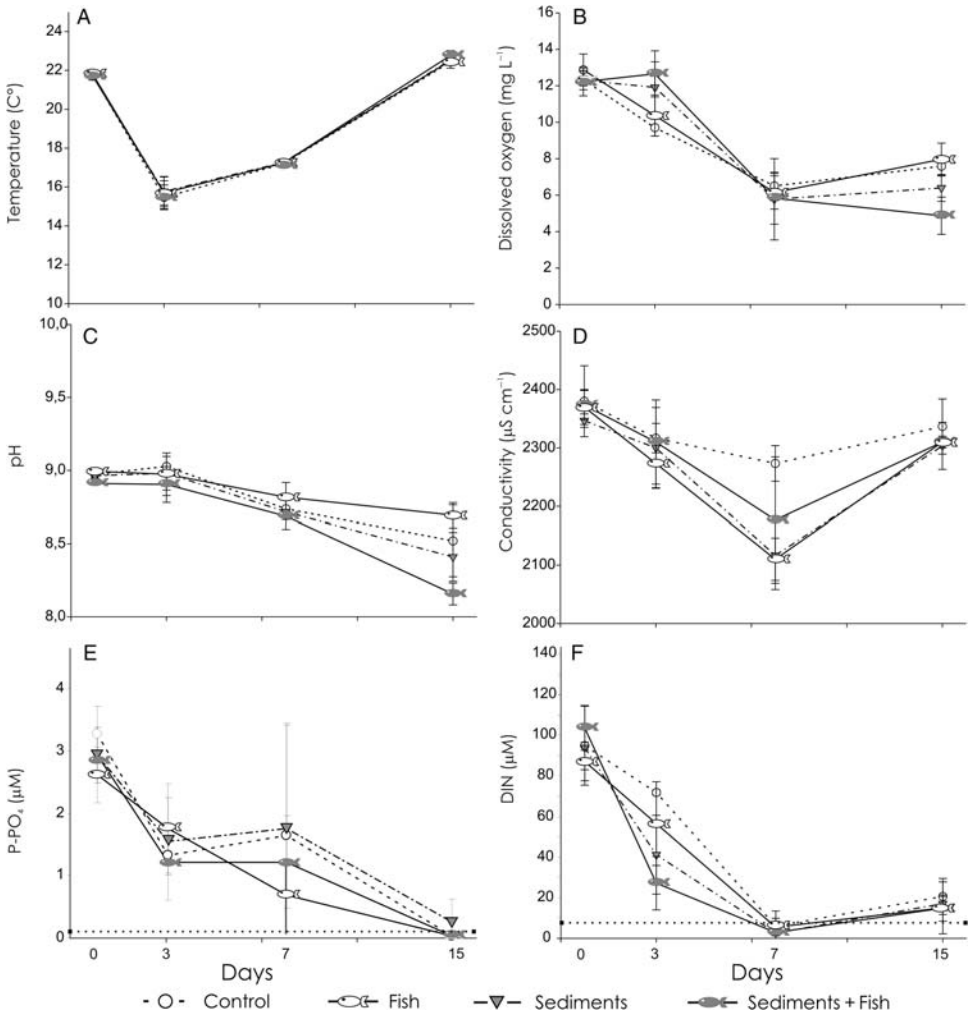

Fig. 2. Variations in the physical and chemical variables analysed in the enclosures during the experiment. (**A**) Water temperature; (**B**) dissolved oxygen; (**C**) pH; (**D**) conductivity; e: $P\text{-}PO_4$; (**F**) (DIN) dissolved inorganic nitrogen. In (**E** and **F**), the line indicates the potentially limiting concentration for phytoplankton growth according Reynolds (Reynolds, 2006).

Cyclopoids and calanoids were the most and the least frequent copepods, respectively. The most abundant cladocerans were *Moina micrura*, *Diaphanosoma* cf. *brevireme*, *Ceriodaphnia* sp., *Bosmina* sp., *Leidigia* sp. and, to a lesser extent, *Daphnia* sp.

Final total zooplankton densities (cladocerans, copepods and rotifers) in the treatments with fish (1.3×10^2 ind. L^{-1} in F and of 3.0×10^2 ind. L^{-1} in SF) were one order of magnitude lower than in the fish-free enclosures (1.1×10^3 ind. L^{-1} in C and of 1.3×10^3 ind. L^{-1} in S), and these differences were significant. No significant differences in zooplankton densities were observed at the beginning and the end of the experiment in treatments with fish (F and SF); conversely, in treatments without fish (C and S), densities increased throughout the experiment (Table I). Independently of fish effects, total zooplankton densities were higher in treatments with sediments than without sediments.

Fishes also impacted on zooplankton community composition. From Day 0 to Day 15, in treatments C and S, the densities of cladocerans increased by an order of magnitude, whereas densities of adult cyclopoids reached values at least seven times higher if compared with initial density. The opposite occurred in treatments F and SF, where cladocerans and adult cyclopoids were almost absent, and significant differences were observed at the end of the experiment. Rotifer abundances did not show significant differences between treatments with and without fishes. Conversely, significant differences in abundances were found in time, being highest on Day 15 in all treatments. Interestingly, calanoid copepods (both adult and nauplii) were scarce in all scenarios both at the beginning and

Table I: Summary of the results of two-way repeated measures analysis of variance performed among the treatments and time for biotic and abiotic variables

	Significant results	MR ANOVA (two-ways) results and *post hoc* comparisons
Temperature	Time; $P < 0.0005$, $F(3,24)=971.0$	Variable over time
Dissolved oxygen	SxT; $P < 0.0005$, $F(3,24)=9.9$	[S] > [S] (3 days); [S] < [S] (15 days)
pH	SxT; $P < 0.005$, $F(3,24)=6.3$	[S] < [S] (15 days)
Conductivity	Time; $P < 0.0005$, $F(3,24)=45.6$	Variable over time
P-PO_4	Time; $P < 0.0005$, $F(3,24)=26.6$	Decline with time
DIN	SxT; $P < 0.005$, $F(3,24)=971.0$	[S] < [S] (3 days)
Nanophytoplankton	FxSxT; $P < 0.05$, $F(3,24) = 3.9$	C < F = S = SF (3 days); C = F < S = SF, C < F (7 days), C = F = S < SF (15 days)
Microphytoplankton	FxSxT; $P < 0.05$, $F(3,24) = 3.6$	C < F = S = SF (3 days), SF = C < S (7 and 15 days)
HNF	FxSxT; $P < 0.005$, $F(3,24) = 7.2$	S > C = F = SF (7 days); S = C > F = SF (15 days)
Ciliates	FxT; $P < 0.05$, $F(3,24)=4.2$	[F] < [F] (3, 7 and 15 days)
Cyclopoids nauplii	FxS; $P < 0.05$, $F(1,8)=6.2$	S > C = F = SF
	FxT; $P < 0.0005$, $F(1,8)=9.8$	[F] > [F] (15 days), [F] ($t_o < t_{15}$)
Calanoids nauplii	—	—
Rotifers	Time; $P < 0.05$, $F(1,8)=12.4$	Increases with time
Cyclopoids	FxT; $P < 0.0005$, $F(1,8)=447.0$	[F] > [F] (15 days), [F] ($t_0 < t_{15}$), [F] ($t_0 > t_{15}$)
Calanoids	Time; $P < 0.05$, $F(1,8)=7.3$	Increases with time
Cladocerans	FxT; $P < 0.005$, $F(1,8)=29.2$	[F] > [F] (15 days), [F] ($t_0 < t_{15}$)
Total zooplankton	Sediments; $P < 0.05$, $F(1,8)=6.9$	[S] < [S]
	FxT; $P < 0.0005$, $F(1,8)=114.6$	[F] > [F] (15 days), [F] ($t_o < t_{15}$)

Only significant interactions are shown; double interactions: SxT (sediment × time), FxT (fish × time), FxS (fish × sediment), triple interactions: FxSxT (fish × sediment × time) and the groups: [S] with sediments (S + SF); [S] without sediments (C + F); [F] with fish (F + SF); [F] without fish (C + S). *F*, degree freedom.

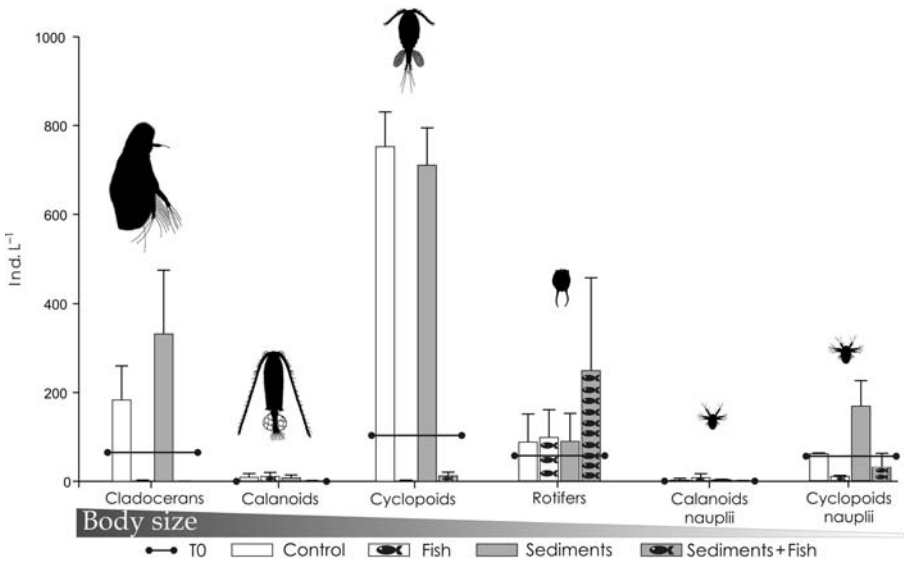

Fig. 3. Zooplankton composition in the enclosures at the beginning (Day 0, represented with a horizontal line) and at the end of the experiment (Day 15, represented with columns).

at the end of the experiment (Fig. 3), even though adult abundances increased over time (Table I).

Nanophytoplankton (algae 3–30 μm)

Mean nanophytoplankton density ranged between 1.4×10^5 and 4.3×10^5 ind. mL^{-1} (Fig. 4A); temporal variation was mainly determined by the Class Chlorophyceae, which represented 90% of this fraction (1.3×10^5 and 3.8×10^5 ind. mL^{-1}) and was dominated by single celled organisms (*Monoraphidium contortum*, *M. circinale*, *M. minutum*, *M. griffithii* and many species of *Chlamydomonas*, *Chlorella*) and coenobial taxa (*Scenedesmus* and *Crucigenia*). The remaining 10% of the nanophytoplankton was composed of cyanobacteria (1.5×10^3 and 2.4×10^4 ind. mL^{-1}), including *Merismopedia tenuissima*, *Woronichinia elorantae* and *Aphanocapsa delicatissima*.

Nanophytoplankton abundances increased in all the enclosures between Day 0 and Day 3, except in the control. In all the treatments, the abundances dropped

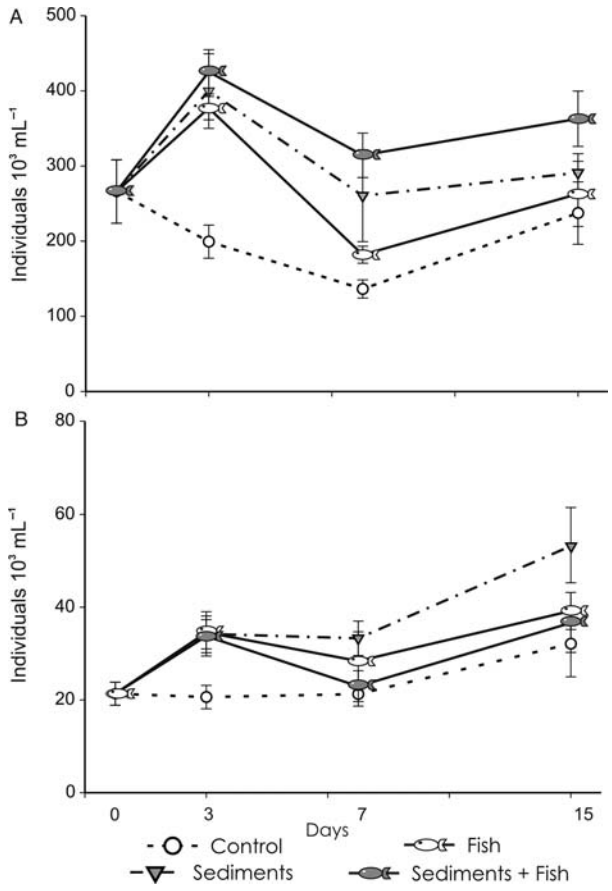

Fig. 4. Phytoplankton community composition in the enclosures. (**A**) Nanoplanktonic algae (3–30 μm); (**B**) large algae (>30 μm). Bars represent standard deviations.

from Day 3 to Day 7 probably owing to a dilution effect caused by a rainfall on the previous days, but slightly increased from Day 7 onwards. The treatment SF had the highest abundance, followed by S, F and C, respectively, along the experiment. Moreover, SF was the only treatment that showed a significant increase in nanophytoplankton abundance from Day 7 until the end of the experiment (Fig. 4A).The MR ANOVA showed significant differences both among treatments and over time (Table I). The *post hoc* comparisons revealed significant differences in density between treatment F, S, SF and C. In all cases, the densities in the treated mesocosms were higher than in the non-treated ones at Days 3 and 7. Also, treatment C had lower nanophytoplankton abundances than F at Day 7; and treatment SF had higher values than C, F and S at Day 15.

Microphytoplankton (algae > 30 μm)

Microphytoplankton density was one order of magnitude lower than the nanophytoplankton (range: 2.1×10^4 and 5.3×10^4 ind. mL^{-1}). Its numbers significantly increased in all the treatments during the experiment. (Fig. 4B). The MR ANOVA showed significant differences over time and among treatments (Table I). The composition was dominated by filamentous cyanobacteria, mainly *Planktolyngbya limnetica* and *Anabaena* sp., and colonial cyanobacteria including *Microcystis* sp. and *Aphanothece* sp. These were followed by chlorophyceans, with the most frequent species being *Closterium acutum* var. *variabile*, *Closterium aciculare*, *Staurastrum* sp., *Pediastrum tetras* and *Actinastrum hantzschii* and the diatom *Nitzschia acicularis*.

The MR ANOVA analysis revealed that when fishes were present (F and SF) the effect of the sediment had no effect on microphytoplankton densities. Without fishes, as mesozooplankton were present, the microphytoplankton fraction was more abundant in the presence of sediments. The microphytoplankton densities increased throughout the experiment (Table I) and the highest values were observed for treatment S, followed by F, SF and finally C.

The densities of eukaryotes and cyanobacteria for nano- and microphytoplankton fractions at the beginning and the end of the experiment are represented in Fig. 5. The nanoplanktonic eukaryotes were the most abundant fraction and thus presented the same trend as the total nanophytoplankton. The densities of the eukaryotic microphytoplankton fraction showed significant differences among treatments: treatments with sediments showed higher abundances than without sediments [$F(3,24)$ 8.5, $P < 0.0005$]; also, treatments with fishes had higher densities than without fishes [$F(3,24)$ 3.4, $P < 0.04$]. Nanoplanktonic cyanobacteria

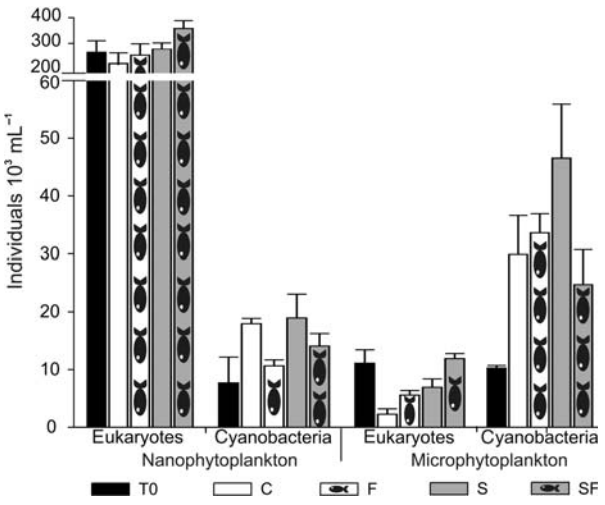

Fig. 5. Abundances of eukaryotes and cyanobacteria of both phytoplankton size fractions analysed at the end of the experiment. Bars represent standard deviations.

abundances showed higher values in treatments without fishes (mesozooplankton present) and in the microplanktonic fraction the highest densities were found in treatment S [$F(3,24)$ 5.2, $P < 0.01$].

HNF and ciliates

Mean HNF densities ranged between 2.0×10^2 and 2.0×10^3 ind. mL^{-1}, and mean ciliate densities between 6.2×10^1 and 4.7×10^2 ind. mL^{-1} (Fig. 6). In the treatments with fish (F and SF), HNF density showed an inverse pattern to that of ciliates (Fig. 6); responding to an increase in the density of ciliates compared with the treatment without fish (C and S).

The MR ANOVA revealed significant differences in HNF densities on Day 7 between C, F and SF versus S. On Day 15, the differences were observed between the treatments with fish (F and SF) versus those without fish (S and C). Ciliate abundances showed an opposite pattern to the HNF densities throughout the experiment. The ciliate densities showed significant differences from Days 3 to 15, where the densities were higher in the treatments F and SF than in C and S (Table I).

DISCUSSION

This study allowed the assessment of the separate and combined effects of both the release of nutrients from the sediment (bottom-up) and of predation (top-down) by zooplanktivorous fish and the consequent cascading effects on various components of the plankton community. Several studies carried out in lakes have acknowledged the impact of fish on plankton community structure (Hrbácek *et al.*, 1961; Brooks and Dodson, 1965; Reinertsen *et al.*, 1990; Holopainen *et al.*, 1992; Mittelbach *et al.*, 1995; Rejas *et al.*, 2005; Acuña *et al.*, 2008; Iglesias *et al.*, 2008). In this study, fish exerted a strong control on total zooplankton densities, particularly on large zooplankton, as has been seen in others works (Meerhoff *et al.*, 2007; Iglesias *et al.*, 2008; Havens *et al.*, 2009). In this sense, in treatments with planktivorous fish the mesozooplankton fraction (cyclopoid copepods and cladocerans) showed very low, almost null, densities, suggesting a strong top-down effect of fishes on mesozooplankton. The abundances of rotifers remained similar among treatments probably owing to the top-down control exerted by fish on rotifers in F and SF treatments as also observed by Gliwicz and Pijanowska (Gliwicz and Pijanowska, 1989). In treatments C and S, the main zooplankton group feeding on rotifers may have been by cyclopoid copepods, as was suggested by Jürgens and Jeppesen (Jürgens and Jeppesen, 2000) or the interference competition by the cladocerans (Gilbert, 1988). While the abundance of rotifers increased significantly in all treatments over time, this increase was not as important as it was for the copepods and cladocerans.

Body size plays a critical role in predator–prey interactions (Scheffer, 1998). Large zooplankton species are vulnerable to visual fish predators (Gliwicz and Pijanowska, 1989), and therefore lakes with abundant planktivorous fish populations may be dominated by small zooplankton species (Järvinen, 2002). In the wetlands of the Natural Reserve Otamendi, zooplankton was dominated by small cladocerans, copepod nauplii and rotifers as observed for tropical and subtropical shallow lakes. This fact may be related to a high predation pressure by small omnivorous–planktivorous fish and by large invertebrate predators over large zooplankton (Iglesias *et al.*, 2008). In the present study, the same was observed in treatments with fish (F and SF), but the

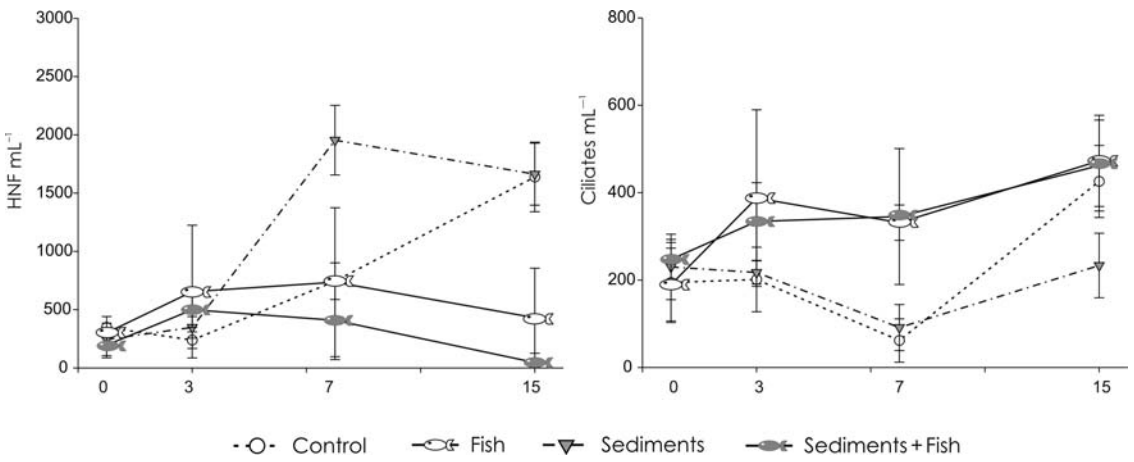

Fig. 6. Temporal variation of the abundance of heterotrophic nanoflagellates (HNF) and ciliates. Bars represent standard deviations.

predation pressure was extreme, because large zooplankton did not have a refuge in the enclosures (Lauridsen and Lodge, 1996; Burks *et al.*, 2002).

Interestingly, fish absence resulted in cascading effects on protozooplankton (ciliates and HNF), as higher zooplankton (mainly cyclopoids copepods) abundances resulted in lower ciliate numbers and resulted in smaller sized organisms and an increased number of ciliate prey (HNF). The same pattern was observed by Jürgens and Jeppesen (Jürgens and Jeppesen, 2000), where the increase in numbers of cyclopoid copepods contributed to the decrease of large ciliates and small-sized organisms were favored. In the treatments with fish (F, SF), the abundances of ciliates and HNF showed an inverse temporal pattern. Under these scenarios, ciliates showed high abundances and enhanced growth throughout the experiment, whereas the opposite occurred with HNF. This difference is probably due to the cascading effect of fish (↑fish−↓mesozooplankton−↑ciliates−↓HNF). Ciliates did not only increase in abundance but also were large. They were either of the same initial species or belonged to different taxa which might have been released from the mesozooplankton grazing pressure. In the fish-free treatments (C and S), the trends between ciliates and HNF were less straightforward. Because of the presence of the mesozooplankton, ciliate abundance remained either unchanged (S) or increased (C). Conversely, HNF abundances increased in both treatments mostly after Day 7. This fact occurred due to the grazing pressure posed by mesozooplanktonon ciliates. Ciliate abundance increased in the control at the end of the experiment probably owing to same increasing pattern of HNF abundance. In addition, ciliates increased in abundance but these were small sized. We have obtained similar results in this wetland (Sinistro *et al.*, 2007). Although many researchers stated that the trophic cascade can be truncated at the level of protozoa (Pace and Funke, 1991); this was not observed in our experiment. The results obtained in the scenarios where fish were added are consistent with the concept of a four-level trophic cascade described in other studies (Sommer *et al.*, 2003; Schnetzer and Caron, 2005).

Whenever fish were absent, zooplankton increased significantly in abundance, and resulted in a community composed of individuals with larger body size, such as cladocerans and copepods. Brooks and Dodson (Brooks and Dodson, 1965) state that large-bodied zooplankters are more efficient phytoplankton grazers than their smaller competitors, which are restricted to consume small particles. This effect was clearly observed on Day 3 of the experiment when the abundance of phytoplankton in treatment F (mesozooplankton absent) was significantly higher than in C (mesozooplankton present).

Notwithstanding, even if total zooplankton (mainly mesozooplankton fraction) abundances were significantly affected by fish occurrence the effect of fishes on total phytoplankton was less pronounced than the effect of sediments. One possible explanation may be that the low effect of zooplankton grazing pressure might reflect their low abundances at the start of the experiment because this fraction is controlled by fish in natural conditions.

The results obtained in treatment SF indicate that both the decrease in zooplankton grazing pressure and the increase in nutrient availability resulted in a positive response of algal growth (mainly for nanophytoplankton). The lower proportion of large algae in this treatment may reflect the adaptive advantage of increased nutrient uptake in small algae in the absence of predation by zooplankton. Conversely, the significant initial decrease in abundance of both fractions of phytoplankton analysed in the scenario C, probably occurred due to zooplankton grazing pressure in combination with no nutrient addition, even if dissolved nutrients were in concentrations above potential limiting values for phytoplankton growth (*sensu* Reynolds, 2006). This result agrees with the previous experimental results carried out in the same wetland where it was shown that zooplankton exerted a considerable grazing pressure on the nanophytoplankton (Sinistro *et al.*, 2007).

In treatment S, the top-down effect of zooplankton on phytoplankton was apparently masked by the bottom-up effect related to the release of nutrients from the sediments. Moreover, the top-down effect was evident in microphytoplankton abundance and composition, as reflected by the increase in total densities and relative proportions of large and colonial filamentous cyanobacteria (*Planktolyngbya limnetica*, *Anabaena* sp., *Microcystis* sp. and *Aphanothece* sp.), algae probably inedible for zooplankton. These changes in microphytoplankton community may be probably related to the fact that in the absence of planktivorous fish, nanophytoplankton was controlled by large sized zooplankton, mainly represented by cladocerans as observed by others authors (Sommer *et al.*, 2003).

By mid-experiment, when dissolved DIN availability was below values acknowledged as potentially limiting phytoplankton growth [7 μM N according Reynolds (Reynolds, 2006)], the scenarios with nutrient release from the sediments (S and SF) were significantly higher in terms of nanophytoplankton densities than the control (C) and F. The significant increase in TP, as this nutrient is released from sediments (Bates and Neafus, 1980; Istvanovics, 1988; Xie *et al.*, 2003), played an important role in determining enhanced nanophytoplankton abundances. It is tempting to suggest that

bottom-up forces play a more important role than zooplankton top-down when dissolved P is sufficient. By the end of the experiment, P availability indicated potential limitation of phytoplankton growth [0.1 μM P according Reynolds (Reynolds, 2006)], whereas DIN was above limiting values. The scenario with increased nutrient release and without grazing effect of zooplankton (SF) was significantly different from all other situations. This suggests that when dissolved phosphorus is scarce, the effect of zooplankton predation becomes more important compared with situations where P availabilities are sufficient to fuel phytoplankton growth. The effect of nutrient input from the sediments (bottom-up) resulted in higher nanophytoplankton densities than the scenario without sediment addition and zooplankton presence. Even if the effect of dissolved nutrients release from sediments was not observed between treatments in the experiment, but in the nutrient dynamics it was reflected in total phosphorous. In isolated water columns, as in laboratory cultures, nutrients are taken up as they are provided and may not be detectable in the medium but be immobilized in the biomass. The lack of significant differences between the concentrations of the principal nutrients in the enclosures with sediments would be explained by the fact that, although nitrogen was continuously released into the water column, it was rapidly captured by algae because of the usually low DIN concentration. In this sense, DIN concentrations showed significant differences at Day 3 (C and F higher than S and SF), when an increase in phytoplankton abundance was observed, probably owing to phytoplankton uptake.

The upper trophic levels may also influence nutrient availability for primary consumers via the stoichiometry of nutrient recycling (Hessen *et al.*, 1994; Sterner *et al.*, 1997; Elser and Urabe, 1999). The C:N and C:P ratios vary among zooplankton species (Järvinen, 2002); for example, cladocerans have a higher proportion of phosphorus and a lower proportion of nitrogen than copepods. Although this differential nutrient uptake could modify the proportion of nutrients in the water column, in this experiment no significant differences in the proportion of both nutrients were found between treatments, because both were present or absent in the same treatment.

Under the conditions including fishes (F and SF) grazing pressure on the mesozooplankton fraction should trigger the increase of nutrient concentrations by excretion (Vanni and Layne, 1997; Attayde and Hansson, 2001a, b), even if part of the nutrients remains captured in fish biomass. Nutrients in excretion should provide a surplus of nutrients to phytoplankton. Thus, fish grazing probably increased phytoplankton densities due to increased nutrient availability, enhancing bottom-up effects.

Although it was expected that algal density would be highest in treatment SF as a result of high nutrient availability and low grazing pressure by zooplankton, the present study leads to some interesting considerations on the top-down and bottom-up forces in this eutrophic system. In these types of environments, where in general the nutrients are not likely to be limiting factors, the phytoplankton can be assumed to be controlled by the top-down effect. However, under certain conditions of strong algal growth, as occurred during the present experiment, the nutrients may eventually become limiting for phytoplankton growth (DIN at mid-experiment and $P\text{-}PO_4$ by the end of the experiment) and thus, be the main factor controlling it. DIN may be limiting in Laguna Grande under certain conditions (Unrein, 2001), as it was also reported for shallow vegetated lakes by several authors (Saunders *et al.*, 2000; Van Donk *et al.*, 2003).

Our results suggest that phytoplankton regulation by zooplankton might be weaker in warm temperate systems than in temperate ones, as it was also reported for tropical ecosystems (Von Rückert and Giani, 2008). This is probably because planktivorous fish predation is the main factor responsible for the low density of zooplankton compared with the phytoplankton in subtropical lakes (Jeppesen *et al.*, 2005; Jeppesen *et al.*, 2007; Meerhoff *et al.*, 2007; Iglesias *et al.*, 2008) and the scarcity of large effective grazers (Hamza *et al.*, 1995; Havens *et al.*, 1996). Moreover, in this study, the phytoplankton appeared to be bottom-up controlled, whereas the zooplankton was mainly top-down regulated.

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