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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

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An experimental study using microcosms was conducted in a South American wetland, Lower Paraná River Basin (Argentina), to analyse the structure of the components of the microbial plankton community and the influence of the light deficiency due to floating macrophytes on this community. Two experiments were run under different light conditions; the decrease of the light penetration due to floating macrophytes was simulated using different nylon mesh covers that resembled natural conditions in the lake. These studies revealed that the light deficiency favoured the replacement of obligate autotrophs by mixotrophic and heterotrophic organisms. Abundances of strictly autotrophic algae along the experiments responded to the light gradient, being maximum in the flasks without cover. Heterotrophic nanoflagellates (HNF) and ciliates increased in the microcosms, probably favoured by the high food availability (picoplankton) and the lack of their predators (zooplankton). The increase of ciliates was higher in the microcosms with more light. In the first experiment, the picoplankton fraction strongly decreased after 24 h in the flasks that included all their potential predators, thus suggesting a grazing pressure on this fraction. Grazing experiments performed with fluorescent-labelled bacteria (FLB) revealed that two Cryptomonas species, which are frequent in the lake (Cryptomonas erosa and Cryptomonas marssonii), can ingest bacteria.

INTRODUCTION

Since the earliest scientific descriptions of the 'microbial loop' (Azam *et al.*, 1983), and the recognition of its role in the recycling of carbon and nutrients in the aquatic ecosystems, increasing importance has been given to the study of the microbial food webs. The microbial components of aquatic food webs (heterotrophic bacteria, autotrophic picoplankton, heterotrophic and mixotrophic flagellates and ciliates) can often be an important, and sometimes dominant, part of aquatic ecosystems (Boenigk and Arndt, 2002). Many studies have stressed that the relative importance of the microbial loop compared to the classical pelagic food chain is greater in oligotrophic environments than in eutrophic systems (Porter, 1988; Weisse, 1991). In particular, del Giorgio and Gasol (del Giorgio and Gasol, 1995) have shown that the heterotrophic/autotrophic ratio of freshwater plankton communities declines along gradients of

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enrichment; thus, in oligotrophic systems, heterotrophic organisms, including bacteria, tend to dominate the total plankton biomass rather than algae. Moreover, studies conducted in humic lakes, with abundant available organic matter, have also demonstrated the importance of the microbial food web in these systems (Amblard *et al.*, 1995; Bergström *et al.*, 2003; Drakare *et al.*, 2003).

Different planktonic organisms, including flagellates, ciliates, rotifers, and even crustaceans have been reported as potential grazers on heterotrophic bacteria and autotrophic picoplankton (Peterson *et al.*, 1978; Güde, 1989; Vaqué and Pace, 1992; Hart and Jarvis, 1993; Jürgens, 1994; Jones, 2000; Sherr and Sherr, 2002). In particular, heterotrophic nanoflagellates (HNF) have been documented as the main grazers on bacteria in many studies (Porter *et al.*, 1985; Sanders *et al.*, 1989; Hahn and Hofle, 2001). Other authors have reported that ciliates as well as certain cladoceran species can also markedly contribute to the grazing on picoplankton (Sherr and Sherr, 1987; Kankaala, 1988; Jürgens, 1994; Langenheder and Jürgens, 2001). Mixotrophic algae may also ingest bacteria and eukaryotic prey (Jones, 1994; Jansson *et al.*, 1999). Phagotrophy may be an important strategy in algae under conditions of light deficiency or when nutrients are the limiting factor (Granéli *et al.*, 1999). When light is favourable, and dissolved organic carbon (DOC) is relatively low, most carbon and phosphorus flux should be through obligate phototrophs to larger zooplankton, with smaller flux through bacteria and obligate heterotrophs. In contrast, conditions of unfavourable light and relatively high DOC should advantage mixotrophs over obligate phototrophs because of both light limitation and increased competition with bacteria for inorganic phosphorus (Jones, 2000).

In wetlands, macrophytes have a pronounced effect on the microbial communities. Particularly, Wetzel and Søndergaard (Wetzel and Søndergaard, 1998) demonstrated that the aquatic plants play an important role in the location of the greatest bacterial growth in the water column. Stanley *et al.* (Stanley *et al.*, 2003) have evaluated the influence of macrophytes on the algal and bacterial production in a wetland, founding that plants had a negative effect on the production of both bacteria and algae, probably because of an allelopathic effect of the macrophytes. The aquatic plants not only provide surface area available for microbial colonization but also create pronounced spatial variation in light, temperature, water current and nutrient conditions within and between macrophyte beds (Wilcock *et al.*, 1999; Stanley *et al.*, 2003).

Whereas numerous studies have focused on pelagic ecosystems, the studies exploring how the submerged and emerged macrophytes affect the structure and functioning of the microbial plankton communities have been relatively limited (Komárková and Komárek, 1975; Kleppel *et al.*, 1980; Middelboe *et al.*, 1998; Mitamura and Tachibana, 1999; Reitner *et al.*, 1999; Scheffer, 1999; Theil-Nielsen and Søndergaard, 1999).

This study was conducted in a wetland from the Lower Paraná River (Reserva Estricta Otamendi, Argentina). Previous investigations in these aquatic environments were focused on the algal communities of several water bodies with different degree of macrophyte cover. In a former study, Izaguirre *et al.* (Izaguirre *et al.*, 2001) observed an interesting algal flora adapted to anoxic conditions and very low light intensities in the water bodies permanently covered by floating plants. Later on, O'Farrell *et al.* (O'Farrell *et al.*, 2003) compared the algal assemblages of different sites of this wetland, analysing their stability and resilience, and concluded that the profusely vegetated relictual oxbow lakes usually contain steady state assemblages well adapted to the extreme ecological conditions. In a more recent study, using a multivariate analysis Izaguirre *et al.* (Izaguirre *et al.*, 2004) showed the variation in the algal assemblages across the transversal dimension of this wetland, and once again the macrophyte cover appears as the stirring factor in the selection of the species. In particular, under the macrophyte cover, the algal community is dominated by species that can optimize the uptake of light, such as small unicellular organisms or very thin filaments, like some chlorococcaleans and cyanobacteria, together with diatoms that tolerate very low light intensities and mixotrophic taxa, mainly cryptophytes and euglenophytes. All these investigations also showed the presence of a rich microbial community, registering high abundances of autotrophic and heterotrophic picoplankton (HPP), and a great variety of HNF, ciliates and mixotrophic algae. Regarding their environmental features, and according to the descriptions given by Williamson *et al.* (Williamson *et al.*, 1999), the aquatic systems of this wetland can be defined as typical 'mixotrophic lake ecosystems', with high DOC and total P contents. These authors have stressed the importance of the DOC, not only as carbon source for the microbial plankton communities but also because high DOC levels can enhance growth of these communities by mitigating the potential UV damage.

The main aim of this study was to analyse the structure of the different components of the microbial plankton community in the main lake of this wetland and to evaluate the effect of the light deficiency due to the floating macrophytes on this community. With this purpose, two field experiments using microcosms under

different light conditions were performed. The following hypotheses were tested:

- The decrease of light penetration produced by the floating macrophytes would favour a replacement of obligate autotrophic algae by mixotrophic species, because these can supplement their nutritional requirements by phagotrophy.
- The presence of potential predators of picoplankton (HNF, ciliates and mixotrophic algae) will decrease their abundance in the microcosms, with respect to the microcosms containing only picoplankton.
- The decrease of picoplankton by predation will be more important in the treatment without light because of the replacement of strictly autotrophic algae by phagotrophic species.
- Different conditions of light attenuation will have a differential effect on the evolution of the microbial plankton communities.

On the other hand, to corroborate the assumption of mixotrophic behaviour of some frequent algae in the lake (mainly *Cryptomonas* spp.), two other *in situ* experiments with microcosms were performed. In these experiments, the bacterial ingestion by mixotrophic algae was examined using fluorescent-labelled bacteria (FLB). We tested the hypothesis that the species of *Cryptomonas* of this wetland are able to prey on bacteria.

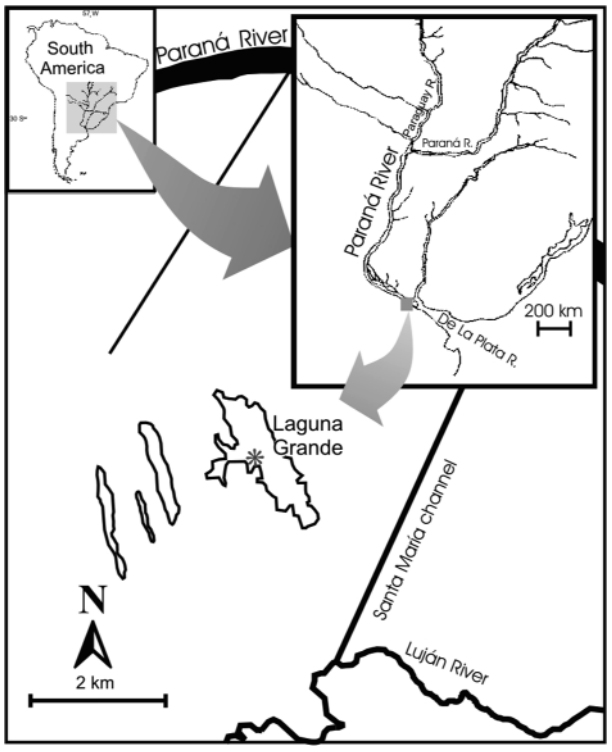

Fig. 1. Map of the Paraná River Basin in Argentina, showing the location of the lake and the site where the experiments were conducted.

METHODS

Study site

The study area is located in a wetland from the Natural Reserve Otamendi, which is delimited by the Paraná de las Palmas and Luján Rivers, Buenos Aires Province, Argentina (34°10′–34°17′ S; 58°48′–58°53′ W) (Fig. 1). The lakes are surrounded by marshy vegetation and temporarily and partially covered by floating macrophytes. The largest lake is Laguna Grande with an area of ∼28 ha. Regarding the definition given by Naiman and Décamps (Naiman and Décamps, 1990), the study site can be described as an area of consecutive water–land ecotones. The dominant macrophytes in the wetland are the rooted *Schoenoplectus californicus* and *Scirpus giganteus* and several floating species such as *Azolla filiculoides*, *Lemna minima*, *Wolffiella oblonga*, *Salvinia rotundifolia* and *Pistia stratiotes*. The area is almost permanently flooded by rainfall, as well as by the river floods, which account for the very poor drainage and consequent reducing conditions of the soils (Chichizola, 1993). The region has a temperate subhumid climate, with a moderate effect due to the Atlantic masses, the Río de la Plata and Paraná de las Palmas Rivers. Precipitations occur during the whole year, with a mean annual value of 950 mm. Mean summer and winter temperatures in this region are 22°C and 9.5°C, respectively.

Light experiments

Experiment I

The first experiment was run in 12 microcosms (2.5-L PVC transparent flasks), which were placed *in situ*, ∼100 m from the shore of Laguna Grande, supported by a floating device. The temporal evolution of the microcosms was daily analysed during the 4 days of the experiment, from 19 to 22 November 2003.

Water samples used to fill the microcosms were obtained from the same shallow lake, and the zooplankton (cladocerans, copepods and rotifers) was previously removed by filtering the water through a 55-μm pore plankton net.

To analyse the influence of the shading produced by the macrophyte cover on the plankton microbial community, two series of treatments were performed: (i) dark microcosms, six flasks that were covered with a dark thick nylon mesh simulating a light attenuation similar

to that produced by the floating plants (98 and 99.9% attenuation for the nylon mesh and floating plants, respectively) and (ii) light microcosms, six flasks that were not covered to allow a normal light penetration. Table I summarizes all the treatments of this experiment.

Experiment II

A second experiment was conducted from 11 to 14 October 2005, using the same experimental flasks and floating device. To analyse the effect of two different conditions of macrophyte cover on the evolution of the microbial plankton components, three treatments were performed:

- Flasks without cover (0-ny mesh)—complete incidence of natural light
- Flasks with a 1-nylon mesh cover—light attenuation of ~81%, equivalent to a natural condition when the lake is partially covered by floating plants
- Flasks with a 2-nylon mesh cover—light attenuation of ~98%, equivalent to a natural condition in the lake when the floating macrophyte cover is very profuse.

In this experiment, the flasks were filled with water filtered through a 55-μm pore plankton net to eliminate the zooplankton. Also, in this case, the temporal evolution in the microcosms was daily analysed during the 4 days.

Grazing experiments

Two grazing experiments were carried out *in situ* on 12 May and 8 August 2005, using microcosms (experimental plastic bottles of 1-L capacity). Rates of bacteria cell removal by mixotrophic algae were measured using FLB, which were prepared according to the protocols described by Sherr *et al.* (Sherr *et al.*, 1987) and Vaqué *et al.* (Vaqué *et al.*, 2001). The FLB were added to natural water samples of the lake previously filtered through a 55-μm pore zooplankton net in the experimental bottles. The concentration of FLB used was ~20% of the natural bacterial density in the lake. The bottles, triplicated, were suspended in the lake with a buoyant device. Subsamples of each bottle were taken immediately after the addition of FLB (t_0), and after 15 min (t_1), 30 min (t_2), 45 min (t_3), 60 min (t_4) and 90 min (t_5), and preserved with cold glutaraldehyde (2% final concentration). In the laboratory, 5 mL of each sample was filtered through a 3-μm polycarbonate black filter (Poretics) to quantify the ingested FLB by epifluorescence microscopy (Zeiss Axioplan). To quantify the natural concentration of bacteria in the lake, 2 mL was filtered through a 0.2-μm polycarbonate black filter (Poretics) and stained with DAPI 4′,6-Diamidino-2-phenyindole. The hourly ingestion rate of FLB by the mixotrophic algae (*Cryptomonas* spp.) was determined according to Šimek *et al.* (Šimek *et al.*, 1995), from the change in the average number of FLB per algae with time over the linear par of the uptake curve (t_0–t_3), using linear regression analysis. The clearance rate was obtained from the FLB ingestion rate divided by the FLB concentration used. Specific ingestion rates for each mixotrophic species were estimated by multiplying the corresponding clearance rate by the bacterial concentration, assuming that native bacteria and FLB were grazed upon the same rates. Grazing impact of the mixotrophic algae on bacterioplankton was estimated by multiplying the specific grazing rate by the *in situ* abundance of mixotrophic algae.

Quantification of the plankton fractions (light experiments)

The abundance of all the plankton fractions was daily analysed taking two small samples (30 mL) from each one of the microcosms. One sample, used for the quantification of microphytoplankton, nanophytoplankton and ciliates, was fixed with acidified lugol 1%, and counting was carried out following the Utermöhl method (Utermöhl, 1958). Chambers of 5 and 10 mL (depending on the plankton abundance) were left to sediment for 24 h,

Table I: Treatments corresponding to the first light experiment (19–22 November 2003)

Dark microcosms with all the fractions (micro-, nano- and picoplankton) (D, all)	Replicates 1, 2 and 3	Water filtered only through a 55-μm pore plankton net to eliminate the zooplankton
Light microcosms with all the fractions (micro-, nano- and picoplankton) (L, all)	Replicates 1, 2 and 3	
Dark microcosms only with picoplankton (D, pico)	Replicates 1, 2 and 3	Water subsequently filtered through a 55-μm pore plankton net, 20- and 3-μm-pore-size polycarbonate filters (diameter 45 mm; Poretics, Osmonics Inc.)
Light microcosms only with picoplankton (L, pico)	Replicates 1, 2 and 3	

and the counting error was estimated according to Venrick (Venrick, 1978), accepting a maximum error of 15%. The separation of the different plankton fractions analysed was based on the greatest axial linear dimension (GALD) (Reynolds, 1986). Phytoplankton was separated into three main fractions: picoplanktonic algae (<3 μm), nanophytoplankton (3–20 μm) and microphytoplankton (>20 μm). Another sample of each flask, preserved with ice-cold filtered glutaraldehyde 2%, was used for picoplankton and HNF counts. Two subsamples of this sample were filtered on 0.2- and 0.6-μm-pore-size black polycarbonate filters Isopore GTPB and DTTP (Millipore) for picoplankton and HNF, respectively. A volume of 2 mL was filtered for picoplankton enumeration and of 5 mL for HNF. The material was stained with DAPI (Porter and 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 (APP euk.) and autotrophic prokaryote picoplankton (APP pro.) were counted from the fluorescence given off by photosynthetic pigments, under blue and green light excitation (Callieri and Pinolini, 1995). The daily evolution of the picoplankton fraction was only analysed for the first experiment. HPP and HNF were counted under UV excitation. A Zeiss Axioplan microscope equipped with a HBO 50-W lamp, a plan-Apochromat ×100 objective and a filter set for blue light excitation (BP 450–490, FT 510 and LP 520 nm), green light excitation (BP 546, FT 580 and LP 590 nm) and UV excitation (BP 365, FT 395 and LP 397 nm) was used.

Physical and chemical data

At the beginning of each experiment, the following physical and chemical variables were measured in the shallow lake: dissolved oxygen, temperature, pH and conductivity, with portable electronic meters Hanna HI9143, HI9025 and HI9033 (Hanna Instruments, USA). Incident and underwater irradiance in the open waters and under the macrophyte cover were measured with a submersible Li-Cor PAR spherical quantum sensor (Li-250). A sample for nutrient analyses was also collected from the lake. Soluble reactive P (SRP), nitrates ($N\text{-}NO_3$) and ammonia ($N\text{-}NH_4$) were measured with a Hach DR/2010 spectrophotometer, using the corresponding kits of Hach reagents.

At time zero (t_0), measurements of temperature, pH, conductivity and dissolved oxygen were also performed in all the microcosms, and a sample for nutrient analyses was taken from each flask. The methodology was the same mentioned above. All these variables were measured every day in all the flasks, during the 4 days.

Data analyses (light experiments)

To analyse the statistical differences between treatments (different light conditions), and among the times, two-way repeated measures (RM) analysis of variance (ANOVA) was performed for each one of the components of the microbial community, with treatment as the main factor and time as the RM. To test for significant differences between treatments, *post hoc* comparisons were made by using Student–Newman–Keuls (SNK) test (Underwood, 1997).

In the case of the picoplankton fraction (Experiment I), the ANOVA was performed on the four treatments (light-all, light-pico, dark-all and dark-pico). It is important to point out that the picoplankton abundance at t_0 was much lower in the flasks containing only picoplankton than in the experimental flasks with all the fractions. This fact was due to the loss provoked by the filtration necessary to retain only the picoplankton in the controls. Although 3-μm-pore-size filters were used, which allow the passage of the picoplanktonic algae, due to the eutrophic condition of the lake, filters were soon covered by the abundant algal material, producing an overlapping of algal types, within which many picoplanktonic algae could be retained. Because the starting point was not the same in the experimental flasks, only in this case the statistical analyses were performed using the densities corresponding to the relation $(Dt_n–Dt_0)/Dt_0$ (where D is density at different times) to obtain independency from the abundance at t_0.

The correlations between pairs of biotic and abiotic variables were estimated using a nonparametric correlation coefficient (Spearman) (Conover, 1980).

RESULTS

Light experiments

Physical and chemical properties inside the microcosms

Table II summarizes the mean values of the physical and chemical variables analysed in the experimental flasks for both experiments at t_0 and t_3.

For the first experiment, the incident light at the beginning of the experiment was 1990 μmol photon m^{-2} s^{-1}; at the subsurface layer, the light was ~822 μmol photon m^{-2} s^{-1}, whereas the light inside the covered flasks (below the nylon mesh) was ~60 μmol photon m^{-2} s^{-1}, which simulated a similar shade as that produced by the floating macrophytes. Under the natural macrophyte cover, light was 25 μmol photon m^{-2} s^{-1}. At the beginning of the second experiment, the incident

Table II: Variation of the mean values of the physical and chemical variables from the beginning (t_0) to the end (t_3) of the light experiments

		Experiment I			Experiment II			
		L-all	L-pico	D-all	D-pico	0-Nylon mesh	1-Nylon mesh	2-Nylon mesh
Water temperature (°C)	t_0	24 (0.0)	24 (0.0)	22 (0.0)	22 (0.0)	25 (0.06)	26 (0.1)	26 (0.21)
	t_3	23 (0.1)	23 (0.1)	24 (0.2)	24 (0.5)	22 (0.01)	22 (0.15)	21 (0.12)
Dissolved oxygen (mg L^{-1})	t_0	8.3 (0.2)	7.7 (0.1)	7.8 (0.2)	7.3 (0.0)	7.6 (0.18)	8.1 (0.11)	7.7 (0.24)
	t_3	11.7 (0.2)	7.9 (0.8)	5.3 (0.3)	4.3 (0.3)	10.2 (0.27)	10.6 (0.24)	9.4 (0.27)
pH	t_0	8.4 (0.02)	8.3 (0.02)	8.3 (0.03)	8.3 (0.05)	8.2 (0.01)	8.3 (0.01)	8.3 (0.01)
	t_3	9.1 (0.00)	8.8 (0.08)	8.7 (0.04)	8.5 (0.05)	9.0 (0.04)	9.0 (0.02)	8.8 (0.01)
Conductivity (μS cm^{-1})	t_0	1289 (1)	1286 (5)	1297 (7)	1289 (2)	2157 (50)	2150 (10)	2127 (31)
	t_3	1517 (109)	1524 (34)	1595 (16)	1633 (60)	2303 (50)	2247 (47)	2170 (10)
Phosphate (μM)	t_0	4.95 (0.32)	5.05 (0.11)	5.05 (0.11)	5.26 (0.11)	11.58 (0.11)	11.58 (1.79)	11.26 (1.26)
	t_3	3.16 (0.32)	5.05 (0.53)	4.32 (0.11)	5.37 (0.42)	12.95 (1.58)	11.89 (0.63)	14.42 (1.26)
Ammonia (μM)	t_0	5.00 (1.67)	13.33 (0.56)	6.67 (5.56)	16.11 (7.78)	21.11 (2.78)	16.67 (2.22)	17.78 (3.33)
	t_3	Not defined	27.78 (2.78)	Not defined	6.11 (7.22)	10.00 (7.78)	13.33 (0.56)	14.44 (1.11)
Nitrate (μM)	t_0	Not defined	Not defined	Not defined	Not defined	Not defined	Not defined	Not defined
	t_3	0.65 (0.16)	0.65 (0.16)	0.65 (0.16)	0.65 (0.16)	Not defined	Not defined	Not defined

SD are indicated between parentheses.

irradiance was 2348 μmol photon m^{-2} s^{-1} and under the macrophyte cover, 46.97 μmol photon m^{-2} s^{-1}. As indicated in *Methods*, light reduction inside the microcosms for the second experiment was of ~81% with 1-ny mesh and ~98% with 2-ny mesh.

Water temperature in the microcosms followed the environmental variations of this parameter, and differences among the experimental bottles were not significant with a significance of 95%.

Dissolved oxygen showed different patterns depending on the treatment in both experiments. Differences among treatments were significant according to the RM ANOVA performed ($P < 0.001$). Although very similar concentrations were measured at t_0 in all flasks, the evolution differed in the microcosms. For Experiment I, the highest values were registered in the flasks without cover (light microcosms) and containing all the components of the microbial community due to the higher photosynthesis by all the autotrophic fractions. Intermediate concentrations were detected in the illuminated microcosms that included only the picoplanktonic fraction, and the lowest figures were measured in the covered flasks (dark microcosms), where a decreasing trend during the experiment was found. During the second experiment, the evolution of dissolved oxygen followed the same pattern, with the lower values also in the flasks less illuminated (2-ny mesh) and higher concentrations in the microcosms with 1- and 0-ny mesh.

Values of pH followed a general increasing trend in all the microcosms in both experiments, and differences among treatments were significant ($P < 0.001$). In the first experiment, the highest values were registered in the flasks with light and containing all the fractions. In the second experiment, pH values were higher in the treatments with 0- and 1-ny mesh.

Differences in conductivity among the treatments were negligible with a significance level of 95% in both experiments.

Phosphate concentrations ($P\text{-}PO_4$) decreased in the microcosms that included all the components of the microbial community (Experiment I), but the decrease was clearly more pronounced in the flasks with light. In the second experiment, no clear temporal pattern was observed. Even when phosphates decreased in some microcosms during the time, the concentrations were always rather high and can be considered as not limiting for the phytoplankton in all instances.

Ammonia ($N\text{-}NH_4$) decreased along the days in the microcosms in both experiments; the flasks containing only picoplankton showed higher values than those that included the whole microbial community. During the second experiment, the concentrations of ammonia were clearly higher than in the former. In this case, the lowest values were registered in the flasks without nylon mesh cover. The RM ANOVA showed significant differences in the ammonia concentrations among times and treatments for the first experiment ($P = 0.001$). In the

case of Experiment II, differences were significant among times ($P = 0.002$) but not significant among treatments.

Nitrate concentrations ($N\text{-}NO_3$) were extremely low in both experiments, and in most of the cases undetectable, which is frequent in this wetland, where the prevalent form of nitrogen is usually the ammonia, because of the high redox conditions.

Autotrophic community >3 μm (nanophytoplankton and microphytoplankton)

Table III summarizes the main phytoplanktonic taxa observed in the Utermöhl algal counts. They were separated according to the size categories indicated in the methodology.

Figure 2 shows the variation of the strictly autotrophic algae (nanophytoplankton and microphytoplankton) for both experiments. Mean densities of the nanophytoplankton fraction (algae 3–20 μm) varied from 9127 to 13 317 individuals mL^{-1} for the first experiment and from 17 227 to 38 989 individuals mL^{-1} for the second one (Fig. 2a and b). For Experiment I, the RM ANOVA revealed that no significant differences in the density of this algal category were registered among times and between treatments (dark and light microcosms), although mean densities were slightly higher in the flasks exposed to the light. These results were more evident in the second experiment, because the variation of the density of nanophytoplankton clearly followed the light gradient; thus, the algal density of this fraction increased in the flasks without nylon mesh and decreased in those with 2-ny mesh and the values were intermediate in flasks with 1-ny mesh. These differences were statistically significant according to the RM ANOVA ($P = 0.017$). The nanoplankton fraction was dominated by Chlorophyceae such as *Monoraphidium contortum*, *Monoraphidium circinale*, *Chlamydomonas* spp., *Oocystis lacustris* and several species of *Scenedesmus* and *Chlorella*. Small cyanobacteria, such as *Aphanocapsa delicatissima* and *Merismopedia tenuissima*, were some of the accompanying species. In both experiments, eukaryotes increased in the microcosms exposed to more light. With respect to the cyanobacteria of this size fraction, the temporal pattern differed in the two experiments.

The microphytoplankton fraction (algae >20 μm) was absolutely dominated by cyanobacteria during the first experiment, with *Planktolyngbya limnetica*, *Cylindrospermopsis raciborskii* and *Planktothrix aghardii* as the dominant species, whose mean densities varied from 79 331 to 215 337 individuals mL^{-1}. This size fraction clearly decreased during the experiment in both treatments (dark and light microcosms) (Fig. 2c). This fact accounts for the significant differences among times obtained from the RM ANOVA ($P = 0.001$). From t_2 to t_3, the abundance of this fraction was lower in the flasks that were exposed

Table III: List of main phytoplanktonic taxa in the enclosure experiments

Prokaryotes 3–20	Eukaryotes 3–20	Eukaryotes >20
Aphanocapsa delicatissima	*Achnanthes exigua* var. *exigua*	*Actinastrum hantzchii*
Aphanocapsa elachista	*Chlamydomonas* spp.	*Aulacoseira granulata* var. *granulata*
Merismopedia punctata	*Chlorella vulgaris*	*Aulacoseira italica*
Merismopedia tenuissima	*Crucigenia quadrata*	*Chlamydomonas* spp.
Romeria leopoliensis	*Didimocystis bicellularis*	*Closterium aciculare*
Woronichinia elorantae	*Diplochloris lunata*	*Cyclotella meneghiniana*
	Monoraphidium circinale	*Dictyosphaerium pulchellum* var. *pulchellum*
Prokaryotes >20	*Monoraphidium contortum*	*Monoraphidium griffithii*
Anabaenopsis elenkini	*Monoraphidium minutum*	*Monoraphidium komarkovae*
Cf. *Arthrospira*	*Oocystis lacustris*	*Nitzschia acicularis*
Cylindrospermopsis raciborskii	*Scenedesmus intermedius*	*Planktonema lauterbornii*
Planktothrix aghardii	*Scenedesmus alternans*	*Scenedesmus acuminatus*
Planktolyngbya limnetica	*Scenedesmus ecornis*	*Scenedesmus quadricauda*
	Sphaerocystis schroeterii	*Scenedesmus bicaudatus*
Mixotrophic >10	*Tetrastrum* spp.	*Scenedesmus spinosus*
Cryptomonas erosa		*Schroederia setigera*
Cryptomonas marssonii		
Cryptomonas ovata		
Euglena variabilis		
Peridinium sp.		

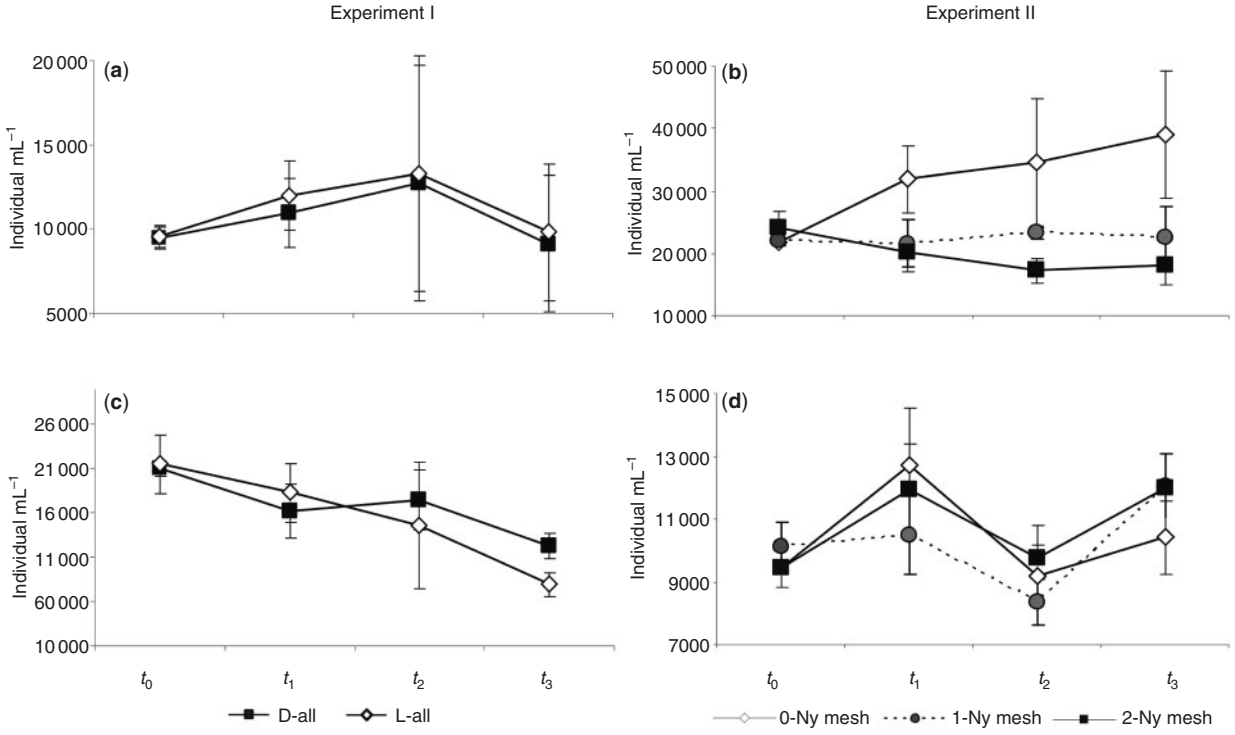

Fig. 2. Variation of the abundance of the autotrophic phytoplankton corresponding to the nano- and microplankton fractions. Experiment I: (**a**) 3–20 μm; (**c**) >20 μm. Experiment II: (**b**) 3–20 μm; (**d**) >20 μm. Bars represent SD.

to the light, although differences between both treatments were not statistically significant. During the second experiment, the microphytoplankton fraction was not absolutely dominated by cyanobacteria as in the first case. On the contrary, the community was conformed by co-dominance of *Planktonema lauterbornii*, *P. limnetica* and some accompanying big diatoms; densities in this case were much lower varying mean values between 7632 and 14 736 individuals mL^{-1} (Fig. 2d). As for the first experiment, no significant differences were observed among treatments, and in this case, the temporal variations did not follow a definite pattern.

As in the case of the other size fraction, eukaryotes >20 μm also exhibited higher densities in the flasks exposed to the light, but the temporal pattern differed in the two experiments. For the first one, the density gradually increased from t_0 to t_3, whereas for the second one a strong increase was observed from t_0 to t_1, and then the abundances decreased in the microcosms. In spite of these differences, in both experiments the abundances of eukaryotes >20 μm remained rather low in the treatments with light deficiency.

In the case of the cyanobacteria >20 μm, the lowest densities were observed in the flasks without cover and the highest ones in the darkness, but also in this case the temporal pattern was different in both experiments.

An inverse correlation between the abundance of algae >20 μm and the concentration of nitrate was found in Experiment I ($r = -0.59$; $P < 0.05$). In Experiment II, the density of this fraction was inversely correlated with ammonia ($r = -0.62$; $P < 0.05$).

Mixotrophic algae

The mixotrophic algae are analysed separately to test one of the hypotheses of this work. Just the recognized phagotrophic species are considered in this section. The dominant mixotrophic algae were *Cryptomonas marssonii*, *Cryptomonas ovata* and *Cryptomonas erosa*, which constituted ~92% of all the recorded phagotrophic species for the first experiment and ~80% for the second one. Figure 3a and b shows the evolution of the mixotrophic algae in the microcosms, which varied between 414 (t_0) and 4024 (t_3) individuals mL^{-1} for Experiment I and between 736 and 2690 individuals mL^{-1} for Experiment II.

During the first experiment, mixotrophic algae exhibited a marked abundance increase in the darkness. This fact has suggested us that these algae were able to have a heterotrophic nutrition, which was corroborated by the grazing experiment. The RM ANOVA revealed significant differences with respect to the time ($P = 0.001$) and between treatments ($P = 0.001$). The interaction

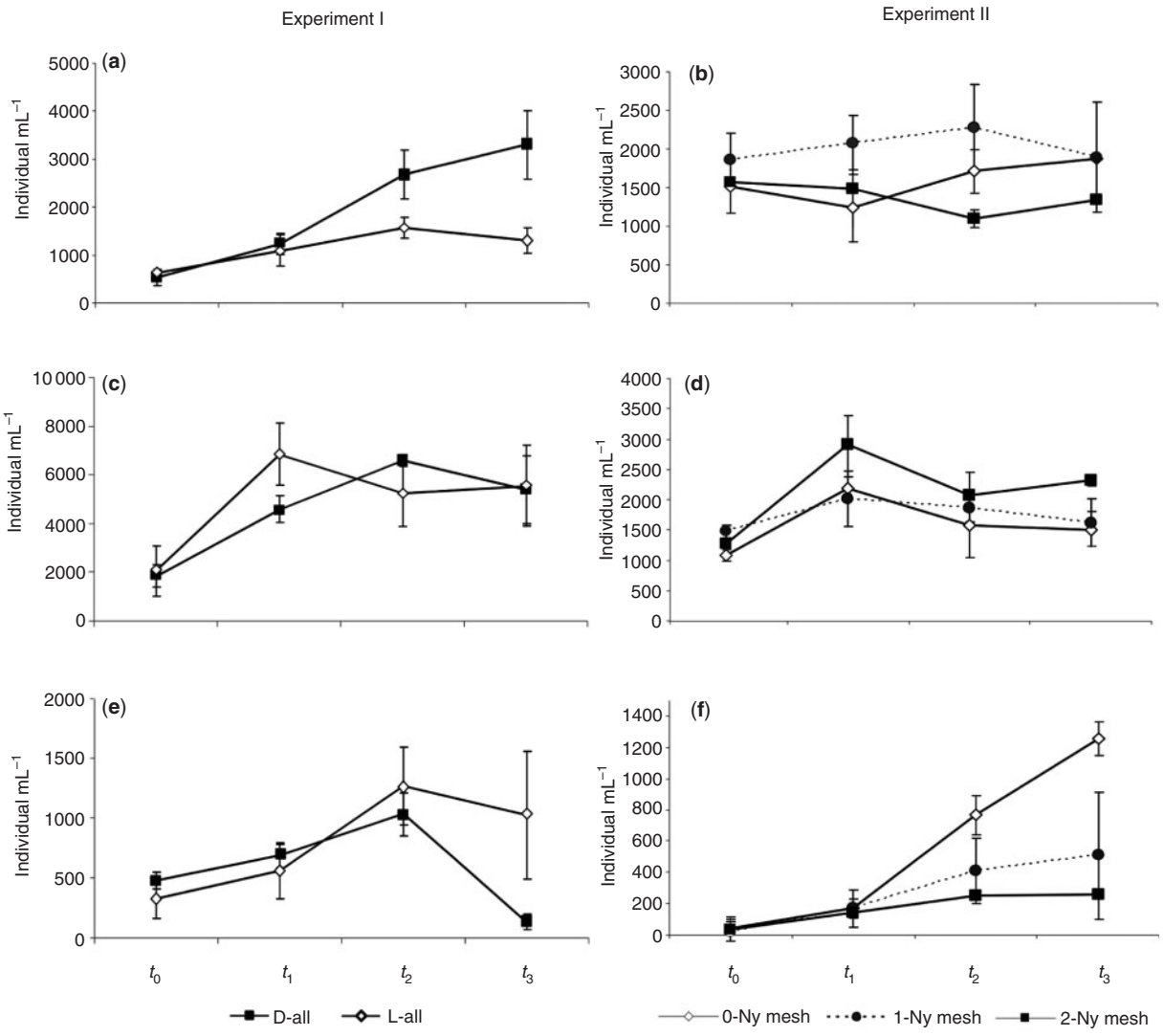

Fig. 3. Temporal variation of the abundance of the potential predators of the picoplankton fraction. Experiment I: (**a**) mixotrophic algae; (**c**) heterotrophic nanoflagellates (HNF); (**e**) ciliates. Experiment II: (**b**) mixotrophic algae; (**d**) HNF; (**f**) ciliates. Bars represent SD.

time–treatments was also significant (P = 0.003). Contrasts showed that the differences between treatments occurred from t_2 (48 h).

At the beginning of the second experiment, the abundance of the mixotrophic algae in the lake was higher than during the previous experience. Densities were of ~1700 individuals mL^{-1} in the natural lake and ranged between 1500 and 1840 individuals mL^{-1} in the microcosms (at t_0). In this case, the increase of these algae along the experiment was not so noticeable as in the former experiment. Thus, no significant differences with respect to time were observed by the RM ANOVA. Nevertheless, differences were significant among treatments (P = 0.005), registering densities slightly higher in the flasks with 1-ny mesh.

To analyse the replacement of obligate autotrophic species by mixotrophic and heterotrophic taxa in the shading microcosms, we calculated the autotrophic/heterotrophic ratio for the nanoplankton fraction (Fig. 4a and b). Figure 4a and b shows that this ratio increased in the flasks with light. The daily evolution of the ratio is particularly evident in the second experiment.

Heterotrophic nanoflagellates

HNF varied from 1445 to 8105 individuals mL^{-1} during the first experiment and from 986 to 3254 individuals mL^{-1} during the second one (Fig. 3c and d), and the abundances increased along the time for both experiments, in all treatments. RM ANOVA showed significant differences with respect to the time (P = 0.0001) for

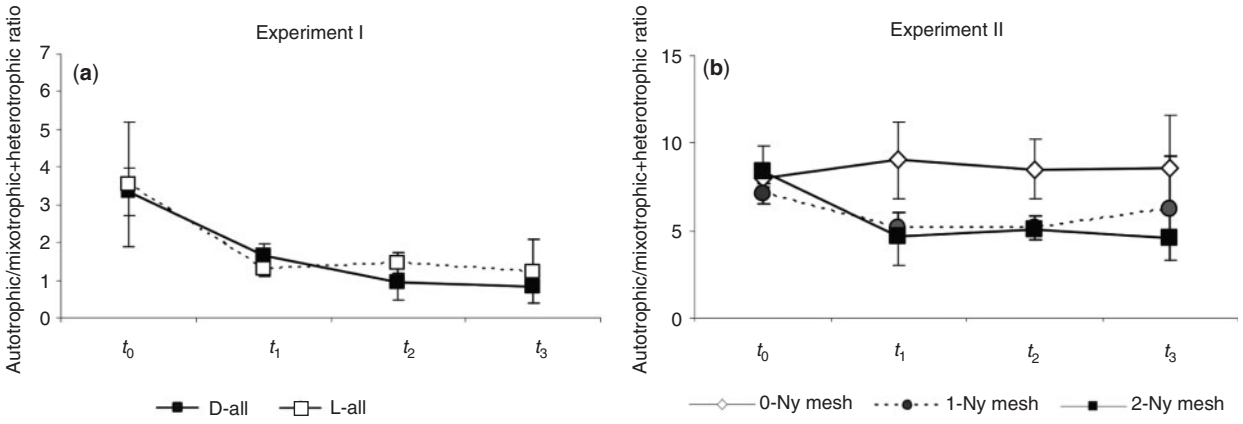

Fig. 4. Variation of the autotrophic/mixotrophic + heterotrophic ratio for the nanoplankton fraction during both light experiments. (**a**) Experiment I; (**b**) Experiment II. Bars represent SD.

both experiments. Comparing the light treatments, differences were not significant for Experiment I, whereas for Experiment II the RM ANOVA showed that significant differences existed among the three treatments ($P = 0.007$). The highest HNF densities were observed in the microcosms with 2-ny mesh.

Ciliates

The temporal of the abundance of ciliates is shown in Fig. 3e and f. Their densities ranged from 80 to 1565 individuals mL^{-1} for Experiment I and from 23 to 1379 individuals mL^{-1} for Experiment II. The community was composed mainly of Oligotrichida. For both Experiment I and II, RM ANOVA showed significant differences between times ($P = 0.003$ and $P < 0.001$, respectively), and the pattern was very similar. In both cases, the abundance of ciliates increased along the time in the flasks with more light. In particular, in the second experiment, the increase of ciliates followed the light gradient: the highest densities were observed in the microcosms without mesh cover, intermediate values in the flasks with 1-ny mesh and lowest densities in flasks with 2-ny mesh.

For the first experiment, the RM ANOVA did not evidence significant differences between the treatments, but the interaction time–treatments resulted significant ($P = 0.021$). Analysing the contrasts, we found that both treatments significantly differed at t_3 ($P = 0.027$), registering a higher abundance of ciliates in the microcosms exposed to the light. For the second experiment, differences among treatments were significant ($P = 0.004$).

In both experiments, the examination of the ciliates under a light microscope at ×1000 magnification evidenced that several organisms contained autotrophic algae *Chlorella*-like inside, which would indicate the existence of mixotrophic species in the ciliate assemblages. Nevertheless, with this study, we are not able to state whether the ciliates have ingested the algae maintaining them as symbionts or whether they retained just the chloroplasts.

Autotrophic and HPP

Figure 5a–d illustrates the variation of the abundance of the different categories of picoplankton analysed (heterotrophic bacteria, picoplanktonic cyanobacteria and picoplanktonic eukaryotes) for the first experiment. As it was detailed in *Methods*, the picoplankton abundance was also evaluated in including only picoplankton, to analyse the potential grazing on this fraction by its main predators (HNF, ciliates and mixotrophic algae).

The abundance of heterotrophic bacteria in the microcosms that included the whole microbial community varied between 6523 and 39 436 bacteria mL^{-1}. The RM ANOVA revealed that no significant differences between treatments existed. Contrarily, this analysis also showed that the differences between times were significant ($P = 0.001$), and the interaction time–treatments was also significant ($P = 0.001$). Contrasts revealed that in the dark microcosms that included all the fractions, the heterotrophic bacteria significantly decreased from t_0 to t_1, and then the abundance remained low until the end of the experiment.

The abundance of heterotrophic bacteria in the flasks including only the picoplanktonic fraction ranged from 6823 to 36 062 bacteria mL^{-1}. In this case, the RM ANOVA showed significant differences between times ($P = 0.035$), between treatments ($P = 0.005$), as well as a significant interaction time–treatments ($P = 0.004$). Differences between treatments were observed at t_1.

The Spearman correlation analysis revealed a significant inverse correlation between bacteria and their three main categories of potential predators: HNF

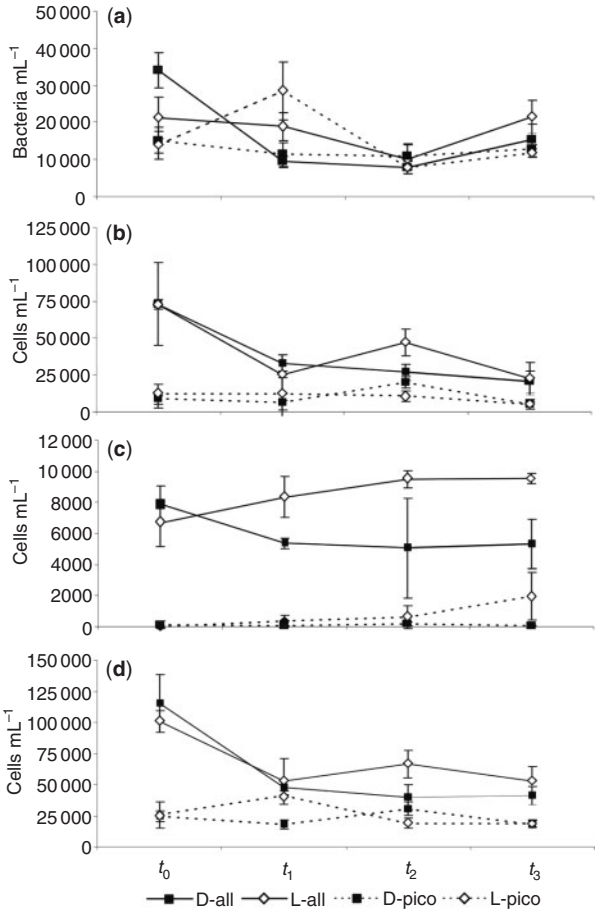

Fig. 5. Variation of the autotrophic and heterotrophic picoplankton (HPP) fraction during the experiment. (**a**) Heterotrophic bacteria; (**b**) pico-cyanobacteria (Pcy); (**c**) picoplanktonic eukaryotes; (**d**) all the picoplankton fractions. Bars represent SD.

($r = -0.41$; $P < 0.05$), ciliates ($r = -0.43$; $P < 0.05$) and mixotrophic algae ($r = -0.64$; $P = 0.0008$). A direct correlation between bacteria and phosphates was also found ($r = 0.54$; $P = 0.005$).

The autotrophic picoplankton was strongly dominated by pico-cyanobacteria (Pcy) algae (*Synechococcus* and *Synechocystis* cells-like), which varied from 10 035 to 95 079 cells mL^{-1} in the microcosms that included all fractions and from 1003 to 23 281 cells mL^{-1} in the controls.

The RM ANOVA showed significant differences between times in the density of Pcy in the flasks that included the whole community ($P = 0.001$). A significant decrease in the abundance of Pcy was found in the flasks containing all the microbial components from t_0 to t_1 ($P = 0.001$), which probably is associated with the grazing pressure on this fraction.

Analysing the flasks that contain only picoplankton, the RM ANOVA also revealed significant differences in relation to the time ($P = 0.001$), but contrasts showed that only at t_2 the density of Pcy was significantly different to the initial density, and the temporal pattern was rather constant, which indicates no important multiplication or losses of this fraction during the experiment (Fig. 5b).

The Spearman correlation analyses showed that Pcy were inversely correlated with the HNF ($r = -0.69$; $P = 0.0002$) and with the mixotrophic algae ($r = -0.66$; $P = 0.0004$).

The picoeukaryotes were relatively more scarce in the microbial community, ranging from 2007 to 10 135 cells mL^{-1} in the flasks that included all the fractions and from 100 to 3312 cells mL^{-1} in the microcosms containing only picoplankton. This fraction was composed essentially of *Chlorella* cells-like. The RM ANOVA for the picoeukaryotes revealed significant differences between treatments ($P = 0.029$) but no differences between times. Regarding the patterns in Fig. 5c, it is clear that the abundance of picoeukaryotes was higher in the microcosms exposed to the light. The abundance of picoeukaryotes was directly correlated with the dissolved oxygen concentration ($r = 0.78$; $P = 0.00001$).

Finally, we also analyse the total abundance of the picoplankton fraction (Fig. 5d). The density varied between 30 857 and 132 835 cells mL^{-1} in the flasks with the whole community and from 13 941 to 44 454 cells mL^{-1} in those with only picoplankton. As it was mentioned above, Pcy constituted the higher proportion of the bulk of the picoplanktonic fraction, and for this reason, the results of the RM ANOVA were very similar to those described for the Pcy. Significant differences were observed between times in the microcosms with the whole community ($P = 0.001$). As in the case of the Pcy alone, a marked decrease from t_0 to t_1 was observed for the total picoplankton. In the same way that was observed for the Pcy, although significant differences were obtained between times in the control flasks ($P = 0.008$), the contrasts revealed that these differences were due to a unique sampling date (in this case t_1), but the general temporal pattern was rather constant.

The bulk of the picoplankton was inversely correlated with the abundance of HNF ($r = -0.68$; $P = 0.00025$) and with the mixotrophic algae ($r = -0.73$; $P = 0.00005$).

Grazing experiments

The experiments performed with the addition of FLB showed that two species of *Cryptomonas* (*C. erosa* and *C. marssonii*), which are frequent in the phytoplankton of the studied shallow lake, can ingest bacteria. In the case of *C. ovata*, our experiments did not show a clear ingestion of FLB, whereas the potential phagotrophy of other *Cryptomonas* species could not be evaluated because of their scarcity during the two grazing experiments.

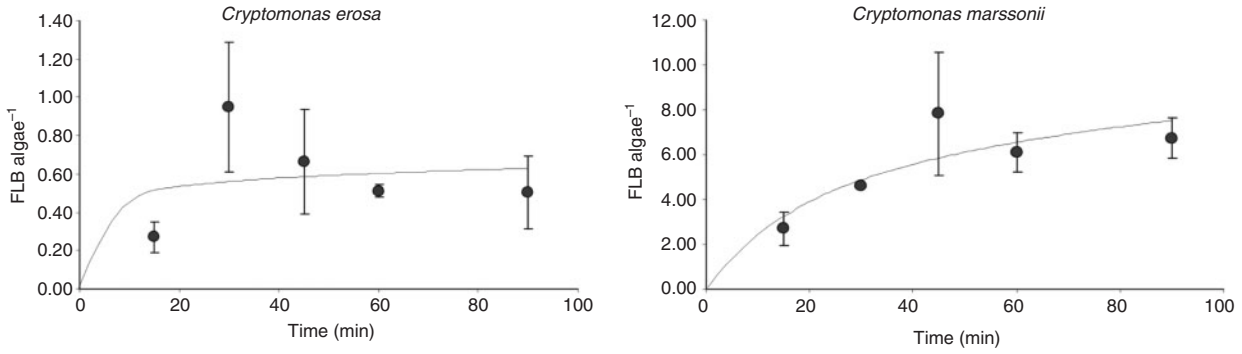

Fig. 6. Average values of fluorescent-labelled bacteria (FLB) ingested by the two *Cryptomonas* species over the time. Bars represent SD.

For *C. erosa*, the mean specific ingestion rate was 3.22 bacteria individual^{-1} h^{-1}, the mean clearance rate 4.27 nL individual^{-1} h^{-1} and the mean grazing impact 9.33×10^3 bacteria mL^{-1} h^{-1}. In the case of *C. marssonii*, mean values were considerably higher, with a specific ingestion rate of 15.38 bacteria individual^{-1} h^{-1}, a clearance rate of 20.38 nL individual^{-1} h^{-1} and a grazing impact of 38.38×10^3 bacteria mL^{-1} h^{-1}. The C consumption by phagotrophy is ~0.044 and ~0.171% h^{-1} of the C biomass for *C. erosa* and *C. marssonii*, respectively. Figure 6 illustrates the average values of FLB ingested by the two *Cryptomonas* species over the time.

DISCUSSION

The microbial community of this wetland is constituted by many species of small algae, among which some are mixotrophic, a great variety of HNF and ciliates, heterotrophic bacteria and autotrophic picoplankton (dominated by Pcy).

In general, the phytoplankton composition (>3 μm) observed in this study was very similar to that reported for this shallow lake in our previous limnological researches (Izaguirre *et al.*, 2001, 2004; O'Farrell *et al.*, 2003). Nevertheless, the algal abundance and the proportion of the algal species strongly differed in the shallow lake between the two light experiments. During the first experiment, an important bloom of filamentous cyanobacteria (>20 μm) occurred in the lake. Contrarily, during the second one, more species co-dominated in the lake (Chlorophyta and Cyanobacteria species) accompanied by diatoms; the abundance of the mixotrophic species (mainly *Cryptomonas* spp.) was higher, and the total phytoplankton density was much lower than during the first experiment.

In spite of these differences in composition, the results of our two light experiments showed that the presence of a dense floating macrophyte cover affects the structure of the microbial community. One of the most important and obvious effects of the floating plants on the water column is the decrease in the light penetration, and the concomitant declination of the photosynthetic activities, which in turn provokes a decrease in the dissolved oxygen. Under these conditions, in natural conditions the reduced chemical forms are the prevalent forms in the lake. Because nutrients are more concentrated near the bottom, and also anoxia is more pronounced in deeper layers, a vertical gradient in ammonia can be observed, due to the uptake by algae and bacteria. These features are typical of the most vegetated water bodies of this wetland and were described in our previous works (O'Farrell *et al.*, 2003; Izaguirre *et al.*, 2004). Another important effect of the macrophytes is that they constitute an important source of dissolved material, which has influence on the availability of nutrients. Franco and Heath (Franco and Heath, 1983) described that the nutrient availability may be decreased in lakes with high dissolved organic matter (DOM) contents because of the formation of humus–metal–P complexes. Nevertheless, these complexes can also be considered as reservoirs of potential P, which can be released from these compounds under P deficiency (Jones, 1998). This study, supported by previous experiments performed by Unrein (Unrein, 2001), showed that phosphate is not a limiting factor for the phytoplankton, but contrarily nitrogen can be limiting under certain conditions. It is important to point out that the floating macrophytes compete with algae for nutrients, because they take them from the water column (Scheffer *et al.*, 2003).

The limitation in light penetration produced by the macrophytes, and the consequent decrease in photosynthesis, was well simulated by means of the nylon mesh used in our experiment. The starting point in all the microcosms of each experiment was almost identical with respect to the oxygen concentration. After 24 h, a decrease in oxygen occurred in the dark flasks because of the decreased photosynthetic activity. Contrarily, in the

microcosms exposed to the light, a higher photosynthesis was evident. Particularly, in the case of the first experiment, the highest values were recorded in the microcosms containing all the size fraction algae.

The relatively high pH values, usually recorded in the shallow lake, are due to the natural alkalinity of its basin (O'Farrell *et al.*, 2003; Izaguirre *et al.*, 2004) on the one hand and to a high photosynthesis in open waters during periods of active algal growing on the other. In this sense, the increase of the pH in the light microcosms is obviously the result of the higher photosynthesis.

The evolution of the nutrient concentrations during the first experiment seems to reflect their uptake by algae and bacteria. In particular, phosphates progressively diminished in those flasks that included all the components of the microbial community. From t_1 to t_3, the decrease was more evident in the light microcosms. During the second experiment, phosphate concentrations were much higher; thus, the variations inside the flasks were not so marked.

The declination of ammonia concentration along the time in both experiments was also probably due to the uptake by algae. Particularly, for the first experiment, the concentration of the dissolved inorganic nitrogen (nitrates + ammonia) sharply decreased after 48 h, showing very low values at the end. Considering that nitrogen could be a limiting nutrient in this wetland, as it was mentioned above, it is probable that this nutrient was limiting for the phytoplankton towards the end of our first experiment. In particular, this assumption seems to be confirmed regarding the inverse correlations between inorganic dissolved nitrogen and the abundances of algae >20 μm in both light experiments. This fact, together with a parallel increase of mixotrophic algae in the dark microcosms, seems to indicate some competition for nutrients among the algae. Under these conditions, mixotrophic species are favoured in the darkness over obligate phototrophic algae because they can ingest particles.

The results of our experiments seem to confirm the first hypothesis postulated in this work. In fact, the darkness favoured the replacement of obligate autotrophic algae by mixotrophic taxa. We think that these algae could be favoured over strictly phototrophic species in the microcosms without light, and under conditions of progressive declination of inorganic nutrients, in particular nitrogen. We only analysed in this study those mixotrophic algae that, according to the description given by Jones (Jones, 2000), are organisms capable of obtaining energy and/or nutrients by both phototrophic autotrophy (using light energy and inorganic nutrients) and phagotrophic heterotrophy (ingesting particles into food vacuoles for subsequent digestion and utilization of derived organic compounds). Within this category of mixotrophic algae, the dominant taxa during our experiments were several species of the genus *Cryptomonas*. Following the classification of Jones (Jones, 2000), these algae can be included within the group of protists whose primary mode of nutrition is phototrophy and that ingest prey only at very low rates during prolonged dark periods. Mixotrophy has an additional cost over the strict autotrophy, and the sum of costs for a cell with both photosynthetic and phagotrophic machinery is greater than for a cell with either apparatus (of equal size) alone (Raven, 1997). Nevertheless, switching between trophic modes may decrease the cost compared with simultaneous phototrophy and phagotrophy (Stoecker, 1998); this author proposed that *Cryptomonas* spp. fits the model of mixotrophy of algae that are primarily phototrophic but that feed to obtain trace organic growth factors.

The results of our grazing experiments with FLB confirmed that at least two *Cryptomonas* species, which are very frequent in the Otamendi aquatic environments (*C. erosa* and *C. marssonii*), are capable of displaying a phagotrophic behaviour. Even though specific grazing rates estimated in this study for *C. erosa* and *C. marssonii* are comparable with previous observations of Porter (Porter, 1988) and Urabe *et al.* (Urabe *et al.*, 2000), they are particularly high. In fact, they are in the highest limit reported for cryptophytes (Tranvik *et al.*, 1989; Roberts and Laybourn-Parry, 1999). However, these rates are in the same range as those estimated by Domaizon *et al.* (Domaizon *et al.*, 2003). According to the results obtained by Urabe *et al.* (Urabe *et al.*, 2000), the diel variation in the phagotrophic behaviour of *Cryptomonas* sp. seems to be related with acquisition of nutrients and some substances essential to their growth when they are in low concentrations in the lake.

In our two light experiments, the growth rate of the HNF was higher than that of the mixotrophic algae during the first day of the experiment. Analysing the data of Experiment I, we observed that the pronounced increase of HNF between t_0 and t_1 coincided with a drastic declination of picoplankton (dominated by Pcy). This fact and the significant inverse correlation between picoplankton and HNF seem to indicate a high ingestion of this fraction by HNF. Porter (Porter, 1988) described that the ingestion rates of non-pigmented flagellates on particles were related with the particle concentration, and in general, they were equivalent or slightly higher than those of the mixotrophic algae. At the beginning of our first experiment, the food supply (picoplankton) was very abundant in the experimental flasks. Pernthaler *et al.* (Pernthaler *et al.*, 1996) demonstrated that HNF, as well ciliates, prefer to consume Pcy rather than heterotrophic bacteria. On the other hand, Callieri *et al.* (Callieri *et al.*,

2002), using fluorescently labelled Pcy, showed that the grazing impact of the HNF community ranged from 1.9 $\times 10^3$ to 8 $\times 10^3$ Pcy mL^{-1}.

As it was mentioned in *Results*, ciliates increased in the microcosms with more light, and their examination showed the presence of *Chlorella* cells-like inside the cytoplasm. Unfortunately, in this study, we cannot assert if the ciliates have ingested the algae and they have retained the cells or the chloroplasts inside, which would be typical of the mixotrophic ciliates. Nevertheless, our results agree with the observations of Amblard *et al.* (Amblard *et al.*, 1995), who found that the biomass of mixotrophic ciliates (Oligotrichida) in a humic lake was positively correlated with light energy. Moreover, Queimaliños *et al.* (Queimaliños *et al.*, 1999) also demonstrated dependence on light in mixotrophic ciliates from a Patagonian lake. According to these authors, some species can even modify their morphology, for example adopting elongated forms, which allows the endosymbiotic *Chlorella* to be arranged to optimize the received light (Modenutti *et al.*, 2004). In the studied wetland, the floating macrophytes usually cause a severe light limitation in the water column during some periods. Thus, the ciliates that inhabit this wetland are probably also well adapted to the changes in irradiance associated with the variations in the macrophyte cover, and their physiological adaptations deserve further experimental studies.

The increase of mixotrophy algae, HNF and ciliates inside the microcosms was probably favoured by the removal of their predators (zooplankton) in accord with the trophic cascade hypothesis (Carpenter *et al.*, 1985). Estimations of total zooplankton abundance (copepods, cladocerans and rotifers) in the shallow lake range between 39 and 813 individuals L^{-1} (R. Sinistro, University of Buenos Aires, unpublished data). The highest densities correspond to a clear water phase in the lake.

Regarding the abundances of heterotrophic bacteria observed for Experiment I, it is evident that they declined in the microcosms containing all the components of the microbial community, probably due to their ingestion by predators. Nevertheless, densities recorded at the beginning of the experiment were relatively low compared with values reported for other aquatic systems of similar trophic status (Sorokin, 1999) and also with concentrations observed in this wetland during other periods (I. Izaguirre, University of Buenos Aires, unpublished data).

Cyanobacteria dominated all the plankton size fractions analysed in the lake during our first experiment. According to data obtained by Smith (Smith, 1983) and Shapiro (Shapiro, 1990), cyanobacteria are favoured at low N–P ratios and high pH.

Although in this work we have not analysed the changes in phytoplankton species composition associated with the different light climate conditions, in both experiments we observed that the proportion of eukaryotes/prokaryotes, as well as the proportion of the dominant taxa, changed under different light attenuations. In general, in our experiments, chlorophytes were benefited by an improvement in the light climate. Huisman *et al.* (Huisman *et al.*, 1999) have shown that the light intensities for phytoplankton growth are species-specific and fall in a very narrow range. Flöder *et al.* (Flöder *et al.*, 2002) have demonstrated that slow light fluctuations in the range 3–12 days affected diversity in phytoplankton communities and that chlorophytes predominated in the treatment with permanent high light, whereas either cyanobacteria or diatoms dominate at low light intensities, which agrees with our observations.

The plankton composition observed at the end of both experiments in the flasks with light deficiency was very similar to that observed in the shallow lake in natural conditions under a macrophyte cover. Together with mixotrophic and heterotrophic taxa, the plankton assemblages included algae well adapted to low light conditions; in particular, several small cyanobacteria and unicellular chlorococcaleans, such as *Chlorella* spp. and *Monoraphidium* spp., which can optimize the uptake of light, as well as diatoms well adapted to light-limited environments, like some *Achnanthes* species. The proportion of bigger chlorophytes, like coenobial and colonial forms (e.g. *Scenedesmus* spp. and *Oocystis* spp.), was higher in the treatments with light, as it occurs in natural conditions in the lake when light conditions improve in the water column.

As it was previously discussed, our experimental study confirmed the first hypothesis postulated in this work. The decrease in the light incidence favoured the replacement of obligate autotrophic species by mixotrophic algae, which are probably better competitors under light-deficient conditions and when nutrients progressively decrease, which occurred towards the end of the experiments. The second hypothesis of this study was corroborated as well. Picoplankton was significantly reduced in the microcosms that included their potential predators (HNF, ciliates and mixotrophic algae) because these strongly increase due to the lack of the metazooplankton. Nevertheless, no significant differences in the ingestion of picoplankton were found between dark and light microcosms. In relation to the fourth hypothesis, this work confirmed that differences in light climate conditions lead to a different microbial plankton composition; for two of the components analysed (autotrophic algae >3 μm and ciliates), the daily evolution of their abundances showed gradual changes associated with the

light gradient analysed. By means of the grazing experiments with FLB, this study also corroborated that two frequent *Cryptomonas* species of this wetland (*C. erosa* and *C. marssonii*) can ingest bacteria. This work constitutes the first approach to the knowledge of the structure and functioning of the microbial food web of this wetland.

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