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Grazing rates of protists in wetlands under contrasting light conditions due to floating plants

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ABSTRACT: We examined the effect of light attenuation, due to floating plants, on the community structure of the main phagotrophic protists and their grazing rates in a wetland in the Lower Paraná Basin. Ingestion experiments (winter and summer) were conducted at 2 sites in the same shallow lake that had contrasting light scenarios: open waters (light) and under profuse macrophyte coverage (dark: light attenuation ~97 %). We compared the rates at which protists ingested 3 types of tracer prey: fluorescently labelled heterotrophic bacteria (FLB), picocyanobacteria (FLC) and picoeukaryotic algae (FLA). Light influenced both the structure of the microbial communities and the protistan grazing rates. Heterotrophic flagellates (HF) were more abundant under the macrophytes, whereas mixotrophic algae (cryptophytes) and autotrophic and heterotrophic picoplankton populations attained higher abundances in open waters. Specific grazing rates (SGRs) of mixotrophs on heterotrophic bacteria (HB) were higher in the light (7.9 to 15.5 prey cells grazer^{-1} h^{-1}), than in darkness (0.1 to 5.1 prey cells grazer^{-1} h^{-1}); the same trend was observed on picocyanobacteria (Pcy) (1.1 and 0.2 prey cells grazer^{-1} h^{-1}, light and dark). SGRs of HF were 1.0 to 7.3 cells grazer^{-1} h^{-1} (on HB) and 0.01 to 1.8 prey cells grazer^{-1} h^{-1} (on Pcy), with highest values in summer and no pattern in relation to light. SGRs of ciliates were higher in summer and in darkness. Clearance rates (CR) on Pcy were higher than on HB, for both HF and mixotrophic algae. In winter, cryptophytes contributed up to 93 % of the microbial grazing in the light, whereas HF were more important in darkness; in summer, bacterivory was dominated by heterotrophs in both light scenarios. Our experimental results highlight the importance of light conditions in structuring bacterial grazing by protists.

KEY WORDS: Phagotrophy · Protists · Mixotrophic algae · Grazing rates · Wetland · Light attenuation

INTRODUCTION

Predation in aquatic microbial food webs is dominated by phagotrophic protists, and this is the major cause of mortality for both heterotrophic and autotrophic bacteria (Sherr & Sherr 2002). During recent decades, numerous studies — conducted in both freshwater and marine ecosystems — have evaluated the impact of grazing by phagotrophic protists on heterotrophic and autotrophic picoplankton in order to elucidate their role in the transfer of matter and energy within food webs (e.g. Porter 1988, Sanders et al. 1989, Pace et al. 1990, Langenheder & Jürgens 2001, Domaizon et al. 2003, Unrein et al. 2007, Massana et al. 2009). Phagotrophic protists include strictly heterotrophic taxa as well as mixotrophic taxa

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(Montagnes et al. 2008, and cites therein). The latter group comprises organisms that are capable of obtaining energy and/or nutrients by both phototrophic autotrophy and phagotrophic heterotrophy (e.g. Stoecker 1998, Jones 2000). Bacterivory has been found to be an important mode of nutrition for heterotrophic flagellates (HF) and ciliates (Sherr & Sherr 1987, Sanders et al. 1989, Callieri et al. 2002), and numerous studies have also revealed the importance of mixotrophs as consumers of bacteria (e.g. Bird & Kalff 1986, Domaizon et al. 2003, Tsai et al. 2007).

Different investigations have shown that protists can ingest both heterotrophic bacteria (HB) and picocyanobacteria (Pcy), but few studies have simultaneously analysed the grazing rates on both types of prey (e.g. Sherr et al. 1991, Pernthaler et al. 1996, Šimek et al. 1997, Hadas et al. 1998, Christaki et al. 2001, Tarbe et al. 2011). Selective feeding by protists has been associated with different characteristics of the prey; among them are the release of dissolved chemical cues, prey motility, prey biochemical composition or nutrient stoichiometry, cell surface characteristics and prey size (Jezbera et al. 2005, Matz & Jürgens 2005, Jezbera et al. 2006, Shannon et al. 2007, Jürgens & Massana 2008). Some studies indicate that prey size is probably one of the main properties influencing the selection of prey (Fenchel 1986, González et al. 1990, Epstein & Shiaris 1992, Jürgens & Matz 2002), although food quality is also observed to exert an important effect on protistan selectivity (Stibor & Sommer 2003, Shannon et al. 2007).

Successive investigations conducted in the Otamendi Nature Reserve wetland have highlighted that floating plants exert a pronounced influence on microbial communities (Sinistro et al. 2006, de Tezanos Pinto et al. 2007, O'Farrell et al. 2009, Izaguirre et al. 2010). Persistent coverage by floating plants reduces the penetration of light into the water column; this strongly affects the proportion of strictly autotrophic vs. heterotrophic taxa, which in turn leads to the depletion of oxygen. Considering that the ingestion rates of protists are affected by light conditions (Porter 1988, Urabe et al. 2000, Pålsson & Granéli 2003, Balseiro et al. 2004, Jakobsen & Strom 2004), contrasting scenarios of light attenuation due to floating macrophytes would be expected to reveal differences in feeding rates of protists in the studied wetland. In some species of mixotrophic algae, ingestion rates can be inversely proportional to light intensity; in other species, phagotrophy seems to depend on photosynthesis, so that ingestion rates can be directly proportional to light (Jones 1997).

Previous information on protistan bacterivory for the Otamendi Nature Reserve wetland is restricted to the experiments carried out by Sinistro et al. (2006). These authors examined the ingestion of bacteria by 2 species of *Cryptomonas* that are usually abundant in this aquatic system; these studies reported relatively high grazing rates. In the present study, we analysed the grazing rates of phagotrophic protists (HF, ciliates and mixotrophic algae) in this wetland under 2 contrasting light scenarios: (1) open waters; (2) waters with a profuse covering of floating macrophytes. We also examined the prey preference of the different protists, comparing 3 types of tracer prey: fluorescently labelled heterotrophic bacteria (FLB), fluorescently labelled picocyanobacteria (FLC) and fluorescently labelled picoeukaryotic (picoplanktonic) green algae (FLA). Within this framework, we postulated the following hypotheses and predictions:

Hypothesis 1: Floating plants affect the structure of the protistan grazing community in the lake. Heterotrophic protists should dominate under conditions of low light penetration.

Hypothesis 2: Ingestion of prey is influenced by the extent of exposure to light in the water column. Thus, grazing rates should differ between light and dark conditions.

Hypothesis 3: Protists show a preference in their ingestion of prey. Thus, the specific grazing rates should be different for the 3 types of tracer prey used.

MATERIALS AND METHODS

Study site

The experiments were conducted in the main shallow lake of the Otamendi Nature Reserve (Buenos Aires Province, Argentina), which is a natural floodplain wetland located in the Lower Paraná Basin (34° 10′ to 34° 17′ S, 58° 48′ to 58° 53′ W). This wetland, delimited by the Paraná and Luján Rivers, encompasses 2 permanent shallow lakes and several semi-permanent relic oxbow lakes. The largest shallow lake (Laguna Grande) has an area of 156 ha (Fig. 1). The littoral areas of the shallow lakes are characterized by the presence of rooted macrophytes (mainly *Schoenoplectus californicus*), and these areas are periodically covered by floating plants, such as *Salvinia rotundifolia, Lemna minima, Pistia stratiotes* and *Azolla filiculoides.* With profuse development of floating plants, light attenuation in the water column is usually extremely high (subsurface

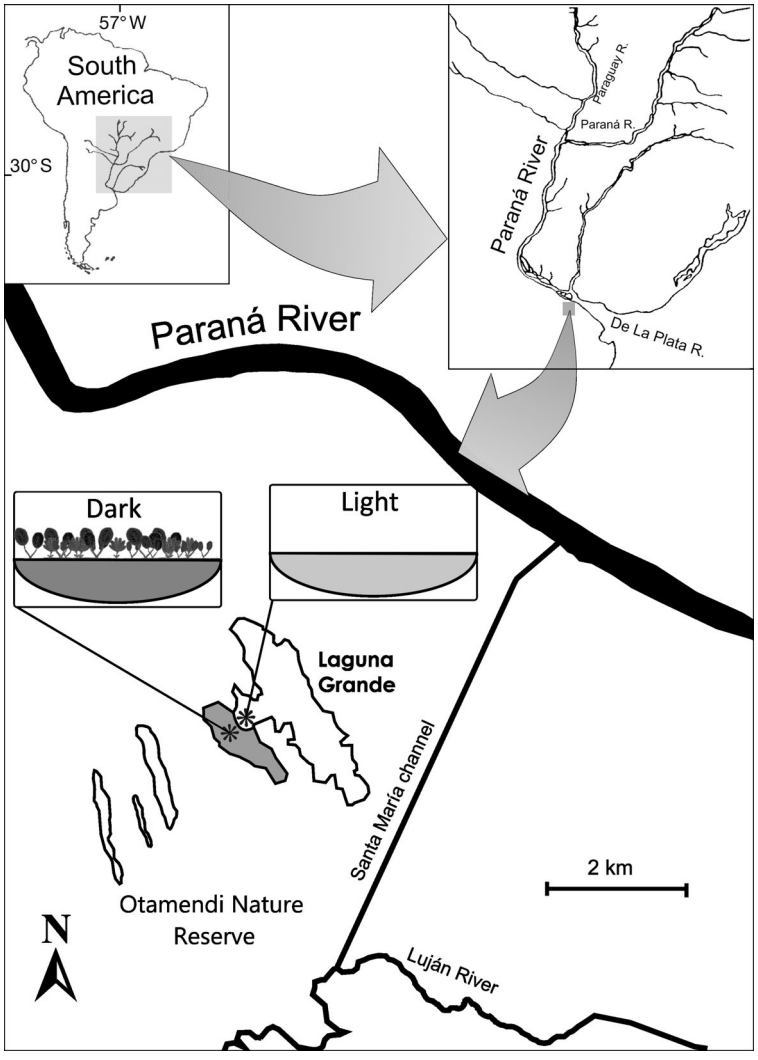

Fig. 1. The Paraná River Basin and the Otamendi Nature Reserve wetland showing the 2 sites in the Laguna Grande where the ingestion experiments were carried out (dark: littoral zone with a profuse covering of floating macrophytes; light: open waters)

light transmittance ~1%), which accounts for very low concentrations of oxygen; however, when the shallow lakes are free of floating plants more than 1% of the incident light reaches the bottom (e.g. Sinistro et al. 2006, de Tezanos Pinto et al. 2007, Izaguirre et al. 2010, Unrein et al. 2010). The climate of the region is defined as 'temperate pampean humid', and precipitations occur throughout the year with an annual mean value of 950 mm. Drainage is poor and is affected by groundwater fluctuations (Chichizola 1993). According to Williamson et al. (1999), this wetland can be classified as a 'mixotrophic lake-ecosystem' characterized by high levels of dissolved organic carbon and total phosphorus.

Ingestion experiments

We performed *in situ* short-term ingestion experiments, in Laguna Grande, in 2 areas of the shallow lake with contrasting underwater light conditions: (1) a littoral area with a high level of coverage by floating plants; (2) open waters (Fig. 1). We ran 2 identical experiments, 1 in winter (July 13, 2009), and 1 in summer (December 28, 2009). We determined the ingestion rates of the main protists in microcosms (experimental plastic bottles of 1 l capacity), using 3 different types of fluorescently labelled prey. We compared the ingestion of FLB, FLC and FLA whose mean biovolumes were, respectively, 0.09 μm^3, 0.72 μm^3 and 13.5 μm^3. FLB were prepared from a strain of *Brevundimonas diminuta* (syn. *Pseudomonas diminuta*) obtained from the Spanish Type Culture Collection (Burjassot, València). For the preparation of FLC we used a culture of a species of *Synechococcus* that had been isolated from a shallow lake in Uruguay, located at a similar latitude, which was provided by Dr. Sylvia Bonilla (Universidad de la República, Uruguay). For FLA we used a culture of a small *Chlorella kessleri* given to us by Dr. Angela Juárez (Universidad de Buenos Aires, Argentina). Each type of prey was prepared according to the protocol described in Unrein et al. (2007). The resulting tracer cells were added to natural water samples which had been taken from the shallow lake and filtered through a 55 µm net (to exclude zooplankton) before use; these water samples were contained in the (1 l) experimental bottles. The mean concentration of each type of prey used was about 18% of its natural population density in the lake. The bottles (triplicate in winter, duplicate in summer) were placed in the lake with buoyant devices. Subsamples were taken from each bottle immediately after the addition of the tracer cells (t_0); subsamples were subsequently taken every 15 min, up to 90 min, and preserved with cold, 10% glutaraldehyde (2% final conc.).

In the laboratory, 5 ml of each sample was filtered through a polycarbonate black filter of pore size 0.8 µm (Poretics) and stained with 4′,6-

diamidino-2-phenylindole (DAPI) to quantify the ingested tracer cells (prey) by epifluorescence microscopy (Olympus BX40) at 1000×. The hourly rate of ingestion of tracer cells by the predators was determined, according to Šimek et al. (1995), from the change in the average number of tracer cells, per protist, with time over the linear part of the uptake curve using regression analysis (generally t_0 to 30 min). Samples from t_0 were also inspected to avoid a potential bias due to the attachment of non-ingested tracers on protistan surfaces. The slope of this regression, divided by the abundance of grazers, is the tracer ingestion rate (tracer cells grazer^{-1} h^{-1}). The clearance rate (CR) (nl grazer^{-1} h^{-1}) was obtained from the tracer ingestion rate divided by the tracer concentration used. Specific grazing rates (SGRs) (prey cells grazer^{-1} h^{-1}) for each protist were estimated by multiplying the corresponding clearance rate by the prey concentration (native plus tracer cells), assuming that both types of cell had been ingested at similar rates. The grazing effects (prey ml^{-1} h^{-1}) of the protists on bacterioplankton and on the autotrophic picoplankton — the Pcy and the picoeukaryotes (Peuk) — were estimated by multiplying the specific grazing rates by the *in situ* abundance of the different protists. The SGR, as a percentage of the cell carbon per day (% d^{-1}), was calculated for each grazer group by dividing the respective specific grazing rates expressed in biomass of prey (µg C prey ind.$^{-1}$ d^{-1}) by the mean protistan biomass (µg C ind.$^{-1}$). We also estimated the contribution of the main protists (HF, mixotrophs and ciliates) to the total bacterivory in winter and summer under both light scenarios (light and dark).

Mean protistan biovolumes were estimated for each group on the basis of the average cell dimensions using appropriate volume formulae by measuring about 150 individuals. The mean cell volume of each group was converted to carbon, assuming a conversion factor of 0.22 pg C µm^{-3} (Børsheim & Bratbak 1987).

To quantify the natural concentration of HB and autotrophic picoplankton populations (Pcy and Peuk) in the lake, 0.5 to 2 ml of each sample was filtered through a polycarbonate black filter of pore size 0.2 µm (Poretics) and stained with DAPI. Natural picoplanktonic fractions and labelled prey cells were counted by epifluorescence.

Mean HB, Pcy and Peuk biovolumes were estimated on the basis of the average cell dimensions using appropriate volume formulae. HB biomass was calculated by using the carbon-to-volume relationship derived by Norland (1993) from the data of Simon & Azam (1989): pg C cell^{-1} = 0.12 pg (µm^3 cell^{-1})$^{0.7}$.

For the estimations of Pcy biomass, we adopted the value calculated by Worden et al. (2004) of 82 fg C cell^{-1}; these authors employed 3 approaches for the conversion of cell biovolume to carbon.

A comparison of CR on FLB and FLC was used to estimate relative preference by HF and *Campylomonas marsonii* (syn. *Cryptomonas marsonii*) for or against 1 of these 2 types of prey.

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; these measurements were carried out with portable electronic meters — Horiba D-54E (Japan) and Hanna HI 9146 (Hanna Instruments). 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). Samples for nutrient analyses were collected just below the surface. Soluble reactive phosphorus (SRP), nitrites+nitrates (N-NO_2+NO_3) and ammonium (N-NH_4) were measured with a Hach DR/2010 spectrophotometer, using the corresponding kits of reagents.

Statistical analyses

We used Student's *t*-test for analysing the significance of the differences in abundance of microbial communities in open waters (light conditions: L) and with a covering of floating macrophytes (dark conditions: D). We compared the specific ingestion rates of the main protists analysed by using the non-parametric Kruskal Wallis test (Zar 1996) in order to analyse the significance of the differences between the rates measured under D and L conditions.

RESULTS

Environmental variables and the natural abundance of grazers and prey types

The 2 sites selected for performing the ingestion experiments were very different in terms of light

penetration in the water column. Under the cover of floating macrophytes, the percentage of light attenuation was extremely high (96 to 98% at the subsurface [~10 cm]); the darkness in this water column was associated with a very low level of dissolved oxygen (0.92 to 3.31 mg l^{-1}). By contrast, in open water, the light attenuation at the subsurface ranged from 24 to 45% (winter and summer, respectively). The water temperature was between 7.7 and 9.1°C for the winter experiment, and between 25 and 26.2°C for the summer experiment; in both seasons, temperatures were slightly lower under the floating plants. Both sites showed relatively high concentrations of phosphate (0.58 to 3.64 mg l^{-1}), with lower values in summer. Concentrations of dissolved inorganic nitrogen (DIN = nitrate + nitrite + ammonium) were considerably low (0.04 to 0.07 mg l^{-1}) at both sites, and were within the range reported as limiting for the growth of phytoplankton according to Reynolds (2006). The environmental variables corresponding to each experiment performed are summarized in Table 1.

We found important differences in the structure of the microbial communities at the 2 studied sites (Table 2). Both in winter and summer, the abundance of HF was significantly higher under the floating plants than in open waters. We did not discriminate among size fractions of HF, but they were within the range 3 to 15 µm (mean value ~ 4 µm). Ciliates smaller than 55 µm were significantly more abundant under the macrophytes in winter; by contrast, in summer, the difference between the sampling sites was not significant. Among the phytoplankton taxa we retrieved 2 cryptophyte species that, according to our previous studies carried out in this wetland (Sinistro et al. 2006), are able to show mixotrophic behaviour by eating bacteria: these were *Campylomonas marsonii* and *Chroomonas* sp. Both cryptophytes were much more abundant in winter (Table 2), and their abundances were significantly higher in open waters than below the plants. Mean biovolumes of the studied grazers were 34 μm^3 for HF, 128 μm^3 for *Chroomonas* sp., 620 μm^3 for *C. marsonii*, and 5200 μm^3 for ciliates.

The abundances of natural heterotrophic and autotrophic picoplankton at the studied sites in winter and summer are indicated in Table 2. HB (mean cell biovolume 0.09 μm^3) were significantly more abundant in open waters than under the macrophytes in both seasons. Pcy, represented by *Synechococcus*-like cells (mean biovolume 0.65 μm^3), were less abundant

Table 1. Environmental variables at the 2 sites where ingestion experiments were performed (winter and summer). ND = non-detectable

	Open waters		Under the macrophytes	
	Winter	Summer	Winter	Summer
Dissolved oxygen (mg l^{-1})	7.2	6.06	0.92	3.31
pH	7.2	7.3	7.2	6.9
Conductivity (µS cm^{-1})	2110	1290	1950	1340
Temperature (°C)	9.1	26.2	7.7	25
N-NH_4 (mg l^{-1})	0.03	0.07	0.05	0.04
N-NO_2+NO_3 (mg l^{-1})	0.01	ND	ND	ND
P-PO_4 (mg l^{-1})	1.66	0.61	3.64	0.58
% Light attenuation (sub-surface)	24	45	96	98

Table 2. Abundance ± SD (cells ml^{-1}) of the picoplanktonic components and of the main bacterivorous protists at the 2 sites where experiments were run. Statistical differences (Student's *t*-test) in the abundance of the different components between the 2 sites are indicated (NS = non-significant)

	Open waters		Under the floating plants		Differences between open and covered waters	
	Winter	Summer	Winter	Summer	Winter	Summer
Heterotrophic flagellates	825 ± 242	2571 ± 695	3183 ± 870	4236 ± 1880	$p < 0.00001$	$p = 0.00068$
Campylomonas marsonii	4763 ± 666	152 ± 54	1105 ± 296	151 ± 74	$p < 0.00001$	NS
Chroomonas sp.	679 ± 291	44 ± 47	593 ± 258	44 ± 55	$p < 0.00001$	NS
Ciliates	107 ± 143	147 ± 68	227 ± 66	150 ± 40	$p = 0.013$	NS
Heterotrophic bacteria	5.31×10^6 ± 5.66×10^4	3.25×10^7 ± 3.81×10^6	8.89×10^5 ± 2.37×10^5	7.39×10^6 ± 1.02×10^6	$p = 0.001$	$p = 0.011$
Picocyanobacteria	2.17×10^5 ± 3.81×10^4	1.58×10^4 ± 9.89×10^2	1.31×10^4 ± 7.07×10^3	4.31×10^4 ± 9.05×10^3	$p = 0.005$	$p = 0.05$
Picoeukaryotes	4.75×10^4 ± 1×10^4	4.5×10^3 ± 1.66×10^3	1.87×10^3 ± 8.84×10^2	6.65×10^3 ± 5.87×10^3	$p = 0.009$	NS

than heterotrophic bacteria, as expected. We found higher abundances of Pcy in open waters in winter, whereas the contrary occurred in summer. Peuk, represented by *Chlorella*-like cells (~ 1.5 to 3 µm diameter; mean biovolume 2.52 μm^3), were much more abundant at the open water site than at the covered site in winter, whereas they had lower and similar abundances at both sites in summer.

Grazing rates of the main predators

Examination of the ingested tracer cells (prey) showed that only FLB and FLC were ingested by the protists that we analysed. FLA were only occasionally observed inside some ciliates, and thus the low numbers did not enable us to make an appropriate evaluation of the grazing rates, but FLA were never observed inside HF and mixotrophic algae, probably because of the relatively large size of this prey. Fig. 2 shows a comparison of the mean abundances of the main protists analysed, as well as their SGRs, CRs and grazing effect on heterotrophic bacteria and picocyanobacteria (FLB and FLC only).

Grazing by HF

The SGRs of HF on HB were significantly higher in summer ($p = 0.009$; chi squared = 6.66). In summer, values varied from 4.3 prey cells ind.$^{-1}$ h^{-1} (light experiment) to 7.3 prey cells ind.$^{-1}$ h^{-1} (dark experiment); in winter, the HF showed a higher SGR in the illuminated microcosms than in darkness (3.3 and 1.0 prey cells grazer^{-1} h^{-1}, respectively), but the difference between the 2 sites was not significant. In the case of the Pcy, owing to their lower abundances in the environment, the SGRs of HF were much lower (0.01 to 1.8 prey cells grazer^{-1} h^{-1}).

The CRs of the HF on HB ranged between 0.1 and 1.15 nl grazer^{-1} h^{-1}; we detected the lowest values under light conditions, and the highest ones in darkness. For the Pcy, the CRs of HF were higher, varying from 0.35 to 8.4 nl grazer^{-1} h^{-1}.

With respect to the grazing effect, the highest values of HF on HB were observed in summer at both sites (11×10^3 to 31.4×10^3 prey cells ml^{-1} h^{-1}), whereas winter estimates were one order of magnitude lower. Values of grazing effect on Pcy were lower than on HB, as expected by the lower abundance of Pcy, and ranged between 0.06×10^3 and 1.5×10^3 prey cells ml^{-1} h^{-1} (dark summer, light winter, respectively).

Grazing by mixotrophic algae

Two species dominate the mixotrophic algal assemblage, and their relatively high abundance allowed us to estimate their grazing rates. The mixotrophic alga *Campylomonas marsonii* showed significantly higher SGRs on HB in the light than in the dark ($p = 0.05$; chi squared = 3.6). For this species the maximum SGR was measured in summer under light conditions and the lowest in summer in darkness (15.5 and 0.1 prey cells grazer^{-1} h^{-1} respectively). SGRs of *C. marsonii* on Pcy varied from 0.06 to 1.1 prey cells grazer^{-1} h^{-1}, and also in this case the ingestion rates were higher in the light experiment, but the differences were not statistically significant. In the case of *Chroomonas* sp., the SGR in the light was 8.3 prey cells grazer^{-1} h^{-1}, but no ingestion was observed in the dark (data not shown).

The highest CRs of *Campylomonas marsonii* on HB were obtained in the winter experiment when the population exhibited a peak of abundance (1.5 to 5.7 nl grazer^{-1} h^{-1}), whereas the lowest CRs were measured in the summer experiment (0.01 to 0.48 nl grazer^{-1} h^{-1}) with very low abundances of these mixotrophic algae. For the Pcy, the CRs were higher than for HB (1.4 to 17.4 nl grazer^{-1} h^{-1}). As mixotrophic algae were much more abundant in winter, their highest grazing effect on HB occurred in this season, with extreme values under light conditions (37.8×10^3 prey cells ml^{-1} h^{-1}); the lowest value was observed in summer in darkness (0.02×10^3 prey cells ml^{-1} h^{-1}). The lowest grazing effect of *C. marsonii* on Pcy was observed in summer at both sites (0.01×10^3 prey cells ml^{-1} h^{-1}), and the highest in winter for the light experiment (5.3×10^3 prey cells ml^{-1} h^{-1}).

Grazing by ciliates

As for ciliates, the SGRs on HB were higher in the darkness than in the light, although differences were not significant. Moreover, values were higher in summer than in winter ($p = 0.0098$, chi squared = 6.66). SGRs were in the range 5.4 to 129.8 prey cells grazer^{-1} h^{-1}, corresponding to the light experiment (winter) and dark experiment (summer) respectively. The low numbers of FLC, together with the lower densities of ciliates, did not permit us to make an appropriate evaluation of their grazing rates on this tracer prey.

The CRs of ciliates on HB showed the same pattern as SGRs, with higher values in darkness (15.8 to 17.6 nl grazer^{-1} h^{-1}, winter and summer, respec-

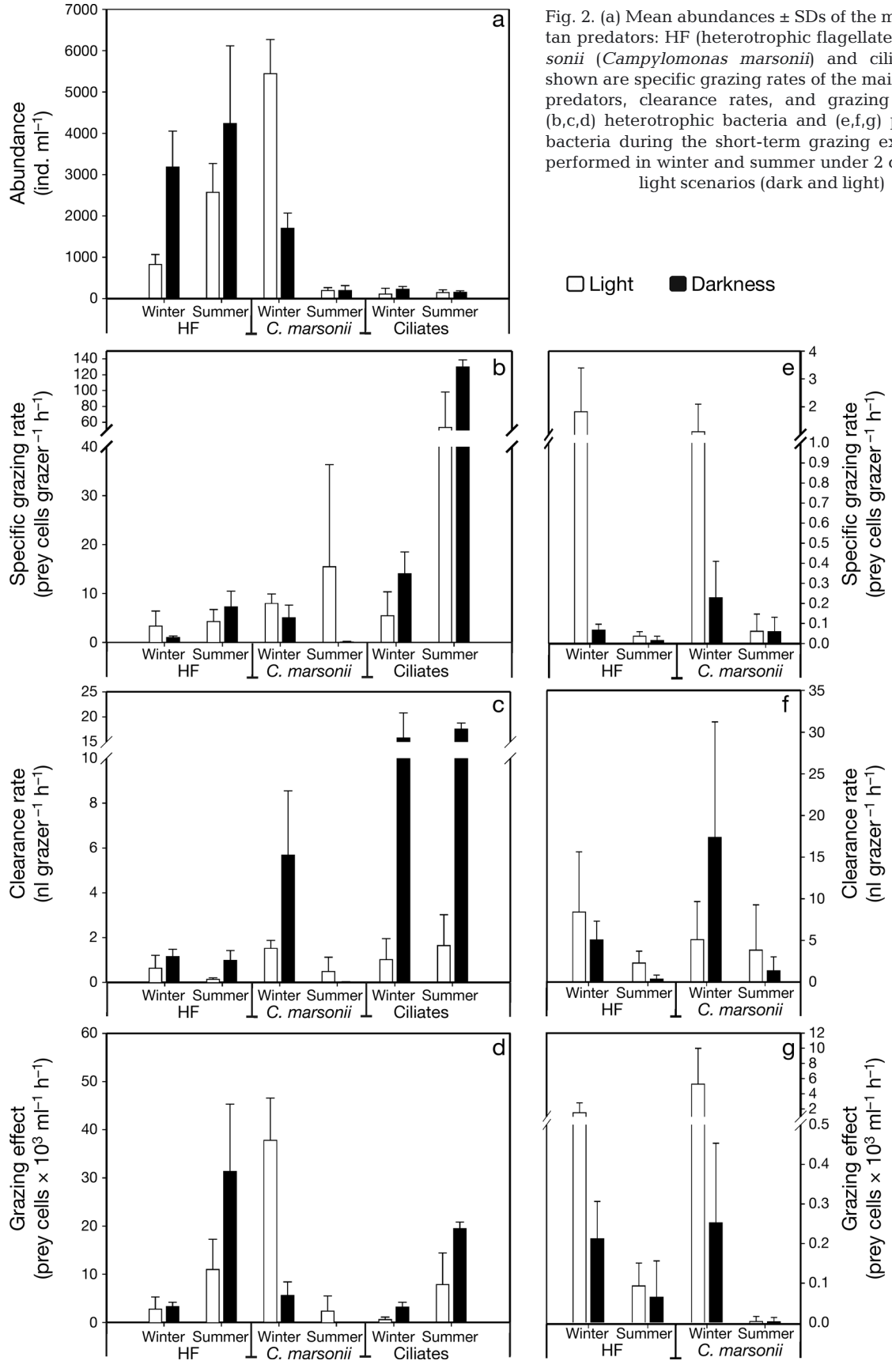

Fig. 2. (a) Mean abundances ± SDs of the main protistan predators: HF (heterotrophic flagellates), *C. marsonii* (*Campylomonas marsonii*) and ciliates. Also shown are specific grazing rates of the main protistan predators, clearance rates, and grazing effect on (b,c,d) heterotrophic bacteria and (e,f,g) picocyanobacteria during the short-term grazing experiments performed in winter and summer under 2 contrasting light scenarios (dark and light)

tively), than in the light (1.02 to 1.64 nl grazer^{-1} h^{-1}, winter and summer, respectively). The grazing effect of ciliates on HB was higher in summer at both sites (7.8 to 19.5 × 10^3 prey cells ml^{-1} h^{-1}). Estimates for Pcy could not be performed for ciliates.

Grazing impact of the main predators

The specific ingestion rates, as a percentage of cell carbon per day, were higher for the smallest predators (HF) for both types of prey (HB and Pcy). Values ranged from 7.41 to 52.53% d^{-1} (for HB) and from 0.40 to 48.73% d^{-1} (for Pcy). The total carbon supply provided by HB plus Pcy varied between 9.19 and 72.85% d^{-1}. As for mixotrophic algae, the specific ingestion rates, as a percentage of cell carbon per day, were comparatively lower, varying from 0.04 to 6.05% d^{-1} (for HB), from 0.08 to 1.6% d^{-1} (for Pcy), and 0.12 to 6.14% d^{-1} (for HB + Pcy). For ciliates, the specific ingestion rates, as a percentage of cell carbon per day, ranged between 0.25 and 6.05% d^{-1} for HB; it could not be calculated for Pcy.

In winter, the contribution of mixotrophic algae to the total grazing of HB under light conditions was very high (~93%). In contrast, under the floating plants heterotrophic protists (HF + ciliates) contributed around 53%. In summer, bacterivory was dominated by the heterotrophic predators (>89%) in both light scenarios (Fig. 3). As the grazing rates of ciliates on Pcy could not be estimated, it was not possible to calculate the contribution of the 3 groups of predators to the total Pcy grazing.

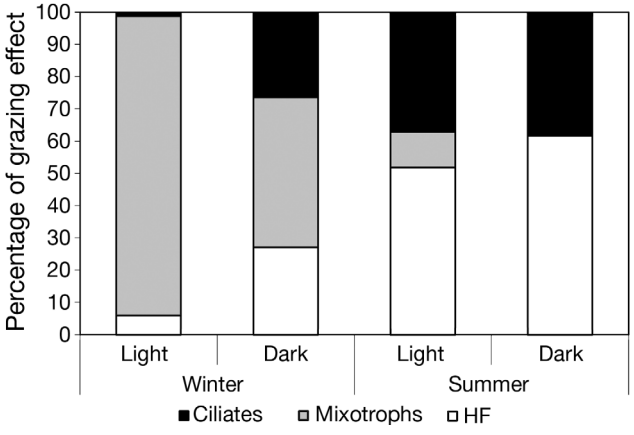

Fig. 3. Contribution of the main protistan predators to total grazing of heterotrophic bacteria expressed as percentages of grazing effect during the short-term experiments performed in winter and summer under 2 contrasting light scenarios (dark and light). HF = heterotrophic flagellates

DISCUSSION

The presence of a covering of dense floating plants exerts a strong influence on the structure of the aquatic microbial communities in the wetland. In past studies, we observed that the floating macrophytes were a key factor in shaping the phytoplankton structure, the dominant photosynthetic picoplankton populations, and the algal primary productivity (O'Farrell et al. 2003, Izaguirre et al. 2004, O'Farrell et al. 2009, Izaguirre et al. 2010). In addition, we showed that the light deficiency, due to a profuse macrophyte coverage, favoured the replacement of strictly autotrophic algae by heterotrophic and mixotrophic protists (Sinistro et al. 2006).

In the present study, independently of the season, the abundance of HF was significantly higher in the darkness under the macrophyte coverage. Simultaneously, HB, Pcy and Peuk were significantly less abundant under the floating plants, probably because they were subjected to an intense grazing pressure by HF, ciliates and mixotrophic algae. The lower abundance of Pcy and Peuk in sites with profuse floating vegetation has been previously reported in a seasonal field survey of this wetland (Izaguirre et al. 2010).

By contrast, in winter, when mixotrophic algae were abundant, they preferred a well illuminated water column, attaining significantly lower abundances under the floating plants. Our results are in agreement with those obtained by Bergström et al. (2003) who compared lakes with different humic contents and observed that the HF were more abundant than the mixotrophic flagellates at very low light levels, whereas mixotrophs dominated over HF when values of effective light climate were >10 µmol m^{-2} s^{-1}. Bergström et al. (2003) argued that the mixotrophs may depend, to a large extent, on energy and carbon derived from photosynthesis. Other studies also demonstrated that higher biomass, cell density or growth rates of mixotrophs are usually achieved in light conditions as compared to darkness (Caron et al. 1993, Hansen & Hjorth 2002). The dominant mixotrophic algae in our system were cryptophytes, which were included in the category of mixotrophs primarily phototrophic that utilize the phagotrophy to survive under prolonged dark conditions (Jones 2000), a behaviour that was observed for some cryptophytes living in Antarctic lakes (Roberts & Laybourn-Parry 1999). Nevertheless, results obtained by Urabe et al. (2000) suggest that *Cryptomonas* sp. would ingest bacteria mainly to acquire some essential substances for their growth. These authors concluded that the

ingestion of bacteria by *Cryptomonas* sp. is not done in order to acquire supplementary energy or carbon when phototrophic activity is low, but to utilize N and P from bacteria as substitutable nutrients when photosynthesis takes place under nutrient-depleted conditions. Our results support this presumption: in winter, when cryptophytes showed a peak of abundance in the shallow lake, their abundances were significantly higher in open waters than under the floating plants, and their specific ingestion rates were also significantly higher in the light than in the dark microcosms. Certainly, this behaviour differs from that of other mixotrophic species, whose ingestion rate is inversely proportional to light intensity, so phagotrophy is believed to primarily provide carbon (Jones 1997). However, some studies also suggest that phagotrophy may provide the same species with more than one element, depending on the prevailing environmental conditions (Pålsson & Granéli 2004, and cites therein).

Previous research has shown that light could also influence phagotrophy in heterotrophic protists. Thus, Jakobsen & Strom (2004) studied the circadian cycles in feeding rates of heterotrophic protists, and observed maximum grazing rates during the day. In our study, we found higher SGRs for HF in the light only in winter, whereas the opposite occurred in summer. With our current data not much can be said on the effect of light on HF grazing.

Water temperature is usually considered one of the most important factors regulating HF phagotrophic activity (e.g. Choi 1994, Vaqué et al. 1994). Our results showed that grazing rates were affected by temperature, because HF and ciliates achieved the maximum SGRs under light conditions in summer. In the case of the cryptophytes, the highest grazing rates were measured in winter, when they were very abundant, whereas in summer other strictly autotrophic phytoplankton taxa were very abundant in the shallow lake — and these probably outcompeted the cryptophytes. In this wetland, cryptophytes are usually more abundant during periods of rising water level and when other phytoplanktonic species are not peaking (O'Farrell et al. 2003).

The specific grazing rates of both HF and *Campylomonas marsonii* were higher for HB than for Pcy, but these differences were due to the higher abundance of HB in the environment. On the contrary, we observed higher CR of these predators on Pcy. These results suggest that the protists were capable of selecting among the 2 types of tracer prey that we used. Our findings are in agreement with those obtained by Šimek et al. (1997) who observed that HF and ciliates had a higher CR on autotrophic picoplankton than on bacteria in a reservoir in the Czech Republic. Similarly, Tarbe et al. (2011), in experiments carried out in Lake Tanganyika, found that protists seemed to discriminate between HB and Pcy, attaining a higher CR on Pcy. Nevertheless, a higher ingestion of Pcy does not necessarily imply that this type of prey is further digested. Boenigk et al. (2001b) examined the ingestion and digestion processes of 6 different food particles for 3 species of HF in different physiological states; they found that digestion was particle-specific — cyanobacteria were excreted a few minutes after ingestion, whereas heterotrophic bacteria were stored and digested in the food vacuoles.

Size-selective uptake of prey by interception-feeding flagellates has been demonstrated in short-term uptake experiments with FLB, and it has been shown that the efficiency of ingestion increases for larger-sized bacteria; in particular, particles of ca. 0.5 µm diameter would be subjected to 4 to 6 times lower grazing mortality than particles of 1 µm (Jürgens & Matz 2002, and cites therein). In freshwater plankton a bimodal size distribution of planktonic bacteria was found during high protist grazing (Pernthaler et al. 1996) because bacteria obtain refuge at the lower end of the size classes as well as at the upper end, when cells become too large to be ingested by small protists. Our results seem to fit this pattern, because the values of CR on HB and Pcy would indicate a preference for Pcy. On the other hand, the largest tracer prey used (FLA) was not ingested by HF and mixotrophic algae. Nevertheless, besides the size, we have to consider other plausible causes, as food quality was not analysed in our study. An experimental investigation carried out by Shannon et al. (2007) found evidence that ingestion rates were affected by both food quality and cell size. On the other hand, other studies have demonstrated that the grazing rates obtained in ingestion experiments using dead bacteria as tracers can be significantly lower than when living bacteria are used (Boenigk et al. 2001a, Massana et al. 2009). Moreover, it is also important to consider that the results obtained using surrogate single genera (as in our study) could be less realistic than when the natural bacterioplankton is employed (e.g. Sherr et al. 1991, Havskum & Riemann 1996).

In our study, specific estimates of grazing rates for cryptophytes are within the range reported by other authors and by ourselves (Urabe et al. 2000, Domaizon et al. 2003, Sinistro et al. 2006), and higher than the grazing rates measured in lakes located at high latitudes (Tranvik et al. 1989, Roberts & Lay-

bourn-Parry 1999). For the HF, our values are also comparable with those reported in the literature for freshwater ecosystems (e.g. Sanders et al. 1989, Pernthaler et al. 1996, Urabe et al. 2000). We observed the highest SGRs for ciliates, which coincides with other studies in which it was reported that some planktonic ciliates (e.g. small oligotrichs) are voracious feeders on bacteria (Sherr & Sherr 1987, Šimek et al. 1995).

The results of our experiments showed that, in winter, when cryptophytes were very abundant, their grazing effect on HB and Pcy exceeded that of the HF. In this season, these mixotrophs accounted for a significant proportion of the total protistan grazing in the shallow lake (>90 % in the light). On the contrary, in summer, the grazing effect on both HB and Pcy was dominated by heterotrophic protists under both light conditions. Our results coincide with the seasonal patterns described for other aquatic systems, where HF were usually the main grazers in summer, whereas the mixotrophic flagellates accounted for a significant proportion of the bacterivory during winter and/or bloom events (e.g. Porter 1988, Sanders et al. 1989, Hitchman & Jones 2000, Domaizon et al. 2003).

According to our data, the specific grazing rates, as a percentage of cell carbon per day, were higher for the smaller predators (HF), and in general within the ranges reported for both freshwater and marine ecosystems (Pernthaler et al. 1996, Unrein et al. 2007). In the case of the cryptophytes, which can also obtain carbon via photosynthesis, the percentage of carbon provided by phagotrophy was generally lower (maximum 6.14 % d^{-1} including both types of prey). For ciliates, the specific grazing rates on HB, as a percentage of carbon, were also relatively low, given that they can also feed on larger prey.

The results of our study reflect that floating macrophytes exert an important influence on the structure of microbial communities in wetlands, and, in particular, on the abundance and composition of the bacterivorous protists. In turn, the light conditions in the water column, which are affected by the floating plants, have a pronounced effect on the protistan grazing rates. On the other hand, seasonal changes in the abundance of the different groups of protists determine important variations in their contribution to the total bacterivory. The main phagotrophic algae in the studied lake (cryptophytes) may be responsible for a significant proportion of the total bacterivory when they are abundant in the wetland at well illuminated sites, whereas heterotrophic protists seem to be more important in summer, and in darkness.

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