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Acta Oecologica 24 (2003) S289–S298
ACTA OECOLOGICA
INTERNATIONAL JOURNAL OF ECOLOGY
www.elsevier.com/locate/actoec

Original article

Impact of different zooplankton structures on the microbial food web of a South Andean oligotrophic lake

Beatriz Modenutti *, Claudia Queimaliños, Esteban Balseiro, Mariana Reissig

Laboratorio de Limnología, Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, Unidad Postal Universidad, 8400 Bariloche, Argentina

Abstract

In oligotrophic Andean lakes, omnivorous calanoid copepods are the dominant zooplankters and, remarkably, phototrophic nanoflagellates and mixotrophic ciliates are the prevailing primary producers. In Lake Rivadavia (Patagonia, Argentina), the centropagids *Boeckella michaelseni* and *Parabroteas sarsi* coexist with the large cladoceran *Daphnia middendorffiana*. The particular feeding modes of these zooplanktonic species probably impact differentially on the microbial community. To determine the effect of predation on the pelagic microbial food web in this lake, we conducted a series of field experiments manipulating different zooplankton structures in 2 l enclosures. The results showed that the presence of *B. michaelseni* and rotifers depressed ciliates and nanoflagellates, but did not affect autotrophic picoplankton and total bacteria abundances. In contrast, the presence of *Daphnia* was decisive in decreasing autotrophic picoplankton abundances. *P. sarsi* was observed to prey on *B. michaelseni* copepodites and adults and a weak cascading effect on the microbial fraction could be detected.

Keywords: Microbial food web; Zooplankton structure; Top down effect: South Andean lakes

1. Introduction

In oligotrophic systems, different studies have shown that planktonic microbial communities (bacteria, autotrophic picoplankton, protists) may be responsible for more of the carbon and energy production in the pelagic zone than the classical phytoplankton-metazooplankton food chains (Azam et al., 1983; Fenchel, 1988; Weisse and MacIssac, 2000; Weisse et al., 1990). Although the organisation of microbial and metazoan food webs is very similar (Riemann and Christoffersen, 1993), the pathway for the transfer of bacterial production within pelagic food webs is still poorly understood (Weisse and MacIssac, 2000).

Microbial food webs are coupled to metazoan food webs, since protists are a suitable food resource for many zooplankton species (Arndt, 1993; Burns and Schallenberg, 1996; Burns and Schallenberg, 1998; Jürgens, 1994). Recent studies in freshwater ecosystems have shown that *Daphnia* exerts a strong impact on picoplankton and nanoplanktonic flagellates and ciliates, consequently altering the microbial food web (Jürgens, 1994). Also, the calanoid copepod *Boeckella dilatata* suppressed ciliate populations in the ultraoligotrophic Lake Wakatipu in New Zealand (Burns and Schallenberg, 1998). Burns and Schallenberg (2001) pointed out that calanoid copepods were clearly more effective per unit biomass than cladocerans in removing protozoa from lakes of different productivity. Therefore, predation by metazooplankton appears to play an important role in structuring microbial food webs.

Recent studies on the microbial communities in South Andean lakes indicated that mixotrophic ciliates and phototrophic nanoflagellates (PNF) are the prevailing primary producers (Modenutti et al., 2000; Queimaliños et al., 1999). In addition, the dominant zooplankters are omnivorous calanoid copepods, which have access to ciliates and flagellates (Balseiro et al., 2001). In Lake Rivadavia (Patagonia, Argentina), the zooplankton assemblage is constituted by the centropagids *Boeckella michaelseni* (Mrázek) and *P. sarsi* Daday, and the large cladoceran *Daphnia middendorffiana* Fischer. *P. sarsi* is a large predaceous calanoid copepod that consumes a great variety of prey, including rotifers, cladocerans and other copepods (Balseiro and Vega, 1994; Diéguez and Balseiro, 1998; Vega, 1995). *B. michaelseni* has been

* Corresponding author.
E-mail address: bmode@crub.uncoma.edu.ar (B. Modenutti).

DOI: 10.1016/S1146-609X(03)00030-4

little researched; however, based on mouthpart morphology and its ecological similarities to *B. gracilipes* Daday, it can be assumed that it is omnivorous and have access to flagellates and ciliates (Balseiro et al., 2001). Due to the particular feeding modes of all these zooplanktonic species, they may produce different effects on the microbial food web. Therefore, the aim of this study is to evaluate the differential effects of *B. michaelseni* and *D. middendorffiana* on the microbial food web of Lake Rivadavia and to determine the potential cascading effect of a predator (*P. sarsi*) on the previous food web structures.

2. Materials and methods

2.1. Study site

Lake Rivadavia is located at 42° 34' S and 71° 39' W at an altitude of 500 m a.s.l. Its surface area is 21.70 $km^2$and has a maximum depth of 142 m. The lake has a glacial origin and drains to Lake Verde and then to Lake Futalaufquen, conforming to the Futaleufú basin of Pacific watershed. The thermal regime of Lake Rivadavia is warm monomictic, with stratification during late spring and summer. Lake Rivadavia planktonic communities, including the microbial fraction were not previously investigated. However, early studies in other lakes of the same basin (lake Futalaufquen) reported a net phytoplankton (cells larger than 25 μm) dominated by the diatom *Synedra nana* Meister, the dinoflagellates *Peridinium* spp. and the chlorophytes *Staurastrum tetracerum* Ralfs and *Oocystis marssonii* Lemm. (Pizzolón et al., 1995), and a zooplankton constituted by the large cladoceran *Daphnia middendorfiana* and two calanoid copepod species, the grazer *B. michaelseni*, and the predator *P. sarsi* (Paggi and Paggi, 1985; Pizzolón et al., 1995). Remarkably, the presence of these large species in the zooplankton appeared as a common feature of this basin.

2.2. Incubation experiments

We carried out two series of field incubation experiments with different zooplankton compositions on two dates: 5-7 December 2000 (late spring, experiment 1) and 7-10 March 2001 (late summer, experiment 2). The lake was sampled on the initial day of each experiment. Temperature and light (Photosynthetically Active Radiation, PAR, 400-700 nm) profiles were measured from 0 to 60 m with a PUV 500B submersible radiometer (Biospherical Instruments). Water samples for nutrient analyses were collected from the epilimnion of the lake, at 15 m depth. In order to determine the vertical distribution, zooplankton was sampled with a Schindler-Patalas trap of 12 l from 0 to 55 m at every 5 m interval.

The experimental design consisted of six treatments with three replicates each. One treatment had no zooplankton (NZ) and in the other five, different zooplankton assemblages were added (Z, D, Z + D, Z + P and Z + D + P, Table 1). Polycarbonate bottles of 2 l were used as experimental units.

Lake water was collected at 15 m depth using a Schindler-Patalas trap and filtered through a 55 μm mesh net. The filtered water was placed in an isolated tank. About 1 h before starting the experiments, zooplankton was collected using a conical plankton net with 55 μm mesh size. According to the observed vertical distribution, *B. michaelseni* and rotifers were collected with vertical tows from 20 m depth to surface, while *D. middendorffiana* and *P. sarsi* were collected from 40 to 20 m depth. After sampling, zooplankton was acclimatised in 1 l beakers containing filtered lake water. *D. middendorffiana* of approximately 2.5 mm body length and *P. sarsi* circa 3 mm were selected for the experiments. *Daphnia* and *Parabroteas* were rinsed with filtered lake water to prevent the occurrence of other zooplankton, and then isolated in 500 ml beakers. Filtered lake water was poured into the 2 l bottles and the different zooplankton species (Table 1) were added using a wide bore pipette.

Experiment 1 (December, late spring) lasted 48 h, therefore a total of 54 bottles were prepared of which 18 were carried immediately to the laboratory to quantify the initial conditions. The remaining 36 bottles were incubated at 10 m depth in a frame at a pelagic lake station. After 24 and 48 h of incubation, 18 bottles were removed from the frame. Experi-

Table 1
Zooplankton initial condition of the experimental design carried out in Lake Rivadavia. *B. michaelseni* and Rotifers are expressed as mean number of individuals per litre, while *D. middendorffiana* and *P. sarsi* are expressed as number per experimental unit

Treatment	Experiment 1		Experiment 2	
	Zooplankton constitution	Zooplankton biomass (μg l^{-1})	Zooplankton constitution	Zooplankton biomass (μg l^{-1})
NZ	–	–	–	–
Z	75 ± 12 *B. michaelseni* and 520 ± 80 Rotifers per litre	302.30 ± 56.60	15 ± 1 *B. michaelseni* and 517 ± 15 Rotifers per litre	63.21 ± 0.35
D	5 *D. middendorffiana*	197.21 ± 5.63	5 *D. middendorffiana*	165.28 ± 2.17
Z + D	75 ± 12 *B. michaelseni* and 520 ± 80 Rotifers per litre + 5 *D. middendorffiana*	302.30 ± 56.60 + 197.21 ± 5.63	15 ± 1 *B. michaelseni* and 517 ± 15 Rotifers per litre + 5 *D. middendorffiana*	63.21 ± 0.35 + 165.28 ± 2.17
Z+P	75 ± 12 *B. michaelseni* and 520 ± 80 Rotifers per litre + 5 *P. sarsi*	302.30 ± 56.60 + 267.85 ± 2.34	15 ± 1 *B. michaelseni* and 517 ± 15 Rotifers per litre + 5 *P. sarsi*	63.21 ± 0.35 + 196.48 ± 2.38
Z + D + P	75 ± 12 *B. michaelseni* and 520 ± 80 Rotifers per litre + 5 *D. middendorffiana* + 5 *P. sarsi*	302.30 ± 56.60 + 197.21 ± 5.63 + 267.85 ± 2.34	15 ± 1 *B. michaelseni* and 517 ± 15 Rotifers per litre + 5 *D. middendorffiana* + 5 *P. sarsi*	63.21 ± 0.35 + 165.28 ± 2.17 + 196.48 ± 2.38

ment 2 (March, late summer) lasted 3 d, therefore 72 bottles were used and the same procedure was followed, removing bottles after 24, 48 and 72 h.

2.3. Laboratory methods

Experimental units were carried from the lake station to the laboratory, in darkness and thermally isolated, within half an hour of removal from the frame. In the laboratory, subsamples of each bottle were obtained. A volume of 60 ml was preserved with buffered formaldehyde (final concentration, 2% v/v) for bacteria, autotrophic picoplankton and nanoflagellate counting. The samples were stored refrigerated in darkness, and quantified within 2 weeks of sampling. Of this volume, 3 ml were stained with fluorochrome 4', 6-diamidino-2-phenylindole (DAPI; final concentration 0.2% w/v) according to Porter and Feig (1980). Counting was done on black membrane filters (Poretics, 0.2 μm pore size) at 1000× in an Olympus BX50 epifluorescence microscope using UV light (U-MWU filter) for total bacteria, and blue light (U-MWB filter) for autotrophic picoplankton. An image analyser was used for counting and size measurements (Image Pro Plus, Media Cybernetic). A volume of 40 ml was filtered onto 1 μm black membrane filters (Poretics) for the enumeration of nanoflagellates. Cells were counted by epifluorescence microscopy at 1000×, using both UV and blue light, to distinguish heterotrophic nanoflagellates (HNF) from phototrophic nanoflagellates (PNF, which include autotrophic and mixotrophic species).

Ciliates and algae were preserved with acid Lugol solution in 250 ml samples and were quantified using an inverted microscope in 50 ml Utermöhl chambers. Algae were counted following Utermöhl technique at 400× and 1000×, while ciliate numbers were assessed scanning the entire surface of the chamber at 200×.

A volume of 150 ml was used for the following analyses: dissolved organic carbon (DOC), total phosphorus (TP), total dissolved phosphorus (TDP) and chlorophyll *a* concentration. TDP was determined on 70 ml of filtered water (GF/F). The samples were digested with potassium persulphate at 125 °C at 1.5 atm for 1 h. The concentrations were calculated applying the ascorbate-reduced molybdenum method (APHA, 1989). TP was measured in the same way as TDP on unfiltered water. DOC was estimated by spectrophotometry through a regression model based on Morris et al. (1995). Chlorophyll *a* concentrations were determined "in vivo" by fluorometry (Turner AU10).

Zooplankton was collected with a 45 μm plankton net and preserved with 4% formaldehyde. Crustaceans were quantified in 5 ml Bogorov chambers under stereomicroscope, and rotifers in 1 ml Sedgwick-Rafter chamber under direct microscope. A minimum of 30 individuals of each species was measured under a microscope with a graduated eyepiece. Zooplankton biomass was calculated according to the method of Bottrell et al. (1976). The growth rates of protists (nanoflagellates and ciliates) were estimated by applying the following equation:

$$r = \ln (N_t / N_0)/t$$

where r is the rate of population growth (d^{-1}), N_0 and N_t are initial and final cell densities, and t is the duration of the incubation.

The effect of zooplankton composition on total bacteria, autotrophic picoplankton, nanoflagellates and ciliates (< 50 μm) was tested by a multi-factor ANOVA. The particular effect of zooplankton on each date was analysed through one way and two way ANOVA. When significant differences were obtained, the multiple comparison test Student-Newman-Keuls was applied. Homoscedasticity and normality were previously tested and data were log transformed when needed.

3. Results

During both experiments, the lake was thermally stratified. In December, the temperature in the epilimnion was 11.5 °C and reached 30 m depth whereas in March, the mixing layer was at 14.7 °C extending up to 32 m depth. The level of 1% of surface PAR irradiance (euphotic zone) was observed to reach 20 m depth on both the dates. Our experiments were incubated at 10 m depth, therefore the light and thermal conditions were those of the lake epilimnion.

Nutrient concentrations were low at both dates (TP: 5 and 7 μg l^{-1}; TDP: 2.5 and 3.5 μg l^{-1} in December and March, respectively). DOC and chlorophyll *a* concentrations were similar in the both occasions (DOC: 1.2 mg l^{-1}, Chl *a* 1.60 μg l^{-1}).

3.1. Experiment 1 (late spring, 5–7 December, 2000)

Zooplankton biomass fluctuated between 197 and 767 μg l^{-1} in the different treatments, depending on the addition of *D. middendorffiana* and/or *P. sarsi* (Table 1). *B. michaelseni* dominated the crustacean assemblage, while the most abundant rotifers were *Keratella cochlearis* Gosse followed by *Synchaeta* aff. *lakowitziana* Lucks. Lake water filtered through 55 μm mesh net contained similar densities and composition of ciliate and algal species than those of the lake. PNF such as *Chrysochromulina parva* Lackeyand *Rhodomonas lacustris* (Pascher & Ruttner) Javornicky dominated the phytoplankton assemblage both in the lake and in all the treatments, reaching 222 cells ml^{-1} at initial time (Table 2). The net phytoplankton of the lake was present in lower abundances (70 cells ml^{-1}), and was composed of *Synedra ulna* Ehr., *S. actinastroides* Lemmermann, *Gymnodinium paradoxum* Schilling, *Aulacoseira granulata* (Ehr.) Simonsen and *Asterionella formosa* Hassel.

The dominant ciliate species was the mixotrophic peritrich *Ophrydium naumanni* Pejler followed by *Limnostrombidium* sp, *Pelagohalteria viridis* (Fromentel) and *Balanion planctonicum* (Foissner, Oleksiv and Müller). This situation

Table 2
Initial conditions of all treatments of experiments carried out in Lake Rivadavia. The values are expressed as mean (±S.E.).*O. naumanni* in NZ and D treatments

	Experiment 1	Experiment 2
Total bacteria (cell ml^{-1})	9.06×10^5 ($\pm 2.50 \times 10^4$)	1.18×10^6 ($\pm 2.40 \times 10^5$)
Autotrophic picoplancton (cell ml^{-1})	2.11×10^4 ($\pm 1.70 \times 10^3$)	2.31×10^4 ($\pm 2.30 \times 10^3$)
PNF (cell ml^{-1})	222.48 (±31.10)	138.65 (±5.60)
HNF (cell ml^{-1})	363.80 (±15.24)	122.28 (±16.79)
O. naumanni (No. l^{-1})	970 (±90)	870 (±90)
460 (±70)*	300 (±70)*	
Ciliates < 50 μm (No. l^{-1})	1080 (±310)	1250 (±210)

was observed both in the lake and in the treatments. However, in the filtered treatments (Table 2, treatments NZ and D), a 50% decrease was observed in *O. naumanni* abundances, from 970 cells l^{-1} in the zooplankton treatments to 460 cells l^{-1} in the filtered lake water.

Manipulation of rotifer and crustacean zooplankton composition was successful. In NZ treatments, up to 95% of zooplankton was removed and in treatments D, Z + D, Z + P and Z + D + P, the survival of *Daphnia* and *Parabroteas* was 100%. Due to the presence of a large predator (*P. sarsi*), the survival of *B. michaelseni* during the experiment differed between treatments. At the end of the incubation in the enclosures where the predaceous *P. sarsi* was present (Z + P and Z + D + P), *B. michaelseni* decreased substantially (from 75 to ≈40 ind l^{-1}) and rotifers were not affected. On the contrary, *D. middendorffiana* did not affect *B. michaelseni* survival, but the abundances of rotifers were slightly lower (≈20%) than in the treatment without *Daphnia*.

Total bacteria abundances did not change substantially among treatments in the first 24 h of incubation (Fig. 1), but a significant decrease was observed at 48 h except in the Z + D + P treatment (ANOVA, $P < 0.05$) (Fig. 1). Bacteria were coccoid-shaped and the diameter ranged from 0.60 to 0.90 μm. A slight decrease in bacterial size was detected in the treatments where *Daphnia* was present (0.54–0.81 μm). Autotrophic picoplankton showed a clear response in relation to zooplankton composition (two way ANOVA, $P < 0.05$) (Fig. 1). The presence of *D. middendorffiana* (D, Z + D and Z + D + P) was crucial in decreasing picoplankton abundances (S-N-K, $P < 0.05$), in particular, after 48 h of incubation (ANOVA interaction T × D, $P < 0.001$) (Fig. 1, Table 3). On the other hand, in the Z + P treatment, picoplankton abundances did not differ from that of NZ treatment. Autotrophic picoplankton were solitary round or rod-shaped cells or small aggregates in one plane, with a cell diameter varying around 1.2 μm and the maximum length reaching up to 4 μm.

PNF slightly increased in the treatment without zooplankton (NZ) and decreased in all treatments with zooplankton, showing the greatest effect at 48 h in the treatment with zooplankton and *Daphnia* (Z + D) (two way ANOVA, $P < 0.05$; S-N-K, $P < 0.05$) (Fig. 2, Table 3). HNF also decreased in zooplankton treatments although the grazing effect on this fraction was less clear (Fig. 2).

Noticeably, within the ciliate assemblage, *O. naumanni* (> 80 μm in length) and the other species, mainly oligotrichs and prostomate holotrichs (< 50 μm in length), showed contrasting responses. *O. naumanni* increased slightly after 48 h of incubation in treatments Z, Z + D and Z + D + P (Fig. 2), although no significant differences were observed among them (two way ANOVA, $P > 0.05$). In treatments NZ and D, we observed a decrease in *O. naumanni* due to the filtration procedure prior to incubation (Table 2). However, during the experiment, the presence of *Daphnia* seemed to cause a

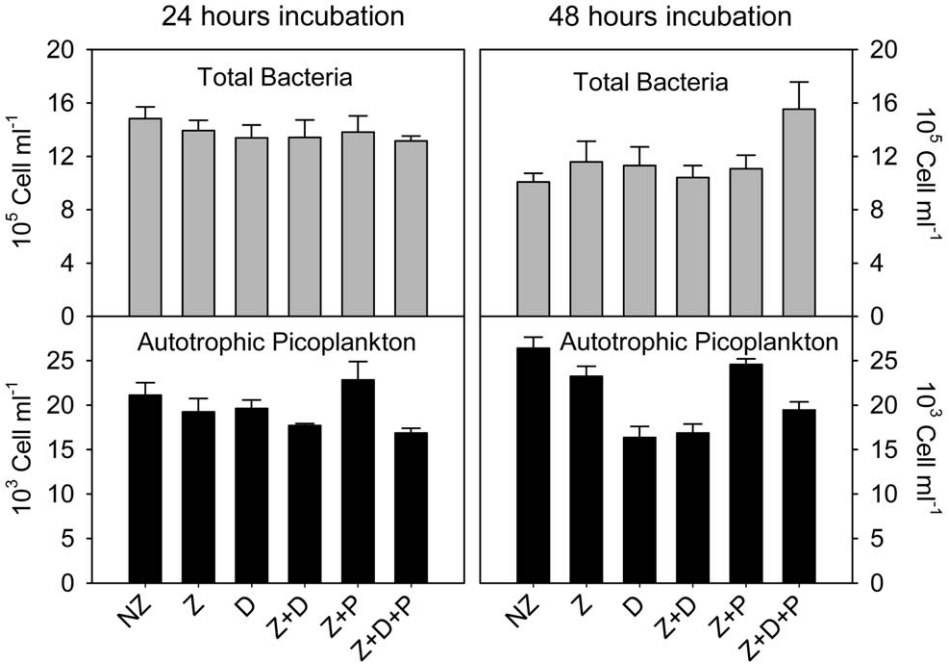

Fig. 1. Total bacteria and autotrophic picoplankton abundances after 24 and 48 h of incubation during experiment 1. References of treatments as in Table 1.

Table 3
Multi-factor ANOVA results of experiment 1. References: APP: autotrophic picoplankton, PNF: phototrophic nanoflagellates, n.s.: non significant

	Total bacteria	APP	PNF	Ciliates (<50 μm)
Without *Parabroteas*				
T (Time)	< 0.0001	n.s	0.033	n.s
Z (Zooplankton)	n.s	n.s	0.023	0.0019
D (*Daphnia*)	n.s	<0.001	0.0008	0.0017
T × Z	n.s	n.s	n.s	n.s
Z × D	n.s	n.s	n.s	n.s
T × D	n.s	0.0008	n.s	n.s
T × Z × D	n.s	n.s	n.s	n.s
With *Parabroteas*				
T (Time)	0.01	0.034	n.s	0.036
D (*Daphnia*)	n.s	< 0.0001	0.0012	n.s
P (*Parabroteas*)	n.s	n.s	n.s	n.s
T × D	0.04	n.s	n.s	n.s
D × P	0.01	n.s	n.s	n.s
T × P	0.02	n.s	0.0091	n.s
T × D × P	0.01	n.s	n.s	n.s

decrease in *Ophrydium* when compared to NZ treatment (*t* test, $P = 0.054$). At the same time, in the treatments with zooplankton, the other ciliate species decreased (ANOVA, $P < 0.05$) (Fig. 2, Table 3). This decline was stronger in treatments Z + D and Z + D + P, indicating a possible synergic effect of *Boeckella*, rotifers and *Daphnia* on medium sized ciliate species.

PNF growth rates were positive only in the treatment NZ, while they were negative in the other treatments (Fig. 3). However, in the treatment with zooplankton and *Parabroteas* (Z+P), PNF growth rates attained values of around 0, indicating that the effect of the predator *P. sarsi* might have induced a decline in grazing pressure by reducing *B. michaelseni* abundances. In the case of HNF and ciliates <50 μm, the growth rates were negative in all treatments. However, in treatments with zooplankton, values were greater than those in the NZ treatment (Fig. 3).

3.2. Experiment 2 (late summer, 7-10 March, 2001)

In this experiment, zooplankton biomass in the different treatments was considerably lower in comparison with the experiment 1, and fluctuated between 63 and 424 μg l^{-1}, depending on the addition of *D. middendorffiana* and/or *P. sarsi* (Table 1). Again, *B. michaelseni* dominated the crustaceans while the most abundant rotifers were *Synchaeta* aff. *lakowitziana* followed by *K. cochlearis*. The presence of *P. sarsi* affected the survival of *B. michaelseni* in the same wayas that in experiment 1; decreasing its density from 15 to 2 ind l^{-1}. Rotifers declined in *D. middendorffiana* treatments as in experiment 1.

The phytoplankton composition changed substantially during summer, since flagellated species dominated both the nanoplankton and the net phytoplankton. *C. parva* and *R. lacustris* exhibited the maximum densities (140 cells ml^{-1}) in the smallest phytoplanktonic size fraction. Large cells were more abundant than in spring, reaching up to 147 cells ml^{-1}, and were dominated by the chrysophycean *Dinobryon sertularia* Ehr., the dinoflagellate *G. paradoxum* and the chlorophyceans *Pandorina morum* Bory and *P. smithii* Chodat.

Ciliate community was dominated by the mixotrophic peritrich *O. naumanni* followed by medium sized oligotrichs *Pelagostrombidium* sp, *Rimostrombidium* sp and the gymnostomatid *Askenasia* sp. The large *O. naumanni* increased its density from 300 cells l^{-1} in the filtered treatments (Table 2, NZ and D) to 870 cells l^{-1} in the zooplankton treatments (Z, Z + D, Z + P and Z + D + P).

Total bacteria abundances showed no significant differences among treatments (Fig. 4) (two way ANOVA, $P > 0.05$). Bacteria were coccoid-shaped with diameters ranging from 0.59 to 0.89 μm and, in this case, no changes in shape or size were detected among treatments or during the experiment.

As in experiment 1, autotrophic picoplankton abundances also showed a direct relationship with zooplankton composition (ANOVA, $P< 0.05$) (Table 4). In treatments with *D. middendorffiana* (D, Z + D and Z + D + P) picoplankton showed a strong decrease after 48 h of incubation (Fig. 4) (ANOVA, $P < 0.001$). After 72 h of incubation, picoplankton remained low in these treatments except for the presence of the predator copepod *P. sarsi* (ANOVA interaction D × P, $P < 0.05$) (Fig. 4, Table 4). Autotrophic picoplankton morphology and size were similar to those observed in experiment 1.

PNF showed a pronounced decrease in the zooplankton treatments (Z, Z + D, D and Z + D + P) (ANOVA, $P < 0.05$) (Table 4) while the Z + P showed no significant differences with NZ treatment at 48 h of incubation (Fig. 5) (S-N-K, $P > 0.05$). In this case, the predaceous copepod *P. sarsi* extended its effect through the food web, and this situation may be considered as a cascading effect. After 72 h of incubation, the decrease in the PNF concentrations was maintained in Z, D, Z + D and Z + D + P treatments (Fig. 5)

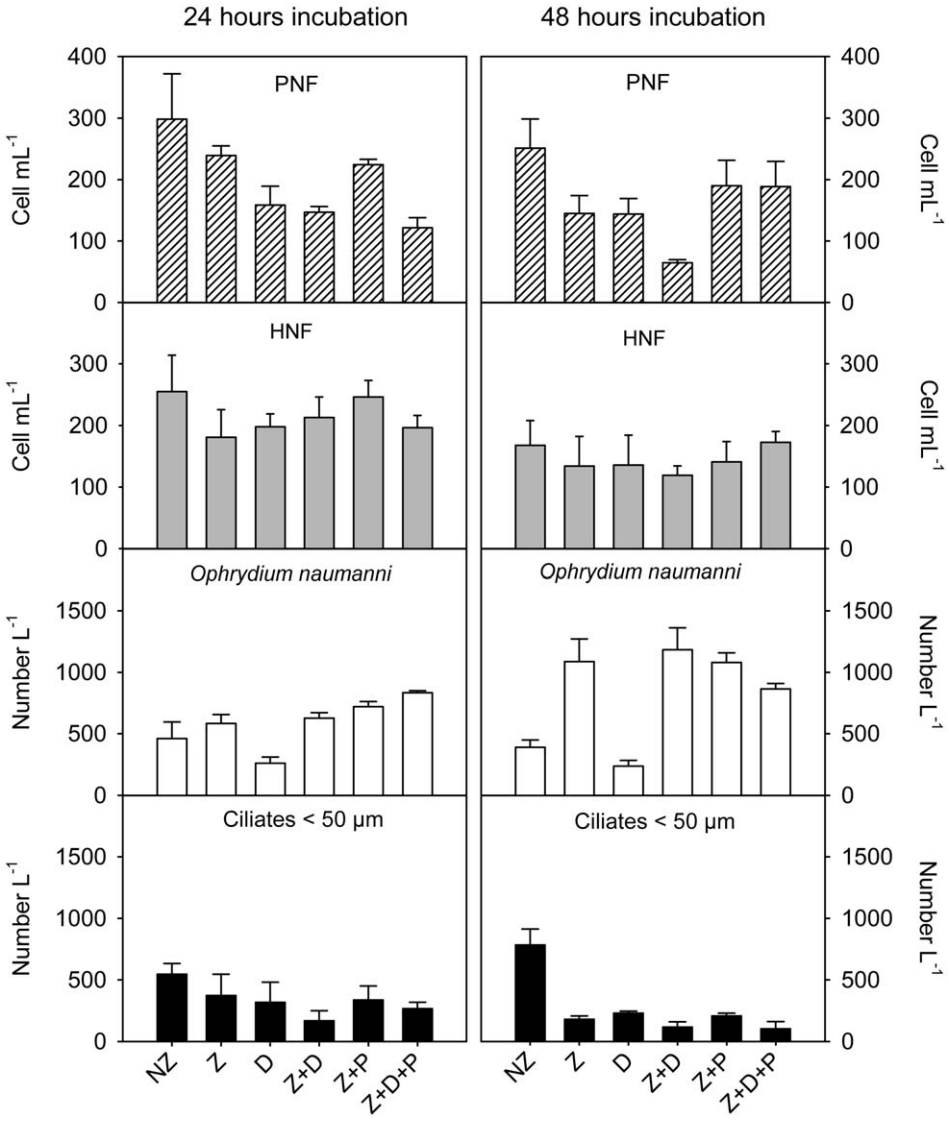

Fig. 2. Phototrophic and HNF (< 20 µm), *O. naumanni*(ciliate > 80 µm) and other ciliate species (< 50 µm) abundances after 24 and 48 h of incubation during experiment 1. References of treatments as in Table 1.

(ANOVA, $P < 0.05$). A similar situation was observed in HNF abundances with a greater grazing effect at 48 h of incubation (Fig. 5).

The ciliate assemblage < 50 µm, dominated by oligotrichs, decreased in all the treatments with zooplankton (ANOVA, $P< 0.05$) (Fig. 5, Table 4). At 24 and 48 h of incubation, the decrease of this ciliate fraction was strongest in treatment Z + D, indicating again, a possible synergic effect of *Boeckella*, rotifers and *Daphnia* on medium sized ciliate species (ANOVA, $P < 0.001$). On the contrary, *O. naumanni* (> 80 µm in length) increased its abundance as in experiment 1, although no significant differences were observed between treatments with zooplankton (two way ANOVA, $P > 0.05$) (Fig. 5).

The growth rates of nanoflagellates (PNF and HNF) were negative in all treatments after 24 h of incubation. However, during the next 48 h of incubation, PNF had positive growth rates in the NZ and Z + P treatments (Fig. 3), while the other treatments remained negative. The growth rates of medium sized ciliates and nanoflagellates (PNF and HNF) in the NZ treatment showed similar trends (Fig. 3). In all the other treatments, including Z + P, ciliates' growth rates were highly negative (Fig. 3).

4. Discussion

Daphnia can exert a significant grazing pressure on different components of microbial food webs due to its ability to filter a large spectrum of particle sizes (Jürgens, 1994). In our experiments, *D. middendorffiana* exhibited a strong top down impact, depressing the autotrophic picoplankton, nanoflagellates and ciliates, including the large *O. naumanni*. In an Alaskan lake, *D. middendorffiana* could feed on the small natural bacterial flora at rates varying between 0.34 and 1.29 ml ind^{-1} h^{-1} (Peterson et al., 1978). The morphology of the filtering apparatus of cladocerans enables them to

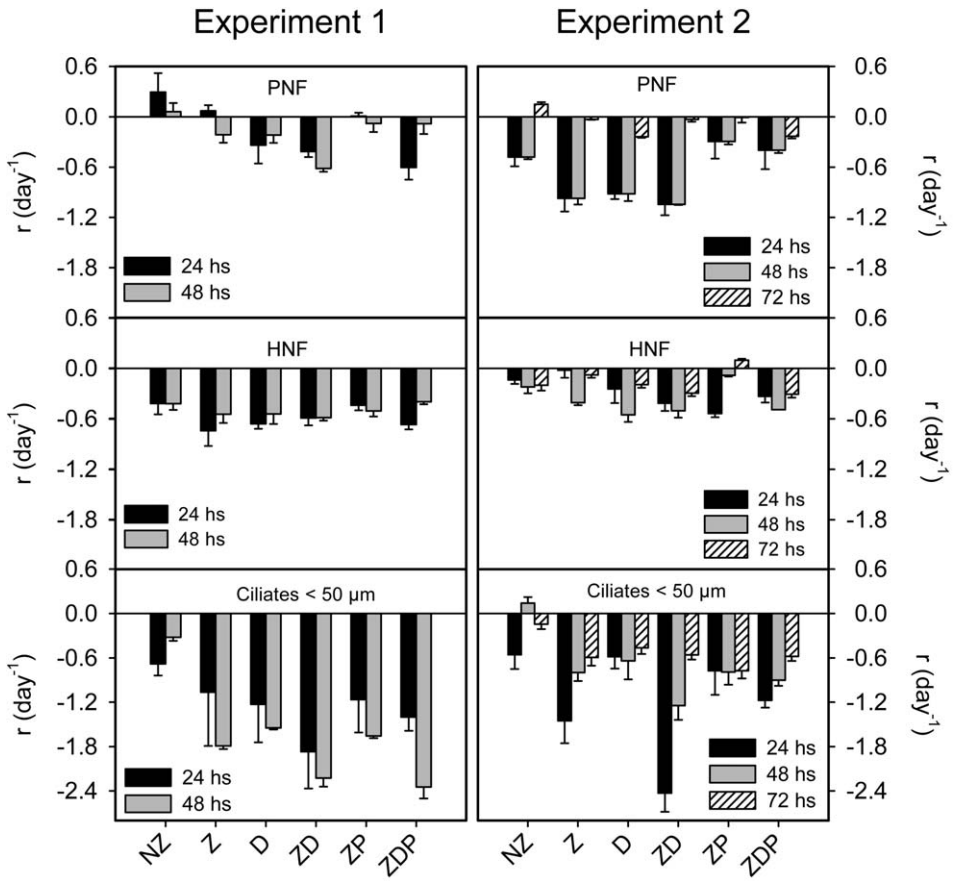

Fig. 3. Growth rates of phototrophic and HNF (< 20 µm) and ciliate species (< 50 µm) during experiments 1 and 2. References of treatments as in Table 1.

exploit small particles (Brendelberger, 1985; Brendelberger and Geller, 1985); nevertheless, body length, food concentration and temperature have a direct relationship with their clearance rates (Lampert, 1987). In the two experiments in Lake Rivadavia, *D. middendorffiana* (2.5 mm body length) showed a selection towards autotrophic picoplankton (Figs. 1 and 4). The differences in sizes between bacteria and autotrophic picoplankton appeared to be the cause of the observed differential grazing impact of *D. middendorffiana*. In other *Daphnia* species, a selection towards particles larger than 0.5 µm with a decrease in filtering rates for the smallest bacterial sizes has been registered (Brendelberger, 1991; Gophen and Geller, 1984). Besides, *Daphnia* can have a positive effect on bacterioplankton through the supply of

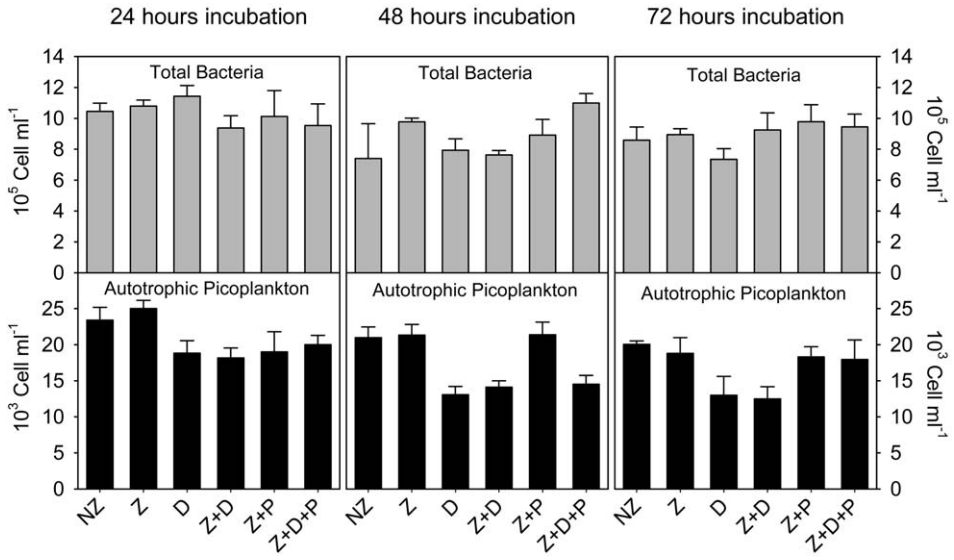

Fig. 4. Total bacteria and autotrophic picoplankton abundances after 24, 48 and 72 h of incubation during experiment 2. References of treatments as in Table 1.

Table 4
Multi-factor ANOVA results of experiment 2. References as in Table 3

	Total bacteria	APP	PNF	Ciliates (< 50 μm)
Without *Parabroteas*				
T (Time)	0.0027	0.0003	< 0.0001	n.s
Z (Zooplankton)	n.s	n.s	< 0.0001	< 0.0001
D (*Daphnia*)	n.s	< 0.0001	< 0.0001	< 0.0001
T × Z	n.s	n.s	0.03	0.02
Z × D	n.s	n.s	< 0.0001	0.001
T × D	n.s	n.s	< 0.0001	0.0023
T × Z × D	n.s	n.s	0.0007	0.0023
With *Parabroteas*				
T (Time)	n.s	0.019	< 0.0001	0.016
D (*Daphnia*)	n.s	0.0002	0.0001	n.s
P (*Parabroteas*)	n.s	n.s	0.0008	0.036
T × D	n.s	n.s	0.018	n.s
D × P	n.s	0.03	0.032	n.s
T × P	n.s	n.s	0.0002	0.013
T × D × P	n.s	n.s	n.s	n.s

DOC as bacterial substrates (Jürgens, 1994). In Lake Rivadavia, we did not observe substantial changes in total bacteria abundances, suggesting that compensation between grazing losses and substrate supply may have occurred. Remarkably, the original bacterial size spectrum remained unaffected or slightly changed towards smaller forms in the presence of *D. middendorffiana*. In microcosm experiments, a shift in bacterial size towards large aggregates and long filaments in relation to protozoan predation was observed, while no changes were registered without such predation or when *Daphnia* suppressed protozoan growth (Jürgens et al., 1997). In our experiments, *D. middendorffiana* seemed to prefer

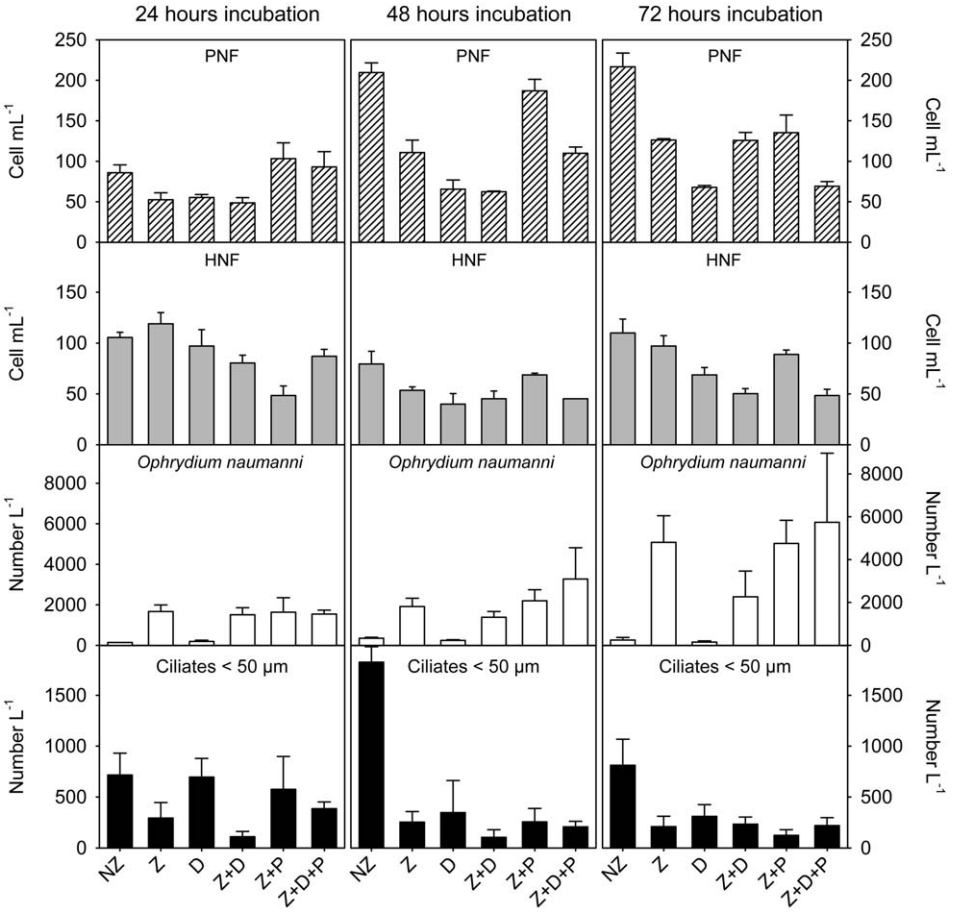

Fig. 5. Phototrophic and HNF, *O. naumanni* (ciliate > 80 μm) and other ciliate species (< 50 μm) abundances after 24, 48 and 72 h of incubation during experiment 2. References of treatments as in Table 1.

larger bacterial forms and therefore the smallest fraction would have been favoured, as was observed in the spring experiment.

Although total bacteria abundances were not heavily suppressed in our experiments, the densities of other members of the microbial food web changed drastically with the presence of *D. middendorffiana*. Nanoflagellates (PNF and HNF) and small ciliates were observed have decreased, just as was indicated in other studies with different *Daphnia* populations (Burns and Schallenberg, 1996; Carrick et al., 1991; Christoffersen et al., 1993; Jürgens et al., 1994; Müller et al., 1991, Weisse, 1991). However, the fact that *D. middendorffiana* might have decreased *O. naumanni* abundances constitutes the first evidence of a grazer affecting this large ciliate; although the mechanisms involved, grazing upon or interference competition, are still unclear.

On the other hand, calanoid copepods are mostly omnivorous with varying tendencies to herbivory or carnivory (Burns and Gilbert, 1993; Vanderploeg, 1990; Williamson and Butler, 1986). The Southern Hemisphere calanoid genus *Boeckella* has been described as consuming small seston (< 10 μm) (Haney and Trout, 1985) and rotifers and crustaceans in addition to algae (Green and Shiel, 1999; Green et al., 1999; Modenutti, 1993). *B. michaelseni* and *B. gracilipes* inhabit the large deep lakes from 39° to 54° S (Bayly, 1992). Recently, it was observed that *B. gracilipes* mouthpart morphology corresponds to the omnivore type and feeding experiments showed that it consumes oligotrich ciliates in comparable rates with those obtained for nanoflagellates (Balseiro et al., 2001). In the case of *B. michaelseni* of Lake Rivadavia, a similar effect on nanoflagellates and ciliates was observed, although ciliate growth rates were more negatively affected than those of nanoflagellates in both the experiments (Fig. 3). Similar results were obtained for *B. dilatata* and *B. hamata* in two New Zealand lakes (Burns and Schallenberg, 1996; Burns and Schallenberg, 1998), where a clear decrease in ciliate abundances in the presence of *Boeckella* was observed.

As was previously determined for *B. gracilipes* (Balseiro et al., 2001), *B. michaelseni* was also unable to depress *O. naumanni*. Recently, it was found that this large ciliate can use bacteria and picoplankton as prey (Modenutti and Balseiro, 2002), and therefore may compete with other bacterivorous protists. In this sense, it seems possible that the decrease in medium sized ciliates and nanoflagellates (Figs. 2, 3 and 5) resulted in an increase of food availability for *O. naumanni*, and this situation might have induced the positive effect observed on this large ciliate population.

Although rotifers may feed on different microbial components (Arndt, 1993), in our experiments, the feeding activityof *D. middendorffiana* and *B. michaelseni* might have obscured rotifers' effect on the microbial food web.

The presence of an invertebrate predator like *P. sarsi* was important for the microbial food web structure. In previous laboratory and field studies, *P. sarsi* was observed to prey efficiently on cladocerans and rotifers (Balseiro and Vega, 1994; Diéguez and Balseiro, 1998; Vega, 1995). In our incubation experiments, the predator was able to consume the copepod *B. michaelseni*, producing a cascading effect in the Z + P treatment. In this case, the top down impact of *P. sarsi* on *B. michaelseni* might have caused a reduction in the overall grazing pressure over nanoflagellates (PNF and HNF) and ciliates. However, the reduced impact appeared more evident on nanoflagellates (Fig. 3), indicating that the effect strongly depends on the ability of prey to recover. Numerous compensatory mechanisms dampen or eliminate cascade effects and therefore, a change in an upper trophic component does not necessarily propagate down the food web (Pace et al., 1999). In our incubation experiments in Lake Rivadavia, we observed that *Parabroteas* reduced *Boeckella* numbers with a consequent cascading effect on nanoflagellates, although in the treatments with *Daphnia* (Z + D + P), such an impact was not observed. On the one hand, *P. sarsi* did not access substantially to the large *D. middendorffiana* (Balseiro and Vega, 1994) and, on the other, the feeding activity of the large grazer does not allow an increase in nanoflagellate populations, in spite of the decrease in *Boeckella* abundances.

5. Conclusion

The large cladoceran *D. middendorffiana* exhibited a strong top down impact on different levels of the microbial food web. *Daphnia* was able to depress the nanoflagellates, ciliates and autotrophic picoplankton. The absence of substantial differences in total bacteria might be due to a compensatory effect combining grazing losses and a positive effect via the supply of DOC as bacterial substrates (Jürgens, 1994).

B. michaelseni had a more restricted impact on protists, since no changes on bacteria and picoplankton were observed. This is in accordance with the model proposed by Burns and Schallenberg (1996) for New Zealand lakes. In the particular case of the large mixotrophic ciliate, *O. naumanni*, no important negative effect caused by zooplankton was observed; however, *D. middendorffiana* would have been able to decrease it. The presence of *B. michaelseni* preying upon other ciliates and nanoflagellates might have caused a reduction of these bacterivorous competitors and, therefore, induced an increase in *Ophrydium*.

The addition of an upper trophic component constituted by the predaceous calanoid *P. sarsi* produced a decrease in grazing on protists by the reduction of *B. michaelseni*, but did not affect *D. middendorffiana* mortality. Nanoflagellates responded positively to the decline of *B. michaelseni*, suggesting that a cascading effect might have occurred.

Acknowledgements

We thank Los Alerces National Park, especially the park ranger Fernando Nahuelpan, for allowing us to conduct this study in Lake Rivadavia, G. Pérez for help during the first

experiment and M. Diéguez for helpful discussions. Financial support for this research was provided by FONCyT PICT 01-06035, NGS # 6620-99 CONICET PIP 0739/98 and PIP 02175/01.

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