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Bacterial grazing by mixotrophic flagellates and *Daphnia longispina*: a comparison in a fishless alpine lake

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ABSTRACT: We compared the bacterivory of mixotrophic flagellates (MxFl) and of *Daphnia longispina* in a fishless high altitude lake with a simple food web. During the ice-free period of Lago Paione Superiore (LPS, Italian Central Alps) we measured species–specific direct uptake of fluorescently labelled bacteria (FLB) by MxFl and *D. longispina*. Bacterial production of DNA and protein and dissolved organic carbon (DOC) were also measured. Between ice melt and September, the most common groups of MxFl in the lake were *Gymnodinium* spp., *Dinobryon sertularia*, *Chromulina* spp. and *Ochromonas* sp. On average they ingested 2.9, 3.7, 9.3 and 14.6 bacterial cells ind.$^{-1}$ h^{-1}, respectively, whereas *D. longispina* ingested 1.9×10^6 bacterial cells ind.$^{-1}$ h^{-1}. The MxFl community ingestion rate was always higher than the rate of *D. longispina*, except in mid August when the *D. longispina* population ingested 2.4 times more bacteria than MxFl. Rates of MxFl and *D. longispina* grazing did not show any significant correlation with bacterial abundance and biomass (ρ Spearman, $p > 0.05$), but a significant correlation was found between MxFl grazing and thymidine uptake at 9 m (ρ Spearman = 0.821, $p < 0.014$, *xy* pairs = 7). It appears that *D. longispina* actively feeds on bacteria when it first appears in the lake, but that overall the *D. longispina* population had a lesser impact on bacteria than did MxFl.

KEY WORDS: Mixotrophic flagellate grazing · *Daphnia longispina* grazing · Fluorescently labelled bacteria · FLB · Bacterial production · Alpine lake · Lago Paione Superiore

INTRODUCTION

The recent classification of mixotrophic flagellates (MxFl), which was based on their behaviour (Jones 1997, 2000), emphasized the ability of these organisms to survive in extreme conditions and to successfully colonize small ponds and lakes. This success is due to the versatility of their behaviour: phagotrophy allows predominantly phototrophic mixotrophs to gain limiting nutrients in both laboratory (Nyagaard & Tobiesen 1993) and field conditions (Stoecker et al. 1997, Olrik 1998), whereas mixotrophs that are mainly heterotrophic can survive in low prey environments by increasing their chlorophyll content (Sanders et al. 1989). In lakes with low bacterial abundances, the ability of MxFl to photosynthesize enables them to dominate over heterotrophs in terms of biomass (Palsson & Daniel 2004).

The phagotrophic activity of MxFl has been studied in humic lakes of northern Europe (Jansson et al. 1996, Jansson et al. 1999), in eutrophic lakes (Hitchman & Jones 2000, Urabe et al. 2000), in Antarctic lakes (Roberts & Laybourn-Parry 1999), and in a few oligotrophic clear-water lakes (Domaizon et al. 2003, Palsson & Granéli 2003). Despite a lack of research, oligotrophic high mountain lakes are optimal environments for mixotrophic algae. MxFl are better adapted than heterotrophic protozoa to low nutrient concentrations (Isaksson 1998) that usually results in low prey (bacteria) abundances. In these lakes MxFl have a competitive advantage, especially during the winter when the bacterial biomass is dramatically reduced (Callieri et al. 1999). When these lakes are also fishless, cladocerans are expected to dominate (Cruz-Pizarro et al. 1994) and, due to their high filtering efficiency

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along a wide spectrum of particle size (Jurgens 1994), they can have a strong impact on microbial communities (Gude 1988, Vaqué & Pace 1992).

Food web manipulation experiments have demonstrated that *Daphnia* spp. and heterotrophic nanoflagellates (HNF) both impact the bacterial community composition by shifting the community towards smaller bacterial cells (Langenheder & Jurgens 2001). In laboratory experiments, *Daphnia* spp. at high densities consumed protozoa and bacteria simultaneously (Jurgens et al. 1997). In addition, there is evidence for an increase in bacterial biomass and production in Arctic lakes containing zooplankton dominated by *Daphnia* spp. (Bertilsson et al. 2003). Nevertheless, in all these experiments, specific metazoan and protozoan predation on bacteria was not compared. There is still a scarcity of species–specific estimates of bacterial grazing rate for MxFl measured in natural communities in the field (Palsson & Granéli 2003).

The main goal of our study was to investigate and compare the bacterivory of *Daphnia longispina* and a single MxFl species in an ultraoligotrophic alpine lake (Lago Paione Superiore, LPS) that has never been colonized by fish. LPS was selected as a study site because it is oligotrophic, fishless, and has a very peculiar and unique planktonic trophic food chain. In this lake, a large population of *D. longispina* (up to 20 ind. l^{-1}) develops annually in late summer (Manca & Comoli 1999). MxFl make up 94% of total phytoplankton biomass (Callieri et al. 2002), exploit bacteria during low nutrient conditions, and can potentially control bacterial production.

When *Daphnia* spp. reach a sufficiently high biomass they can become the major bacterial consumer (Jurgens 1994). But what happens in a system where *Daphnia* spp. competes for bacteria with one of their own favourite prey (i.e. MxFl)? To address this and other questions, we quantified the bacterivory of *D. longispina* and some of the most common species of mixotrophic flagellates using the same fluorescently labelled bacteria (FLB) technique. We worked directly on the shore of the lake to avoid lengthy sample transportation from a high to a low elevation site (from 2269 m at the lake to 193 m a.s.l. at our laboratory), which could have led to a reduction in the survival or performance of the organisms and thus to unrealistically low estimates of grazing rates.

MATERIALS AND METHODS

Study site. We performed *in situ* grazing experiments in LPS, an alpine lake located in the Italian Central Alps (46° 10′ N, 8° 11′ E). The lake area is 0.014 km^2 and the maximum depth is 12 m. The main zooplankton component of this lake is a population of *Daphnia longispina* (Manca & Comoli 1999). The lake has a phytoplankton composition comprised of 94% MxFl (Callieri et al. 2002), and there are several historical data sets on the chemical and biological evolution of the lake (Tonolli 1949, Tonolli & Tonolli 1951, The MOLAR Water Chemistry Group 1999, Pugnetti & Bettinetti 1999). The lake experiences extreme environmental conditions. It is ice-covered for about 9 mo every year, and photosynthetically active radiation (PAR) is on average 80% of the surface irradiance at 1 m and 10% near the bottom (C. Callieri unpubl. data). It is a dimictic lake with a weak stratification near the bottom in August (Corno 2000). It is slightly acidic (pH 5.82), has low alkalinity (0.01 mEq l^{-1}), and is oligotrophic (total phosphorus 3.1 µg l^{-1}, nitrate 350 µg l^{-1}; The MOLAR Water Chemistry Group 1999).

Sampling, analysis, counts and biomass calculations. During the 1999 ice-free period, beginning immediately after ice melt (July 21) and extending to the end of September (see Table 2), sampling for phytoplankton and counting of bacteria was performed weekly at depths 1 m below the surface (mean percentage irradiance: 80%) and 1 m above the bottom (mean percentage irradiance: 10%; from here on we refer to this depth as '9 m'). Sampling was performed in the morning from a boat in the centre of the lake, using a 1.5 l van Dorn bottle. Samples were kept cold for grazing experiments, whereas samples for counting were preserved in acetic Lugol's solution (for phytoplankton) or in 2% formaldehyde (for bacteria) (Straskrabová et al. 1999).

On the same dates, zooplankton samples were collected by duplicate vertical net hauls (126 µm) towed from the bottom to the surface (~600 l), at the point of maximum depth (Riccardi 2002). One sample was fixed with 4% formaldehyde and the other was kept cold, to keep the animals alive for grazing experiments performed on the lake shore (Riccardi 2002).

Phytoplankton abundance was quantified in 10 ml chambers with an inverted microscope following the Utermohl technique (Utermohl 1958). Individual cell volumes were calculated from the volume of geometrical bodies that approximated the shapes of the cells, and were transformed to biomass by assuming a density of 1 g cm^{-3} (Straskrabová et al. 1999). Bacteria were counted after DAPI staining using 0.2 µm pore size black polycarbonate filters (Nuclepore) following Porter & Feig (1980). At least 400 bacterial cells were counted on at least 10 fields of individual filters. Between 100 and 200 cells were sized using a semiautomatic image analysis system and the volumes calculated (Image Pro Plus). Bacterial carbon biomass was calculated according to the allometric relationship between cell volume (V) and

carbon content (C) reported by Norland (1993): $C = 120V^{0.72}$, in fg cell^{-1}.

DOC was measured at the 2 depths with a TOC analyzer (TOC-5000A, Shimadzu) after filtration on precombusted GF/F (Whatman) glass fibre filters. The TOC analyser (ASI-5000A, Shimadzu) was calibrated in the range of 0.5 to 1.5 mg organic carbon before each sample run. The autosampler, after acidifying and purging the samples to remove inorganic carbon, ran at least 5 replicates per sample until a CV of 2% was reached. Statistical analyses (non parametric correlation, ρ Spearman) were performed using Sigma Stat (Systat Software).

Bacterial activity. Bacterial activity was evaluated from ^{3}H-thymidine (specific activity: Thy 84 Ci mmol^{-1}, Amersham) and 14C-Leucine (specific activity: Leu 310 mCi mmol^{-1}, Amersham) uptake rates (Fuhrman & Azam 1980, Kirchman et al. 1985). We decided to use both methods as they can provide different information; Thy uptake estimates DNA biosynthesis and is therefore more related to cell division (Riemann et al. 1990), whereas Leu uptake is a constant fraction of bacterial protein and its bacterial uptake is related to the rate of carbon production. The labelled substrates were added to triplicate 10 ml samples plus 1 control, up to 20 nM final concentration. The saturation level for both tracers was ascertained in preliminary experiments. The incubation (1 h in the dark) took place on the lake shore, to maintain temperature conditions similar to those of the epilimnion (in a thermic container with lake water, temperature range 11.7 to 15.5°C). Formalin (4% v/v) was added to controls prior to the tracer addition, and to the samples directly after incubation to terminate reactions. In the lab, approximately 4 h after the incubation period, samples were shaken and then filtered onto 0.2 µm polycarbonate membrane filters (Nuclepore). Filters were rinsed 3 times with 5 ml of ice-cold 5% trichloroacetic acid, 3 times with 5 ml ice-cold 80% ethanol, and put into scintillation vials. Samples were counted in a Beckman LS 5000 TD liquid scintillation counter, and preset to count 14C and ^{3}H channels simultaneously after adding 6 ml of liquid scintillation cocktail (Ready Safe Beckman). Bacterial production was estimated from ^{3}H-Thymidine uptake according to Smits & Riemann (1988) and that from 14C-Leucine uptake according to Simon & Azam (1989) using the empirical factor of 7×10^{16} cells produced per mole of leucine uptake (Riemann et al. 1990).

Grazing experiments. The short term direct-uptake experiments with MxFl and *Daphnia longispina* were performed separately using the same technique of direct-uptake of FLB (labelled with 5-[4,6-dichlorotriazin-2-yl] aminofluorescein: DTAF) (Sherr et al. 1987, Riccardi 2002). Nine experiments were performed for MxFl at 9 m, where their abundance was higher, and 5 were performed at 1 m in order to compare the relative activity of grazers. Only 5 experiments were carried out for *D. longispina*, due to problems with animal sampling and handling. FLB were composed of natural lake-water bacterial assemblages with an average cell volume of 0.47 ± 0.14 µm^3. This mean cell volume is in the range (0.01 to 1.46 µm^3 cell^{-1}) of alpine lake bacterial cell volumes (Straskrabová et al. 1999).

Experiments were conducted at noon, on the shore of the alpine lake, which maintained temperature conditions as similar to those of the epilimnion (in a thermic container with lake water), and were conducted in dim light that was artificially reduced to the range of radiation intensity measured at the bottom of the lake (4 to 13 µmol photons m^{-2} s^{-1}).

Mixotrophic flagellates. FLB (in amounts up to 10–25% of the total number of bacteria) were added to two 1.5 l samples collected from 1 and 9 m. We took 100 ml from the 2 incubation flasks at time zero (t_0) and after 6, 10, 20, and 30 min.

All these samples were fixed by adding acid Lugol's solution (0.5% final concentration), immediately followed by 1% buffered formaldehyde and a few drops of sodium thiosulphate to clear the Lugol (Sherr & Sherr 1993). The MxFl vacuole content was inspected with epifluorescence microscopy (Axioplan equipped with UV and blue-light filter set; 1250× magnification) following the method described by Sherr et al. (1987). Depending on relative abundances, 30 to 400 cells for each MxFl species were checked for FLB uptake in each sample. Hourly ingestion rate of FLB by MxFl was determined from the change in the average number of FLB ind.$^{-1}$ over time for the linear part of the uptake curve, using linear regression analysis (following Šimek et al. [1995]). Species-specific grazing rate was measured for the main MxFl species at the 2 depths. We calculated the amount of natural bacteria ingested from the percentage of tracer (FLB) addition and the *in situ* abundance of bacteria. The clearance rate was obtained from the FLB ingestion rate divided by the concentration of FLB used in the grazing experiment. The community ingestion rate was calculated by multiplying the ingestion rate of bacteria by the *in situ* abundance of MxFl.

***Daphnia longispina*.** Five experiments were carried out on adult *Daphnia longispina* specimens using a density of 20 ind. l^{-1}, which is the density of the maximum population peak (Manca & Comoli 1999). The MxFl and *Daphnia* grazing experiments both started when *Daphnia* first appeared in the lake (July 27), but some of the experiments were unsuccessful. Daphnids were added to 4 replicate bottles and to 1 control bottle filled with 126 µm filtered lake-water. A second control was set up that contained no *D. longispina*. FLB

were added in amounts up to 10–25% of the total number of bacteria. Treatments and controls were incubated in the near-shore water, protected from direct sun (temperature 11.7 to 15.5°C), for 5 to 20 min. Four incubation times (5, 10, 15 and 20 min) were used, based on laboratory tests and according to the gut retention times for cladocerans (Geller 1975, Cauchie et al. 2000). After each experiment daphnids were gently collected by filtration on a 25 µm mesh, narcotized in carbonated water to prevent egestion of gut content, rinsed with sterile Milli-Q water, and preserved in 1% formaldehyde. In the laboratory, after microscopic observation to confirm the removal of FLB from the animals' body surface, the daphnids were sonicated

Table 1. Main phytoplankton taxa identified in Lago Paione Superiore, with cell volume, size, and ranges of specific clearance and ingestion rates. Where genera are represented by 2 or more species, size measurements refer to smaller species. *Mixotrophs; **potential mixotrophs not directly observed to feed on fluorescently labelled bacteria (FLB)

Main taxa	Cell volume (μm^3)	Length × width (µm)	Clearance rate (nl ind.$^{-1}$ h^{-1})	Ingestion rate (bact. cells ind.$^{-1}$ h^{-1})
Chrysophytes				
Chromulina spp.*	11	2.7 × 2.8	1.37–43.1	1.17–30.4
*Dinobryon sertularia**	427	12 × 5	1.32–15.9	1.25–11.2
Ochromonas spp.*	61	7.5 × 4.2		3.78–34.0
Mallomonas alveolata	153	9.9 × 4.9	–	–
Cryptophytes				
Cryptomonas sp.**	2377	30.6 × 12.5	–	–
Rhodomonas sp.*	202	10.7 × 5.6	24.8	17.5
Green Algae				
Chlamydomonas spp.	68	8.1 × 4.3	–	–
Dinoflagellates				
Gymnodinium spp.*	613	11.8 × 9.3	1.27–8.97	1.05–4.88
Peridinium sp.**	19529	30.3 × 23.4		

Table 2. (A) Species–specific ingestion rate (Ing., bacterial cells ind.$^{-1}$ h^{-1}) and (B) species–specific clearance rate (Cl., nl ind.$^{-1}$ h^{-1}) of main mixotrophic flagellate (MxFl) species at 1 and 9 m depth. Sampling dates (d/mo) provided. –: measurement not performed; np: not present; 0: species present but not observed ingesting fluorescently labelled bacteria (FLB)

(A)	*Dinobryon sertularia*				*Chromulina* spp.				*Gymnodinium* spp.				*Rhodomonas* sp.				*Ochromonas* sp.			
	1 m		9 m		1 m		9 m		1 m		9 m		1 m		9 m		1 m		9 m	
Date	Ing.	±SD	Ing.	±SD	Ing.	±SD	Ing.	±SD	Ing.	±SD	Ing.	±SD	Ing.	±SD	Ing.	±SD	Ing.	±SD	Ing.	±SD
21/7	1.71	0.02	1.25	0.08	5.25	3.59	16.24	0.94	3.71	2.34	4.88	–	np		np		np		np	
27/7	–		1.38	0.50	–		3.38	1.91	–		1.14	–	–		np		–		np	
4/8	2.29	0.70	9.22	6.03	8.45	7.73	12.56	8.64	3.43	–	3.72	0.20	np		np		np		np	
11/8	–		5.72	2.86	–		14.45	2.22	–		2.21	–	–		np		–		np	
17/8	–		1.94	–	–		0		–		1.05	–	–		np		–		np	
31/8	0		11.25	1.55	12.90	8.67	30.40	7.28	np		np		0		17.50	–	18.5	11.59	30.15	0.39
8/9	3.06	1.03	1.30	1.00	1.88	1.02	7.48	1.88	np		np		0		0		3.78	–	6.50	2.08
21/9	0		1.57	0.24	1.17	0.51	2.58	0.72	np		np		0		0		34.0	–	5.16	2.18
24/9	–		np		–		3.66	–	–		np		–		0		–		4.39	–

(B)	*Dinobryon sertularia*				*Chromulina* spp.				*Gymnodinium* spp.				*Rhodomonas* sp.				*Ochromonas* sp.			
	1 m		9 m		1 m		9 m		1 m		9 m		1 m		9 m		1 m		9 m	
Date	Cl.	±SD	Cl.	±SD	Cl.	±SD	Cl.	±SD	Cl.	±SD	Cl.	±SD	Cl.	±SD	Cl.	±SD	Cl.	±SD	Cl.	±SD
21/7	5.27	0.07	2.30	0.14	16.15	11.05	29.85	1.72	11.41	7.18	8.97	–	np		np		np		np	
27/7	–		1.55	0.55	–		3.78	2.14	–		1.27	–	–		np		–		np	
4/8	4.48	1.38	13.38	8.74	16.55	15.14	18.21	12.54	6.71	–	5.39	0.29	np		np		np		np	
11/8	–		8.68	4.35	–		21.93	3.37	–		3.36	–	–		np		–		np	
17/8	–		2.89	–	–		0		–		1.56	–	–		np		–		np	
31/8	0		15.94	2.19	17.58	11.82	43.07	10.31	np		np		0		24.80	–	25.3	15.80	42.71	0.55
8/9	3.66	1.24	1.32	1.02	2.25	1.22	7.60	1.90	np		np		0		0		4.47	–	6.60	2.11
21/9	0		1.75	0.27	1.37	0.59	2.87	0.80	np		np		0		0		46.4	–	5.75	2.43
24/9	–		np		–		4.15	–	–		np		–		0		–		4.98	

(3 times for 15 s) in 10 ml sterile water at 50 W with a Branson sonifier-cell disrupter; 1 ml of this suspension was filtered onto a 0.2 µm black Nuclepore filter, stained with DAPI, and FLB were counted as for bacteria. The ingestion and clearence rate was calculated as for the MxFl. Other details are available in Riccardi (2002).

RESULTS

Chrysophytes are the most common algal group in LPS, and composition here includes 2 species of *Chromulina* and *Dinobryon sertularia*. Two species appeared for the first time during the summer of 1999: the chrysophycean *Ochromonas* sp., already well known as a voracious mixotroph (Caron 1987, Sanders 1991, Posch et al. 1999), and the cryptophyte *Rhodomonas* sp. The genus *Gymnodinium* is present in the lake and was represented by 2 species: the small *G. ordinatum* (cell diameter 12 to 16 µm) and a larger *Gymnodinium* sp. (25 µm). Some autotrophic phytoflagellates and potentially mixotrophic organisms (not observed as bacterial grazers during this study) were also present: *Mallomonas alveolata* and other occasional species, *Chlamydomonas* spp., and *Peridinium* sp. (see Table 1 for a list of the main phytoplankton taxa found during this study). Detailed MxFl species abundances during the summer are presented in Fig. 1. Total phytoplankton abundance and biomass was higher at 9 m, where light ranged from 8 to 18% (16 to 160 µmol photons m^{-2} s^{-1}) of the underwater PAR measured at the surface.

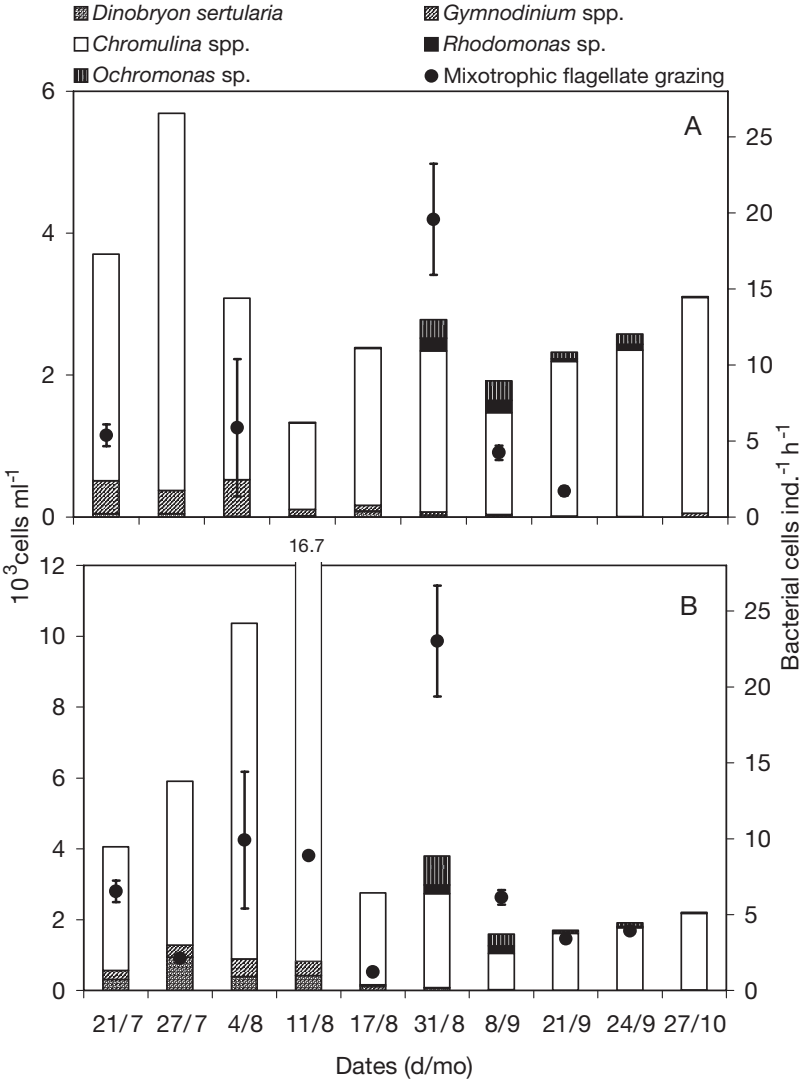

Fig. 1. Abundances of main mixotrophic flagellate (MxFl) species (bars), and bacterivory by MxFl (±SD) at (A) 1 and (B) 9 m, in Lago Paione Superiore

MxFl population dynamics at 1 and 9 m were compared to MxFl grazing (Fig. 1). At 9 m, where there was the highest abundance of organisms, there were 2 grazing peaks: one in early August and the other (higher) peak at the end of August, in correspondence with the appearance of *Ochromonas* sp. and *Rhodomonas* sp.; *Gymnodinium* spp. almost disappeared after sampling on August 17.

Specific grazing rates at 1 and 9 m (Table 2) showed differences among species and throughout the season. *Chromulina* spp. ingested on average 5.9 and 10.1 bacterial cells $ind.^{-1}$ h^{-1} at 1 and 9 m, respectively. This grazing rate on bacteria was higher than of *Dinobryon sertularia*, which grazed a mean of 1.4 and 4.2 bacterial cells $ind.^{-1}$ h^{-1} at 1 and 9 m respectively. The voracious *Ochromonas* sp. ingested on average 14.6 bacterial cells $ind.^{-1}$ h^{-1} at both depths, and *Gymnodinium* spp. took up about 2.9 bacterial cells $ind.^{-1}$ h^{-1}. For *Chromulina* spp., the decrease in grazing activity was coupled with the drop in abundance. *Ochromonas* sp. reached the highest mean clearance rate (19.5 nl $ind.^{-1}$ h^{-1}) followed by *Chromulina* spp., which cleared 14.3 nl $ind.^{-1}$ h^{-1} (averaged over both depths). *Rhodomonas* sp. was present from August 31 to the end of the study, but only actively grazed on 1 occasion (at 9 m, see Table 2). It is noteworthy that among all species, the highest grazing and clearance rates were attained on August 31, the date of highest *Daphnia* population density.

Bacterial abundance ranged between 0.3 and 0.9 × 10^6 cells ml^{-1} (mean 0.7 ± 0.03), and bacterial biomass

Table 3. Bacterial abundances, biomass and dissolved organic carbon (DOC) at 1 and 9 m depth

Date (d/mo)	Bacterial abundance (10^6 cells ml^{-1}) 1 m	±SD	9 m	±SD	Bacterial biomass (µg C l^{-1}) 1 m	±SD	9 m	±SD	DOC (µg C l^{-1}) 1 m	±SD	9 m	±SD
21/7	0.29	–	0.51	–	18.2	–	47.1	–	1404	28.4	706	17.5
27/7	0.69	0.02	0.77	0.03	43.2	1.4	71.7	2.3	529	16.6	408	13.0
4/8	0.46	0.01	0.64	0.02	44.3	1.4	39.2	1.3	667	15.3	234	5.1
11/8	0.77	0.02	0.58	0.02	48.6	1.6	36.7	1.2	647	16.8	547	13.7
17/8	0.62	0.08	0.54	0.02	50.2	6.6	43.7	1.4	336	9.1	477	9.5
31/8	0.70	0.02	0.68	0.02	43.1	1.4	39.5	1.3	427	9.5	700	16.1
8/9	0.70	0.02	0.84	0.03	39.0	1.3	45.6	1.5	473	9.0	610	12.2
21/9	0.66	0.02	0.72	0.02	40.5	1.3	45.2	1.5	1377	41.3	1513	43.9
24/9	0.93	–	0.77	0.05	56.5	–	51.6	3.6	1484	46.0	2473	79.1

Table 4. Bacterial production (BP) (DNA [Thy: thymidine] and protein [Leu: leucine] biosynthesis), mixotrophic flagellate (MxFl) grazing, and MxFl grazing impact on bacterial population, measured at t_0 as % of bacteria ingested h^{-1}. Data for BP and MxFl grazing are mean ± SD

Date (d/mo)	BP Thy (10^3 cells ml^{-1} h^{-1}) 1 m	±SD	9 m	±SD	BP Leu (10^3 cells ml^{-1} h^{-1}) 1 m	±SD	9 m	±SD	MxFl grazing (10^3 cells ml^{-1} h^{-1}) 1 m	±SD	9 m	±SD	MxFl grazing impact (% grazed cells h^{-1}) 1 m	9 m
21/7	–		–		–		–		19.9	2.7	26.5	8.4	6.88	5.21
27/7	0.7	0.1	5.1	0.1	0.9	0.4	9.9	2.1	–		12.6	2.9		1.59
4/8	3.2	0.2	10.3	1.6	4.8	1.6	5.6	0.5	18.1	13.9	102.7	67.3	3.88	15.81
11/8	1.3	0.0	6.3	1.0	2.8	0.4	2.5	0.2	–		148.7	14.8		25.07
17/8	2.3	0.3	3.1	0.2	3.0	0.7	3.3	1.4	–		3.3	–		0.60
31/8	1.1	0.1	3.4	1.0	3.7	0.1	0.9	0.6	54.4	10.2	87.4	11.2	7.57	12.63
8/9	1.7	0.4	3.0	0.1	2.0	0.1	1.6	0.2	8.1	0.9	9.8	2.2	1.14	1.14
21/9	2.0	0.6	2.2	0.2	0.8	0.4	1.1	0.3	3.9	0.7	5.8	1.4	0.58	0.79
24/9	–		–		–		–		–		7.5	–		0.93

ranged between 18 and 72 µg C l^{-1} (mean 45 ± 1.9) (Table 3). Bacterial abundance and biomass remained relatively stable over time despite large fluctuations in bacterial production and grazing. DOC concentrations ranged from 0.2 to 2.4 mg C l^{-1}, with higher values at 9 m in September after some days of heavy rain (Bertoni et al. 2002). Thy estimates gave higher values with respect to Leu at 9 m (mean: 4.8 vs. 3.5×10^3 cells ml^{-1} h^{-1}) and lower values at 1 m (mean: 1.8 vs. 2.6×10^3 cells ml^{-1} h^{-1}), indicating a difference in the bacterial population growth/division ratio at the 2 depths (Table 4). Percent of bacteria ingested by MxFl measured at t_0 ranged from 0.6 to 25% (Table 4). Bacterial abundance and biomass did not show any significant correlation to DOC and MxFl grazing (ρ Spearman, $p > 0.05$; data pooled for both depths). Nevertheless, bacterial production (Thy) was significantly correlated to MxFl grazing at 9 m (ρ Spearman = 0.821, $p = 0.014$, *xy* pairs = 7), but not to *Daphnia longispina* grazing or to DOC.

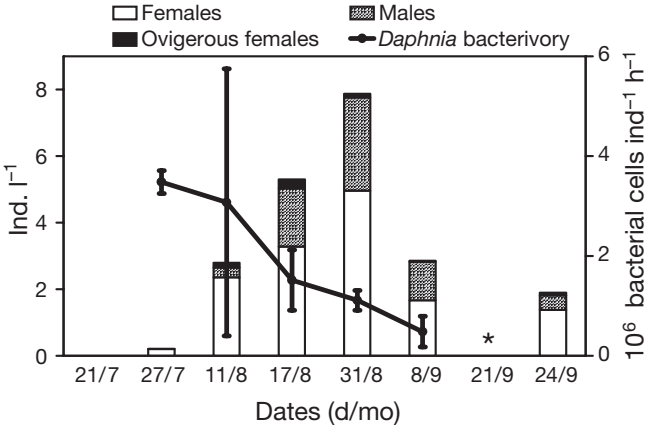

Fig. 2. *Daphnia longispina*. Population structure (bars) and bacterivory (±SD) at 9 m in Lago Paione Superiore. July 21: *D. longispina* not found; *sample not available

Population structure of *Daphnia longispina* showed a peak in males and females (around 8 ind. l^{-1}) at the end of August, but ovigerous females were most abundant in mid August (Fig. 2). The individual bacterivory of *D. longispina* was higher (but with a high standard deviation) at the beginning of August when numbers were around 3 ind. l^{-1}. On average, *D. longispina* ingested 1.9×10^6 bacterial cells ind.$^{-1}$ h^{-1} (range 0.5 to

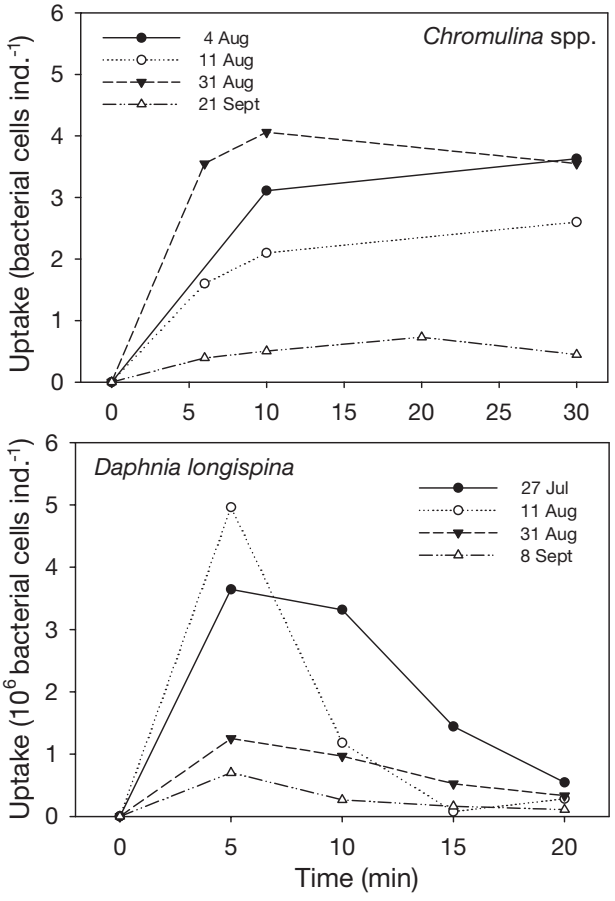

Fig. 3. *Chromulina* spp. and *Daphnia longispina*. Bacterial uptake rate vs. time (note different time scales)

3.5×10^6 bacterial cells ind.$^{-1}$ h^{-1}). Comparison of bacterial uptake (bacteria plus FLB) vs. time by *D. longispina* and *Chromulina* spp. showed many differences (Fig. 3). Due to size and filtering ability, each *D. longispina* ingested roughly 6 orders of magnitude more bacteria than *Chromulina* spp., and they also exhibited a different ingestion curve pattern. The small mixotrophic cell reached maximum bacterial uptake after 10 min and maintained a plateau for at least the following 30 min. *D. longispina* reached maximum ingestion in the first 5 min, and then reduced ingestion as egestion increased.

As *Daphnia longispina* was sampled with vertical hauls, we compared its bacterivory with the predatory activity of MxFl as calculated from the average MxFl community grazing rate in the entire water column (Fig. 4). The community ingestion rate of MxFl was always higher than that of *D. longispina*, except on August 17 when the *D. longspina* population ingested 2.4 times more bacteria than MxFl. The highest peak in MxFl grazing was attained on August 11 (148.7×10^3 bacterial cells ml^{-1} h^{-1}; Fig. 4).

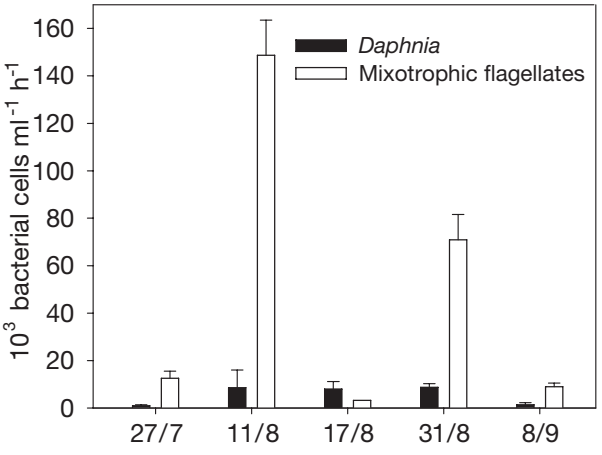

Fig. 4. *Daphnia longispina* and MxFl. Comparison of bacterivory

DISCUSSION

In LPS the MxFl community ingestion rate was higher than that of *Daphnia longispina*. MxFl and *D. longispina* removed between 0.6 and 25% and between 0.04 and 1.4% of natural bacterioplankton abundance h^{-1}, respectively. The impact of *Dinobryon sertularia*, *Chromulina* spp. and *Gymnodinium* spp. was high in the first part of the summer, exactly during the period in which the *D. longispina* population developed. Many studies have been published on the grazing activity of different species of *Dinobryon* both in laboratory and in the field, but the results are often contrasting (Caron et al. 1993, Jones 1997, Palsson & Graneli 2003). For example, in an oligotrophic Swedish lake, the grazing rate of *Dinobryon crenulatum* and *Dinobryon divergens* measured with microspheres reached a maximum of 0.27 bacterial cells ind.$^{-1}$ h^{-1} (Palsson & Graneli 2003), whereas *Dinobryon* spp. ingestion rate ranged from 1.2 to 95 bacterial cells ind.$^{-1}$ h^{-1} in natural plankton communities (Bird & Kalff 1987). Caron et al. (1993) found that the FLB ingestion rates of *Dinobryon cylindricum* in cultures were at the lower end of the range observed in natural populations; however, when calculated for bacterial biomass instead of absolute abundance, the results were comparable due to the smaller average cell size in natural bacterial assemblages.

Our estimates performed with FLB *in situ* for *Dinobryon sertularia* (1.2 to 11.2 bacterial cells ind.$^{-1}$ h^{-1}) were much more similar to those measured for *D. divergens* in laboratory experiments by Jones & Rees (1994). They observed an ingestion rate of 6 fluorescent microsphere cells^{-1} h^{-1} and a maximum clearance rate of 10.8 nl ind.$^{-1}$ h^{-1} (in our study, rates ranged between 1.3 and 15.9 nl ind.$^{-1}$ h^{-1}). In our study,

Gymnodinium spp. ingested FLB at all times when they were present. Previously published data on the bacterial ingestion rate of *Gymnodinium* spp. (Hitchmann & Jones 2000) refer to a small artificial pond and are not expressed as bacteria ingested, but as FLB ingested. Considering that FLB addition to the sample was around 10% of the bacterial abundance, the maximum ingestion rate found in that study (0.27 FLB ind.$^{-1}$ h^{-1}) is near to the mean value that we measured (2.9 bacterial cells ind.$^{-1}$ h^{-1}).

The only estimate of *Chromulina* spp. clearance rate reported in the literature is lower than our measurements (maximum 15 nl ind.$^{-1}$ h^{-1}), but it was performed with fluorescent microspheres instead of FLB. FLB are more similar to natural bacteria and are therefore more edible. We noticed that both *Chromulina* spp. ingested bacteria voraciously, except on August 17 (the peak in *Daphnia longispina* density) when the MxFl population—in particular *Chromulina* spp.—was noticeably low.

A drastic change in phytoplankton community composition was observed after August 17. *Gymnodinium* spp. were absent and *Dynobrion sertularia* almost disappeared. Two new species appeared, *Ochromonas* sp. and *Rhodomonas* sp., and *Chromulina* spp. increased again. During August, the bacterivory of *Daphnia longispina* was quite constant, around 8 × 10^3 bacterial cells ml^{-1} h^{-1}, even though individual bacterial uptake changed a lot and decreased as the number of daphnids increased. The control of *Daphnia* spp. on phytoplankton biomass, suggested by previous studies from observations of mirror-shaped curves for phyto- and zooplankton (Cammarano & Manca 1997), was confirmed by direct measurements using fluorescently labelled prey (Riccardi 2002). To our knowledge there are no specific measurements of *D. longispina* grazing on MxFl; therefore, we think the best approximation could be the range of algal ingestion rates reported by Riccardi (2002) (19 to 192 × 10^3 cell ind.$^{-1}$ h^{-1}) performed in LPS. From these estimates, a *D. longispina* population could consume 0.3 to 17.6% of phytoplankton abundance h^{-1} (Riccardi 2002) and strongly affect community composition.

The presence of *Ochromonas* sp. in the second part of the summer contributed to a second peak of grazing by MxFl. *Ochromonas* sp. is commonly used as a grazer to estimate predation effects on the bacterial community (Hahn & Hofle 1999, Boenigk et al. 2001a,b, Wu et al. 2004), but previously these studies were always conducted in laboratory cultures. *Ochromonas* sp. ingestion rates, measured using different bacterial strains or fluorescent microspheres, ranged between 2.7 and 175.7 particles ind.$^{-1}$ h^{-1} (Boenigk et al. 2001b) or between 6.8 and 27.8 bacterial cells ind.$^{-1}$ h^{-1} (Wu et al. 2004). In these laboratory experiments, the number of predator-prey contacts—and thus the number of successful ingestions—was artificial because the initial bacterial population was >10^6 bacterial cells ml^{-1}. This density is about 10 times higher than natural bacterial abundances measured in LPS. In this lake, the ratio bacteria/MxFl is on average 260/1.

Cryptomonads can feed on particles (Porter 1988, Tranvik et al. 1989); therefore, *Rhodomonas minuta* and *R. lacustris* can be considered to be bacterivorous (Jansson et al. 1996, Olrik 1998). We were not able to identify the species of *Rhodomonas* from LPS, but we were able to distinguish it from the chrysophyte *Ochromonas* sp. based on differences in morphology. *Rhodomonas* sp. ingested FLB only once out of the 4 sampling times when it was present, but on that occasion it grazed 17.5 bacterial cells ind.$^{-1}$ h^{-1}.

In LPS, zooplankton biomass was dominated by *Daphnia longispina* (which comprised >80% of total zooplankton biomass), whereas *Cyclops abyssorum tatricus* was only abundant at the very beginning of the summer (Riccardi 2002). *D. longispina* bacterivory was in the range of literature values (Jurgens 1994), and this gave us confidence in applying the FLB method to the estimation of *D. longispina* grazing (Wiedner & Vareschi 1995, Riccardi 2002). When observing *D. magna*, Langenheder & Jurgens (2001) obtained a grazing rate of 0.75 to 6.4 × 10^6 bacterial cells ind.$^{-1}$ h^{-1} using natural bacterioplankton. We observed a grazing rate of 0.5 to 3.5 × 10^6 bacterial cells ind.$^{-1}$ h^{-1} for *D. longispina*. Results of *D. longispina* grazing in LPS, reported elsewhere (Riccardi 2002), showed that bacteria represented a relatively small fraction of the diet of *D. longispina* compared to algae, but that their importance increased when phytoplankton biomass decreased. In other environments it has been observed that bacteria can be the dominant food source when algal food is limiting (Porter 1984, Kankaala 1988).

Contrary to our expectations, bacterial biomass and abundance were not directly related to MxFl and *Daphnia longispina* grazing or to DOC concentrations. However, we observed some indications of a possible effect of MxFl grazing on bacterial division at 9 m, as estimated by Thy uptake. At this depth, higher bacterial production estimated from Thy compared to Leu uptake could indicate a higher DNA synthesis rate at the depth at which bacteria grazing was also higher. The stimulation of bacterial production due to grazing activity by protists has been postulated from observations in laboratory experiments (Šimek et al. 1997). We suggest that nutrient regeneration through grazing could also be effective in the presence of mixotrophic predation, and could explain our field data results. Higher Leu uptake observed at 1 m may have reflected

enhanced protein synthesis at a temperature higher than that observed at 9 m.

High flow episodes that carry fresh DOC have been found to stimulate bacterial production in humic lakes (Jansson et al. 1999, Drakare et al. 2002). This dynamic was not evident in the oligotrophic clear water lake that we studied, possibly because of low bacterial numbers and activity. Similarly, the allochthonous input from the catchment to the lake observed in September did not show any immediate effect on bacterial production. Chryptophytes dominated the phytoplankton during this time, in response to phosphorous mobilization due to *Daphnia longispina* feeding activity (Bertoni et al. 2002). DOC concentration measurements in LPS were quite high and unusual for a clear oligotrophic alpine lake (V. Straskrabová pers. comm.), but were similar to values provided in other studies on this lake (Callieri & Bertoni 1999). Nevertheless, its effect on planktonic organisms and in particular on bacteria was not clear in this study.

Bacterial abundances and biovolumes stayed at a constant level. Compared to other freshwater systems, the observed range of bacterial abundance (from 0.3 to 0.9×10^6 bacterial cells ml^{-1}) is at the lower end of the range (Riemann & Cristoffersen 1993). However, this range is typical for high mountain lakes: e.g. values reported from Gossenkoellensee (Austrian Alps) and for Joeri Lake III (Switzerland) range from 0.2 to 0.6 and 0.7 to 1.7×10^6 bacterial cells ml^{-1}, respectively (Hinder et al. 1999, Wille et al. 1999). The presence of *Chromulina* spp. and *Dynobrion sertularia* at the peak of bacterial production may depress bacterial abundance but lead to a rapid turnover of newly produced bacterial biomass.

In conclusion, we characterized the events taking place during the short open-water period in LPS through the study of key organisms: bacteria, mixotrophs, and *Daphnia longispina*. Bacterial abundance did not fluctuate a great deal, and was not strictly dependent on DOC, but division was correlated with the grazing activity of MxFl. Mixotrophic flagellates actively grazed on bacteria and were responsible for up to 25% of bacterial mortality. *Daphnia longispina* was also able to feed on bacteria but probably utilized this food only during the period of algal-food limitation. In the absence of fish, an important element of *D. longispina*'s success, daphnid survival and development was strictly connected to the microbial food web.

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LITERATURE CITED

Bertilsson S, Hansson LA, Graneli W, Philibert A (2003) Size-selective predation on pelagic microorganisms in Arctic freshwaters. J Plankton Res 25:621–631

Bertoni R, Callieri C, Corno G (2002) The mixotrophic flagellates as key organisms from DOC to *Daphnia* in an oligotrophic alpine lake. Verh Int Ver Limnol 28:392–395

Bird DJ, Kalff J (1987) Algal phagotrophy: regulating factors and importance relative to photosynthesis in *Dinobryon* (Chrysophyceae). Limnol Oceanogr 32:277–284

Boenigk J, Matz C, Jurgens K, Arndt H (2001a) Confusing selective feeding with differential digestion in bacterivorous nanoflagellates. J Eukaryot Microbiol 48:425–432

Boenigk J, Matz C, Jurgens K, Arndt H (2001b) The influence of preculture conditions and food quality on the ingestion and digestion process of three species of heterotrophic nanoflagellates. Microb Ecol 42:168–176

Callieri C, Bertoni R (1999) Organic carbon and microbial food web assemblages in an oligotrophic alpine lake. J Limnol 58:136–143

Callieri C, Pugnetti A, Manca M (1999) Carbon partitioning in the food web of a high mountain lake: from bacteria to zooplankton. J Limnol 58:144–151

Callieri C, Bertoni R, Corno G (2002) Dynamics of bacteria and mixotrophic flagellates in an Alpine lake in relation to *Daphnia* population development. J Limnol 61:177–182

Cammarano P, Manca M (1997) Studies on zooplankton in two acidified high mountain lakes in the Alps. Hydrobiologia 345:97–109

Caron DA (1987) Grazing of attached bacteria by heterotrophic microflagellates. Microb Ecol 13:203–218

Caron DA, Sanders RW, Lim EL, Marrasé C, Amaral LA, Whitney S, Aoki RB, Porter KG (1993) Light-dependent phagotrophy in the freshwater mixotrophic chrysophyte *Dinobryon cilindricum*. Microb Ecol 25:93–111

Cauchie HM, Joaquim-Justo C, Hoffmann L, Thomé JP, Thys I (2000) A note on the use of fluorescently labelled algae for the determination of gut passage time in *Bosmina* and *Daphnia*. Verh Int Ver Limnol 27:2987–2991

Corno G (2000) Ruolo dei flagellati mixotrofi nel microbial loop di un lago alpino oligotrofo (Lago Paione Superiore, Alpi Centrali). MSc dissertation, University of Milan

Cruz-Pizarro L, Reche I, Carrillo P (1994) Plankton dynamics in a high-mountain lake (Las Yeguas, Sierra Nevada, Spain)—indirect evidence of ciliates as food source of zooplankton. Hydrobiologia 274:29–35

Domaizon I, Vibaud S, Fontvieille D (2003) Taxon-specific and seasonal variations in flagellates grazing on heterotrophic bacteria in the oligotrophic lake Annecy—importance of mixotrophy. FEMS Microbiol Ecol 46:317–329

Drakare S, Blomqvist P, Bergstrom AK, Jansson M (2002) Primary production and phytoplankton composition in relation to DOC input and bacterioplankton production in humic Lake Örträsket. Freshw Biol 47:41–52

Fuhrman JA, Azam F (1980) Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl Environ Microbiol 39: 1085–1095

Geller W (1975) Die Nahrungsaufnahme von *Daphnia pulex* in Abhängigkeit von der Futterkonzentration, der Temperatur, der Körpergrösse und dem Hungerzustand der Tiere. Arch Hydrobiol Suppl 48:47–107

Gude H (1988) Direct and indirect influences of crustacean zooplankton on bacterioplankton of Lake Constance. Hydrobiologia 159:63–73

Hahn MW, Hofle MG (1999) Flagellate predation on a bacte-

rial model community: interplay of size-selective grazing, specific bacterial size, and bacterial community composition. Appl Environ Microbiol 65:4863–4872
Hinder B, Baur I, Hanselmann K, Schanz F (1999) Microbial food web in an oligotrophic high mountain lake (Joeri Lake III, Switzerland). J Limnol 58:162–168
Hitchman RB, Jones HLJ (2000) The role of mixotrophic protists in the population dynamics of the microbial food web in a small artificial pond. Freshw Biol 43:231–241
Isaksson A (1998) Phagotrophic phytoflagellates in lakes—a literature review. Arch Hydrobiol Spec Issues Adv Limnol 51:63–90
Jansson M, Blomqvist P, Jonsson A, Bergstrom AK (1996) Nutrient limitation of bacterioplankton, autotrophic and mixotrophic phytoplankton, and heterotrophic nanoflagellates in Lake Örträsket. Limnol Oceanogr 41: 1552–1559
Jansson M, Bergstrom AK, Blomqvist P, Isaksson A, Jonsson A (1999) Impact of the allochthonous organic carbon on microbial food web carbon dynamics and structure in Lake Örträsket. Arch Hydrobiol 144:409–428
Jones HLJ (1997) A classification of mixotrophic protists based on their behaviour. Freshw Biol 37:35–43
Jones RI (2000) Mixotrophy in planktonic protists: an overview. Freshw Biol 45:219–226
Jones RI, Rees S (1994) Influence of temperature and light on particle ingestion by the freshwater phytoflagellate *Dinobryon*. Arch Hydrobiol 132:203–211
Jurgens K (1994) Impact of *Daphnia* on planktonic microbial food web. A review. Mar Microb Food Webs 8:295–324
Jurgens K, Arndt H, Zimmermann H (1997) Impact of metazoan and protozoan grazers on bacterial biomass distribution in microcosm experiments. Aquat Microb Ecol 12: 131–138
Kankaala P (1988) The relative importance of algae and bacteria as food for *Daphnia longispina* (Cladocera) in a polyhumic lake. Freshw Biol 19:285–196
Kirchman DL, K'nees E, Hodson RE (1985) Leucine incorporation and its potential as a measure of a protein synthesis by bacteria in natural aquatic systems. Appl Environ Microbiol 49:599–607
Langenheder S, Jurgens K (2001) Regulation of bacterial biomass and community structure by metazoan and protozoan predation. Limnol Oceanogr 46:121–134
Manca M, Comoli P (1999) Studies on the zooplankton of Lago Paione Superiore. J Limnol 58:131–135
Norland S (1993) The relationship between biomass and volume of bacteria. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, FL, p 303–308
Nyagaard K, Tobiesen A (1993) Bacterivory in algae: a survival strategy during nutrient limitation. Limnol Oceanogr 38:273–279.
Olrik K (1998) Ecology of mixotrophic flagellates with special reference in Chrysophyceae in Danish Lake. Hydrobiologia 369/370:329–338
Palsson C, Granéli W (2003) Diurnal and seasonal variations in grazing by bacterivorous mixotrophs in an oligotrophic clear-water lake. Arch Hydrobiol 157:289–307
Palsson C, Daniel C (2004) Effects of prey abundance and light intensity on nutrition of a mixotrophic flagellate and its competitive relationship with an obligate heterotroph. Aquat Microb Ecol 36:247–256
Porter KG (1984) Natural bacteria as food resources for zooplankton. In: Klug MJ, Reddy CA (eds) Current perspectives in microbial ecology. American Society for Microbiology, Washington, DC, p 340–345
Porter KG (1988) Phagotrophic phytoflagellates in microbial food webs. Hydrobiologia 159:89–97
Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–948
Posch T, Šimek K, Vrba J, Pernthaler J, Nedoma J, Sattler B, Sonntag B, Psenner R (1999) Predator-induced changes of bacterial size-structure and productivity studied on an experimental microbial community. Aquat Microb Ecol 18: 235–246
Pugnetti A, Bettinetti R (1999) Biomass and species structure of the phytoplankton of a high mountain lake (Lake Paione Superiore, Central Alps, Italy). J Limnol 58: 127–130
Riccardi N (2002) In situ measurement of *Daphnia longispina* grazing on algae and bacteria in a high mountain lake (Lake Paione Superiore, Northern Italy) using fluorescently labeled cells. Water Air Soil Pollut Focus 2:343–357
Riemann B, Christoffersen K (1993) Microbial trophodynamics in temperate lakes. Mar Microb Food Webs 7:69–100
Riemann B, Bell RT, Jorgensen NOG (1990) Incorporation of thymidine, adenine and leucine into bacterial assemblages. Mar Ecol Prog Ser 65:87–94
Roberts EC, Laybourn-Parry J (1999) Mixotrophic cryptophytes and their predators in the Dry Valley lakes of Antarctica. Freshw Biol 41:737–746
Sanders RW (1991) Trophic strategies among heterotrophic flagellates. In: Patterson D, Larsen J (eds) The biology of the free-living heterotrophic flagellates. Clarendon Press, Oxford, p 21–38
Sanders RW, Porte KG, Bennett SJ, DeBiase AE (1989) Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. Limnol Oceanogr 34:673–687
Sherr EB, Sherr BF (1993) Protistan grazing rates via uptake of fluorescently labeled prey. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis, Boca Raton, FL, p 695–701
Sherr BF, Sherr EB, Fallon RD (1987) Use of monodispersed, fluorescently labeled bacteria to estimate in situ protozoan bacterivory. Appl Environ Microbiol 53:958–965
Šimek K, Bobkova J, Macek M, Nedoma J, Psenner R (1995) Ciliate grazing on picoplankton in a eutrophic reservoir during the summer phytoplankton maximum: a study at the species and community level. Limnol Oceanogr 40: 1077–1090
Šimek K, Vrba J, Pernthaler J, Posch T, Hartman P, Nedoma J, Psenner R (1997) Morphological and compositional shift in an experimental bacterial community influenced by protists with contrasting feeding modes. Appl Environ Microbiol 58:3715–3720
Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. Mar Ecol Prog Ser 51:201–213
Smits JD, Riemann B (1988) Calculation of cell production from [^{3}H]thymidine incorporation with freshwater bacteria. Appl Environ Microbiol 54:2213–2219
Stoecker DK, Li A, Coats DF, Gustafson DE, Nannen MK (1997) Mixotrophy in the dinoflagellate *Prorocentrum minimum*. Mar Ecol Prog Ser 152:1–12
Straskrabová V, Callieri C, Carrillo P, Cruz-Pizarro L and 6 others (1999) Investigation on pelagic food web in mountain lakes—aims and methods. J Limnol 58:77–87
The MOLAR Water Chemistry Group (1999) The MOLAR project: atmospheric deposition and lake water chemistry. J Limnol 58:88–106
Tonolli V (1949) Gli alti laghi della Val Bognanco. Parte II. Mem Ist Ital Idrobiol 5:39–93

Tonolli V, Tonolli L (1951) Osservazioni sulla biologia ed ecologia di 170 popolamenti zooplanctonici di laghi alpini d'alta quota. Mem Ist Ital Idrobiol 6:53–136

Tranvik LJ, Porter KG, Sieburth JM (1989) Occurrence of bacterivory in Cryptomonas, a common freshwater phytoplankter. Oecologia 78:473–476

Urabe J, Gurung TB, Yoshida T, Sekino T, Nakanishi M, Maruo M, Nakayama E (2000) Diel changes in phagotrophy by *Cryptomonas* in Lake Biwa. Limnol Oceanogr 45: 1558–1563

Utermohl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton Methodik. Mitt Int Ver Limnol 9:1–38

Vaqué D, Pace LM (1992) Grazing on bacteria by flagellates and cladocerans in lakes of contrasting food-web structure. J Plankton Res 14:307–321

Wiedner C, Vareschi E (1995) Evaluation of a fluorescent microparticle technique for measuring filtering rates of *Daphnia*. Hydrobiologia 302:89–96

Wille A, Sonntag B, Sattler B, Psenner R (1999) Abundance, biomass and size structure of the microbial assemblage in the high mountain lake Gossenkollensee (Tyrol, Austria) during the ice-free period. J Limnol 58:117–126

Wu QL, Boenigk J, Hahn MW (2004) Successful predation of filamentous bacteria by a nanoflagellate challenges current models of flagellate bacterivory. Appl Environ Microbiol 70:332–339

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