Journal of Plankton Research Vol.21 no.9 pp.1659–1679, 1999

Bacterivory of metazooplankton, ciliates and flagellates in a newly flooded reservoir

A.Thouvenot, M.Richardot, D.Debroas and J.Devaux

Laboratoire de Biologie Comparée des Protistes, Unité associée CNRS, Université Blaise Pascal Clermont Ferrand II, 63117 Aubière Cedex, France

Abstract. Bacterial consumption by metazoan zooplankton and phagotrophic protists was measured *in situ* during the period of thermal stratification in the epilimnion (1 m) and metalimnion (7 m) of a newly flooded reservoir (Sep reservoir, France). The mean bacterial consumption was 2.53×10^6 bacteria l^{-1} h^{-1} at 1 m and 0.97×10^6 bacteria l^{-1} h^{-1} at 7 m. The main consumers over the whole study period were the cladocerans *Daphnia longispina* and *Ceriodaphnia quadrangula*, accounting on average for 72% of the potential total predation of bacteria at 1 m and 56% at 7 m, especially during the months of May–June and August. Heterotrophic nanoflagellates (HNF), which accounted for 12% estimated total predation of bacteria at 1 m and 13% at 7 m, only exerted a limited predation, mainly by a *Monas*-type cell. Ciliates, dominated in terms of abundance by *Pelagohalteria viridis*, accounted for 4% of total predation in the epilimnion ($0.00–0.42 \times 10^6$ bacteria l^{-1} h^{-1}). In a newly flooded reservoir, metazoan zooplankton appear to be the main consumers of bacteria. Predation of ciliates and HNF by zooplanktonic crustaceans could account for the low contribution of components of the microbial loop to bacterial consumption.

Introduction

Heterotrophic bacteria form a large proportion of the biomass in pelagic ecosystems (Cho and Azam, 1988; Simon *et al*., 1992). The small variations in bacterial abundance, despite the fact that bacterial generation times are of the order of a day in temperate regions (Pace, 1988), suggest that there is an effective regulatory system. Predation by protozoa (Sanders *et al*., 1989), metazoan zooplankton (Güde, 1988; Arndt 1993; Jürgens, 1994), mortality caused by viruses (Proctor and Führman, 1992) and the quantity of dissolved organic carbon produced by algae (Cole *et al.*, 1988) are the most frequently cited regulatory mechanisms. Heterotrophic flagellates are generally thought to be the main consumers of bacteria in marine ecosystems (Fenchel, 1982) and fresh waters (Riemann, 1985; Güde, 1986), but phagotrophic phytoflagellates can also have an impact on bacteria and have grazing rates that are similar to those of heterotrophic flagellates (Bird and Kalff, 1986). In addition, ciliates can be important consumers of bacteria in freshwater (Simek *et al*., 1995) and marine environments (Sherr *et al*., 1989). Although protozoans, or more generally protists, are thought to be the main consumers of bacteria, some metazoans can nevertheless play an important role in regulating bacterial communities (e.g. Jürgens *et al*., 1994). Among these, Cladocera, rather than Copepoda, are thought to be effective consumers of bacteria (Peterson *et al*., 1978; Geller and Müller, 1981; Pace *et al.*, 1983; Güde, 1988; Jürgens, 1994), which can be an important food source during the summer (Pace *et al*., 1983). Some field studies have highlighted their important predation activity (Børsheim and Olsen, 1984; Riemann, 1985; Bjørnsen *et al.*, 1986), whereas others concluded that these crustaceans were only of minor importance in controlling bacterial populations (Toth, 1980;

Pedros-Alio and Brock, 1983). Although some rotifers do consume heterotrophic bacteria (Pourriot, 1977; Bogdan *et al.*, 1980; Ricci, 1984; Boon and Shiel, 1990; Ooms-Wilms *et al.*, 1995; Ooms-Wilms, 1997), their contribution to regulating the bacterial plankton is, however, modest (Sanders *et al.*, 1989; Pace *et al.*, 1990; Pernie *et al.*, 1990).

The literature therefore clearly shows that metazoans do consume bacteria, but little is known of the importance of this predation compared to that of heterotrophic and mixotrophic protists. Simultaneous observation of the succession of bacterial consumption by metazoans and protozoans has only rarely been attempted (Sanders *et al.*, 1989; Vaqué and Pace, 1992). Their role could also depend on the type of ecosystem being studied. For example, Paterson *et al.* (1997) suggested that metazoan zooplankton in a newly flooded reservoir could use organisms other than phytoplankton, such as bacteria, as an energy source.

In this study, we determined which taxa consumed bacteria-sized particles, estimated the filtration and ingestion rate of individual organisms on bacteria, and calculated the impact of grazing by protozoans and metazoans on the bacterial community. This study, therefore, provided information on the relative roles of the different predators in controlling the bacterial abundance in an ecosystem in a transitional stage.

Method

The oligomesotrophic Sep reservoir (33 ha) situated in the Massif Central of France (46°2′N and 3°1′E) was first flooded in 1995 to provide irrigation water for crops. During the study period (April–August 1997), lake water was sampled every week using an 8 l Van Dorn-type bottle in the epilimnion (1 m) and metalimnion (7 m) at the deepest point in the lake. Estimations of grazing on bacteria by protozoans and metazooplankton were made every week from May to August. At the same time, the metazoan zooplankton in the water column were collected by taking three vertical hauls from the bottom to the surface using a Juday type net of 55 μm mesh size.

Abiotic and biotic variables

The water temperature, dissolved oxygen content and pH were determined with a multiparameter probe (YSI GRANT 3800). Water transparency was estimated from the Secchi disc depth. Phosphorus (PO_4-P), nitrates (NO_3-N) and ammonium (NH_4-N) contents were analysed using standard American Public Health Association (1992) methods. The chlorophyll *a* content was determined by spectrophotometry (Lorenzen, 1967; Strickland and Parsons, 1968). Dissolved organic matter was quantified after filtration through a 0.2 μm polycarbonate filter (Millipore). The dissolved protein concentration (DPROT) was determined using the micro BCA Protein Assay Reagent Kit (Pierce) and that of total dissolved carbohydrates (TDCHO) by Burney and Sieburth's (1977) and Johnson and Sieburth's (1977) method, after acid hydrolysis (1 N HCl; 100°C, 15 h).

Sample storage

The samples were fixed immediately after collection and stored at 4°C. Formaldehyde was used as fixative for bacteria (final concentration 2%), glutaraldehyde for flagellates (final concentration 1%), mercuric chloride for ciliates (final concentration 2.5%), and a mixture of formaldehyde and sucrose (final concentration 4%) for metazoans, the latter preventing release of eggs and physical deformation (Prepas, 1978).

Counting organisms

Heterotrophic bacteria were stained with DAPI (1 µg/l), then filtered through black polycarbonate membranes of 0.2 µm pore size (Millipore), using the protocol described by Porter and Feig (1980). After staining with primulin (final concentration 200 µg/ml) (Caron, 1983), flagellates were recovered on black polycarbonate filters of 0.8 µm pore size (Nuclepore). Both preparations were made within 24 h of sampling and were stored at –25°C to minimize losses of autofluorescence (Bloem *et al*., 1986). Counts were made under an Olympus HBS epifluorescence microscope equipped with an epifluorescent HB2-RFL light source, an HBO-100 W mercury lamp and a neofluar 100/1.25 objective lens. Two types of light filters were used: UG-1, DM 400, L 435 (UV light) for heterotrophic bacteria and heterotrophic nanoflagellates (HNF), and BP 545, O 590 (blue light) for autotrophic nanoflagellates. In a preliminary series of triplicate counts of bacteria and flagellates (magnification ×1250) and ciliates (magnification ×500), the coefficient of variation for bacteria was <5%, when counting 500–800 bacteria in 30–60 fields, with an eyepiece fitted with a graticule delimiting the field. The coefficient of variation was 6% for pigmented flagellates (PF), 9% for HNF after counting 200–300 cells, and 12% for ciliates after counting 100–200 individuals. Large-sized and/or colonial ciliates and flagellates were counted using Utermöhl's (1958) method with a Leitz (Wild M40) inverted microscope. The entire counting field was examined at a magnification of ×500 for ciliates, within 2 months of sample fixation as recommended by Sime-Ngando and Grolière (1991). Ciliates were identified to genus or species level by reference to Kahl (1930–1935), Kudo (1966) and by consulting the works of Foissner (1994). The metazoan zooplankton were counted under a binocular microscope (Wild M3 Z) in a Dolfuss chamber. The dry weight of each taxon was calculated from the formulae of Bottrell *et al*. (1976).

Estimating the predation rates of metazoan zooplankton and protozoans on the bacterial communities

A stock solution of tracer particles 0.5 µm in diameter was prepared from a concentrated solution of Fluoresbrite Plain Microspheres (Polysciences). To ensure that the microspheres were well dispersed, the solution was treated with bovine serum albumin (BSA) at a concentration of 5 mg l^{-1} (Pace and Bailif,

1987). The concentration of microspheres was estimated by epifluorescence microscopy, after filtration onto polycarbonate filters of 0.2 μm pore size.

The plankton from 1 and 7 m were acclimatized for 5 min in the lake in glass bottles with a capacity of 1 l for metazoan zooplankton (three replicates) and of 250 ml for protozoans (1–2 replicates). A concentration of microspheres of between 2 and 5% of the bacterial abundance in the lake (Ooms-Wilms *et al.*, 1995) was injected into each metazooplankton bottle, which was then turned upside down several times to mix. Preliminary experiments under laboratory conditions (20°C) showed that an incubation time of 5 min was less than the digestion time for the various metazoans. After incubation, each bottle was filtered through a nylon sieve of 55 μm pore size. To prevent any regurgitation, the metazooplankton were anaesthetized with carbonated water before fixation in a final concentration of 4% formaldehyde and 60 g l^{-1} sucrose. For protozoans, a concentration of microspheres of between 8 and 12% (MacManus and Okubo, 1991; Carrias *et al.*, 1996) of the mean bacterial abundance in the lake was added to the 250 ml bottles. Preliminary time series experiments showed that bead uptake by protozoans was linear between 0 and 30–40 min. Thus, *in situ* incubations were stopped at 30 min by adding a cold solution of glutaraldehyde (2% final concentration) to prevent egestion of tracer particles (Sanders *et al.*, 1989). The plankton in the control bottles were fixed immediately after adding the tracer particles, to determine the concentration of particles adhering to organisms (background noise). The numbers of microspheres ingested by the metazooplankton, ciliates, microflagellates and colonial flagellates were determined under an epifluorescence microscope Leitz fluovert FU, filter A (513593, UV light) and in transmitted light after sedimenting 75–100 m of sample in counting cells. The counts of microspheres in protozoa were conducted using the same method as that described for ciliates. The numbers of microspheres ingested by metazooplankton were estimated by examining the entire alimentary tract at a magnification of ×125–250. The ingestion of tracer particles by phagotrophic nanoflagellates was determined after filtering two subsamples (10–25 ml) onto a black polycarbonate filter of 0.8 μm pore size. Two hundred to 300 cells were then examined using the method described above. Preliminary triplicate experiments showed that the coefficients of variation for the abundance of microspheres in protist organisms were 37% for HNF, 32% for pigmented flagellates and 26% for ciliates, whereas those in metazoans were 22% for *Daphnia longispina*, 8% for *Ceriodaphnia quadrangula*, 6% for *Bosmina longirostris* and 45% for rotifers.

The filtration [TF; μl individual (ind.)$^{-1}$ h^{-1}], ingestion (TI; bacteria ind.$^{-1}$ h^{-1}) and grazing rates (TGR; bacteria l^{-1} h^{-1}) for each taxon were calculated as follows:

$$\mathrm{TF} = (M_t - M_0)/M \times T \text{ and } \mathrm{TI} = \mathrm{TF} \times (B + \text{microspheres})$$

$$\mathrm{TGR} = \mathrm{TI} \times \text{abundance of the taxon } (l^{-1})$$

where M_t is the number of microspheres ingested per individual (microspheres ind.$^{-1}$) at incubation time t, M_0 is the number of microspheres ingested per individual (microspheres ind.$^{-1}$) at incubation time 0 (background noise), M is the

concentration of microspheres during incubation (microspheres μl^{-1}), T is the incubation time (h) and B is the bacterial concentration during incubation (bacteria μl^{-1}).

Results

Abiotic and biotic variables

Vertical oxygen and temperature profiles were measured at every metre. The values presented are those obtained at the depths of the experiments (Figure 1). The mean temperature in the epilimnion was 19.2 ± 4.3°C and that of the metalimnion 14.0 ± 4.2°C. The dissolved oxygen concentration at 1 m depth only changed slightly with time, with a mean value of 8.1 ± 1.3 mg l^{-1}. At 7 m, the oxygen content declined from the start of measurements to reach a minimum value of 1.20 mg l^{-1} on 25 June; it then varied between 2.20 and 5.03 mg l^{-1} (mean 3.4 ± 1.0 mg l^{-1}). The mean concentrations of nitrates, ammonium and orthophosphates were 1.59 mg N l^{-1} (0.87–2.3 mg N l^{-1}), 0.006 mg N l^{-1} (0.003–0.009 mg N l^{-1}) and 0.030 mg P l^{-1} (0.015–0.047 mg P l^{-1}), respectively. The mean chlorophyll *a* concentration was 1.9 µg l^{-1}, and fluctuated during the study period between 0.6 and 10.5 µg l^{-1} (Figure 2). The DPROT and TDCHO concentrations varied

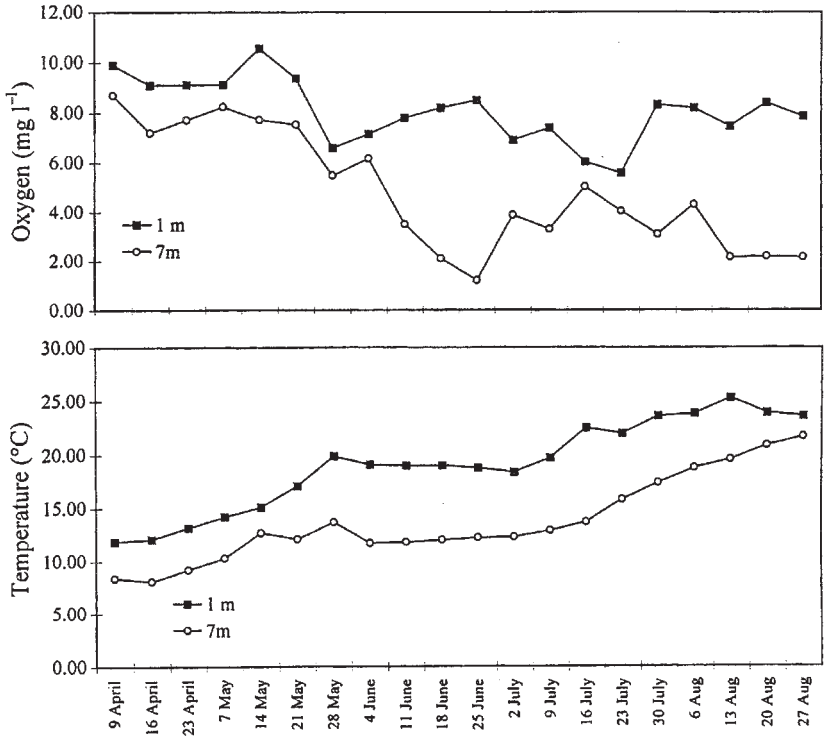

Fig. 1. Temporal changes in dissolved oxygen (mg l^{-1}) and temperature (°C) at 1 and 7 m depths.

between 4.11 and 7.64 mg l^{-1} (mean 5.88 mg l^{-1}) and from 1.21 to 3.13 mg l^{-1} (mean 2.04 mg l^{-1}), respectively.

Plankton community succession

Bacteria. The abundance of heterotrophic bacteria (Figure 2) varied from 1.4 to 4.5 × 10^6 cells ml^{-1} (mean = 2.7 × 10^6 cells ml^{-1}). The abundance was slightly higher at 1 m than at 7 m, except in April. Most of the bacteria were in the form of cocci, with a size between 0.4 and 0.6 µm.

Flagellate and ciliate protists. The various taxa (HNF, pigmented flagellates and ciliates) encountered in the Sep reservoir are shown in Table I. The mean

Table I. Size, biovolume, filtration rate and ingestion rate of the various taxa of protists

Protists	Mean length (µm)	Biovolume (µm^3)	Bead uptake	Clearance (10^{-3} µl ind.$^{-1}$ h^{-1})			Ingestion (bacteria ind.$^{-1}$ h^{-1})		
				Min.	Max.	Mean	Min.	Max.	Mean
Ciliates									
Oligotrichida									
Pelagohalteria viridis	29.0	3450	Y	3.1	47.5	26.9	10.1	236.7	125.1
Halteria sp.	28.0	2520	Y	0.0	24.7	9.9	0.0	118.8	46.8
Strobilidium caudatum	21.5	3111	N						
Strombidium viride	51.0	23 459	Y			4.0			13.6
Prostomatida									
Urotricha furcata	20.5	1251	N						
Balanion sp.	13.5	1294	N						
Scuticociliatida									
Cyclidium sp.	13.0	618	N						
Undetermined Scuticociliatida	37.5	1193	N						
Haptorida									
Askenasia volvox	30.0	7650	N						
Didinium sp.	42.5	12 750	N						
Paradileptus elephantinus	280.0	714 737	N						
Peritrichida									
Vorticella sp.	55.5	17 028	Y	35.1	52.3	43.7	192.1	195.1	193.5
Suctorida	50.1	11 780	N						
Undetermined	35.0	22 444	N						
Pigmented flagellates									
Chrysococcus sp.	10.0	524	N						
Chrysidalis sp.	4.5	31	N						
Cryptomonas ovata	24.5	1856	Y	0.0	4.3	0.7	0.0	14.0	2.6
Dinobryon cylindricum	11.5	220	Y	28.4	51.6	31.8	63.2	137.6	103.8
Mallomonas sp.	20.0	1097	N						
Pandorina morum	12.5	1023	N						
Peridinium volzii	50.5	65 449	N						
Rhodomonas minuta	9.5	173	N						
Synura sp.	13.0	1050	N						
Heterotrophic nanoflagellates									
Kathablepharis sp.	5.8	65	N						
Monas-like cells	6.0	72	Y	0.0	3.3	0.7	0.0	14.1	2.5
Chrysomonadine (2–5 µm)	3.0	17	N						
Undetermined	4	26	N						

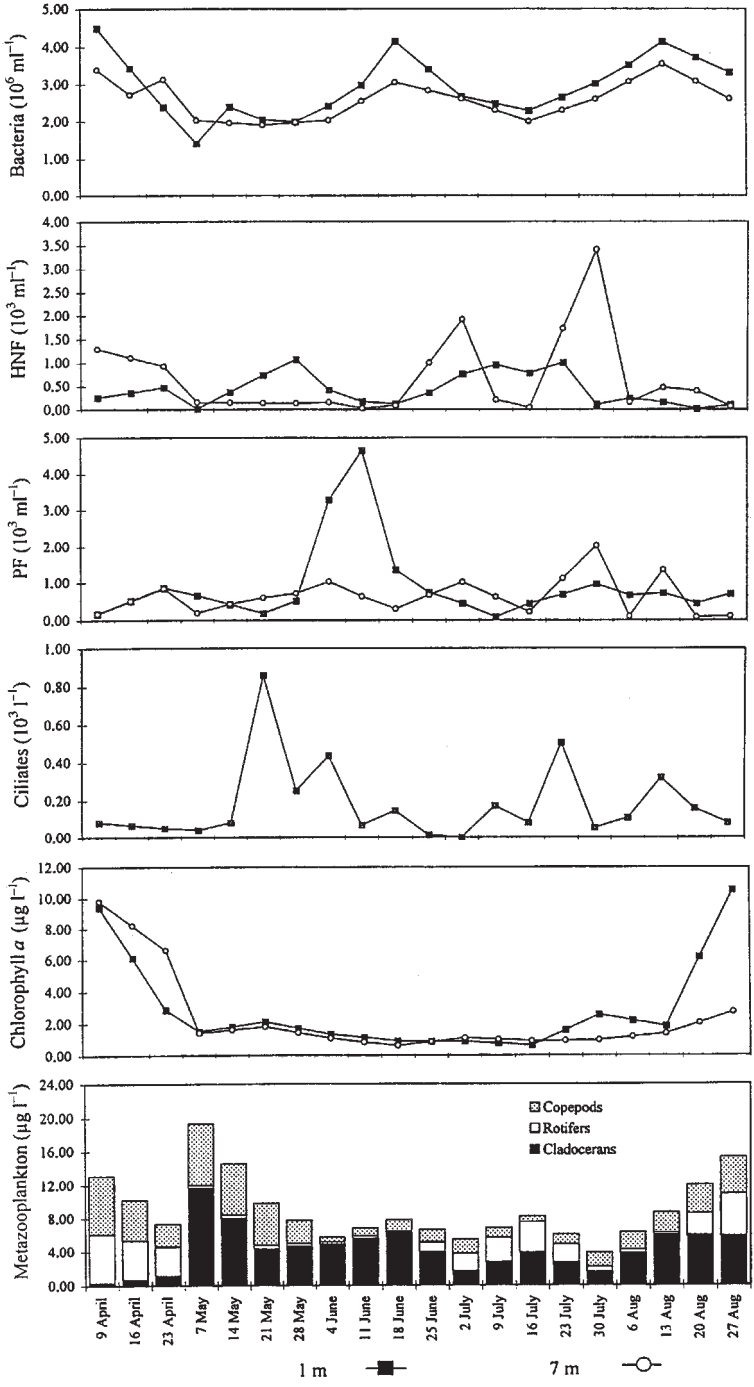

Fig. 2. Abundances of bacteria, heterotrophic nanoflagellates (HNF), pigmented flagellates (PF) and ciliates, and chlorophyll *a* at 1 and 7 m, and metazooplankton biomass in the water column.

densities of HNF measured during the study periods at 7 m (0.7×10^3 cells ml^{-1}) were slightly higher than those at 1 m (0.4×10^3 cells ml^{-1}). The highest densities of HNF in the epilimnion were recorded in May and July (Figure 2). In the metalimnion, the highest density was recorded at the end of July. This community (Figure 3) was dominated by cells of the chrysomonadine, *Monas*-like cells (46% at 1 m and 14% at 7 m) and a small unidentified heterotrophic flagellate (unidentified sp. 2) with a size of between 2 and 5 μm (41% at 1 m and 73% at 7 m). *Kathablepharis* sp. was mainly present in spring when it accounted for 13% of total HNF abundance (Figure 3). The density of pigmented flagellates (Figure 2), which fluctuated from 0.09 to 4.6×10^3 cells ml^{-1}, was higher at 1 m (mean 1.0 ×

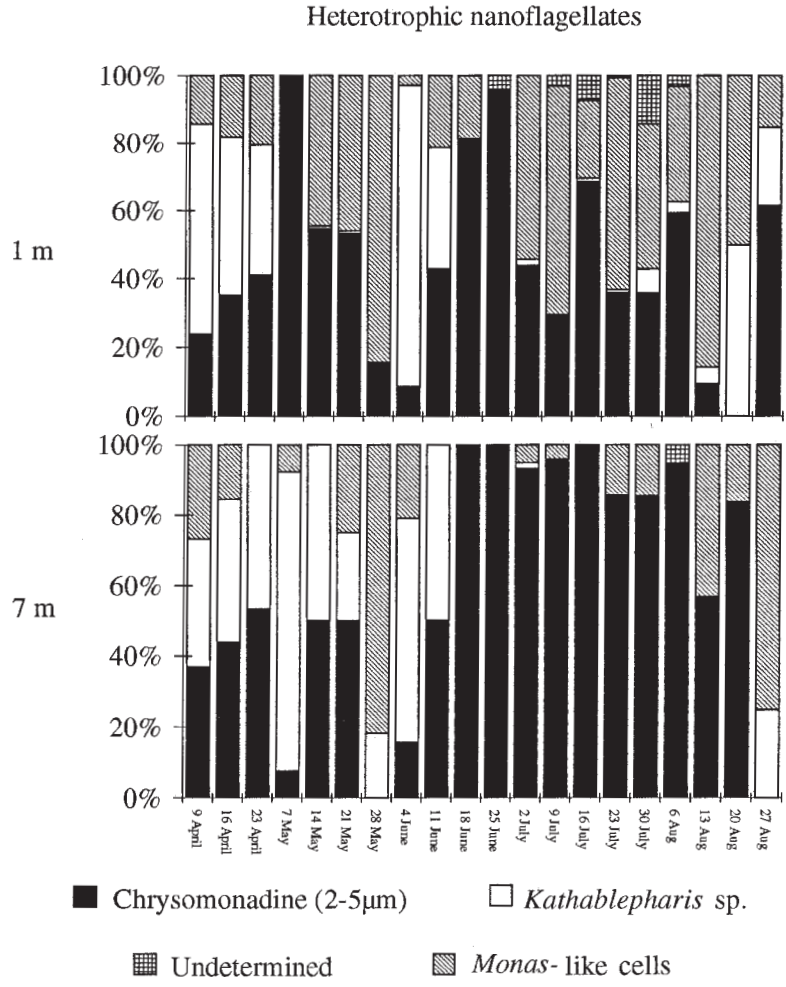

Fig. 3. Relative abundance of heterotrophic nanoflagellates (HNF) at 1 and 7 m depths.

10^3 cells ml^{-1}) than at 7 m (mean 0.7×10^3 cells ml^{-1}). The highest density was recorded in June in the epilimnion and in July in the metalimnion. *Chrysidali*s sp. (57% of abundance at 1 m and 6% at 7 m) and *Rhodomonas minuta* (36% of abundance at 1 m and 82% at 7 m) accounted for most of the total abundance of pigmented flagellates. *Cryptomonas ovata* was especially abundant in April–May and at the end of summer (Figure 4). Densities of ciliates varied from 0.04 to 0.86 $\times 10^3$ cells l^{-1} (mean 0.18×10^3 cells l^{-1}) (Figure 2), the highest densities being recorded in May. These protozoans were dominated in terms of density by a mixotrophic oligotrich, *Pelagohalteria viridis*, which on average accounted for 67% of cell abundance during the study period.

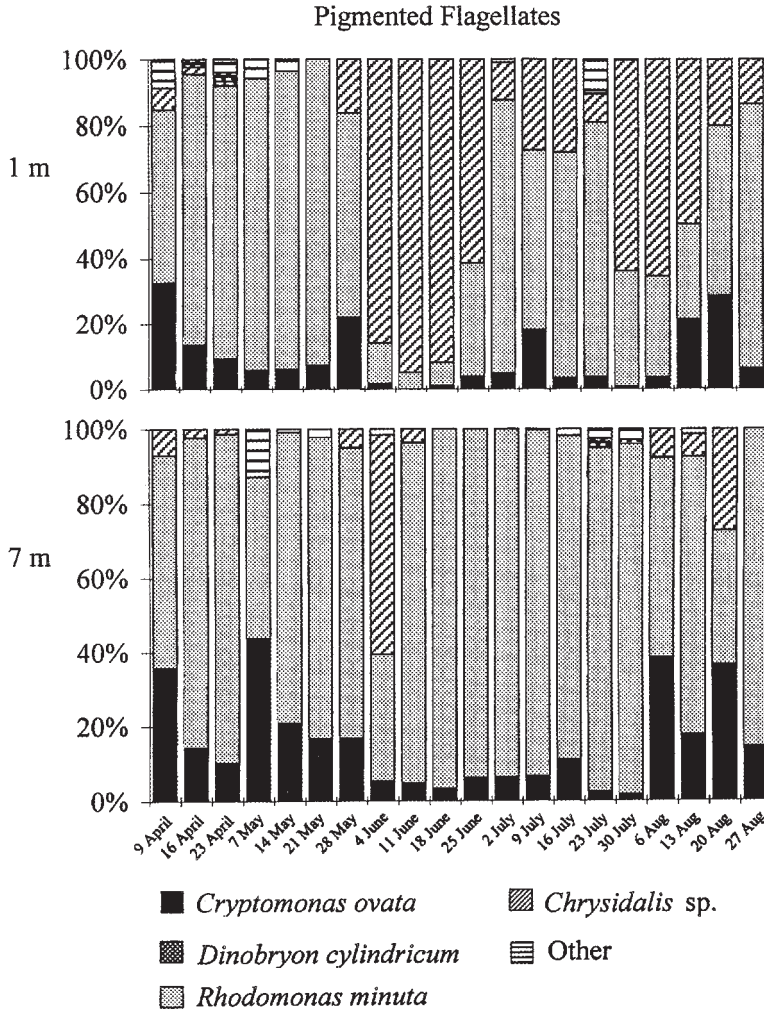

Fig. 4. Relative abundance of pigmented flagellates (PF) at 1 and 7 m depths.

Metazooplankton. The various taxa (crustaceans and rotifers) counted in the Sep reservoir are shown in Table II. The total biomass of metazoan zooplankton in the water column (Figure 2) varied from 3.9 to 19.3 µg l^{-1} (mean 9.10 µg l^{-1}). In April, copepods (*Eudiaptomus gracilis* and *Cyclops vicinus*) and the rotifer *Polyarthra* sp. dominated the community in terms of biomass. Over the whole year, copepods consisted mainly of juvenile stages (nauplii and copepodites). The calanoid *E.gracilis* was the most abundant copepod from May onward, but most of the metazooplankton biomass was composed of Cladocera and especially *D.longispina*, which accounted for 99.7% of the biomass of these crustaceans from May to June. In July and August, *Hexarthra mira* and *Polyarthra* sp. were the dominant species of rotifers. *Ceriodaphnia quadrangula*, which only occurred in August, accounted for 36% of the cladoceran biomass at this time. When chlorophyll *a* reached values of <2 µg l^{-1} (Figure 2), from the end of May to July, the metazooplankton biomass remained low. This period coincided with the peaks in the abundance of HNF, ciliates, PF and bacteria. At the start of the study, the decrease in bacterial abundance also coincided with an increase in the biomass of *D.longispina*.

Estimated consumption of bacteria by flagellate and ciliate protists and metazooplankton

The range and mean values for filtration and ingestion rates for each taxon of flagellate and ciliate protists and metazooplankton are shown in Tables I and II. Among the heterotrophic nanoflagellates, only cells of the *Monas* type ingested

Table II. Filtration and ingestion rate of the various taxa of metazooplankton

Metazooplankton	Bead uptake	Clearance (µl ind.$^{-1}$ h^{-1})			Ingestion ($\times 10^3$ bacteria ind.$^{-1}$ h^{-1})		
		Min.	Max.	Mean	Min.	Max.	Mean
Crustaceans							
Cladocerans							
Bosmina longirostris	Y	1.0	17.0	9.0	3.3	138.0	28.0
Ceriodaphnia quadrangula	Y	7.0	144.0	76.0	2.9	456.2	175.8
Daphnia longispina	Y	10.0	290.0	90.0	10.6	704.8	229.1
Copepods							
Cyclops vicinus	N						
Acanthocyclops robustus	N						
Eudiaptomus gracilis	N						
Rotifers							
Asplanchna priodonta	N						
Conochilus unicornis	Y	0.8	2.3	2.2	2.2	14.2	6.5
Filinia terminalis	N						
Hexarthra mira	Y	11.2	17.2	14.2	30.2	46.1	38.1
Kellicottia longispina	N						
Keratella cochlearis	N						
Keratella quadrata	N						
Polyarthra sp.	N						
Synchaeta sp.	N						

bacteria-sized particles, whereas, among pigmented flagellates, only *Cryptomonas ovata* and *Dinobryon cylindricum* ingested bacteria-sized particles (Table I). The ciliates consuming bacteria-sized particles were Oligotrichida and Peritrichida. Cladocera all consumed bacteria-sized particles, whereas *Polyarthra* sp., which accounted for most of the rotifer biomass, and Copepoda never ingested microspheres.

Estimated total consumption of bacteria in the epilimnion varied between 0.08 and 10.27 $\times$ 10^6 bacteria l^{-1} h^{-1} (Figure 5A). Predation of bacteria-sized particles by heterotrophic and mixotrophic flagellates averaged 0.64 $\times$ 10^6 bacteria l^{-1} h^{-1}. The highest grazing rates by protists were recorded on 13 and 20 August (2.54 and 2.47 $\times$ 10^6 bacteria l^{-1} h^{-1}), when they were caused by a heterotrophic flagellate (*Monas*-like cells) and then by a pigmented flagellate (*Cryptomonas ovata*)

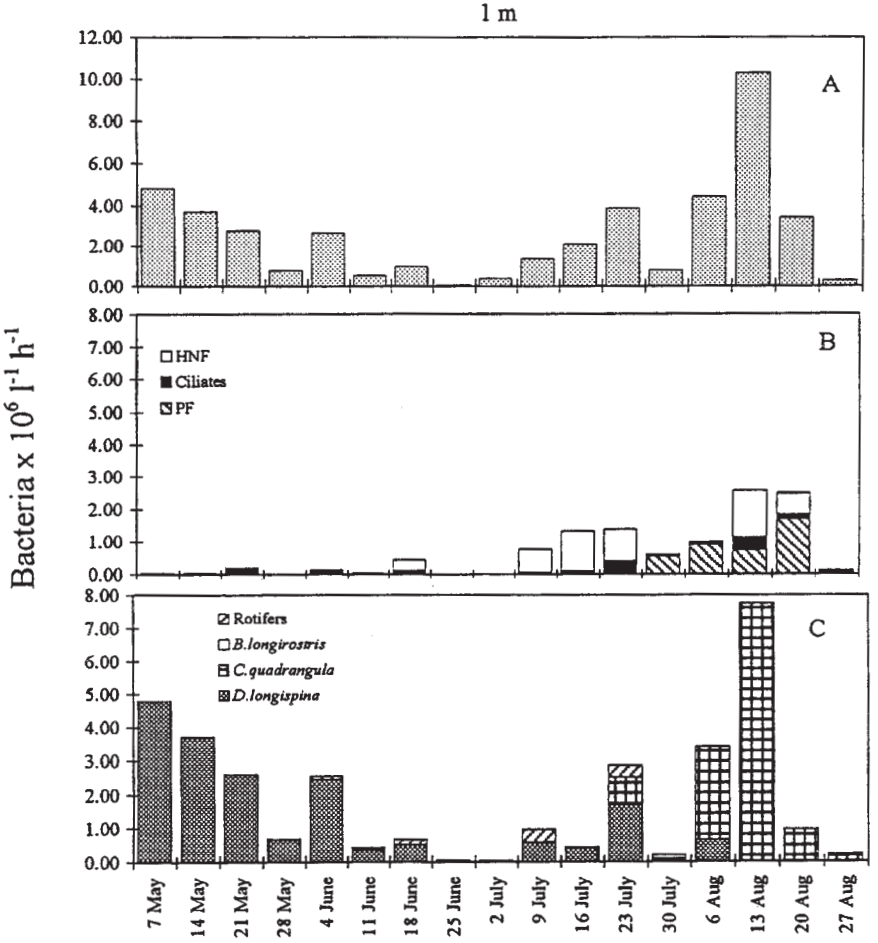

Fig. 5. Temporal changes in total bacterial consumption (**A**), by phagotrophic protists (**B**) and by metazoan zooplankton (**C**) at 1 m depths.

(Figure 5B). There was no consumption of bacteria-sized particles by protists on 25 June and 2 July. The mean consumption of bacteria-sized particles by ciliates was 0.10×10^6 bacteria l^{-1} h^{-1}, accounting for 15% of the total grazing by protists for all the measurements combined. Two oligotrich species, *Pelagohalteria viridis* and *Halteria* sp., were responsible for 95% of the ingestion of microspheres. *Vorticella* were only encountered on a single date, on 4 June, when their potential predation of bacteria was 0.022×10^6 bacteria l^{-1} h^{-1}. The impact of scuticociliates was negligible because of their low density throughout the study.

The average predation by metazooplankton was 1.89×10^6 bacteria l^{-1} h^{-1}. In spring, *D.longispina* was the main bacterial consumer, whereas *Ceriodaphnia quadrangula* became the main consumer in summer. These two species accounted for 57 and 39% of bacterial predation by metazoans, respectively, whereas *B.longirostris*, which was only counted at 1 m, only accounted for 2% of total metazoan activity. *Hexarthra mira* and *Conochilus unicornis*, with mean filtration rates of 14.2 and 2.2 µl ind.$^{-1}$ h^{-1}, respectively, were the two rotifer species that ingested microspheres in June and July.

Bacterial consumption in the metalimnion fluctuated between 0 and 3.07×10^6 bacteria l^{-1} h^{-1} (mean 0.97×10^6 bacteria l^{-1} h^{-1}) (Figure 6A). The mean grazing rate of ciliates and total flagellates decreased slightly with depth and was only 0.43×10^6 bacteria l^{-1} h^{-1} at 7 m (Figure 6B). The numbers of ciliates counted in each replicate throughout the study period, and of crustaceans and rotifers in early May and in July, were too low in the metalimnion to calculate the grazing impact.

From the start of the study until 16 July, bacterial consumption by flagellates was $<0.31 \times 10^6$ bacteria l^{-1} h^{-1}. Pigmented phagotrophic flagellates (*Dinobryon cylindricum* and *Cryptomonas ovata*), which only accounted for 12% of consumption at 1 m, had a higher contribution at 7 m (32%). This was due to their high consumption rates on 23 and 30 July (2.06 and 1.82×10^6 l^{-1} h^{-1}) (Figure 6B). The grazing impact of protists was highest on 30 July (2.78×10^6 bacteria l^{-1} h^{-1}) and was mainly due to the pigmented flagellate *Dinobryon cylindricum* whose consumption accounted for 75% of grazing on bacteria-sized particles by protists at this depth. As in the epilimnion, most of the bacterial consumption by metazooplankton in the spring was by *D.longispina* and mainly by *Ceriodaphnia quadrangula* in summer (Figure 6C).

Over the entire study, cladocerans accounted on average for 72% of predation at 1 m and 56% at 7 m, and were the main consumers of bacteria-sized particles, especially in May, June and August. HNF accounted for 12% of bacterial consumption at 1 m and 13% at 7 m, and therefore only had a limited predation mainly due to a *Monas*-type cell. Ciliates and rotifers accounted for 4 and 2% of total predation, and were the organisms with the lowest grazing impact.

Discussion

Various methods have been used to quantify bacterial consumption by individual planktonic organisms in the field. The use of fluorescent microspheres is one of the standard methods used for estimating the ingestion rate of protists (e.g. MacManus and Fürhman, 1988), rotifers (e.g. Ooms-Wilms *et al.*, 1995; Ooms-Wilms, 1997)

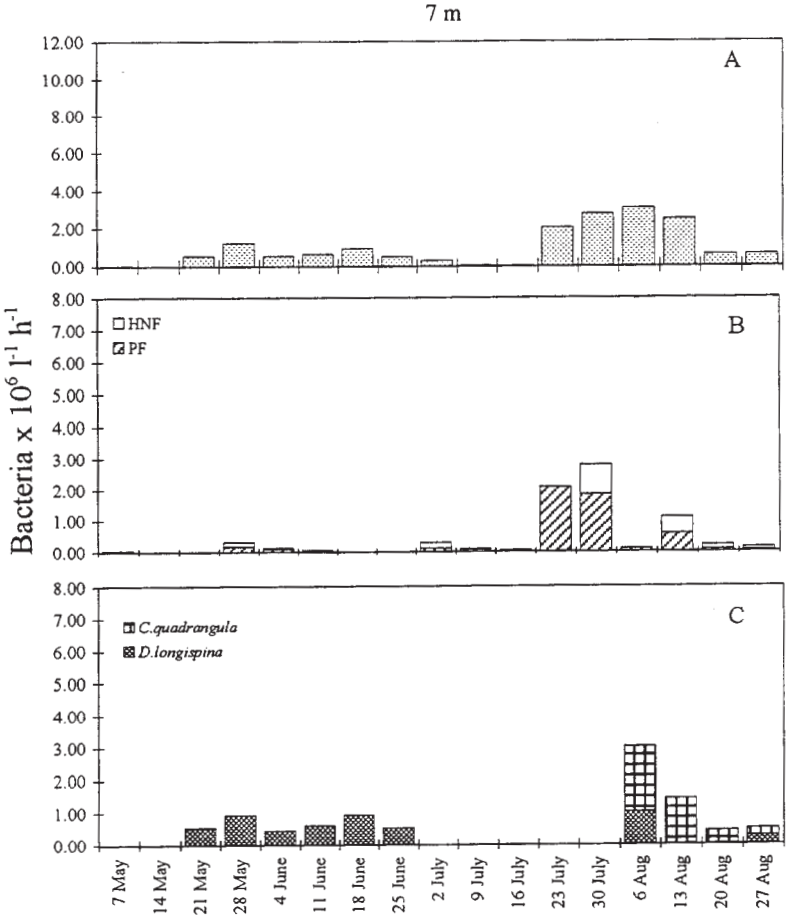

Fig. 6. Temporal changes in total bacterial consumption (**A**), by phagotrophic protists (**B**) and by metazoan zooplankton (**C**) at 7 m depths.

and cladocerans (Børsheim and Andersen, 1987; Wiedner and Vareshi, 1995). Protozoans and copepods are organisms that are capable of selecting their food (Monger and Landry, 1992; Gonzales *et al*., 1993), in contrast to rotifers (Arndt, 1993) and cladocerans (DeMott, 1986; Lampert, 1987) which are thought to be relatively unselective in terms of the bacterial particle size. Various studies have shown that using fluorescent microspheres underestimates predation by some ciliates and flagellates that preferentially ingest bacteria labelled with fluochrome (FLB) rather than the microspheres (Sherr *et al.*, 1987; Simek and Straskrabova, 1992), whereas other studies have shown that there are no significant differences in predation between the different types of particles (e.g. Sanders *et al*., 1989; Jones and Rees, 1994).

For metazoans, the method used has an influence on the calculation of the ingestion rates (Ooms-Wilms *et al*., 1995). For example, the rapidly digested FLB

tend to underestimate grazing by Cladocera. Furthermore, although some authors have determined bacterial consumption in rotifers using FLB (Ooms-Wilms, 1991; Turner and Tester, 1992), Boon and Shield (1990) demonstrated that there are methodological problems. Copepods are capable of selecting their prey (DeMott, 1988) and could, therefore, be sensitive to the method used. However, to our knowledge, there are no experimental data to support such a hypothesis. In fact, the work on food selection by these organisms was conducted using particles of a size between 2 and 20 μm (DeMott, 1988; Kerfoot and Kirk, 1991), which is much greater than the length of bacteria occurring in lake ecosystems. Fluorescent microspheres also have the advantage of being easy to see in the alimentary tract of the different metazoans, in contrast to labelled bacteria (FLB or minicells).

The use of microspheres would, therefore, appear to be the best compromise for estimating simultaneous consumption of bacteria by both protists and metazooplankton. The predation on bacteria-sized particles measured in this study is obviously only representative of the two zones that were studied, and especially in the case of metazoans that can migrate over long distances through the water column.

Metazoans appeared to be the main consumers of bacteria in the epilimnion and metalimnion in this study. The measurements of predation at 1 and 7 m, and the highly significant negative correlation ($P < 0.001$) between the abundance of the cladoceran *D.longispina* in the water column and the mean density of bacteria in the zones studied, demonstrate the predator–prey relationships that exist between these two communities. The consumption, which at maximum reached 7% of the standing stock of bacteria, was similar to that recorded by Vaqué and Pace (1992) in a dystrophic lake. The potential bacterial consumption is low and cannot at first sight satisfactorily explain the control of bacterial production, even if we accept a very conservative estimate of one doubling of total bacteria every 5 days. However, in this ecosystem, few of the bacteria are active (0.2–3.2%) (Jugnia *et al.*, 1998) and only these are capable of dividing (Roszak and Calwell, 1987). It should also be noted that the experiments were conducted during daylight hours, which certainly led to an underestimate of the impact of organisms on bacteria, since the metazooplankton populations undertake vertical migrations and are usually more concentrated at the surface during the night (Lampert, 1989). Our results, in general, confirm studies conducted in the natural environment, which emphasize the importance of the bacterial grazing activity of Cladocera (Jürgens, 1994). *Daphnia longispina* and *Ceriodaphnia quadrangula*, which were of small average size in this ecosystem (720 ± 220 and 381 ± 78 μm, respectively), have a filter mesh size of <0.5 μm (Geller and Müller, 1981; Brendelberger and Geller, 1985) which makes them efficient at retaining small-sized particles (Jürgens, 1994; Toth and Kato, 1997) and suggests that they are effective consumers of bacteria (Peterson *et al.*, 1978; Geller and Müller, 1981; Pace *et al.*, 1983; Güde, 1988; Jürgens, 1994). These results are confirmed by grazing measurements which showed that *D.longispina* was the species with the highest bacterial consumption activity. The calculated filtration rate values (Table II) for this species are close to those measured by Børsheim and Andersen (1987) using

microspheres and by Kankaala (1988) using bacteria labelled with tritiated thymidine. *Ceriodaphnia quadrangula* was the main bacterial consumer in August. The filtration rate measured for this species (7–144 µl ind.$^{-1}$ h^{-1}; mean 76 µl ind.$^{-1}$ h^{-1}) was higher than that recorded by Bern (1987) with radioactively labelled bacteria, but the incubation temperature (14.0°C) was lower than in this study (22.5 ± 2.1°C). The filtration measured for *B.longirostris* (between 1 and 17 µl ind.$^{-1}$ h^{-1}; mean 9.0 µl ind.$^{-1}$ h^{-1}) was close to that found by Toth and Kato (1997) (13.4 ± 4.9 µl ind.$^{-1}$ h^{-1}), and was the lowest of all the species of Cladocera recorded in this study. This result confirms the many studies that have shown that Bosminidae usually have a low bacterial grazing activity (Bogdan and Gilbert, 1982, 1984; DeMott and Kerfoot, 1982; Schoenberg and Maccubin, 1985; Ross and Munawar, 1987; Gulati *et al*., 1991).

Few studies have been conducted on the consumption of bacteria by rotifers under natural conditions (Bogdan and Gilbert, 1982; Sanders *et al*., 1989; Ooms-Wilms, 1997). Heterotrophic bacteria could represent a food source for these organisms which are relatively non-specific micro filter feeders over a size range of between 0.5 and 2.0 µm (Pourriot, 1977; Rothhaupt, 1990), but rotifers only accounted for 2% of total predation on bacteria. This is in agreement with studies which have shown that their contribution to total bacterial consumption is generally <10% (Sanders *et al*., 1989; Pace *et al*., 1990; Pernie *et al*., 1990). Some of the species present in the lake (e.g. *Asplanchna priodonta*, *Polyarthra* sp. and *Synchaeta* sp.) never ingested the fluorescent particles and probably do not consume bacteria (Bogdan *et al*., 1980; Boon and Shiel, 1990; Ooms-Wilms *et al*., 1995). Other species, such as *Hexarthra mira* and *Conochilus* sp., had a similar bacterial consumption activity to that measured by Sanders *et al.* (1989) and Ooms-Wilms *et al*. (1995). In agreement with the studies of Brett *et al*. (1994) and Sanders *et al*. (1989), this study also showed no evidence of bacterial consumption by copepods, in contrast to that of Roff *et al.* (1995) in the marine environment.

Among the phagotrophic protists, pigmented flagellates were the most important consumers, followed by HNF and ciliates. Pigmented phagotrophic flagellates accounted for 32% of grazing on bacteria at 7 m. Among these organisms, *Cryptomonas ovata* and *Dinobryon cylindricum* showed a similar activity to that recorded by Tranvik *et al.* (1989) and Bird and Kalf (1986). The estimated filtration rate for *D.cylindricum* was similar to that recorded by Carrias *et al*. (1996), whereas that obtained for *C.ovata* (mean 0.7 × 10^{-3} µl ind.$^{-1}$ h^{-1}) was slightly higher than the value determined by Tranvik *et al.* (1989) using FLB in a humic mesotrophic lake (0.27–0.55 × 10^{-3} µl ind.$^{-1}$ h^{-1}).

The low density of ciliates encountered in the epilimnion of the Sep reservoir was similar to that reported from oligotrophic environments (e.g. Laybourn-Parry, 1994). The community was mainly dominated by a mixotrophic oligotrich *Pelagohalteria viridis*, which is frequently found in lake plankton (Carrias *et al*., 1996). On average, ciliates accounted for 15% of total bacterial consumption by protists (4% of total grazing). This result is similar to that reported for the eutrophic Lake Oglethorpe (Sanders *et al.*, 1989) and for the oligomesotrophic Lake Pavin (Carrias *et al.*, 1996). *Pelagohalteria viridis*, which contains symbiotic

algae (Foissner *et al.*, 1988), paradoxically had a higher mean ingestion rate than the heterotrophic oligotrich *Halteria* sp.

The structure of the HNF community was similar to that encountered in lakes of oligomesotrophic to mesotrophic trophic status (Salbrechter and Arndt, 1994; Mathes and Arndt, 1995; Carrias *et al.*, 1998). Among these microorganisms, only the *Monas*-like cells ingested microspheres, with a filtration rate ($0.4–3 \times 10^{-3}$ µl ind.$^{-1}$ h^{-1}) similar to that obtained by Sanders *et al.* (1989) in the eutrophic Lake Oglethorpe and close to that of Carrias *et al.* (1996) in the oligomesotrophic Lake Pavin. The ingestion rates that were measured for these HNF (0–14 bacteria ind.$^{-1}$ h^{-1}) were similar to those obtained by Bloem *et al.* (1989) in a eutrophic lake (2–17 bacteria ind.$^{-1}$ h^{-1}).

This study showed that metazoans, and particularly Cladocera, were partly responsible for controlling the abundance of bacteria in a newly flooded lake. However, predation by *Ceriodaphnia quadrangula* on bacteria in August paradoxically coincided with an increase in bacterial abundance. It seems likely that bacterial production, which is strongly temperature dependent (White *et al.*, 1991), exceeded predation at this time. At times of low chlorophyll *a* concentrations, bacteria could be an important food supplement for some metazoans (Pace *et al.*, 1983), but they are poorly assimilated relative to algae (Hessen *et al.*, 1989) and they lack the long-chain unsaturated fatty acids that may be crucial for zooplankton nutrition (Muller-Navarra, 1995).

Furthermore, as shown by Thouvenot *et al.* (1999) in field experiments in the same ecosystem, metazoans could exert a heavy predation on some protozoans, which would result in a reduction in the overall predation on bacteria by these organisms. This predation by metazoans could be exerted on ciliates of the size measured in this study, which was frequently <30 µm, and which correspond with metazooplanktonic nutritional requirements (Wiackowski *et al.*, 1994; Rabette *et al.*, 1998). The dominance of *Pelagohalteria viridis* could then be attributed to its ability to move by leaps (Tamar, 1979), enabling it to escape from predators and be one of the few ciliates that is capable of existing in the presence of Cladocera (Jürgens *et al.*, 1994). The inverse relationship ($P < 0.01$) between the biomass of copepods and rotifers in the water column and of PF in the euphotic zone suggests that there is a predator–prey relationship between these metazoans and pigmented flagellate protists.

Several facts also tend to indicate that HNF were subjected to heavy predation. Firstly, the density of heterotrophic flagellate protists ($0.5–3.6 \times 10^3$ cells ml^{-1}) was slightly lower than the values generally reported for oligotrophic or mesotrophic environments ($0.2–5.6 \times 10^3$ cells ml^{-1}) (Pick and Hamilton, 1994; Tzaras and Pick, 1994) and, secondly, there was a negative correlation ($P < 0.01$) between the abundance of the cladoceran *D.longispina* in the water column and these flagellates. The ratios of bacterial abundance to HNF abundance (7026:1 at 1 m and 3835:1 at 7 m) were greater than those recorded by Amblard *et al.* (1995) (2590:1) and Simek *et al.* (1995) (1000:1), and could be interpreted as the result of predation. The low bacterial consumption by HNF could also be explained by the structure of this community. *Kathablepharis ovalis* is a species that in fact prefers larger sized particles such as nanophytoplankton (Carrias *et al.*, 1996). In

addition, the chrysomonadine (2–5 μm), which was difficult to identify because of its small size (Finlay *et al.*, 1988) and which dominated the HNF community, never consumed bacteria and we were never able to detect autotrophic picoplankton in its cells. Osmotrophic activity of HNF (Sherr and Sherr, 1987; Marchant and Scott, 1993) could have been favoured by the high DOM concentrations that were greater than those usually found in lakes of this trophic level (Striquer-Soares and Chevolot, 1996). These findings could explain why protists, and particularly HNF, which are generally considered to be the main microorganisms capable of transferring picoplankton production towards higher trophic levels (Güde, 1986; Cole *et al*., 1988; Sherr and Sherr, 1988; Sanders *et al*., 1989; Carrias *et al*., 1996), only played a limited role in regulating the bacterial communities in this type of ecosystem.

The low abundance of phagotrophic protists in this newly flooded reservoir and the low proportion of HNF capable of consuming bacteria have therefore resulted in the metazoan zooplankton becoming the main consumers of bacteria. These results, combined with those of Thouvenot *et al.* (1999), demonstrate that these organisms have a high predation impact on bacteria, HNF and ciliates, thus confirming the hypothesis of Paterson *et al.* (1997), who suggested that some components of the microbial loop could be an important source of food for metazoans in a newly flooded reservoir. Nevertheless, the proportion of carbon coming from these organisms compared to that from strict autotrophs remains to be determined.

References

Amblard,C., Carrias,J.F., Bourdier,G. and Maurin,N. (1995) The microbial loop in a humic lake: seasonal and vertical variations in the structure of the different communities. *Hydrobiologia*, **300/301**, 71–84.

American Public Health Association (1992) *Standard Methods for the Examination of Water and Wastewater*, 18th edn. APHA, Washington, DC, 1134 pp.

Arndt,H. (1993) Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, cilates)—a review. *Hydrobiologia*, **255/256**, 231–246.

Bern,L. (1987) Zooplankton grazing on (methyl-3-H)thymidine labelled natural particles assemblages: determination of filtering rates and food selectivity. *Freshwater Biol.*, **17**, 151–159.

Bird,D.F. and Kalff,J. (1986) Bacterial grazing by planktonic algae. *Science*, **231**, 493–495.

Björnsen,K.Y., Larsen, J.P., Geertz-Hansen,O. and Olesen,M. (1986) A field technique for the determination of zooplankton grazing on natural bacterioplankton. *Freshwater Biol.*, **16**, 644–648.

Bloem,J., Bar-Gilissen,M.-J.B. and Carpenter,T.E. (1986) Fixation, counting, and manipulation of heterotrophic nanoflagellates. *Appl. Environ. Microbiol.*, **52**, 1266–1272.

Bloem,J., Ellenbroeck,M., Bar-Gilissen,M.-J.B. and Cappenberg,T.E. (1989) Protozoan grazing and bacterial production in stratified lake Vechten estimated with fluorescent labeled bacteria and thymidine incorporation. *Appl. Environ. Microbiol.*, **55**, 1787–1795.

Bogdan,K.G. and Gilbert,J.J. (1982) Seasonal patterns of feeding by natural population of *Keratella*, *Polyarthra* and *Bosmina*: clearance rates, selectivities, and contributions to community grazing. *Limnol. Oceanogr*., **27**, 918–934.

Bogdan,K.G. and Gilbert,J.J. (1984) Body size and food size in freshwater zooplankton. *Proc. Natl Acad. Sci. USA*, **81**, 6427–6431.

Bogdan,K.G., Gilbert,J.J. and Starkweather,P.L. (1980) *In situ* clearance rates of planktonic rotifers. In Dumont,H.J. and Green,J. (eds), *Rotatoria. Developments in Hydrobiology I*. Dr W.Junk, The Hague, pp. 73–77.

Boon,P.I. and Shiel,J.J. (1990) Grazing on bacteria by zooplankton in Australian billabongs. *Aust. J. Mar. Freshwater Res*., **41**, 247–257.

Börsheim,K.Y. and Andersen,S. (1987) Grazing and food selection by crustacean zooplankton compared to production of bacteria and phytoplankton in a shallow Norwegian mountain lake. *J. Plankton Res.*, **9**, 367–379.
Børsheim,K.Y. and Olsen,Y. (1984) Grazing activities by *Daphnia pulex* on natural populations of bacteria and algae. *Verh. Int. Ver. Limnol.*, **22**, 644–648.
Bottrell,H.H., Duncan,A., Gliwicz,Z.M., Grygierek,E., Herzig,A., Hillbricht-Ilkowska,A., Kurasawa,H., Larsson,P. and Weglenda,T. (1976) A review of some problems in zooplankton production studies. *Norw. J. Zool.*, **5**, 419–456.
Brendelberger,H. and Geller,W. (1985) Variability in eight *Daphnia* species: mesh sizes and filtering areas. *J. Plankton Res.*, **7**, 473–487.
Brett,M.T., Wiackowski,K., Lubnow,F.S., Mueller-Solger,A., Elser,J.J. and Goldman,C.R. (1994) Species-dependent effects of zooplankton on planktonic ecosystem processes in Castle Lake, California. *Ecology*, **75**, 2255–2263.
Burney,C.M. and Sieburth,J.McN. (1977) Dissolved carbohydrates in sea water. II, a spectrophotometric procedure for total carbohydrate analysis and polysaccharide estimation. *Mar. Chem.*, **5**, 15–28.
Caron,D.A. (1983) Technique for enumeration of heterotrophic and phototrophic nanoplankton, using epifluorescence microscopy, and comparison with other procedures. *Appl. Environ. Microbiol.*, **46**, 491–498.
Carrias,J.F., Amblard,C. and Bourdier,G. (1996) Protistan bacterivory in an oligomesotrophic lake: importance of attached ciliates and flagellates. *Microb. Ecol.*, **31**, 249–268.
Carrias,J.F., Amblard,C., Quiblier-LLoberas,C. and Bourdier,G. (1998) Seasonal dynamics of free living and attached heterotrophic nanoflagellates in an oligotrophic lake. *Freshwater Biol.*, **39**, 91–101.
Cho,B.C. and Azam,F. (1988) Major role of bacteria in biogeochemical fluxes in the ocean's interior. *Nature*, **333**, 441–443.
Cole,J.J., Findlay,S. and Pace,M.L. (1988) Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar. Ecol. Prog. Ser.*, **43**, 1–10.
DeMott,W.R. (1986) The role of taste food selection by freshwater zooplankton. *Oecologia (Berlin)*, **69**, 334–340.
DeMott,W.R. (1988) Discrimination between algae and artificial particules by freshwater and marine copepods. *Limnol. Oceanogr.*, **33**, 397–408.
DeMott,W.R. and Kerfoot,W.C. (1982) Competition among cladocerans: nature of interaction between *Bosmina* and *Daphnia*. *Ecology*, **63**, 1949–1966.
Fenchel,T. (1982) Ecology of heterotrophic microflagellates. II. Bioenergetics and growth. *Mar. Ecol. Prog. Ser.*, **8**, 225–231.
Finlay,B.J., Clarke,K.J., Cowling,A.J., Hindle,R.M. and Rogerson,A. (1988) On the abundance and distribution of protozoa and their food in a productive freshwater pond. *Eur. J. Protistol.*, **23**, 205–217.
Foissner,V.W. (1994) Progress in taxonomy of planktonic freshwater ciliates. *Mar. Microb. Food Webs*, **8**, 9–35.
Foissner,V.W., Skogstad,A. and Pratt,J.R. (1988) Morphology and infraciliature of *Trochiliopsis australis* n.sp., *Pelagohalteria viridis* (Fromentel, 1876) n.g., comb., *Strobilidium lacustris* n.sp. (Protozoa: Ciliophora) from stagnant waters. *J. Protozool.*, **35**, 489–497.
Geller,W. and Müller,H. (1981) The filtration apparatus of cladocera: filter mesh-sizes and their implications on food selectivity. *Oecologia (Berlin)*, **49**, 316–321.
Gonzales,J.M., Sherr,E.B. and Sherr,B.F. (1993) Differential feeding by marine flagellates on growing vs. starving, and on motile vs. non-motile, bacterial prey. *Mar. Ecol. Prog. Ser.*, **102**, 257–267.
Güde,H. (1986) Loss processes influencing growth of planktonic bacterial populations in lake Constance. *J. Plankton Res.*, **8**, 795–810.
Güde,H. (1988) Direct and indirect influences of crustacean zooplankton on bacterioplankton of Lake Constance. *Hydrobiologia*, **159**, 63–73.
Gulati,R.D., Vuik,C., Sierwertsen,K. and Postema,G. (1991) Clearance rates of *Bosmina* species in response to changes in trophy and food concentration. *Verh. Int. Ver. Limnol.*, **24**, 745–750.
Hessen,D.O., Andersen,T. and Lyche,A. (1989) Differential grazing and ressource utilization of zooplankton in a humic lake. *Arch. Hydrobiol.*, **114**, 321–347.
Johnson,K.M. and Sieburth,J.McN. (1977) Dissolved carbohydrate in seawater. I, a precise spectrophotometric analysis for monosaccharides. *Mar. Chem.*, **5**, 1–13.
Jones,R.I. and Rees,S. (1994) Characteristics of particle uptake by the phagotrophic phytoflagellate, *Dinobryon divergens*. *Mar. Microb. Food Webs*, **8**, 97–110.
Jugnia,L.B., Richardot,M., Tadonléké,R.C., Sime-Ngando,T., Debroas,D. and Dévaux,J. (1998)

Variations saisonnières des bactéries actives dans la couche euphotique d'un réservoir récemment mis en eau: la retenue de la Sep, Puy de Dome, France. A.F.L., April 1998, Chambéry, France.
Jürgens,K. (1994) Impact of *Daphnia* on planktonic microbial food webs. A review. *Mar. Microb. Food Webs*, **8**, 295–324.
Jürgens,K., Arndt,H. and Rothaupt,K.O. (1994) Zooplankton-mediated changes of bacterial community structure. *Microb. Ecol.*, **27**, 27–42.
Kahl,A. (1930–1935) Urtiere order Protozoa 1: Wimpertier oder Cilata (Infusoria), eine bearbeitung der freibenden und ectocommensalen Infusorien der Erde, unter Ausschluss der marinen Tintinnidae. In Dahl,F. (ed.), *Die Tierwelt Deutschlands.* G.Fisher, Jena, Parts 18 (year 1930), 21 (1931), 25(1932), 30 (1935), 886 pp.
Kankaala,P. (1988) The relative importance of algae and bacteria as food for *Daphnia longispina* (Cladocera) in a polyhumic lake. *Freshwater Biol.*, **19**, 285–296.
Kerfoot,W.C. and Kirk,K.L. (1991) Degree of taste discrimination among suspension-feeding cladocerans and copepods. Implication for detritivory and herbivory. *Limnol. Oceanogr.*, **36**, 1107–1123.
Kudo,R. (1966) *Protozoology*. Charles C.Thomas, Springfield, IL.
Lampert,W. (1987) Feeding and nutrition in *Daphnia*. *Mem. Ist. Ital. Idrobiol.*, **45**, 143–192.
Lampert,W. (1989) The adaptative significance of diel vertical migration of zooplankton. *Funct. Ecol.*, **3**, 21–27.
Laybourn-Parry,J. (1994) Seasonal successions of protozooplankton in freshwater ecosystems of different latitudes. *Mar. Microb. Food Webs*, **159**, 99–110.
Lorenzen,C.J. (1967) Determination of chlorophyll *b* in ocean. *Deep-Sea Res.*, **28**, 265–275.
MacManus,G.B. and Fürhman,J.A. (1988) Clearance of bacteria-sized particles by natural populations of nanoplankton in the Chesapeake Bay outflow plume. *Mar. Ecol. Prog. Ser.*, **42**, 199–206.
MacManus,G.B. and Okubo,A. (1991) On the use of surrogate food particles to measure protistan ingestion. *Limnol. Oceanogr.*, **36**, 219–222.
Marchant,H.J. and Scott,F.J. (1993) Uptake of submicrometer particles and dissolved organic material by antartic choanoflagellates. *Mar. Ecol. Prog. Ser.*, **92**, 59–64.
Mathes,J. and Arndt,H. (1995) Annual cycle of protozooplankton (ciliates, flagellates and sarcodines) in relation to phyto- and metazooplankton in Lake Neumühler See (Mecklenburg, Germany). *Arch. Hydrobiol.*, **134**, 337–358.
Monger,B.C. and Landry,M.R. (1992) Size-selective grazing by heterotrophic nanoflagellates: an analysis using live-bacteria and dual-beam flow cytometry. *Arch. Hydrobiol. Beih.*, **37**, 173–185.
Muller-Navarra,D.C. (1995) Evidence that highly unsaturated fatty acids limits *Daphnia* growth in nature. *Arch. Hydrobiol.*, **132**, 297–307.
Ooms-Wilms,A.L. (1991) Ingestion of fluorescently labelled bacteria by rotifers and cladocerans in Lake Loosdrecht as measures of bacterivory: preliminary results. *Mem. Ist. Ital. Idrobiol.*, **48**, 269–278.
Ooms-Wilms,A.L. (1997) Are bacteria important food source for rotifers in eutrophic lakes? *J. Plankton Res.*, **19**, 1125–1141.
Ooms-Wilms,A.L., Postema,G. and Gulati,R.D. (1995) Evaluation of bacterivory of rotifera based on measurement of *in situ* ingestion of fluorescent particles, including some comparisons with Cladocera. *J. Plankton Res.*, **17**, 1057–1077.
Pace,M.L. (1988) Bacterial mortality and the fate of bacterial production. *Hydrobiologia*, **159**, 41–49.
Pace,M.L. and Bailif,M.D. (1987) Evaluation of fluorescent microsphere technique for measuring grazing rates of phagotrophic microorganisms. *Mar. Ecol. Prog. Ser.*, **40**, 185–193.
Pace,M.L., Porter,K.G. and Feig,Y.S. (1983) Species- and age-specific differences in bacterial resource utilization by two co-occurring cladocerans. *Ecology*, **64**, 1145–1156.
Pace,M.L., McManus,G.B. and Findlay,S.E.G. (1990) Planktonic community structure determines the fates of bacterial production in a temperate lake. *Limnol. Oceanogr.*, **35**, 795–808.
Paterson,M.J., Findlay,D., Beaty,K., Findlay,W., Schindler,E.U., Stainton,M. and McCullough,G. (1997) Changes in the planktonic food web of a new experimental reservoir. *Can. J. Aquat. Sci.*, **54**, 1088–1102.
Pedros-Alio,M.L. and Brock,T.D. (1983) The impact of zooplankton feeding on the epilimnic bacteria of an eutrophic lake. *Freshwater Biol.*, **13**, 227–239.
Pernie,G.L., Scavia,D., Pace,M.L. and Carrick,H.J. (1990) Micrograzer impact and substrate limitation of bacterioplankton in Lake Michigan. *Can. J. Fish. Aquat. Sci.*, **47**, 1836–1841.
Peterson,B.J., Hobbie,J.E. and Haney,J.F. (1978) Daphnia grazing on natural bacteria. *Limnol. Oceanogr.*, **23**, 1039–1044.
Pick,F.R. and Hamilton,P.B. (1994) A comparison of seasonal and vertical patterns of phagotrophic flagellates in relation to bacteria and algal biomass in temperate lake. *Mar. Microb. Food Webs*, **8**, 201–215.

Porter,K.J. and Feig,Y.S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr*., **34**, 943–948.
Pourriot,R. (1977) Food and feeding habits of Rotifera. *Arch. Beih. Ergebn. Limnol*., **8**, 243–260.
Prepas,E. (1978) Sugar-frosted *Daphnia*: an improved fixation technique for Cladocera. *Limnol. Oceanogr*., **23**, 557–559.
Proctor,L.M. and Führman,J.A. (1992) Mortality of marine bacteria in response to enrichment of the virus size from seawater. *Mar. Ecol. Prog. Ser*., **87**, 283–293.
Rabette,C., Thouvenot,A. and Lair,N. (1998) Laboratory experiments on trophic relationship and remote detection between two ciliates and *Cyclops vicinus vicinus*. *Hydrobiologia*, **373/374**, 157–167.
Ricci,C. (1984) Culturing of some bdelloid rotifers. *Hydrobiologia*, **112**, 45–51.
Riemann,B. (1985) Potential importance of fish predation and zooplankton grazing on natural populations of freshwater bacteria. *Appl. Environ. Microb.*, **50**, 187–193.
Roff,J.C., Turner,T.T., Webber,M.K. and Hopcroft,R.R. (1995) Bacterivory by tropical copepod nauplii: extend possible significance. *Aquat. Microb. Ecol.*, **9**, 165–175.
Ross,P.E. and Munawar,M. (1987) Zooplankton feeding rates at offshore stations in the North American Great Lakes. *Arch. Hydrobiol. Ergebn. Limnol*., **25**, 157–164.
Roszak,D.B. and Calwell, R.R. (1987) Survival strategies of bacteria in the natural environment. *Microb. Rev*., **51**, 365–379.
Rothhaupt,K.O. (1990) Changes of the functional responses of the rotifers *Brachionus rubens* and *Brachionus calyciflorus* with particle sizes. *Limnol. Oceanogr*., **35**, 16–23.
Salbrechter,M. and Arndt,H. (1994) The annual cycle of protozooplankton in the alpine, mesotrophic lakes. *Mar. Microb. Food Webs*, **8**, 217–234.
Sanders,R.W., Porter,K.G., Bennett,S.J. and Debiase,A.E. (1989) Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. *Limnol. Oceanogr*., **34**, 673–687.
Schoenberg,S.A. and Maccubin,A.E. (1985) Relative feeding rates on free and particle bound bacteria by freshwater macrozooplankton. *Limnol. Oceanogr*., **30**, 1084–1090.
Sherr,E.B. and Sherr,B.F. (1988) Roles of microbes in pelagic food webs: a revised concept. *Limnol. Oceanogr*., **33**, 1225–1227.
Sherr,B.F., Sherr,E.B. and Fallon,R.D. (1987) Use of monodispersed, fluorescently-labeled bacteria to estimate *in situ* protozoan bacterivory. *Appl. Environ. Microbiol.*, **53**, 958–965.
Sherr,E.B., Rassoulzadegan,F. and Sherr,B.F. (1989) Bacterivory by pelagic choreotrichous ciliates in coastal waters of the NW Mediterranean Sea. *Mar. Ecol. Prog. Ser*., **55**, 235–240.
Simek,K. and Straskrabbova,V. (1992) Bacterioplankton production and protozoan bacterivory in a mesotrophic reservoir. *J. Plankton Res*., **14**, 773–787.
Simek,K., Bobkova,J., Macek,M., Nemoda,J. and Psener,R. (1995) Ciliates grazing on picoplankton in a eutrophic reservoir during summer phytoplankton maximum: A study at the species and community level. *Limnol. Oceanogr*., **40**, 1077–1090.
Sime-Ngando,T. and Grolière,C. (1991) Effet quantitatif des fixateurs sur la conservation des ciliés planctoniques d'eau douce. *Arch. Protistenkd.*, **140**, 109–120.
Simon,M., Cho,B.C. and Azam,F. (1992) Significance of bacterial biomass in lakes and the ocean: comparaison to phytoplankton biomass and biogeochemical implications. *Mar. Ecol. Prog. Ser*., **86**, 103–110.
Strickland,J.D.H. and Parsons,T.R. (1968) *A Practical Handbook for Sea Water Analysis*. *Fish. Res. Board Can. Bull*., pp. 167–311.
Striquer-Soares,F. and Chevolot,L. (1996) Particulate and dissolved carbohydrates and proteins in Lobo reservoir (Sao Paulo State, Brazil): relationships with phytoplankton. *J. Plankton Res*., **18**, 521–537.
Tamar,H. (1979) The movements of jumping ciliates. *Arch. Protistenkd*., **122**, 290–327.
Thouvenot,A., Richardot,M., Debroas,D. and Dévaux,J. (1999) Impact of natural metazooplankton assemblage on planktonic microbial communities in a newly flooded reservoir. *J. Plankton Res.*, **21**, 179–199.
Toth,G.L. (1980) The use of dialyzing sacks in estimation of production of bacterioplankton and phytoplankton. *Arch. Hydrobiol*., **81**, 474–482.
Toth,L.G. and Kato,K. (1997) Size selective grazing of bacteria by *Bosmina longirostris*—an image-analysis study. *J. Plankton Res*., **19**, 1477–1493.
Tranvik,L.J., Porter,K.G. and Sieburth,J.McN. (1989) Occurrence of bacterivory in Cryptomonas, a common freshwater phytoplankter. *Oecologia (Berlin)*, **78**, 473–476.
Turner,J.T. and Tester,P.A. (1992) Zooplankton feeding ecology: bacterivory by metazoan microzooplankton. *J. Exp. Mar. Biol. Ecol*., **160**, 149–167.

Tzaras,A. and Pick,F.R. (1994) The relationship between bacterial and heterotrophic flagellate abundance in oligotrophic to mesotrophic temperate lakes. *Mar. Microb. Food Webs*, **8**, 347–355.
Utermöhl,H. (1958) Zur Vervollkommung der quantitativen Phytoplankton. *Meth. Mitt. Int. Ver. Limnol.*, **9**, 1–38.
Vaqué,D. and Pace,M.L. (1992) Grazing on bacteria by flagellates and cladocerans in lakes of contrasting food-web structure. *J. Plankton Res.*, **14**, 307–321.
Wiackowski,K., Brett,M.T. and Goldman,C.R. (1994) Differential effects of zooplankton species on ciliate community structure. *Limnol. Oceanogr.*, **39**, 486–492.
White,P.A., Kalff,J., Rasmussen,J.B. and Gasol,J.M. (1991) The effect of temperature and algal biomass on bacterial production and specific growth rate in freshwater and marine habitats. *Microb. Ecol.*, **21**, 99–118.
Wiedner,C. and Vareshi,E. (1995) Evaluation of a fluorescent microparticle technique for measuring filtering rates of *Daphnia*. *Hydrobiologia*, **302**, 89–96.

Received on November 1, 1998; accepted on May 7, 1999