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Impact of natural metazooplankton assemblage on planktonic microbial communities in a newly flooded reservoir

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Abstract. The grazing impact of a natural assemblage of metazoan zooplankton on pigmented flagellates (PF), heterotrophic nanoflagellates (HNF), ciliates, and non-flagellate algae and microcyanobacteria (NFAM) was measured *in situ* during the period of thermal stratification in a newly flooded reservoir (Reservoir de la Sep, France). Experiments were conducted with diffusion chambers in the meta- and epilimnion over a period of 7 h. The mean mortalities of PF in the epi- and metalimnion (0.08 ± 0.02 and 0.06 ± 0.03 h^{-1}, respectively), of HNF (0.04 ± 0.02 and 0.05 ± 0.02 h^{-1}) and ciliates (0.09 ± 0.04 and 0.11 ± 0.04 h^{-1}) demonstrate the impact of the metazoan zooplankton, and particularly of the rotifer *Asplanchna priodonta*, on the components of the microbial loop. The mortality of NFAM, accounting for 14 and 18% of total mortality, remained low throughout the study. The taxa with the highest mortality were pigmented flagellates of 4–19 μm, HNF, and small-sized ciliates such as *Halteria* sp. (0.10 ± 0.02 h^{-1} at 1 m and 0.30 ± 0.38 h^{-1} at 7 m) and *Urotricha furcata* (0.11 ± 0.05 h^{-1} at 1 m and 0.12 ± 0.06 h^{-1} at 7 m). Large-sized ciliates (*Paradileptus elephantinus*) and sessile ciliates (Suctorida, *Vorticella* sp.) had a very low mortality (<0.04 h^{-1}). After reservoir flooding, the organisms in the microbial trophic loop, favoured by the high quantities of allochthonous organic matter, are subject to a higher mortality than the phytoplankton.

Introduction

A large part of the matter and energy flow in pelagic ecosystems does not follow the linear trophic pathway phytoplankton → zooplankton, but transits by a microbial food web known as the 'microbial loop' (Azam *et al.*, 1983; Porter *et al.*, 1985; Sanders *et al.*, 1989). In recent years, an increasing number of studies have underlined the importance of flagellate and ciliate protozoans in this trophic web. These, and particularly flagellates, are involved in controlling bacterial communities (Beaver and Crisman, 1989; Pace *et al.*, 1990; Sanders *et al.*, 1992; Riemann and Christoffersen, 1993) and play a key role in recycling nutrients accumulated by bacteria (Caron, 1991). Although the role of protozoans in predation on picoplanktonic cells has been studied in lake and marine environments, little is as yet known on the impact of predation by metazoan plankton on Protozoa. Protozoans do, however, seem to be the main micro-organisms 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). With the exception of some Cladocera (Jürgens, 1994) and Rotifera (Arndt, 1993; Ooms-Wilms *et al.*, 1995; Ooms-Wilms, 1997), the metazoan zooplankton are generally incapable of consuming cells of picoplanktonic size (Sanders and Wickham, 1993), but they can however exert a strong grazing pressure on Protozoa (Arndt and Nixdorf, 1991; Sanders and Wickham, 1993). Knowledge of the relationships between protozoans and metazoan zooplankton is essential for understanding the energy and material flows in aquatic food webs. For example, various authors have stressed the importance of predation on protozoans by calanoid copepods in the

marine (Jonsson and Tisselius, 1990; Stoecker and McDowell-Capuzzo, 1990) and lacustrine environments (Burns and Gilbert, 1993; Hartmann *et al.*, 1993; Carrias *et al.*, 1998) and of cyclopoids (Brett *et al.*, 1994; Wiackowski *et al.*, 1994; Dobberfuhl *et al.*, 1997; Rabette *et al.*, 1998). Cladocera, and particularly Daphniidae, have been the subject of many studies relating to their role in predation on organisms belonging to the microbial loop in lake environments (reviewed by Jürgens, 1994). On the other hand, few authors have taken an interest in predation by rotifers on protozoans (reviewed by Arndt, 1993).

Moreover, most of these studies have been conducted under experimental conditions, or only concern a single group of organisms among the metazoan zooplankton and planktonic protists (Atkinson, 1996; Jürgens *et al.*, 1996; Jack and Gilbert, 1997; Pérez *et al.*, 1997). There have been very few studies on seasonal changes in the impact of the entire metazoan zooplankton assemblage on protists (Kleppel *et al.*, 1988; Carrick *et al.*, 1991; Uitto, 1996; Paffenhöfer, 1998).

The aim of this study was, therefore, to measure the impact of the natural zooplankton assemblage on the main components of the microbial loop (pigmented and heterotrophic flagellates and ciliates) and on non-flagellate micro- and nanoalgae and microcyanobacteria, during the period of thermal stratification. The study was conducted on a recently flooded reservoir, where, as suggested by Paterson *et al.* (1997), the microbial trophic network would tend to be favoured compared to the linear trophic pathway of phytoplankton → zooplankton.

Method

The Sep Reservoir, lying at an altitude of 500 m in the Massif Central of France (46°2′N and 3°1′E), was built to irrigate croplands. With an area of 33 ha and a mean depth of 14 m, this reservoir has a catchment of 27 km^2 whose vegetation consists of oak and beech forests and grasslands. After being partially flooded in 1995, the reservoir was completely emptied in September. Sampling and experiments were conducted in 1996, at fortnightly intervals, during the period of thermal stratification from May until the reservoir was emptied in September, at the deepest point in the lake (35 m when it is full of water). Experiments were conducted at depths of 1 m (epilimnion) and 7 m (metalimnion).

Abiotic and biotic variables

Water temperature, dissolved oxygen and pH were measured with a multiparameter probe (YSI GRANT 3800). The water transparency was estimated by measuring the Secchi disc depth. Phosphorus (PO_4-P), nitrates (NO_3-N) and ammonium (NH_4-N) were analysed in water samples using standard methods (American Public Health Association, 1992). The chlorophyll *a* content was determined by spectrophotometry (Lorenzen, 1967; Strickland and Parsons, 1968). The dissolved organic matter (DOM) concentration was determined after filtration through a 0.2 μm polycarbonate membrane. Dissolved proteins

(DPROT) were measured using the 'micro BCA Protein Assay Reagent' Kit (Pierce) with bovine serum albumin (BSA) as a standard. The total dissolved carbohydrate (TDCHO) concentration was measured after acid hydrolysis (1 N HCl; 100°C, 15 h) according to Burney and Sieburth's (1977) and Johnson and Sieburth's (1977) method.

Measuring the impact of the metazoan zooplankton on protists

These measurements were carried out in box diffusion chambers of dimensions 17 × 17 × 17 cm, made of plexiglass 6 mm thick and having a volume of 4.9 l. Each face was equipped with a nylon mesh of 5 μm pore size, accounting for 42.5% of the total surface area of the cube. Openings on two of the faces, fitted with stoppers, were used to fill and empty the chambers. Measurements of the nitrogen and phosphorus concentrations (NH_4-N, NO_3-N, NO_2-N and PO_4-P), and of temperature, pH and oxygen, showed that the water quality in the diffusion chambers was the same as that of the natural environment after 7 h incubation, which was the chosen duration for these experiments (e.g. Hartmann, 1991; Moncef *et al*., 1994).

A 32 l volume of water was collected from the epi- and metalimnion, using a 10 l Van Dorn type bottle, and was mixed in a container before being used to fill the diffusion chambers. This type of sampling can lead to an underestimate of copepods. At each depth, two diffusion chambers were filled with untreated water and two with water filtered through a 100 μm pore size mesh which in preliminary tests was shown to remove almost all of the metazoan zooplankton (controls). The abundance of flagellates, ciliates, and non-flagellate algae and microcyanobacteria (NFAM) was determined at $t = 0$ and $t = 7$ h in each diffusion chamber. The bacterial abundance was determined at the start of each experiment on a sample of untreated water.

Sample preservation

Heterotrophic bacteria were fixed in formaldehyde solution (final concentration 2%). Flagellates were preserved in a solution of glutaraldehyde (final concentration 1%) (Bloem *et al*., 1986). Ciliate protozoans were fixed in mercuric chloride solution (final concentration 2.5%) (Sime-Ngando and Grolière, 1991) and NFAM in Lugol's iodine (Bourrelly, 1966). The metazoan zooplankton remaining in the diffusion chambers at the end of the incubation period were collected on a 100 μm sieve and then fixed in a sucrose/formaldehyde solution (final concentration 4%) to prevent the release of eggs and physical deformation (Prepas, 1978). The samples were fixed immediately after collection and stored at 4°C.

Counting organisms

Heterotrophic bacteria were stained with DAPI (1 μg l^{-1}), then filtered onto black polycarbonate membranes of 0.2 μm pore size (Millipore) using the protocol

described by Porter and Feig (1980). Flagellates were first stained with primulin (final concentration 200 µg ml^{-1}) (Caron, 1983) and were then collected onto black polycarbonate filters of 0.8 µm pore diameter (Nuclepore). Both preparations were made 24 h after sampling and stored at –25°C to minimize autofluorescence losses (Bloem *et al.*, 1986). Counts were then conducted using an Olympus HBS epifluorescence microscope equipped with an HB2-RFL epifluorescence illuminator, an HBO-100W mercury vapour lamp and a neofluar 100/1.25 objective lens. Two types of filter were used: UG-1, DM 400, L 435 (UV light) for heterotrophic nanoflagellates and heterotrophic bacteria, and BP 545, O 590 (blue light) for pigmented nanoflagellates. During a series of preliminary experiments, triplicate counts were conducted on bacteria and flagellates (×1250 magnification) and ciliates (×500 magnification). For bacteria, the coefficient of variation (CV) was <5% when counting 500–800 bacteria (30–60 fields) using a micrometer eyepiece that defined the extent of the field. The CV was 6% for pigmented flagellates (PF) and 9% for heterotrophic nanoflagellates (HNF) after counting 200–300 cells, and 12% after counting 100–200 ciliates. Ciliates, large-sized and/or colonial flagellates and NFAM were counted using Utermöhl's method (1958) with a Leitz type inverted microscope (Wild M40). The total area of each counting chamber was examined at ×500 magnification for ciliates within 2 months of fixing the samples, as recommended by Sime-Ngando and Grolière (1991). Pigmented flagellates were identified to genus or species. Heterotrophic flagellates were classified into two size classes (2–5 and 5–10 µm). Ciliates were identified to genus or species. The metazoan zooplankton were counted under a binocular microscope (Wild M3 Z) in a Dolfuss chamber. The dry weight of each taxon was calculated using the formulae of Bottrell *et al.* (1976). Triplicate experiments were conducted at each change in the composition of the metazooplankton (in May, June and July) in order to determine the variability in counts between the diffusion chambers containing zooplankton (untreated water). We obtained coefficients of variation of 7% for the biomass of calanoids, of 9% for cyclopoids, 5% for rotifers and 8% for cladocerans.

Data processing

Differences in the net growth rates of flagellates, ciliates, and non-flagellated algae and microcyanobacteria in treatments with and without zooplankton reflect mortality due to zooplankton as follows:

$$a_{wz} - d_i = (\ln Nwz_t - \ln Nwz_0)/t = r_{wz} \quad (1)$$

$$a_z - (d_i + d_z) = (\ln Nz_t - \ln Nzt_0)/t = r_z \quad (2)$$

a is the growth rate with (z) and without (wz) zooplankton. We assume that the loss of organisms <5 µm by passing through the membrane was the same whether zooplankton were present or absent, and that a_{wz} and a_z were equal. r is the exponential growth rate with (z) and without (wz) zooplankton. d_i is the incidental mortality rate (mortality not due to zooplankton), d_z is the mortality due to

zooplankton and t is the experimental time. N_t and N_0 are the abundance (Nz_t, Nzt_0 in the enclosure with zooplankton, and Nwz_t, Nwz_0 in that without) at the beginning and at the end of the experiment. The differences between the treatment with and without zooplankton reduce to:

$$d_z = r_{wz} - r_z \tag{3}$$

The experiments were run in duplicate, the reported mortalities are the means of the two replicates.

For all experiments, we used one-tailed *t*-tests to determine whether the growth of flagellates, ciliates and phytoplankton was greater in treatments without zooplankton relative to treatments with zooplankton.

Results

Abiotic and biotic variables

The oxygen and temperature profiles were measured at every metre. The values shown are those of the depths at which the experiments were conducted.

The mean temperature (Figure 1) in the epilimnion was 18.6 ± 3.2°C, the maxima being 13.7 and 23.6°C. The mean temperature in the metalimnion was 14.0 ± 3.5°C. The dissolved oxygen concentration at 1 m depth reached a peak value of 11.6 mg l^{-1} on 31 July and then fell to 5.2 mg l^{-1} on 10 September. At 7 m, the oxygen content decreased from the start of measurements to reach 1.10 mg l^{-1} on 3 July. It then increased again from 31 July to reach 4.7 mg l^{-1} on 10 September. For the studied period, the mean Secchi depth was 2.4 ± 0.8 m. The mean nitrate, ammonium and orthophosphate concentrations were 1.86 mg N l^{-1} (0.91–3.95 mg N l^{-1}), 0.05 mg N l^{-1} (0.01–0.82 mg N l^{-1}) and 0.01 mg P l^{-1} (0.001–0.068 mg P l^{-1}), respectively. The mean chlorophyll *a* concentration was 5.6 µg l^{-1} and fluctuated during the study period from 0.9 to 13.4 µg l^{-1}. The DPROT content varied from 3.5 to 7.5 mg l^{-1} at 1 m, and between 3.0 and 8.3 mg l^{-1} at 7 m. The mean TDCHO concentrations were 3.3 (1.1–5.4) mg l^{-1} at 1 m and 2.6 (0.7 –5.6) mg l^{-1} at 7 m.

Seasonal dynamics and composition of the different planktonic communities

The densities correspond to the mean abundance of organisms collected in the water that was used to fill the two diffusion chambers containing untreated water at each depth.

Bacteria. The mean bacterial abundance in the epilimnion was higher in summer than in spring (Figure 2), the respective means being 6.2 ± 1.6 × 10^6 and 2.4 ± 0.6 × 10^6 bacteria ml^{-1}. The mean density in the metalimnion was almost constant until 14 August at 2.5 ± 0.6 × 10^6 bacteria ml^{-1} and then increased to reach a peak value of 7.2 × 10^6 bacteria ml^{-1} on 28 August.

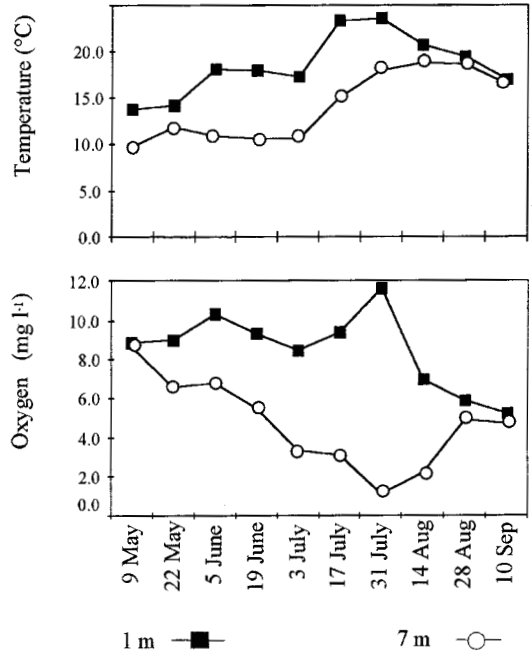

Fig. 1. Temporal changes in dissolved oxygen (mg l^{-1}) and temperature (°C) in the epilimnion and metalimnion.

Flagellates. The total abundance of pigmented flagellates at 1 m (Figures 2 and 3) varied between 0.2 and 15.0 × 10^3 cells ml^{-1} (mean = 4.4 ± 4.9 × 10^3 cells ml^{-1}). At 7 m, the density was between 0.1 and 3.7 × 10^3 cells ml^{-1} (mean = 1.0 ± 1.0 × 10^3 cells ml^{-1}). *Dinobryon* sp., a colonial pigmented flagellate, accounted for most of the community from the start of the study until 22 May at 1 m and until 5 June at 7 m. *Chrysidalis* sp. (4.5 µm) then largely dominated the pigmented flagellate community (4–19 µm) at 1 m until 17 July and then at 7 m from 3 July onwards. From 31 July at 1 m and from 14 August at 7 m, two small-sized pigmented flagellates, *Cryptomonas* sp. 2 and *Rhodomonas minuta*, were most abundant.

The abundance of heterotrophic flagellates (Figures 2 and 3) varied from 0.04 to 0.74 × 10^3 cells ml^{-1} at 1 m (mean = 0.26 ± 0.23 × 10^3 cells ml^{-1}), accounting for 9 ± 11% of total flagellates. At 7 m, the cell density varied from 0.02 to 1.07 × 10^3 cells ml^{-1} (mean = 0.31 ± 0.32 × 10^3 cells ml^{-1}) and they accounted for 20 ± 12% of total abundance. From mid-July, their concentration decreased strongly at both depths to a mean of 0.23 ± 0.12 × 10^3 cells ml^{-1} at 1 m and 0.26 ± 0.06 × 10^3 cells ml^{-1} at 7 m.

Non-flagellate nano- and microalgae and microcyanobacteria (NFAM). A first period of growth was recorded in the epilimnion at the start of the study (Figures 2 and 3), with 1058 × 10^3 cells l^{-1}. The assemblage then consisted mainly of one colonial species, *Sphaerocystis schroeteri*. During the period from 5 June to 14

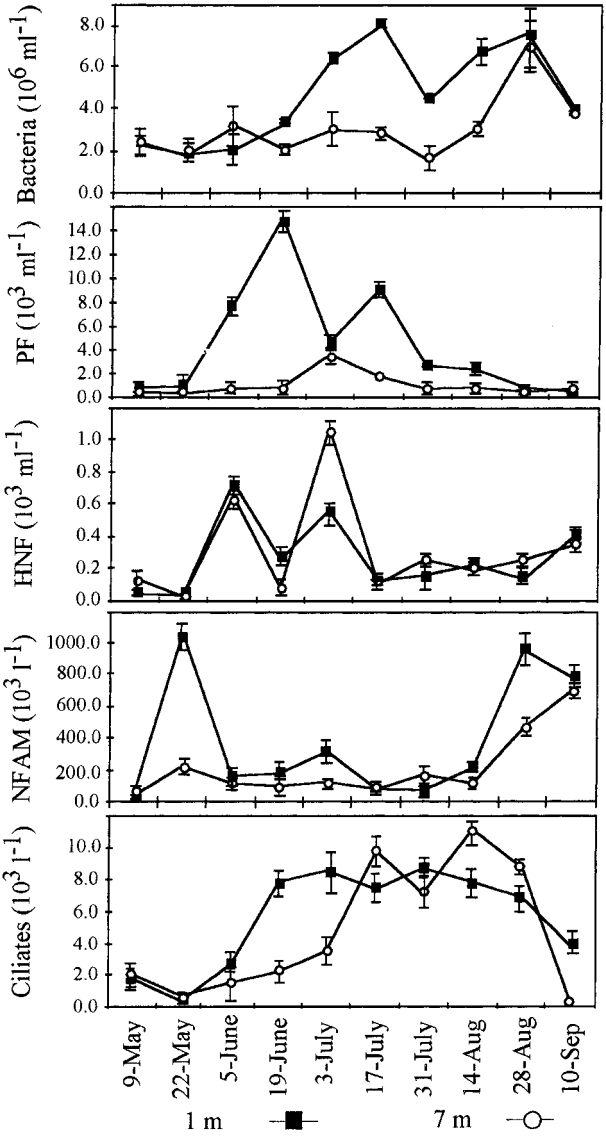

Fig. 2. Abundances of pigmented flagellates (PF), heterotrophic flagellates (HNF), non-flagellate algae and microcyanobacteria (NFAM), ciliates and bacteria at 1 and 7 m depths measured in the diffusion chambers.

August, the abundance of NFAM was low, with a mean of 168 ± 92 × 10^3 cells l^{-1}. Bacillariophyceae dominated the community in June with large-sized species such as *Fragilaria crotonensis* and *Synedra acus*, these being supplanted on 31 July and 14 August by Euchlorophyceae, *Botryococcus braunii* and *Scenedesmus opoliensis*. A second phase of algal growth started on 28 August, with 953 × 10^3 cells l^{-1}, consisting of small-sized species such as *Cyclotella* sp. and *Chlorocloster* sp.

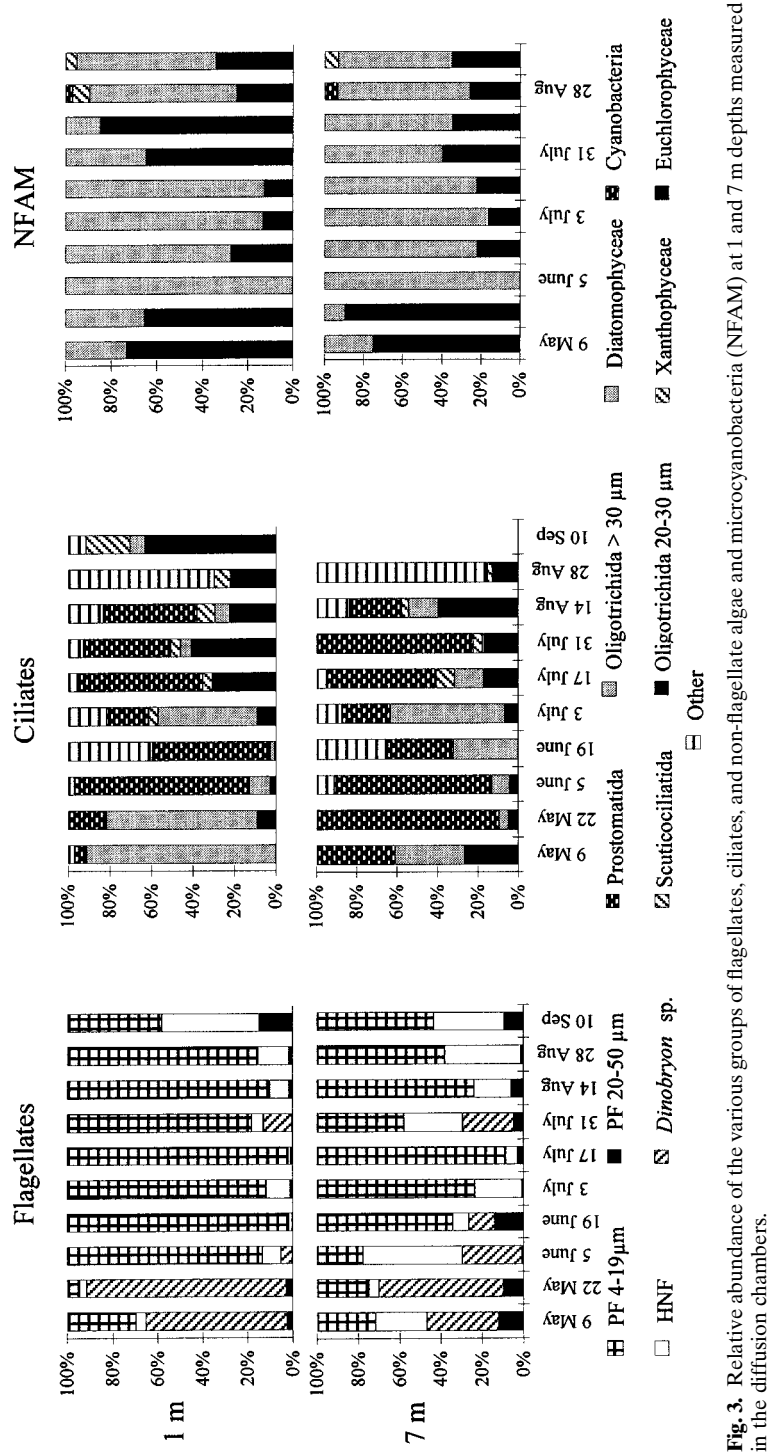

Fig. 3. Relative abundance of the various groups of flagellates, ciliates, and non-flagellate algae and microcyanobacteria (NFAM) at 1 and 7 m depths measured in the diffusion chambers.

The mean abundance of NFAM was low in the metalimnion (Figures 2 and 3) at 120 ± 56 × 10^3 cells l^{-1} until mid-August. In May, Euchlorophyceae, represented by *Ankistrodesmus gelificatum* and then *Sphaerocystis schroeteri*, comprised almost all the NFAM community. From June onwards, Bacillariophyceae (several species of the genus *Synedra*) dominated the assemblage. At the end of August, the density of NFAM increased to reach a peak value of 698 × 10^3 cells l^{-1} on 10 September. The assemblage then consisted mainly of *Cyclotella* sp.

Ciliates. From 19 June to 28 August, the mean ciliate density at 1 m (Figure 2), 7.9 ± 0.7 × 10^3 cells l^{-1}, was relatively constant. At 7 m, the total ciliate density varied from 2.2 × 10^3 cells l^{-1} on 19 June to 8.8 × 10^3 cells l^{-1} on 28 August. No ciliates were found in the samples taken at 7 m on 10 September.

With the exception of 19 June and 28 August (Figure 3), the community was mainly composed of Prostomatida (mainly *Urotricha furcata*), and Scuticociliatida were infrequent. Oligotrichida were represented at first by large-sized species such as *Srombidium viride*, but these were replaced by smaller species (*Strobilidium* sp. and *Halteria* sp.) from 17 July. Throughout the rest of the study, ciliates belonging to the 'other' group (Haptorida, Peritrichida, Suctorida and unidentified) had a relatively high density with *Vorticella* sp. at 1 m and *Askenasia volvox* at 7 m.

Zooplankton biomass

The total biomass measured in the diffusion chambers in the epilimnion (Figure 4) decreased from 9 May [212.4 µg dry weight (dw) l^{-1}] to 3 July (20.1 µg dw l^{-1}).

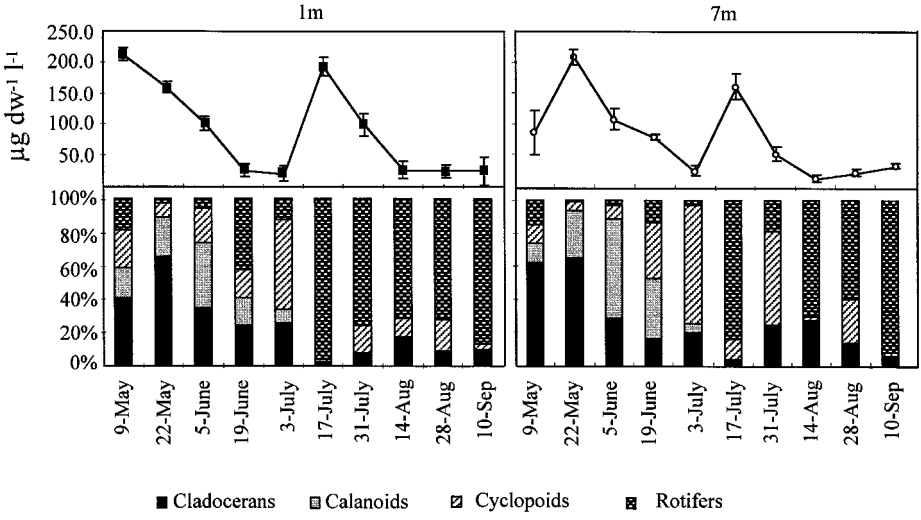

Fig. 4. Mean zooplankton biomass, in µg dw^{-1} l^{-1}, of cladocerans, calanoids, cyclopoids and rotifers in the experimental diffusion chambers at 1 and 7 m depth, and relative biomass of the various metazooplankton group.

Table I. Mean growth rates ± SD of HNF, PF, ciliates and NFAM in the diffusion chambers in the absence (wz) and presence of metazoan zooplankton (z) at 1 and 7 m depth

Depth (m)	Taxa	Mean ± SD of net growth rate (wz) (h^{-1})	Mean ± SD of net growth rate (z) (h^{-1})
1	HNF	0.02 ± 0.10	–0.07 ± 0.11
	PF	0.02 ± 0.05	–0.03 ± 0.02
	Ciliates	0.07 ± 0.09	0.01 ± 0.06
	NFAM	0.04 ± 0.03	0.01 ± 0.02
7	HNF	0.07 ± 0.14	–0.07 ± 0.15
	PF	0.00 ± 0.04	–0.07 ± 0.04
	Ciliates	0.03 ± 0.07	–0.06 ± 0.06
	NFAM	0.03 ± 0.03	0.00 ± 0.05

The pattern of change in the zooplankton biomass was different in the metalimnion. It decreased in spring from 22 May onwards (208.8 µg dw l^{-1}), and during the change in the population on 17 July it was slightly lower (160.3 µg dw l^{-1}) than at 1 m. The zooplankton biomass was lowest on 14 August at 11.4 µg dw l^{-1}. In May, cladocerans, consisting mainly of *Daphnia longispina*, dominated the zooplankton biomass at both depths. These metazoans were replaced by 5 June by a calanoid *Eudiaptomus gracilis* (copepodite stages at 1 m and adults at 7 m). On 3 July at both depths and 31 July at 7 m, copepodite stages (I–IV) of the cyclopoid *Acanthocyclops robustus* dominated the metazoan zooplankton biomass. The rotifer *Asplanchna priodonta* was dominant from July to September.

Metazooplankton grazing on protozoans and NFAM

The mean growth rates of HNF, PF, ciliates and NFAM in the presence and absence of metazoan zooplankton are shown in Table I. Results of one-tailed *t*-tests of the hypothesis that growth rates (h^{-1}) were higher in treatments where zooplankton were removed [equation (1) > equation (2)] are grouped for flagellates, ciliates and NFAM in Table II. With the exception of 5 June at both depths, when calanoids dominated the biomass, and 28 August and 10 September at 1 m, the growth rate of flagellates (Table II) was significantly lower in the presence of zooplankton. The growth rate of ciliates (Table II) did not differ significantly between the two treatments until 22 May in the epilimnion, and until 19 June in the metalimnion and then again on 28 August at 1 m, when Suctorida numerically dominated the community (Figure 3). When the concentration of non-flagellate algae was low (9 May at 7 m) and/or the non-flagellate algae present were of large size and/or colonial (22 May and 5 June at 7 m, 19 June at 1 m and 17 July at both depths), there was no difference in growth whether zooplankton were present or absent.

The mortality [equation (3); Figure 5, Table III] was only calculated when there was a significant difference in growth rates between the two treatments.

Table II. Results of one-tailed *t*-tests of the hypothesis that the growth rates of the various taxa (h^{-1}) were higher in the diffusion chambers not containing zooplankton

Depth (m)	Taxa	9 May	22 May	5 June	19 June	3 July	17 July	31 July	14 Aug.	28 Aug.	10 Sept.
1	Flagellates	<0.05	<0.05	NS	<0.05	<0.05	<0.05	<0.05	<0.05	NS	NS
	Ciliates	NS	NS	<0.05	<0.05	<0.05	<0.05		<0.05	NS	0.08
	NFAM	<0.1	<0.05	<0.1	NS	<0.05	NS	<0.1	NS	<0.05	<0.05
7	Flagellates	<0.05	<0.07	NS	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
	Ciliates	NS	NS	NS	NS	<0.05	0.09	0.08	<0.05	<0.05	NP
	NFAM	NS	NS	NS	<0.05	0.09	NS	<0.05	<0.05	<0.05	0.07

NS, not significant; NP, not present.

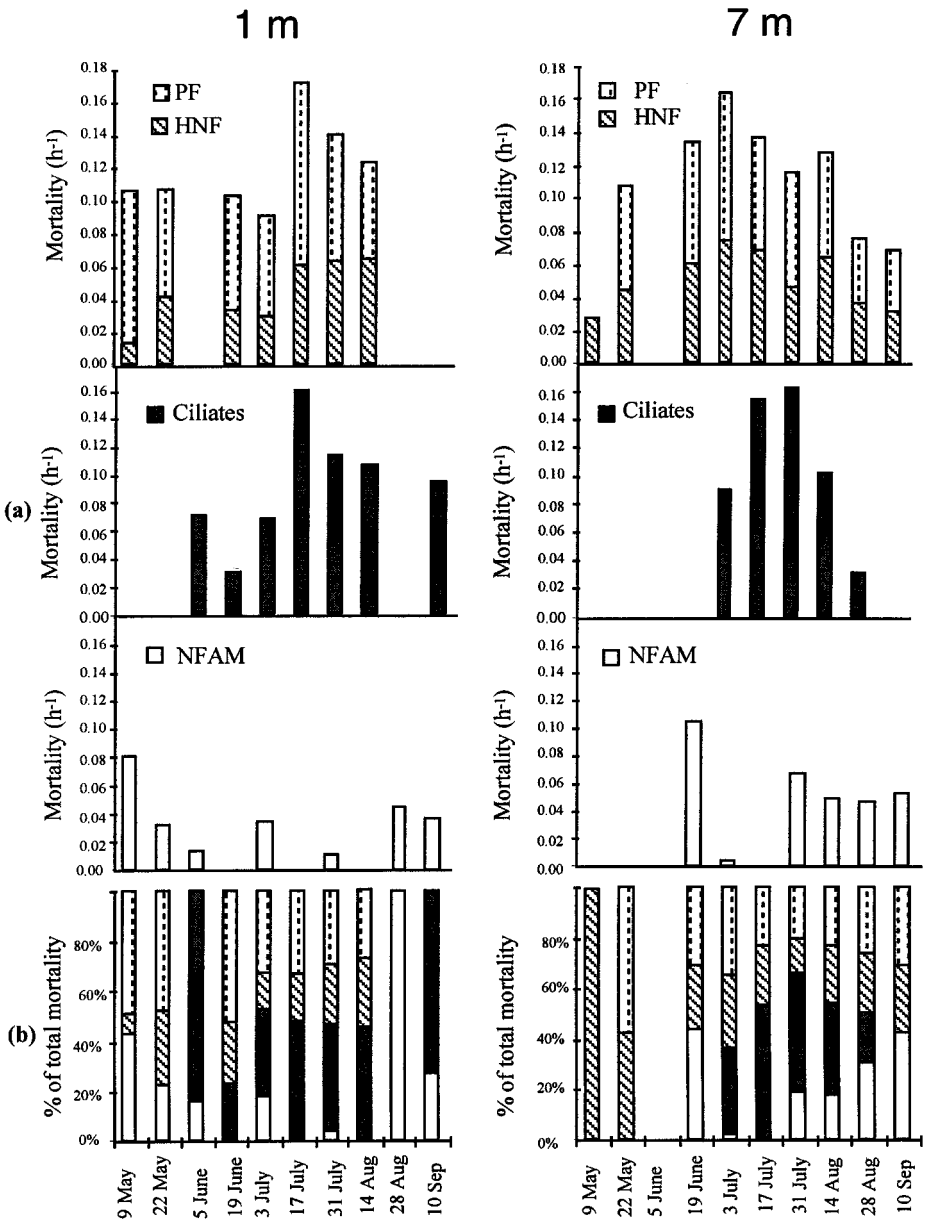

Fig. 5. Mean mortality of pigmented and heterotrophic flagellates, ciliates, non-flagellate algae and microcyanobacteria (NFAM) (**a**) and relative contribution (%) to total mortality of the various groups (**b**) at 1 and 7 m depth.

Table III. Mortality, length and mean biovolume of the various taxa in the Sep Reservoir at 1 and 7 m depth

Taxa	Mean length (µm)	Biovolume ($µm^3$)	Mortality 1 m (±SD) (h^{-1})	Mortality 7 m (±SD) (h^{-1})
Non-flagellated nano- and microalgae				
Euchlorophyceae				
Ankistrodesmus gelificatum	16.0	334	0.08 ± 0.05	0.05 ± 0.07
Botryococcus braunii	81.0	268	0.01 ± 0.00	
Crucigenia tetrapedia	10.5	55	0.03 ± 0.04	0.05 ± 0.13
Pediastrum duplex	17.0	480	*	0.02 ± 0.02
Scenedesmus apiculatus	17.0	840	0.03 ± 0.013	0.07 ± 0.11
Scenedesmus opoliensis	23.0	976	0.10 ± 0.19	0.01 ± 0.06
Sphaerocystis schroeteri	8.3	268	0.10 ± 0.19	0.14 ± 0.20
Tetraedron minimum	13.0	845	0.01 ± 0.07	
Diatomophyceae				
Asterionella formosa	45.5	180	0.04 ± 0.05	0.08 ± 0.06
Cyclotella sp.	15.0	530	0.05 ± 0.04	0.14 ± 0.12
Fragilaria crotonensis	70.0	1050	0.01 ± 0.01	0.02 ± 0.03
Synedra acus	165.0	4665	0.02 ± 0.02	*
Synedra rumpens	48.5	238	0.01 ± 0.01	0.01 ± 0.04
Synedra ulna	265.0	7493	*	
Tabellaria fenestrata	85.0	3060	*	0.01 ± 0.08
Tabellaria floculosa	31.0	3417	*	*
Xanthophyceae				
Chlorocloster sp.	19.5	373	0.04 ± 0.04	0.12 ± 0.00
Microcyanobacteria				
Microcystis sp.	5.0	65	*	*
Ciliates				
Oligotrichida				
Halteria sp.	28.0	2520	0.10 ± 0.02	0.30 ± 0.38
Strobilidium caudatum	21.5	3111	0.05 ± 0.12	†
Strombidium viride	51.0	23 459	0.04 ± 0.03	0.05 ± 0.02
Prostomatida				
Urotricha furcata	20.5	1251	0.11 ± 0.05	0.12 ± 0.06
Balanion sp.	13.5	1294	†	†
Scuticociliatida				
Cyclidium sp.	13.0	618	†	†
Undetermined Scuticociliatida	37.5	1193	0.06 ± 0.05	0.08 ± 0.04
Haptorida				
Askenasia volvox	30.0	7650	†	†
Didinium sp.	42.5	12 750	0.04 ± 0.20	†
Paradileptus elephantinus	280.0	714 737	*	*
Peritrichida				
Vorticella sp.	55.5	17 028	0.01 ± 0.01	†
Suctorida	50.1	11 780	0.03 ± 0.06	*
Undetermined	35.0	22 444	0.04 ± 0.03	†
Flagellates				
Pigmented				
Chrysococcus sp.	10.0	524	0.17 ± 0.10	0.10 ± 0.03
Chrysidalis sp.	4.5	31	0.10 ± 0.07	0.09 ± 0.06
Cryptomonas sp1	24.5	1856	0.12 ± 0.10	0.07 ± 0.07
Cryptomonas sp2	15.0	513	0.09 ± 0.11	0.22 ± 0.27
Dinobryon sp.	11.5	220	*	*
Mallomonas sp.	20.0	1097	0.11	0.12 ± 0.05
Pandorina morum	12.5	1023	0.02 ± 0.00	*
Peridinium volzii	50.5	65 449	0.04 ± 0.01	
Rhodomonas minuta	9.5	173	0.09 ± 0.12	0.11 ± 0.10
Synura sp.	13.0	1050	0.08 ± 0.03	0.18 ± 0.09
Heterotrophic				
5–10 µm	6.0	72	0.11 ± 0.10	0.10 ±0.06
2–5 µm	3.0	17	0.07 ± 0.10	0.19 ± 0.07

*Taxa too rare for analysis.
†Taxa growth is not different with and without zooplankton.

The mean mortality of flagellates in the epilimnion was 0.12 ± 0.03 h^{-1} (0.04 ± 0.02 for HNF and 0.08 ± 0.02 for PF). During the period from 9 May to 3 July, when the assemblage was composed of cladocerans and copepods, the mean flagellate mortality was around 0.10 ± 0.01 h^{-1} (Figure 5). In contrast, when rotifers dominated from 17 July to the end of the study, the mean mortality was higher (0.14 ± 0.02 h^{-1}). The mortality in the metalimnion varied between 0.03 and 0.16 h^{-1} with a mean of 0.11 ± 0.04 h^{-1} (0.05 ± 0.02 for HNF and 0.06 ± 0.03 for PF). This mortality steadily increased to reach a peak value on 3 July of 0.16 h^{-1} and then decreased to the end of the study. The ciliate mortality (Figure 5) could also be separated into two distinct periods, at both depths. Before 17 July, it was 0.06 ± 0.02 h^{-1} at 1 m and 0.09 h^{-1} at 7 m, whereas after this date it was 0.12 ± 0.03 and 0.11 ± 0.06 h^{-1}.

The mortality of NFAM (Figure 5) varied from 0.01 to 0.08 h^{-1} (mean 0.04 ± 0.03 h^{-1}) in the epilimnion and from 0.004 to 0.10 h^{-1} (mean 0.06 ± 0.03 h^{-1}) in the metalimnion. It accounted for 14% of the total mortality at 1 m and 18% at 7 m.

The relative mortality of PF differed little between the epilimnion and metalimnion. It varied from 26 to 52% at 1 m and from 23 to 58% at 7 m (Figure 5). The mortality of heterotrophic flagellates ranged between 7 and 28% at 1 m and from 14 to 100% at 7 m. The relative mortality of ciliates fluctuated from 23 to 84% in the epilimnion and from 20 to 53% in the metalimnion. The relative mortality of NFAM varied widely, from 4 to 100% at 1 m and from 23 to 58% at 7 m.

Discussion

Limits of the method

The calculation that we used to determine the mortality of protists caused by metazooplankton grazing is a standard method in limnology (e.g. Pace and Vaqué, 1994). However, this approach is only valid if the growth rates (a) are equal ($a_z = a_{wz}$). However, even in exploitive competition, Wiackowski *et al.* (1994) showed that there was no overestimation of mortality. In order to overcome the artefacts commonly known as 'bottle effects', we used diffusion chambers that allowed exchanges of water and nutrients with the lake water. Some indirect feedback processes, such as excretion and sloppy feeding, that can stimulate growth were therefore probably of a limited extent in the control diffusion chambers. Thus, the calculated mortality [equation (3)] probably corresponded to the true mortality caused by predation or interference competition and inequities in the realized birth rates ($a_z \neq a_{wz}$). On the other hand, it was impossible to distinguish what proportion of this mortality resulted from grazing by other organisms, such as ciliate and flagellate protists, rather than the metazoan zooplankton. Nevertheless, the fact that the growth rate was always greater in the diffusion chambers without metazoan zooplankton suggests that these had a preponderant role in the mortality of the taxa that we measured. A non-quantifiable artefact can result from the fact that organisms were unable to migrate horizontally or vertically beyond the confines of the diffusion chamber. The incubation was therefore conducted over a short period (7 h) either side of solar midday in order to minimize disturbance to organisms.

The mortality values obtained in this study on a newly flooded reservoir were generally higher (Table III) than those measured in the oligotrophic Lake Michigan, where the mortality rates of nano- and microprotozoans were 0.1–0.5 and 0.02–0.17 day^{-1}, respectively (Carrick *et al.*, 1991; values cited in Pace and Vaqué, 1994), but were similar to those recorded by Pace and Vaqué (1994) in three different lakes.

Abundance of the various communities

The mean density of heterotrophic flagellates throughout 1996 (0.02×10^3–5.76×10^3 cells ml^{-1}; mean 1.01×10^3 cells ml^{-1}) was similar to that reported for oligo-mesotrophic environments (Pick and Hamilton, 1994; Tzaras and Pick, 1994; Carrias *et al.*, 1996). Some PF are mixotrophic species (Laybourn-Parry, 1992). Consequently, they can be considered to be part of the microbial trophic loop and were distinguished from the rest of the phytoplankton. The abundance of pigmented flagellate protists is difficult to compare with that recorded in other lake ecosystems, since those studies that have dealt with this community in natural environments have either focused on the nanoplanktonic component of these flagellate protists (Carrick and Fahnenstiel, 1989; Laybourn-Parry, 1994), or on the total pigmented nanoplankton including flagellate and non-flagellate species (Happey-Wood, 1991) or on a particular group of pigmented flagellate protists (Carrick and Fahnenstiel, 1990). The abundance of ciliate protozoans (0.37×10^3–11.08×10^3 cells l^{-1}; mean 5.13×10^3 cells l^{-1}) was lower than that recorded in oligo-mesotrophic (Amblard *et al.*, 1993) or mesotrophic lakes (Beaver and Crisman, 1989; Müller, 1989; Carrias *et al.*, 1994). Although the abundance of flagellates and ciliates in the study site was of the same order of magnitude as that found in oligo-mesotrophic environments, the maximum values of bacterial abundance were greater than those recorded in lakes of this trophic status (Wetzel, 1983). The high bacterial abundance could partly be explained by the high DOM content. Only Chrost *et al.* (1986), in a study of the hypereutrophic Lake Glekobie, recorded DPROT concentrations similar to ours, whereas these are usually between 0.1 and 1.5 mg l^{-1} (Striquer-Soares and Chevolot, 1996). Furthermore, the TDCHO concentrations were similar to those recorded by Münster (1985) in the eutrophic lake Plußsee. Bacterial abundance is usually correlated with that of flagellates and ciliates (Fenchel, 1982; Sanders and Wickham, 1993; Sherr and Sherr, 1994). However, the mean ratios of bacterial abundance to HNF (3535:1) and of bacteria to total ciliates (7×10^5:1) were higher than in the oligo-mesotrophic Lake Pavin (France), where the ratios were 2590:1 and 0.1×10^5:1, respectively (Amblard *et al.*, 1993), and than in the eutrophic Rimov reservoir, where the ratio of bacteria to HNF was 1000:1 (Simek *et al.*, 1995). This low abundance of HNF and ciliates and the measurements of mortality demonstrate the importance of metazoan zooplankton in regulating these communities in this newly flooded reservoir. This mortality was higher than that of NFAM, which are usually difficult to ingest because of their large size (Table III) and their shape (Neale *et al.*, 1991).

Impact of the metazooplankton on flagellate and ciliate protists

The ciliates that were subjected to the highest mortality were Prostomatida and Oligotrichida of 20–30 μm, such as *Urotricha furcata* (20.5 μm) and *Halteria* sp. (28 μm) (Table III). These results demonstrate the high mortality of small-sized ciliates in accordance with the findings of Carrick *et al.* (1991) and Wickham and Gilbert (1993). Large-sized ciliates, such as *Paradileptus elephantinus* and sessile ciliates (Suctorida, *Vorticella* sp.) are difficult to consume (Jack and Gilbert, 1997) and were only subject to very low mortality (Table III) even when they were at peak abundance (19 June at 1 m, 14 and 28 August at both depths). The mortality of PF and HNF, whose size range in this study varied from 2 and 50 μm, depended on the type of predator and their feeding methods.

At the start of the study, the zooplankton assemblage in the experimental diffusion chambers consisted at both depths mainly of the cladoceran *Daphnia longispina*. The genus *Daphnia* is thought to be unselective in terms of the nature of its prey (DeMott, 1986; Lampert, 1987), and is capable of ingesting particles with a lower size limit of ~1 μm (Brendelberger, 1991). Nanoplanktonic protozoans (flagellates and small ciliates) in the size range suitable for *Daphnia* feeding methods (e.g. Jürgens, 1994; Montel and Lair, 1998) were very heavily consumed when these metazoans dominated. The resulting mortality on heterotrophic flagellates by the zooplankton assemblage at 1 and 7 m ($0.03 \pm 0.0.1$ h^{-1}) can be compared with the results of Pace and Vaqué (1994), 0.04 h^{-1}, in the presence of the species *Daphnia pulex*, in containers submerged in the field. During this period, ciliates were not subjected to any significant mortality (Table III and Figure 3). These protozoans were represented mainly by an oligotrichida, *Strombidium viride*, which is too large to be consumed by the genus *Daphnia* which prefers small-sized ciliates (Jack and Gilbert, 1993, 1997; Wickham and Gilbert, 1993; Jürgens, 1994).

When calanoid copepods dominated the metazoan zooplankton biomass, PF and HNF mortality was recorded at either depth (Figure 4), even though small-sized PF (*Chrysidalis* sp.; mean size = 4.5 μm) were present at high concentrations. These results confirm those of Burns and Schallenberg (1996) with *Boeckella hamata* in experiments with submerged enclosures. They confirm the hypothesis put forward by Carrias *et al.* (1998) concerning the calanoid *Acanthodiaptomus denticornis* which did not consume free-living HNF in an oligo-mesotrophic lake. The ability of calanoid copepods to consume small-sized particles depends particularly on their developmental stage (e.g. Verity and Paffenhöfer, 1996). The absence of flagellate mortality in the metalimnion can be explained by the fact that the copepods sampled consisted almost entirely of adults that are incapable of capturing prey of <10 μm (Paffenhöfer, 1998). The copepods occurring in the epilimnion were mainly juveniles (nauplii and copepodites I–IV) which were able to consume particles with a diameter of 4–5 μm (Paffenhöfer, 1984; Bergreen *et al.*, 1988; Jürgens *et al.*, 1996) using a passive feeding method for small-sized particles (Vanderploeg and Paffenhöfer, 1985). The absence of mortality at this depth could perhaps be attributed to the size of their dominant potential prey, *Chrysidalis* sp., which is at the lower limit of the

size range consumed by calanoids. In contrast, when the copepods were dominated by copepodite stages of cyclopoids, there was a high mortality of PF and HNF in the 5–10 μm size range. Although the calanoid *Eudiaptomus gracilis* did not cause any mortality among PF and HNF in the epilimnion in June, it did consume ciliates (*U.furcata*) which are larger sized than HNF and PF. This result confirms the findings of Hartmann *et al.* (1993) in laboratory experiments with the calanoid *Acanthodiaptomus denticornis* and the results obtained in field experiments with submerged enclosures by Burns and Schallenberg (1996) on *Boeckella* sp. The mortality that we measured, 0.07 h^{-1}, is greater than that recorded by Burns and Gilbert (1993), 0.02 h^{-1}. However, their measurements were made under laboratory conditions with two species of *Diaptomus* and *Espichura lacustris* on cultures of ciliates. The fact that we were unable to demonstrate any significant ciliate mortality on 5 June at 7 m could have been due to the high variance in ciliate counts at this depth and to the much lower abundance of calanoid copepods.

When rotifers dominated the zooplankton assemblage biomass, they consisted mainly of *Asplanchna priodonta*, a species that feeds preferentially on prey with a size of between 10 and 15 μm (e.g. Ejsmont-Karabin, 1974; Garreau *et al*., 1988). Nevertheless, flagellates were subjected to mortality, even though they are usually smaller than 10 μm. As the review by Arndt (1993) has shown, there have been few studies on the consumption of HNF by rotifers. In a series of *in vitro* experiments, Jürgens *et al*. (1996) did however show that *Brachionus rubens* and *Keratella cochlearis* were capable of consuming HNF. Sorokin and Pavelja (1972), in field experiments, showed that *Asplanchna priodonta*, the dominant rotifer in Lake Dalnee, was capable of consuming 72% of the protozoan production, but the authors did not distinguish between flagellates and ciliates.

Asplanchna priodonta, the dominant species of rotifer, is capable of consuming ciliates (Sorokin and Pavelja, 1972; Ejsmont-Karabin, 1974; Garreau *et al.*, 1988; Arndt, 1993). The ciliate mortality, when *Asplanchna priodonta* dominated the assemblage at both depths, was between 0.04 and 0.16 h^{-1}. These values are higher than those obtained with *Asplanchna girodi* (0.04–0.08 h^{-1}) by Jack and Gilbert (1993) using a mixture of four ciliates under laboratory conditions. The different experimental conditions, and also the fact that a small proportion of the biomass was represented by cyclopoids that are capable of causing mortality to ciliate protozoans (Dobberfuhl *et al.*, 1997; Nakamura and Turner, 1997), could explain the difference in mortality rates.

The high quantities of allochthonous organic matter measured after flooding the reservoir seem to have favoured the microbial trophic loop compared to the standard food chain. The relative biomass of all the microbial planktonic communities in the Sep Reservoir, including bacteria, flagellates (heterotrophs and mixotrophs) and ciliates, was thus higher (78% at 1 m and 76% at 7 m) than the non-flagellate algal compartment. This study confirmed the works of Paterson *et al*. (1997) who hypothesized that in a newly flooded reservoir the metazoan zooplankton could depend on other energy sources than the phytoplankton, and would thereby exert a strong predation on ciliates and flagellates. The heavy predation by the metazoan zooplankton on components of the microbial loop (PF

and heterotrophic flagellate protists, and ciliate protists) would probably lead to different trophic relationships than those normally found. Thus, the HNF, which are generally thought to be the main predators on bacteria (Sanders *et al.*, 1992), would be subject to heavy predation by *Daphnia longispina* and *Asplanchna priodonta*. Consequently, the maintenance of low bacterial abundance cannot be directly attributed to heterotrophic flagellates, which, with the exceptions of a few dates, occurred at low densities. The pigmented mixotrophic flagellate *Dinobryon* sp., which is capable of ingesting large quantities of bacteria (Bird and Kalff, 1986; Carrias *et al*., 1996), and the cladoceran *Daphnia longispina* (Jürgens *et al.*, 1994), could therefore control bacterial abundance. Thus, by the predation that they exert on these protozoans and by their ability to ingest bacteria, the reservoir's metazoan zooplankton had a strong direct or indirect influence on the carbon flows passing through the microbial trophic loop in this newly flooded reservoir.

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