

Trophic links in the lowland River Meuse (Belgium): assessing the role of bacteria and protozoans in planktonic food webs

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Abstract: Trophic interactions within the plankton of the lowland River Meuse (Belgium) were measured in spring and summer 2001. Consumption of bacteria by protozoa was measured by monitoring the disappearance of ³H-thymidine-labelled bacteria. Metazooplankton bacterivory was assessed using 0.5- μ m fluorescent microparticles (FMPs), and predation of metazooplankton on ciliates was measured using natural ciliate assemblages labelled with FMPs as tracer food. Grazing of metazooplankton on flagellates was determined through *in situ* incubations with manipulated metazooplankton densities. Protozooplankton bacterivory varied between 6.08 and 53.90 mg C m⁻³ day⁻¹ (i.e. from 0.12 to 0.86 g C⁻¹ bacteria g C⁻¹ protozoa day⁻¹). Metazooplankton, essentially rotifers, grazing on bacteria was negligible compared with grazing by protozoa (~1000 times lower). Predation of rotifers on heterotrophic flagellates (HFs) was generally low (on average 1.77 mg C m⁻³ day⁻¹, i.e. 0.084 g C⁻¹ flagellates g C⁻¹ rotifers day⁻¹), the higher contribution of HF in the diet of rotifers being observed when *Keratella cochlearu* was the dominant metazooplankton. Predation of rotifers on ciliates was low in spring samples (0.56 mg C m⁻³ day⁻¹, i.e. 0.014 g C⁻¹ ciliates g C⁻¹ rotifers day⁻¹) in contrast to measurements performed in July (8.72 mg C m⁻³ day⁻¹, i.e. 0.242 g C⁻¹ ciliates g C⁻¹ rotifers day⁻¹). The proportion of protozoa in the diet of rotifers was low compared with that of phytoplankton (<30% of total carbon ingestion) except when phytoplankton biomass decreased below the incipient limiting level (ILL) of the main metazooplanktonic species. In such conditions, protozoa (mainly ciliates) constituted ~50% of total rotifer diet. These results give evidence that microbial organisms play a significant role within the planktonic food web of a eutrophic lowland river, ciliates providing an alternative food for metazooplankton when phytoplankton becomes scarce.

INTRODUCTION

Although the importance of protozoa in rivers has not been extensively investigated, studies in the last decade report high abundances, biomasses and productions of both ciliates and flagellates in these systems (Garlough and Meyer, 1989; Lair *et al.*, 1999; Scherwab, 2001; Weitere and Arndt, 2002a, 2002b). How protozoa intervene in food webs of large rivers is still very poorly understood, but given the high productivity measured in such ecosystems, fluxes of matter through these organisms, and hence the role they play in microbial food webs, are likely to be important.

The role of protozoa, and in particular heterotrophic flagellates (HFs), in controlling bacterial production in rivers has been demonstrated by different authors (Iriberry *et al.*, 1993; Servais *et al.*, 2000; Vörös *et al.*, 2000). By efficiently feeding on bacteria, protozoa can channel bacterial production into upper trophic levels, provided that metazooplankton actually prey on protozoa in their natural environment. On the contrary, if metazooplankton can themselves feed efficiently on bacteria, protozoan bacterivory and subsequent predation by metazooplankton on protozoans will result in a less efficient transfer of bacterial carbon to the larger planktonic grazers. Laboratory and field studies have provided evidence that both cladocerans and rotifers can graze bacteria and that some species can use them as a significant food source (Starkweather *et al.*, 1979; Bogdan *et al.*, 1980; Urabe and Wanatabe, 1990; Jürgens *et al.*, 1997; Work *et al.*, 2005). However, *in situ* measurements of metazooplankton bacterivory in temperate environments generally yield low food inputs to metazooplankton and low percentages of bacterial standing stock or production grazed (Hart and Jarvis, 1993; Ooms-Wilms, 1997; Thouvenot *et al.*, 1999a; Kim *et al.*, 2000).

So, whether significant ingestion of protozoa by metazooplankton actually takes place in rivers is virtually unknown. By contrast, studies in lowland rivers have shown that the part of primary production used by bacteria (dissolved and particulate organic carbon resulting from algal exudates, algal lysis or sloppy feeding) can be substantial and may even exceed algal carbon assimilated by metazooplankton (Servais *et al.*, 2000). Many

laboratory studies have provided evidence that, in freshwaters, metazooplankton comprises potential predators of both HF's (Arndt, 1993; Jürgens *et al.*, 1996) and ciliates (Arndt, 1993; Jack and Gilbert, 1997; Mohr and Adrian, 2000; Weisse and Frahm, 2002). Nevertheless, field measurements of such trophic interactions are still scarce, especially in rivers. Studies carried out in lakes report increased mortality rates of heterotrophic protozoa in the presence of metazooplankton, giving evidence of top-down control of protozoa (Thouvenot *et al.*, 1999b; Hansen, 2000). Some authors estimated that copepod and cladoceran carbon intake through ingestion of heterotrophic protozoa was comparable with that of phytoplankton (Garrick *et al.*, 1991; Adrian and Schneider-Olt, 1999). In rivers, Weitere and Arndt (Weitere and Arndt, 2002b) and Scherwab (Scherwab, 2001) reported, for the lower River Rhine, a relatively low impact of planktonic predators on protozoa (mean annual of 32% of HNF gross production lost due to grazing by rotifers and ciliates). By contrast, the role of benthic predators seems to be considerable when discharge decreases in this river. However, the observed transfer of planktonic bacterial carbon to benthos via predation on planktonic HNF by benthic organisms may be related to the bottom structure of the River Rhine, which offers hard substrata allowing considerable development of biofilm-dwelling organisms (Weitere, 2001). In addition to that study on benthic predators, there is a clear need for further investigations in other river systems to assess the impact of planktonic predators on protozoans.

The goal of this study was to provide experimental data with which to assess the role planktonic protozoa play in riverine food webs and their potential impact on the transfer of bacterial production to upper trophic levels. In this article, we report measurements of bacterial consumption by protozoa and of metazooplankton grazing on bacteria, HF's and ciliates, and we compare observed grazing rates on heterotrophic plankton with estimates of grazing on autotrophic plankton.

METHOD

Study site

The River Meuse rises in the East of France and flows through Belgium and the Netherlands, where it meets the lower Rhine, forming the Dutch Delta, which opens into the North Sea. Total length of the river is 885 km, and its catchment is about 36 000 km², 40% of it in Belgium. In its Belgian course, the River Meuse is regulated for navigation, with weirs and locks distributed along its length. The study site, Tailfer, is situated 521 km from the source (Fig. 1). At this site, the mean depth is ~4 m and the mean width is ~100 m. In the studied stretch, the river has a mean annual discharge of 150 m³ s⁻¹ and is eutrophic, supporting for most of the growing season a phytoplankton production comparable with that of eutrophic lakes, for example, from 1.1 to 6.7 g C m⁻² day⁻¹ with an average of 3 g C m⁻² day⁻¹ in 1996 (Servais *et al.*, 2000). The metazooplankton are dominated by rotifers, with maximal abundance of 4000 rotifers L⁻¹ (Viroux, 2000).

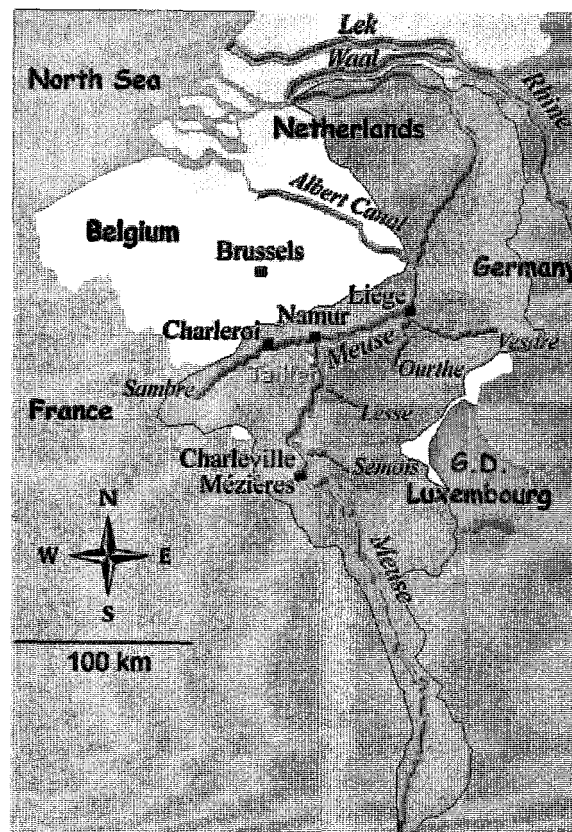
Monitoring of plankton

Planktonic communities (abundance and biomass of phytoplankton, bacteria, protozooplankton and metazooplankton) were monitored from March to early October 2001; phytoplankton and metazooplankton were sampled fortnightly, whereas protozooplankton and bacteria were sampled from 8 to 11 times over the 8 months period of study.

Phytoplankton were sampled at the subsurface (0-1 m) from the middle of the river using a 2.5-L Van Dorn bottle and filtered on Nylax sieves with mesh sizes of 10 and 28 µm. The fractions obtained were further filtered onto Whatman GF/C glass fibre filters in triplicate, and pigments on filters were extracted during 15 min in 10 mL of 90% acetone, using a Branson 2210 sonicator. Two sonications were performed, separated by overnight extraction at 4°C. Pigment extracts were processed by high-performance liquid chromatography (HPLC), following Descy and Métens (Descy and Métens, 1996). Algal biomass, initially expressed in µg chlorophyll *a* L⁻¹, was converted to carbon assuming a constant C : chlorophyll *a* ratio of 37.5 (Descy and Gosselain, 1994).

Bacterial abundance was assessed using epifluorescence microscopy at x1000 magnification, following the procedure of Porter and Feig (Porter and Feig, 1980). After fixation of 20 mL of river water with buffered formaldehyde (2.5 v/v percentage final concentration) and staining with 4,6 diamidino-2-phenylindole (DAPI, 1 µg mL⁻¹ final concentration), a minimum of 500 cells were counted in each sample and two measurements were carried out per sample. Bacteria were classified among 24 size classes using a calibrated eyepiece graticule, and cell volume among each size class was calculated. Biomass was estimated from abundance and biovolume distribution, using the equation linking carbon content per cell (C in fg C cell⁻¹) and biovolume (V in µm³ cell⁻¹), which was determined by Simon and Azam (Simon and Azam, 1989), i.e. $C = 92 \times V^{0.598}$.

Fig. 1: Location of the River Meuse, its drainage area and the sampling point (Tailfer).



For enumeration of protozoa, 200 mL of river water was fixed with 0.5% (v/v) glutaraldehyde and stained with DAPI ($1-3 \mu\text{g mL}^{-1}$) for 10 min. For ciliates, between 10 and 20 mL of river water was filtered on 3- μm filters; flagellates were collected on 0.8- μm filters, and the filtered volume was adjusted between 5 and 13 mL depending on the abundance of phytoplankton in samples. Filtrations were carried out under a maximal backpressure of 130 mbars onto Millipore polycarbonate filters that had been previously stained with Irgalan Black (2 g L^{-1}) in a small volume of acetic acid (2% v/v). Each filter used to retain the ciliates was examined at $\times 400$ magnification in epifluorescence. Between 100 and 200 randomly selected fields were scanned, depending on density. Flagellates were counted at a magnification of $\times 1000$ from 100 fields. Biovolumes for each form encountered were determined by approximating geometric shapes after measurement of relevant dimensions with an eyepiece graticule. Biovolume was converted to carbon using a factor of $0.11 \text{ pg C } \mu\text{m}^{-3}$ for ciliates (Turley *et al.*, 1986) and $0.22 \text{ pg C } \mu\text{m}^{-3}$ for flagellates (Børsheim and Bratbak, 1987).

Metazooplankton were sampled using the transect method of Viroux (Viroux, 1999). A transparent 10-L Schindler-Patalas plankton trap was used to obtain spatially integrated subsurface samples, the volume of which ranged from 90 to 120 L depending on plankton concentration and sampling conditions. A 63- μm mesh size was used to concentrate plankton into 250-mL plastic containers. Samples were fixed with 0.4% (v/v) acidified Lugol iodine. In the laboratory, samples were settled for a few days in glass sedimentation columns so that the volume of samples was reduced to a maximum of 25 mL before examination, and formaldehyde was gradually added to a final concentration of 4%. From these highly concentrated samples, subsamples of 500-1000 μL were taken using a pipette with a cut-off tip and examined under an inverted microscope at a magnification of $\times 100$. Enumeration was limited to rotifers, given the extremely low numbers of cladocerans and copepods observed in the samples. Taxonomical identification of rotifers was based on the keys of Ruttner-Kolisko (Ruttner-Kolisko, 1974), Pontin (Pontin, 1978) and Pourriot and Francez (Pourriot and Francez, 1986). Rotifer biomass was estimated using published values for individual dry weight (DW) taken from a variety of sources (Dumont *et al.*, 1975; Leimeroth, 1980; Cajander, 1983; Andrew and Fitzsimons, 1992).

A carbon/DW ratio of 0.5 was used for conversion to carbon.

Grazing rates

Measurements of grazing rates were carried out on two occasions in spring (May) and three occasions in summer (July) 2001.

Ingestion of bacteria by protozooplankton

Measurements of bacterial mortality due to grazing by protozoans were carried out using the method based on the disappearance of the radioactivity from the genetic material of bacteria previously labelled with ^3H -thymidine (Servais *et al.*, 1985, 1989). A 200-mL water sample from the river was inoculated with (methyl- ^3H)-thymidine (Amersham, 1480-1850 GBq mmol $^{-1}$) at a concentration of 4 nM and incubated in the dark at field temperature for 20 h. This incubation period was sufficient for thymidine depletion from the medium. The sample was then divided into two subsamples, one of which was filtered on a 63- μm pore-size membrane to remove metazooplankton, whereas the other was filtered through a 2- μm pore-size membrane to retain most of the eukaryotic microorganisms. A mixture of cycloheximide-colchicine at respective concentrations of 200 and 100 mg L $^{-1}$ was added in the subsample filtered on 2- μm membrane. This mixture has been reported as an efficient inhibitor of protozoan reproduction and feeding while having no direct effect on bacterial growth (Sherr *et al.*, 1986). The radioactivity was measured twice a day for 2-3 days on 5 mL aliquots from both subsamples. Trichloroacetic acid (final concentration 5%) was added to the 5 mL aliquots, which were then filtered on a 0.2- μm pore-size cellulose nitrate membrane, and the radioactivity associated with the filter was measured by liquid scintillation (Packard-USA TriCarb 2100TR liquid scintillation Analyzer) after addition of 10 mL of scintillation cocktail (Filter Count, Perkin Elmer, USA). A linear decrease of the radioactivity in both subsamples was usually observed on semilog plots of radioactivity versus time. The slope of this decrease in the subsample filtered on 63 μm gave the first-order rate of mortality expressed in h $^{-1}$; the slope of radioactivity versus time in the subsample filtered on 2 μm allowed the calculation of the mortality rate not attributable to grazing by protozoa. The difference between the two rates yielded the mortality rate resulting from grazing by protozoa. Fluxes of bacterial mortality and grazing were obtained by multiplying the first-order rates by bacterial biomass.

Ingestion of bacteria by metazooplankton

Fluorescent microparticles (FMPs) have been used to determine metazooplankton bacterivory by others before (Ooms-Wilms *et al.*, 1995; Thouvenot *et al.*, 1999a). Ooms-Wilms and collaborators (Ooms-Wilms *et al.*, 1995) showed that metazooplankton preference for fluorescently labelled bacteria (FLB) over FMPs is not the rule and depends strongly on the grazer species. FMPs are brightly fluorescent, and their quantification in the guts of metazooplankton is more reliable than that of FLB, especially in large species like Brachionids, hence our choice to use FMPs as tracer of bacterial consumption.

Seven-litre polyethylene cubitainers (Kartell, Milano, Italy) were filled with subsurface river water and inoculated with 0.5- μm fluorescent microspheres (FMPs) (FluoresbriteYG carboxylate microspheres; Polysciences, Eppelheim, Germany) at a concentration of ~2% of the natural bacterial abundance. Preliminary results showed an increasing ingestion rate of the FMPs over the first 15 min. This artefact was attributed to a stress on metazooplankton organisms due to the pouring of the water into the cubitainers, so the animals were allowed to recover for 20 min before the addition of FMPs. The medium was then gently mixed and incubated *in situ* for 5, 10, 15, 20 and 30 min. At the end of each incubation, the content of one cubitainer was filtered through a 37- μm plankton net, and metazooplankton collected on the net was narcotized in soda water and fixed with formaldehyde (final concentration 2% v/v). The numbers of FMPs in the guts of metazooplankton grazers were counted under epifluorescence microscopy. Controls where formaldehyde had been added to the medium before the addition of FMPs were used to check for possible unwanted adherence of FMPs to the cuticle of predators.

Specific filtration rates of metazooplankton feeding on bacteria (F_{bact} ; individual $^{-1}$ h $^{-1}$) were calculated with the equation:

$$F_{\text{bact}} = \frac{FMP_{\text{gut}} \times 60}{FMP_{\text{med}} \times t},$$

where FMP_{gut} is the mean number of FMPs in the gut of animals belonging to one species, FMP_{med} is the number of FMPs per μL of experimental medium and t (in minutes) is the duration of incubation, F_{bact} was calculated for species with numbers of animals observed higher than 20, whereas those taxa with numbers observed lower than

20 were pooled in a single category ('others'). Specific contributions to total metazooplankton community ingestion of bacteria (CIR_{bact} ; $\mu\text{g C m}^{-3} \text{day}^{-1}$) were calculated as:

$$CIR_{bact} = F_{bact} \times Bact \times Meta \times C_{bact} \times 24,$$

where F_{bact} is the specific filtration rate, $Bact$ is the number of bacteria per μL of river water, $Meta$ is the number of animals per m of river water and C_{bact} is the mean carbon content of bacterial cells in the river ($26.5 \text{ fg C cell}^{-1}$).

Ingestion of ciliates by metazooplankton

To measure ingestion of ciliates by metazooplankton, we chose to use ciliates labelled with FMPs. The method is described in detail in the work of Joaquim-Justo *et al.* (Joaquim-Justo *et al.*, 2004). In brief, river water was sampled in the field and immediately brought back to the laboratory. Ciliates were labelled with FMPs and fed to metazooplankton sampled simultaneously in the river and concentrated 10 times. Incubations were carried out at *in situ* temperature.

Specific ingestion rates of ciliates by metazooplankton (IR_{cil} ; $\text{ng C individual}^{-1} \text{h}^{-1}$) were calculated using the equation:

$$IR_{cil} = \frac{FMP_{gut} \times Cil_{insitu/exp} \times C_{cil} \times 60}{FMP_{cil} \times Cil_{lb} \times t},$$

where FMP_{gut} is the mean number of FMPs in the gut of animals belonging to one species minus FMP numbers in controls, FMP_{cil} is the mean number of FMPs per labelled ciliate at the onset of measurements, $Cil_{in situ/exp}$ is the abundance of ciliates in the river or in experimental medium (ciliates L^{-1}), Cil_{lb} is the abundance of labelled ciliates in experimental medium (ciliates L^{-1}), C_{cil} is the mean carbon content of ciliates from the river as calculated for each date on the basis of biovolume estimates using a conversion factor to carbon of $0.11 \text{ pg C } \mu\text{m}^{-3}$ (Turley *et al.*, 1986) and t (min) is the duration of the incubation. IR_{cil} was calculated for species with numbers of animals observed higher than 20, whereas those taxa with numbers observed lower than 20 were pooled in a single category ('others').

Total metazooplankton community ingestion of ciliates (CIR_{cil} ; $\mu\text{g C m}^{-3} \text{day}^{-1}$) was calculated as the sum of all IR_{cil} multiplied by the respective specific abundance of metazooplankton species (individual m^{-3}).

Ingestion of HF's by metazooplankton

Ingestion rates of flagellates were quantified by manipulating metazooplankton densities. This methodology is commonly used for assessing feeding of predators on protozoans (see e.g. Weisse and Frahm, 2002; Weitere and Arndt, 2002b; Liu *et al.*, 2005). Polystyrene cubitainers of 7 L were filled with river water and subsequently inoculated with densities of metazooplankton comprised between $1 \times$ and $7 \times$ *in situ* abundance. Two cubitainers were inoculated for each metazooplankton density, and flagellate abundances were counted in triplicate samples for each cubitainer. All containers were incubated *in situ* for 24 h. HF's were sampled at the beginning and end of incubation periods, fixed with glutaraldehyde (final concentration 2%), stained with DAPI, and three subsamples of 10 mL were filtered on black $0.8\text{-}\mu\text{m}$ pore-size polycarbonate membranes and enumerated under epifluorescence microscopy. Abundances and sizes of flagellates were determined and biovolumes were calculated assuming simple geometric forms; carbon biomasses were estimated from biovolumes using a conversion factor of $0.22 \text{ pg C } \mu\text{m}^{-3}$ (Børsheim and Bratbak, 1987). Net observed growth rates (r , day^{-1}) were estimated as

$$r = \frac{(\ln B_t - \ln B_0)}{t},$$

where B_t and B_0 are flagellate carbon biomass at the end and beginning of incubations, respectively, and t is the duration of the experiments (expressed in days). The slope of the linear regression between net observed growth rates and metazooplankton abundance (individual m^{-3}) yields an estimate of the grazing rates (G) on flagellates expressed in $\text{m}^3 \text{individual}^{-1} \text{day}^{-1}$. Grazing rates were converted into community ingestion rates (CZS_{fl} ; $\text{ng C m}^{-3} \text{day}^{-1}$) using the following equation:

$$CIR_{\text{a}} = G \times BHF_0 \times Meta,$$

where G is the grazing rate, BHF_0 is the *in situ* carbon biomass of HFs (ng C m^{-3}) at the beginning of the incubation and $Meta$ is the abundance of metazooplankton (number per m^3) in river water.

Ingestion of phytoplankton by metazooplankton

Ingestion of phytoplankton by metazooplankton was estimated on the basis of specific ingestion rate parameters, metazooplankton abundances, edible algal biomass and temperature. Parameters of the functional grazing response were obtained from previous campaigns of *in situ* measurements in the same river stretch (Gosselain, 1998), normalized to 20°C assuming a doubling of ingestion rates with a 10°C temperature increase (Gosselain *et al.*, 1996). The calculations were made according to a type I functional response (i.e. linear), using as edible algal biomass the algal fraction $<28 \mu\text{m}$, and the incipient limiting levels (ILLs) and maximal ingestion rates of the most abundant rotifer taxa, that is, *Brachionus* spp. (mainly *B. calyciflorus* Pallas) and *Keratella* spp. (mainly *Keratella cochlearis* Gosse). For less abundant taxa (such as Synchaetidae), we used ingestion rates from the literature. Details of calculations and literature data used can be found in the work of Gosselain (Gosselain, 1998). Total metazooplankton ingestion of phytoplankton is the sum of estimated specific ingestion rates multiplied by their respective specific abundances.

RESULTS

Monitoring of plankton

The fluctuations in biomass of planktonic compartments are shown in Fig. 2. Phytoplankton was the compartment presenting the highest biomass throughout the study: most phytoplankton biomass values were between 1 and 4 mg C L^{-1} . A phytoplankton bloom was first observed at mid-May followed by subsequent higher peaks in early June and early July. These peaks corresponded to low discharge periods ($<200 \text{ m}^3 \text{ s}^{-1}$) during which temperature increases were observed (Fig. 3, data supplied by the Compagnie Intercommunale Bruxelloise des Eaux, Tailfer). Phytoplankton units with greater axial linear dimension (GALD) $<10 \mu\text{m}$ dominated during most of the growing season; HPLC analysis showed these algae to be mainly diatoms. Exception to this dominance was observed at the end of May and throughout July when phytoplankton particles of GALD comprised between 10 and $28 \mu\text{m}$ were most abundant. In July, the shift in size was parallel to a shift towards higher proportion of Chlorophytes.

Fig. 2: Variations in the biomass of major compartments of the planktonic food web in the River Meuse in 2001. All values are expressed in $\mu\text{g C L}^{-1}$. Outlying values are indicated. Vertical lines indicate dates when carbon fluxes were measured.

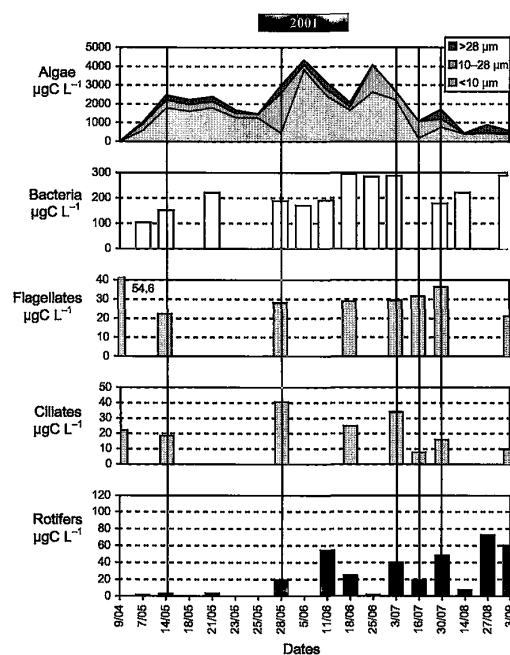
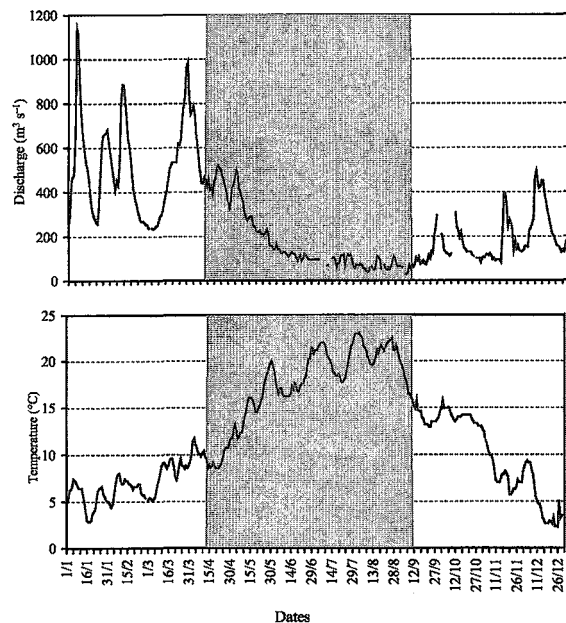


Fig. 3: Daily variations of discharge (top diagram) and water temperature (bottom) measured at Tailfer in 2001. Sampling season is situated in time by shaded area. Data are from the *Compagnie Intercommunale Bruxelloise des Eaux* and used with permission.



Bacterioplankton was the second most important group in terms of biomass, with a range of 100-300 $\mu\text{g C L}^{-1}$. Biomasses of flagellates, ciliates and metazooplankton were all $<100 \mu\text{g C L}^{-1}$, and the biomass of metazooplankton displayed the largest variations. Discontinuous sampling of heterotrophic assemblages does not allow comparing temporal variations of all planktonic compartments.

Composition of metazooplankton is presented in Fig. 4. Only rotifers are reported, as numbers of other zooplankters were very low during the whole period of study. Rotifer abundance was generally low, with total numbers varying from 6 to 254 individual L^{-1} . *Keratella cochlearis* was the most abundant species except at the end of the developing season (from mid-August on) when *Synchaetids* and *Brachionids* reached higher numbers.

Bacterivory

Protozooplankton bacterivory ranged from 6.08 to 53.90 $\text{mg C m}^{-3} \text{day}^{-1}$ (Table I), that is, from 0.12 to 0.86 g C^{-1} bacteria g C^{-1} protozoa day^{-1} and was lowest on 30 July. Metazooplankton bacterivory measurements with EMPs implied the determination of gut passage time (GPT, determined here as the time lapse after which ingestion rates of FMPs stop increasing linearly with time). GPT was either >20 min (two first sampling campaigns when field temperatures were $\sim 15^\circ\text{C}$) or >15 min (three last sampling campaigns when field temperatures were $\sim 20^\circ\text{C}$) for all metazooplankton bacterivorous species. Metazooplankton comprised mainly rotifers, among which most individuals of the species *K. cochlearis*, *Euchlanis dilatata* Ehrb. and *Brachionus* spp. ingested 0.5 μm FMPs (Table II). By contrast, *Trichocerca pusilla* Lauterborn, *Synchaeta* spp. and *Polyarthra* spp. virtually never ingested EMPs. Worth mentioning, despite their in Table II due to their rarity in samples, is the high bacterivory of *Anuraeropsis fissa* Gosse which when observed always had high numbers of FMPs in its gut. Conversely, we never found FMPs in the guts of *Cephalodella* spp. Specific filtration rate on FMPs was the highest for *Brachionus angularis* Gosse (Table I), with an activity twice as high as other main bacterivorous species. Nonetheless, *K. cochlearis* was the species with the highest contribution to total community ingestion rate of bacteria on all occasions (Table I).

Protozoan bacterivory was much higher (by three orders of magnitude) than bacterivory by metazooplankton at all times.

Fig. 4: Composition of rotifer communities at Tailfer in 2001. Upper panel shows absolute abundances; relative contribution of the species to total biomass is shown in lower panel (Pol, *Polyarthra* spp.; Syn, *Synchaeta* spp.; Br. an, *Brachionus angularis*, Br. ca, *Brachionus calyciflorus*; Ker. co, *Keratella cochlearis*).

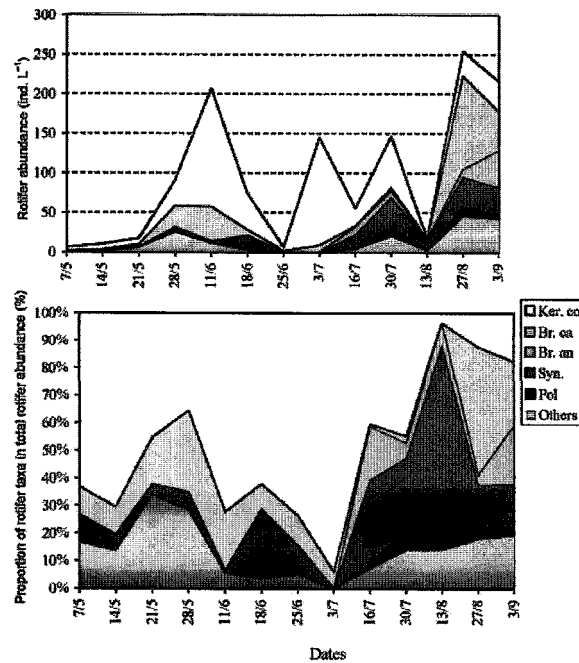


Table I: Bacterivory in the river

Metazooplankton bacterivory					
Species	F_{bact} ($\mu\text{L individual}^{-1} \text{ h}^{-1}$)				
	Mean \pm SE				
<i>Keratella cochlearis</i>	0.064 \pm 0.020				
<i>Euchlanis dilatata</i>	0.065 \pm 0.040				
<i>Brachionus calyciflorus</i>	0.057 \pm 0.009				
<i>Brachionus angularis</i>	0.152				
Others	0.018 \pm 0.015				
Dates					
Species [CIR_{bact} ($\mu\text{g C m}^{-3} \text{ day}^{-1}$)]	14 May	28 May	3 July	16 July	30 July
<i>Keratella cochlearis</i>	0.94	6.65	51.55	6.68	32.18
<i>Euchlanis dilatata</i>		2.22			
<i>Brachionus calyciflorus</i>		5.20	3.87		
<i>Brachionus angularis</i>					
Others	0.14	2.07		4.38	2.51
Total CIR_{bact}	1.08	16.13	55.42	11.06	34.69
Total protozooplankton bacterivory ($\mu\text{g C m}^{-3} \text{ day}^{-1}$)	21 678	42179	53901	18301	6084

Table II: *Bacterivory of metazooplankton species*

Species	Dates					Global trend
	14 May	28 May	3 July	16 July	30 July	
<i>Keratella cochlearis</i>	••	••	••	••	••	••
<i>Euchlanis dilatata</i>		••	••			••
<i>Brachionus calyciflorus</i>		••	•			•/••
<i>Brachionus angularis</i>			••			
<i>Brachionus urceolaris</i>						
<i>Brachionus quadridentatus</i>			•			
<i>Trichocerca pusilla</i>				o	o	o
<i>Synchaeta</i> spp.	o	•	o	o	o	o
<i>Polyarthra</i> spp.	•			o	o	o

Bacterivory is expressed as percentages of individuals that ingested 0.5 μm fluorescent microparticle (FMP). Only species with $n \geq 15$ were reported. ••, >50% of individuals observed with FMP in gut. •, 10-50% of individuals observed with FMP in gut. •, <10% of individuals observed with FMP in gut.

Predation of metazooplankton on protozoa

GPT of ciliates in metazooplankton was >25 min on all occasions and >35 min on one occasion (28 May). Ingestion rates of rotifers feeding on ciliates could only be calculated for three species owing to the low abundance of rotifers observed in the samples and the low numbers of FMPs observed in the gut of metazooplankton. Metazooplankton species that had ingested labelled ciliates and for which the number of individuals observed in the samples was higher than 20 are *K. cochlearis*, *E. dilatata* and *Synchaeta* spp. (Table III). *Keratella cochlearis* always ingested ciliates and exhibited higher ingestion rates in the two last sampling campaigns. *Synchaeta* spp. only showed a quantifiable ciliate predation during the last two campaigns. Total community ingestion of ciliates by metazooplankton was very low in May (mean 0.56 $\text{mg C m}^{-3} \text{ day}^{-1}$ or 0.014 g C^{-1} ciliates g C^{-1} rotifers day^{-1}) and much higher in July (mean 8.72 $\text{mg C m}^{-3} \text{ day}^{-1}$ or 0.242 g C^{-1} ciliates g C^{-1} rotifers d^{-1}) (Fig. 5).

Grazing of metazooplankton on flagellates was very low in general, with significant community ingestion only on 14 May and 3 July, when predation on flagellates expressed as carbon was similar to predation on ciliates (Figs 5 and 6).

Metazooplankton community ingestion rates on microbial compartments and phytoplankton

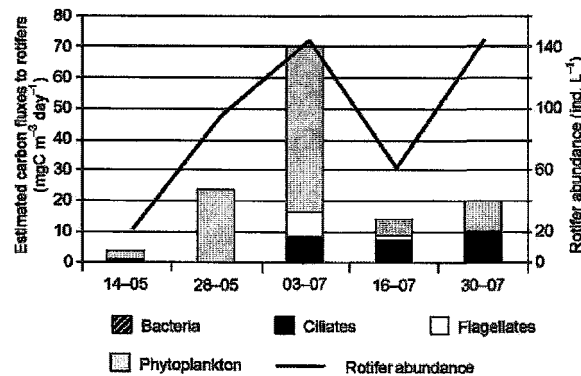
Total metazooplankton community ingestion on bacteria, flagellates, ciliates and phytoplankton on the five sampling occasions is depicted in Fig. 5. Total ingestion reached a maximum of 70 $\text{mg C m}^{-3} \text{ day}^{-1}$ on 3 July; otherwise, it was comprised between 4 and 27 $\text{mg C m}^{-3} \text{ day}^{-1}$ and roughly followed rotifer abundance except on 30 July when the increase in carbon ingested by metazooplankton was lower than the increase in rotifer abundance. Phytoplankton constituted >73% of metazooplankton diet on all dates except on the last two when ciliates contributed 50% of metazooplankton diet (Fig. 6).

Table III: *Specific ingestion of ciliates by metazooplankton*

Species	IR_{cil} ($\text{ng C individual}^{-1} \text{ h}^{-1}$)				
	14 May	28 May	3 July	16 July	30 July
<i>Keratella cochlearis</i>	1.89	0.366	2.614	12.514	5.206
<i>Euchlanis dilatata</i>		0.731			
<i>Synchaeta</i> spp.	0	0*	0	0.522	0.551

Ingestion rates (IR_{cil}) were only calculated for predator species with $n > 20$ to the exception of * $n = 9$.

Fig. 5: Total community ingestion of planktonic organisms by metazooplankton (expressed as carbon fluxes) measured in the river.



DISCUSSION

Methodology

Methods used to measure grazing rates on field samples are generally of two main types. A first type is based on long-term incubations (from some hours to several days) with manipulated natural planktonic assemblages (modification of prey : predator ratio by dilution or concentration using size fractionation). The main advantage of this kind of method is its technical simplicity. Nevertheless, separation of predators and preys through filtrations is not always unambiguous, as their sizes sometimes overlap or are very similar (Zimmermann, 1996; Paffenhöfer, 1998). This is particularly problematic in ecosystems where rotifers dominate metazooplankton, as is so in most rivers (Marneffe *et al.*, 1996; Kobayashi, 1997; Viroux, 1999; Kim *et al.*, 2001; Baranyi *et al.*, 2002). Moreover, low grazing rates are difficult to detect with such methods, inter-replicate variations in prey growth rates often blotting out effects of predators (Dolan *et al.*, 2000). These methods also virtually exclude the assessment of grazing activities of different taxa.

For these reasons we opted, when possible, for the second type of methodology: the food-tracer approach. This technique relies on the use of labelled tracer food for measuring ingestion rates by predators during short incubations. Grazing rates of metazooplankton on bacteria and ciliates were measured using FMPs as food-tracer and as a way to label food, respectively. Flagellates from the river, however, very rarely ingested FMPs; that is why, for measuring predation of flagellates by metazooplankton, we had to use long-term incubations with manipulated rotifer densities.

Protozoans have been shown to display considerable differences in the way they ingest and/or process living bacteria, dead bacteria (FLBs) and latex beads (Verity, 1991; Boenigk *et al.*, 2001a, 2001b); these differences are likely to affect estimates of grazing rates of bacteria by protozoa. In addition, the bias is likely to fluctuate throughout seasons as the taxonomic composition of protozooplankton changes (Jacquet, 2003). As a consequence, to measure bacterivory by protozooplankton, we decided to use a method based on measurements of the disappearance of the radioactivity of bacterioplankton previously labelled with ³H-thymidine, validated by Servais and his co-workers (Servais *et al.*, 1985, 1989; Menon *et al.*, 2003).

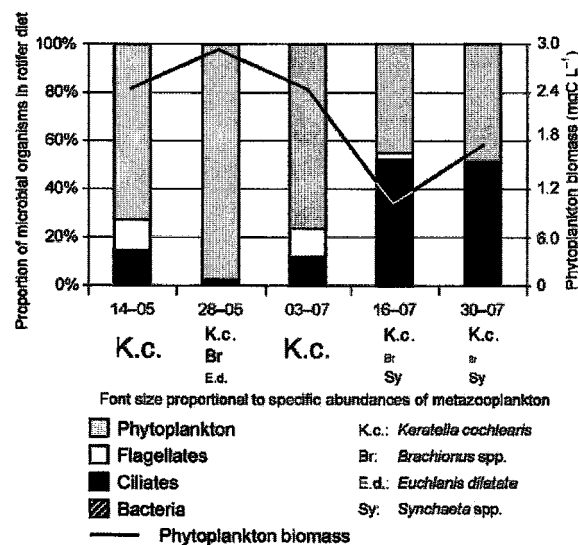
Fluxes in the planktonic food web of the river

Protozoa consumption rates of bacteria in our study were in the range reported by others (Servais *et al.*, 1998; Callieri *et al.*, 2002; Weitere and Arndt, 2002a). It is to be noted that flagellates are widely considered as the main grazers of bacteria (Arndt *et al.*, 2000; Jacquet *et al.*, 2005; Weitere *et al.*, 2005). Accordingly, parallel investigations in the River Meuse showed that grazing of bacteria by flagellates accounted on average for $91 \pm 10\%$ of total grazing by protozoa over the study period. Lower consumption of bacteria by protozoa on 30 July was thus most probably due to lower flagellate numbers ($3.6 \times 10^6 \text{ L}^{-1}$ as compared with $6.2 \pm 0.4 \times 10^6 \text{ L}^{-1}$ on average for the other sampling campaigns).

Our observations of bacterivory by *K. cochlearis*, *B. calyciflorus*, *B. angularis* and *Anuraeopsis fissa*, but not by *Trichocerca* spp., *Polyarthra* spp. or *Synchaeta* spp., are in agreement with previous studies (Starkweather *et al.*, 1979; Sanders *et al.*, 1989; Ooms-Wilms *et al.*, 1995; Hwang and Heath, 1999; Kim *et al.*, 2000). Low ingestion

of FMPs by these two latter taxa on 14 May and 28 May, respectively (Table II), was most probably due to indirect ingestion of FMPs through predation on ciliates that had ingested FMPs. Indeed, the longer GPTs of most metazooplankton species on those dates might have allowed protists (ciliates) present in cubitainers to ingest FMPs and their subsequent ingestion by *Polyarthra* spp. and *Synchaeta* spp. Both genera are indeed considered as specialist feeders on large (~30-40 µm) particles and are thus potential predators for ciliates (Pourriot, 1977). This would explain the presence of low numbers of FMPs in the guts of rotifers on these dates. This artefact might also explain why Kim and co-workers (Kim *et al.*, 2000) unexpectedly observed low bacterivory in *Polyarthra* spp., *Synchaeta* spp. and *Trichocerca* spp., as these authors also used incubation times of up to 30 min. *Euchlaris dilatata* was never reported to be bacterivorous, but it was absent from all taxa lists presented by authors who conducted *in situ* bacterivory experiments.

Fig. 6: Relative proportions of microbial organisms in the diet of metazooplankters (rotifers). Abundance of main rotifer species is indicated by font size of rotifer acronyms in figure. Line shows fluctuations in phytoplankton biomass.



Specific filtration rates of metazooplankton on bacteria (F_{bact}) as measured with 0.5 µm FMPs (Table I) were in the range of values reported by other authors, for example, from 0.009 to 0.018 µL individual⁻¹ h⁻¹ for *K. cochlearis* and 0.034-0.061 µL individual⁻¹ h⁻¹ for *B. angularis* in Lake Loosdrecht (Ooms-Wilms, 1997) and 0.375 ± 0.333 µL individual⁻¹ h⁻¹ for *K. cochlearis* and 30.250 ± 11.875 µL individual⁻¹ h⁻¹ for *B. angularis* in the Nakdong River (Kim *et al.*, 2000). Specific ingestion rates of bacteria by metazooplankton were all low in comparison with their food requirements which amount to at least 20% of body mass ingested per day (Pourriot *et al.*, 1982 in Ooms-Wilms, 1997) with a mean body mass of 82 ng C for *K. cochlearis* (Andrew and Fitzsimons, 1992) and a mean ingestion rate of 0.30 ± 0.13 ng C individual⁻¹ h⁻¹ in our samples. Metazooplankton community bacterivory measurements were in the lower end of the range reported by other authors (Sanders *et al.*, 1989; Ooms-Wilms, 1997; Hwang and Heath, 1999; Kim *et al.*, 2000), this being largely because of low metazooplankton abundance (maximum of 147 individual L⁻¹ on dates when fluxes in microbial food webs were measured). When compared with bacterial standing stock, and bacterial grazing by protists, grazing of bacteria by metazooplankton in the River Meuse appears to be negligible (<0.1%) suggesting the irrelevance of the direct link between bacteria and metazooplankton. Weitere and co-workers (Weitere *et al.*, 2005) also estimated very low carbon fluxes from bacterioplankton to metazooplankton in a study on the River Rhine. This contrasts with the findings by some authors for metazooplankton dominated by cladocerans or rotifers (Wylie and Currie, 1991; Jürgens *et al.*, 1997; Hwang and Heath, 1999; Levine *et al.*, 1999; Work *et al.*, 2005).

Predation of rotifers on ciliates was only quantifiable for three species owing to low abundance of rotifers in the samples. Specific ingestion of *K. cochlearis* on ciliates in May (Table III) was comparable with ingestion rates measured by Weisse and Frahm (Weisse and Frahm, 2002) with *Urotircha furcata* and *Balanion plancticum* as food (I max of 0.44 ng C individual⁻¹ h⁻¹ and 1 ng C individual⁻¹ h⁻¹, respectively, using a conversion factor of 0.11 pg C µm⁻³). In July it was considerably higher. This could be due to the smaller size of ciliates in the River Meuse in July (~10 µm in length as compared with 10-25 µm for *U. furcata* and *B. plancticum*), which could make them easier to handle for small rotifers like *Keratella*. But other factors such as swimming/escape

behaviour of ciliates and the presence of *Cryptomonas* cells in *in vitro* experiments might have interfered as well. Predation of *Synchaeta* spp. on ciliates was in the upper range of values reported in the literature (from 0.2 to 5 ciliates individual⁻¹ h⁻¹) (Zimmermann, 1996) as compared with 3.6-4.5 ciliates individual⁻¹ h⁻¹ in our experiments.

If growth rates of ciliates in the river are considered to be around 0.5 day⁻¹ as might be expected according to literature data (Gilbert and Jack, 1993; Scherwab, 2001), then production of ciliates in the river varied between 3.5 and 20 mg C m⁻³ day⁻¹; these values are in the range of the predation exerted by metazooplankton on ciliates (Fig. 5) and may indicate effective top-down control of these protists on 16 and 30 July at least.

Predation by metazooplankton on flagellates was close to zero (Fig. 5). It is to be noted that the majority of HFs in the River Meuse are very small [from 1 to 6 µm equivalent spherical diameter (ESD), with a dominance of organisms with 2-3 µm ESD]. This might explain the low predation exerted by species such as *B. calyciflorus*, *B. angularis*, *E. dilatata* and *Synchaeta* spp. whose feeding optima have been described for particles of sizes >5 µm (Pourriot, 1977; Rothhaupt, 1990; Walz, 1997). *Keratella cochlearis*, on the contrary, besides efficient feeding on bacteria-sized particles in the 0.4-1.6 µm range (Bogdan and Gilbert, 1984; Ronneberger, 1998), has also been successfully cultured on the 2.4-4.2-µm green alga *Stichococcus bacillaris* (Walz, 1983) and the 5-6-µm Cryptophyte *Rhodomonas minuta* (Stemberger, 1981). Odier studies report efficient feeding on larger items such as 30-40 µm *Cryptomonas* spp. (Pourriot, 1977; Vancil, 1983). These observations however do not address filter feeding but rather raptorial feeding on these prey items with a size similar to *Keratella* body width (35 µm). So it appears that food items in the size range of 2-3 µm should be ingested by this rotifer. If the proportion of flagellates in metazooplankton diet is compared with the contribution of *K. cochlearis* to total rotifer abundance (Fig. 6), it appears that the proportion of flagellates in the diet of rotifers is indeed higher when this species dominates the community. Another reason that might explain that even *K. cochlearis* kept the HF proportion in their diet rather low is a possible preference for co-occurring autotrophic food or heterotrophic species that feed on algae (i.e. mainly ciliates). Mohr and Adrian (Mohr and Adrian, 2002) give evidence of such preference in the rotifer *Brachionus calyciflorus*, which ingested preferentially an algivorous ciliate as compared with a bacterivorous one, on the one hand, and preferred an autotrophic flagellate over a bacterivorous flagellate, on the other hand. Autotrophic flagellates such as Cryptophytes (included in this study within phytoplankton) have been found to be of high nutritional value for rotifers (Lubzens *et al.*, 1985; Fernández-Reiriz and Labarta, 1996). Interestingly, Boersma and Stelzer (Boersma and Stelzer, 2000) showed the growth of *K. cochlearis* was HUFA-limited (highly unsaturated fatty acid-limited) in the presence of a natural *Scenedesmus*-dominated seston. Higher content of essential HUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of Cryptophytes as compared with Chlorophytes (Vanderploeg *et al.*, 1996; Von Elert and Stampfl, 2000) could explain the success of this rotifer species when fed with Cryptophytes and the difficulties generally encountered with cultures based on other food. This is further supported by *in vitro* studies that show *K. quadrata* displays increased reproduction when fed with DHA-supplemented food (Boëchat *et al.*, 2005). These particular requirements might lead *K. cochlearis* to a high selectivity towards its preys. Indeed, results by Mohr and Adrian (Mohr and Adrian, 2002) with *Brachionus calyciflorus* tend to support the concept that prey selection has evolved to discriminate food of good versus poor nutritional value as rotifers selected preys that allowed them higher growth rates.

An interesting result of our study lies in the difference of the proportion of protozoa (mainly ciliates) and phytoplankton in the diet of metazooplankton. As seen in Fig. 6, the proportion of heterotrophic protists in the diet of rotifers on the three first occasions was ≤27% of total ingestion, whereas it was >50% thereafter. When compared with the variations of phytoplankton biomass (Fig. 6), it appears that there was a decrease in the proportion of phytoplankton ingested when phytoplankton biomass in the river was lower than or close to the ILL determined for *K. cochlearis* and *B. calyciflorus* in field measurements performed in the River Meuse (1.5 mg C L⁻¹ and 1.7 mg C L⁻¹, respectively) (Gosselain, 1998). This observation suggests that when phytoplankton standing stock is higher than the ILL of the dominant rotifer species in the river, the contribution of protozooplankton to the diet of metazooplankton is low. By contrast, when phytoplankton availability drops below the ILL of the main predator species, which implies increased energy expenditure to collect these food items, heterotrophic microorganisms (mainly ciliates) become a food source for metazooplankton of equal importance compared with phytoplankton. This hypothesis is reinforced by the observation that Synchaetidae found in samples from 3 July had ingested numerous large autotrophic cells (observed as large red spheres under epifluorescence microscope) in contrast to what was observed thereafter, indicating a possible switch to heterotrophic food items after this date, as further supported by the increased ingestion rates on ciliates' measurements (Table III). This shift in the main source of carbon to metazooplankton from phytoplankton when the latter is blooming, to protozooplankton when phytoplankton becomes relatively scarce, was also observed by Levine and collaborators (Levine *et al.*, 1999) in pelagic lake Champlain (USA-Canada). It is to be noted that the

size of phytoplankton on 16 and 30 July, dates on which ciliates constituted >50% of the rotifer diet, was essentially of GALD > 10 µm, contrary to the situation on previous dates of measurements (Fig. 2). This shift in phytoplankton size might also have contributed to the shift towards higher ingestion of ciliates.

It should be noted that all carbon fluxes presented here are sensitive to conversion factors to carbon mass. For phytoplankton, the conversion factor is based on an extensive data set from the River Meuse (Descy and Gosselain, 1994). For other groups, factors are those found and widely used in the literature and should be considered with more caution. Nevertheless, it is to be kept in mind that in microorganisms the ratio of key components to carbon can be affected when nutrient depletion occurs in their environment or in their resources. The lowland River Meuse is an environment where nutrients are virtually never limiting for the growth of primary producers (Gosselain *et al.*, 1994). It is thus likely that if a bias in conversion to carbon mass existed, it was constant throughout the study period; it should thus play no part in the differences observed between the fluxes during the early and late season and should not significantly interfere with our conclusions.

Moreover, if most of the results presented here are *in situ* or semi-*in situ* measurements, carbon fluxes from phytoplankton to metazooplankton are based on a model. This model that integrates abiotic and biotic factors is based on data collected in the studied site for many years and provides reliable estimates of phytoplankton grazing for Brachionids and Keratella-like rotifers as shown by Everbecq and coworkers (Gosselain, 1998; Everbecq *et al.*, 2001). Nevertheless, in the case of feeding rates of Synchaetids, estimates were based on literature data. Even if this group never dominated the rotifer community on experimentation days, it was well represented on the last two dates of measurements, implying a possible bias in the estimation of the carbon flux from phytoplankton on these dates.

In conclusion, this study provides evidence that bacterial production in the lowland River Meuse is not likely to be significantly channelled up to upper trophic levels when phytoplankton concentrations are higher than the ILL of the main rotifer species. Direct grazing on bacteria by metazooplankton has been shown to be negligible at all times, and indirect grazing through heterotrophic protists is low in such conditions. This contrasts with what happens when phytoplankton concentrations decrease enough to affect rotifer ingestion rate: in that case, carbon input from predation on ciliates can be equivalent to grazing on phytoplankton. The efficiency of carbon transfer from bacteria to metazooplankton can be questioned though, as predation of flagellates (the main consumers of bacterial production) by metazooplankton was shown to be low, and ciliates are apparently the main predators of flagellates in the river during the studied period. Nevertheless, low proportions of HF in the diet of rotifers can be nutritionally advantageous, as there is evidence that these organisms are a poor food for rotifers. Moreover, predation on heterotrophic protists that fed on phytoplankton (i.e. mainly ciliates) may be nutritionally advantageous for rotifers; it would also bypass long (and thus presumably less efficient) microbial food webs that involve bacteria and bacterivorous HF. These considerations emphasize the need to include trophic interactions between HF and ciliates on the one hand and phytoplankton and heterotrophic protists on the other hand in studies of microbial food webs in rivers. Regardless of their efficiency, such food webs seem to play a significant role within the plankton of the eutrophic River Meuse, ciliates apparently providing an alternative food source for rotifers when phytoplankton becomes scarce.

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