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Feeding of planktonic rotifers on ciliates: a method using natural ciliate assemblages labelled with fluorescent microparticles

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Abstract: *A method was developed to allow direct measurements of predation exerted by metazooplankton on ciliates. The method relied on the use of ciliates labelled with fluorescent microparticles (FMP). Optimal labelling conditions were determined with ciliates from cultures (Tetrahymena pyriformis) and with natural ciliate assemblages sampled in a river. Labelled T. pyriformis were used as tracer food to determine gut passage time (GPT) and ingestion rates of the rotifer Brachionus calyciflorus in the laboratory. Predation of metazooplankton from the lowland river Meuse (Belgium) was determined by labelling natural assemblages of ciliates and using them as tracer food for metazooplankters sampled in the river. Optimal labels of ciliates, i.e. sharp distribution of FMP in cells, were obtained with short incubations (10 min) and low FMP concentrations (1×10^5 mL^{-1}). GPT varied between 30 and 45 min for B. calyciflorus and from 25 up to >35 min for rotifers from the river. The ingestion rate of B. calyciflorus fed with T pyriformis was 3.3 ± 0.6 ciliate rot^{-1} h^{-1}, ie. 1.4 ±0.3 ng C rot^{-1} h^{-1}. Metazooplankton species for which the ingestion of ciliates could be measured were the rotifers Keratella cochlearis, Euchlanis dilatata and Synchaeta spp. Ingestion rates measured ranged fiom 0.4 to 12.5 ngC rot^{-1} h^{-1}. The method proposed proved to be useful in estimating the predation of microplankton on ciliates in semi- in situ conditions; in further developments, labelled natural assemblages of ciliates could be used for in situ incubations with the Haney chamber.*

INTRODUCTION

Ciliates have been reported in high numbers and biomasses in many freshwater aquatic ecosystems where they constitute from ~5 to 90% of total zooplankton biomass, with values around 50% being common (Massana *et al.,* 1996; Kobayashi *et al.,* 1998; Biyu, 2000). (For a short review in lakes, see also Gilbert and Jack, 1993.)

Both laboratory and field studies give evidence that many metazooplankton organisms feed on ciliates (Gilbert and Jack, 1993; Jürgens *et al.,* 1994; Hansen, 2000; Mohr and Adrian, 2000; Weisse and Frahm, 2002) at rates sometimes comparable to feeding on phytoplankton (Froneman *et al,* 1996; Nakamura and Turner, 1997; Adrian and Schneider-Olt, 1999; Thouvenot *et al.,* 1999a). Moreover, ciliates are considered as efficient feeders on heterotrophic flagellates and bacteria (Weisse, 1990; Šimek *et al.,* 1996; Hadas *et al.,* 1998; Premke and Arndt, 2000), which is not often the case for metazooplankters (Thouvenot *et al.,* 1999b; Kim *et al.,* 2000). Consequently, significant predation of metazooplankton on ciliates could result in high matter fluxes from microbial food webs to metazooplankton, thereby establishing a link between microbial productions and upper trophic levels of the food chain.

Most field studies investigating such links were based on long-term incubations (from ~7 h to several days) with manipulated natural planktonic assemblages (dilution method, size-fractionation method and incubations with metazooplankton densities varying from 1 x up to 16x the ambient levels). The main advantage of these kinds of methods is their technological simplicity. Nevertheless, separation of predators and preys through filtration is not always unambiguous as their sizes sometimes overlap or are very similar (Zimmermann, 1996; Paffenhöfer, 1998). It is noteworthy that the majority of studies investigating *in situ* predation on ciliates with such methods concern predation by microcrustaceans. It is indeed very rare to find results concerning predation by rotifers, and the few studies providing such data concern the genus *Asplanchna* that includes the largest species. Should these methods be used in ecosystems where small 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), separation difficulties are to be expected (C. Joaquim-Justo, personal observation, with samples from the river Meuse). Moreover, similar to what has been reported with dilution experiments, 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 the grazing activities of different taxa, which, as in the present study, could be important since it is to be expected that ingestion of ciliates by rotifers will vary greatly according to their species (Pourriot, 1977).

An advantageous alternative is provided by food-tracer techniques. Among possible labelling techniques for ciliates, indirect labelling through ingestion of fluorescent microparticles (FMP), as proposed by, for example, Dolan and Coats (Coats, 1991) and Cleven (Cleven, 1996), is particularly interesting. Indeed, unlike radiolabelling of tracer preys, this method allows the assessment of inter-individual variations and leaves no uncertainties concerning loss of label with preserved samples. It also offers the advantage over labelling with fluorescent dyes in that preys crushed in the feeding process can still be counted in the guts of predators through enumeration of FMPs and that it allows the quantification of ingested preys. Besides, labelling of prey organisms with fluorescent dyes often requires heat killing of cells to ensure proper staining, which in the case of motile organisms like ciliates is problematic. Vital dyes, on the other hand, pose the technical problem that residual quantities of dyes added to experimental media with labelled preys are sufficient to cause fluorescence of predators even when no prey is ingested, and this phenomenon renders counting of labelled preys in their gut difficult if not impossible (unpublished results). This phenomenon is to consider particularly in the case of labelled protozoans, as these are very susceptible to filtrations implied in the washing out of the dye, and thus, filtrations have to be kept as limited as possible.

An important concern common to all tracer techniques is the choice of the food items to be labelled. Indeed, these are supposed to be representative of all food particles of the investigated category. Using a single species as tracer food should be considered with caution, though, for many metazooplankton species have been shown to be selective about their potential prey on the basis of multiple factors such as size (Rothhaupt, 1990), taste (DeMott, 1986), surface texture (Mohr and Adrian, 2000), swimming/escape behaviour of their prey (Starkweather and Bogdan, 1980; Gilbert and Jack, 1993), not to mention the still poorly understood species-specific interactions (Wickham, 1995; Weisse and Frahm, 2001). Consequently, the use of a single labelled species (generally from cultures) might lead to bias. To avoid such discrepancies, natural assemblages should be preferred to prepared tracer food whenever possible. In this instance, however, homogenous labelling of the samples should be evaluated, as label can be taken up at very different rates by the various taxons comprised in the same category. This is yet another advantage of using FMPs for labelling purposes, since it allows through a simple epifluorescence observation a straightforward and thorough assessment of the labelling quality of all types of food present in the samples. Moreover, as the label is included in food vacuoles, it is expected that there should be no discrimination against labelled ciliates.

The aim of the present study was therefore to develop a method for measuring *in situ* predation of planktonic rotifers on ciliates using natural ciliate assemblages labelled with FMPs. Preliminary experiments were carried out with ciliates from cultures *(Tetahymena pyriformis)* and natural ciliate assemblages sampled from the river Meuse to determine proper labelling conditions. The method was implemented to measure ingestion of labelled *T. pyriformis* by the rotifer *Brachionus calyciflorus* in the laboratory. The rotifer species was chosen as it is widely distributed in freshwater ecosystems (Marneffe *et al.,* 1996; Lair and Reyes-Marchant, 1997; Viroux, 1997); it has also been shown to be a potential predator of ciliates (Gilbert and Jack, 1993). The ciliate was chosen because it is easily cultured and its considerable size (~40 µm) allows its easy handling; moreover, it has been reported to be preyed on by *B. calyciflorus* (Gilbert and Jack, 1993; Mohr and Adrian, 2000). The method was also implemented in field measurements carried out in the lowland river Meuse (Belgium) to determine the predation of zooplankton on the ciliate community of the river.

METHOD

Culture of test organisms

The ciliate *T. pyriformis* from the Culture Collection of Algae and Protozoa (CCAP) (Windermere, UK) was grown (axenic cultures) in proteose peptone yeast (PPY, CCAP) extract medium and kept in dim light at a temperature of 23 ± 2°C. Typical dimensions of the cells were ~35 µm in length and ~15 µm in width. Log-phase growth lasted for ~80 h after inoculation of medium; hence, experiments were carried out with ciliates sampled within 48 h after inoculation of PPY medium.

Rotifers of the species *B. calyciflorus* hatched from cysts (Microbiotest, Deinze, Belgium) were reared with *Dictyo-sphaerium ehrenbergianum,* a chlorella-like alga, at 23 ± 2° C in a light cycle of 16 h of illumination and 8 h of darkness.

Labelling of the ciliates

Preliminary experiments were set to determine the optimal FMP concentrations and incubation time ensuring a suitable labelling of ciliates. *Tetrahymena pyriformis* was incubated with FMPs (0.5 µm fluorescent

microspheres; Fluoresbrite YG carboxylate microspheres, Polysciences, Eppelheim, Germany) at concentrations of 1 x 10^6, 2 x 10^6 and 4 x 10^6 per mL for 5, 10, 15, 20, 30 and 60 min. Natural assemblages of ciliates were sampled from the river Meuse at Tailfer, Belgium (521 km from the source), and incubated at *in situ* temperature with 1 x 10^5, 5 X 10^5 and 1 x 10^6 FMPs mL^{-1} for times varying from 10 to 180 min. At the end of the incubations, protozoans were fixed with glutaraldehyde (final concentration 2%), stained with 4,6-diamidino-2-phenylindole (10 µg mL^{-1}) for 10 min and collected on 0.8 µm polycarbonate filters. Filtrations were carried out under a maximum pumping pressure of 130 mbar on filters stained beforehand with Irgalan Black (2 g L^{-1}) in a small volume of acetic acid 2% (v/v). Between 100 and 200 ciliates were examined at a x400 magnification under an epifluorescence microscope.

Label residence time was tested with *T. pyriformis.* Labelled cells were filtered on a 10 µm mesh plankton net, rinsed several times and resuspended in Volvic mineral water. Numbers of ciliates in the water and their FMP content were checked after 30, 60, 120 and 180 min.

Grazing experiments

Prior to all grazing experiments, labelled ciliates were separated from the FMPs by filtration on 3 µm polycarbonate filters (5.5 cm in diameter). To ensure satisfactory elimination of FMPs, a maximum of 50 mL of ciliate suspension was passed through each filter, rinsed twice with Volvic water and resuspended in 55 µm filtered river water. Filtration was carried out under a maximum pumping pressure of 130 mbar to avoid cell damage. It is to be noted that this procedure did not ensure total separation of labelled ciliates and non-ingested FMPs, residual concentrations generally being comprised between 1000 and 10 000 FMPs mL^{-1} in experimental media, i.e. approximately 1 % of the initial labelling concentration. These residual concentrations match those reported by Dolan and Coats (Dolan and Coats, 1991) for similar experiments.

Ingestion of *T. pyriformis* by *B. calyciflorus* was measured with 10 rotifers mL^{-1} and 600 ciliates mL^{-1} in six replicates. Rotifers were acclimated to experimental conditions (i.e. to the density of rotifers and ciliates to be used in the test) for 2 h prior to the measurements. After the acclimation period, rotifers were isolated from unlabelled ciliates through filtration on a 55 µm plankton net and resuspended in Volvic water, and labelled ciliates were added to the experimental media. Gut passage time (GPT) of *B. calyciflorus* was tested in these conditions, with incubation times varying from 5 to 120 min.

For measuring the ingestion rate of natural ciliate assemblages by metazooplankters, water collected from the river Meuse at km 521 (Tailfer, Belgium) was brought back to the laboratory immediately after sampling and placed in an incubator set at *in situ* temperature. Metazooplankton was concentrated 10 times and allowed to feed on the labelled ciliates for 15, 25 and 35 min. Two measurements were carried out in May 2001 and three in July 2001.

As it was not possible to wash out all the free FMPs from the suspensions of labelled ciliates, controls with concentrations of residual FMPs equivalent to those measured in experimental media were run in parallel to the experiments to account for direct ingestion of residual free FMPs by metazooplankton. To do so, the concentration of residual FMPs was measured in experimental media at the end of each incubation with labelled ciliates and the measured concentration (generally between 1000 and 10 000 FMPs mL^{-1}) was then added to a control which consisted of zooplankton and unlabelled ciliates in the same concentrations as in experimental media.

At the end of incubations, rotifers were narcotized in carbonated water, fixed with formaldehyde (final concentration 2%), collected on a 37 µm mesh and examined under an epifluorescence microscope for FMPs in their guts.

Specific ingestion rates of ciliates by metazooplankton (IR_{cil}; ngC ind^{-1} h^{-1}) were calculated using the equation:

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

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 *T. pyriformis* or ciliates from the

river, as calculated for each date on the basis of biovolume estimates using a conversion factor to carbon of 0.11 pgC μm^{-3} (Turley *et al.*, 1986), and *t* (minutes) is the duration of the incubation. IR_{cil} were calculated for species with numbers of animals observed higher than 20, while those taxa with numbers observed lower than 20 were pooled in a single category ('others').

RESULTS

Labelling of the ciliates

As can be seen in Fig. 1, the number of FMPs in *T. pyriformis* increased with time up to 14 FMPs per ciliate; the higher the concentration of FMP used, the steeper the slope. The proportion of labelled *T. pyriformis* varied from 58 to 100% and from 7 to 91 % with ciliates from the river Meuse (Fig. 2). In both the cases, the proportion of labelled ciliates increased with FMP concentration and with time.

The distribution of FMPs in T. *pyriformis* given in Fig. 3 shows that at the highest FMP concentration tested, the range of FMP number per ciliate was wide (from 1 to 15), with low proportions of individuals that had ingested the same number of FMPs. This was also the case with lower FMP concentrations when incubation times were long. On the contrary, with low FMP concentrations and incubation times of 10-20 min, a majority (~75%) of ciliates had ingested 1, 2 or 3 FMPs.

Label of *T. pyriformis* decreased with time, parallel to the division of the cells (Fig. 4); the FMP number in the ciliates decreased by a factor of ≤1.4 within the first hour of incubation in FMP-free medium.

Similar trends were observed with ciliates from the river (Fig. 5), the FMP distribution in the individuals tending to be broader at similar FMP concentration and incubation time though. Ciliates observed in river samples were divided into four categories: oligotrichs; small (~10 µm in length) ovoid forms that belonged to various taxa but that were impossible to identify on the basis of samples fixed and filtered; vorticellids; and 'others', i.e. a group comprising diverse and less abundant forms like *Monodinium* spp. and *Didinium* spp., tintinids, amoeba, etc. Oligotrichs and small ovoid forms accounted for ~75% of individuals in all samples; these two dominant categories seemed to ingest FMP at similar rates, contrary to vorticellids, whose bodies were often packed with high numbers of FMPs (~50) when other ciliates had only ingested <10. Nevertheless, the difference of label between these latter and most ciliates was such that metazooplankters that had ingested vorticellids were easily detected and excluded from the calculations. Importantly, it is to be noted that virtually no flagellate (also present in the samples) had ingested FMPs.

Fig. 1: *Label of the ciliate Tetrahymena pyriformis with different fluorescent microparticle (FMP) concentrations and incubation times.*

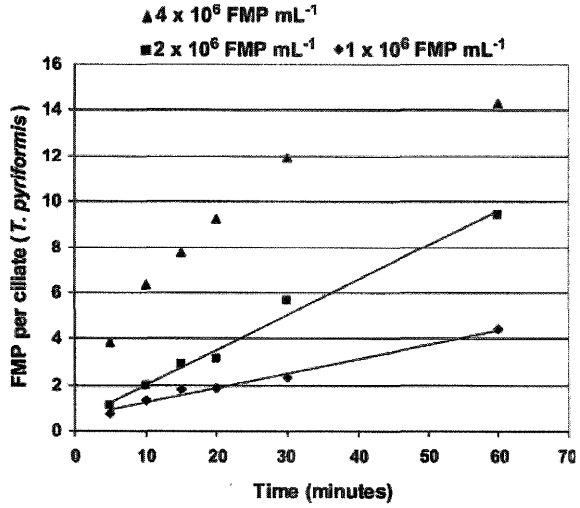
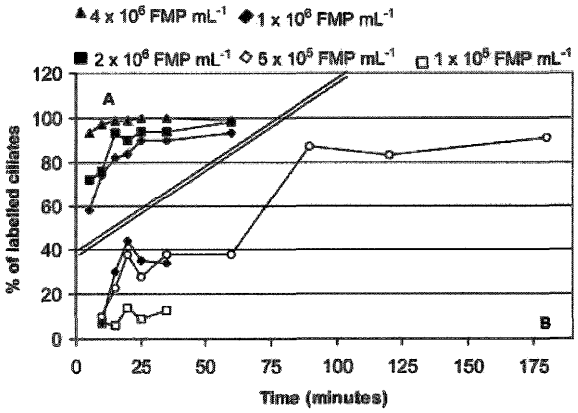


Fig. 2: *Proportion of ciliates labelled with different fluorescent micro-particle (FMP) concentrations in incubation medium and incubation times, a, Tetrahymena pyriformis, b, natural assemblage of ciliates from the river Meuse.*



Grazing experiments

To determine the GPT of *B. calyciflorus* feeding on *T. pyriformis*, two observations noted during the experiments need to be reported. First, as shown in Fig. 6a, it appeared that not all rotifers started feeding simultaneously; indeed, the proportion of individuals that had ingested ciliates increased with time, up to a maximum of ~80%, a maximum sometimes reached only after 80 min of incubation. Second, direct observation of the rotifers revealed that egestion of FMPs was not a progressive phenomenon where FMPs ingested replace FMPs egested but that animals emptied their guts of all FMPs at once. As a consequence, the mean number of FMPs in the gut of rotifers was not constant after GPT was reached but fluctuated and continued to increase. In this case, GPT is the incubation time corresponding to the last point before a decrease in mean FMP number in the gut of rotifers is observed. As can be seen in Fig. 6b, it was comprised between 30 and 45 min; consequently, incubations of 30 min were run for grazing experiments.

Fig. 3: *Distribution of fluorescent microparticles (FMPs) in Tetrahymena pyriformis according to FMP concentration and incubation time. Graph shaded indicate label conditions used in grazing experiments.*

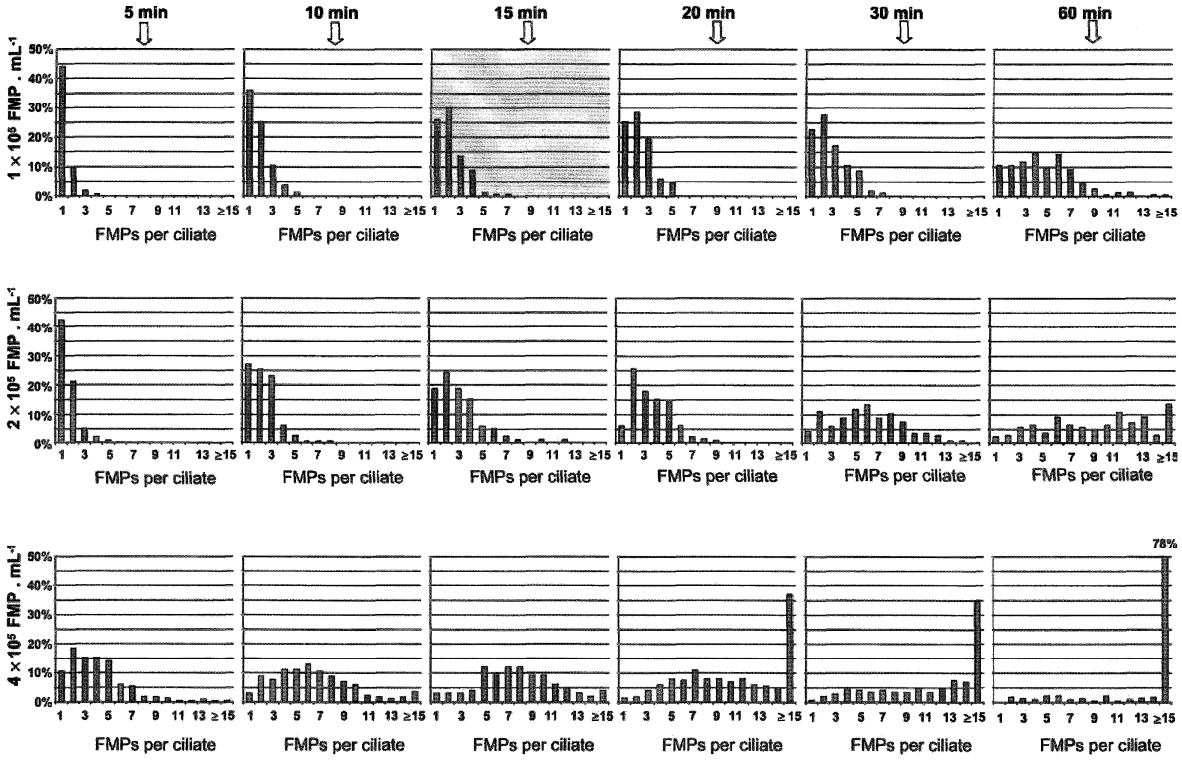


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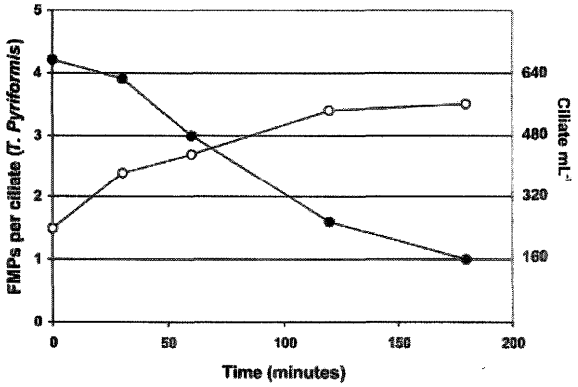
Fig. 4: *Evolution of Tetrahymena pyriformis label in fluorescent micro-particle (FMF)-free medium.*



For practical reasons, GPT of metazooplankton sampled in the river Meuse was estimated on the basis of less frequent sampling. Figure 7 shows the evolution of the ratio between the mean FMP numbers ingested by rotifers (all taxa pooled) and the maximum mean FMP number ingested on all incubation times; when this ratio is 1, it means that GPT is reached. It appears that this ratio was maximal after 35 min in May, while it was maximal after 25 min in July. GPT was thus assumed to be >35 min in May and comprised between 25 and 35 min in July; consequently, ingestion rates of metazooplankton were calculated on the basis of measurements at 35 min in May and at 25 min in July.

The ingestion rate of *B. calyciflorus* fed with *T. pyriformis* was 3.3 ± 0.6 ciliate rot^{-1} h^{-1}, i.e. 1.4 ± 0.3 ngC rot^{-1} h^{-1}, calculated assuming a biovolume of 3950 μm^3 per cell and a carbon content of 0.11 pgC μm^{-3} (Turley *et al.,* 1986). For metazooplankters from the river, feeding on ciliates could only be calculated for three species of rotifers due to the low abundances of other groups in the samples. Metazooplankton species for which the number of individuals observed in the samples was higher than 20 are *Keratella cochlearis, Euchlanis diltata* and *Synchaeta* spp. (Table I). *Keratella cochlearis* always ingested ciliates and exhibited ingestion rates varying from 0.4 to 12.5 ngC ind^{-1} h^{-1}; *Synchaeta* spp. only showed a quantifiable ciliate consumption during the last two campaigns, and its ingestion rate was a maximum 0.6 ngC ind^{-1} h^{-1}. A general trend emerging for other taxa when individuals of all sampling occasions were pooled is the absence of FMPs in the guts of *Polyarthra* spp. (*n* = 23), bdelloïds (*n* = 11), *Trichocerca pusilla* (*n* = 20), all Brachionids but *Brachionus angularis* (*n* = 28) and copepod nauplius larvae (*n* = 15). *Brachionus angularis* (*n* = 11) occasionally ingested FMPs, but the total FMPs ingested in control were in the same order.

DISCUSSION

Proportions of labelled ciliates were high with longer incubations times (Fig. 2); nevertheless, the distribution of FMPs in ciliates *(both* from cultures and from the field) proved to be more advantageous with shorter incubations and low FMP concentrations (Fig. 3 and 5). Indeed, the sharper the distribution of FMPs in the tracer food, the more accurately the numbers of FMPs counted in the guts of predators will be equated to the number of ciliates ingested. Consequendy, we considered incubations >10-15 min as inadequate for labelling of the ciliates. As higher FMP concentrations did not yield sharper distribution of label in the ciliates and separation of non-ingested FMPs and labelled ciliates was difficult, we advise against using such concentrations ($>1.10^5$ FMP mL^{-1}) in the labelling procedure. This is especially critical for ciliates from the river as they belong to various taxa and display a wider range of ingestion rates as compared with the inter-individual variations observed with *T. pyriformis.*

It is to be noted that, in similar experimental conditions, the proportion of labelled individuals observed with *T. pyriformis* was considerably higher than with ciliates from the river. This discrepancy may reflect the high ingestion rate of T. *pyriformis* compared to species found in the river, but it can also be due to a discrimination of some ciliates against artificial particles. Whatever the cause, the proportion of labelled ciliates in natural assemblages was generally ~10% (after 10 min with 1 x 10^5 FMP mL^{-1}). The low proportion of labelled preys was not a major problem in our experiments, as the labelled individuals belonged to the most abundant forms observed, but it implied that great numbers of ciliates had to be counted to ensure a proper estimation of mean FMPs in the cells. One should be cautious about the representativeness of labelled ciliates though, especially if the proportion of labelled preys is low.

An important observation to note is the absence of FMPs in flagellates also present in samples collected in the river; this indeed ensured that the measurements performed only concerned ingestion of ciliates by metazooplankters. It has to be noted though that in other environments, flagellates were shown to ingest such FMPs even if in low proportions (Jacquet, 2003).

GPT determinations are always crucial for ingestion-rate measurements based on tracer-food techniques; this is particularly true with the method developed here, since proportions of labelled ciliates are rather low (~ 10%) when label is qualitatively optimal, and numbers of ciliate ingested by rotifers are not very high on an hourly basis (Table II). Consequently, to observe statistically significant numbers of FMPs in metazooplankton, incubation times should be as long as possible. GPT measured with both *T. pyriformis* and ciliates from the river (from 25 to 50 min) are in the range of values reported for rotifers, i.e. from 16 to 45 min (Starkweather and Gilbert, 1977; Haney *et al*, 1986; Korstad *et al*, 1989). Rather than running short incubation times (~10 min), as is usually done with tracer-food techniques and rotifers, we recommend considering longer incubations combined with three different times to check for GPT, as described here for samples from the river. It is generally considered that the quantity of labelled food accumulated in the gut of Brachionidae and other filter-feeding zooplankters increases in time-course experiments up to a maximum, which corresponds to GPT, and stays constant further on. Our observations though imply that regular fluctuations of food in the gut should be observed in the case of *B. calyciflorus.* It is interesting to note that such a pattern was indeed observed by Haney *et al.* (Haney *et al.,* 1986) and Korstad *et al.* (Korstad *et al.,* 1989) who studied GPT of *B. calyciflorus* and *Brachionus plicatilis* respectively.

Fig. 5: *Distribution of fluorescent microparticles (FMPs) in ciliates from the river Meuse according to FMP concentration and incubation time. Graph shaded indicate label conditions used in grazing experiments.*

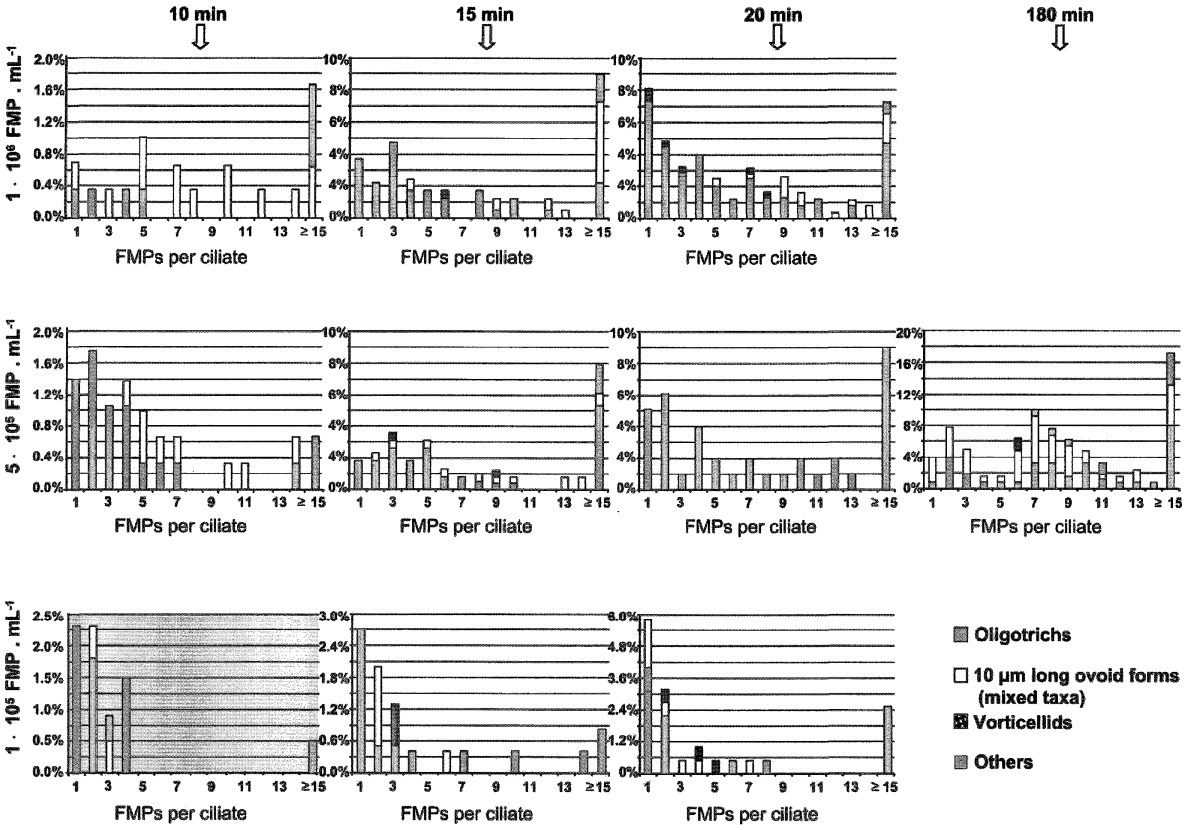


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Fig. 6: *Gut passage time of Brachionus calyciflorus fed with labelled Tetrahymena pyriformis. (a) Evolution of the proportion of B. calyciflorus feeding on labelled T. pyriformis with fluorescent microparticles (FMPs) in the gut with time. Different symbols are for different experiments. (b) Evolution of mean FMP number in B. calyciflorus fed with labelled T. pyriformis with time. Different symbols are for different experiments. Arrows: Gut passage time.*

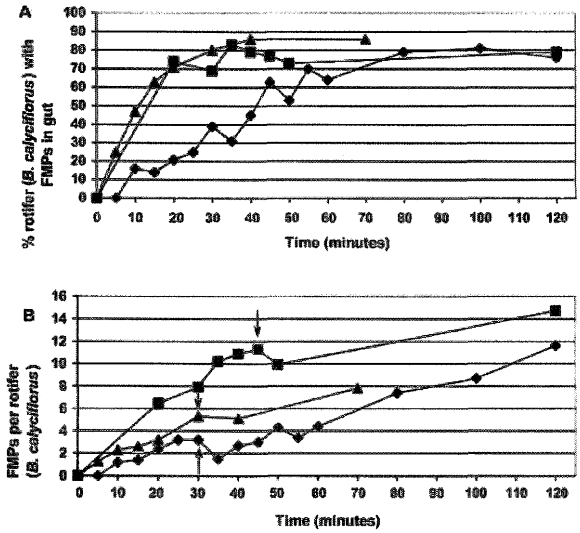
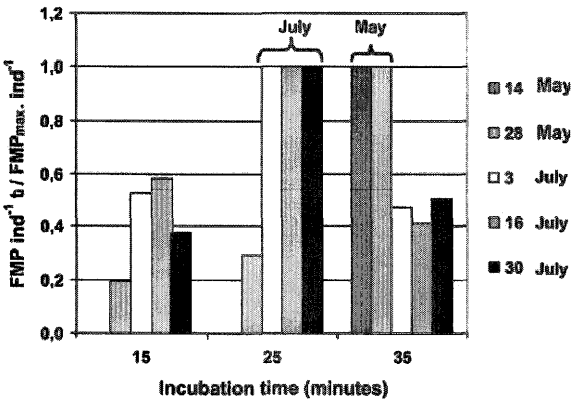


Fig. 7: *Ratio between ingestion rate of metazooplankters from the river Meuse at t_i (15, 25 and 35 min) and maximal ingestion rate.*



Ingestion rates of *B. calyciflorus* feeding on *T. pyriformis* measured with the FMP method match the range of values reported by others; most ingestion rates measured with field samples for other species are also comprised in the range of values reported in the literature (Table II). Rates measured for *K. cochlearis* in mid and late July are considerably higher though. 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 *Urotricha furcata)*, which could make them easier to tackle for a small species like *K. cochlearis* (width of ~35 µm). But other factors such as swimming/escape behaviour of ciliates and the presence of *Cryptomonas* cells in the *in vitro* experiments performed by Weisse and Frahm (Weisse and Frahm, 2001) might have interfered as well. Indeed, the two river samples in which this rotifer species exerted the highest predation rates on ciliates also coincided with samples with phytoplankton biomass lower than or close to the incipient limiting level determined for *K. cochlearis* in field measurements performed in the river Meuse, i.e. 1.5 mg C L^{-1} (Gosselain, 1998), which suggests the importance of ciliates in the diet of rotifers of this species when phytoplankton becomes rarefied.

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Table I: *Specific ingestion of ciliates by rotifers*

Species	**On *Tetrahymena pyriformis***	**On natural ciliate assemblage**				
		14 May	28 May	3 July	16 July	30 July
		IR_{cil} (ngC ind^{-1} h^{-1})				
Brachionus calyciflorus	1.4 ± 0.3[a]					
Keratella cochlearis		1.9	0.4	2.6	12.5	5.2
Euchlanis dilatata			0.7			
Synchaeta spp.		0		0	0.5	0.6
		IR_{cil} (cell ind^{-1} h^{-1})				
B. calyciflorus	3.3 ± 0.6[a]					
K. cochlearis		9.8	1.7	3.2	86.3	35.9
E. dilatata			3.4			
Synchaeta spp.		0		0	4.1	3.8

[a]Standard deviation of six replicates (n = 50 for each replicate). Ingestion rates (IR_{cil}) were only calculated for predator species with $n \geq 20$.

Table II: *Ingestion of ciliates by rotifers reported in the literature*

Rotifer species	**Prey**	**Ingestion rate**		**Source**
		Cells ind^{-1} h^{-1}	**ngC ind^{-1} h^{-1}**	
Brachionus calyciflorus	*Tetrahymena pyriformis* (46 µm)	0.8-1.4	0.5-0.8[a]	Gilbert and Jack (1993)
	T. pyriformis (45-15 µm)	6-6 ($/_{max}$)	3.9[a]	Mohr and Adrian (2000)
	Strobilidium gyrans (58 µm)	0.4-1.3		Gilbert and Jack (1993)
	Coleps sp. (45-15 µm)	7 ($/_{max}$)	4.1[a]	Mohr and Adrian (2000)
	T. pyriformis (40-20 µm)	3.3	1.3	This study
Brachionus angularis	*Cyclidium* sp. (20 µm)	1.1		Arndt, Jurgens and Zimmermann, unpublished in Arndt (1993)
Brachionus rubens	*Cyclidium* sp. (20 µm)	4.8		Arndt, Jurgens and Zimmermann, unpublished in Arndt (1993)
	T. pyriformis (45-15 µm)	6.6 ($/_{max}$)	3.9[a]	Mohr and Adrian (2000)
Euchlanis dilatata	River ciliate assemblage	3.4	0.7	This study
Keratella cochlearis	*Urotricha furcata* (2000 µm^3)	0.3-2.2	0.1-0.5[b]	Weisse and Frahm (2002)
	River ciliate assemblage	1.7-86.3	0.4-12.5	This study
Keratella quadrata	*Balanion planctonicum* (1820 µm^3)	1.5-5.1	0.3-1.0[b]	Weisse and Frahm (2002)
Synchaeta littoralis	*Strombidium* sp. (35 µm) field	1.5		Burckhardt, 1986 in Arndt (1993)
	Paramecium sp. (135 µm)	0.2-0.4		Burckhardt and Arndt, 1987 in Arndt (1993)
Synchaeta pectinata	*T. pyriformis* (46 µm)	1.2-2.1	0.7-1.2[a]	Gilbert and Jack (1993)
	S. gyrans (58 µm)	1-2.1		Gilbert and Jack (1993)
	Colpidium striatum (81 µm)	0.1-0.9		Gilbert and Jack (1993)
Synchaeta vorax	*Chilodonella* sp. (30 µm)	0.5-3		Arndt *et al.*, 1990 in Arndt (1993)
Synchaeta spp.	River ciliate assemblage	3.8-4.1	0.5-0.6	This study
Asplanchna girodi	*T. pyriformis* (46 µm)	1.4	0.8[a]	Gilbert and Jack (1993)
	Strombidium sp. (35 µm)	1.5		Burckhardt, 1986 in Arndt (1993)
Asplanchna brightwelli	*Paramecium aurelia* (140 µm)	0.6-5		Maly (1969)

[a]Values converted to biovolumes using a length of 45 µm and a width of 15 µm.
[a] and [b]Values converted to carbon using a conversion factor of 0.11 pgC µm^{-3} (Turley *et al.*, 1986).

It is to be noted that ingestion-rate estimations will be strongly impacted by significant standard deviation from mean FMP content of ciliates. Indeed, the assemblages sampled in the river Meuse were rather uniform, with 75% of cells being either small oligotrichs or 10 µm long ovoid forms. Moreover, ingestion rates of FMPs by individuals of these categories were quite similar (87% of individuals having ingested one or two FMPs). One should be cautious though that in environments where natural assemblages would comprise many different

categories of ciliates displaying ingestion rates of FMPs varying over a wide range, estimation of ingestion of ciliates by metazooplankton using the method described here would be very hazardous. Here we chose to express ingestion rates as carbon ingested per rotifer per unit of time to allow the comparison of feeding rates on different types of preys. This, however, implies a further bias in the estimation of the rates reported, as the carbon content of ciliates was not measured but estimated on the basis of biovolume measurements and a single conversion factor from biovolume to carbon found in the literature was used.

As mentioned in *Introduction*, in ecosystems where metazooplankton is dominated by small rotifers and where both predators and preys are present in low numbers, which is often the case in rivers (Scherwaβ, 2001), the use of community manipulation techniques for the study of predation of small metazooplankters on natural ciliates is problematic. This led us to opt for direct measurements to assess this potential trophic link between microbial food webs and upper trophic levels in the river Meuse.

The method described here proved to be an advantageous development of classical food-tracer technique. Indeed, the use of FMPs to label natural assemblages of ciliates allowed to avoid the killing of tracer cells, as would have been necessary if a fluorescent dye had been used, and to choose a single species as tracer food, as would have been the case if radiolabel had been considered. These advantages are particularly relevant as rotifers have been shown to exert different ingestion rates on food items with differences in mobility (Starkweather and Bogdan, 1980; Gilbert and Jack, 1993) and on ciliates of similar size, shape and mobility but different species (Weisse and Frahm, 2002). Moreover, as label was included in food vacuoles of preys, it is also expected that labelling did not alter their surface properties. This is an important factor since even the rotifer *B. calyciflorus*, generally considered as a species mainly selecting its food on the basis of size (DeMott, 1986), showed a marked preference for untreated *D. ehrenbergianum* as compared with 5-(4,6-dichlorotriazin-2-yl)aminofluorescein (DTAF)-stained algae (unpublished results).

A critical step of the method is the separation of free FMPs and labelled ciliates. As ciliates are fragile organisms, filtrations have to be gentle and limited, but on the other hand, separation has to be performed within reasonable lapse of time as vacuole turnover leads to significant egestion of label by ciliates after, for example, 1 h (Fig. 4), and even several rinses of the filters did not ensure total elimination of free FMPs in labelled ciliate suspensions. Overestimation of ingestion rate due to the presence of residual FMP concentrations in experiments could be estimated by running controls. However, it has to be mentioned that these difficulties multiplied the number of observations. Indeed, label of ciliates had to be assessed at the beginning and at the end of incubations, and for at least 200 cells, FMPs had to be enumerated in all experimental media, and considering that a minimum of a few hundreds of metazooplankters had to be examined per sample and that for each sample at least four different lots have to be analysed (three for GPT optimization and one control with residual FMP), time allocated to the countings and observations turned out to be considerable.

Nevertheless, the method proved to be promising and provided valuable measurements of predation exerted by small rotifers on ciliates. A further improvement of the method would be the inclusion of flagellates labelled with smaller FMPs of different fluorescence, provided that a sufficient proportion of flagellates would ingest such FMPs. This would indeed enable the measurement of the predation on both ciliates and flagellates simultaneously. Moreover, in the presence of sufficient metazooplankton abundances, this method could easily be extended to field incubation chambers as well.

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