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Clearance rates of the bdelloid rotifer, *Habrotrocha thienemanni*, a tree-hole inhabitant

Miloslav Devetter

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Abstract Bdelloid rotifers are basal consumers in aquatic and limnoterrestrial communities that feed primarily on small bacteria. Unfortunately, we know only a little of the role they play in the trophic dynamics in some unusual habitats they inhabit. *Habrotrocha thienemanni* is a typical example; it is a typical tree-hole inhabitant, commonly achieving dense populations. Filtering rates of *H. thienemanni* were estimated using fluorescent microspheres of a size close to natural bacterial community (0.5 μm in diameter) at two temperatures (15 and 20°C). This microspheres artificial food had been coated with BSA protein. Mean clearance rates of this rotifer varied between 1.65 and 3.79 μl ind^{-1} h^{-1} under different temperatures. Uptake of particles coated with protein was significantly higher than that on uncoated particles ($t = 2.85$; $P = 0.005$). Particle uptake also was correlated to the body size of the animal ($r = 0.44$; $P = 0.004$,). The clearance rate of the natural *H. thienemanni* population (56,800 ind l^{-1}) ranged from 981 to 5170 ml l^{-1} d^{-1}.

Keywords Temperature · Population CR · Filtering rates · Fluorescent particles

M. Devetter (✉)
Institute of Soil Biology, Academy of Sciences of the Czech Republic, Na sádkách 7, 370 05 Ceske Budejovice, Czech Republic
e-mail: devetter@upb.cas.cz

Introduction

Bdelloid rotifers are an important component of aquatic habitats, especially semiaquatic habitats, such as mosses, lichens and soil, and fallen leaf mass in forests (Bartoš 1959; Donner 1965). Unfortunately, the importance of bdelloid rotifers to the trophic dynamics of these habitats is rarely studied (Wallace et al. 2006). The rotifers are considered to be an important constituent of the microbial food web in freshwater habitats (Starkweather 1980; Arndt 1993). Monakov (2003) stated that Bdelloidea are microphagous and generally filter feeders and play a crucial role in the consumption of bacteria in soils and in the consequent carbon cycle. Pourriot (1977) suggested that many species of bdelloids are exclusively bacteriophagous (e.g., *Habrotrocha thienemanni*), while some are phytophagous (e.g., *Philodina citrina*) and others feed on both bacterial and plant matter (e.g., *Adineta vaga* and *Philodina roseola*). In contrast to monogononts from open water environments (Bogdan et al. 1980; Bogdan and Gilbert 1982; Boon and Shiel 1990; Ooms-Wilms et al. 1995), the feeding efficiency of bdelloids and their role in systems have not been well studied (Erman 1956; Wallace and Starkweather 1983).

In this study, I assess the efficiency of bdelloid filtering rates in comparison with monogononts, evaluate the potential of natural bdelloid population to control of bacterial communities, and test filtering effectivity of bacteria-sized particles.

Methods

Study organism

Habrotrocha thienemanni Hauer 1924 is a bdelloid rotifer typical of water-filled tree-holes (Bartoš 1959). The species is highly modest and adaptable to conditions and is easy to culture in lab conditions. Very dense, monospecific populations (tens of thousands l^{-1}) usually develop in tree holes. The mean size of experimental organisms, measured as width of contracted body was 76.2 ± 19.6 μ.

Grazing measurements

Animals were collected on 24 June 2004 from three adjacent maple tree-holes of similar conditions (*Acer pseudoplatanus* var. *arthropurpureum*) and the samples were mixed. The mean conductivity of the water was 711 ± 199 (SD) µS cm^{-1} and pH 6.7 ± 0.6 (SD) (Devetter 2004). The animals, in their source water, were placed into round experimental bottles (100 ml) and acclimated in the dark to experimental temperature of 15 or 20°C for 24 h. A grazing experiment was done using carboxylated fluorescent particles (Fluoresbrite, Polysciences©) in a size close to the size of the natural bacterial community (Table 1). Microspheres had been coupled by carbodiimide in the environment of carbonate (pH 9.6) and phosphate (pH 4.5) buffer respectively. Bovine serum albumin (BSA) protein was then coupled to microspheres in borate buffer (pH 8.5), stabilized by 2-aminoethanol and stored in storage buffer (pH 7.4). A pool of fluorescent particles (microspheres) measuring 0.5 µm in diameter was added into the experimental vessel to a final concentration of approximately 10^5 ml^{-1}. This density is an order lower than the natural bacterial community present in experiments. Uncoated microspheres serves as a control. After a 6-min exposure time, boiling water was added to stop the experiment. In the blank control, boiling water was added immediately after the microspheres were added. The content of the experimental bottle was filtered through a 40-µm filter, washed with distilled water and fixed with formaldehyde to 2% final concentration. Samples were analyzed under an epifluorescent microscope and microspheres ingested by animals were counted (Olympus BX 61). Natural bacterial community was analysed in epifluorescent microscope by Lucia © image analysis software (Laboratory Imaging Ltd.) after the bacteria were stained with DAPI.

Clearance rate (CR, µl ind^{-1} h^{-1}), the water volume filtered by an animal per unit time, was calculated from the following equation:

$$CR = (M_t - M_0)/C \times T$$

where M_t is the mean number of particles ingested by an individual, M_0 is the mean number of particles counted inside the animal in the blank (incubation time 0), C is the tracer particle concentration (µl^{-1}) and T is incubation time (= 0.1 h). Ingestion rate was calculated:

$$IR = CR \times B$$

where B is abundance of bacteria. It was assumed that the rotifers did not discriminate between tracer particles (microspheres) and natural bacteria (McManus and Fuhrman 1986).

Results

Habrotrocha thienemanni showed a significant uptake of bacteria-sized fluorescent particles through the experiments. Ingestion rate of the fluorescent

Table 1 Structure of particles subjected to filtration by *Habrotrocha thienemanni* in experiment (20°C, coated with BSA)

Abundance (10^3 ind ml^{-1})	Natural bacterial community $N = 220$		Tracer beads	
	1386		47	
	Mean	SD	Mean	SD
Width (µm)	0.29	0.08	0.49	0.02
Length (µm)	1.57	0.74	0.49	0.02
Volume (µm^3)	0.11	0.09	0.06	0.00
Carbon (fg ml^{-1})	22.56	12.84	–	

Natural bacteria were dyed by DAPI and analysed by image analysis as described in the text

Table 2 Ingestion (I) of bacteria-sized fluorescent particles coated by BSA and clearance rates (CR) by *Habrotrocha thienemanni* in tree-holes

Exp.	Temperature (°C)	*N*	Tracer conc. (part ml^{-1})	I mean (part ind^{-1} h^{-1})	CR mean ± SD (µl ind^{-1} h^{-1})
1	20	25	47,226	149.5	3.79 ± 2.1
2	15	25	91,512	154.4	1.65 ± 1.4

Zero values were omitted

particles in both the temperatures varied around 150 part. Ind^{-1} h^{-1}. The calculated mean clearance rate (CR) at the two temperatures is shown in Table 2. The mean clearance rate at 20°C was 3.8 µl ind^{-1} h^{-1} (±2.1 SD) and was more than twice that at 15°C (1.7 ± 1.4 SD µl ind^{-1} h^{-1}). However, clearance rate values up to 9.6 µl ind^{-1} h^{-1} were recorded in same trials.

Variation in clearance rate may reflect differences in the size of the animals. Particle uptake by *H. thienemanni* is significantly related to body size (Fig. 1), with larger rotifers ingesting more particles ($P = 0.004$, $r = 0.44$).

The clearance rates of particles coated with BSA protein and of uncoated particles showed minor but significant differences ($t = 2.85$, $P = 0.005$) (Table 3). The somewhat lower CR of uncoated particles might be due to the partial rejection of these particles. Using a population density of 56,800 ind l^{-1} the population clearance rate of *H. thienemanni* at 20°C in a tree hole was estimated to be 5306 ml l^{-1} d^{-1}.

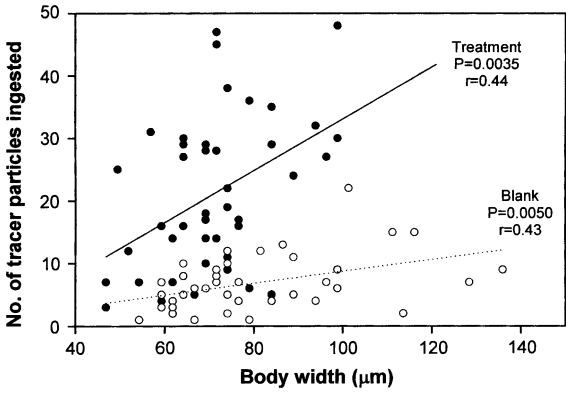

Fig. 1 Dependence of the number of ingested particles (coated) on body size of *Habrotrocha thienemanni* in experiments at 20°C. Body size was measured by width of the animals killed with hot water. Full symbol shows treatment, open symbol is blank

Discussion

Several published studies (Bogdan and Gilbert 1982; Boon and Shiel 1990) have estimated filtering rates of rotifers in lakes, but only a few have examined this activity in food of sizes <1 µm (Vadstein et al. 1993; Ooms-Wilms et al. 1995). In these studies, the clearance rate of planktonic monogononts varied from 0.01 to 0.3 µl ind^{-1} h^{-1}, except for *Brachionus plicatilis* (CR = 4.7 µl ind^{-1} h^{-1}) (Table 3). Notably, the filtering rates of *H. thienemanni*, measured by me were much higher than in almost all previous studies of planktonic monogononts. Consequently, the present study provides evidence of high filtering efficiency of the bdelloids feeding on the smallest size categories of potential food (Table 4).

Only two studies give data on the filtering rates of bdelloid rotifers. First, Erman (1956), measured the CR of *Philodina roseola* 0.88 µl ind^{-1} h^{-1}, and found it constant when feeding on *Scenedesmus acuminatus* culture. Second, Wallace and Starkweather (1983) estimated the CR of unidentified bdelloids from a small lake at 1.2 µl ind^{-1} h^{-1} using live, radioactively labeled *Enterobacter*. However, size of the food particles in the study of Erman is significantly larger than the food examined in present study, which corresponds to that available in tree-holes. A higher uptake of fluorescently labeled bacteria than of uncoated particles by rotifer species also was reported by Ooms-Wilms et al. (1995).

Table 3 Clearance rates (CR) of *Habrotrocha thienemanni* on particles coated and not coated with BSA protein at 20°C

Exp.	*N*	CR mean (µl ind^{-1} h^{-1})	DF	*t*	*P*
Coated with BSA	42	3.79	87	2.848	0.005
Not coated	47	2.32			

Two sample *t*-test

Zero values were omitted

Table 4 Clearance rates of planktonic rotifers from various studies and of *H. thienemanni*

Species	Food particles	CR µl ind^{-1} h^{-1}	Reference
Brachionus plicatilis	Small bacteria	4.7	Vadstein et al. (1993)
Pompholyx sulcata	Fluor. part. (0.5 µm)	0.01–0.04	Ooms-Wilms et al. (1995)
Brachionus angularis	Fluor. part. (0.5 µm)	0.03–0.06	Ooms-Wilms (1997)
Keratella cochlearis	Fluor. part. (0.5 µm)	0.01–0.02	Ooms-Wilms (1997)
Keratella cochlearis	Latex beads	0.3	Ronneberger (1998)
Habrotrocha thienemanni	Fluor. part. (0.5 µm)	1.7–9.6	This Study

Clearance rates reflect also specific preferences of different clones (Ooms-Wilms et al. 1993).

The estimation of CR of the entire population with particles coated by BSA at 20°C (highest number) was higher than values reported for planktonic animals in many classic and recent papers (Gliwicz 1969; Haney 1973; Jeppesen et al. 2002). This study evidenced the high potential of bdelloid rotifers to be the control of small-sized microbial communities in nature.

Although *H. thienemanni* was already described by Hauer (1924), it has reported thereafter only a few times (Bartoš 1959; Donner 1965), so that little is know of the ecological requirements of this bdelloid. This is despite the fact that this bdelloid species is probably common in tree holes or other phytotelmata. However, most studies dealing with tree hole habitats have focused mainly on insect larvae (Kitching 1971; Devetter 2004).

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