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PRIMARY RESEARCH PAPER

Live labeling technique reveals contrasting role of crustacean predation on microbial loop in two large shallow lakes

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Abstract We tested the hypotheses that the ciliate assemblages in moderately eutrophic lake are controlled by the effective crustacean predation, and the high abundances of planktonic ciliates in highly eutrophic and turbid lake are due to insufficient regulation by crustacean zooplankton. A food tracer method coupled with natural assemblage of microciliates labeled with fluorescent microparticles was used to measure the cladoceran and copepod predation rates on planktonic ciliates and to estimate the carbon flow between the ciliate–crustacean trophic links. The results revealed that the microciliates (15–40 μm) were consumed by all dominant cladoceran and copepod species in both the lakes studied, mainly by *Chydorus sphaericus* and cyclopoid copepods in Lake Võrtsjärv, and by *Daphnia* spp. and *Bosmina* spp. in Lake Peipsi. The grazing loss in moderately eutrophic Peipsi indicated strong top-down control of ciliates mainly by cladocerans. The extraordinary abundant population of planktonic ciliates having a predominant role in the food web in highly eutrophic and turbid Võrtsjärv is explained by the measured low crustacean predation rates on ciliates. The estimated carbon flow from the ciliates to crustaceans suggest that in eutrophic lakes majority of the organic matter channeled via metazooplankton to higher trophic levels may originate from the microbial loop.

Keywords Zooplankton feeding · Microbial loop · Ciliates · Predation · Trophic link

Introduction

With increasing system productivity, ciliates may become important members of the microbial loop (Sherr & Sherr, 2002). They can be the major bacteriovores, the most important early spring algivores, and the main consumers on heterotrophic nanoflagellates in lakes (Šimek et al., 1990; Weisse et al., 1990). Ciliates, in turn, serve as food for various metazooplankters (Wiackowski et al., 1994; Adrian & Schneider-Olt, 1999; Joaquim-Justo et al., 2004), thus forming an important linkage between the microbial production and upper trophic levels.

Both laboratory and field studies have highlighted the complex nature of interactions between ciliates and metazooplankton in natural communities, including the morphological and behavioral responses of ciliates to predators (Gilbert, 1994; Mohr & Adrian,

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2000; Ueyama et al., 2005) and the species-specific feeding, demonstrated especially for copepods (Wiackowski et al., 1994; Wickham, 1995a) and rotifers (Weisse & Frahm, 2002). Among crustaceans, the strongest structuring effect on ciliate assemblages is caused by selective feeding of copepods, capable of inducing a shift in size and composition of ciliates (Hansen, 2000; Zöllner et al., 2003). Large cladocerans (*Daphnia* spp.) as relatively unselective feeders influence a wide size range of ciliates and, if constituting a major component of zooplankton biomass, can substantially suppress ciliate abundances (Wickham & Gilbert, 1991; Pace & Vaqué, 1994; Zöllner et al., 2003). Other studies pointed out also a significant predation pressure by cyclopoid copepods (Wiackowski et al., 1994; Wickham, 1995a), which temporarily could even exceed the ciliate growth rate (Hansen, 2000). Therefore, seasonal and long-term shifts in ciliate and metazooplankton assemblages can lead to variable predation rates in natural communities. So far, very few direct predation estimations of different predator groups and species on ciliates in naturally occurring communities are available (Adrian & Schneider-Olt, 1999; Hansen, 2000; Joaquim-Justo et al., 2004). The estimation of the ciliate–metazooplankton link in terms of carbon transfer is crucial for understanding the regulation of pelagic food webs in lakes with different productivity.

Various types of food tracers (such as radioactively and fluorescently labeled cells and artificial fluorescent particles) have been used to directly estimate zooplankton grazing on phytoplankton and bacteria (e.g., Sanders et al., 1989; Hwang & Heath, 1999; Sommer et al., 2000). Planktonic ciliates are actively moving organisms that make the common application of food tracer techniques complicated. Among potential ciliate predators, the cyclopoid and calanoid copepods detect their prey mainly by mechanoreception (DeMott & Watson, 1991), and actively select for motile prey species. Therefore, for studying ciliate consumption, it is important that the tracer particles maintain the characteristics of motile living cells.

In this study, we applied the live labeling technique. Ciliates were labeled with fluorescent microparticles (FMP), as proposed by Dolan & Coats (1991), Smalley et al. (1999), and Joaquim-Justo et al. (2004) to directly measure the cladoceran and copepod predation on a natural assemblage of planktonic ciliates. The study was carried out in two large and shallow lakes with contrasting food webs—Peipsi and Võrtsjärv in Estonia. Both lakes can be characterized as eutrophic with the difference of high nutrient and seston concentration in Võrtsjärv referring to a highly eutrophic state, and with relatively lower nutrient level referring to a moderately eutrophic state in Peipsi. Earlier protozoans feeding experiments in Võrtsjärv showed that the FMP were effectively consumed by the dominant ciliate species belonging to oligotrichs and scuticociliates (Zingel et al., 2007; Zingel & Nõges, 2008); therefore, the method with natural ciliates labeled with FMP was assumed to be adequate to measure metazooplankton predation. The biomass of planktonic ciliates in these two lakes differs approximately by an order of magnitude being generally low in Peipsi, but forming more than a half of the total zooplankton biomass in Võrtsjärv (Zingel & Haberman, 2008). Also earlier grazing measurements have shown the higher importance of ciliates compared to metazoan zooplankton in the food web of Võrtsjärv (Kisand & Zingel, 2000; Zingel et al., 2007). Therefore, the specific objectives of this study were (1) to indentify the main metazooplankton species predating on ciliates, and (2) to test the hypotheses that (a) high abundances and the predominant role of ciliates in the food web of Võrtsjärv are due to a lack of top-down regulation by predators; and (b) that ciliate assemblages in Peipsi are controlled by crustacean predation.

Methods

Study sites

Lake Võrtsjärv is a large (270 km^2) and shallow (mean depth 2.8 m; maximum depth 6 m) highly eutrophic lake in central Estonia (58°05′–58°25′N and 25°55′–26°10′E). The average annual total phosphorus (dissolved + particulate) concentration is 54 μg l^{-1}, total nitrogen (dissolved + particulate) concentration 1.60 mg l^{-1}, and mean Secchi depth 1.1 m. The shallowness of the lake and the wave-induced resuspension of bottom sediments together with the algal blooms contribute to the formation of high seston concentrations and high turbidity during summer (Nõges et al., 1998a). The mean annual chlorophyll *a* concentration is 27 μg l^{-1} (Nõges et al., 2008).

Lake Peipsi (3,555 km^2) is located on the border between Estonia and Russia (57°51′–59°01′N and 26°57′–28°10′E). The lake consists of three basins of which the largest (2,611 km^2) and deepest (average depth 8.3 m) northern part, Peipsi s.s. is an unstratified eutrophic lake with mesotrophic features. The average annual total phosphorus concentration is 35 µg l^{-1}, total nitrogen concentration 0.7 mg l^{-1}, and mean Secchi depth 2.2 m (Starast et al., 2001). The mean annual chlorophyll *a* concentration is 19 µg l^{-1} (Nõges et al., 2008).

Several articles have dealt with the trophic relations in the Peipsi and Võrtsjärv (e.g., Haberman, 1998, 2001; Nõges et al., 1998b; Zingel & Haberman, 2008). In both lakes, the phytoplankton biomass is dominated by diatoms and cyanobacteria with relatively higher total biomass in Võrtsjärv (mean annual algal biomasses are 6.9 and 13.1 gWW m^{-3} in Peisi s.s. and Võrsjärv, respectively; Nõges et al., 2008). In Võrtsjärv, the filamentous phytoplankton are dominating all the year round: species of *Aulacoseira* in spring, *Plaktolyngbya limnetica, Limnothrix planktonica*, and *Aphanizomenon skujae* in summer and autumn. Algal blooms are common in the lake. In Peipsi s.s., the blue-green *Gloeotrichia echinulata* dominate in summer causing occasionally the water-blooms (Nõges et al., 1998a; Laugaste & Haberman, 2005). In zooplankton, the species characteristic to both eutrophic waters (*Daphnia cucullata*, *Bosmina c. goregoni*, *Anuraeopsis fissa*, etc.) and oligo-mesotrophic waters (*B. berolinensis*, *Bythotrephes longimanus*, etc.) occur concurrently in Lake Peipsi (Haberman, 2001), while relatively small metazooplankters (*Anuraeopsis fissa*, *Chydorus sphaericus*, *Bosmina longirostris*, etc.) known to be eutrophication indicators (Jeppesen et al., 2000) predominate in Võrtsjärv (Haberman, 1998). The mean weight of cladocerans is significantly larger in Lake Peipsi (mean individual wet weight 25 µg) than those of Võrtsjärv (8 µg) indicating that the metazoan zooplankton in Peipsi can be more effective transporters of energy than in Võrtsjärv (Haberman et al., 2007). In highly eutrophic Võrtsjärv, the benthic detrital food chain and the microbial loop are responsible for most of the transformation of organic matter. This is reflected also in the high bacterial production (21.5% of the total primary production) and the presence of an extremely abundant protist community (Nõges et al., 1998b; Zingel & Haberman, 2008). The average long-term bacterial abundances in Võrtsjärv and Peipsi are 3.7 × 10^6 cell ml^{-1} and 2.4 × 10^6 cell ml^{-1}, respectively (Lokk et al., 2001; Tammert & Kisand, 2004). The most abundant fish species in Võrtsjäv are bream, pikeperch, and pike (Järvalt et al., 2004). The fish community in Peipsi is dominated by smelt (Pihu & Kangur, 2001).

Sampling and counting of zooplankton communities

The study lakes were sampled at monthly intervals from June to September in 2005 in Peipsi, and in 2008 in Võrtsjärv. Lake water for ciliate and metazooplankton samples and for the grazing experiments was integrated by pooling the water collected with a 2-l Ruttner sampler at 1-m intervals over the entire water column. The depth of the sampled water column was 3–4 m in Võrtsjärv and 4–5 m in Peipsi depending on the water level. The sampling stations were located in the middle of the lake in Võrtsjärv and near-shore in Peipsi. We used one depth-integrated sample per lake and sampling time to estimate ciliate and metazooplankton abundances and biomasses. The samples were fixed with acidified Lugol's solution (0.5% final concentration). Ciliate biomass and community composition were determined using the Utermöhl (1958) technique. Volumes of 50 ml were allowed to settle for at least 24 h in plankton chambers. Ciliates were enumerated and identified with an inverted microscope at ×400–×1000 magnification. The entire content of each Utermöhl chamber was surveyed. Ciliate carbon content was determined from the measured biovolumes by adopting a conversion factor of 190 fg C µm^{-3} (Putt & Stoecker, 1989). Metazooplankton composition and biomass were analyzed in a Bogorov chamber in triplicate subsamples of 2.5 or 5 ml under a dissecting microscope at ×60 magnification. A fraction of 10–25% of the total sample size was counted. Crustacean carbon weight was estimated by means of length–dry weight relationships (Dumont et al., 1975) and a carbon/dry weight ratio of 0.5.

Labeling of the ciliates and grazing experiments

Metazooplankton predation rates on planktonic ciliates were determined in short-term grazing experiments using natural assemblages of ciliates labeled with FMP and using them as a tracer food for natural metazooplankton community. In the grazing experiments, no manipulations were made with metazooplankton community, and the feeding rates were measured with ambient metazooplankton composition and abundance.

For labeling the ciliates, the 15–40 μm plankton fraction (containing ciliates, phytoplankton, and detrital particles) was gently separated from the natural lake water by plankton nets and suspended into a small volume of filtered (GF/C, Whatman) lake water. FMP of 0.5-μm diameter (Fluoresbrite; Polysciences Inc.) were added to this suspension to reach a final concentration of 6×10^5 ml^{-1} and incubated for 10 min. The FMP concentration in the labeling media represented approximately 10% of the mean bacterial abundance in the studied lakes. The labeling was stopped by filtering the ciliates gently trough a 15-μm net to separate the ciliates from the non-ingested FMP. Ciliates were rinsed several times with GF/C-filtered lake water to eliminate the non-ingested FMP, and finally suspended into GF/C-filtered lake water. From this suspension, subsamples to estimate the ciliate labeling efficiency and the residual non-ingested FMP were taken and fixed with buffered formalin (1% final concentration). The state of ciliates was evaluated simultaneously with labeling efficiency. As the ciliates were able to feed and collect FMPs, their state was considered satisfactory. No additional state check was done. Still we must bear in mind that filtering may affect some of the ciliate species and thus the experimental ciliate community may differ from the in situ community. The labeling estimation confirmed no occurrence of heterotrophic nanoflagellates: the labeled microorganisms contained solely planktonic ciliates in both studied lakes. The residual FMP concentration (<0.5% of the initial labeling concentration) showed that the non-ingested FMPs were sufficiently eliminated and were assumed not to influence the feeding rates of predators in the following grazing experiments. To start the grazing experiments, the labeled ciliate suspension was added to 5-l integrated lake water containing the natural metazooplankton community at ambient abundance. The tracer cells formed approximately 20% of the natural lake ciliate numbers in the experimental water. The incubation container (polyethylene carboy, 5-l capacity) was kept in laboratory with temperature and light conditions similar to in situ and rotated gently during the incubation period. To determine appropriate incubation periods, time course measurements (up to 30 min) were made. Incubation lasted for 10–30 min, after which the water was filtrated through a 48-μm plankton net. Metazooplankton was anesthetized with carbonated water and fixed in 4% formaldehyde. Grazing experiments were carried out in 2005 in Peipsi, and in 2008 in Võrtsjärv once per month from June to September. On each date, one labeling and grazing experiment was made. As we did not measure the ciliate abundances after the incubations, there is a slight possibility of potential underestimation of metazooplankton grazing rates due to food limitation at the end of the experiment.

For FMP counting in ciliates, the samples were stained for 1–2 min with DAPI to reach a final concentration of 2 μg ml^{-1} and gently filtered through 0.8-μm pore-size black isopore filters (Poretics Inc.). Contents of the food vacuoles were examined with an Olympus BX60 fluorescence microscope under ×1000 magnification using blue light (470/505 nm, OG 515). At different dates, a total of 141–181 ciliates were analyzed for FMPs in Võrtsjärv, and 154–193 in Peipsi. For FMP counting in metazooplankton, individual zooplankters were collected from the samples and filtrated onto polycarbonate membranes of 10-μm pore size (Poretics Inc.). The number of FMPs in the guts of metazooplankters was counted at ×1600 magnification under an epifluorescence microscope (Zeiss Axiovert S 100). Whenever it was possible, 50 individuals of both copepods and cladocerans were analyzed in each experiment, and the total number of analyzed crustacean individuals varied from 73 to 110 in Võrtsjärv, and from 85 to 113 in Peipsi. Specific ingestion rates of ciliates by metazooplankton (IR; ciliates ind^{-1} h^{-1}) were calculated as follows:

$$\mathrm{IR} = \frac{\mathrm{FMP_{gut}} \times \mathrm{Cil_{lake}} \times 60}{\mathrm{FMP_{cil}} \times \mathrm{Cil_{exp}} \times t}$$

where $\mathrm{FMP_{gut}}$ is the mean number of FMPs in the gut of animals belonging to one species, $\mathrm{FMP_{cil}}$ is the mean number of FMPs per labeled ciliate at the onset of the experiments, $\mathrm{Cil_{lake}}$ is the abundance of ciliates in the lake (ciliates l^{-1}), $\mathrm{Cil_{exp}}$ is the abundance of labeled ciliates in the experimental medium (ciliates l^{-1}), and t is the incubation time (minutes). The analyzed metazooplankton individuals, which had no FMPs in their guts, were included into the calculations. The program STATISTICA for Windows was used for statistical

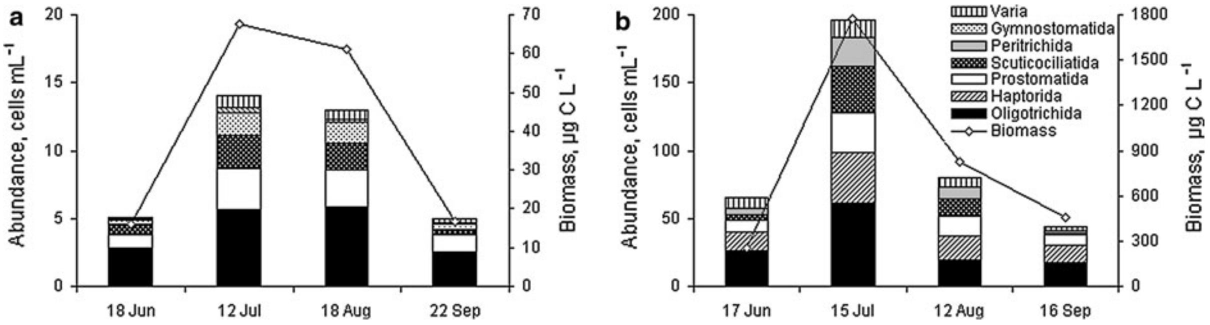

Fig. 1 Abundance, biomass, and composition of planktonic ciliates in Lake Peipsi in 2005 (**a**) and Lake Võrtsjärv in 2008 (**b**)

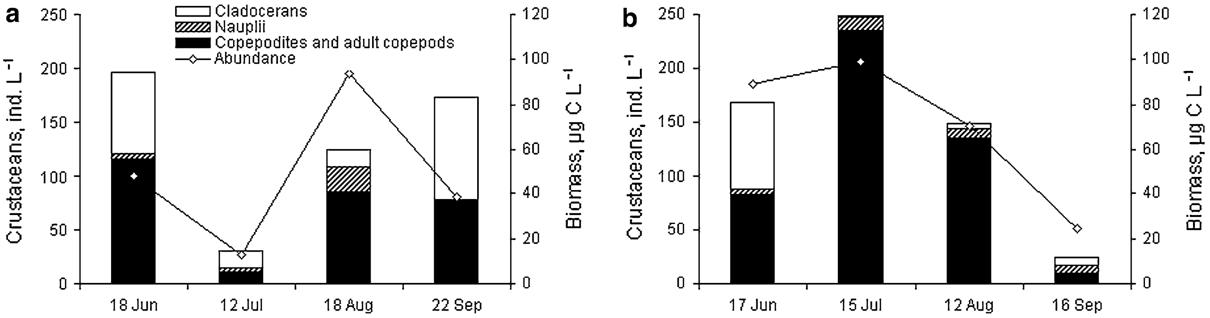

Fig. 2 Abundance and biomass of crustaceans in Lake Peipsi in 2005 (**a**) and Lake Võrtsjärv in 2008 (**b**)

analyses. The comparison between lakes was performed using the Mann–Whitney *U*-test.

Results

Ciliate and crustacean abundance, biomass, and composition

As shown in Fig. 1, the ciliate numbers and biomasses were significantly higher in Võrtsjärv (44–196 cells ml^{-1} and 252–1,774 µg C l^{-1}, respectively) than in Peipsi (5–14 cells ml^{-1} and 16.2–67.6 µg C l^{-1}; Mann–Whitney *U*-test, $P < 0.05$). In both lakes the maximum ciliate numbers occurred in July. Altogether, 28 ciliate taxa were identified in Peipsi where the community of ciliates was dominated by oligotrichs (*Rimostrombidium* spp., *Pelagostrombidium* sp., *Limnostrombidium* sp., *Codonella cratera*, and *Tintinnidium fluviatile*) and scuticociliates (*Uronema nigricans* and *Cyclidium claucoma*). Prostomatids (*Urotricha* spp. and *Balanion planktonicum*), peritrichs (*Vorticella* spp. and *Epistylis procumbens*), and gymnostomes (*Askenasia volvox* and *Mesodinium pulex*) were less abundant. Among the 62 ciliate taxa found in Võrtsjärv, the oligotrichs were most abundant, especially *Rimostrombidium* spp. and *Halteria grandinella*, followed by scuticociliates (*Uronema* sp. and *Cyclidium* spp.), prostomatids (*Urotricha* spp. and *Coleps* spp.), and gymnostomes (*Askenasia volvox* and *Mesodinium pulex*, Dileptus sp.). Ciliates within the size range of 15–40 µm, subjected for labeling in this study, formed an average 84% and 57% of total ciliate numbers in Peipsi and Võrtsjärv, respectively.

The crustacean numbers and biomasses did not differ significantly between the lakes (Mann–Whitney *U*-test, $P > 0.05$) and ranged during the study period from 27 to 195 ind l^{-1} and from 14.6 to 94.6 µg C l^{-1} in Peipsi, and from 51 to 207 ind l^{-1} and from 11.8 to 119.1 µg C l^{-1} in Võrtsjärv, respectively (Fig. 2). In

Võrtsjärv, the maximum numbers of cladocerans occurred in June (119 ind l^{-1}), followed by relatively low numbers onward. The cladoceran community was dominated by small *Chydorus sphaericus*. In Peipsi, the higher numbers of cladocerans occurred in June (35 ind l^{-1}) and September (43 ind l^{-1}). In Peipsi, several species of *Daphnia* (*D. cucullata*, *D. galeata*, and *D. cristata*) and *Bosmina* (*B. berolinensis*, *B. c. coregoni*, and *B. gibbera*) dominated in the cladoceran community. The copepod communities in both lakes were mostly formed by *Mesocyclops* and *Termocyclops*. In Peipsi, the calanoid *Eudiaptomus gracilis* also occurred in small numbers.

FMP ingestion by ciliates

The proportion of ciliates that contained FMPs did not differ significantly between the lakes (Mann–Whitney *U*-test, $P > 0.05$) and varied depending on the experimental dates, from 14.7 to 23.4% in Peipsi and from 18.3 to 24.7% in Võrtsjärv. The average number of FMPs per ciliate was 0.28 ± 0.11 (±SD) and 0.44 ± 0.09 in Peipsi and Võrtsjärv, respectively. The distribution of FMPs in labeled cells showed the highest proportion of ciliates containing 1 FMP, and the maximum ingestion of six FMPs per ciliate during 10-min incubation time (Fig. 3). The main grazers of FMP in Võrtsjärv were the oligotrichs (*Rimostrombidium* sp. and *Halteria grandinella*). The scuticociliates (*Uronema* sp. and *Cyclidium* sp.), prostomatids (*Urotricha* spp.), and peritrichs (*Vorticella* spp. and *Epistylis procumbens*) were less important. In Peipsi, scuticociliates (*Uronema* sp.) and peritrichs (*Vorticella* spp.) were the most effective picograzers.

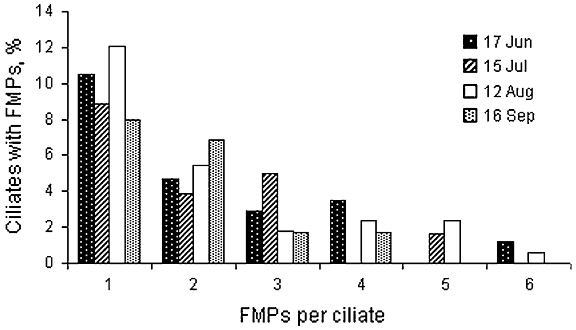

Fig. 3 Distribution of FMPs in natural planktonic ciliates during 10-min incubation in experiments in Võrtsjärv

Crustacean grazing on ciliates

Fluorescent microparticles were observed in guts of all dominant crustacean taxa in both studied lakes. Specific ingestion rates on ciliates differed between lakes and varied between sampling dates (Table 1). Considerably higher ingestion rates were measured for cladocerans in Peipsi compared to Võrtsjärv. *Chydorus sphaericus* was the cladoceran with the highest specific ingestion rate (up to 28.6 ciliates ind^{-1} h^{-1}) in Võrtsjärv and *Daphnia galeata* in Peipsi (up to 144 ciliates ind^{-1} h^{-1}). In Võrtsjärv, the copepodite stages of cyclopoids had generally lower ingestion rates than their adults; in Peipsi, only adult copepod ingestion on ciliates was observed. The crustacean community consumption did not differ significantly between lakes (Mann–Whitney *U*-test, $P > 0.05$) and ranged during the study period from 905 to 2,381 ciliates l^{-1} h^{-1}, and from 122 to 4,618 ciliates l^{-1} h^{-1} in lakes Peipsi and Võrtsjärv, respectively (Fig. 4). In both lakes, the highest ingestion rates occurred in June, followed by rather low rates, especially in Võrtsjärv. In Peipsi, cladocerans were the dominant ciliate predators throughout the study period (58.7–100% of total crustacean predation), whereas in Võrtsjärv, their predation prevailed only in June and September. The estimated carbon flux rates from ciliates to metazooplankton ranged from 74.6 to 154 µg C l^{-1} day^{-1} in Peipsi and from 21.4 to 370 µg C l^{-1} day^{-1} in Võrtsjärv (Fig. 4).

Discussion

Cladoceran and copepod predation on ciliates

The 15–40-µm size class of ciliates subjected to feeding experiments in this study formed the major part of the natural ciliate-standing stocks in the studied lakes and are known to be within the edible size range for both cladocerans and cyclopoid copepods (Wickham, 1995a; Adrian & Schneider-Olt, 1999; Zöllner et al., 2003). The uniform and sufficient labeling of the prey ciliates is a crucial aspect of the labeling method that we applied for studying metazooplankton grazing, given the different selectivity of natural ciliates belonging to various groups, and the use of artificial particles for labeling. The communities of planktonic ciliates in the study lakes were

Table 1 Specific ingestion on natural 15–40 μm ciliate assemblage by crustaceans in Lake Võrtsjärv and Lake Peipsi

Species	Ingestion rate (±SD) (cells ind^{-1} h^{-1})				
	June	July	August	September	*N*
Lake Võrtsjärv					
Daphnia cucullata			6.2 ± 12.3	17.2 ± 42.5	40
Chydorus sphaericus	28.6 ± 37.8	4.2 ± 10.3	5.4 ± 11.2	4.6 ± 11.1	100
Cyclopoid copepodites	33.1 ± 53.7	0.0	0.7 ± 3.6	4.9 ± 17.1	92
Adult cyclopoids	22.6 ± 62.3	5.8 ± 24.9	4.7 ± 22.8	7.4 ± 20.9	70
Lake Peipsi					
Daphnia galeata		144 ± 138		95.9 ± 114	32
Daphnia cucullata	135 ± 130	98.5 ± 80.2	40.1 ± 82.9	35.4 ± 67.3	65
Diaphanosoma brachyurum			85.6 ± 127		10
Bosmina spp.	32.7 ± 57.0	52.8 ± 67.9		21.9 ± 54.9	84
Adult copepods	26.0 ± 64.8	14.1 ± 31.5	23.8 ± 54.7	0.0	56

N number of observations

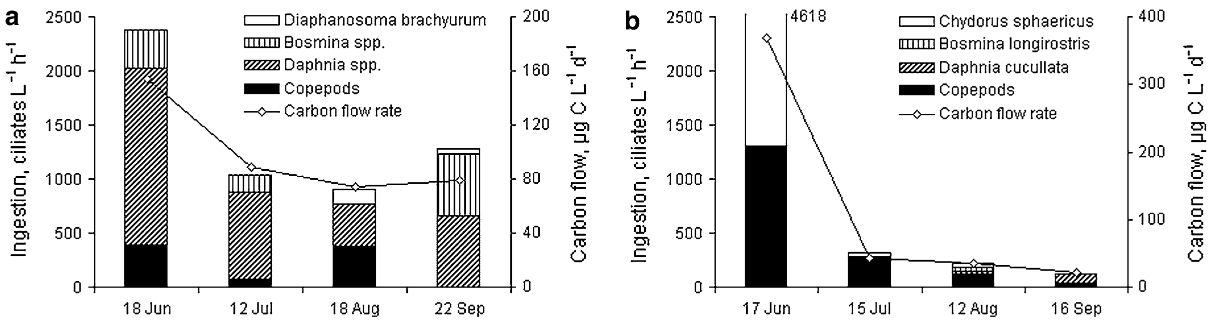

Fig. 4 Crustacean ingestion on natural 15–40 μm ciliate assemblages and carbon flow rate in Lake Peipsi in 2005 (**a**) and Lake Võrtsjärv in 2008 (**b**)

dominated by filter-feeding oligotrichs among which at least some species are reported as unselective feeders (e.g., Jürgens & Šimek, 2000). The labeling procedure yielded a rather uniform result: the best-labeled oligotrichs and scuticociliates belonged to dominant species and to the 15–40-μm size group. The proportion of labeled ciliates formed about 20% of the ciliate population and, among the labeled ciliates, 71% contained 1 or 2 microparticles. The ciliate labeling was sufficient to detect the feeding signals in potential crustacean predators.

Grazing experiments revealed that the 15–40-μm sized ciliates were ingested by all dominant cladoceran and copepod species in the studied lakes (Table 1). On a temporal scale, the highest specific ingestion rates occurred in both lakes in June, which is the period of rapid production and high biomass of metazooplankton in the studied lakes (Haberman, 1998, 2001). Comparable predation rates (1.7–86.3 cells ind^{-1} h^{-1}) with high seasonal variability measured with the same method are reported also for a rotifer species feeding on natural ciliate assemblage in river Meuse in Belgium (Joaquim-Justo et al., 2004). In natural environments, the ciliate ingestion may be severely affected by the size and species composition of ciliates (e.g., Wiackowski et al., 1994), and by the presence of alternative food for predators (Wickham, 1995a), consequently, this can lead to seasonally highly fluctuating ingestion rates. The overall specific ingestion rates for crustacean species measured in this study (Table 1) lay in the reported range for metazooplankton in both field and laboratory studies (Wickham, 1995b; Adrian & Schneider-Olt, 1999; Joaquim-Justo et al., 2004).

In Peipsi, substantially higher specific ingestion rates were measured for cladocerans compared to

Võrtsjärv. This can be explained by larger cladoceran species (Daphnia galeata, D. cucullata, *Bosmina berolinensis*, *B. c. coregoni* and *B. gibbera*) in Peipsi that probably have higher filtering rates, and are obviously capable of ingestion of ciliates of a wider size range (Zöllner et al., 2003) compared to relatively small cladoceran species (such as *Chydorus sphaericus* and *D. cucullata* and *B. longirostris*) in Võrtsjärv. Furthermore, in highly eutrophic and turbid Võrtsjärv with its abundant filamentous phytoplankton and resuspended particles, the cladocerans' encountering of and selectivity for ciliates could be severely interfered compared to moderately eutrophic Peipsi. This could especially be the case from July to September in Võrtsjärv, explaining the substantially reduced specific and total ingestion rates (Table 1; Fig. 4b) despite even higher number of ciliates and potential grazers (mainly cyclopoids) at that time. Intensive development of filamentous cyanobacteria (*Limnothrix planktonica*, *L. redekei* and *Planktolyngbya limnetica*; up to 30 mgWW l^{-1}) occurring in Võrtsjärv over summer (Nõges et al., 1998a) generates a high concentration of particles that may disturb feeding, cause lowered feeding rates (Chow-Fraser & Sprules, 1986; Levine et al., 2005), and create additional survival options for ciliates supporting also their high numbers in this lake. However, other factors such as the different ciliate community composition (e.g., Wiackowski et al., 1994), and the presence of alternative prey items for cyclopoids (Wickham, 1995a), such as algal cells and small metazoans, could play a role in the low predation rates. From June onward, the planktonic ciliate assemblage in Võrtsjärv was largely formed by small (<30 µm) picovorous ciliates, which may be too small prey or too hard to catch for copepods (Wiackowski et al., 1994; Wickham, 1995b; Adrian & Schneider-Olt, 1999; Hansen, 2000). Among dominant oligotrichs, the *Halteria grandinella* (that was also the main grazers on FMP in that lake) is known to be one of the most rapidly swimming ciliates, moving in swift jumps (Ueyama et al., 2005), which makes it probably hard to catch by metazooplankters (Gilbert, 1994). In epifluorescent microscopy, the copepods guts were usually packed with algal cells (observed as red spherical cells under fluorescence microscope—personal observations), which means, that copepods might prefer algal food instead of hunting small fast swimming ciliates. Furthermore, the lower ingestion rates from June onward (Table 1; Fig. 4b) suggest also that the crustaceans had to rely more on food items other than ciliates during the summer progress in Võrtsjärv.

Although copepod naupliar ingestion was not measured in this study, their role in ciliate predation cannot be neglected. Naupliar ingestion on ciliates can be expected to be lower than that of copepodites or adult stages (Merrell & Stoecker, 1998), but owing to their relatively high abundances (10–134 ind l^{-1} during the study period), the total crustacean predation on ciliates in lakes Võrtsjärv and Peipsi could be somewhat underestimated.

Quantitative importance of the crustacean-ciliate trophic link

Former studies have shown that the ciliate biomass in Peipsi is negatively correlated with the biomass of metazooplankton (Zingel & Haberman, 2008). In this study, the direct measurement in Peipsi revealed that the 15–40 µm ciliates were mainly ingested by cladoceran species of *Daphnia* (*D. galeata*, *D. cucullata*) and *Bosmina* (*B. berolinensis* and *B. coregoni*) (Fig. 4a). The increase of cladoceran numbers and biomass and their predation rates in June and September (Figs. 2a, 4a) coincided with the minimum ciliate abundances at the same time, showing the importance of metazooplankton grazing, and especially the cladoceran–ciliate trophic link, in regulating ciliate dynamics in Peipsi. The results are consistent with many other studies, showing that cladocerans, and especially the large-sized *Daphnia*, can effectively suppress ciliate abundances (e.g., Pace & Vaqué, 1994).

In Võrtsjärv, the correlation between ciliate and metazooplankton biomasses is positive (Zingel & Haberman, 2008), implying that the protozoan zooplankton dominating in this lake is clearly bottom-up controlled. The low crustacean ingestion rates on ciliates from July to September measured in this study (Fig. 4b) support our working hypothesis that the extremely abundant ciliate community and their dominant role in the food web of Võrtsjärv is due to the lack of top-down predation by metazooplankton. The metazooplankton of Võrtsjärv is mostly composed of small-sized grazers, such as *Chydorus sphaericus, Bosmina longirostris*, and small cyclopoids incapable of high ingestion rates (Agasild et al., 2007). Ciliates are shown to be the dominant consumers on small phytoplankton and bacteria in Võrtsjärv, consuming ca. 20% of the standing stock of nanoplankton, and nearly 100% of biomass production of bacteria during the vegetation period (Zingel et al., 2007), and thereby creating a high importance of microbial food web in that lake. In the light of the present results, however, the link between the classical grazing food chain and the microbial loop remains relatively weak in Võrtsjärv and a large proportion of energy transferred via the picoplankton–ciliate link remains unused by larger zooplankton. The situation may be somewhat different in the beginning of the vegetation period. In June, the relatively higher specific and community ingestion rates (Table 1; Fig. 4b) allowed for higher matter flux from the microbial loop to the higher trophic levels; the population of the abundant small cladoceran *Chydorus sphaericus* (116 ind l^{-1}) consumed more than 3,000 ciliates per hour and formed more than 70% of the total crustacean ingestion on ciliates. This confirms that these small cladocerans often dominating in eutrophic systems, and mostly considered as consumers of bacteria and small algal cells (e.g., Oom-Wilms, 1997), can also be important predators on ciliates. Ventelä et al. (2002) studied the effect of small zooplankton community dominated by *C. sphaericus* on microbial loop organisms and found significant species-specific response of planktonic ciliates; however, they could not establish the direct relationship between *Chydorus* and protozoans.

Despite the relatively low ingestion rates, ciliates can contribute a significant part of the diet of metazooplankton in Võrtsjärv. The estimated carbon flux rates from ciliates to metazooplankton in Võrtsjärv were comparable to those previously measured from phytoplankton to metazooplankton (8.1–206.4 μg C l^{-1} day^{-1}), which were calculated from the data of Agasild et al. (2007) using a biovolume conversion factor of 100 fg C μm^{-3} (Nixdorf & Arndt, 1993), and substantially higher than those published by Zingel et al. (2007) on carbon flux rates from bacteria to metazooplankton (0.01–0.6 μg C l^{-1} day^{-1}). Carbon flux rates from ciliates to metazooplankton estimated in our study lakes were significantly higher than the consumption of crustaceans on heterotrophic protozoa in oligotrophic Lake Michigan (0.3–11.2 μg C l^{-1} day^{-1}; Carrick et al., 1991) and in hypertrophic Lake Søbygård (2.8–23.5 μg C l^{-1} day^{-1}; Hansen, 2000). Assumingly, the relatively high crustacean biomass containing effective cladoceran predators enabled high flux rates in Peipsi. In Võrtsjärv, the very high ciliate densities resulted in a significant amount of carbon channeled through the ciliate–crustacean link, despite even the dominance of small-sized crustacean grazers.

The control of lower trophic levels via cascading effects is a widely occurring phenomenon in aquatic food webs. In some cases, planktonic ciliates have been shown as dominant phytoplankton grazers in lakes (e.g., Weisse et al., 1990); therefore, the effective control of the ciliate community by metazoans could favor phytoplankton growth. Based on the present result, it can be assumed that, despite ciliates being the dominant algal consumers in Võrtsjärv (Zingel et al., 2007), metazooplankton probably does not have significant control on algal dynamics via cascading effects, as they are not able to control ciliate abundance. Hence, in Peipsi where the crustaceans can effectively suppress the ciliate abundance, a positive effect on algal growth might be expected. Although not supported experimentally, the relatively larger contribution of small presumably edible algal cells (<30 μm) in Peipsi (on average 20% of total phytoplankton biomass; Laugaste & Haberman, 2005) compared to that in Võrtsjärv (on average only 10%; Agasild et al., 2007), may be an indication of the mediated (through ciliates) effect of metazooplankton on algae in lakes.

In conclusion, the specific predation rates determined in the present study demonstrate the numerous connections existing between the planktonic ciliate populations and crustacean zooplankton in lakes. The results revealed contrasting crustacean predation impacts on ciliate abundances in eutrophic lakes with an effective top-down control mainly by cladocerans in the moderately eutrophic lake, and relatively weak predation effect in the highly eutrophic and turbid lake. The further importance of the ciliate and crustacean link in present study is demonstrated by the carbon flow suggesting that, in eutrophic lakes, a significant amount of the organic matter channeled via metazooplankton to higher trophic levels may originate from the microbial loop. However, before making a judgment of this, more quantitative information on feeding links between ciliates and metazooplankton is needed for understanding the regulation of planktonic ciliate communities and more generally the pelagic food webs in lakes with different productivities.

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