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THE ROLE OF ZOOPLANKTON GRAZING IN SHALLOW EUTROPHIC LAKE ECOSYSTEMS

ZOOPLANKTONI TOITUMISE MÕJU MADALATE EUTROOFSETE JÄRVEDE ÖKOSÜSTEEMILE

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following papers, which are referred to by Roman numerals in the text. The papers are reproduced by kind permission of the publishers, Springer Science and Business Media (I, II, IV) and the Journal of Plankton Research (III).

I Tõnno, I., Künnap, H. & Nõges, T. 2003. The role of zooplankton grazing in the formation of 'clear water phase' in a shallow charophyte-dominated lake. *Hydrobiologia*, 506-509: 353–358.

II Agasild, H., Zingel, P., Tõnno, I., Haberman J. & Nõges, T. 2007. Contribution of different zooplankton groups in grazing on phytoplankton in shallow eutrophic Lake Võrtsjärv (Estonia). *Hydrobiologia*, 584: 167–177.

III Agasild, H. & Nõges, T. 2005. Cladoceran and rotifer grazing on bacteria and phytoplankton in two shallow eutrophic lakes: *in situ* measurement with fluorescent microspheres. *J. Plankton Res.*, 27(11): 1155–1174.

IV Zingel, P., Agasild, H., Nõges, T. & Kisand, V. 2007. Ciliates are the dominant grazers on pico- and nanoplankton in a shallow, naturally highly eutrophic lake. *Microb. Ecol.* 53(1): 134–142.

INTRODUCTION

The water column as well as the littoral zone of a lake is inhabited by numerous and various types of small unicellular and multicellular heterotrophic organisms called zooplankton. The role of these in the ecosystem is to process energy and organic matter in food webs. Depending on the composition of the community, their feeding has a marked impact on other plankton communities. Thus, zooplankton can affect the entire ecosystem through trophic relationships.

Since many shallow lakes experienced eutrophication during the 20th century, studies of the functioning of the ecosystem as well as food webs in shallow lakes have received increasing attention during recent decades. Investigations have revealed that shallow lakes alternate between macrophyte- and plankton-dominated states with different groups of primary producers. The great variability of ecological conditions in shallow lakes also results in more complex food webs. Several aspects of the role of zooplankton in the functioning of food webs in shallow eutrophic lakes, especially those involving microorganisms, are still poorly understood.

The present thesis is based on studies of zooplankton feeding in shallow eutrophic lakes in Estonia, with particular attention to a large, naturally highly eutrophic lake, Võrtsjärv. The impact of grazing by both proto- and meta-zooplankton was studied and compared to their impacts in two other small shallow eutrophic lakes in different alternative states.

1. REVIEW OF THE LITERATURE

1.1. The role of zooplankton in pelagic food webs

In freshwater systems the zooplankton consists primarily of protozoans (flagellates and ciliates), with rotifers, copepods and cladocerans among the metazooplankton. They inhabit the water column and exploit food sources from the surrounding environment, so they contribute to matter and energy flow in pelagic ecosystems.

The traditional view of aquatic ecosystems considers the grazing of metazooplankton on phytoplankton to be the most important carbon pathway from primary production to zooplanktivorous fish (the classic "grazer food chain") (Steele, 1974). Nowadays the concept of a "grazing food chain" is supplemented by the concepts of a "microbial loop", starting with bacteria consuming dissolved organic matter, and a "microbial food web", in which small ciliates together with heterotrophic nanoflagellates play a key role in transferring carbon from bacteria and pico- and nanoplanktonic autotrophs to higher trophic levels (ciliates, rotifers and larger zooplankton) (Azam et al., 1983; Sherr & Sherr, 1988). With increasing knowledge of the basic structure and function of the pelagic ecosystem, it has become evident that the continuum of various organisms living in the water column acts as a complex system, and the classic "grazer food chain" and "microbial food web" pathways interact through several direct (predator-prey relationships) and indirect (regeneration and consumption of nutrients) connections to become integrated components of a single planktonic food web (Riemann & Christoffersen, 1993) (Figure 1). The role of heterotrophs in the microbial food web is to mediate the transfer of dissolved and particulate organic matter to higher trophic levels; this matter might otherwise be lost to the food web (Sherr & Sherr, 1988). Because more trophic levels are involved in such energy transfer, and because of losses due to respiration at each step, the "microbial food web" is regarded as a less efficient energy pathway than the classic "grazer food chain" (Pomeroy & Wiebe, 1988). However, there are "shortcuts" that make it possible to reduce carbon losses. In some eutrophic lakes, dense populations of cladocerans (e.g. *Daphnia*) (Pace et al., 1990; Christoffersen et al., 1993) and even rotifers (Hwang & Heath, 1999) have been found to consume a substantial portion of bacterial production, thus bypassing the microbial food web and transferring the bacterial carbon directly to higher consumers.

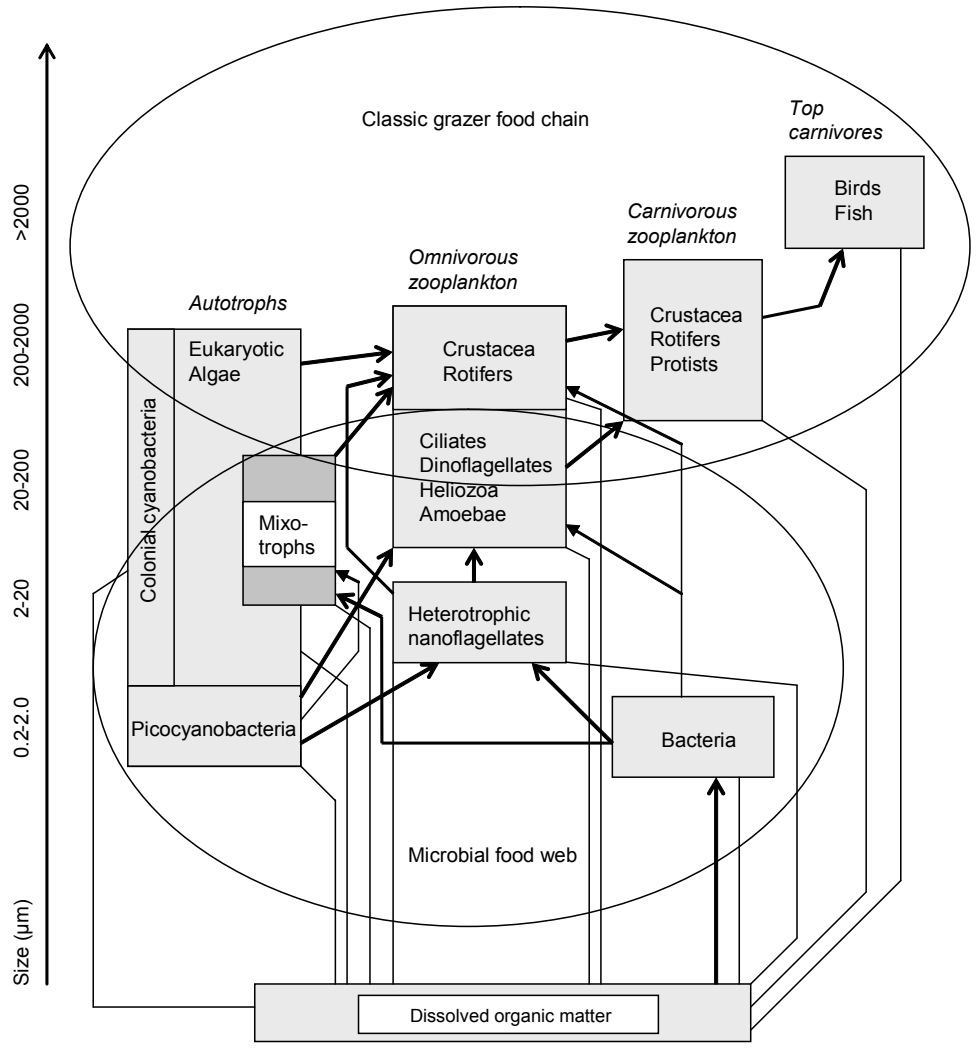

Figure 1. Structure of the planktonic food web in lakes. Algal production is transferred along the classic "grazer food chain" via omnivorous and carnivorous zooplankton to top predators. Microorganisms form the "microbial food web", which in part overlaps with the "grazer food chain". Feeding interactions and bacterial substrate uptake are indicated by solid lines and arrow heads. The pool of dissolved organic matter is replenished by various release processes (excretion, exudation, cell lysis, "sloppy" feeding) and used as substrate by bacteria (modified from Weisse, 2005).

Schematic representations of food webs are mostly simplified, showing aggregation of species into major functional groups or guilds and interactions between them. Within each group, however, organism sizes range widely (Figure 1). An important characteristic of the food web is that the functioning processes in it (e.g. size-selective predation, feeding and nutrient excretion rates) are to a large extent based on size-dependent relationships (Bergquist et al., 1985; Hansen et al., 1994; Hansen et al., 1997; Jeppesen et al., 2000).

1.2. Effect of zooplankton feeding on food web structure

Plankton communities are structured by the simultaneous effects of selective feeding and competition for resources. Several issues concerning food, such as threshold food concentrations (Duncan, 1989; Kirk, 2002), feeding overlap and competition between consumers (Weisse and Frahm, 2002), and nutritional quality (Ahlgren et al., 1990; Mohr & Adrian, 2002), are noted as important factors in regulating seasonal species and community successions of zooplankton. Predation is considered to be the major cause of mortality, affecting the biomass, structure and average size of the zooplankton community (Gliwicz & Pijanovska, 1989; Jürgens & Jeppesen, 2000). A general feature of more eutrophic systems is that top-down regulation is a stronger shaping factor for the plankton community than food resources (bottom-up regulation) (Jürgens et al., 1999; Jeppesen et al., 2000). In turn, through feeding, zooplankton alter the plankton communities along trophic relationships. Cascading trophic interactions are known to play an important role in transferring the effects of fish predation via zooplankton to lower trophic levels (Carpenter & Kitchell, 1993; Christoffersen et al., 1993).

As a whole, the zooplankton community is adapted to utilize a wide range of food organisms. Because they live in a medium of suspended particles, among which small unicellular algae, bacteria and detritus are the most abundant food resources, suspension feeding is the most common mode of food collection among zooplankton; it is used by most cladocerans, calanoid copepods, and by rotifers and ciliates. A raptorial type of feeding is generally used for capturing larger and less abundant food objects such as other animals (ciliates and small metazoans) or larger algal cells (Gliwicz, 2005). However, within groups in the zooplankton community, feeding is more variable. Detailed laboratory studies have

revealed several modes of food collection and selectivity for cladocerans (DeMott & Kerfoot, 1982; Vanderploeg, 1990; Kerfoot & Kirk, 1991), copepods (Williamson, 1980; Vanderploeg & Paffenhöffer, 1985; DeMott; 1986), rotifers and protozoans (Starkweather, 1980; Fenchel, 1987; Wetzel, 2001). As ciliates prefer a similar size-feeding spectrum of about 3-20 µm, they are the protozoans that compete with metazoan zooplankton for the same food size basis (Weisse & Frahm, 2002). Although it has often been demonstrated that particle size is the most important factor in food selection (DeMott, 1989), other properties of a food particle such as shape, taste, texture and biochemical composition are also involved in the selection process, making food choice more complex (Knisely & Geller, 1986; DeMott & Moxter, 1991; Kerfoot & Kirk, 1991; Mohr & Adrian, 2002).

Owing to their divergent feeding abilities and food selection, differently-dominated zooplankton communities can differ markedly in grazing rates and impact on plankton communities (Pace et al., 1990; Cyr & Pace, 1992; Jürgens & Jeppesen, 2000). Strong group-specific impacts on planktonic protozoa and bacteria have been reported for daphnids, copepods and rotifers (e.g. Arndt, 1993; Jürgens, 1994; Zöllner et al., 2003). In the trophic cascade, high metazoan grazing pressure may result in significantly altered plankton communities. Most significant changes have been associated with the presence/absence of *Daphnia* species. Large *Daphnia* can graze down phytoplankton biomass and affect the species composition (Vanni & Temte, 1990; Vakkilainen et al., 2004), and have a strong influence on the structure of microbial food webs both by suppressing the biomass of heterotrophic flagellates and ciliates and by grazing on bacteria (Christoffersen et al., 1993; Jürgens, 1994; Jürgens & Jeppesen, 2000). On the other hand, low metazooplankton grazing, which is usually associated with zooplankton communities dominated by small-size cladocerans, cyclopoid copepods and rotifers, can lead to uncontrolled ciliate growth and consequently to low biomass of small nanoplankton, but also to an increase in the biomass of large phytoplankton (Berquist et al., 1985; Jürgens & Jeppesen, 2000; Ventelä et al., 2002; Stephen et al., 2004).

Seasonal shifts in species composition of freshwater zooplankton are also reflected in structural and functional changes in the food web.

1.3. Impact of zooplankton grazing in shallow eutrophic lakes

Studies of shallow lake ecosystems have received increasing attention since the late 1980s. Since then, basic theories of the functioning of shallow lakes have emerged (Moss et al., 1997; Sheffer, 1998; Jeppesen et al., 1998). According to the state-of-art theory, shallow lakes have two alternative states, a vegetation-dominated clear-water state and a turbid plankton-dominated state (Sheffer, 1998). Switching between these states is abrupt; the ecosystem's response to increasing eutrophication is not linear but follows a sigmoidal hysteresis curve. Both states are stabilized by a number of feedback mechanisms, among which macrophytes are most important for stabilizing the clear water state by maintaining water transparency by direct and indirect effects on phytoplankton growth. The turbid water state is further stabilized by planktivorous fish, which suppress the larger filter feeders and permit extensive phytoplankton growth (Moss et al., 1997; Sheffer, 1998, Jeppesen et al., 1998). The effects are thus transmitted throughout the ecosystem. The originally identified shifts between a clear and turbid state remains one of the most contrasting examples. In the light of recent studies (Sheffer & van Nes, 2007), temperate shallow lakes may be dominated alternatively by charophytes, submerged angiosperms, green algae or cyanobacteria as the ecosystem responds to environmental changes. The large variability of ecological conditions also results in more complex food webs in shallow lakes.

Most studies of shallow lake food web functioning have dealt with the issue of phytoplankton-zooplankton interactions. Through refugees and suitable algal food, macrophyte-rich lakes support the development of communities of large zooplankton (often dominated by *Daphnia*) that are effective in phytoplankton grazing (Schriver et al., 1995). Therefore, in many plant-dominated shallow eutrophic lakes, zooplankton grazing has a major effect on the phytoplankton biomass and plays a major role in maintaining clear water conditions (Jeppesen et al., 1999). However, there are examples of lakes in which even a relatively low zooplankton grazing pressure (10-20% of the phytoplankton biomass per day) could contribute to low phytoplankton density (Blindow et al., 2000). In these cases, grazing would not be the main limiting factor for phytoplankton, but would play a role in combination with other limiting factors.

In plankton-dominated lakes, various different factors may affect zooplankton grazing. The high abundances of cyanobacteria, which often

dominate in algal communities at higher nutrient levels (Jeppesen et al., 2000), can prevent an effective control of the phytoplankton by zooplankton grazing. Cyanobacteria are known to be of poor food quality for zooplankton in respect to their low nutritional value (Ahlgren et al., 1990), potential toxity (DeMott & Moxter, 1991) and filamentous structure resulting in interference with feeding process (DeMott et al., 2001). A similar disturbing effect on zooplankton food gathering has been observed for resuspended sediments in shallow lakes (Kirk, 1991; Levine et al., 2005). As substantial part of phytoplankton appears not to be available as suitable food for zooplankton in shallow eutrophic lakes (Nõges et al., 1998a), the alternative food sources such as bacteria and detrital particles, which often occur in higher concentrations than living algal cells (Mann, 1988), would be an important food supplement to metazoan grazers, especially at times with limited supply of edible phytoplankton (Christoffersen et al., 1990; Kerfoot & Kirk, 1991). Both bottom-up and top-down effects force the zooplankton community towards the dominance of small-bodied zooplankters (Schriver et al., 1995; Jeppesen et al., 2000; DeMott et al., 2001), small cladocerans (e.g. *Bosmina* spp. and *Chydorus sphaericus*), rotifers (e.g. *Keratella cochlearis* and *Anuraeopsis fissa*) and cyclopoid copepods (Ooms-Wilms et al., 1995; Haberman, 1998), supporting also the development of large populations of protozoans (Mayer et al., 1997; Jürgens et al., 1999).

Although small zooplankters have occasionally been reported to reduce total algal biomass in shallow lakes (Jeppesen et al., 1990), small-bodied zooplankton cannot generally control the biomass of a blue-green-dominated phytoplankton community (Christoffersen et al., 1990; Jeppesen et al., 1996; Ventelä et al., 2002). Most primary production in shallow eutrophic lakes is subject to bacterial decomposition (Nõges et al., 1998a) and will be channelled via the microbial food web. Heterotrophic microorganisms may constitute a substantial amount of zooplankton biomass in eutrophic lakes (Zingel, 1999), and in some cases protists, especially heterotrophic nanoflagellates, have been shown to consume most of the bacterioplankton as well as autotrophic picoplankton production (Šimek et al., 1997). Therefore, the microbial food web may be very important in the functioning of turbid and shallow eutrophic lakes (Zingel et al., 2007). However, simultaneous observation of the succession of bacterial and phytoplankton consumption by metazoans and protozoans has rarely been attempted and only in few occasions in eutrophic lakes (Work & Havens, 2003). As in eutrophic systems, the role of the microbial food

web seems to be strongly connected to the structure of the zooplankton community (Riemann & Christoffersen, 1993; Zöllner et al., 2003); its impact on autotrophic and heterotrophic food sources and the carbon flow to higher trophic levels is quite poorly predictable.

Despite the increasing number of papers on the functioning of food webs in shallow lake ecosystems, there are still open questions about the role of zooplankton in food webs, primarily in the microbial food webs of shallow lakes in different ecological states. Also, only a few studies have considered different zooplankton groups, including those from microbial communities, as grazers, and have addressed their consumer-effect on standing stocks and the production of main food sources. For understanding of the structure and functioning of the pelagic ecosystems, it is therefore important to consider all the major components of the planktonic food web.

2. AIMS OF THE STUDY

The principal aim of the present study was to evaluate the impact of zooplankton grazing on bacteria and phytoplankton in shallow eutrophic lakes. The main questions addressed were:

1. What is the community impact and seasonal pattern of zooplankton grazing in shallow eutrophic lakes with variable ecological states? (**I, II**)

2. How do different zooplankton groups (heterotrophic nanoflagellates, ciliates, rotifers, cladocerans, zooplankton size-groups) act as grazers on pico- and nanoparticles in shallow eutrophic lakes? (**II, III, IV**)

3. Which zooplankton taxa are the main grazers on bacteria and phytoplankton in shallow eutrophic lakes? (**III**)

3. MATERIALS AND METHODS

3.1. Study area

Studies **II, III** and **IV** were carried out in Võrtsjärv, a large (270 km^2) and shallow (mean depth 2.8 m, maximum depth 6 m) eutrophic lake situated in Central Estonia. The average total phosphorus concentration (TP) is 0.054 mg L^{-1} and the total nitrogen concentration (TN) is 1.6 mg L^{-1}. The shallowness of the lake and the wave-induced resuspension of bottom sediments contribute to the formation of high seston concentrations and high turbidity during summer (Haberman et al., 1998). The large and open northern and central part of the lake acts as a typical plankton-dominated lake. The southern part of the lake is narrow, very shallow (<1.5 m) and filled with submerged macrophytes. Nutrient concentrations increase slightly in the north-south direction because the largest inflow from the Väike Emajõgi River in the south (Tuvikene et al., 2004).

Paper **II** focuses on the plankton-dominated part of Võrtsjärv. In paper **IV** the plankton-dominated and the southern macrophyte-dominated compartments of Võrtsjärv are studied separately. In paper **III** Võrtsjärv is compared to a smaller lake, Kaiavere, which is a shallow plankton-dominated lake in East Estonia with an area of 2.51 km^2, a mean depth of 2.8 m and a maximum depth of 4.5 m (Mäemets, 1968, 1977). Kaiavere is an eutrophic lake, with average TP and TN of 0.042 mg L^{-1} and 1.52 mg L^{-1}, respectively (Nõges et al., 2003). Paper **I** focuses on the small (0.399 km^2) and shallow (mean depth 2.2 m, maximum depth 4.2 m) eutophic Lake Prossa in the trumline area in East Estonia (Mäemets, 1977). The average TP is 0.022 mg L^{-1} and the TN is 1.0 mg L^{-1}. The entire bottom of Prossa is covered by macrovegetation, overwhelmingly dominated by charophytes (Nõges et al., 2003).

3.2. Sampling

Integrated samples for proto- and metazooplankton were obtained by mixing the water collected via a 2 L Ruttner sampler at 1 m intervals through the entire water column. For metazooplankton samples, 10 L of integrated water was filtered through a 48 µm plankton net and concentrated to 100 mL. In the studies described in paper **II**, a Juday net (mesh size 85 µm) was used for metazooplankton sampling. The lake water used for zooplankton grazing experiments was obtained from the integrated water samples.

In Võrtsjärv, either one stationary sampling station **(II, III)** near to the deepest part of the lake, or two stations (**IV**) - the stationary sampling station and a station in the southern part - were sampled biweekly. In Kaiavere (**III**), one sampling station was studied monthly. In Prossa, the samples were collected weekly (April to June) or monthly (July to October) (**I**). Depth-integrated water samples were taken from five sampling points on the transect from the lake centre to the shore, and mixed together.

3.3. Sample fixation and microscopy

Metazooplankton samples for analyses of composition, abundance and biomass were fixed with acidified Lugol solution (0.5% final concentration). Triplicate subsamples (2.5 or 5 mL) were counted under a binocular microscope in a Bogorov chamber at 32× and 56× magnification. For calculations of biomass, average body lengths of at least 20 individuals of each taxon were measured. The individual weights of rotifers were estimated from average lengths according to Ruttner-Kolisko (1977). The lengths of crustaceans were converted to wet weights according to Studenikina & Cherepakhina (1969) for nauplii, and to Balushkina & Winberg (1979) for other groups (**I, II, III**). The dry weight biomass of metazooplankton (**IV**) was estimated by length/weight relationships according to Dumont et al. (1975) and Bottrell et al. (1976). The carbon biomass of metazooplankton was calculated assuming that wet weight constitutes 10% of dry weight and the latter contains 40% carbon (Zingel et al., 2007).

Ciliate samples were fixed with acidified Lugol solution (0.5% final concentration). Ciliate biomass and community composition were determined using the Utermöhl (1958) technique (**I, II, IV**). Volumes (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.

The abundance of heterotrophic nanoflagellates (HNF) and small ciliates (<50 µm) (**IV)** was determined on the same filters. Preserved samples (buffered formalin, 1% final concentration) were stained for 1–2 min with

DAPI at a final concentration of 2 µg mL^{-1} and gently filtered through 0.8 µm pore-size black isopore filters (Poretics Inc.). Protists were examined with an Olympus BX60 fluorescence microscope under 1000× magnification (for details see **IV**). Where possible, the dominant taxa among the fluorescently stained ciliates were identified on the basis of the compositions of parallel Lugol-fixed samples. Ciliate and HNF biomasses were calculated from the average biovolume (i.e. wet weight) of particular groups of organisms using conversion factors from biovolume to carbon biomass. These were 220 fg C $µm^{-3}$ for HNF (Borsheim & Bratbak, 1987), and 190 fg C $µm^{-3}$ for ciliates (Putt & Stoecker, 1989).

3.4. Grazing experiments

Zooplankton communities and size fractions grazing on phytoplankton were studied using a radiotracer technique (**I, II**). A monoculture (50 mL) of *Scenedesmus brasiliensis* Bohlin (cell size 2.5-4 µm) was labeled with $NaH^{14}CO_3$ for 36 hours at 120 W m^{-2}. A small volume (10 mL) of washed (filtered lake water, Whatman GF/C) and concentrated radioactively-labeled algal cells was added to 3 or 4 L of integrated lake water and incubated in the lake for 7 minutes. In the experimental vessels, the concentration of labeled *S. brasiliensis* monoculture did not exceed 5-10% of the total suspended solid content of the lake water. After incubation, the water was filtered through the plankton net to collect the zooplankton. To obtain two size fractions of zooplankton (**II**), 48-100 µm and >100 µm, the incubated water was filtered through mesh size 100 µm and the filtrate through mesh size 48 µm. The filtered zooplankton was anesthetized with carbonated water and stored on ice. To measure the radioactivity of food, 30 mL of filtrate was retained on the GF/C filters. In the laboratory, zooplankton was washed from the net to the GF/C filters. The radioactivity of the filters containing zooplankton and food particles was measured using a RackBeta 1211 liquid scintillation counter (LKB Wallac) with the scintillation cocktail OptiPhase HiSafe 3 (Perkin Elmer). One measurement series consisted of three replicates. For calculations see **I** and **II**.

Proto- and metazooplankton grazing on various sizes of food particles was determined from the rate of uptake of fluorescently-labeled microparticles (**III, IV**). Fluorescent microspheres of four sizes were used in feeding experiments: diameters 0.5 µm (Fluoresbrite®; Polysciences Inc.) for bacteria

and autotrophic picoplankton (APP), and 3, 6 and 24 µm (Duke Scientific Corporation) for unicellular phytoplankton and other unicellular organisms of corresponding size. An amount of tracer not exceeding 10% of the density of the corresponding size groups of natural pico-and nanoplankton in the lake water was added to integrated lake water. For metazoans, a 3 L incubation vessel was used. Incubations lasted for 7 min (the grazing rate was linear, the vials were shaken gently twice during incubation), after which the water was filtered through a 48 µm mesh size plankton net, anesthetized with carbonated water (submerging the net together with animals in carbonated water for 10 s), and fixed in formaldehyde [final concentration 4% (vol/vol)]. For microsphere counting, individual metazooplankters were collected from the samples on a polycarbonate filter of 10 µm pore size (Poretics). The number of microspheres in animal guts was counted at 1000× magnification using an epifluorescence microscope (Zeiss Axiovert S100). In experiments with protozoans (**IV**), the incubation was carried out in 20 mL vials. Incubations lasted for 7 min, after which the samples were fixed with buffered formalin (1% final concentration). The preserved samples were stained for 1–2 min with DAPI at a final concentration of 2 µg mL^{-1} and gently filtered through 0.8 µm pore-size black isopore (Poretics Inc.) filters. Protists and the contents of their food vacuoles were examined with a Olympus BX60 fluorescence microscope under 1000× magnification using blue light (470/505 nm, OG 515). For details of calculations see **IV**.

4. RESULTS AND DISCUSSION

4.1. Food base and grazer composition in studied lakes

In this study, three lakes differing in ecological status were investigated with the main focus on a large, naturally highly eutrophic lake, Võrtsjärv. The habitats of macrophyte- and plankton-dominated lakes provided different feeding conditions for zooplankton.

Võrtsjärv is a highly turbid lake (average seston concentration 16.5 mg L^{-1}) mainly because of wind-induced resuspension of the partly mineralized sediment particles. During the vegetation period, Secchi depth does not usually exceed 1 m in the plankton-dominated part (Nõges et al., 1999). The algal communities in the plankton-dominated lakes of Kaiavere and the central part of Võrtsjärv mostly comprised an association of filamentous algae, with cyanobacteria (*Planktolyngbya limnetica*, *Limnothrix planktonica* and *Aphanizomenon* spp.) and diatoms (mostly *Aulacoseira* spp.) forming the highest total biomass in central part of Võrtsjärv (average 20.8 gWW m^{-3}) (**III**). The southern part of Võrtsjärv, although covered with macrophytes, harbors also an abundant phytoplankton community in summer (**IV**). In the macrophyte-dominated Prossa, only a scanty phytoplankton (average biomass 2.3 gWW m^{-3}) was formed, mainly comprising chrysophytes and cryptophytes; forms that are presumably ingestible for grazers (algae <30 µm) constituted most of the total phytoplankton biomass (average 54%) (**I**). The plankton-dominated lakes also provided more bacteria and autotrophic picoplankton than the macrophyte-dominated lakes (**I, III, IV**).

The metazooplankton prevailing in these lakes are typical of eutrophic lakes (Ooms-Wilms et al., 1995; Jeppesen et al., 1996; Vijverberg & Boersma, 1997) with abundant populations of rotifers (most commonly *Keratella* spp. and *Polyarthra* spp.) and small cladocerans such as *Chydorus sphaericus* (in plankton-dominated lakes) and *Bosmina longirostris* (in the macrophyte-dominated Prossa), and with low numbers of *Daphnia*. The copepod community consisted mostly of cyclopoids, except in Prossa where calanoids (*Eudiaptomus* sp.) dominated. The highest metazooplankton abundance occurred in Kaiavere (average 2626 ind. L^{-1}) (**III**), while the highest biomass was measured in the Prossa (average 2.5 gWW m^{-3}) (**I**). Among zooplankton, the ciliate community was most successful in establishing the highest numbers and biomass in Võrtsjärv, contributing

on average more than 50% of the total zooplankton biomass (Figure 1). The spring peak was made up of large herbivorous oligotrichs (mostly *Rimostrombidium* sp., *Pelagostrombidium* sp. and *Codonella cratera*) and the summer maximum was dominated by small bacterivorous scuticociliates (*Uronema* sp.) and oligotrich species (*Cyclidium* sp., *Rimostrombidium* sp.) (**II, IV**).

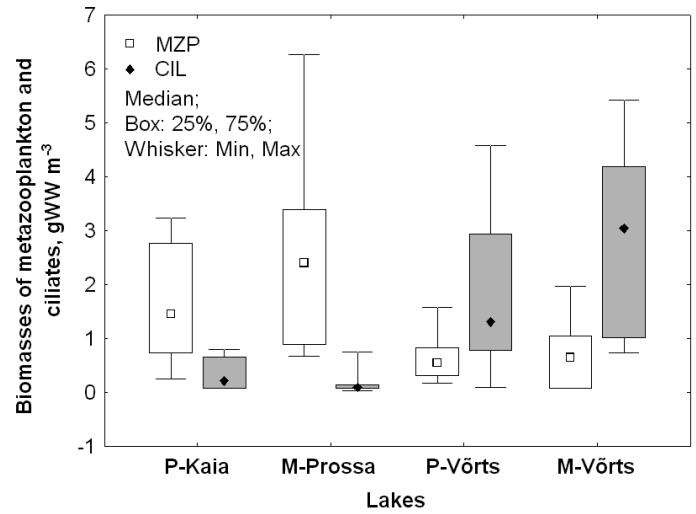

Figure 1. Biomasses of metazooplankton (MZP) and ciliates (CIL) in the lakes studied: plankton-dominated Kaiavere (P-Kaia), macrophyte-dominated Prossa (M-Prossa), the plankton-dominated part of Võrtsjärv (P-Võrts) and the macrophyte-dominated part of Võrtsjärv (M-Võrts).

4.2. Impact of zooplankton grazing on phytoplankton

The filtration rate of the zooplankton community (>100 µm) was similar in macrophyte-dominated Prossa and in the plankton-dominated part of Võrtsjärv (averages 0.20 and 0.21 d^{-1}, respectively; **I, II**), but because the proportion of ingestible size algae was significantly higher and the total phytoplankton lower in Prossa, the grazing loss of phytoplankton was considerably greater in the macrophyte-dominated lake than the plankton-dominated lake (averages 9% and 2.4% of total phytoplankton biomass, respectively). In both lakes the highest rates of phytoplankton

removal were observed in spring (27.6% d^{-1} in Prossa at the beginning of May and 16.4 d^{-1} in Võrtsjärv in April), during a period of low total phytoplankton biomass and a high proportion of ingestible phytoplankton. Thereafter, the patterns of seasonal development of the phytoplankton were different.

In the macrophyte-dominated Prossa, the high zooplankton grazing rates induced the collapse of the spring phytoplankton community in late May, followed by an increase in water transparency **(I).** The Secchi depth increased to 2.6 m by mid-June, indicating the achievement of the 'clear water phase'. The amount of 'edible' phytoplankton started to decrease before the pools of inorganic nitrogen and phosphorus became depleted while the total amount of phytoplankton continued to increase. This shows that the reduction of at least the small phytoplankton fractions could not be caused by nutrient limitation, but could be ascribed to zooplankton grazing. In this lake, zooplankton was responsible for the formation of the 'clear water phase'. At the time of phytoplankton collapse, the zooplankton community consisted mainly of rotifers (*Keratella cochlearis*). Their importance in the spring phytoplankton decline has been reported in several studies (Mayer et al., 1997; Kim et al., 2000). In summer, the zooplankton grazing rates remained generally low (below 5%) and from mid-June onward, phytoplankton was controlled by other factors such as the lack of inorganic nitrogen and phosphorus, which remained very low throughout summer. The entire bottom of Prossa is covered with dense *Chara* beds that most probably kept the mineral nutrients low and did not allow phytoplankton to develop. The ability of macrovegetation, primarily *Chara*, to stabilize the 'clear water state' by intensive nutrient consumption and the potential allelopathic effect against phytoplankton is well known (Hosper & Meijer, 1993; Meijer, 2000).

The zooplankton grazing pattern in the plankton-dominated part of Võrtsjärv was followed seasonally over two years (1998 and 2000) **(II)**. Relatively high grazing on 'edible' phytoplankton was observed in most of the study periods (average 44% d^{-1}), and in some periods zooplankton grazing exceeded the primary production rate (137% d^{-1}), but owing to the high total phytoplankton biomass, the overall grazing loss remained low and no significant consumption effect on the total phytoplankton biomass was observed (Figure 2). Average daily consumption by the zooplankton community (>48 µm) was only 4% of the total phytoplankton biomass. The low grazing values measured are consistent with the results of previous zooplankton feeding studies in Võrtsjärv (Nõges, 1998). Considering the algal and grazer composition in Võrtsjärv, i.e. mainly filamentous forms of cyanobacteria and diatoms, and a low proportion of ingestible size forms (on average 10% of the total phytoplankton biomass), and the abundant populations of ciliates and small rotifers (*Keratella* spp., *Polyarthra* spp., *Anuraeopsis fissa*), small-bodied cladocerans (*C. sphaericus*) and cyclopoid copepods (*Mesocyclops* spp.), a high grazing pressure on phytoplankton would not be expected.

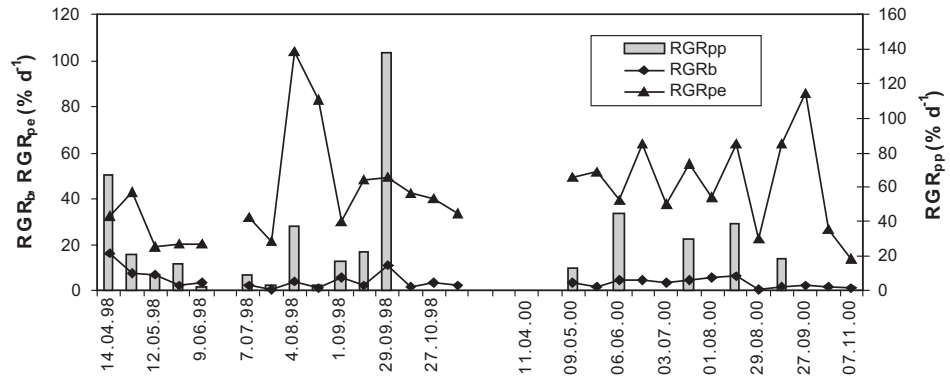

Figure 2. Relative rates of zooplankton grazing of total phytoplankton biomass (RGR_b) and 'edible' phytoplankton biomass (RGR_{pe}), and relative rate of grazing of primary production (RGR_{pp}), in Võrtsjärv in 1998 and 2000 (**II**).

In both the macrophyte- and plankton-dominated lakes, statistical analyses revealed that the nanoplankton size-fraction (5-15 µm) was the most important algal food size for main grazers (*B. longirostris* and *Eudiaptomus* sp. in Prossa, and ciliates, *Polyarthra* spp., *C. sphaericus* and *Daphnia cucullata*, in Võrtsjärv) (**I, II**). In Võrtsjärv in 1998, that size fraction of the phytoplankton comprised relatively more grazable and higher-quality food objects for zooplankton such as flagellates (Bogdan & Gilbert, 1982; Knisely & Geller, 1986), in contrast to 2000, when *Scenedesmus* sp. and *Dinobryon* sp. were mostly represented. Higher food quality might have supported the higher biomass of small-sized grazers such as ciliates and rotifers in that lake in 1998 (**II**).

4.3. Comparison of different zooplankton groups and species as grazers on bacteria and phytoplankton

4.3.1 Zooplankton size-groups (II)

In view of the existence of detailed long-term information about zooplankton species composition, abundance and biomass dynamics (Haberman, 1998; Nõges et al., 1998b; Zingel, 1999), the question was posed whether variable and abundant small zooplankton or larger crustaceans have a greater impact on phytoplankton grazing in Võrtsjärv. Comparing feeding among the zooplankton size-groups, our study revealed that 48-100 µm organisms comprising ciliates and rotifers are the primary consumers of algae in the plankton-dominated part of Võrtsjärv. Their feeding accounted for between 46 and 85% (average 68%) of the rates of filtering and grazing by the total zooplankton. A prevalence of ciliates and rotifers among the phytoplankton consumers was observed over most of the study period during the two-year investigation (1998 and 2000). Studies on grazing by zooplankton size-groups including protozoa are scarce. Few studies have reported a dominance of rotifers over crustaceans in phytoplankton grazing (Quiblier-Lloberas et al., 1996; Kim et al., 2000).

4.3.2. Metazooplankton grazers (III)

Studies of cladoceran and rotifer grazing in Võrtsjärv and Kaiavere revealed that the lakes differed in the leading group of bacterivores: rotifers (mainly *Filinia longiseta* and *Keratella* spp.) in Kaiavere and cladocerans (mainly *C. sphaericus*) in Võrtsjärv (average 64% and 74% of the daily total zooplankton grazing on bacterial size class, respectively) (Figure 3). The leading role of rotifers in bacterial consumption observed in Kaiavere could be attributed to the higher density of bacterivorus rotifers and the presence of efficient bacterivores such as *F. longiseta, Pompolyx complanata* and *Conochilus unicornis*, which were not found or only rarely observed in Võrtsjärv. Their efficiency, due to higher species-specific filtering rates, is also mentioned in other studies (Ooms-Wilms et al., 1995; Hwang & Heath, 1999).

In terms of phytoplankton grazing, cladocerans were generally the main consumers of the edible phytoplankton size class, contributing on average 72% and 94% of the total ingestion in Kaiavere and Võrtsjärv, respectively (Figure 5). *D. cucullata* was the dominant algal grazer in Kaiavere.

In Võrtsjärv, phytoplankton was mostly consumed by the cladocerans *C. sphaericus* and *D. cucullata* (Figure 4). The dominant cladoceran, *C. sphaericus*, alone accounted for an average of 52% of the total rotifer and cladoceran grazing on phytoplankton in Võrtsjärv.

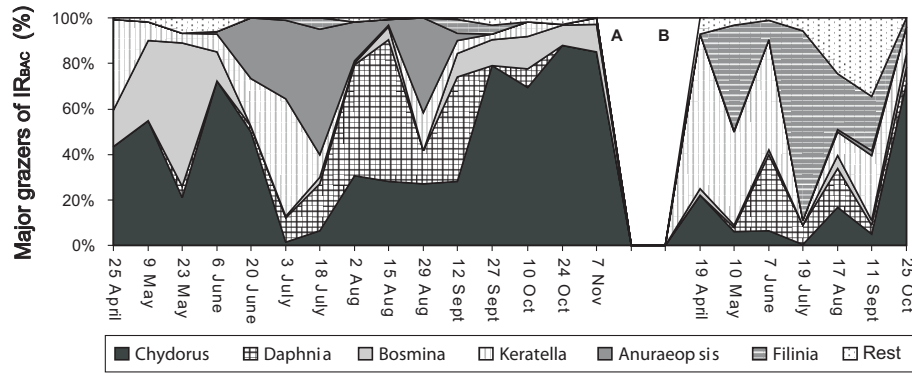

Figure 3. Contributions of major grazers (%) to metazooplankton bacterivory (IR_{BAC}) in Võrtsjärv (A) and Kaiavere (B) **(III).**

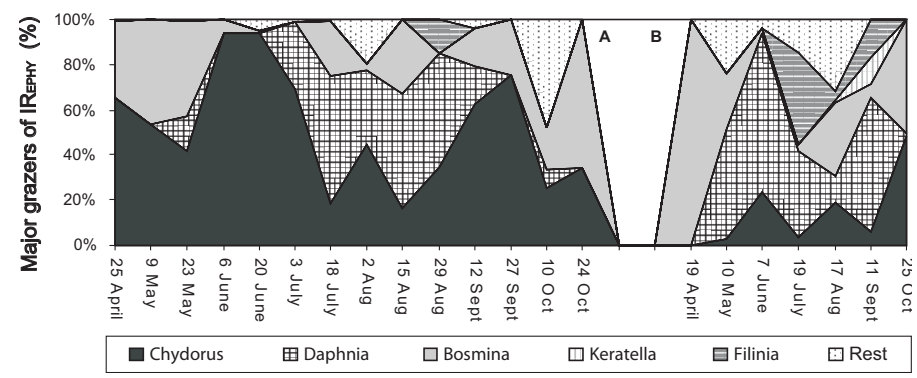

Figure 4. Contributions of major grazers (%) to metazooplankton phytoplankton grazing (IR_{EPHY}) in Võrtsjärv (A) and Kaiavere (B) **(III).**

In Kaiavere, the rates of ingestion of bacteria and phytoplankton (average 1140 cells mL h^{-1} and 15112 cells L h^{-1}, respectively) were several times higher than in Võrtsjärv (average 174 cells mL h^{-1} and 1611 cells L h^{-1}, respectively). The greater number of grazers (mainly rotifers and *Daphnia*) and the presence of efficient bacterivores among the metazooplankton in Kaiavere most probably contributed to this difference.

However, the consumption of fluorescent microparticles showed that the impact of grazing on production and standing stocks of bacteria and ingestible phytoplankton (<30 µm) was low. Metazooplankton was estimated to graze only 0.5% and 0.1% of the standing stock of bacteria daily on average, and 2.6% and 1.0% of standing stock of ingestible phytoplankton, in Kaiavere and Võrtsjärv, respectively. The average consumption of primary production was less than 1% and grazing on bacterial production was around 4%. The daily removal of bacterial production observed in this study is comparable to the results of Jeppesen et al. (1996) in a hypertrophic lake (0.4-19.2% d^{-1}) during a period of strong fish predation and with zooplankton composition similar to the lakes in this study. The low estimated metazooplankton grazing suggests that most of the consumption of bacterial and phytoplankton production in these lakes is probably channeled through the microbial food web.

4.3.3. Protozooplankton and metazooplankton grazers (IV)

Comparison of grazing by protozooplankton (ciliates and heterotrophic nanoflagellates) and metazooplankton (cladocerans and rotifers) showed that small ciliates (<30 µm) were the dominant consumers of bacteria and small phytoplankton in Võrtsjärv. Ciliates were the most important grazers throughout the productive season in both the plankton-dominated and macrophyte-dominated compartments of the lake. The total rates of grazing of bacteria by ciliates were accordingly 8-270× and 100-1000× higher than the total rates of grazing by HNF and metazooplankton. In the plankton and macrophyte compartments, ciliates accordingly consumed 5-250× and 8-7000× more nanoplankton than the metazooplankton community (Figure 5 and 6).

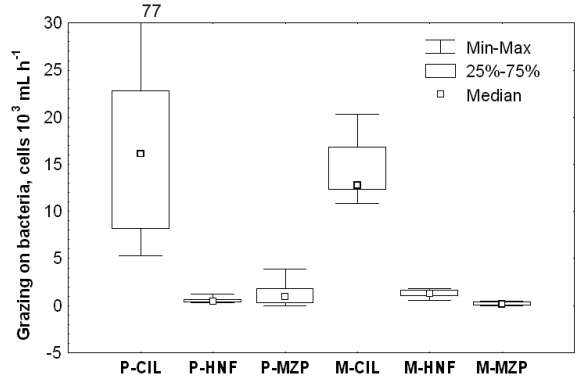

Figure 5. Total grazing of bacteria by ciliates (CIL), heterotrophic nanoflagellates (HNF) and metazooplankton (MZP) in the plankton-dominated (P-CIL, P-HNF, P-MZP) and macrophyte-dominated (M-CIL, M-HNF, M-MZP) compartments in Võrtsjärv.

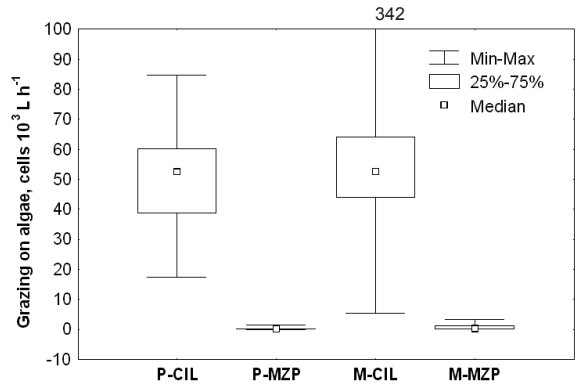

Figure 6. Total grazing of algae by ciliates (CIL) and metazooplankton (MZP) in the plankton-dominated (P-CIL, P-MZP) and macrophyte-dominated (M-CIL, M-MZP) compartments in Võrtsjärv.

In the phytoplankton-dominated compartment of Võrtsjärv the average daily removal of bacterial standing stock was 9.3% by ciliates, 0.5% by HNF, 0.01% by rotifers and 0.07% by cladocerans. Ciliates were able to graze more than 100% of the bacterial biomass production. Although they grazed only on the smaller fraction of autotrophic nanoplankton (2-5 µm), their average daily consumption constituted 20.7% of the standing stock of the respective size class of algal cells, while metazooplankton consumed on average only 1% of the ingestible phytoplankton (<30 µm).

Calculation of the carbon flow in Võrtsjärv showed that the flux was more balanced in the phytoplankton-dominated part. In both compartments of the lake, most of the autochthonously produced organic carbon was not taken up directly by herbivores but was channeled to heterotrophic bacteria. Carbon flux via bacterivory was substantial in the phytoplankton-dominated part of lake and bacterial production was balanced by grazing, whereas in the macrophyte-dominated compartment the total bacterial grazing by all zooplankton groups constituted only 10% of bacterial production.

The dominance of ciliate grazing in bacterial and nanoplankton consumption observed in Võrtsjärv has seldom been reported elsewhere (Tadonleke et al., 2005) and only in systems different from the highly turbid and shallow eutrophic Võrtsjärv. Typically, HNF are considered the major bacteriovores in most lakes because of their efficiency and because they are more abundant than small ciliates (Rothhaupt & Güde, 1996; Gasol, 1995). In Võrtsjärv, the ciliate community has high abundances (during the study period 28 - 158 cells mL^{-1}) and clearly dominates numerically over HNF (<90 cells mL^{-1}). The biomass of ciliated protozoans in this lake is comparable to the metazoan zooplankton and usually forms more than 50% of the whole zooplankton biomass (Zingel, 1999). In eutrophic systems, ciliates are believed to be controlled top–down by metazooplankton rather than by food availability (Jürgens et al., 1999). Considering the low abundance of metazooplankton and their low rates of grazing on nano-sized particles, it seems that ciliates dominate in Võrtsjärv because of the lack of top–down regulation by predators. Owing to the strong grazing pressure by ciliates, HNF are established in a low numbers and have no significant effect on bacteria.

5. CONCLUSIONS

1. In shallow eutrophic macrophyte-dominated lakes, zooplankton grazing can significantly affect the seasonal dynamics of phytoplankton, initiating the 'clear water phase' (e.g. in Prossa). In plankton-dominated shallow eutrophic lakes, zooplankton is able to consume only a minor part of the phytoplankton (e.g. less than 5% of the total phytoplankton biomass in Võrtsjärv) because of the prevalence of 'inedible' algae. The impact of grazing is most significant in the nanoplankton fraction, among which the size range 5-15 µm seems to be the prevailing algal food for the main grazers (**I, II**).

2. In shallow eutrophic lakes, zooplankton grazing generally has a high impact on phytoplankton in spring when the phytoplankton biomass is low but contains relatively more 'edible' algal cells. During that period, small phytoplankton is readily used by the first peak of herbivorous grazers such as ciliates and rotifers (**I, II**).

3. In shallow eutrophic lakes, dominant small zooplankton assemblages such as ciliates and rotifers can dominate phytoplankton grazing throughout the vegetation period and can be responsible for more than 60% of total phytoplankton biomass consumption (**II**).

4. In shallow eutrophic lakes, the abundant rotifer community comprising species with high specific filtration rates (e.g. *Filinia longiseta, Pompolyx complanata, Conochilus unicornis*) can be the most efficient group of grazers on bacteria among the metazooplankton. At the species level, the small cladoceran *Chydorus sphaericus*, which frequently dominates in shallow eutrophic lakes, may be the major metazooplankter grazer on phytoplankton and bacteria (**III**).

5. In shallow eutrophic lakes, ciliates could form an important component of the food web because they are the predominant grazers on small phytoplankton and bacteria, and consume a substantial proportion of bacterial production; the impact of grazing by heterotrophic nanoflagellates and metazooplankton might remain negligible **(IV)**.

6. Low metazooplankton grazing, and the high proportion of ciliates and their consumption of bacteria, indicate the great importance of the microbial food web in shallow eutrophic lakes (**III, IV**).

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SUMMARY IN ESTONIAN

Zooplanktoni toitumise mõju madalate eutroofsete järvede ökosüsteemile

Käesoleva töö eesmärgiks oli uurida zooplanktoni toitumise mõju füto- ja bakterplanktoni kooslustele erinevas tasakaaluseisundis olevate madalate eutroofsete järvede toiduahelas. Sesoonselt hinnati zooplanktoni toitumise mõju fütoplanktoni üldhulgale ja primaarproduktsioonile. Süvendatult uuriti ja võrreldi erinevate zooplanktoni suurus- ja taksonoomiliste rühmade (heterotroofsed viburloomad, ripsloomad, keriloomad ja vesikirbulised) toitumise mõju bakterplanktonile ja väikesemõõtmelisele fütoplanktonile. Töö üheks eesmärgiks oli täiendada suhteliselt puudulikku informatsiooni peamiste füto- ja bakterplanktonit tarbivate metazooplanktoni liikide kohta madalates eutroofsetes parasvöötmejärvedes. Põhiline osa uurimustest teostati Võrtsjärvel, tulemusi võrreldi kahe teise madala järvega (Prossa ja Kaiavere). Töö tulemusena leiti järgmist:

1. Madalates makrofüüdienamusega järvedes võib zooplanktoni toitumine oluliselt mõjutada fütoplanktoni sesoonset dünaamikat ning põhjustada selgevee perioodi tekkimist (näiteks Prossa järves). Seevastu fütoplanktonienamusega madalates järvedes, kus fütoplanktoni koosluse moodustavad valdavalt suured zooplanktonile söödamatud vetikad, suudab zooplankton süüa vaid väikese osa kogu fütoplanktoni biomassist (Võrtsjärves vähem kui 5%). Tugevamat ärasöömismõju avaldab zooplankton nano-suurusega fütoplanktonile, millest suurusvahemik 5-15 µm tundub olevat tähtsaim vetikatoit peamistele fütoplanktonit söövatele zooplankteritele madalates eutroofsetes järvedes (ripsloomad, *Polyarthra* spp., *Bosmina longirostris*, *Chydorus sphaericus* ja *Daphnia cucullata*; **I, II**).

2. Madalates eutroofsetes järvedes avaldab zooplankton tugevamat ärasöömismõju fütoplanktonile enamasti kevadel kui vetikate biomass on veel madal ning sisaldab suhteliselt rohkem väikeseid zooplanktonile söödava suurusega rakke. Esimesteks fütoplanktoni sööjateks sel ajal on arvukalt planktonis esinevad ripsloomad ja keriloomad **(I, II)**.

3. Madatalate eutroofsete järvede zooplanktonis domineeriv ripsloomade ja keriloomade kooslus võib olla peamine fütoplanktoni sööja kogu vegetatsiooniperioodi vältel ning nende rühmade osakaal fütoplanktoni biomassi vähendamisel võib moodustada üle 60% kogu zooplanktoni mõjust (**II**).

4. Madatalates eutroofsetes järvedes esinev arvukas keriloomade kooslus, mis sisaldab kõrge toitumiskiirusega liike (nagu *Filinia longiseta, Pompolyx complanata, Conochilus unicornis*) võib olla tähtsaimaks bakterite sööjaks metazooplanktoni rühmade hulgas. Vesikirbuliste koosluses sageli domineeriv väikesekehaline *C. sphaericus* võib olla tähtsaimaks fütoplanktonit ja baktereid söövaks metazooplankteriks liigi tasandil (**III**).

5. Madalates eutroofsetes järvedes võivad ripsloomad olla oluliseks toiduahela komponendiks, olles peamised bakterite ja väikeste vetikate sööjad ning tarbides märgatava osa bakteriproduktsioonist, samas kui metazooplanktoni osa vetikate ja bakterit söömisel võib jääda tagasihoidlikuks (**IV**).

6. Madal metazooplanktoni toitumise mõju ning kõrge ripsloomade osakaal zooplanktonis ja toiduallikate tarbimisel näitab mikroobse toiduahela suurt tähtsust madalate eutroofsete järvede ökosüsteemi funktsioneerimisel (**III, IV**).

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I

PUBLICATIONS

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The role of zooplankton grazing in the formation of 'clear water phase' in a shallow charophyte-dominated lake

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Key words: clear water phase, shallow charophyte-dominated lake, zooplankton grazing, nutrient depletion

Abstract

In *Chara*-dominated shallow eutrophic Lake Prossa (Estonia), the collapse of spring phytoplankton community occurred in late May after which both primary production (PP) and phytoplankton biomass (B_p) stayed at a very low level. By mid-June the Secchi depth had increased up to 2.6 m indicating the achievement of the 'clear water phase', which persisted thoughout the rest of the vegetation period. The biomass of 'edible' phytoplankton formed on average 53% of the total phytoplankton biomass, and the share of herbivorous zooplankton was on average 61% of the total zooplankton biomass. In spring zooplankton removed daily 27% of the total B_p and 29% of PP by grazing while in summer these values rarely exceeded 5%. Zooplankton grazing was responsible for the decrease of 'edible' (<31 μm) phytoplankton after its spring peak as well as for maintaining its biomass at a very low level during the whole vegetation period. Depletion of mineral forms of nitrogen and phosphorus that occurred most probably because of the development of charophytes by the end of May supported the collapse of the whole phytoplankton community and kept the water clear throughout the summer and autumn.

Introduction

Zooplankton–phytoplankton interactions are a central topic in plankton ecology. In the majority of lakes the rapid phytoplankton growth in spring is usually followed by a 'clear water period' which is induced mainly by zooplankton (ZP) grazing (Jeppesen et al., 1999). However, nutrient (N, P) limitation may also cause phytoplankton collapse (Vanni & Temte, 1990). A 'clear water phase' (CWP) forms if algal standing stock consists of small 'edible' cells, and the biomass of filter-feeding ZP is high. CWP can persist for a short time as up to two weeks (Lampert et al., 1986) or for a longer period (Kasprzak et al., 1999). In many cases, cladocerans as predominant filter feeders control phytoplankton composition and biomass in eutrophic lakes (Ghadouani et al., 1998). Well established submerged vegetation is important for their maintenance (Blindow et al., 2000) and can contribute to the formation of CWP as well.

In the present paper, we studied zooplankton community grazing rate in a shallow eutrophic charophyte-dominated lake with the aim to analyse whether zooplankton grazing could initiate the formation of the CWP and guarantee its persistence throughout the vegetation period. The role of zooplankton grazing *vs.* nutrient depletion by charophytes is discussed and prioritised.

Description of study site

Lake Prossa is a shallow lake in East-Estonia. Its area is 0.399 km^2, volume 723 800 m^3, maximum depth 4.2 m, mean depth 2.2 m, maximum length 1470 m and maximum width 300 m. The bottom of the lake is covered with nearly 1.5 m thick mud layer (Mäemets, 1977). L. Prossa is an eutrophic lake, characterised by an average 1.0 mg l^{-1} of total nitrogen and 0.22 mg l^{-1} of total phosphorus. The whole bottom area

of the lake is covered by macrovegetation, dominated overwhelmingly by charophytes.

Materials and methods

Water samples were collected weekly between 24.04.01 and 13.06.01 and monthly from July to October. All analyses were made from depth-integrated water taken from five sampling points from the lake centre to the shore and mixed together. Water chemistry was analysed according to the methods described by Grasshoff et al. (1983). Phytoplankton and ciliate samples were preserved with acidified Lugol's solution (0.5% final concentration), and studied using the Utermöhl (1958) technique for species composition and biomass. Phytoplankton presumably edible for metazooplankton was split into three size classes (SC1, SC2 and SC3) by the maximum linear dimension: 2–5 μm, 5–15 μm and 15–31 μm, respectively. Chlorophyll *a* (Chl *a*) was measured spectrophotometrically in 96% ethanol extracts. Metazooplankton (MZP) was collected by filtering 10 l integrated lake water through the plankton net (48 μm), fixed with Lugol's solution and counted in three 2.5–5 ml subsamples, which formed 10–20% of the whole sample volume. At least 20 individuals of each species were measured in every sample for biomass calculation. The individual weights of rotifers were estimated from average lengths according to Ruttner-Kolisko (1977). The lengths of crustaceans were converted to wet weights according to Studenikina & Cherepakhina (1969) for nauplii, and to Balushkina & Winberg (1979) for other groups. Bacteria and picophytoplankton was retained on black nucleopore filters (0.2 μm pore size, Poretics), stained by DAPI and counted under the epifluorescence microscope at 1000× magnification (Porter & Feig, 1980). Primary production (PP) was measured *in situ* at six depths during 2 h in midday by the ^{14}C technique (Steemann Nielsen, 1952). The trapeze integration over depth was applied for calculating PP (mg C m^{-2} h^{-1}). Daily values (PP_{day}; mg C m^{-2} day^{-1}) were calculated as PPday = PP /(0.230 – 890 · 10^{-5} · DL), where DL is the length of the light day in hours (Nõges & Nõges, 1998).

To assess zooplankton (ZP) community grazing, 50 ml of *Scenedesmus brasiliensis* Bohlin monoculture (cell size 2.5–4 μm) were labelled with $NaH^{14}CO_3$ during 36 h at 120 W m^{-2}. Then cells were centrifuged at 3000 rpm, washed with filtered lake water (Whatman GF/C) and suspended in the same solution. Final quantity of the obtained algal suspension was 30 ml. Ten ml of this suspension was added into 4 l of integral lake water and incubated in the lake for 7 min. In experimental vessels, the concentration of labelled *S. brasiliensis* monoculture did not exceed 5–10% of the total suspended solid concentration of lake water that varied between 1.0 and 5.5 mg DW l^{-1}. After incubation, water was filtered through the plankton net (mesh size 100 μm) to collect the ZP. Filtered ZP was anaesthetisized with carbonated water and stored on ice. To measure the radioactivity of food, 30 ml of filtrate was retained on the GF/C filters. In the laboratory ZP was washed from the net to the GF/C filters. The radioactivity of the filters with ZP and food particles was measured with a liquid scintillation counter RackBeta 1211 (LKB Wallac). ZP community filtration rate (F, ml l^{-1} h^{-1}) was calculated according to the formulae developed by Lampert & Taylor (1985). One measurement series consisted of three replicates. Grazing rate (G, mg l^{-1} h^{-1}) was obtained by multiplying F with the biomass of the 'edible' phytoplankton (B_{pe}, 2.0-31.0 μm). Relative grazing rate of phytoplankton biomass (RGR_b,% d^{-1}) was found as

$$RGR_b = 24^{*}G/B_p,$$

where B_p is total phytoplankton biomass (mgWW l^{-1}), and relative grazing rate of primary production (RGR_{pp},% d^{-1}) as $RGR_{pp} = 24{*}G/PP_{day}$ assuming 10% carbon content of phytoplankton wet biomass.

The program STATISTICA FOR WINDOWS version 6.0 was used for statistical analysis.

Results

Seasonally two main groups, chrysophytes and cryptophytes, dominated in the phytoplankton (Phy) of L. Prossa. Diatoms were abundant only in early spring. The maximum of phytoplankton biomass (6.92 g WW m^{-3}) occurred on May 15th, thereafter B_p decreased sharply and stabilized at a low level (Fig. 1A). Among the three Phy size classes potentially edible for ZP (Fig 1A), SC1 – covered mainly green algae, SC2 – mainly chrysophytes, cryptophytes and some green algae, and SC3 – mainly cryptophytes, diatoms and also some chrysophytes. On average, 54% of the phytoplankton biomass belonged to the 'edible' size range of 2.0–31.0 μm (SC1, SC2 and SC3 together), the maximum proportion of this size range was 86%. Among MZP rotifers dominated in spring and in July, while

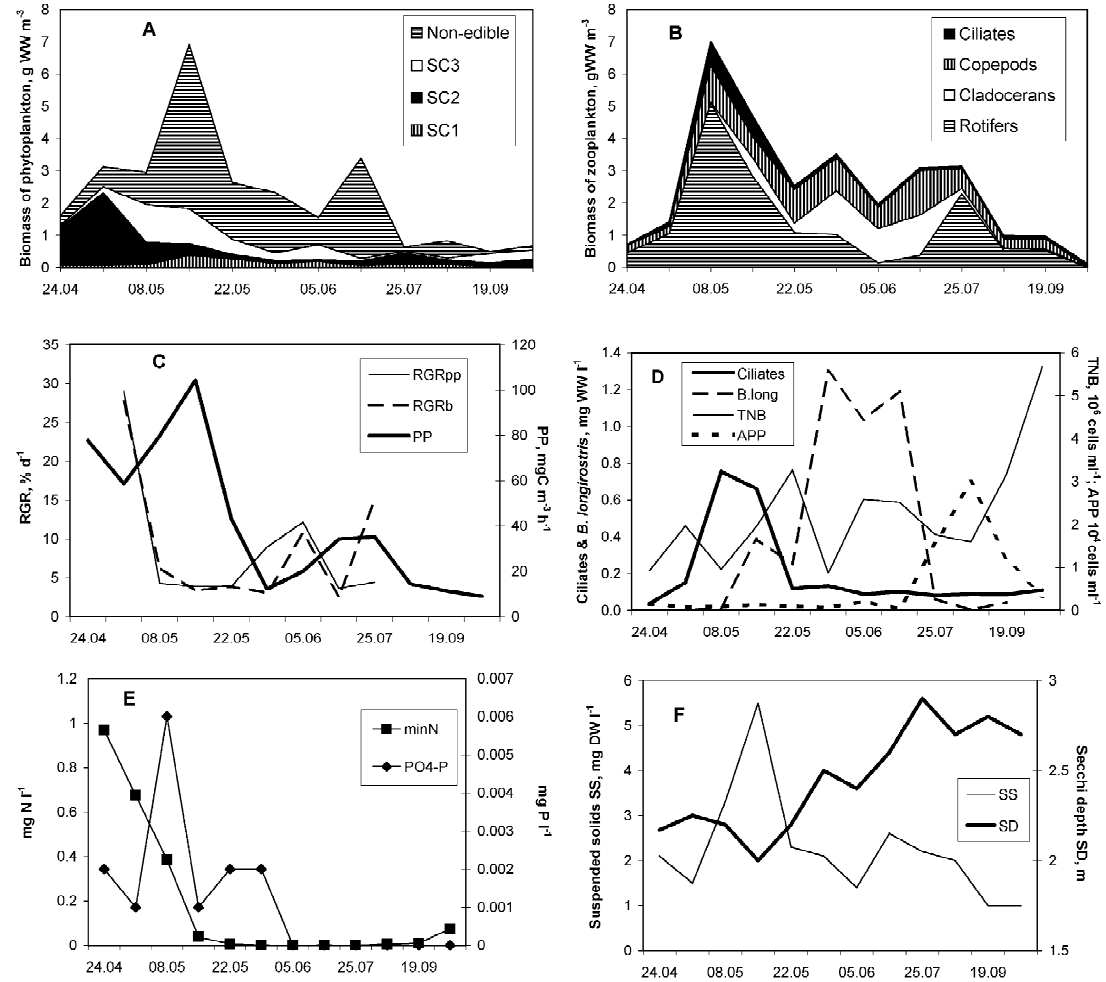

Figure 1. Seasonal dynamics of plankton community and related indices in L. Prossa in 2001. (A) Phytoplankton size classes – SC1 (2–5 μm), SC2 (5–15 μm), SC3 (15–31 μm); (B) Zooplankton; (C) Primary production (PP) and relative grazing rate on phytoplankton biomass (RGRb) and PP (RGRpp); (D) Total number of bacteria (TNB) and picophytoplankton (APP), biomass of ciliates and *Bosmina longirostris*; (E) Mineral nitrogen (minN) and phosphates (PO4-P);. (F): Suspended solids (SS) and Secchi depth (SD).

cladocerans and copepods were abundant at the end of May and in June (Fig. 1B). The herbivorous *Bosmina longirostris* O.F. Müller dominated among cladocerans and among copepods (*Eudiaptomus* sp.). After the spring maximum (6.3 gWW m^{-3}) on May 8th, metazoan biomass stabilized and decreased only in August. Herbivorous rotifers were abundant already at the beginning of May, the increase of the biomass of other filtering zooplankton occurred with a time lag of some weeks after the peak of the 'edible' phytoplankton. After the collapse of phytoplankton at the end of May, a rapid decrease followed also among herbivorous ZP. Herbivores formed on the average 61% (maximum 86%) of the total MZP biomass. The filtration rate (F) of zooplankton, larger than 100 μm (cladocerans, copepods, large rotifers) and their relative grazing rate on algal standing stock (RGR_b) and primary production (RGR_{pp}) increased rapidly after the spring peak of B_{pe} (Fig. 1C). The highest F and RGR_b and RGR_{pp} (14.4 ml l^{-1} h^{-1}, 27.6% d^{-1}and 29% d^{-1}, respectively) occurred on May 2nd when rotifers (*Keratella cochlearis* Gosse) were dominating.

The maximum of the ciliate biomass occured on May 8th (0.75 mgWW l^{-1}), thereafter it decreased to a low level while the biomass of *B. longirostris* showed an opposite pattern being high from late May until early August (Fig. 1D). The total number of bacteria (TNB) fluctuated throughout the vegetation period, and increased sharply (from 1.6 to 5.7 * 10^6 cells ml^{-1}) in autumn. Picophytoplankton (APP) remained on a low level from April to June (average 1000 cells ml^{-1}), followed by a sharp increase (maximum 3.0 * 10^4 cells ml^{-1}) in July (Fig. 1D). After the spring maximum of inorganic nitrogen and phosphorus concentrations (0.97 mgN l^{-1} and 0.006 mgP l^{-1}, respectively) they declined to a low level by mid-May (Fig. 1E). The maximum concentration of suspended solids (5.5 mg DW l^{-1}) occurred on May 15th while in summer and autumn the concentration remained low (average 1.7 mg DW l^{-1}). The highest Secchi depths (maximum 2.9 m) were measured in summer (Fig. 1F).

Correlation and factor analyses were performed to estimate phytoplankton–zooplankton interactions in spring (24.04.01–13.06.01). The biomass of the cladoceran *B. longirostris* correlated negatively with the biomasses of cryptophytes ($r = -0.82$; $P = 0.045$), SC3 ($r = -0.83$; $P = 0.04$) and with the whole 'edible' phytoplankton ($r = -0.85$; $P = 0.032$). F had a strong negative correlation with the biomass of cryptophytes ($r = -0.86$; $P = 0.027$). A positive correlation occurred between RGR and the biomass of SC2 ($r = 0.91$; $P = 0.004$). The two strongest factors determined altogether 79.3% of the variability of the analysed spring data set. In the first factor (F1), which accounted for 42.5% of the total variability, the biomass of SC2 had the greatest negative factor weight (−0.76) while the biomass of *B. longirostris* and the sum of the biomasses of herbivorous copepods and cladocerans had the greatest positive factor weights (>0.7). In the second factor (F2) responsible for 36.8% of the total variability, the biomass of SC3 and the concentration of chlorophyll *a* (Chl *a*) had greatest negative, and F had the greatest positive (>0.7) factor weights.

Discussion

In *Chara*-dominated Lake Prossa the collapse of the spring phytoplankton community occurred in late May after which both primary production (PP) and B_p stayed on very low level (Fig. 1A,C). By mid-June the Secchi depth had increased up to 2.6 m (Fig. 1F) clearly indicating the achievement of the CWP that persisted thoughout the rest of the vegetation period. At the time of the phytoplankton collapse, the zooplankton community consisted mainly of rotifers being generally known as ineffective grazers on bigger phytoplankton forms (Gliwicz, 1969; Mayer et al.,1997).

Our main question addressed was to find out the role of zooplankton in the phytoplankton collapse. According to Mayer et al. (1997), rotifers can play an important role as herbivores in spring and early summer when the phytoplankton community is composed of small forms like chlorococcal green algae or centric diatoms. The low biomass of SC 1 in L. Prossa throughout the whole vegatation period (Fig. 1A) gives the basis to presume that rotifers being the most abundant group of zooplankton controlled SC1 and kept its biomass at a low level. The role of rotifers should have been especially high in early May as they dominated in this period of the highest zooplankton grazing activity (Fig. 1C) after which the biomass of all 'edible' phytoplankton size classes declined. A similar phenomenon could be noticed also in late June when the community grazing rate increased together with the biomass of rotifers. The two first factors revealed by the factor analysis also stressed the importance of ZP grazing for phytoplankton abundance and community composition. The increase of the biomass of herbivores simultaneously with the decrease of the amount of edible phytoplankton indicates their direct relationship in the food chain (Lampert et al., 1986; Jeppesen et al., 1990). The amount of 'edible' phytoplankton started to decrease before the pools of inorganic N and P got depleted while the total amount of phytoplankton continued to increase (Fig. 1A,E). This shows that the reduction of at least the small phytoplankton fractions could not be caused by nutrient limitation. However, nutrient depletion can be considered as one possible reason for the collapse of the total phytoplankton community at the end of May, though, even a parasite attack on phytoplankton can not be neglected (Reynolds, 1984).

Another question is, which factors enabled the CWP to last until the end of the vegetation period? In summer zooplankton biomass was about a half of that during the spring peak, though the most effective phytoplankton grazers like cladocerans and copepods were more abundant (Fig. 1B). Cladocerans are particularly successful in establishing large populations during spring and summer. This success is based on their ability to utilize a wide spectrum of food particles (bacteria, picoalgae, protozoans and phytoplankton) and to achieve high growth rates at high food concentrations (Christoffersen & Bosselmann, 1997). The upper size limit of phytoplankton edible for cladocerans is considered to be 20–30 μm (Bern, 1990; Zurek & Bucka, 1994). According to that assumption, nearly all three phytoplankton size classes considered 'edible' in L. Prossa were consumable for zooplankton. The dominant cladoceran in L. Prossa, *B. longirostris*, has two different feeding modes, one for small particles, and the other for large ones (DeMott, 1982, 1985). As shown by our statistical analyses, *B. longirostris* seems to be able to consume all three size classes of Phy in L. Prossa, that is probably the reason for its success in this lake where suitable food is scarce throughout the summer. The lacking of statistically significant correlations of *B. longirostris* with bacteria, picoalgae and protozoans indicates that cladocerans rely mostly on nanoplankton in L. Prossa while picoplankton is circulating mainly in the microbial loop. Calanoid copepods form another important group of phytoplankton grazers in L. Prossa. Still, according to the factor analysis, only the summary biomass of calanoid copepods and cladocerans was negatively related with SC2. Basing on the literature data (Sterner, 1989; Christoffersen & Bosselmann, 1997), calanoid copepods feed selectively on a narrow food spectrum of small phytoplankton species and, due to lower maintenance cost, can maintain growth at lower temperature and lower food concentration than cladocerans. According to Lampert & Taylor (1985), copepods feed very selectively on *Scenedesmus*. In L. Prossa *Scenedesmus* was unimportant throughout the whole investigation period, and herbivorous copepods had to compete for small phytoplankton with rotifers and also cladocerans. At the end of May when B_{pe} decreased (Fig. 1A) and the water temperature increased, calanoid copepods probably lost the advantage they had at lower temperature, and cladocerans started to dominate. Still the role of calanoid copepods in creating high grazing pressure in spring that initiated the CWP can not be neglected.

Considering that in summer RGR remained generally below 5%, it is hard to assume that the long-lasting CWP in L. Prossa could be caused only by grazing. It seems that from mid-June onward, Phy was controlled by other factors like the lack of inorganic nitrogen and phosporus that remained very low in summer (Fig. 1E). The whole bottom of L. Prossa is covered with dense *Chara* beds that most probably keep mineral nutrients low during the whole summer and do not allow phytoplankton to develop. The ability of macrovegetation, and first of all *Chara*, to stabilize clear water states is well known (Hosper & Meijer, 1993). The expansion of the *Chara* meadows usually results in a shift of the whole lake to a lasting clear-water state (Meijer & Hosper, 1997; Ruggiero et al., 2003). The water above the *Chara* meadows might be kept clear due to the intensive nurient consumption by macrophytes. Besides the competition for nutrients, the influence of allelochemicals against phytoplankton (Meijer, 2000) as well as the increased net sedimentation rate in the macrophyte areas (Søndergaard & Moss, 1997; Meijer & Hosper, 1997; Pluntke & Kozerski, 2003) can also play a role in persisting CWP in L. Prossa. The role of charophytes in this lake is discussed in more detail by Nõges et al. (2003).

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II

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SHALLOW LAKES

Contribution of different zooplankton groups in grazing on phytoplankton in shallow eutrophic Lake Võrtsjärv (Estonia)

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Abstract The grazing impact of different sized zooplankton on 'edible' and total phytoplankton biomass and primary production was measured in L. Võrtsjärv during a seasonal study in 1998 and 2000. The organisms of 48–100 μm size class, composed of ciliates and rotifers, contributed significantly to the total grazing of zooplankton community throughout the study period (average 68%). The average daily filtering and grazing rate of the whole zooplankton community (micro- and macro-zooplankton) remained low, corresponding to a filtration of 44% of the water volume, 4% of the total phytoplankton biomass and 29% of primary production. However, a strong grazing pressure on small-sized phytoplankton (<30 μm) was estimated in most of the study period (average 44% d^{-1}). Among size classes of 'edible' phytoplankton, the size range 5–15 μm was the most important algal food for the dominant zooplankton grazers (herbivorous ciliates, *Polyarthra* spp., *Chydorus sphaericus* and *Daphnia cucullata*) in L. Võrtsjärv.

Keywords Micro-zooplankton · Macro-zooplankton · Filtration rate · Grazing rate · Edible phytoplankton · Primary production

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Shallow lakes in a changing world

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Introduction

Cladocerans are typically considered as predominant phytoplankton grazers in lakes, mainly due to their high ingestion rates and abilities to filter particles of wide size spectrum and to consume a great range of food types (Lampert & Sommer, 1993). In zooplankton grazing estimations, mostly cladocerans and copepods are taken into account while the impact of rotifers and protozoa is usually neglected. In shallow eutrophic lakes, however, the zooplankton is often dominated by small-sized forms like the protozoa and the rotifers (Mayer et al., 1997; Zingel, 1999), while the large-sized cladocerans (i.e. *Daphnia* spp.) and the copepods are suppressed by the size selective fish predation (Jeppesen et al., 1996) and unfavourable feeding conditions like filamentous cyanobacteria and high seston concentration (Porter & McDonough, 1984; Tóth, 1992). In such lakes small-sized zooplankton like ciliates and rotifers may compose a considerable part of total zooplankton biomass (Zingel, 1999) and may

even dominate in phytoplankton grazing (Quiblier-Lloberas et al., 1996), altering the frequency of distribution and amount of food available to larger zooplankton due to highly selective feeding (Starkweather, 1980).

The aim of the present study was to evaluate the zooplankton grazing impact on 'edible' and total phytoplankton biomass and primary production during a seasonal cycle, and to find out the relative importance of small (ciliates and rotifers) and large (mainly cladocerans and copepods) zooplankton in grazing on phytoplankton in L. Võrtsjärv. Nevertheless, the algal and zooplankton communities occurring in L. Võrtsjärv are well studied (Haberman, 1998; Nõges et al., 1998a), including grazing studies (Nõges, 1998; Agasild & Nõges, 2005), there is still debate as to whether small-bodied or large-bodied zooplankton have a greater impact on phytoplankton grazing.

Study site

Lake Võrtsjärv is a large (270 km^2) and shallow (mean depth 2.8 m, maximum depth 6 m) eutrophic lake in Central Estonia (58°05′—58°25′ N, and 25°55′–26°10′ E). The average total phosphorus concentration is 54 $\mu g\ l^{-1}$ and total nitrogen concentration is 1.6 mg l^{-1} (Haberman et al., 1998). The shallowness of the lake and the wave-induced resuspension of bottom sediments contribute to the formation of high seston concentrations and high turbidity during summer.

Methods

Zooplankton grazing experiments, zooplankton and phytoplankton biomass estimations, and primary production measurements were conducted in 1998 and 2000 from April to November at bi-weekly intervals. One stationary sampling station was sampled for the integrated lake water obtained by mixing up the water collected by a 2-l Ruttner sampler from the entire water column at 1 m intervals from the surface to the bottom (maximum depth 3–4 m depending on the water level). Subsamples for phytoplankton, ciliates, primary production and chlorophyll *a* concentration analyses, and for zooplankton grazing experiments were taken from this water. Metazooplankton samples were collected with a quantitative Juday net of 85 μm from the same monitoring station.

Phytoplankton and ciliate samples were preserved with acidified Lugol's solution (0.5% final concentration), and studied using Utermöhl (1958) technique for the species composition and biomass. For determining the 'edible' phytoplankton biomass (B_{pe}), the algal samples were counted by splitting the presumably edible size fraction for zooplankton (<30 μm) into three size classes (SC5, SC15 and SC30) by the maximum linear dimension: 2–5 μm, 5–15 μm and 15–30 μm, respectively.

Chlorophyll *a* was measured spectrophotometrically in 96% ethanol extracts. Primary production (PP) of phytoplankton was estimated in situ using the $^{14}CO_2$ assimilation technique introduced first by Steeman-Nielsen (1952) on 13 times in 1998 and seven times in 2000. Depth integrated lake water was poured into 24 ml glass scintillation vials, 50 μl of sterile $NaH^{14}CO_3$ (VKI, Denmark) solution (1.7 μCi per vial) was added to achieve final activity 0.07 $\mu Ci\ ml^{-1}$. The vials were incubated for 2 h at six depths: 0 m, 0.25 * Secchi depth (S), 0.5 * S, 1 * S, 2 * S and 3 * S in the lake. Then 6 ml of water from each sample was poured into a clean glass scintillation vial and acidified (pH < 2) by adding 150 μl of 0.5 N HCl. Inorganic ^{14}C was assumed to be removed during 24 h (Niemi et al., 1983). Next, 5 ml subsamples were poured into new plastic vials. The radioactivity was assessed by LSC RackBeta 1211 (Wallac, Finland) using external standardization for DPM calculations. Scintillation cocktail OptiPhase HiSafe 3 (Perkin Elmer) was applied. PP was calculated according to the standard formula (Guidelines, 1984). Non-photosynthetic carbon fixation was measured in dark vials and subtracted from light assimilation. The trapeze integration over depth was applied for calculating PP (mg C $m^{-2}\ h^{-1}$). Daily values (PP_{day}; mg C $m^{-2}\ day^{-1}$) were calculated according Nõges & Nõges (1998) as $PP_{day} = PP/(0.230–890 * 10^{-5} * DL)$, where DL is the length of the light day in hours.

Metazooplankton samples were fixed with acidified Lugol's solution (0.5% final concentration), counted under a binocular microscope in a Bogorov chamber and enumerated at ×32–56 magnification. For biomass calculations, average body lengths of at least 20 individuals of each taxon were measured. The individual weights of rotifers were estimated from average lengths according to Ruttner-Kolisko (1977). The lengths of crustaceans were converted to wet weights according to Studenikina & Cherepakhina (1969) for nauplii, and to Balushkina & Winberg (1979) for other groups. In order to estimate the biomass of herbivorous and non-herbivorous zooplankton, adult cyclopoid copepods (Hansen & Santer, 1995), cladoceran *Leptodora kindtii* (Focke) (Herzig, 1995), and rotifer *Asplanchna priodonta* Gosse (Thouvenot et al., 1999) were considered as predominantly non-herbivorous organisms among metazooplankton. Ciliates were divided into herbivores and non-herbivores using data, which was gained by the simultaneous grazing experiments using fluorescently labelled microspheres performed in L. Võrtsjärv in 2000.

To assess zooplankton community grazing, 50 ml of *Scenedesmus brasiliensis* Bohlin monoculture (cell size 2.5–4 μm) was labelled with $NaH^{14}CO_3$ during 36 h at 120 W m^{-2}. Then the cells were centrifuged at 3000 rpm, washed with filtered lake water (Whatman GF/C) and suspended in the same solution. The final quantity of the obtained algal suspension was 30 ml. Ten milliliters of this suspension was added into 3 l of integral lake water and incubated in the lake for 7 min. In experimental vessels the concentration of labelled *S. brasiliensis* monoculture did not exceed 5–10% of the total suspended solid concentration of the lake water that varied between 5.5 mg DW $l^{-1}$and 39.5 mg DW l^{-1}.

After incubation, water was filtered through the plankton net to collect the zooplankton. To obtain two size fractions of zooplankton: microzooplankton (MicroZ, 48–100 μm) and macrozooplankton (MacroZ, >100 μm), the incubated water was first filtered through the mesh size of 100 μm and the filtrate through the mesh size of 48 μm. The filtered zooplankton was anesthetized with carbonated water and stored on ice. To measure the radioactivity of food, 30 ml of filtrate was retained on the GF/C filters. In the laboratory, zooplankton was washed from the net to the GF/C filters. The radioactivity of the filters with zooplankton and food particles was measured by LSC RackBeta 1211 (Wallac, Finland) using scintillation coctail OptiPhase HiSafe 3 (Perkin Elmer).

Zooplankton community (MicroZ + MacroZ) filtration rate (F; ml $l^{-1}\ h^{-1}$) was calculated according to the formulae developed by Lampert & Taylor (1985):

$$F = \frac{R(\text{animals})}{[R(\text{water}) - R(\text{filtrate})]/v} * \frac{60}{t * V},$$

where F = community filtration rate (ml $l^{-1}\ h^{-1}$), t = feeding time (min), V = volume of water in experiment (l), R = measured radioactivity (becquerels), and v = volume of the water samples (ml).

One measurement series consisted of three replicates. Grazing rate (G; mg $l^{-1}\ h^{-1}$) was obtained by multiplying F with the biomass of the 'edible' phytoplankton (B_{pe}, 2.0–30.0 μm). Relative grazing rate of total phytoplankton biomass (RGR_b; % d^{-1}) was found as $RGR_b = 24 * G/B_p$, where B_p is total phytoplankton biomass (mgWW l^{-1}), and relative grazing rate of 'edible' phytoplankton biomass (RGR_{pe}; % d^{-1}) as $RGR_{pe} = 24 * G/B_{pe}$. Relative grazing rate of primary production (RGR_{pp}; % d^{-1}) was found as $RGR_{pp} = 24 * G/PP_{day}$ assuming 10% carbon content of phytoplankton wet biomass. Zooplankton grazing experiments were performed on 15 occasions in 1998 and on 14 times in 2000. The MicroZ grazing was not measured in the first part of the 1998 season until July.

Statistical analyses

The program STATISTICA FOR WINDOWS version 5.0 was used for statistical analyses. Spearman's correlation coefficients were used to determine the relations of the grazers and phytoplankton variables.

Results

Phytoplankton and zooplankton communities

During the study period, water temperature varied from 2.6°C to 20°C in 1998 and from 2.5°C to 19.3°C in 2000 (Fig. 1). The highest chlorophyll *a* concentrations were measured in September in both studied years (Fig. 1).

The average total phytoplankton biomass (B_p) was similar in both studied years (Fig. 2a), forming 21.8 gWW m^{-3} and 21.6 gWW m^{-3} in 1998 and 2000, respectively. Cyanobacteria *Limnothrix redekei* (Van Goor) Meffert and *L. planktonica* (Wolosz.) dominated in 1998 and *Planktolyngbya limnetica* (Lemm.) Kom.-Legn., *L. planktonica* (Wolosz.) Meffert., and *Aphanizomenon skujae* Kom.-Legn. et Cronb. dominated in 2000. Diatoms were the second most abundant group, mostly *Melosira* sp. in 1998 and *Aulacoseira* sp. in 2000.

In 1998 the 'edible' phytoplankton biomass (B_{pe}) formed on the average 14% of B_p but its share was considerably higher (up to 50%) in April and May due to low B_p at the same time (Fig. 2a). During the study period in 2000 the B_{pe} formed an average two times less (7%) of B_p compared to 1998. In 1998 the B_{pe} stayed relatively low until mid-August, in 2000 the period of minimum B_{pe} occurred from late August until the end of September (Fig. 2a).

In 1998 among the size classes of B_{pe}, the SC5 covered mainly green algae (*Scenedesmus* sp.) and crysophytes (*Dinobryon* sp.), SC15 comprised of mainly crysophytes (*Synura* sp.), cryptophytes (*Rhodomonas* sp.), diatoms (*Stephanodiscus* sp., *Fragillaria* sp.) and green algae (*Tetraedron* sp., *Pediastrum* sp.), and SC30 involved mainly diatoms (*Stephanodiscus* sp.), cyanobacteria and cryptophytes. In 2000 SC5 covered mainly small unidentified phytoplankton, SC15—mainly green algae (*Scenedesmus* sp.), crysophytes (*Dinobryon* sp.) and some cryptophytes, and SC30—mainly diatoms (*Stephanodiscus* sp., *Fragillaria* sp.), dinophytes (*Peridinium* sp.) and green algae (*Scenedesmus* sp.).

In 1998 the highest rates of primary production (PP) were measured in June and August. Five PP measurements available in 2000 yielded a maximum rate in May (Fig. 2b).

In 1998 and 2000 the mean zooplankton biomass was 2.7 gWW m^{-3} and ciliates contributed on average 58% of total zooplankton biomass (Fig. 3a). High biomasses of metazooplankton and ciliates occurred in spring from April until the end of June, and in July–August.

The ciliates dominated in MicroZ fraction (Fig. 3b) and cladocerans in MacroZ fraction (Fig. 3c). Herbivores contributed on average 66% and 68% to the MicroZ and MacroZ biomasses, respectively. In seasonal dynamics short seasonal delay between herbivorous micro- and macrozooplankton peak abundances was observed on several occasions. In spring of 1998 higher MicroZ biomass occurred than in spring of 2000, while in spring of 2000 MacroZ biomass was higher than in the spring of 1998 (Fig. 3b, c). During the spring period, MicroZ main grazers

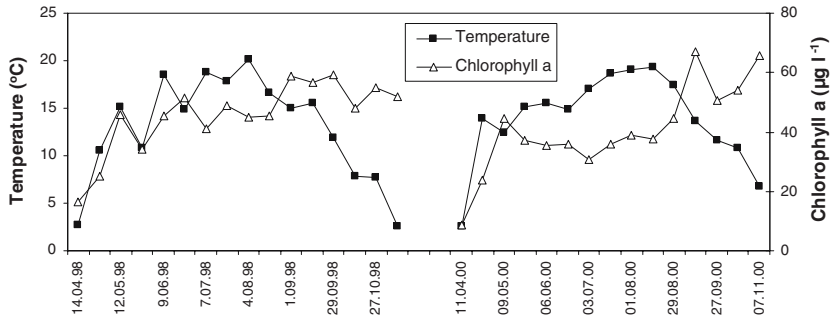

Fig. 1 Changes in temperature and chlorophyll *a* in L. Võrtsjärv in 1998 and 2000

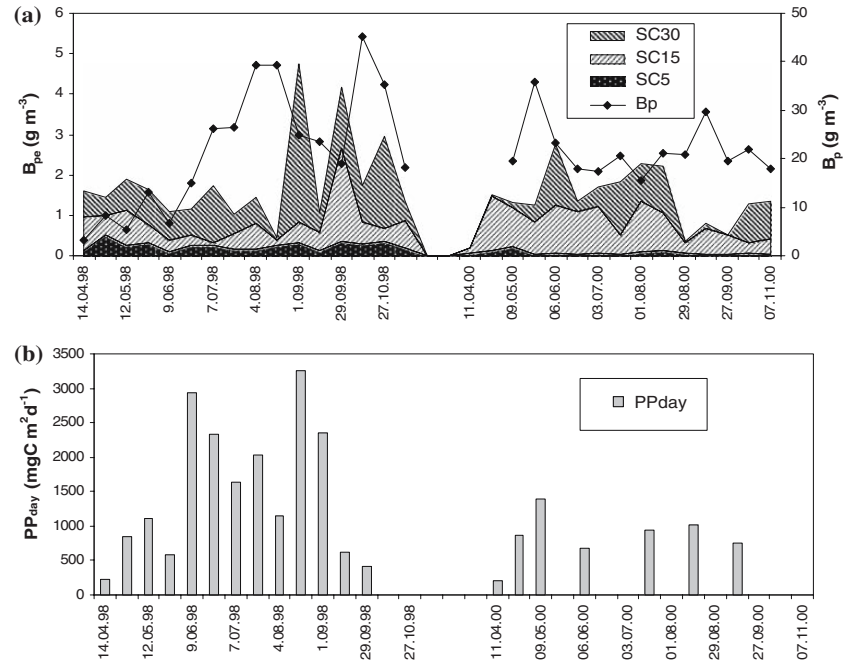

Fig. 2 (**a**) Temporal changes in biomasses of total phytoplankton (B_p) and 'edible' phytoplankton (B_{pe}, 2.0–30.0 µm) and its contributing size classes (SC5, SC15, SC30); (**b**) phytoplankton primary production rate (PP_{day}), in L. Võrtsjärv in 1998 and 2000

were *Polyarthra dolichoptera* Idelson and *Keratella cochlearis* (Gosse) among rotifers, and *Codonella cratera* (Leidy), *Rimostrombidium* sp., *Pelagostrombidium* sp. and *Tintinnidium fluviatile* (Stein) among ciliates. In the spring of 1998 small-sized picovores (mainly scuticociliates) were also abundant. Among MacroZ, cladocerans *Bosmina longirostris* (O.F. Müller) and *Chydorus sphaericus* (O.F. Müller) dominated in spring period, and rotifers also contributed significantly in May 1998 (Fig. 3c). In summer the peaks of MicroZ and MacroZ biomasses were lower than in spring, and the species composition was also different (Fig. 3b, c). In this period the community of ciliates was dominated by small-sized bacterivorous species like *Cyclidium* sp., *Uronema* sp. and *Halteria* sp., and *Anuraeopsis fissa* (Gosse), *Keratella c. tecta* (Gosse) among the rotifers. In August the larger filter-feeder cladoceran *Daphnia cucullata* Sars also peaked.

Grazing influence on 'edible' and total phytoplankton biomass and primary production

Zooplankton filtering rates (F) in both studied years were quite similar forming on average 41% and 47% of the water volume per day in 1998 and 2000, respectively (Fig. 4a).

The highest measured total zooplankton F in August 1998 and in September 2000 coincided with the highest relative grazing rates of 'edible' phytoplankton biomass (RGR_{pe}) (104% d^{-1} and 86% d^{-1}, respectively) (Fig. 4a, b), and with the decreased B_{pe} at the same time (Fig. 2a). According to F, on average 44% of B_{pe} was grazed daily by zooplankton in 1998 and 2000. Among the size classes of B_{pe}, a significant inverse relationship between SC15 and the main grazers: herbivorous ciliates ($r = -0.6$, $P < 0.05$), *Polyarthra* spp. ($r = -0.5$, $P < 0.05$), *C. sphaericus*

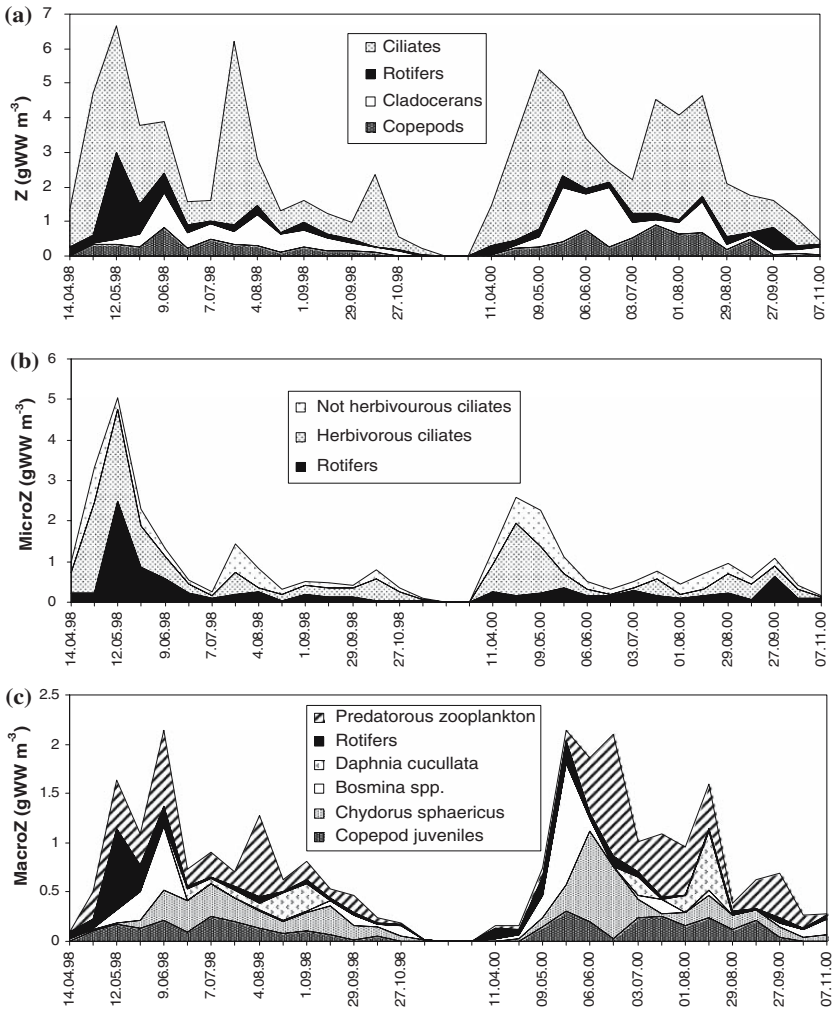

Fig. 3 Zooplankton biomasses in L. Võrtsjärv in 1998 and 2000. (**a**) Total zooplankton (Z) and the contributing groups (ciliates, rotifers, cladocerans, copepods); (**b**) micro-zooplankton (MicroZ, 48–100 μm) and the contributing groups (not herbivorous ciliates, herbivorous ciliates and rotifers); (**c**) macro-zooplankton (MacroZ, >100 μm) and the contributing taxa

($r = -0.6$, $P < 0.05$) and *D. cucullata* ($r = -0.5$, $P < 0.05$) was found.

In 1998 relative grazing rates of the total phytoplankton biomass (RGR_b) formed 0.85–16.4% d^{-1} (average 4.7% d^{-1}). In 2000 somewhat lower RGR_b values occurred (0.4–6.6% d^{-1}, average 3.3% d^{-1}). During the study period the highest RGR_b occurred on 14 April 1998 when 16% of B_p was consumed by the MacroZ alone (Fig. 4b). The highest RGR_b by both MicroZ and MacroZ formed 11% d^{-1} at the end of September 1998 and 6.6% d^{-1} in the middle of August in 2000.

In both studied years the relative grazing rate of phytoplankton primary production (RGR_{pp}) formed on an average 29% d^{-1}. In 1998 the highest RGR_{pp} was measured at the end of September (137% d^{-1}) and 2000 yielded a maximum rate in June (39% d^{-1}) (Fig. 4b).

During the measurement period of both MicroZ and MacroZ feeding (August–November in 1998 and May–November in 2000), the grazer fraction 48–100 μm had major importance in zooplankton grazing. That group of organisms, including ciliates and rotifers, accounted for between 46% and 85% (average 68%) of the filtering and grazing rates by the total zooplankton (Fig. 4a).

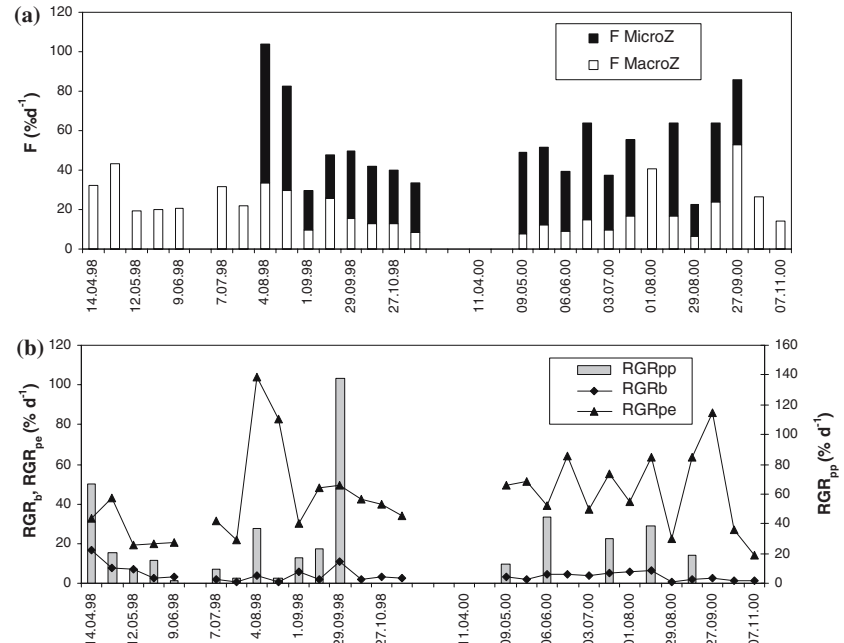

Fig. 4 (**a**) Filtration rates of micro-zooplankton (*F* MicroZ) and macro-zooplankton (*F* MacroZ); (**b**) relative zooplankton grazing rates of total phytoplankton biomass (RGR_b) and 'edible' phytoplankton biomass (RGR_{pe}), and relative grazing rate of primary production (RGR_{pp}) in L. Võrtsjärv in 1998 and 2000. Note that the MicroZ grazing was not measured in the first part of the 1998 season

Discussion

Although the phytoplankton and zooplankton communities occurring in L. Võrtsjärv are well studied (Haberman, 1998; Nõges et al., 1998a; Zingel, 1999), the relative importance of small-bodied and large-bodied zooplankton in grazing on phytoplankton has been under speculation. This study revealed MicroZ as the primary consumer on phytoplankton in L. Võrtsjärv. This fraction, formed by the ciliates and rotifers, dominated in zooplankton filtering and grazing activity over a major part of the seasonal cycle, while larger zooplankton (>100 μm) composed mainly of crustaceans formed on average one-third of the zooplankton feeding contribution (Fig. 4a).

Considering the zooplankton composition in L. Võrtsjärv, the greater impact of small-sized grazers in phytoplankton consumption was expected, as more than 60% of zooplankton biomass is formed by ciliates and rotifers (Zingel, 1999). Among the crustaceans, mainly cyclopoid copepods (mostly *Mesocyclops* spp.) and small-bodied filter-feeding cladocerans like *C. sphaericus* and

Bosmina spp. dominated. In many eutrophic lakes, particularly, *Daphnia* spp. have been shown to be the key species in controlling the phytoplankton (Sterner, 1989). In L. Võrtsjärv the genus *Daphnia* is represented by only one relatively small-bodied species *D. cucculata*, which occurs in rather low numbers in a short period (Haberman, 1998).

In shallow eutrophic lakes, the formation of zooplankton composition is strongly forced from two sides—by food quality and by size selective feeding of fish (Porter & McDonough, 1984; Jeppesen et al., 1996). In L. Võrtsjärv the zooplankton community inhabits the environment with high concentrations of inedible filamentous cyanobacteria and diatoms, while the food base for zooplankton contains large quantities of bacteria and detritus and only low amounts of 'edible' phytoplankton (Nõges et al., 1998b). On average, only 10% of B_p could presumably be ingested by zooplankton. A strong feeding pressure on zooplankton might be assumed, as the lake is rich in fish—bream, perch, and roach being the most abundant species (Nõges et al., 1998b).

As a result of algal and grazer composition, their grazing impact on total phytoplankton is quite weak in L. Võrtsjärv. On average only 4% of the B_p and 29% phytoplankton PP was consumed daily by zooplankton (larger than 48 μm) during the study period in 1998 and 2000. Although in some periods zooplankton filtered the whole water volume (104%) during 1 day and grazing exceeded the primary production rate (137%), the consumption was never enough to influence considerably the B_p. The maximum RGR_b (16% d^{-1}) occurred in April 1998 at very low level of B_p (Figs. 2a and 4b).

Quite similar results were obtained also in earlier studies in L. Võrtsjärv, where the consumption of only cladocerans was investigated. In 1984 and 1985 average cladoceran RGR_b and RGR_{pp} formed 2.5% d^{-1} and 28.5% d^{-1}, respectively, and the maximum values formed 12% d^{-1} and 133% d^{-1}, respectively (Nõges, 1998).

Assuming that zooplankton was feeding only on the 'edible' algae, however, heavy grazing on small-sized phytoplankton (<30 μm) was estimated in most of the study period (average 44% d^{-1}) (Fig. 4b). The direct phytoplankton–zooplankton relationship was observed with the clearest evidence in August 1998 and September 2000 when the highest zooplankton filtering rates coincided with maximum RGR_{pe} (104% d^{-1} and 86% d^{-1}, respectively) (Fig. 4), and with the lowest B_{pe} at the same time (Fig. 2a). In 1998 the biomass of small algae was kept low due to zooplankton grazing until mid August (Fig. 2a). MicroZ grazing was not measured during that period, but considering the high biomass of herbivorous ciliates and their theoretic daily grazing rate of 150% of the body mass (Arndt et al., 1990), most of the B_{pe} was assumed to be consumed by the MicroZ and macroZ in the first half of the study period.

Probably the low B_p and high contribution of small algae suitable for zooplankton grazing (Fig. 2a) supported the formation of abundant populations of grazers (Fig. 3) and therefore stronger grazing pressure on phytoplankton in the first half of the year 1998. During this period different types of phytoplankton grazers peaked in the zooplankton community. The spring population of ciliates (mainly *Rimostrombidium* sp., *Pelagostrombidium* sp. and *C. cratera*) and rotifers (mainly *P. dolichoptera, Keratella* spp.) formed the first peak of grazers in the middle of May, followed by the high biomass of cladocerans (*B. longirostris* and *C. sphaerius*) and copepods in early June (Fig. 3b, c).

Among the size classes of B_{pe}, the algal size class SC15 biomass from spring to August was twice lower in 1998 than in 2000 (Fig. 2a). Supported by the statistical analysis, the 5–15 μm sized phytoplankton is an important algal food source for the main zooplankton grazers as herbivorous ciliates, *Polyarthra* spp., and cladocerans *C. sphaericus* and *D. cucullata* in L. Võrtsjärv. Therefore, a strong food competition between ciliates and metazooplankton might be assumed over that size of algae. In 1998 the SC15 was largely composed of flagellates like *Rhodomonas*, which are more easily ingested by zooplankton than non-flagellated algae. The flagellates are supposed to be well grazable, because these cells may adhere by their flagella to the feeding appendages during the collection process, and because they don't often have sturdy

cell walls (Knisely & Geller, 1986). In feeding studies with different algal types or with natural phytoplankton mixture, flagellates (like *Cryptomonas* and *Chlamydomonas*) are often found to be preferred algal food for zooplankton (Bogdan & Gilbert, 1982; Knisely & Geller, 1986). Larger sized (>5 μm) flagellated algae are an especially important component in the diet of *Polyarthra* (Bogdan & Gilbert, 1982). The high quality food base probably supported the development of abundant populations of small-sized grazers (herbivorous ciliates, *Polyarthra* spp.) responsible for the low SC15 of B_{pe} in 1998.

In 2000 the SC15 was composed primarily of less 'edible' algae (mainly *Scenedesmus* sp. and *Dinobryon* sp.), which may also be the reason for lower MicroZ biomass that year. Another cause may be the abundant population of crustaceans in 2000 (Fig. 3c). An exploitative food competition between ciliates and metazooplankton (Sanders et al., 1989; Weisse & Frahm, 2002) may cause the niche separation in seasonal dominance of grazer types. Metazooplankton are also known to prey intensively on ciliates and can affect their numbers (Adrian & Schneider-Olt, 1999; Jürgens et al., 1999).

Considering zooplankton community studies, there always remains a question about the method of measurement as the zooplankton community consists of variety of organisms, which are diverse in both feeding mechanisms and in modes of food selection (Pourriot, 1977; DeMott, 1986). In the present investigation commonly used radioactive method was applied to determine the zooplankton filtration rate. The tracer cells (2.5–4 μm diameter) were chosen to be of ingestible size for both smaller and larger zooplankton organisms in the studied lake. Though, the given sized tracer particles might not be equally preferred by all species and might be assumed to be more ingestible for MicroZ (Hansen et al., 1994). However, in crustacean community in L. Võrtsjärv, the dominating organisms are small cladocerans like *C. sphaericus* and naupliar stages of cyclopoid copepods, which are known as efficient grazers on small-sized food objects (Hwang & Heath, 1999; Hansen & Santer, 1995; Agasild & Nõges, 2005). Therefore we assume, that the tracer particle size used in the experiments did not seriously alter the outcome of MicroZ and MacroZ grazing results and general findings of this study.

In case of large lakes like L. Võrtsjärv, the horizontal heterogenity may influence the plankton communities between different parts. The grazing pattern studied here may be assumed to be valid in the northern and central plankton dominated part of the lake where the study was performed. Due to its shallowness and large opened area the water mass is well exposed to the wind action keeping it in movement and stirred. In the narrow and macrophyte covered southern part, however, the different species composition of plankton community (Nõges et al., 2004) may also involve different phytoplankton–zooplankton interactions.

In conclusion, the small-sized zooplankton such as ciliates and rotifers are the dominant phytoplankton consumers in L. Võrtsjärv. Due to specific algal (mainly filamentous forms) and grazer composition, the zooplankton community has generally low grazing impact on the total phytoplankton biomass in this lake. However, a strong feeding pressure is evident on the small-sized phytoplankton. Grazing impact is most significant on nano-plankton fraction, among which the size range of 5–15 μm seems to be the most important algal food for the main phytoplankton grazers (ciliates, *Polyarthra* spp., *C. sphaericus* and *D. cucullata*) in L. Võrtsjärv. Although a lot of attention has been paid on zooplankton grazing of phytoplankton community, there are few studies comparing the grazing impact of zooplankton of different size fractions or even taking into account protozooplankton feeding. The main results obtained in this study showing the major importance of MicroZ in phytoplankton grazing might be extrapolated to other eutrophic lakes, where small-sized organisms like protozoa and rotifers dominate in zooplankton assemblage.

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Cladoceran and rotifer grazing on bacteria and phytoplankton in two shallow eutrophic lakes: *in situ* measurement with fluorescent microspheres

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Metazooplankton grazing on bacteria and on the phytoplankton of various sizes was estimated in shallow eutrophic lakes Kaiavere and Võrtsjärv (Estonia) by in situ feeding experiments with fluorescent microspheres (diameters 0.5 μm for bacteria and 3, 6 and 24 μm for phytoplankton). Zooplankton community composition, abundance and food density were important factors determining grazing rates in these lakes. Cladocerans and rotifers filtering rates (FR) and ingestion rates (IR) on bacteria and phytoplankton were several times higher in Lake Kaiavere where bacterivorous rotifers and Daphnia contributed more to zooplankton assemblage. While cladocerans were generally the main phytoplankton consumers, both lakes differed with respect to the groups of bacterivores. Based on consumption of fluorescent microspheres, the metazooplankton grazing rates were relatively low and had low impact on production and standing stock of bacteria and ingestible phytoplankton (<30 μm). On average, 0.5 and 0.1% of standing stock of bacteria and 2.6 and 1.0% of standing stock of ingestible phytoplankton was grazed daily by metazooplankton in lakes Kaiavere and Võrtsjärv, respectively. That corresponded to daily grazing of 4.1% of the bacterial production and 0.43% of the total primary production (PP) by metazooplankton in Lake Kaiavere compared with 4.3 and 0.06% in Lake Võrtsjärv, respectively. The results suggest that the majority of consumption of the bacterial and phytoplankton PP is most likely channelled through the microbial loop.

INTRODUCTION

The zooplankton communities in lakes consist of variety of organisms with different feeding ability, which exploit a wide diversity of food available in the environment. Analyses on large data sets have indicated that grazing rates measured in different communities vary with zooplankton biomass, food concentration and zooplankton taxonomic composition (Cyr and Pace, 1992), as well as with the body size of grazers (Peters and Downing, 1984; Lampert, 1988). It is already a well-known pattern, that communities of large-bodied zooplankton, i.e. *Daphnia* can graze more intensively on phytoplankton than communities of smaller species (e.g. rotifers and *Bosmina*) (Sterner, 1989; Cyr and Pace, 1992). Good evidence for this view are biomanipulated lakes, where the increase in the individual crustacean size and zooplankton mass have resulted in higher grazing activity, often exceeding phytoplankton growth rate (Gulati, 1990). Moreover, many studies recently have demonstrated that zooplankton communities with large cladocerans, particularly Daphniidae, can strongly influence the entire microbial community [bacteria, autotrophic picoplankton, heterotrophic nanoflagellates (HNF), ciliates], by both direct and indirect consumption (Jürgens, 1994; Jürgens and Jeppesen, 2000). Size-selective predation by fish (Jeppesen *et al.*,

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1996) together with resource partitioning because of different feeding modes and selectivities of zooplankters (Pourriot, 1977; Gilbert and Bogdan, 1984; DeMott, 1986) in environment with fluctuating resource availability are important factors affecting the zooplankton community structure in lakes. The abundance and biomass of planktivorous fish increase with the increasing productivity of lake ecosystem, which consequently leads to intensive predation on the zooplankton community (Jeppesen *et al.*, 1997). In shallow eutrophic lakes, smaller bodied individuals and species of rotifers, cladocerans and cyclopoid copepods often dominate in metazooplankton communities (Mayer *et al.*, 1997; Haberman, 1998). Also the habitat of shallow eutrophic lake with large populations of inedible filamentous phytoplankton and large populations of bacteria favour development of protists and small metazoa (Porter and McDonough, 1984; Gulati, 1990; Nõges *et al.*, 1998; Jeppesen *et al.*, 2000).

Owing to the important ecological role of zooplankton in energy and matter transfer in food webs, its grazing studies are of great importance. Much attention has been paid to the grazing of phytoplankton (Lampert *et al.*, 1986; Sterner, 1989; Vanni and Temte, 1990). However, in recent times, consumption on bacterioplankton has been more intensively studied in freshwater ecosystems (Sanders *et al.*, 1989; Vaqué *et al.*, 1992; Hwang and Heath, 1999). Although in natural environments many of zooplankton species feed on both bacteria and algae (Bogdan and Gilbert, 1982; Børsheim and Olsen, 1984; Ooms-Wilms, 1997), grazing experiments have mostly been conducted on a single food object, and relatively little information is still available about the simultaneous grazing of zooplankton on phytoplankton and bacteria (Wylie and Currie, 1991; Kim *et al.*, 2000). This is partly because of methodological insufficiencies. However, a variety of methods for measuring zooplankton grazing have been developed. As a direct method, several tracers have been used to assess and quantify *in situ* zooplankton consumption of bacteria and phytoplankton. Radioactively and fluorescently labelled cells being the most commonly applied methods (Bogdan and Gilbert, 1982; Lampert and Taylor, 1985; Telesh *et al.*, 1995 Ooms-Wilms *et al.*, 1995). Another approach of a tracer method is the use of fluorescently labelled inert particles. It has several methodological advantages—experiments are simple in concept, fluorescent microspheres are highly visible inside the consumer and they do not grow, decompose or fade. The method with fluorescently labelled inert particles allows a study of the more detailed aspects of zooplankton feeding, including the size and taste discrimination mechanisms (DeMott, 1986; Bern, 1990; Rothhaupt, 1990b; Kerfoot and Kirk, 1991), as well as the variation among individuals and between species (Ooms-Wilms *et al.*, 1995). A combination of spheres with different diameter in the experiment enable implications for food-type selection, e.g. bacteria and different size of phytoplankton (Ooms-Wilms, 1997; Kim *et al.*, 2000). Kerfoot and Kirk (Kerfoot and Kirk, 1991) in their experiment covered the spheres with exudates of different species of phytoplankton and studied the simultaneous selection of size and taste by various freshwater suspension feeders. They concluded that many cladocerans are functioning more as detritovores than herbivores. Moreover, a new method by labelling natural ciliate assemblage with fluorescent microspheres enables a study of *in situ* predation on protozoa in short-term incubation experiments without changing the motility and surface properties of prey organisms (Joaquim-Justo *et al.*, 2004). However, possible different consumption of natural food items like bacteria and phytoplankton and inert particles is most often described as a shortcoming of the fluorescent microsphere method (DeMott, 1988; Ooms-Wilms *et al.*, 1993, 1995). The method has commonly been used to determine and estimate the ingestion of bacteria mostly by protozoans (Pace and Bailiff, 1987; Šimek *et al.*, 1990) and for selectivity studies on separated species of rotifers (Rothhaupt, 1990b; Ronneberger, 1998) and crustaceans (DeMott, 1986, 1988; Bern, 1990; Kerfoot and Kirk, 1991) among the metazoan zooplankton. However, a limited number of community studies on zooplankton grazing activity by fluorescent microsphere technique are available so far (Sanders *et al.*, 1989; Ooms-Wilms *et al.*, 1995; Thouvenot *et al.*, 1999; Kim *et al.*, 2000).

The aim of the present study was, therefore, to estimate metazooplankton grazing activity on phytoplankton and bacterioplankton. The main objectives of this study were (i) to test fluorescent microspheres as tracers for measuring zooplankton grazing in communities of high contribution of rotifers and copepods; (ii) to estimate the simultaneous grazing impact on bacterioplankton and (edible) phytoplankton; (iii) to compare the relative importance of rotifers and cladocerans as the consumers of bacteria and various phytoplankton size fractions and (iv) to determine the major species of grazers on bacteria and phytoplankton in shallow eutrophic lakes.

METHOD

Study sites

The study was carried out in two shallow eutrophic lakes Kaiavere (58°36′ N and 26°39′ E) and Võrtsjärv (58°05′–58°25′ N and 25°55′–26°10′ E) (Estonia), which were included into the framework of the European Commission project ECOFRAME, which considered the ecological status criteria and the implementation of water framework directive in shallow lakes of Europe.

Lake Võrtsjärv is a large (270 km^2) and shallow (mean depth, 2.8 m; maximum depth, 6 m) plankton-dominated eutrophic lake situated in central Estonia. The average total phosphorus (dissolved + particulate) concentration is 1.74 $\mu M\ L^{-1}$; total nitrogen (dissolved + particulate) concentration is 114.3 $\mu M\ L^{-1}$. The shallowness of the lake and the wave-induced resuspension of bottom sediments contribute to the formation of high-seston concentrations and high turbidity during summer (Nõges *et al.*, 1998).

Lake Kaiavere is a shallow plankton-dominated lake in east Estonia. Its area is 2.51 km^2, the mean depth is 2.8 m and the maximum depth is 4.5 m (Mäemets, 1968, 1977). Lake Kaiavere is an eutrophic lake, with the average total phosphorus and total nitrogen concentration of 1.35 $\mu M\ L^{-1}$ and 108.6 $\mu M\ L^{-1}$, respectively.

Sample collection

The data for this study were gathered in the frame of the field-work program of ECOFRAME, which allowed to use the background data from this project. In April–November, 2000, Lake Võrtsjärv was studied biweekly and Lake Kaiavere monthly. The integrated lake water was obtained by mixing up the water collected by 2-L Ruttner sampler from the entire water column at 1-m intervals. Subsamples for phytoplankton, metazooplankton, total number of bacteria, bacterial and primary production (PP), seston and chlorophyll concentration analyses and for zooplankton grazing experiments were taken from this water. For metazooplankton samples, 10 L of the integrated water was filtrated trough 48-μm plankton net. Phytoplankton and metazooplankton samples were fixed with acidified Lugol's solution. Bacterial samples were preserved with formaldehyde (2% final concentration).

Plankton abundance, biomass and production

The total number of bacteria was determined by fluorescence microscope at ×1000 magnification (Leica DM RB) on DAPI stained 0.22 μm black membrane filters (Osmonics Inc., Livermore, USA), according to Porter and Feig (Porter and Feig, 1980). [^{3}H]Thymidine incorporation was applied to estimate bacterial heterotrophic activity and transformed to bacterial cell production (cells $mL^{-1}\ h^{-1}$) by applying the empirical conversion factor 3.4×10^{18} (Kisand and Nõges, 1998). A LSC RackBeta 1211 (LKB Wallac) was used for radioactive assays. Bacterial production was measured on eight dates in Lake Võrtsjärv and on five dates in Lake Kaiavere.

Phytoplankton species composition and biomass was analysed using Utermöhl's technique (Utermöhl, 1958). Cells were enumerated with an inverted microscope Hund Wilovert S at ×400 magnification. The samples were counted until reaching at least 400 counting units (filaments, cells, colonies), which gives a counting error of ±10% for the total biomass. For grazing experiments, the number of phytoplankton cells (<30 μm), presumably edible for metazooplankton, were counted separately. Size classes were split by the maximum linear length measure 2–5 μm as the first, 5–15 μm as the second and 15–30 μm as the third size class (SC1, SC2 and SC3, respectively).

For chlorophyll analysis, plankton was filtered onto Whatman GF/C filters. Pigments were extracted with 96% ethanol and analysed spectrophotometrically. The equation of Jeffrey and Humphrey (Jeffrey and Humphrey, 1975) was applied for the calculation of chlorophyll *a* (Chl *a*). *PP* was measured *in situ* at six depths during 2 h in midday by ^{14}C technique (Steeman-Nielsen, 1952). The trapeze integration over depth was applied for calculating *PP* (mg C $m^{-2}\ h^{-1}$). Daily values (PP_{day}; mg C $m^{-2}\ day^{-1}$) were calculated as $PP_{day} = PP/(0.230 - 890 \cdot 10^{-5} \cdot DL)$, where *DL* is the length of the light day in hours (Nõges and Nõges, 1998).

The metazoan zooplankton was counted under a binocular microscope (MBC-9) in a Bogorov chamber and enumerated at ×32–56 magnification. The individual wet weights of rotifers were estimated from average lengths, according to Ruttner-Kolisko (Ruttner-Kolisko, 1977). The lengths of crustaceans were converted to wet weights, according to Studenikina and Cherepakhina (Studenikina and Cherepakhina, 1969) for nauplii and Balushkina and Winberg (Balushkina and Winberg, 1979) for other groups.

Concentration of seston (suspended solids) was determined on preweighed Whatman GF/C filters after drying at 105°C for 2 h (Nõges *et al.*, 2003).

Measurements of zooplankton grazing rates

Zooplankton grazing was measured by *in situ* feeding experiments with fluorescent microspheres of diameters 0.5 μm (Fluoresbrite, Polysciences Inc.) for bacteria and 3, 6 and 24 μm (Duke Scientific Corporation) for phytoplankton. The density of phytoplankton size groups was counted before each grazing experiment, and the used tracer amount corresponded to 10% of the density of the respective phytoplankton size group. For determination of grazing on bacteria, the amount of tracer corresponded to 10% of mean annual bacterial abundance during the ice-free period (April to October) of years 1982 and 1996 in Lake Kaiavere, 1998 and 1999 in Lake Võrtsjärv. Microspheres of different size were

added to integrated lake water in a 3-L glass vessel at the same time and mixed with a plankton sample by shaking the incubation vessel. Incubations lasted for 7 min, after which the water was filtered through plankton net of 48-μm mesh size, anaesthetized with carbonated water and fixed in 4% formaldehyde. For microsphere counting, individual zooplankters were collected from the samples and filtered onto polycarbonate membranes of 10-μm pore size (Poretics). The numbers of microspheres in their guts were counted at ×1000 magnification using an epifluorescence microscope (Zeiss Axiovert S 100). The filtration and ingestion rates (*FR*, μL individual^{-1} h^{-1} and *IR*, bacteria individual^{-1} h^{-1}, respectively) on different size microspheres for each taxon, and the metazooplankton *FR*s and the *IR*s (*CFR*, mL L^{-1} day^{-1}; *CIR*, cells L^{-1} h^{-1}) were calculated as follows:

$$FR = \frac{M_t}{M \times T}; \quad IR = FR \times P;$$

$$CFR = FR \times N; \quad CIR = IR \times N$$

where M_t is the number of microspheres ingested per one individual during the incubation time (microspheres individual^{-1}); M is the concentration of microspheres in incubation vessel (mL^{-1}); T is the incubation time (h); P is the concentration of food particles (bacteria, phytoplankton) in incubation vessel (cells mL^{-1}) and N is the abundance of the zooplankton taxon (L^{-1}). The *CFR* and *CIR* were determined as the sum of species-specific FR and species-specific *IR* for all representing taxa observed. The number of individuals, which had empty guts were included into the calculations.

Statistical analysis

The program Statistica for Windows version 5.0 was used for statistical analyses. Student *t* test was used to compare the average values of measured indices in different lakes. Regression analysis and Spearman's correlation coefficients were used to determine the relations of the grazing intensity and other variables.

RESULTS

Physical environment and plankton communities

During the study period, water temperature varied from 8.5 to 19.6°C in Lake Kaiavere and from 6.7 to 19.3°C in Lake Võrtsjärv. The average Secchi depth in Lake Võrtsjärv was significantly more shallow, and the concentration of suspended matter and phytoplankton higher than in Lake Kaiavere (Table I). The lowest transparency occurred in June, August and September and the highest seston concentration in June and September–November (Fig. 1).

In Lake Võrtsjärv, cyanobacteria [*Planktolyngbya limnetica* (Lemm.) Kom.-Legn., *Limnothrix planktonica* (Wolosz.) Meffert. and *Aphanizomenon skujae* Kom.-Legn. et Cronb.] dominated in phytoplankton followed by diatoms (mostly *Aulacoseira* sp.), which formed peaks in late May and in the middle of September. In Lake Kaiavere, diatoms (mostly *Aulacoseira* sp.) dominated in spring and summer and cyanobacteria (mostly *Aphanizomenon* sp.) in summer and autumn; maximum phytoplankton biomass occurred in September (Fig. 2). The zooplankton communities in lakes Kaiavere and Võrtsjärv were exposed to different densities of food. In Lake Kaiavere, the abundance of edible phytoplankton (<30 μm) was on average two times higher than in Lake Võrtsjärv (Table I), corresponding to 27.5 and 8.5, respectively, of total phytoplankton biomass. The highest density of edible phytoplankton in both lakes was measured in May and August (Fig. 2). Among the size classes, the 2–5 μm was the most abundant.

The rate of algal *PP* and the abundance of heterotrophic bacteria in Lake Kaiavere were slightly higher, while the Chl *a* concentration and the bacterial production were slightly lower than in Lake Võrtsjärv (Table I). However, those differences were not statistically significant. The highest Chl *a* concentration in Lake Võrtsjärv was measured in September–October and in Lake Kaiavere in July (Fig. 1); maximum *PP* values occurred in May and in June, respectively. Bacterial standing stock and productivity were the highest in June (Fig. 2).

Metazooplankton abundance and biomass in Lake Kaiavere were substantially higher than those in Lake Võrtsjärv (Table I). Zooplankton numbers in Lake Kaiavere were highest in May and in Lake Võrtsjärv in July. Relatively high biomasses were measured in Lake Võrtsjärv in May–June and August, and in Lake Kaiavere in May and August (Fig. 3). Among metazooplankton, cladocerans and copepods had no significant difference in abundance and biomass between lakes, while the amount of rotifers, among them bacterivorous, was higher in Lake Kaiavere. Rotifers were the dominating group in both lakes, contributing on average 91 and 56% of total metazooplankton density and biomass in Lake Kaiavere and 75 and 15.6% in Lake Võrtsjärv, respectively. The corresponding values for cladocerans were 3 and 15% in Lake Kaiavere and 10 and 46% in Lake Võrtsjärv. Species compositions of the zooplankton community were similar in both lakes. In spring (May and June), *Keratella cochlearis* (Gosse), *Keratella cochlearis tecta* (Gosse) (in Lake Kaiavere) and *Polyarthra* spp. dominated in number. In summer, *K. c. tecta*, *Polyarthra* spp. and *Trichocerca* spp. were abundant in both lakes, in Lake Võrtsjärv also *Anuraeopsis fissa* (Gosse) and in Lake Kaiavere *Filinia longiseta* (Ehrb.). The dominant cladocerans were *Chydorus sphaericus* (O.F. Müller), *Daphnia cucullata* Sars and bosminids. *Bosmina longirostris* (O.F. Müller) was numerous in Lake Võrtsjärv, while *Bosmian coregoni* (Baird) contributed more in Lake Kaiavere where also *D. cucullata* was significantly (*t* test, $P < 0.01$) more abundant (mean 16 individual L^{-1}) than in Lake Võrtsjärv (mean 4 individual L^{-1}). The highest abundance of cladocerans was observed in June (Fig. 3). Copepods consisted mainly of juvenile stages (nauplii and copepodites) of cyclopoid copepods *Mesocyclops leuckarti* Claus and *Thermocyclops oithonoides* Sars. The calanoid *Eudiaptomus* sp. was found in rather low numbers in Lake Kaiavere.

Table I: Secchi depth, seston and chlorophyll a *(Chl* a*) concentration, variables of phytoplankton, bacterioplankton and zooplankton*

Variables	Lake Kaiavere			Lake Võrtsjärv			*P*
	Minimum	Maximum	Mean ± SE	Minimum	Maximum	Mean ± SE	
Secchi depth, m	0.85	2.80	1.29 ± 0.69	0.5	1.2	0.82 ± 0.16	**
Seston, mg L^{-1}	4.83	17.7	10.8 ± 4.91	6.0	24.0	16.5 ± 4.45	*
Chl *a*, mg L^{-1}	26.5	57.8	41 ± 9.78	25.0	71.1	44.6 ± 13.6	NS
B_{PHY}, gWW m^{-3}	1.86	13.1	6.85 ± 3.58	9.06	35.9	20.8 ± 6.1	***
A_{EPHY}, 10^6 cells mL^{-1}	5.67	81.6	30.7 ± 25.0	5.19	22.9	11.9 ± 4.69	*
A_{SC1}, 10^3 cells mL^{-1}	1.10	76.3	20.5 ± 26.9	1.53	18.9	8.10 ± 5.11	NS
A_{SC2}, 10^3 cells mL^{-1}	3.67	18.8	9.27 ± 5.53	1.05	12.1	3.44 ± 2.55	***
A_{SC3}, 10^3 cells mL^{-1}	0	2.01	1.00 ± 0.84	0.07	1.45	0.42 ± 0.33	NS
A_{BAC}, 10^6 cells mL^{-1}	1.17	6.38	3.95 ± 2.04	0.54	7.68	3.45 ± 2.42	NS
PP, mg C m^{-3} day^{-1}	133	1041	461 ± 314	243	494	354 ± 95.0	NS
BP, 10^6 cells mL^{-1}	2.39	94.2	33.7 ± 37.7	0.03	165	47.4 ± 60.1	NS
B_{MZ}, gWW m^{-3}	0.24	3.23	1.66 ± 1.06	0.18	1.58	0.66 ± 0.43	*
A_{MZ}, 10^5 individual m^{-3}	3.04	72.2	26.3 ± 25.3	0.56	22.0	6.88 ± 5.48	*
B_{RO}, gWW m^{-3}	0.07	2.16	1.00 ± 0.80	0.02	0.16	0.08 ± 0.05	***
A_{RO}, individual L^{-1}	245	6597	2431 ± 2351	33	1967	538 ± 494	**
B_{CL}, gWW m^{-3}	0.01	0.6	0.23 ± 0.20	0.03	1.13	0.32 ± 0.35	NS
A_{CL}, individual L^{-1}	1	115	43 ± 39	6	178	46 ± 55	NS
B_{CO}, gWW m^{-3}	0.15	1.32	0.43 ± 0.42	0.02	0.63	0.26 ± 0.19	NS
A_{CO}, individual L^{-1}	10	609	152 ± 208	3	225	104 ± 83	NS

Biomass of phytoplankton (B_{PHY}), abundances of standing stock of 'edible' phytoplankton (<30 μm) (A_{EPHY}) and its size classes (A_{SC1}, 2–5 μm; A_{SC2}, 5–15 μm; A_{SC3}, 15–30 μm) and the abundance of bacteria (A_{BAC}). Productions of bacteria (*BP*) and phytoplankton (*PP*). Biomasses and abundances of total metazooplankton (B_{MZ}; A_{MZ}), rotifers (B_{RO}; A_{RO}), cladocerans (B_{CL}; A_{CL}) and copepods (B_{CO}; A_{CO}). Significance level (*P*) of the *t* test of the comparison between lakes. NS, not significant.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Zooplankton grazing on different-size microspheres

Many species of rotifers took up 0.5 and 3 μm microspheres, and all species of cladocerans took up 0.5, 3 and 6 μm tracer particles in grazing experiments. Among cladocerans, 90% of the tested individuals grazed microspheres. The microspheres of 24-μm size were grazed very rarely, found only once in the gut of *D. cucullata* and *Asplanchna priodonta* Gosse. Cyclopoid copepods and nauplii never had fluorescent particles in their guts. Only on a few occasions were some tracers ingested by calanoid copepods. Several abundant species of rotifers, like *Polyarthra* spp. and *Trichocerca* spp. did not ingest microspheres. Copepods and the rotifers, which did not consume microspheres formed altogether a considerable part of the total metazooplankton biomass, on an average 53 and 82% in lakes Võrtsjärv and Kaiavere, respectively (Fig. 4). Among the group of rotifers, the taxa not consuming microspheres formed on an average 88% of biomass and 57% of abundance in Lake Võrtsjärv and 90% of biomass and 45% of abundance in Lake Kaiavere. In Lake Kaiavere, the big predator rotifer *A. priodonta* contributed one half of the biomass of rotifers not consuming microspheres. In June and July, the rotifer species, which did not consume microspheres formed only a minor part of rotifers abundance, 6–13% (Fig. 4).

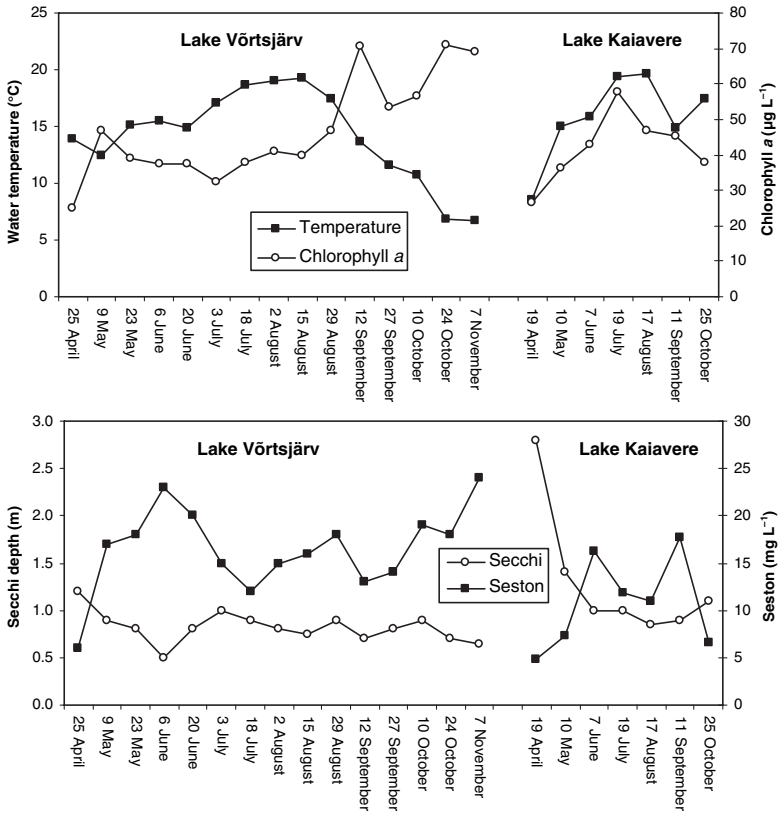

Fig. 1. Temperature, Secchi depth and concentration of chlorophyll *a* (Chl *a*) and seston in lakes Võrtsjärv and Kaiavere during the study period.

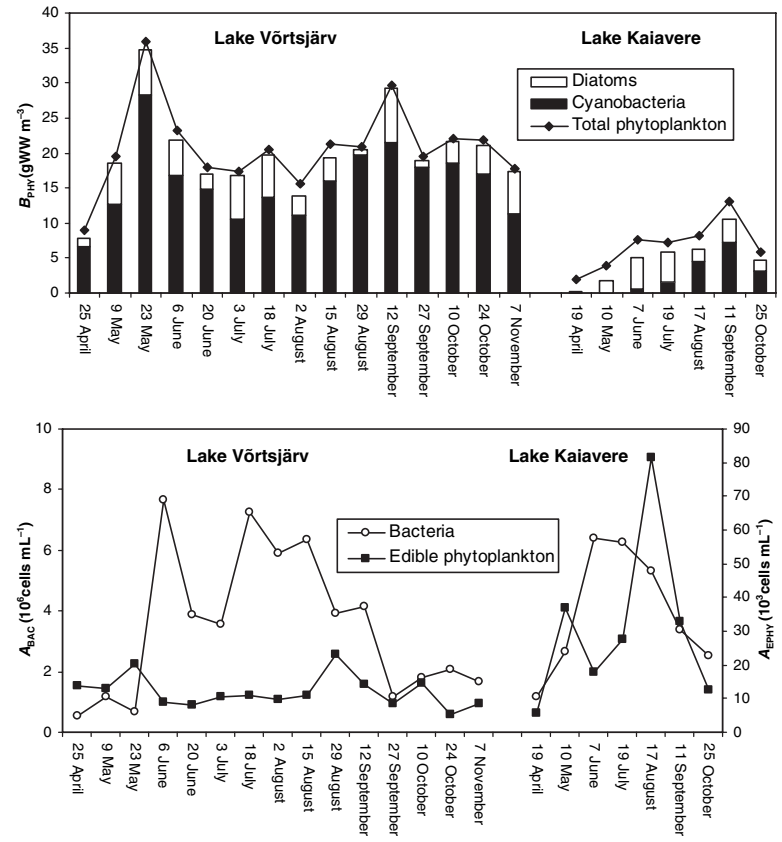

Fig. 2. Phytoplankton biomass (B_{PHY}), the abundance of bacteria (A_{BAC}) and 'edible' phytoplankton (A_{EPHY}) in lakes Võrtsjärv and Kaiavere.

The average metazooplankton (cladocerans + rotifers) *FR* (mL L^{-1} day^{-1}) and the *IR* (particles L^{-1} h^{-1}) in Lake Kaiavere was several times higher than in Lake Võrtsjärv (Table II). In both lakes, the *FR*s on size groups of 3 and 6 µm were generally substantially higher than the *FR* on the smallest (0.5 µm) particles, only in few dates (in Lake Kaiavere in May and in Lake Võrtsjärv in July and September) the situation was somehow reversed. Relatively high grazing rates occurred in Lake Võrtsjärv in May–June, August and October and in Lake Kaiavere in May–September (Fig. 5). In both lakes, the higher *FR* and *IR* coincided with higher numbers of grazers. In Lake Võrtsjärv, the metazooplankton *FR* and the *IR* on 0.5-µm particles was higher in warmer water ($r = 0.621$, $P < 0.05$ and $r = 0.769$, $P < 0.001$, respectively, $n = 15$). The population average species-specific FRs (*PSFR*, involving all observed individuals, including those with empty guts) on 3 and 6 µm microspheres was somewhat higher in Lake Võrtsjärv than in Lake Kaiavere (Table III). Also, the *PSFR* on 0.5-µm microspheres were slightly higher in Lake Võrtsjärv. Cladocerans *D. cucullata*, *C. sphaericus* and *B. longirostris* were the species with the highest *SFR* (involving the individuals that ingested microspheres) on the tested tracer sizes. Among rotifers *Pompolyx complanata* Gosse, *F. longiseta* and *Conochilus unicornis* Rousselet were the species with the highest *SFR* on bacteria-sized microspheres.

DISCUSSION

Metazooplankton grazing impact on different food sources

In our study, the seasonal variation of cladoceran and the rotifers grazing rate followed the changes in the density of the main grazers. Generally, zooplankton exert a much stronger negative effect on phytoplankton biomass in spring when phytoplankton species susceptible to zooplankton grazing tend to be more abundant, while relatively resistant species are more common in summer (Vanni and Temte, 1990). Also in this study, the high density of edible phytoplankton might support the grazer's development in May followed by high-grazing rates in May and June. From spring onwards, relatively lower *FR*s were observed. Although relatively high zooplankton *FR* occurred in early summer (Fig. 5) in the both studied lakes, the grazing rates were never high enough to influence considerably the phytoplankton and bacterioplankton densities (Table IV). The highest removal rates on phytoplankton corresponded to the grazing of 6.2 and 3.7% of the standing stock of ingestible phytoplankton (<30 µm) in lakes Kaiavere and Võrtsjärv, respectively. The highest daily grazing rates on total PP occurred in Lake Võrtsjärv in June and in Lake Kaiavere in September and resulted in consumption of only 0.23 and 0.81% of *PP*, respectively. The daily maximum bacterial consumption corresponded to 1.61 and 0.26% of the standing stock of bacteria in lakes Kaiavere and Võrtsjärv, respectively. In Lake Kaiavere, the maximum consumption on bacterial production of 9.5% occurred in August. In Lake Võrtsjärv, the maximum grazing of 21% on bacterial production was recorded in April at very low bacterial production level while in summer the highest removal rate of bacterial production occurred in July (Fig. 6). The daily removal of the production of bacteria in lakes Kaiavere and Võrtsjärv is close to those obtained by Jeppesen *et al.* (Jeppesen *et al.*, 1996) in eutrophic Lake Søbygaard during the years (1984–1985) of higher fish predation pressure on zooplankton (Table V). The real grazing on bacteria

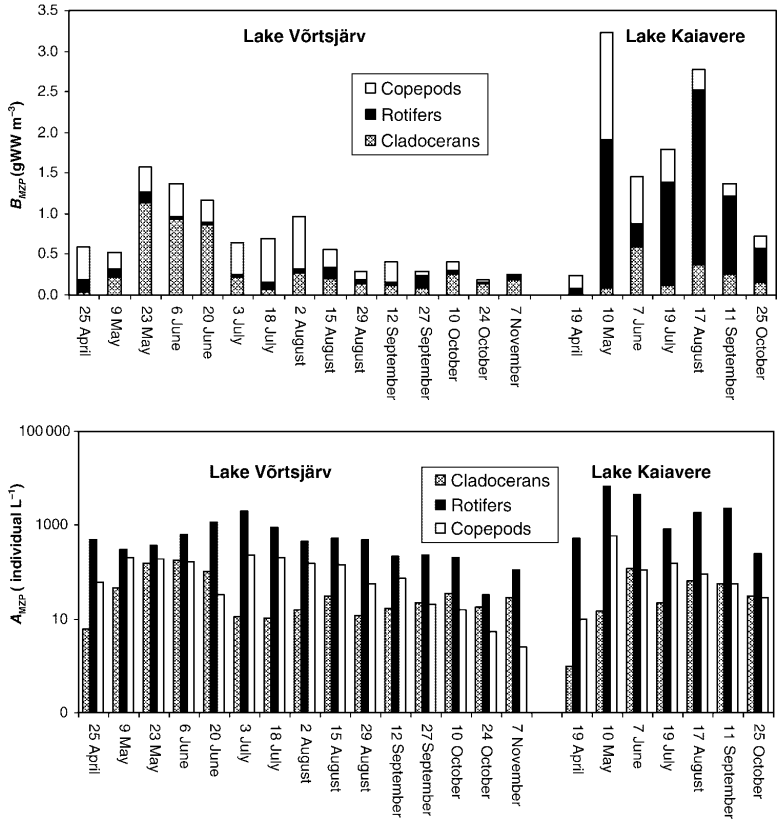

Fig. 3. Metazooplankton biomass (B_{MZ}) and abundance (A_{MZ}) in lakes Võrtsjärv and Kaiavere.

should certainly be higher because metazooplankton are not the main consumer of bacteria as many studies have shown protists as the main bacterivores (Güde, 1986; Bloem *et al.*, 1989). Kisand and Zingel (Kisand and Zingel, 2000) reported that ciliates are the main bacterial grazers in Lake Võrtsjärv in spring, contrary to many other works showing that HNF are generally the major grazers on bacteria (Sanders *et al.*, 1989; Vaqué *et al.*, 1992). In Lake Võrtsjärv, the main reason of the leading role of ciliates is the very low abundance of HNF and very high number of the planktonic ciliates, which can form >50% of the whole zooplankton biomass (Zingel, 1999), and their grazing impact should be considerable.

Based on the consumption of fluorescent microspheres, the higher metazooplankton *FR* and the *IR* on both bacteria and phytoplankton were estimated in Lake Kaiavere, compared with Lake Võrtsjärv (Table II). The elevated rates resulted in higher daily average grazing rate on standing stock of bacteria and edible phytoplankton as well as PP while grazing loss of bacterial production was similar in both lakes (Table IV). The different feeding conditions and different number of grazers seem to be the main reason of higher metazooplankton *FR* and the *IR* in Lake Kaiavere, compared with Lake Võrtsjärv. The composition of metazooplankton in lakes Kaiavere and Võrtsjärv is typical for eutrophic lakes where small cladocerans such as *C. sphaericus* and bosminids dominate, and the genus *Daphnia* is of minor importance. Among rotifers, *Keratella* spp. and *Polyarthra* spp. are most common. Being quite similar considering zooplankton taxonomic composition, the biomass and abundance occurred to be significantly higher in Lake Kaiavere. This difference was mainly caused by rotifer group. Also the large filter-feeding cladoceran *D. cucullata* was significantly more abundant in Lake Kaiavere compared with Lake Võrtsjärv. Considering the living

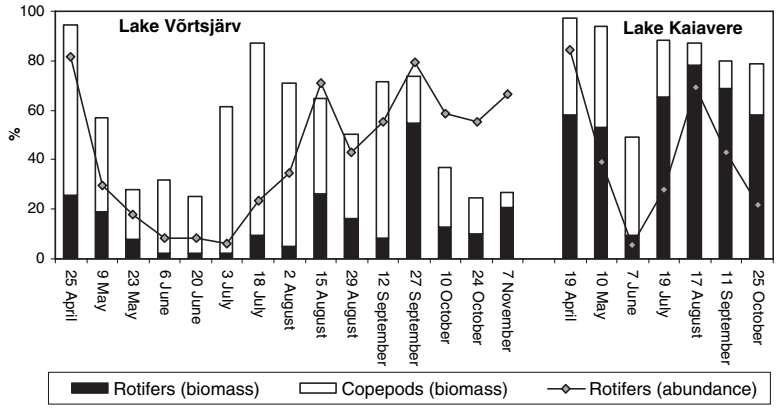

Fig. 4. Relative proportion (% in total metazooplankton biomass) of rotifers and copepods, which did not consume microspheres in the experiments, and the contribution of rotifers (% in total metazooplankton abundance) not consuming microspheres in studied lakes.

*Table II: Filtering rate (*FR*) and ingestion rate (*IR*) on microspheres of different size (0.5, 3 and 6 μm) by metazooplankton (MZ), rotifers (RO) and cladocerans (CL)*

Variables	Microsphere size	Grazers	Lake Kaiavere			Lake Võrtsjärv			*P*
			Minimum	Maximum	Mean ± SE	Minimum	Maximum	Mean ± SE	
FR, mL L^{-1} day^{-1}	0.5 μm	MZ	0.07	16.6	6.47 ± 6.72	0.14	2.56	0.99 ± 0.79	*
		RO	0.05	15.7	4.83 ± 5.65	0.001	1.05	0.27 ± 0.33	**
		CL	0.02	6.7	1.64 ± 2.28	0.14	2.33	0.72 ± 0.74	NS
	3 μm	MZ	0	33.9	12.3 ± 12.1	0	14.0	4.51 ± 4.53	NS
		RO	0	13.5	4.43 ± 5.76	0	0.83	0.11 ± 0.28	NS
		CL	0	33.8	7.88 ± 11.8	0	14.0	4.21 ± 4.42	NS
	6 μm	MZ	0.44	46.7	13.7 ± 17.2	0	35.7	6.08 ± 9.30	NS
		RO	0	45.1	6.44 ± 17.0	0	8.8	0.59 ± 2.27	NS
		CL	0.44	27.6	7.26 ± 9.49	0	35.7	5.49 ± 9.37	NS
IR, 10^5 cells L^{-1} h^{-1}	0.5 μm	MZ	0.03	42.9	11.4 ± 14.9	0.07	8.18	1.74 ± 2.18	NS
		RO	0.02	25.1	7.63 ± 9.16	0.001	2.16	0.50 ± 0.69	*
		CL	0.008	17.8	3.77 ± 6.30	0.04	7.46	1.23 ± 1.95	NS
IR, 10^3 cells L^{-1} h^{-1}	3 μm	MZ	0	49.6	10.5 ± 17.7	0	3.32	1.15 ± 0.97	NS
		RO	0	26.6	5.67 ± 9.81	0	0.57	0.05 ± 0.15	*
		CL	0	22.9	4.82 ± 8.16	0	3.32	1.05 ± 0.92	NS
IR, 10^3 cells L^{-1} h^{-1}	6 μm	MZ	0.08	15.4	4.61 ± 5.43	0	1.73	0.54 ± 0.57	*
		RO	0	6.89	0.99 ± 2.61	0	0.69	0.05 ± 0.18	NS
		CL	0.08	15.4	3.63 ± 5.51	0	1.73	0.49 ± 0.59	*

Significance level (*P*) of the *t* test of the comparison between lakes. NS, not significant.
*$P < 0.05$, **$P < 0.01$.

and feeding conditions for zooplankton, Lake Võrtsjärv seems to be the much worse environment with a higher density of seston particles and large 'inedible' phytoplankton than in Lake Kaiavere where the number of small phytoplankton cells is relatively high (Table I; Fig. 2). High concentration of seston particles including filamentous algae is known to negatively affect food collection process especially that of the large filter-feeding cladocerans and, therefore, cause lowered filtering and feeding activity (Porter and McDonough, 1984;

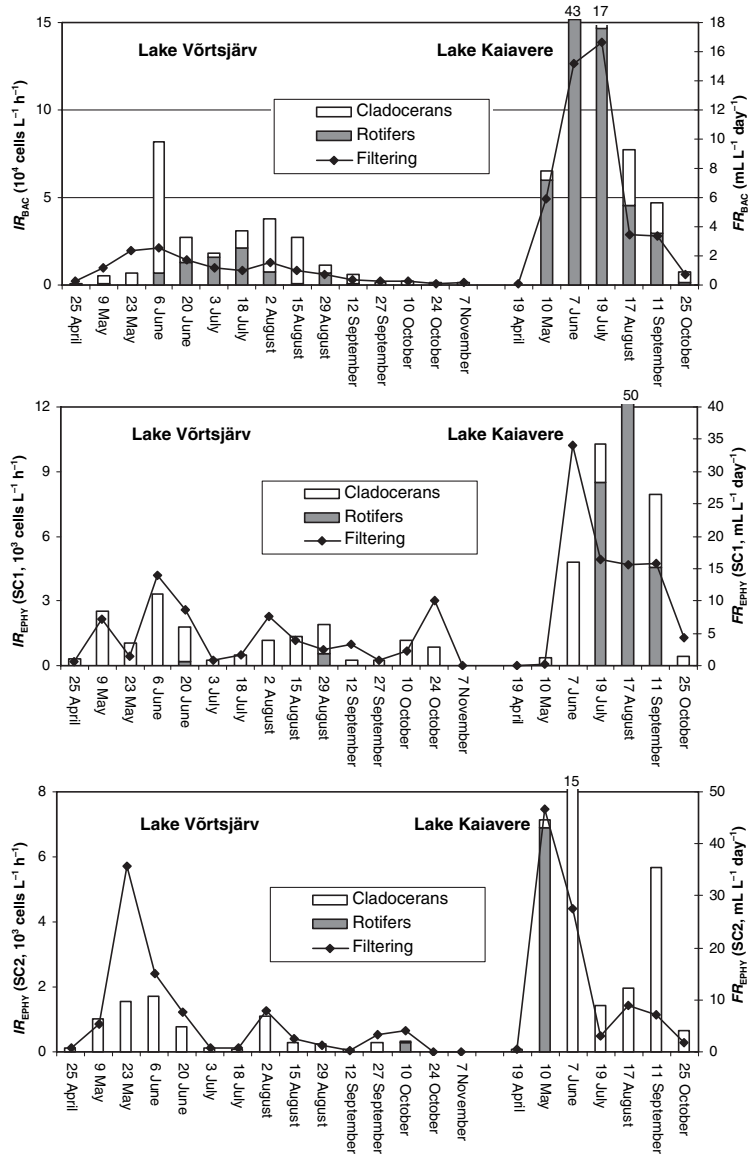

Fig. 5. Temporal changes in ingestion rates of cladocerans and rotifers on bacteria (IR_{BAC}) and 'edible' phytoplankton (IR_{EPHY}) of size class 2–5 μm (SC1) and 5–15 μm (SC2) and their total (cladocerans + rotifers) filtering rates (FR_{BAC}; FR_{EPHY}) in lakes Võrtsjärv and Kaiavere.

Chow-Fraser and Sprules, 1986; Tóth, 1992). The inhibiting effect as well as the low nutritional value of seston and filamentous cyanobacteria may cause food limitation in large filter-feeding cladocerans followed by decrease in their number (Infante and Abella, 1985; Tóth, 1992). The feeding process of small-bodied cladocerans (e.g. *Bosmina* and *Chydorus*) is less inhibited by the presence of large filamentous algae; probably because of their narrower

*Table III: The major grazer's population average species-specific filtering rates (*PSFR*—including individuals with empty guts) (μL individual^{-1} h^{-1}) and maximum and minimum* SFR *(microspheres consumed individuals) (μL individual^{-1} h^{-1}) on different size of microspheres in lakes Võrtsjärv and Kaiavere*

Species	0.5-μm microspheres			3-μm microspheres			6-μm microspheres		
	PSFR	*SFR*		*PSFR*	*SFR*		*PSFR*	*SFR*	
	Mean ± SE	Minimum	Maximum	Mean ± SE	Minimum	Maximum	Mean ± SE	Minimum	Maximum
Lake Kaiavere									
Cladocerans									
Chydorus sphaericus	0.81 ± 0.26	0.15	2.36	3.13 ± 3.32	1.12	50.1	2.72 ± 2.18	6.41	32.5
Daphnia cucullata	2.59 ± 2.07	0.04	11.9	8.59 ± 10.6	1.12	100	8.05 ± 9.62	4.55	64.1
Bosmina longirostris	0.85 ± 0.23	0.50	1.13	5.63 ± 3.81	2.68	7.10	2.39 ± 2.28	4.55	10.6
Bosmina coregoni	0.29 ± 0.15	0.02	0.92	3.10 ± 3.96	1.12	67.9	7.57 ± 6.46	6.41	32.5
Rotifers									
Keratella cochlearis	0.04 ± 0.03	0.02	0.31	0.04 ± 0.11	7.10	7.10	Not found		
Keratella cochlearis tecta	0.04 ± 0.02	0.02	0.27	0.01 ± 0.03	1.12	1.12	Not found		
Anuraeopsis fissa	0.05 ± 0.01	0.04	0.08	Not found			Not found		
Filinia longiseta	0.69 ± 0.17	0.36	1.30	1.23 ± 1.86	3.37	21.3	Not found		
Pompolyx complanata	1.33 ± 0.17	0.94	2.51	Not found			Not found		
Conochilus unicornis	0.65 ± 0.03	0.63	0.67	2.81 ± 3.97	5.62	5.62	Not found		
Lake Võrtsjärv									
Cladocerans									
Chydorus sphaericus	0.69 ± 0.31	0.04	3.67	5.77 ± 7.79	10.5	120	4.67 ± 5.76	1.93	104
Daphnia cucullata	2.65 ± 1.43	0.18	9.24	10.3 ± 4.43	1.74	62.7	9.35 ± 8.40	13.4	63.1
Bosmina longirostris	0.45 ± 0.39	0.04	2.92	5.07 ± 11.4	4.53	40.7	3.89 ± 4.45	19.3	82.0
Bosmina coregoni	0.20 ± 0.16	0.04	1.37	5.95 ± 8.39	5.21	47.5	2.41 ± 2.80	7.05	31.4
Rotifers									
Keratella cochlearis	0.03 ± 0.03	0.04	0.35	Not found			Not found		
Keratella cochlearis tecta	0.02 ± 0.02	0.04	0.27	Not found			Not found		
Anuraeopsis fissa	0.06 ± 0.02	0.04	0.31	0.02 ± 0.06	4.68	4.68	Not found		
Filinia longiseta	0.67 ± 0.47	0.41	1.37	Not found			Not found		

carapace gape there is a lower risk of filaments entering the food chamber and entangling the thoracic appendages (Porter and McDonough, 1984). Both qualitative and quantitative aspects of food sources are the factors shaping the composition and abundance of zooplankton assemblage (Rothhaupt, 1990a; Cordova *et al.*, 2001), which in turn influence the grazing activity (Cyr and Pace, 1992). That might also be the reason for lower grazing activity in our lakes, particularly which might be assumed for Lake Võrtsjärv, where the seston concentration and the phytoplankton biomass are considerably higher, and cyanobacteria contributed 76% of the total phytoplankton biomass during the study period. However, fish predation is known to affect grazer's community composition as well as abundance and biomass and, therefore, control lower trophic levels (Christoffersen *et al.*, 1993; Jürgens and Jeppesen, 2000). Generally, its influence is stronger in eutrophic lakes where the abundance and biomass of planktivorous fish is higher (Jeppesen *et al.*, 1997). In the present study, both investigated lakes are rich in fish, roach, perch and bream as the most frequent species; however, greater abundance and biomass can be found in Lake Võrtsjärv (P. Zingel *et al.*, Estonian Agricultural University, unpublished data). Therefore, more intensive predation pressure on zooplankton in Lake Võrtsjärv could be assumed.

Relative importance of major groups and species of grazers on various size fractions of food

Considering the similar zooplankton taxonomic composition, the lakes differed by the leading group of bacterivores,

Table IV: Grazing rate on standing stock of bacteria (GR_{BAC}) and 'edible' phytoplankton (<30 μm) (GR_{EPHY}), primary production (GR_{PP}) and bacterial production (GR_{BP}) by metazooplankton (MZ), rotifers (RO) and cladocerans (CL)

Variables	Grazers	Lake Kaiavere			Lake Võrtsjärv			*P*
		Minimum	Maximum	Mean ± SE	Minimum	Maximum	Mean ± SE	
GR_{BAC}, % day^{-1}	MZ	0.007	1.61	0.52 ± 0.54	0.008	0.26	0.09 ± 0.08	NS
	RO	0.0002	0.039	0.015 ± 0.014	0.000003	0.004	0.001 ± 0.001	**
	CL	0.00007	0.03	0.007 ± 0.01	0.00032	0.01	0.003 ± 0.003	NS
GR_{EPHY}, % day^{-1}	MZ	0.04	6.16	2.60 ± 2.16	0	3.71	0.98 ± 1.09	NS
	RO	0	0.19	0.05 ± 0.07	0	0.04	0.003 ± 0.009	NS
	CL	0.002	0.26	0.06 ± 0.09	0	0.16	0.04 ± 0.05	NS
GR_{BP}, % day^{-1}	MZ	0.13	9.47	4.10 ± 3.43	0.06	20.9	4.34 ± 7.21	NS
GR_{PP}, % day^{-1}	MZ	0.004	0.81	0.43 ± 0.33	0.006	0.23	0.06 ± 0.08	*

Significance level (*P*) of the *t* test of the comparison between lakes. NS, not significant.
*$P < 0.05$, **$P < 0.01$.

rotifers (mainly *F. longiseta* ja *Keratella* spp.) in Lake Kaiavere and cladocerans (mainly *C. sphaericus*) in Lake Võrtsjärv (Fig. 7), accounting on average for 64 and 74% of the daily total zooplankton bacterivory, respectively. The higher rate of bacterivory observed in Lake Kaiavere (Table IV) is probably because of higher density of bacterivorous rotifers, and the presence of efficient bacterivores as *P. complanata*, *F. longiseta* and *C. unicornis* (Ooms-Wilms *et al.*, 1995; Hwang and Heath, 1999), which were not found or only rarely observed in Lake Võrtsjärv. A similar pattern was shown as a potential reason for different rate of bacterivory between the coastal and offshore sites in Lake Erie (Hwang and Heath, 1999).

Cladocerans were generally the main consumers of phytoplankton, contributing on the average 72 and 94% of the total ingestion of edible phytoplankton in lakes Kaiavere and Võrtsjärv, respectively. In Lake Võrtsjärv, the dominant grazer *C. sphaericus* accounted alone for an average 52% of total rotifer and cladoceran grazing on phytoplankton (Fig. 7). Though cladocerans were the dominant grazers on bacteria in Lake Võrtsjärv and on phytoplankton in both lakes, their abundance was low in July. The reason for that might be in size-selective grazing by fish as well as because of the changed feeding conditions in summer period as the contribution of filamentous cyanobacteria was high in that period, while edible phytoplankton was relatively scarce (Fig. 2). It is known that large zooplankton may be disadvantaged during blooms of cyanobacteria and replaced by smaller species (Zánkai and Ponyi, 1986; Christoffersen *et al.*, 1993). Also in this study, small bacterivorous rotifers (mainly *K. c. tecta* and *A. fissa*) became abundant and contributed on an average 68% of total metazoan bacterivory in Lake Võrtsjärv in July and at the end of August (Fig. 7). In Lake Kaiavere, rotifers (*F. longiseta*, *C. unicornis*, *Keratella* spp.) formed >50% of total grazing of SC1 of phytoplankton in July, August and September (Fig. 5). This indicates that rotifers may be temporarily even more important grazers than cladocerans in these lakes.

Phytoplankton of SC2 was consumed mostly by cladocerans *C. sphaericus* and *D. cucullata* in Lake Võrtsjärv and mostly by *D. cucullata* in Lake Kaiavere. According to *PSFR* in this study, SC2 was more readily used by *D. cucullata*, while *C. sphaericus* preferred smaller phytoplankton (Table III). Although only a minor part of the standing stock of phytoplankton SC2 was removed, the negative correlation ($P < 0.05$) between cladocerans abundance and the standing stock of SC2 support the assumption of active use and importance of that size fraction as food for zooplankton. Cladocerans are considered to occupy the key position in food webs of freshwater systems and are generally the major consumers on phytoplankton in lakes (Lampert *et al.*, 1986; Sterner, 1989; Cyr and Pace, 1992). In lakes Võrtsjärv and Kaiavere, cladocerans were represented mainly by small-bodied forms incapable to exert considerable grazing loss of food sources (Jeppesen *et al.*, 1996; Nõges, 1998a). *Daphnia cucullata* was the species with the highest *PSFR* (Table III) being the main phytoplankton consumer in Lake Kaiavere. In Lake Võrtsjärv, its abundance was too low to have any significant effect on phytoplankton in the lake where *C. sphaericus* was the main consumer on both bacteria and phytoplankton. This small cladoceran, originally restricted to the littoral zone (Pejler, 1975) is well adapted to conditions of eutrophic lake with

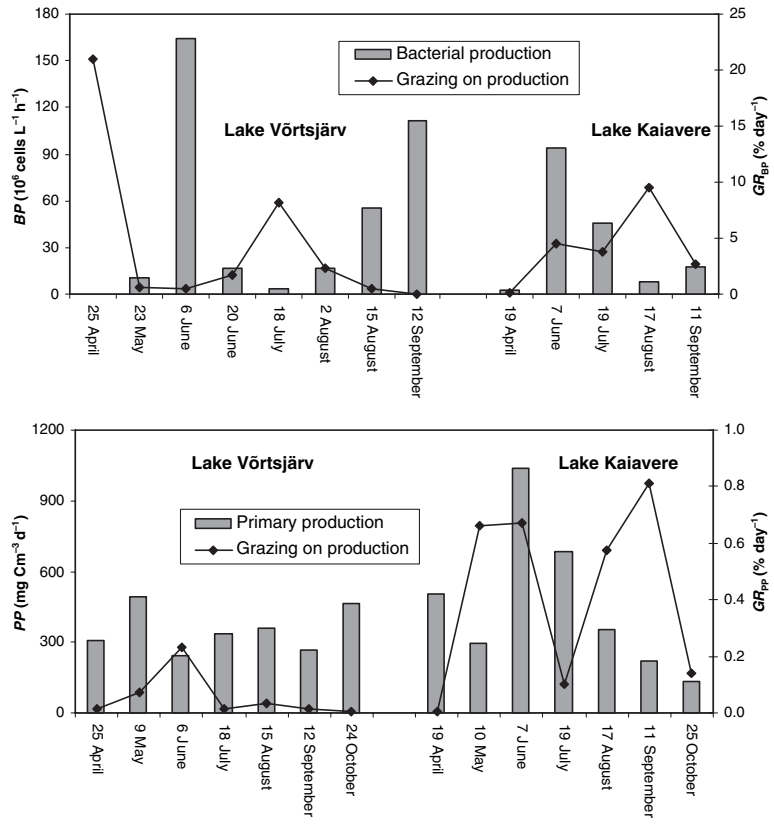

Fig. 6. Bacterial production (*BP*) and primary production (*PP*) and metazooplankton grazing rate on them (GR_{BP}; GR_{PP}) in lakes Võrtsjärv and Kaiavere.

high concentration of detritus and filamentous phytoplankton and is often the dominant cladoceran (Porter and McDonough, 1984; Vijverberg and Boersma, 1997; Haberman, 1998).

Unlike relatively poorly selective feeding cladocerans like *Daphnia* and *Chydorus* (Bern, 1990; Kerfoot and Kirk, 1991), the feeding of *Bosmina* is far more complex as it is strongly taste and size selective (Bogdan and Gilbert, 1982; DeMott, 1982; Kerfoot and Kirk, 1991). This can be attributed to *Bosmina* dual-feeding mode, using different thoracic limbs one mode for small-particle filtering and another for large-particle grasping (Bleiwas and Stokes, 1985; DeMott, 1986; Kerfoot and Kirk, 1991). The dual-feeding option enables the bosminids to employ widely varying sizes of food particles and is apparently the important factor in the success of *Bosmina* in lakes of different trophy (Gulati *et al.*, 1991). Bosminids are common cladocerans in eutrophic lakes with the seasonal replacement pattern, *B. coregoni* replaces *B. longirostris* as summer progresses (Gulati *et al.*, 1991). *Bosmian coregoni* is better adapted to waters with high-food concentration dominated by large food particles as it is in summer plankton of eutrophic lakes (Gulati *et al.*, 1991). In this study, *B. coregoni* expressed lower *IR* and *FR* on bacteria than *B. longirostris*; however, the difference was statistically not significant. This result is consistent to Irvine (Irvine, 1986) that *B. longirostris* is more efficient feeder on smaller-size food, whereas *B. coregoni* prefers larger food particles. Irvine (Irvine, 1986) also found a niche separation for coexistence of *D. cucullata* and *B. coregoni* as the latter preferentially feeds on food particles >7 μm, whereas *D. cucullata* is more efficient on particles <7 μm. Also in this study, *FR*s of *D. cucullata* on tested tracer

Table V: Zooplankton grazing rates on standing stock of bacteria (GR_{BAC}) and bacterial production (GR_{BP}) and primary production (GR_{PP}) from various studies

System	GR_{BAC} (% day^{-1})	GR_{BP} (% day^{-1})	GR_{PP} (% day^{-1})	Grazers	Source
Hudson estuary (May–October)	10.1 (3–16)			PMZ	Vaqué *et al.* (1992)
Lake Paul (May–August)	3.2 (2–5)			PMZ	Vaqué and Pace (1992)
Lake Thuesday (May–August)	3.4 (1–22)			PMZ	Vaqué and Pace (1992)
Lake Oglethorpe (February–September)	5.8 (2–35)	80.0 (11–162)		PMZ	Sanders *et al.* (1989)
Lake Erie coastal (May–August 1993)		28 (8–46)		MZ	Hwang and Heath (1999)
Lake Erie coastal (May–August 1994)		116 (16–374)		MZ	Hwang and Heath (1999)
Lake Erie offshore (May–August 1993)		147 (32–291)		MZ	Hwang and Heath (1999)
Lake Erie offshore (May–August 1994)		228 (104–407)		MZ	Hwang and Heath (1999)
Lake Frederiksborg Slotssø		21–87		PMZ	Christoffersen *et al.* (1990)
Lake Frederiksborg Slotssø		5	26	>200 µm	Jespersen *et al.* (1988)
Lake Frederiksborg Slotssø		27		*Daphnia*	Riemann and Bosselmann (1984)
Lake Søbygaard (May–October 1984–1985)		5.4–11.8 (0.4–19.2)	8 (0.4–32)	MZ	Jeppesen *et al.* (1996)
Lake Søbygaard (May–October 1986)		43	30	MZ	Jeppesen *et al.* (1996)
Lake Prossa (May–July)			8.8 (3.6–29)	>100 µm	Tõnno *et al.* (2003)
Lake Võrtsjärv (May–November 1984–1985)			28.5 (0.3–133)	Cladocerans	Nõges (1998a)
Lake Võrtsjärv (April–November 1998)			15 (3–68.2)	>100 µm	Nõges and Künnap (2003)
Lake Võrtsjärv (August–November 1998)			17.6 (6–40)	>48–100 µm	Nõges and Künnap (2003)
Lake Võrtsjärv (April–November 2000)	0.1 (0.008–0.26)	4.3 (0.06–20.9)	0.06 (0.006–0.23)	MZ	This study
Lake Kaiavere (April–October 2000)	0.5 (0.007–1.6)	4.1 (0.13–9.5)	0.43 (0.004–0.81)	MZ	This study

MZ, metazooplankton; PMZ, protists and MZ.

sizes (0.5, 3 and 6 µm) were higher than those measured for *Bosmina* species (Table III). The *FR* and grazing rate of *B. coregoni* on the smallest food particles were significantly different than those found for *Daphnia* and *Chydorus*, supporting the idea of a food niche separation for coexistence with other cladocerans. The strongest difference was found between *B. coregoni* and *Daphnia* ($P < 0.0001$).

PSFR on bacteria (0.5-µm microspheres) obtained in this study (Table III) was quite close to those estimated by the group of Ooms-Wilms *et al.* (Ooms-Wilms *et al.*, 1993, 1995), Ooms-Wilms (Ooms-Wilms, 1997) and Sanders *et al.* (Sanders *et al.*, 1989) using fluorescent microspheres of the same size (Table VI), but substantially lower than measured by Thouvenot *et al.* (Thouvenot *et al.*, 1999). Grazing of *A. fissa* and *F. longiseta* on phytoplankton (particle size 3 µm) in our experiments was generally in the same range as found by Ooms-Wilms (Ooms-Wilms, 1997). Although the edible phytoplankton density was lower in Lake Võrtsjärv, the cladocerans *PSFR* on phytoplankton was slightly higher than in Lake Kaiavere (Table III). At least partly, this may be explained by lower food concentration, which can cause elevated *FR*s (Ringelberg and Royackers, 1985; Rothhaupt and Lampert, 1992). At times of low algal biomass, bacteria could be an important food supplement or become even dominant food source for some metazoans (Børsheim and Olsen, 1984; Christoffersen *et al.*, 1990; Wylie and Currie, 1991). Considering the low density of 'edible' phytoplankton in Lake Võrtsjärv, bacteria in this lake are probably more important food source for metazooplankton than in Lake Kaiavere. Also the regression analysis suggested that during the period of low transparency and scarce edible phytoplankton, the bacteria were more readily ingested by metazooplankton in Lake Võrtsjärv ($r = 0.9$, $P < 0.001$). The food shortage might also be a reason for lower zooplankton abundance in Lake Võrtsjärv as the food density is shown to be important factor affecting zooplankton reproduction and population increase (Rothhaupt, 1990a; Cordova *et al.*, 2001). However, in our study, substantially higher average zooplankton *FR*s were measured on phytoplankton size groups than those on bacteria (Table II), indicating greater importance of algae as food source for zooplankton in these lakes. Christoffersen *et al.* (Christoffersen *et al.*, 1990) and Kim *et al.* (Kim *et al.*, 2000) obtained similar results in eutrophic Frederiksborg Slotssø (Denmark) and in regulated large river Nagdong (Korea), respectively.

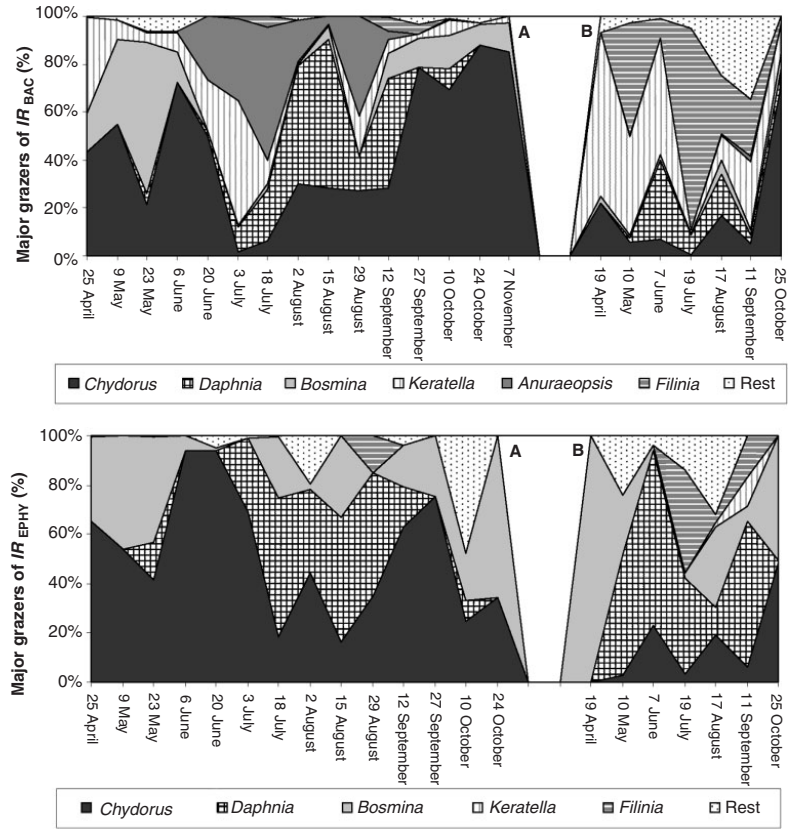

Fig. 7. Major grazers' contribution (%) to metazooplankton bacterivory (IR_{BAC}) and phytoplankton grazing (IR_{EPHY}) in Lake Võrtsjärv (**A**) and Lake Kaiavere (**B**).

Critical analysis of applied method

It was the first attempt to estimate bacterial consumption by metazooplankton in Lake Võrtsjärv, hence grazing on phytoplankton has been measured several times. Also other studies have supported the evidence of low consumption of PP by secondary producers in Lake Võrtsjärv and assumed that the consumption of phytoplankton biomass proceeds most likely through the microbial loop (Nõges, 1997; Nõges *et al.*, 1998; Nõges, 1998a). Comparative studies have shown that the values of the clearance and grazing rates can vary depending on the method of the measurement (Nõges, 1998b; Ooms-Wilms *et al.*, 1995). In Lake Võrtsjärv, earlier studies with different methods (radioactive, particle counting and Chl *a* concentration change) have resulted in substantially higher grazing rates on phytoplankton (Nõges, 1998a; Nõges and Künnap, 2003) than our study (Table V). In 1984 and 1985, the average cladoceran grazing accounted for 2.5% of the phytoplankton biomass and 28.5% of the PP with maximum community grazing of 12% of the phytoplankton biomass and 133% of the PP (Nõges, 1998a). Similar rates were obtained in 1998 when large zooplankton (>100 µm, mainly crustaceans) with the average *FR* 9.4 mL L^{-1} h^{-1} consumed 2.9% of the phytoplankton biomass and 15% of the *PP* (Nõges and Künnap, 2003). Further rough calculations of the results of the present study are based

*Table VI: Species-specific filtering rates (*SFR, *μL individual^{-1} h^{-1}) of some zooplankton taxa from various studies*

Species	0.5-μm microspheres	Published source
Cladocerans		
Chydorus sphaericus	<0.4	Ooms-Wilms *et al.* (1995)
Daphnia cucullata	1–3	Ooms-Wilms *et al.* (1995)
Bosmina longirostris	<0.4, 9.0	Ooms-Wilms *et al.* (1995); Thouvenot *et al.* (1999)
Bosmina coregoni	<0.4	Ooms-Wilms *et al.* (1995)
Rotifers		
Keratella cochlearis	0.009–0.017, 0.006–0.097	Ooms-Wilms *et al.* (1995); Ooms-Wilms (1997); Sanders *et al.* (1989) (*Keratella* spp.)
Anuraeopsis fissa	0.009–0.068, 0.011–0.029	Ooms-Wilms *et al.* (1995); Ooms-Wilms (1997); Sanders *et al.* (1989)
Filinia longiseta	0.16–0.44, 0.07–0.29	Ooms-Wilms *et al.* (1993, 1995); Ooms-Wilms (1997); Sanders *et al.* (1989)
Conochilus unicornis	0.009, 2.2	Ooms-Wilms (1997); Thouvenot *et al.* (1999)

Measured by fluorescent microspheres as tracers.

on the assumption that zooplankton consumes phytoplankton SC1, SC2, SC3 and the bacteria with same efficiency as the microspheres of diameter 3, 6, 24 and 0.5 μm, respectively. If that is assumed, then the average metazooplankton daily grazing could be estimated as 0.5 and 0.1% of the standing stock of bacteria and 2.6 and 1.0% of the standing stock of ingestible phytoplankton in lakes Kaiavere and Võrtsjärv, respectively. That corresponds to average daily grazing of 4.1% of the bacterial production and 0.43% of the total PP in Lake Kaiavere, and, 4.3 and 0.06%, respectively, in Lake Võrtsjärv. Comparing the results with some values from the literature (Table V), a really quite a low grazing intensity on bacteria and phytoplankton was measured in our lakes.

The unique advantage of using fluorescent particles is that they can directly be observed within individual zooplankter, thereby allowing the determination of relative and absolute grazing rates. Though natural particles and microspheres may not be equally preferred by all species (DeMott, 1988; Ooms-Wilms *et al.*, 1993, 1995), the selectivity is generally considered to be low for bacteria-size particles (Kerfoot and Kirk, 1991), but increases with the increasing particle size (DeMott, 1986; Bern, 1990; Rothhaupt, 1990b). The zooplankton community consists of variety of organisms, which are diverse in both feeding mechanisms and in modes of food selection (Pourriot, 1977; Gilbert and Bogdan, 1984; DeMott, 1986). Thus results for total grazing may strongly depend on both method of measurement and the species composition of the zooplankton (Ooms-Wilms *et al.*, 1993, 1995). The low phytoplankton grazing in this study may also be the result of some underestimation. In Lake Võrtsjärv, rotifers generally did not consume phytoplankton. Only in a few occasions were *A. fissa* and *Keratella* spp. observed to ingest the smallest phytoplankton tracers in experiment. There is, however, no reason to expect that zooplankton in Lake Võrtsjärv is more selective than that in Lake Kaiavere. Probably, as the tracer level used in Lake Võrtsjärv was lower because of lower edible phytoplankton density, rotifers might not encounter phytoplankton-size tracer particles in the short-term feeding experiments and the grazing of rotifers on phytoplankton was somewhat underestimated. Several abundant rotifer species in our lakes, e.g. *Polyarthra* spp., *Trichocerca* spp., *Synchaeta* spp. never ingested any size of microspheres, which is also affirmed by other studies for bacteria size microspheres (Sanders *et al.*, 1989; Ooms-Wilms *et al.*, 1995; Thouvenot *et al.*, 1999). This support the view of Gilbert and Bogdan (Gilbert and Bogdan, 1984) that raptorial feeders do not consume bacteria, contrasting with the activity of the filter-feeding rotifers. Generally, the filter-feeding rotifers like *Keratella* are able to collect and process numerous small cells simultaneously (Gilbert and Bogdan, 1984), and prey selection can mainly be described as a passive, mechanical process in which the morphological structure of the filtering apparatus results in size preferences (Pourriot, 1977; Starkweather, 1980; Rothhaupt, 1990b). Probably their poorly selective feeding mode makes them also less discriminative against artificial food tracers. But most of the abundant rotifer species in these lakes, including *Polyarthra* spp. and *Synchaeta* spp. have been shown to consume algae (Pourriot, 1977; Bogdan and Gilbert, 1982; Telesh *et al.*, 1995) and also phytoplankton-size microspheres (Ooms-Wilms, 1997; Kim *et al.*, 2000). Though authors using fluorescent inert particles as tracers have mentioned the rotifer species not consuming microspheres (Sanders *et al.*, 1989; Ooms-Wilms *et al.*, 1995; Thouvenot *et al.*, 1999), none of the studies have estimated their portion of total zooplankton community. In this study, a significant contribution of rotifer species, which did not ingest microspheres to the total biomass and the abundance of metazooplankton (Fig. 4) may have resulted in very low estimated phytoplankton consumption. However, in June and July, most of the rotifers consumed microspheres in the experiments. Therefore, we assume that quite realistic values for rotifers grazing on phytoplankton were achieved in summer in Lake Kaiavere where the rotifers readily ingested phytoplankton-size tracers.

Also copepods generally did not ingest microspheres in our study. This is supported by other studies (Sanders *et al.*, 1989; Ooms-Wilms *et al.*, 1995; Hwang and Heath, 1999) while, however, in experiment of Kim *et al.* (Kim *et al.*, 2000), cyclopoid copepodids and nauplii ingested microspheres, indicating consumption of phytoplankton and very low-level bacterivory. In case of calanoid copepods, plastic beads have been successfully used in size and taste selectivity studies of zooplankton (DeMott, 1988; Kerfoot and Kirk, 1991; Zánkai, 1994). Generally, copepods are considered to be more selective against microspheres than rotifers and cladocerans (DeMott, 1986; Kerfoot and Kirk, 1991). Studies on copepods feeding mechanisms with morphological examination of the feeding appendages, laboratory selectivity studies and new approaches with video techniques have indicated them as highly selective organisms in plankton community (DeMott, 1986, 1988; Kerfoot and Kirk, 1991; Makridis and Vadstein, 1999; Bundy and Vanderploeg, 2002). Copepods examine their food items individually and are able to discriminate between different foods on the basis of particle size, taste and nutritional quality, as well as select on the basis of biochemical composition in terms of toxity (DeMott, 1986, 1988; DeMott and Moxter, 1991; Kerfoot and Kirk, 1991). Copepods discriminate not only between natural food particles and inert particles, depending on the nutritional environment (Kerfoot and Kirk, 1991), but also between inert particles with and without the flavour of algae (DeMott, 1986). The relatively dense copepod assemblages in lakes Kaiavere and Võrtsjärv are mainly formed by cyclopoid copepods of the genus *Mesocyclops* and *Thermocyclops*. Though developmental stages of cyclopoid copepods nauplii and copepodites feed mostly on phytoplankton, they are predominantly carnivorous when adult (Hansen and Santer, 1995). Although copepods (both developmental stages and adults) did not consume microspheres in our experiments, their consumption of protozoa and small metazoa can have significant 'indirect' grazing impact on bacteria and phytoplankton (Adrian and Schneider-Olt, 1999; Hansen, 2000).

In our study, grazing on the particles >24 μm was not estimated. Our largest, 24-μm particles were ingested only on few occasions and thus seemed to be almost inedible. It is, however, possible that hard spheres cannot be ingested if they are bigger than the mouth opening, while the softer and more flexible algae of the same size can still be eaten (Rothhaupt, 1990b). Algae are softer than polystyrene beads and could be broken and compressed during ingestion. DeMott (DeMott, 1995) showed in his experiment with *Daphnia* that algae that are 2–5 times larger than the largest ingestible bead can still be effectively consumed.

In summary, based on consumption of fluorescent microspheres relatively low zooplankton grazing rates were measured in lakes Kaiavere and Võrtsjärv. The low consumption of standing stocks and productions of bacteria and phytoplankton in this study support the results of many other studies that in eutrophic lakes a major part of the bacterial and phytoplankton PP is channelled through the microbial food web. The metazooplankton grazing rate was strongly influenced by the metazooplankton taxonomic composition. The grazing rate on phytoplankton could be higher than estimated in our study as microsphere method may underestimate the *IR* of copepods and some rotifer species. However, the method provided valuable results on differential use of variable food sizes and types on species level of cladocerans and many rotifer species in shallow eutrophic lakes. Our results suggest that phytoplankton was generally the most important food source for cladocerans and rotifers. In the periods of high-seston concentration and scarce edible phytoplankton, bacteria were more readily ingested.

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IV

Microbial Ecology

Ciliates are the Dominant Grazers on Pico- and Nanoplankton in a Shallow, Naturally Highly Eutrophic Lake

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Abstract

Abundance and biomass of the microbial loop members [bacteria, heterotrophic nanoflagellates (HNF), and ciliates] were seasonally measured in the naturally eutrophic and shallow (2.8 mean depth) Lake Võrtsjärv, which has a large open surface area (average 270 km^2) and highly turbid water (Secchi depth <1 m). Grazing rates (filter feeding rates) on 0.5-, 3-, and 6-μm-diameter particles were measured to estimate pico- and nanoplankton grazing (filter feeding) by micro- and metazooplankton. Among grazers, HNF had a low abundance (<50 cells mL^{-1}) and, due to their low specific filtering rates, they only grazed a minor fraction of the bacterioplankton (≤4.2% of total grazing). Ciliates were relatively abundant (≤158 cells mL^{-1}) and, considering their high specific feeding rates, were able to graze more than 100% of the bacterial biomass production in the open part of the lake, whereas the average daily grazing accounted for 9.3% of the bacterial standing stock. Ciliates were potentially important grazers of nanoplanktonic organisms (on average, approximately 20% of the standing stock of 3-μm-size particles was grazed daily). Metazooplankton grazed a minor part of the bacterioplankton, accounting for only 0.1% of standing stock of bacteria. Grazing on nanoplankton (3–6 μm) by metazooplankton was higher (0.4% of standing stock). The hypothesis is proposed that ciliates dominate due to a lack of top–down regulation by predators, and HNF have a low abundance due to strong grazing pressure by ciliates.

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Introduction

Although a shift toward ciliate-dominated grazing on pico-sized particles in eutrophic systems has been previously described (e.g., [40, 25]), heterotrophic nanoflagellates (HNF) are typically the main bacterial grazers in most of aquatic ecosystems (e.g., [4, 12]). Beaver and Crisman [2] also described the compositional shift from large ciliates toward small ciliates (<30 μm) in oligo- and eutrophic environments, respectively. Ciliates are believed to become important members of the microbial loop when their food is abundant (e.g., [48, 37]) and the importance of their competitors and predators has also been recognized (e.g., [34]). In eutrophic systems, ciliates are believed to be controlled top–down by metazooplankton rather than by food availability [17].

An earlier short-term study in Lake Võrtsjärv has shown the importance of ciliate grazing on bacteria [20]. Bacteriovorous ciliates (*Uronema* sp., *Cyclidium* sp., *Urotricha furcata*, *Rimostrombidium* sp.) were able to efficiently control the production of heterotrophic bacterioplankton over the short period of the spring bloom of diatoms. The total abundance of HNF was extremely low and thereby their total grazing on bacteria remained minor compared to grazing by ciliates (approximately 2400 bacteria h^{-1} L^{-1} compared to approximately 90 bacteria h^{-1} L^{-1}, respectively).

The objectives of the present study were to compare grazing on pico-sized [bacteria and autotrophic picoplankton (APP) with a diameter <2 μm] and nano-sized (nanoalgae and protozoa with a diameter from 2 to 20 μm) particles by the grazers HNF, ciliates, rotifers, and cladocerans, in a shallow lake with intensive resuspension. For comparison, the water column of marcrophyte-dominated compartment of the same lake was studied. This was done to assess the differences of grazing in the

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water column of the open part (intensive resuspension, abundant filamenous phytoplankon) and macrophyte-dominated part of the lake. The direct aim of the study was to prove whether ciliates dominate by numbers and grazing on pico- and nanoplankton in phytoplankton-dominated part of this lake over longer periods than short occasions described earlier. The reasons for a shift from HNF grazing toward ciliate grazing in a eutrophic and shallow lake ecosystem are briefly discussed.

Materials and Methods

Sampling. Lake Võrtsjärv is a large (270 km^2) and shallow (mean depth 2.8 m, maximum depth 6 m) eutrophic lake situated in Estonia (coordinates between latitudes 58°05′N, 58°25′N and approximate longitude 26°00′). Station1 (St1) was near the deepest area of the lake, which has the typical limnological characteristics of a phytoplankton-dominated lake (Fig. 1). The southernmost part of the lake—station 2 (St2)—is narrow, very shallow (depth <1.5 m), and filled with submerged macrophytes that display typical macrophyte-dominated characteristics.

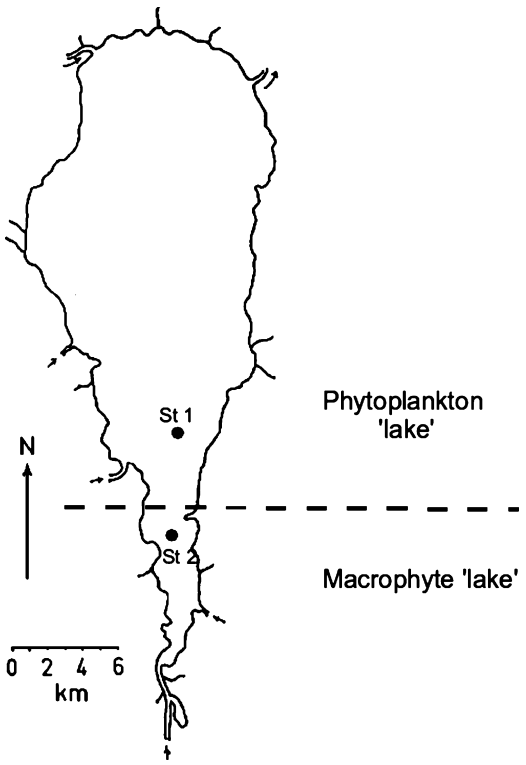

Figure 1. Sampling sites in Lake Võrtsjärv. Station 1 (St1) was situated in the typically phytoplankton-dominated area ("Phytoplankton lake"), and station 2 (St2) was in the macrophyte-dominated area ("Macrophyte lake").

From April to October 2000, St1 and St2 were sampled biweekly. The integrated lake water was obtained by mixing the water collected via a 2-L Ruttner sampler at 1-m intervals through the entire water column. For metazooplankton samples, 10 L integrated water was filtrated through a 48-μm plankton net and concentrated to 100 mL. Samples of phytoplankton, zooplankton, and planktonic ciliates were fixed with acidified Lugol's solution [0.5% (vol/vol) final concentration]. Bacterial samples (20 mL) were preserved with formaldehyde [2% (vol/vol) final concentration].

Counting the Abundance of the Planktonic Organisms. The total number of bacteria (TNB) (means±SD presented) was determined by fluorescence microscopy at 1000× magnification (Leica DM RB) on DAPI-stained 0.22-μm black membrane filters (Osmonics Inc., Livermore, CA, USA) according to Porter and Feig [31]. Filters were obtained from volumes of 1–5 mL of fixed subsamples. At least 400 cells were determined per filter. Autofluorescent picoautotrophs were detected and counted with epifluorescence microscopy by blue and green excitation. In total, at least 200 cells were counted per filter.

The abundance of HNF and small ciliates (<50 μm in size) was determined on the same filters. Samples were fixed with buffered formalin (1% final concentration). Preserved samples were stained for 1–2 min with DAPI at a final concentration 2 μg mL^{-1} and gently filtered through 0.8-μm pore-size black isopore filters (Poretics Inc., Livermore, CA, USA). Protists were examined with a Olympus BX60 fluorescence microscope under 1000× magnification using blue light (470/505 nm, OG 515). Auto- and mixotrophy of flagellates was checked on the basis of autofluorescence of chloroplasts under green (546/565 nm, OG 590) and blue light (470/505 nm, OG 515). A total of 250 (if more than 30 cells were encountered) to 400 (if less than 15 cells were encountered) fields of view were counted on each filter. Where possible the dominant taxa of fluorescently stained ciliates were identified based on the composition of parallel Lugol-fixed samples.

Ciliate biomass and community composition were determined using the Utermöhl technique [46]. Volumes (50 mL) were settled 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. For metazooplankton samples, 10 L integrated lake water was filtrated trough 48 mm plankton net and concentrated to about 100 mL. The samples were fixed with acidified Lugol's solution. Triplicate samples (2.5 or 5 mL) for each metazooplankton were counted under a binocular microscope in a chamber (dimensions 13×6 cm, capacity 8 mL) at 32 and 56× magnifications.

Phytoplankton species composition and biomass were also analyzed using the Utermöhl technique [46]. Cells were enumerated with an inverted microscope (Hund Wilovert S) at 400× magnification. Samples were counted until at least 400 counting units (filaments, cells, or colonies) were reached giving a counting error of ±10% for the total biomass. For grazing experiments, the number of phytoplankton cells (<30.0 μm; presumably edible for zooplankton) was counted separately. Size classes (SC) were split by the maximum linear length measure: 2.0–5.0, 5.0–15.0, and 15.0–30.0 μm for SC1, SC2, and SC3, respectively.

Production Measurements. Total net primary phytoplankton production (particulate + soluble) was estimated by $^{14}CO_2$ assimilation [27, 22]. Samples were incubated for 2 h at constant light intensity (120 W m^{-2}) and at the same temperature as the lake. Non-photosynthetic carbon fixation was measured in dark and subtracted from light assimilation values.

Net heterotrophic bacterial biomass production was measured using tritiated leucine incorporation (TLI). TLI was measured in three 10-mL replicates and two formalin killed negative controls with 59.0 Ci L-(4,5-^{3}H)-leucine $mmol^{-1}$ (Amersham Ltd., Arlington Heights, IL, USA) [41]. The samples were incubated for 60 min at *in situ* temperature in the dark. Incubations were stopped by addition of a final concentration of 2% (vol/vol) formaldehyde. The filters were rinsed with cold trichloroacetic acid [TCA, 5% (vol/vol)] and washed with cold 80% (vol/vol) ethanol. Filters were radioassayed in a LSC Rackbeta 1211 (LKB Wallac, Turku, Finland) and quenching corrected using the external standard ratio method giving a counting efficiency of about 40%. Net bacterio- plankton production (BP) was calculated from leucine incorporation rates by using the empirically derived conversion factors (4×10^{16} cells mol^{-1}) suggested by Kisand and Nõges [19] for L Võrtsjärv. Gross BP was calculated from net BP by using the bacterial growth efficiency (BGE) values (average 30%) suggested by Biddanda *et al.* [3] and del Giorgio *et al.* [8].

Calculation of Planktonic Organism's Biomass. Biomass was calculated from average biovolume (i.e., wet weight) of particular groups of organisms using conversion factors from biovolume to carbon biomass. These were 240 fg C μm^{-3} for APP [49], 100 fg C μm^{-3} for the remaining algae [28], 380 fg C μm^{-3} for bacteria [23], 220 fg C μm^{-3} for HNF [5], and 190 fg C μm^{-3} for ciliates [33]. Dry weight biomass of metazooplankton was estimated by length/weight relationships according to Dumont *et al.* [10] and Bottrell *et al.* [6]. Carbon biomass of metazooplankton was calculated assuming that wet weight constitutes 10% of dry weight and the latter constitutes 40% of carbon.

Grazing Experiments. Grazing by proto- and metazooplankton was measured by *in situ* feeding experiments with fluorescent microspheres of diameters 0.5 μm (Fluoresbrite®; Polysciences Inc., Warrington, PA, USA) for bacteria and APP, and of 3, 6, and 24 μm (Duke Scientific Corporation, Palo Alto, CA, USA) for unicellular phytoplankton and other unicellular organisms of corresponding size. The tracer amount that did not exceed 10% of the density of corresponding size groups of natural pico- and nanoplankton in lake water was added to integrated lake water.

For metazoans, a 3-L incubation vessel was used. Incubations lasted for 7 min (the grazing rate was linear, the vials were shaken gently twice during incubation), after which the water was filtered through a 48-μm mesh size plankton net, anesthetized with carbonated water (submerging the net together with animals into carbonated water for 10 s), and fixed in formaldehyde [final concentration, 4% (vol/vol)]. For microsphere counting, individual metazooplankters were collected from the samples on a polycarbonate filter of 10 μm pore size (Poretics). The number of microspheres in animal guts was counted at 1000× magnification using an epifluorescence microscope (Zeiss Axiovert S100). The ingestion rates (IR; cells $individuals^{-1}$ h^{-1}) for each taxon and community ingestion rates (CIR; cells L^{-1} h^{-1}) were calculated as follows:

$$\mathrm{IR} = M_t \times T \times P/M;$$

$$\mathrm{CIR} = \mathrm{IR} \times N;$$

where M_t is the number of microspheres ingested per individual during the incubation time (microspheres $individual^{-1}$); M is the concentration of microspheres in incubation vessel (mL^{-1}); T is the incubation time (h); P is the concentration of food particles (bacteria, phytoplankton) in the incubation vessel (cells mL^{-1}), N is the abundance of the grazer taxon (individuals mL^{-1}). CIR were determined as the sum of species specific IR for all representative taxa observed. The individuals that had empty guts were included.

In experiments with protozoans, the incubation was carried out in 20-mL vials. Incubations lasted for 7 min, after which samples were fixed with buffered formalin (1% final concentration). Preserved samples were stained for 1–2 min with DAPI at a final concentration 2 μg mL^{-1} and gently filtered through 0.8 μm pore-size black isopore (Poretics Inc.) filters. Protists and the contents of their food vacuoles were examined with a Olympus BX60 fluorescence microscope under 1000× magnification using blue light (470/505 nm, OG 515). Where possible

Table 1. Abundance and total grazing rates in the L. Võrtsjärv water column

		Abundance (cells mL^{-1})		
TNB	St1	0.31–7.86×10^6		
	St2	1.27–5.35×10^6		
APP	St1	5.42–179.5×10^4		
	St2	4.8–31.6×10^4		
		Abundance (individuals L^{-1})	Grazing on picoplankton (particles mL^{-1} h^{-1})	Grazing on nanoplankton (particles L^{-1} h^{-1})
HNF	St1	9.4–39.7×10^3	284–1243	–
	St2	56.2–84.3×10^3	573 – 1824	–
Ciliates	St1	28–158×10^3	5.31–77.14×10^3	17.3–84.7×10^3
	St2	13–106×10^3	10.8–20.3×10^3	5.3–342×10^3
Rotifers	St1	32.9–1967	0.065–215.8	0–685
	St2	10–1187	0.382–186.6	0–578
Cladocerans	St1	6–178	0.123–0.746	0–5043
	St2	0–91	2.0–275.3	52–5099

TNB: total number of bacteria; APP: autotrophic picoplankton; HNF: heterotrophic nanoflagellates.

the dominant taxa of fluorescently stained ciliates were identified based on the composition of parallel Lugol fixed samples. To estimate total ciliate grazing rate, their uptake rates were multiplied by their total *in situ* abundances as in the case of the metazooplankton.

Carbon Consumption by Grazers. Gross grazer production was calculated from the grazing rates and biomass of the grazed food. Biomass of the food was calculated on the bases of the average biovolume of particular size of particles; for 0.5 μm fluorescent microspheres the carbon content of bacteria (380 fg C μm^{-3}) was used and for SC1, 2, and 3 the carbon content of algae (100 fg C μm^{-3}) was used. The biovolume of the SC was calculated from the average diameter of spherical particles in the corresponding SC.

Results

Environment. At station 1 (Fig. 1), temperature varied from 6.7 to 19.3°C, Secchi depth from 65 to 100 cm, total phosphorus (TP) concentration from 50 to 71 μg L^{-1}, and total nitrogen (TN) concentration from 0.65 to 1.20 mg L^{-1}. At station 2 (St 2), TP and TN concentrations were slightly higher compared to St 1 (the highest values were 98 μg L^{-1} and 1.70 mg L^{-1}, respectively).

Abundance of Plankton Organisms. The TNB was statistically equal (*t*-test, p=0.81) at St 1 (3.45× 10^6± 2.42×10^6 cells mL^{-1}, number of replicates n=15) compared to St 2 (3.16×10^6±1.68×10^6 cells mL^{-1}, n=7) (Table 1 and Fig. 2). APP was also less abundant (*t*-test, p=0.04) at St 2 (16.7×10^4±10.1×10^4 cells mL^{-1}; n=7) compared to St 1 (50.5× 10^4±49.8×10^4 cells mL^{-1}; n=15). The abundance of HNF was very low at both sampling sites [22.9±8.6 cells mL^{-1} (n=11) and 70±10 cells mL^{-1} (n=6) at Sts 1 and 2, respectively]. Total abundance of ciliates at St 1 (77.8±47.5 cells mL^{-1}; n=11) was equal (*t*-test, p=0.36) to St 2 (66.8±38.4 cells mL^{-1}; n=6).

During the investigated period the average population of ciliated protozoans was dominated by oligotrichs

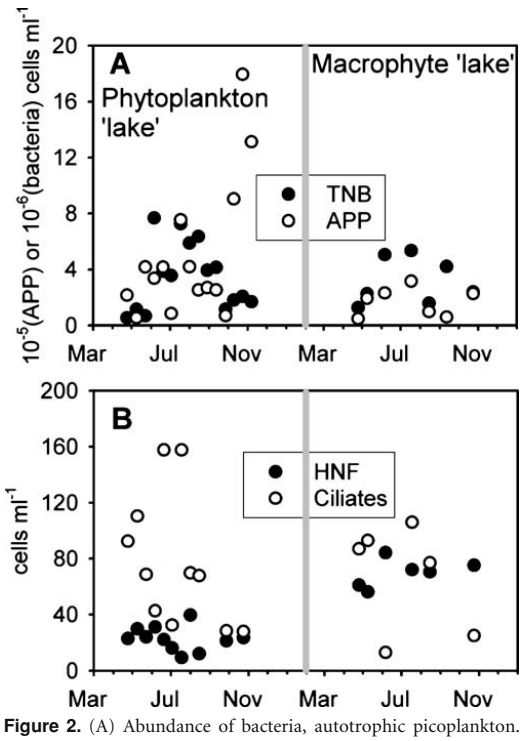

Figure 2. (A) Abundance of bacteria, autotrophic picoplankton. (B) Heterotrophic nanoflagellates and total abundance of ciliates.

(57.4%), scuticociliates (14.6%), prostomatids (11.3%), and gymnostomatids (10.8%). The most common oligotrichs were *Rimostrombidium* sp. (1.2–33.5 cells mL^{-1}, average 10.5), *Halteria* sp. (0.0–8.5 cells mL^{-1}, average 1.1), *Pelagostrombidium* sp. (0.6–8.5 cells mL^{-1}, average 2.5), *Limnostrombidium* sp. (0.4–12.6 cells mL^{-1}, average 1.0), and *Codonella cratera* (0.0–25.1 cells mL^{-1}, average 4.9). Scuticociliates were dominated by *Uronema* sp. (0.5–18.3 cells mL^{-1}, average 7.2) and *Cyclidium* sp. (0.5–28.6 cells mL^{-1}, average 6.4). The most common prostomatids were *Urotricha* sp. (0.2–15.3 cells mL^{-1}, average 6.7), and *Balanion planktonicum* (0.8–9.7 cells mL^{-1}, average 3.2) and most common gymnostomatids were *Mesodinium pulex* (4.6–27.4 cells mL^{-1}, average 8.7) and *Dileptus* sp. (0.0–0.2 cells mL^{-1}, average 0.1). The spring peak was dominated by large sized herbivorous oligotrichs (44.8% of total numbers) with the most abundant species being *C. cratera* (up to 25.1 cells mL^{-1}). The summer peak consisted of small bacterivorous scuticociliates and oligotrich species (29.7% and 26.6% of total number, respectively). The most abundant species were *Cyclidium* sp. (28.6 cells mL^{-1}) and *Rimostrombidium* sp. (33.5 cells mL^{-1}).

Among the metazooplankton, bacterivorous rotifers dominated, whereas cladocerans were considerably less abundant (Table 1). In May and June, *Keratella cochlearis* (127–495 individuals L^{-1}, with an average of 249) and *Polyarthra* spp. (18–123 L^{-1}, average 79) dominated. In July and August, *K. cochlearis tecta* (7–1509 L^{-1}, average 360), *Polyarthra* spp. (3–439 L^{-1}, average 186), *Anuraeopsis fissa* (20–292 L^{-1}, average 173), and *Trichocerca* spp. (35–135 L^{-1}, average 82) were abundant. The highest abundance of cladocerans was observed in June (up to 142 individuals L^{-1}) and the dominant cladoceran was *Chydorus sphaericus* (up to 131 individuals L^{-1}). For more details about metazooplankton, see Agasild and Nõges [1].

Filamentous phytoplankton (diatoms and especially cyanobacteria, *Aulacoseira* spp., *Oscillatoria amphibia* f. *tenuis*, *Planktolynbya limnetica*, *Limnotrix redekei*, and *Aphanizomenon* sp.) dominated in L. Võrtsjärv. Edible phytoplankton for grazers (mostly algae <30 μm in size) comprised only a minor part of the phytoplankton biomass and was the most abundant in June and August. Size class 1 members (2–5 μm) were mainly green algae (*Pediastrum* sp.) and small unidentified algae; SC2 (5–15 μm) were mainly green algae (*Scenedesmus* sp.), crysophytes (*Dinobryon* sp.), and some chryptophytes; meanwhile, SC3 (15–30 μm) was mainly made up of diatoms (*Stephanodiscus* sp. and *Fragillaria* sp.), dinophytes (*Peridinium* sp.), and green algae (*Scenedesmus* sp.).

Grazing Rates at the Community Level. In L. Võrtsjärv the main grazers of picoplankton (bacteria and APP) and nanoplankton were ciliates (Table 1, Fig. 3). Total grazing rates of bacteria by ciliates was considerably higher compared to grazing by metazooplankton (about 100- to 1000-fold) and HNF (8- to 270-fold). Compared to metazooplankton, ciliates grazed 5- to 250-fold more nanoplankton (NP) at St 1, and 8- to 7000-fold more NP at St 2. Ciliates grazed only the smaller fraction of NP (SC1) and none from the larger fraction (SC2 or SC3).

Community-level Loss of Bacteria Due to Protozoan Grazing. Bacterioplankton production calculated from ^{3}H-leucine incorporation rates ranged from 31 to 164,500 cells mL^{-1} h^{-1}. As calculated from measured grazing rates, ciliates could potentially graze from 6% over 100% (median of about 100%) of the bacterial production. HNF grazed from only small fraction (median of 4.8%) of the bacterial production.

Carbon Fluxes in the Microbial Loop. Median carbon flux rates were compared in the phytoplankton (St 1; Fig. 4a) and macrophyte-dominated areas (St 2; Fig. 4b) of the lake. In the phytoplankton-dominated area, phytoplankton biomass was double that observed at the macrophyte dominated compartment (2.28 and

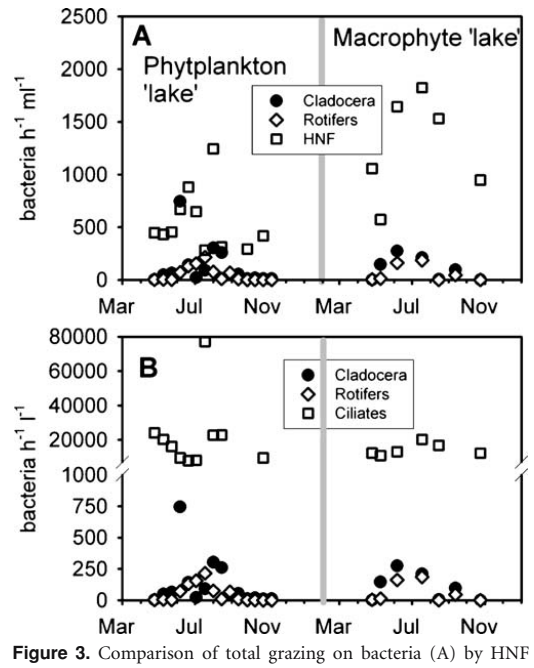

Figure 3. Comparison of total grazing on bacteria (A) by HNF with grazing by rotifers and cladocerans, and (B) by ciliates with grazing by rotifers and cladocerans.

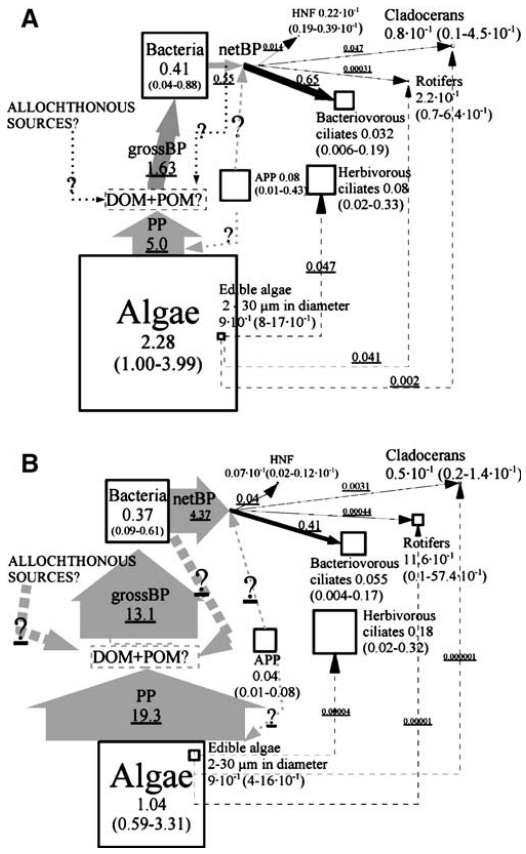

Figure 4. Average carbon flux charts for the (A) phytoplankton- and (B) macrophyte-dominated compartments of the L. Võrtsjärv water column. Diagonal length of the boxes equals the median biomass of planktonic organisms (µg C l^{-1}, numbers represent median carbon biomass, in brackets the absolute range) and the width of the arrows equals the median flux rate (µg C l^{-1} h^{-1}, underlined numbers represent median flux rate). Grey arrows represent production and black arrows show the grazing intensity of planktonic organisms on the different compartments of the food web (e.g., phytoplankton and bacteria).

1.04 mg C L^{-1}, respectively, statistically higher by *t*-test, p=0.02). Smaller sizes of algae (2–30 µm) edible to zooplankton formed a small proportion of the total phytoplankton (0.04% and 0.9% in the open and macrophyte areas, respectively). Primary production (PP) at St 2 (19.4 µg C L^{-1} h^{-1}) was about 4-fold higher than at St 1 (5.0 µg C L^{-1} h^{-1}, statistically different *t*-test, p=0.05). Bacterial biomass was roughly equal in both compartments (0.46 and 0.36 mg C L^{-1} at Sts 1 and 2, respectively, *t*-test, p=0.71). Similarly to PP, the net bacterial production (netBP) at St 2 (4.37 µg C L^{-1} h^{-1}) was approximately 8 times higher compared to St 1 (0.54 µg C L^{-1} h^{-1}, statistically different, *t*-test, p=0.037). At St 1, the netBP was nearly balanced by grazing, which equaled an average of 0.65 µg C L^{-1} h^{-1}. At St 2, total bacterial grazing (sum of all groups) was estimated to consume only 10% of the netBP.

Average daily removal of bacterial standing stock by ciliates was 9.3%, and 0.5% by HNF. Ciliates grazed 20.7% of the standing stock of autotrophic nanoplankton (SC1). In the phytoplankton-dominated compartment of the lake, within 24 h rotifers and cladocerans were able to graze about 0.01% and 0.07% of the standing stock of bacteria, respectively. Estimates for the macrophyte-dominated compartment were 0.04% and 0.14%, respectively.

Discussion

The aim of the study was to compare the role of HNF and ciliates in grazing of pico- (bacteria and APP) and nano-sized (large microorganisms such as unicellular flagellates and algae) particles in a turbid, shallow, and eutrophic lake. The major source of turbidity is resuspension of the partly mineralized sediment particles; the seston concentration varied between 6 and 24 mg L^{-1} of DW during the study period [1], and the carbon content of seston was in average 22% [29]. Our results showed that ciliates, rather than heterotrophic flagellates, were the predominant grazers on the bacteria and APP all over the productive season. Outcompetition of HNF by ciliates over the whole seasonal period in nonhumic eutrophic ecosystems has not been described earlier. In addition, filter feeding metazooplankton did not graze a significant portion of 0.5- to 6-µm-diameter particles. The importance of ciliates in terms of grazing did not differ between the water columns of macrophyte- and phytoplankton-dominated parts of the lake. Flux rates of organic carbon were different between the macrophyte- and phytoplankton-dominated parts. In the macrophyte-dominated compartment, most of the autochthonously produced organic carbon was channeled to heterotrophic bacteria but was not taken up by protists, whereas in the phytoplankton-dominated part of lake the BP was balanced by ciliate bacteriovory.

Dominance of Ciliates. The L. Võrtsjärv ciliate community is extremely rich and usually contains >50% of the whole zooplankton biomass as planktonic ciliates [43]. The annual maximum abundance usually occurs in late July or early August, when the community of ciliates is mostly dominated by small (<30 µm) bacterivorous taxa. The species composition of ciliates in L. Võrtsjärv is highly variable with altogether 70 taxa of ciliates, more than two-thirds of which are considered to be rare, for instance, various benthic species (e.g., *Uroleptus piscis*, *Stichotricha aculeata*, *Stylonychia mytilus*, *Euplotes* spp.) that are probably stirred up by intensive wave action. However, their abundance in the water column remains low (<1 individual mL^{-1}) and their ecological impact to the plankton community is therefore small. There have been few reports of benthic ciliates being resuspended into the water column, e.g., in Kattegat [26] and in a shallow subtropical lake [36]. Therefore, this mechanism in the change in diversity can be considered as unimportant. It is also known that most ciliate species are rare and are only present a few weeks of the year [11]. Thus, it must be acknowledged that many important shifts in protozoan plankton dynamics may be missed due to the low sampling frequency [44]. The most abundant species in L. Võrtsjärv are euplanktonic pico- and nanovores, which seem very well adapted to the shallow and highly turbid ecosystem.

Ciliates are usually strongly regulated by metazooplankton communities [13, 17]. However, this might not be the case in shallow and turbid environments with high abundance of filamentous algae and strong resuspension. Although several ciliate species have an active chemical defense against rotifers [48], we speculate that in turbid environments with a highly abundant filamentous phytoplankton community resuspended particles disturb the grazing by metazooplankton. Therefore, it seems that metazooplankton are not able to regulate the ciliate community.

Low Numbers of Heteretrophic Nanoflagellates. HNF are the major bacteriovores in most lakes because of their efficiency and higher abundance compared to small ciliates (e.g., [34]). In L. Võrtsjärv, consumption of HNF by ciliate grazing may explain why their abundance was so low in the water column and, consequently, why they only played a minor role in bacteriovory. It has been reported that small algivorous ciliates are able to regulate HNF dynamics (e.g., [42, 47]), and filter feeding daphnids and rotifers are also considered to be highly efficient grazers of HNF [15]. However, in light of the low grazing rates of metazooplankton on nano-sized particles in L. Võrtsjärv, this mechanism can be excluded. The domination of ciliate grazing on bacteria (as observed in L. Võrtsjärv) has only been rarely observed. Therefore, we have to compare conditions that are very different from L. Võrtsjärv ecosystems. For instance, a relatively low ratio of HNF to ciliate biomass was reported in the small experimental bog lake L. Fuchskuhle [38, 24], and a study from Canadian boreal lakes demonstrated that ciliates (particularly *Cyclidium glaucoma*) played a major role in grazing on bacteria [45]. Low abundances of HNF have mainly been reported from humic lakes, reaching several hundreds of cells per mL (compared to <100 mL^{-1} in L. Võrtsjärv). In Canadian boreal lakes, the HNF and ciliate carbon biomasses represented approximately 5% and 13% of the bacterial carbon biomass, respectively (mean abundance of 5400 cells mL^{-1}). Similar to L. Võrtsjärv, ciliates were more effective consumers of nanoplankton than metazooplankton (grazing approximately 20% and 1% of the 3-µm-size particles, respectively). However, these ecosystems were different compared to the turbid eutrophic L. Võrtsjärv where the abundance of ciliates and grazing rates were much higher than in Canadian lakes. In L. Võrtsjärv the grazing rates were comparable to lower range in some eutrophic ecosystems, e.g., in the Římov Reservoir in south Bohemia where ciliate grazing values remained in the range of 10–100×10^3 bacteria mL^{-1} h^{-1} [39]. Comerma *et al.* [7] found that ciliates were voracious grazers of bacteria in the eutrophic Sau Reservoir (northeast Spain), consuming 12–146% of the bacterial production. In L. Võrtsjärv the respective values were even higher, with a median of 127% of the bacterial production. In the Sau Reservoir the ciliate abundance was in the same range as in L. Võrtsjärv, and the ciliate grazing rates mostly remained below 40×10^3 bacteria mL^{-1} h^{-1} with only one exceptional peak (180×10^3 bacteria mL^{-1} h^{-1}). In a turbid system where the seston concentrations are very high, the food capture rate may be somewhat reduced. Thus, irrespective of high food supply, the IR of ciliates may stay lower than expected.

Minor Role of Metazooplankton. In shallow and turbid lakes the high concentration of large inedible filamentous and colonial forms of algae does not support the development of a metazooplankton community. Considering the metazooplankton composition in L. Võrtjärv, i.e., abundant population of small rotifers (*Keratella* spp., *Polyarthra* spp., *A. fissa*, and *Trichocerca* spp.), small-bodied cladocerans (mainly *C. sphaericus*), and cyclopoid copepods (*Mesocyclops* spp.), a high grazing pressure on phytoplankton would be unexpected. Based on consumption of fluorescent microspheres, relatively low zooplankton grazing rates on both bacteria and phytoplankton were measured in L. Võrtsjärv. On average, only 0.1% and 1.0% of the standing stock of bacteria and ingestible phytoplankton were grazed daily by metazooplankton, respectively (see also [1]). Therefore, it was concluded that metazoan grazing was unimportant in loss of both bacteria and nanoalgae.

In many lakes, large cladocerans (particularly Daphniidae) have been shown to be the key species in controlling phytoplankton as well as influencing the entire microbial community [21, 15, 16]. In the shallow, turbid, and plankton-dominated L. Võrtsjärv, the genus *Daphnia* was represented by low numbers of *D. cucculata* (median, 4 individuals L^{-1}) of relatively small individuals (median, 0.015 mg individual^{-1}). A high concentration of seston particles (including filamentous algae) is known to negatively affect food collection processes, especially of large filter-feeding cladocerans (e.g., [32]).

In addition to the inhibiting effect, the low nutritional value of seston and filamentous cyanobacteria may initially limit growth of large filter-feeding cladocerans followed by a decrease in their number [14]. Another explanation could be that in L. Võrtsjärv, the low numbers of *Daphnia* can be attributed to size selective predation by the abundant fish including roach, ruff, perch, and bream ([30]; Salujõe and Haberman, personal communication).

The experimental framework, although on the single species level for a three-link food chain: prey–intraguild prey–intraguild predator, was described by Diehl and Feissel [9]. The basal food and top species are predicted to increase with enrichment (i.e., eutrophication). We speculate that in the eutrophic L. Võrtsjärv the cascading effects in the microbial loop are as follows: low abundance of the metazooplankton allows the development of a large community of ciliates (intraguild predator) that grazes on both nano and pico-sized particles; HNF community (intraguild prey) is suppressed and does not have a significant effect on bacteria.

Carbon Fluxes. Carbon biomass estimates showed that filamentous phytoplankton and bacteria were the important reservoirs of the living carbon biomass in both compartments of L. Võrtsjärv (Fig. 4). However, the carbon fluxes were different between the macrophyte- and phytoplankton-dominated compartments, and the ratio of the phytoplankton to bacteria biomass was higher in the macrophyte-dominated part of the lake. Carbon flux was more balanced in the "phytoplankton lake." Calculation of the carbon consumption (bacterivory and herbivory) showed that the bacterivory carbon flux was substantial in the phytoplankton-dominated part of the lake. In the "macrophyte lake," the majority of planktonic PP was neither channeled through planktonic bacteria to planktonic protozoa, nor passed directly by herbivory to higher trophic levels. However, these differences did not change the main range of HNF, and ciliates abundance and their grazing on bacteria and HNF remained unimportant in the food web.

Methodological Remarks. A tracer method using fluorescently labeled inert particles has several methodological advantages. Experiments with particles are conceptually simple, fluorescent microspheres are highly visible inside the consumer, and they do not grow, decompose, or fade. A combination of different diameter spheres in the experiment enabled implications for food type selection to be discerned, e.g., bacteria and different size of phytoplankton [18]. It has been shown that some grazers prefer labeled bacteria to microspheres (e.g., [35]). The size spectrum of natural food is much more variable and this significantly influences the rates of particle uptake; and also, the fluorescent microspheres and labeled bacteria fit only for measuring the grazing rates of unselective filter feeders. This method does not enable measurements of the grazing rates of organisms that actively recognize, select and catch specific prey.

Conclusion

Results from the present study support the hypothesis that small ciliates (<30 μm) are the major consumers of bacteria and algae in such shallow turbid ecosystems as L. Võrtsjärv. Considering the eutrophic conditions, grazing rates were not too high and resembled the more oligo- or dystrophic conditions of highly humic water bodies; in the latter, a similar dominance of ciliates over HNF has been reported. We hypothesize that ciliates do not dominate in L. Võrtsjärv due to favorable eutrophic feeding conditions, but rather due to the lack of top–down regulation by predators. However, the influence of feeding conditions can not be completely excluded. In most eutrophic and hypereutrophic lakes dominated by filamentous algae, phytoplankton edible to zooplankton is scarce, but due to their greater feeding flexibility ciliates seem to dominate in L. Võrtsjärv where the concentration of other seston particles is high.

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1998-2000, Eesti Teadusfondi grant 3579 „Suvise temperatuurikihistuse mõju aineringele järve ökosüsteemis“, magistrand

LIST OF PUBLICATIONS

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1.2. Papers published in other peer-reviewed international journals with a registered code:

Zingel, P., Nõges, P., Tuvikene, L., Feldmann, T., Järvalt, A., Tõnno, I., Agasild, H., Tammert, H, Luup, H., Salujõe, J. & Nõges, T. (2006). Ecological processes in macrophyte- and phytoplankton-dominated shallow lakes. *Proc. Estonian Acad. Sci. Biol. Ecol.*, 55(4): 280–307.

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Nõges, T., Haberman, J., Kangur, A., Kangur, K., Kangur, P., Agasild, H., Timm, H., Zingel, P. & Nõges, P. (2004). Food webs in Lake Võrtsjärv. Haberman, J., Pihu, E., Raukas, A. (eds.). *Lake Võrtsjärv*. Estonian Encyclopaedia Publishers, pp. 335–345.

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3.2. Papers published in books by Estonian or foreign publishers not listed in the ISI Web of Proceedings:

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