The influence of zooplankton enrichment on the microbial loop in a shallow, eutrophic lake

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Abstract

With increasing primary productivity, ciliates may become the most important members of the microbial loop and form a central linkage in the transformation of microbial production to upper trophic levels. How metazooplankters, especially copepods, regulate ciliate community structure in shallow eutrophic waters is not completely clear. We carried out mesocosm experiments with different cyclopoid copepod enrichments in a shallow eutrophic lake to examine the responses of ciliate community structure and abundance to changes in cyclopoid copepod biomass and to detect any cascading effects on bacterioplankton and edible phytoplankton. Our results indicate that an increase in copepod zooplankton biomass favours the development of small-sized bacterivorous ciliates. This effect is unleashed by the decline of predaceous ciliate abundance, which would otherwise graze effectively on the small-sized ciliates. The inverse relationship between crustacean zooplankton and large predaceous ciliates is an important feature adjusting not only the structure of the ciliate community but also the energy transfer between meta- and protozooplankton. Still we could not detect any cascading effects on bacterio- or phytoplankton that would be caused by the structural changes in the ciliate community.

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Introduction

Zooplankton plays an important role in the food web of freshwater lakes, serving as a link between lower (phyto-, protozooplankton) and higher (fish) trophic levels. Selective feeding pressure by different planktivorous fish larvae is one of the most important factors shaping zooplankton communities, affecting the structure, abundance and biomass of different organism groups (Jeppesen et al. 1992; Mills and Forney 1983). Alterations in metazooplankton community structure can in turn influence algal and protozoan communities (Gilbert and Jack 1993; Jürgens and Jeppesen 2000; Wickham 1995a). Thus the ability of zooplankters to prey on phytoplankton and/or different protist groups and the suitability of zooplankton as food for fish determines the efficiency of matter and energy transformation through the food web. Depending on zooplankters food source the energy is transported to upper trophic levels via two different
pathways—through the classical grazing food chain and the microbial loop, which are connected to each other in many indirect and direct ways (Riemann and Christoffersen 1993). When phytoplankton cannot be grazed by metazoans, they enter the less efficient microbial loop that is mediated by bacteria and protists. As a result, planktonic protists are currently the subject of greater scrutiny in aquatic food web studies.

The microbial loop is widely studied in lakes of contrasting trophic status and different geographical areas (Ambland et al. 1995; Gobler et al. 2008; Kisand et al. 1998; Peštova et al. 2008; Zingel 2005; Zingel et al. 2006). With increasing primary productivity, ciliates may become the most important members of the microbial loop (Sherr and Sherr 2002). They can be a significant food source for metazooplankters (Adrian and Schneider-Olt 1999; Hansen 2000; Wiackowski et al. 1994) and in turn major bacterivores, the most important early spring algivores and the main consumers of heterotrophic protozoans (Šimek et al. 1990; Weisse et al. 1990; Zingel and Nóges 2008), thus forming a central linkage in the transformation of microbial production to upper trophic levels.

The cascading effect of copepod and cladoceran dominated zooplankton community on microbial assemblages has been studied quite extensively in freshwater systems (Jürgens et al. 1994; Jürgens and Jeppesen 2000; Wickham 1998; Zöllner et al. 2003). In particular, cyclopoid and calanoid copepods are known to be efficient selective grazers of planktonic ciliates (Burns and Gilbert 1993; Wiackowski et al. 1994; Wickham 1995b), while relatively unselective filter-feeding cladocerans (e.g. Daphnia spp.) have strong top-down impacts on protozoa as well as phytoplankton and large sized bacterial communities (Jürgens et al. 1994; Porter et al. 1988). Still, there are few studies (Jürgens et al. 1994; Jürgens and Jeppesen 2000; Zöllner et al. 2003, 2009) that have examined if the predatory effect of zooplankton on protists cascade down to heterotrophic bacterial communities. In most studies the protozoan communities were usually strongly controlled by metazoan predation but changes in the amount of ciliates did not significantly alter the abundance and biomass of bacterial communities (Sipura et al. 2003; Ventelä et al. 2002; Wickham 1998). In contrast, strong direct effects on bacterial abundances were found when zooplankton community was represented by unselective filter-feeding cladoceran Daphnia spp. in lakes (Jürgens 1994; Pace et al. 1999). Still, most of the experiments indicate that changes in grazer community might cause alterations in cell morphology and community composition rather than change the abundance and biomass (Jürgens et al. 1999, 1994; Zöllner et al. 2003).

Our recent works have shown the importance of planktonic ciliates in the food web of the shallow eutrophic Lake Võrtsjärv (Southern Estonia, Northern Europe) because of their high abundances (reaching values up to 191 cells ml⁻¹) and biomass values (constituting more than half of the total zooplankton biomass (Zingel and Nóges 2010). The positive correlation between the biomass of ciliates and metazooplankters implies that the small-sized protozooplankters are regulated by bottom-up (food availability) rather than top-down factors (Zingel and Haberman 2008; Zingel 1999). Live labeling experiments conducted in Võrtsjärv (Agasild et al. 2012) have shown that microciliates (15–40 µm) were consumed by all dominant cladoceran and copepod species (mainly by Chydorus sphaericus and cyclopoid copepods) but their predation effect on ciliates was relatively weak. Our further enclosure studies (Agasild et al. 2013) in Võrtsjärv revealed that the removal of a large fraction of crustaceans also initiated a decrease in the total abundance of ciliates. At the community level, we observed a substantial increase in large-sized predaceous ciliates (>100 µm) and a simultaneous decrease in the abundance of smaller ciliates, which however did not cascade down to the level of bacteria and edible phytoplankton. It was suggested that an important trophic link exists between cyclopoid copepods and the large-sized predatory ciliates that triggered the trophic cascade in the planktonic ciliate assemblage.

In the present study, mesocosm experiments with different cyclopoid copepod enrichments were conducted to (i) examine the responses of ciliate community structure and abundance to changes in cyclopoid copepod biomass; (ii) detect cascading effects on bacterioplankton and on edible phytoplankton. Our hypothesis was that in a shallow eutrophic lake, the ciliate community structure is controlled by copepods. Copepods can suppress predaceous ciliates, leading to an increase in small-sized ciliate abundance.

Material and Methods

Study site

Võrtsjärv, the second largest lake in Estonia, is situated in the South-Estonian pre-glacial basin, centred at 58°15′7″ N and 26°1′47″ E (Fig. 1). The lake has an elongated shape, with a surface area of 270 km², length of 34.8 km and width of 14.8 km. Despite the large surface area, the lake is shallow with a mean depth of 2.8 m and a maximum depth of 6 m. Lake water is well mixed and turbid due to surface waves and currents. During the growing season Secchi depth usually does not exceed 1 m in the central parts of the lake (Nõges et al. 1998). The average concentrations of total phosphorus (0.05 mg P L⁻¹) and nitrogen (2 mg N L⁻¹) characterise the lake mostly as a eutrophic to hypertrophic water body (Tuukkene et al. 2004).

The shallowness of the lake together with wave-induced resuspension of bottom sediments is responsible for algal blooms and contributes to the formation of high seston concentrations and high turbidity during summer. The phytoplankton community is mainly dominated by two groups, the narrow filamentous cyanobacteria and the diatoms. Among the cyanobacteria, four filamentous species Planktolyngbya limnetica [(Lemm.) Kom.-Legn.], Limnothrix planktonica [(Wolosz.) Meffert], Limnothrix redekei [van Goor Meffert] and Aphanizomenon skujae (Kom.-Legn. et Cronb.) are the
most numerous in summer and autumn. The diatoms are dominated by the genus *Aulacoseira* in spring. The mean annual chlorophyll a concentration is 27 µg L⁻¹ (Nõges et al. 2008), but maximum values may reach up to 100 µg L⁻¹.

The ciliate community accounts for 64% of the total zooplankton biomass and is dominated by large-sized herbivorous oligotrichs and prostomatids in spring and by small bacterivorous oligotrichs and scuticociliates in summer (Zingel 1999). The metazooplankton community is dominated by small-sized rotifers (mainly *Polyarthra* spp., *Keratella* spp. and *Anuraeopsis fissa*), small-bodied cladoceran species *Chydorus sphaericus* (O.F. Müller), *Bosmina longirostris* (O.F. Müller), *Daphnia cucullata* (Sars), and by cyclopoid copepods of genera *Mesocyclops* and *Thermocyclops* (Haberman 1998). Metazooplankton biomass is relatively low, rarely exceeding 100 µg C L⁻¹; biomass maximum usually occurs in June.

**Sampling design and collection of samples**

The mesocosm experiment of cyclopoid copepod enrichment was carried out from August 20th to September 11th, 2009. This period was selected as cyclopoid copepods are generally dominant in the metazooplankton in this time of the year. This allowed for a more efficient collection of these animals to substantially increase their concentration in the experimental mesocosms and create a clear difference between the treated and control mesocosms with naturally low zooplankton concentration. Moreover, a high abundance of small bacterivorous and large predatory ciliates was expected in this time period offering a suitable time frame for studying cyclopoid and ciliate interactions. Six transparent plastic enclosures (polyethylene bags with glass-fibre reinforcement: diameter 1.5 m, depth 4 m, volume 5 m³), closed at the bottom and fixed in the wooden frame were filled with natural lake water. Enclosures 1 and 2 (subsequently referred to as M1) were left intact containing zooplankton of natural concentration and serving as control media. The remaining four enclosures were treated with two different copepod concentrations. Zooplankton for enrichment treatments was collected with 145-µm-mesh plankton net from the same lake 1 day prior to the experiment. The collected fraction of the metazooplankton community comprised an overwhelming amount of copepods (adult and copepodite stages); the rotifer assemblage, which was composed of...
small-sized species, mainly *Anuraeopsis fissa* and *Keratella tecta*, was not represented in this fraction. To obtain different levels of cyclopoid copepod predation impact in experimental treatments, the collected copepods were added to the experimental media. Enclosures 3 and 4 (subsequently referred to as treatment M2) yielded an enrichment of approximately 2.5× of natural copepod biomass, and the enclosures 5 and 6 (treatment M3) yielded an enrichment of approximately 5 times the natural copepod biomass. The enrichment by 2.5 times approximately corresponds to the highest observed yearly biomass of cyclopoid copepods in Vörtsjärv; the enrichment by 5 times was chosen to follow the response of ciliate assemblages to the extreme biomass peak of copepods.

The experiment lasted for 22 days, wherein different plankton communities (bacteria, phytoplankton, ciliates, metazooplankton) were sampled 8 times (on days 1, 4, 7, 9, 13, 16, 19, 22). Bacterial production was measured on 7 dates (days 4, 7, 9, 13, 16, 19, 22) and phytoplankton primary production was measured in 4 mesocosms (1 M1, 1 M2 and 2 M3) on 5 dates (days 9, 13, 16, 19, 22). To analyse the plankton dynamics, integrated water samples were collected from each mesocosm—water was collected from three depths (0.5 m, 1 m and 2 m) and integrated in a barrel. For metazooplankton samples 10 L of water was poured through a 48-μm-mesh net. These concentrated samples as well as 200 mL of unfiltered water for ciliate and phytoplankton analyses were preserved with acidified Lugol’s solution (0.5% final concentration). 10 mL water samples were fixed with glutaraldehyde (1% final concentration) for bacterioplankton analyses.

**Sample analysis**

The taxonomic composition, abundance and biomass of phytoplankton and ciliates were determined by *Utermöhl technique* (1958). For ciliates, volumes of 50 mL were allowed to settle for at least 24 h in counting chambers. Ciliates were enumerated and identified with an inverted microscope (Nikon Eclipse Ti-U; Nikon Instruments Europe B.V., Amstelveen, the Netherlands) at 400–1000× magnification. The entire contents of each *Utermöhl* chamber were surveyed. Abundances were counted in two size classes: 20–40 μm and >40 μm. Ciliate carbon content was determined from the measured volumes by using a conversion factor of 190 fg C μm⁻³ (Putt and Stoecker 1989).

Phytoplankton cells were enumerated and measured with an inverted microscope (Ceti Versus, Kontich-Antwerp, Belgium) at 100× or 400× magnification. Samples were counted until at least 400 counting units (filaments, cells, colonies) had been processed, which gives a counting error of ±10% for the total biomass. The phytoplankton community of Vörtsjärv is mainly dominated by large filamentous species, therefore a reduced impact of zooplankton grazing on the phytoplankton community is obvious. To still determine any cascading effects on the algal community, phytoplankton cells (<30 μm), presumed edible for zooplankton, were counted separately. Size classes were split according to the maximum linear length: 2.0–5.0 μm as the first, 5.0–15.0 μm as the second and 15.0–30.0 μm as the third size class (SC1, SC2 and SC3, respectively). Phytoplankton biomass in carbon units was calculated using a biolume conversion factor of 0.22×mgC×mm⁻³ (Reynolds 1984).

Phytoplankton primary production (PP) was measured 5 times in situ with ¹⁴C-assimilation technique (Steemann-Nielsen 1952) (30th August, 2nd, 5th, 8th, 11th September). The radioactivity of the sample was measured with a scintillation counter (LSC RackBeta 1211, Wallac, Finland) using external standardisation for decays per minute (DPM) calculations and Optiphase ‘HiSafe 3’ scintillation cocktail (Wallac, Finland). Integral primary production (PPint) was calculated by integrating measured PP values over depth. For more precise description about the measuring technique refer to Nöges et al. (2011).

The abundance of heterotrophic bacteria was determined by DAPI (4’,6’-diamidino-2-phenylindole) direct count (Porter and Feig 1980). Samples (3 mL) were filtered through 0.2-μm pore-size black polycarbonate membrane filters (Poretics Inc., Livermore, CA, USA) and stained with DAPI for 5 min at a final concentration of 10 μg mL⁻¹. Filters were stored at 20°C until counting with an inverted microscope (Zeiss Axiovert S100; Carl Zeiss MicroImaging GmbH, Jena, Germany) at 1000× magnification using violet light. At least 200 cells were counted per filter.

Bacterial production was determined by leucine (Leu) incorporation as specified in Kirchman et al. (1985) with modification of cold trichloroacetic (TCA) treatment reported by Wicks and Robarts (1988). Six 10 mL replicates including three formalin-killed blanks per sample were incubated at *in situ* water temperature for 1 h with ¹⁴C-Leucine (306 mCi mmol⁻¹, Amersham Ltd.). The incubation was terminated by the addition of 2% formalin (final concentration). The precipitate for radioactivity assessment was achieved from cold 100% TCA treatment, filtered onto cellulose acetate filters (Millipore Inc.), and washed by cold 5% TCA and cold 80% ethanol. Filters were radioassayed by LSC Rackbeta 1211 (LKB Wallac). Leucine incorporation was converted to protein production using the fractions 0.073 Leu/protein and 0.86 C/protein according to Simon and Azam (1989).

Metazooplankton samples were counted under dissecting microscope (Olympus SZ40; Olympus Deutschland GmbH, Hamburg, Germany) at 60× magnification. Crustacean and rotifer carbon biomasses were estimated by length-carbon relationships (Manca and Comoli 2000; Telesh et al. 1998), and by length-dry weight relationships (Bottrell et al. 1976; Dumont et al. 1975) using the carbon conversion factor of 0.48 mg C per mg dry weight (Andersen and Hessen 1991).
Fig. 2. The average biomass (a) and abundance (b) of different metazooplankton groups in mesocosms (M1 = natural copepod biomass concentration, M2 = 2.5 × of natural copepod biomass, M3 = 5 × of natural copepod biomass).

Statistical analysis

The program STATISTICA 8.0 for Windows (StatSoft, Inc. 2007) was used for statistical analyses. The simultaneous comparison of all different treatments (control enclosures (M1), copepod-enriched treatments (M2 and M3)) was performed using the Friedman’s ANOVA and Kendall’s concordance nonparametric analysis of variance for multiple dependent samples. The nonparametric Wilcoxon matched pairs test was used for comparing two dependent samples. In more detail, the Friedman’s test shows only the general difference between all treatments but does not show which treatments exactly differed from which. Therefore also the Wilcoxon matched pairs test was applied for revealing the difference between treatment pairs. Spearman’s correlation analysis was used to determine the relationships between the different biotic indices. As the replicate mesocosms did not show any significant differences in biotic indices, the values of replicate indices were averaged.

Results

Metazooplankton

Metazooplankton (MZP) community composition and dominants were taxonomically quite similar in all mesocosms. During the whole experiment copepods (mostly *Mesocyclops leuckarti*) dominated, constituting 18–88% of the total MZP biomass (Fig. 2A). Rotifers (mainly *Anuraeopsis fissa* Gosse, *Keratella tecta* Gosse, *Polyarthra luminosa* Kutikova,) were numerically dominant, accounting 68–95% of the total MZP abundance (Fig. 2B). Cladoceran community was dominated by *Daphnia cucullata* but its abundance was low. Copepod biomass in the enriched enclosures was initially clearly higher than in the control enclosure. At the beginning of the experiment copepods formed 71% and 85% of total metazooplankton biomass in treatments M2 and M3 respectively, and 54% in control media M1. Thereafter the biomass of copepods started to decrease in all treatments and...
at the end of the experiment the biomass of copepods in M2 and M3 was quite similar with the M1 enclosures. Maximum and minimum values of copepod biomass in M1, M2 and M3 were 35.2 and 7.1, 81.2 and 14.6, and 133.6 and 15.7 μg CL⁻¹, respectively.

We found statistically significant differences (Friedman’s ANOVA, p < 0.05) between all the different treatments in the total biomass of MZP, in the total biomass and abundance of copepods, in the biomass and abundance of adult copepods + copepodites as well as in the biomass and abundance of *Leptodora kindtii* and *Polychaera luminosa*. On average, the abundance and biomass of cladocerans were slightly higher in M2 (8.9 ind.L⁻¹; 12.2 μg CL⁻¹) and M3 (7.5 ind.L⁻¹; 8.8 μg CL⁻¹) compared to M1 (6.7 ind.L⁻¹; 7.5 μg CL⁻¹), however this difference was statistically not significant (Friedman’s ANOVA, p > 0.05). Among cladoceran species the abundance and biomass of *Leptodora kindtii* differed between M1 and M2 and M2 and M3 (Wilcoxon test, p < 0.05). As the rotifer community remained intact in the enrichment process their abundance and biomass did not differ significantly between the treatments (Friedman’s ANOVA, p > 0.05). Among rotifer species the abundance of *Polychaera* spp. differed between M1 and M3 and its biomass differed between M1 and M3 and M2 and M3 (Wilcoxon test, p < 0.05).

**Ciliates**

The total abundance of ciliates followed a decreasing trend in all the studied treatments (Fig. 3B), ranging between 41.2 and 87.3 ind. mL⁻¹ and being highest in M3. During the whole experiment, the ciliate community was dominated mostly by bacterivorous species (*Rimostrombidium* spp., *Cyclidium* spp., *Uronema* spp.), accounting for averagely 64% of the total ciliate abundance (Fig. 3B). The less abundant (on average 1.42 ind. mL⁻¹) species were predaceous ciliates (mainly *Paradideptus elephantinus* Kahl) but their abundance and biomass increased at the end of experiment. This led to the slight increase also in the total ciliate biomass (Fig. 3A), which changed during the experiment only in a small range (709–889 μg CL⁻¹). During the whole experiment bacterivorous ciliates generally dominated, constituting

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**Fig. 3.** The average biomass (a) and abundance (b) of ciliates in different functional groups in mesocosms (legend for M1, M2, M3; see Fig. 2).
on an average 40.5% of the total protozooplankton biomass. Only at the end of experiment the biomass of predaceous ciliates increased sharply, leading to great changes in ciliate functional composition with the final community in M1 and M2 being dominated by predaceous species and the proportion of bacterivorous ciliates decreased (Fig. 4). We found also significant differences in the biomass and abundance of predaceous and bacterivorous ciliates considering all different treatments (Friedman’s ANOVA, \( p > 0.05 \)). Wilcoxon test showed significant differences \( (p < 0.05) \) in predaceous ciliate biomasses between the treatments M1 and M3 and M2 and M3 and in their abundances between the treatments M1 and M2 and M1 and M3; differences in bacterivorous ciliate abundances and biomasses occurred between the treatments M1 and M3 and M2 and M3. The abundances and biomasses of herbivorous and bacterio-herbivorous ciliates did not differ significantly between the treatments (Wilcoxon test, \( p > 0.05 \)).

The ciliate community in all mesocosms was dominated by smaller size class (15–40 \( \mu \)m; mostly bacterivorous ciliates) that comprised averagely 80.5% of the total ciliate abundance. The abundance of larger (>40 \( \mu \)m; mostly predaceous ciliates) species remained quite low (on the average 12 ind. mL\(^{-1}\)) during the whole experiment. However, in different treatments we found significant differences in the total abundance of ciliates and in the total abundance of their smaller (15–40 \( \mu \)m) fraction (Friedman’s ANOVA, \( p < 0.05 \)). Wilcoxon test showed that the total abundance of ciliates as well the total abundance of 15–40 \( \mu \)m size class differed between M1 and M3 and M2 and M3 \( (p < 0.05) \). In abundances of the larger (>40 \( \mu \)m) fraction we found significant differences between the treatments M1 and M2 and M2 and M3 (Wilcoxon test, \( p < 0.05 \)).

In both manipulated mesocosm types (M2 and M3) a negative correlation was found between the biomasses of predaceous and bacterivorous ciliates (Spearman rank order \( r = -0.90, p < 0.05 \)). In M1 the biomass of predaceous ciliates was negatively correlated with bacterio-herbivorous ciliates \( (r = -0.81; p < 0.05) \). Pooled data from all mesocosms and treatments showed a clearly significant inverse relationship between large predaceous and small bacterivorous ciliates (Fig. 5A) indicating the predator-prey regulation.

The biomass of MZP was positively correlated with the abundance of smaller (15–40 \( \mu \)m) ciliates in all different treatments, and most strongly with the biomass of bacterivorous ciliates in M1 and M2 (Spearman \( r = 0.71; p < 0.05 \)). Pooled data from all mesocosms and treatments showed that copepods had a generally negative effect on the predaceous ciliate abundance and an overall positive effect on the total ciliate abundance (Fig. 5C, B, respectively).
Bacterioplankton

The total number of bacteria ranged from 3.12 to 4.82 × 10^6 cells mL⁻¹ in the control mesocosm, a similar range was observed also in treatments M2 (3.08–4.43 × 10^6 cells mL⁻¹) and M3 (3.35–4.87 × 10^6 cells mL⁻¹), decreasing slightly during the whole experiment in all different treatments (Fig. 6A). Bacterial abundances were slightly higher in the M3 treatment and control mesocosms but the difference between all treatments was not statistically significant (Friedman’s ANOVA, p > 0.05). The biomass of the total ciliate community was negatively correlated with the abundance of bacteria in all treatments but this correlation was statistically significant only in M3 (r = −0.73; p < 0.05). Contrarily, the total abundance of ciliates, bacterivorous ciliates and ciliates in smaller size class (15–40 µm) was positively correlated with the numbers of bacteria in all treatments but again the correlations were significant only in M3. Considering bacterial production, the lowest values occurred in M2 (Fig. 6B). Overall, there appeared statistically significant differences (Friedman’s ANOVA, p < 0.05) in bacterial production comparing all the different treatments, whereby Wilcoxon test showed a clear difference (p < 0.05) only between M2 and M3.

Phytoplankton

Phytoplankton biomass in all the treatments was rather similar (Fig. 7A) amounting to an average of 5.8 ± 1.0 (st.dev.) mg CL⁻¹. Different zooplankton amount did not have an evident impact on total biomass and abundance of phytoplankton as no statistically significant differences were found between the treatments (Friedman’s ANOVA, p > 0.05). In all treatments the majority of phytoplankton biomass (87.7 ± 3.7%; 4.9 ± 0.8 mg CL⁻¹) was formed by filamentous cyanobacteria (Limnothrix planktonica (Wolosz.) Meffert, L. redekei Van Goor (Meffert), Aphanizomenon skujae Kom.-Legn. et Cronb., Planktothrix agardhii sp.) (Fig. 7A). Diatoms (Syndered spp., Navicula sp., Nitzschia spp., Aulacoseira ambiguax (Grunow) Simonsen)
were numerically dominant (Fig. 7B). Chryso-, chloro-, euglenophytes and dinoflagellates formed only a minor part of the community.

The edible fraction of phytoplankton (SC1, SC2, SC3) was dominated by smaller diatoms, crypto-, chloro-, chryso-
phytes, euglenophytes and cyanobacteria, forming altogether 14.4–36.3% of phytoplankton biomass and 4.6–13.5% of its abundance. There appeared no statistically significant differences between the treatments in the abundances and biomasses of any phytoplankton size-classes (Friedman’s ANOVA, \( p > 0.05 \)). With respect to integral primary production the lowest values occurred in M2 and highest value in M1 (Fig. 8).

**Discussion**

In Vörtsjärv copepods have two seasonal peaks, first in May and the second usually in July or August, co-occurring with the highest ciliate abundance and the dominance of small-sized bacterivores in the planktonic ciliate assemblage (Zingel 1999). Cyclopoid copepods form an important and often the dominant group among zooplankton in eutrophic lakes (e.g. Hansen 2000; Santer et al. 2006). Predatory cyclopoids have a relatively broad feeding spectrum and

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**Fig. 7.** The biomass (a) and abundance (b) of presumably edible phytoplankton (PP) size classes (SC1 2.0–5.0 µm, SC2 5.0–15.0 µm, SC3 15.0–30.0 µm), and total phytoplankton biomass and abundance in mesocosms (legend for M1, M2, M3; see Fig. 2).

**Fig. 8.** Integral primary production in mesocosms (legend for M1, M2, M3; see Fig. 2).
they can have a strong direct top-down effect on ciliate abundances (Hansen 2000; Wiackowski et al. 1994; Wickham 1995a). As the current study was performed in large enclosure volumes with ambient zooplankton to keep the conditions close to natural as much as possible (Sarnelle 1997), we cannot totally rule out the effect of other metazoan zooplankton–cladocerans and rotifers. Still, the biomass of copepods dominated overwhelmingly in metazooplankton community during the experiment, making them the most likely group among metazoan zooplankters to impact planktonic ciliates.

We found clear differences between all different treatments in the total abundance and biomass of cyclopoid copepods. However, the increased abundance and biomass of copepods in the experimental treatments did not suppress ciliate abundances. Instead, the copepods had a clear positive effect on the total ciliate abundance and a negative effect on the preda-
ceous ciliate numbers (Fig. 5B, C, respectively). The biomass of copepods was positively correlated with the biomass of small-sized bacterivorous ciliates. In our former short-term experiment in Võrtsjärv, the removal of large crustaceans (in contrast to their increased number in the present study), did not cause the increase in ciliate abundances, which was the expected reaction to a weakened top-down control by metazoans. Instead the total ciliate abundance decreased because only predatory ciliates profited from weakened metazoan control while their feeding pressure on more abundant smaller ciliates increased substantially (Agasild et al. 2013). Studies from the eutrophic lake systems have shown that the cyclopoid copepods can effectively control the protozoan communities and thereby influence the efficiency of the microbial food web (Jürgens et al. 1999; Wickham 1995a). Dominant species in the Võrtsjärv crustacean community are relatively small cyclopoids (e.g. Mesocyclops leuckarti and Thermocyclops oithonoides G. O. Sars) and small-bodied cladocerans (Clydorus sphaericus, Daphnia cucullata and Bosmina longirostris). We have formerly carried out crustacean feeding studies in Võrtsjärv, using labelled natural ciliates (Agasild et al. 2012). This study revealed that though the small ciliates (15–40 μm) are ingested by all dominant crustacean taxa in Võrtsjärv, the overall impact of the crustacean community on the ciliate total number was low. From July to September, the crustacean feeding comprised only 3.9–6.8% of the ciliate standing stock.

In the present experiment all cyclopoid communities experienced also a noticeable temporal decline. The decrease in copepod abundance and biomass in treatments M2 and M3 after the onset of experiment was apparently caused by high predation pressure on their juvenile stages and by food limitation caused by substantial copepod enrichment compared to control conditions. Mesocyclops leuckarti, the dominant cyclopoid in the experiment, is commonly predatory already from the early copepodite stages (Hansen and Santer 1995). For cyclopoids, the cannibalistic feeding is also described and their predation can cause a substantial mortality of juveniles, affecting thereby also their population size (Bosch et al. 1993). In our treatment enclosures the biomass of nauplii was substantially decreased on the experimental days 4 and 7; among rotifers during the same period, a twice lower abundance and biomass of soft-bodied Polyarthra spp. was observed compared to control media indicating clearly an increased predation. Similar dynamics was not evident among the rotifer dominants with thick lorica, such as Ana-
rueopsis fissa and Keratella tecta. Soft-bodied rotifers are known to suffer strongly from copepod predation and their population dynamics are tightly coupled (Devetter and Seda 2006; Dieguez and Gilbert 2001). As the copepodites and females of M. leuckarti are strongly dependent on animal food (Hansen and Santer 1995), the decline in their major food items might have caused the shortage of food sources and subsequent decline in copepod populations in our experiment.

Still, in case of food shortage, one might expect cope-
pods to switch to protozoans as an alternative food source as the ciliate community in our experiment was very abundant. However, this was not the case and the underly-
ing reason probably lies in the ciliate composition. From June onward, the planktonic ciliate assemblage in Võrt-
sjärv was largely formed by small (<30 μm) picovorous ciliates (such as Cyclidium spp., Uronema spp. and small-sized Rimostrombidium, also dominating in the experiment), which may be too small prey or too hard to catch for cyclopoid copepods (Adrian and Schneider-Olt 1999; Hansen 2000; Wiackowski et al. 1994; Wickham 1995b). Also the former crustacean feeding studies in Võrtsjärv (Agasild et al. 2012) showed that cyclopoid copepod predation rates on micro-sized ciliates (15–40 μm) were substantially reduced (0.7–7.4 cells ind.−1 h−1) from July to September compared to rates in June (22.6–33.1 cells ind.−1 h−1) when the ciliate community was dominated by relatively large herbivorous species. Copepod preference to larger prey types among ciliates over small ones is often described, which implies a better detection and capture of larger motile prey compared to small ones (Adrian and Schneider-Olt 1999; Nishibe et al. 2010; Vincent and Hartmann 2001; Wickham 1995a; Zöllner et al. 2003). Thus, beside the bottom-up effects, such as the increased supply from bacterioplankton co-occurring the cyanobacterial bloom, the dominance of small picovorous ciliates in late summer in Lake Võrtsjärv (Nöges et al. 1998; Zingel and Nöges 2010) is partly an adaption to increased copepod predation. This trade-off is resulting also in relatively weak transfer of microbial loop carbon through the food web during that phase of plankton seasonal succes-
sion.

The predator-prey species-specific interactions, noticed especially for cyclopoid copepods (Wiackowski et al. 1994; Wickham 1995a) turned out to be more important in our experiment than the overall direct feeding on small ciliates. Similarly to Agasild et al. (2013) we found in the current experiment that ciliate abundance was predominantly regulated by internal community structure. The increase in the cyclopoid biomass led to a decreasing number of
large predacious ciliates, which are known to actively feed on small-sized ciliates. Towards the end of the experiment the overall biomass of crustaceans decreased leading to a concurrent increase in a predacious ciliate biomass. Most probably a trophic link exists between cyclopoid copepods and the large predacious ciliates. As raptorial feeders, the genera *Mesocyclops* and *Thermocyclops* have been reported to ingest various metazooplankters and insect larvae (Blumenshine and Hambright 2003; Panagadía-Reyes et al. 2004). Therefore, attacking large-sized (>300 μm) and relatively slow-moving ciliates is also likely as it has recently been showed for epibenthic cyclopoid copepod (Reiss and Schmid-Araya 2011). A strong inverse relationship between cyclopoid copepods and large ciliates has been likewise observed, for example, in the Salton Sea (Tiffany et al. 2007).

Our experiment demonstrated that there seems to be a clear pattern of ciliate community regulation in conditions of low abundance of crustaceans and in the presence of large ciliate predators. These predacious species most probably declined the abundances of small-sized ciliates and shifted dramatically the size structure and species composition of the ciliate community. Dolan and Coats (1991) estimated the feeding of predacious ciliates on small bacterivorous species at prey abundances similar to those in our experiment and found ingestion rates comparable to those measured for crustaceans in Võrtsjärv (Agasild et al. 2012). Owing to their substantially increased abundance towards the end of the experiment, the predacious ciliates most probably reduced the abundances of small-sized ciliate species.

Due to their high abundances in Võrtsjärv, ciliates have been found to be the dominant predators of bacteria consuming nearly 100% of biomass production of bacteria (Zingel et al. 2007). Despite that, the decrease in bacterivorous ciliate abundance was not reflected in higher bacterial numbers. Contrarily, the bacterial abundances also declined towards the end of the experiment together with small-sized ciliates. This can be partially explained with bacterial production rates, which also declined simultaneously (Fig. 6B). Besides, several experiments have shown that changes in grazer’s community might cause taxonomic shifts in the bacterial assemblage rather than changes in abundance (Zöllner et al. 2003).

There were also no apparent linkages between ciliates and phytoplankton biomass. Our former studies have shown that in Võrtsjärv the major consumers of edible algae (Zingel et al. 2007), are not metazooplankters but ciliates, consuming about 20% of the standing stock of nanoplankton. The small-size edible phytoplankton, which is under strong grazing pressure by herbivorous ciliates only constitutes an average of 10% of total phytoplankton biomass in Võrtsjärv. Metazooplankton community has only weak grazing impact on phytoplankton mainly due to small-sized grazer composition and the algal assemblage dominated by inedible filamentous forms of diatoms and cyanobacteria. The average daily consumption forms only 4% of the total phytoplankton biomass (Agasild et al. 2007). As phytoplankton forms most of the planktonic biomass in Võrtsjärv and 90% of phytoplankton is made up by inedible species, some inertia in the general food web structure is expected. This was reflected also in our experiment where phyto- and bacterioplankton showed a relatively stable population and community structure (Figs. 6, 7). The decline in primary production (Fig. 8) in the middle of the experiment was not due to increased grazing, but because of poor light conditions during that day. We do not know the exact reason for the slightly lower bacterial and phytoplankton production in treatment M2 compared with M1 and M3 (Figs. 6B, 8). Still, one explanation can be that as the M3 had the highest share of bacterivorous ciliates also the grazing pressure on bacteria was accordingly highest on that treatment. Similarly the M1 had highest share of herbivorous ciliates and therefore also the highest grazing pressure on edible algae (Zingel et al. 2007). It is a well-known fact that higher grazing pressure can generally stimulate higher productivity (e.g. Sommer 2012) and this could be the case also in our experiment. The exact mechanisms linking bacterial and phytoplankton production, ciliate community composition and metazooplankton grazing should be studied in more detail in further experiments.

The results of the present study reveal the regulatory linkages of the ciliate community taking place during the late summer phase in a highly eutrophic Võrtsjärv. However, due to the seasonality of plankton communities a different crustacean-ciliate regulation mechanism might exist earlier in the season, for example in June, when small-bodied cladocerans (mainly *Chydomus sphaericus*) form the majority of the crustacean biomass. Owing to their different feeding modes compared to cyclopoids, and, more importantly, due to ciliate community domination by relatively larger herbivorous forms, a relatively weaker top-down regulation of ciliate community via the linkage of crustaceans and predatory ciliates might be expected in the early summer phase compared to the strong interactions between cyclopoid copepod, predatory ciliate, and bacterivorous ciliate later in the season. Therefore, the interaction pathways and its impact on the ciliate community may depend largely on the species composition of both, ciliates and their predators.

**Conclusions**

Our study indicated that an increase in copepod zooplankton biomass in a shallow eutrophic lake favours the development of small-sized bacterivorous ciliates. This effect is unleashed by the decline of predacious ciliate abundance, which would otherwise graze effectively on the small-sized ciliates. The inverse relationship between a crustacean zooplankton and large predaceous ciliates is an important feature not only adjusting the structure of the ciliate community but also the energy transfer between meta- and protozooplankton.
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