Eesti Maaülikool
Estonian University of Life Sciences

EFFECTS OF MACROPHYTES, FISH AND METAZOOPLANKTON ON A MICROBIAL FOOD WEB

Veetaimestiku, kalade ja metazooplanktoni mõju
mikroobsele toiduahelale

KATRIT KARUS

A Thesis
For applying for the degree of Doctor of Philosophy in Hydrobiology

Väitekiri
Filosoofiadoktori kraadi taotlemiseks hüdrobioloogia erialal

Tartu 2014

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LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following papers, which are referred to by Roman numerals in the dissertation.

I. Karus, K., Paaver, T., Agasild, H. & Zingel, P. 2014. The effects of predation by planktivorous juvenile fish on the microbial food web. European Journal of Protistology, 50: 109–121.

II. Agasild, H., Zingel, P., **Karus, K.,** Kangro, K., Salujõe, J. & Nõges, T. 2013. Does metazooplankton regulate the ciliate community in a shallow eutrophic lake? Freshwater Biology, 58: 183–191.

III. Zingel, P., Paaver, T., **Karus, K.,** Agasild, H. & Nõges, T. 2012. Ciliates as the crucial food source of larval fish in a shallow eutrophic lake. Limnology and Oceanography, 57: 1049–1056.

IV. Zingel, P., Agasild, H., **Karus, K.,** Kangro, K., Tammert, H., Tõnno, I., Feldmann, T. & Nõges, T. The influence of zooplankton enrichment on the microbial loop in a shallow, eutrophic lake. Submitted to Freshwater Biology.

V. Karus, K., Feldmann, T., Nõges, P. & Zingel, P. 2014. Ciliate communities of a large shallow lake: association with macrophyte beds. European Journal of Protistology, 50: 382–394.

Author's contribution to the papers:

	I	II	III	IV	V
Concept	*	*			*
Study design	*				*
Data collection				*	
Data analysis	*	*	*	*	*
Manuscript preparation	*	*	*	*	*

1. INTRODUCTION

Up to the 1980s, or more precisely before the formulation of microbial loop theory, it was thought that all primary production is channelled through a classical grazing food web from phytoplankton to zooplankton to fish (Steele, 1974). However, several decades of studies have demonstrated that this simple idea of orderly trophic level nutrient transfers and energy fluxes from phytoplankton to zooplankton and finally to fish is inadequate and misleading because approximately half of algal production can bypass the traditional food web and directly channel through a microbial loop (Berman, 1990; Fenchel, 1988; Pomeroy & Wiebe, 1988; Sherr & Sherr, 1991). In this loop, a crucial part of the primary production is not directly consumed by zooplankton but is channelled through a supply of organic matter before it becomes available via bacterial production to protozoans. More particularly, the loop describes a trophic pathway in a food web, where phytoplankton-derived dissolved organic matter is absorbed by heterotrophic bacteria, their production is grazed by ciliates and heterotrophic nanoflagellates, and this "dissolved organic matter-bacteria-protozoa" loop is then coupled with a classical grazing food web by metazoan grazing on protozoans. Therefore, it follows that protozoans form a trophic nexus in microbial food webs. The efficiency with which zooplankton communities consume primary production and their own suitability as food for larger zooplankton and/or fish determines the efficacy of the food web. As the microbial loop usually consists of more trophic levels and the energy loss is higher owing to respiration at each level, this kind of food web is considered a less efficient pathway for energy and nutrients than the classical grazing food web (Pomeroy & Wiebe, 1988). Moreover, this loop theory has long been a debating issue because it implicates another system, which can be separated from the classical grazing food web. However, the microbial loop should be considered an integral part of the pelagic food webs as there is no separate loop or appendage beside the classical food web and no firm border between them; they are connected to each other in many direct and indirect ways (Porter et al., 1988; Riemann & Christoffersen, 1993). For these various reasons, the importance of microbial loop in the functioning of ecosystems as well as food webs in lakes is underestimated. Consequently, most food web studies have focused on classical grazing food webs, studying interactions from fish and macrophytes to zooplankton and phytoplankton. The cascading effects of fish on whole microbial loop

organisms have been studied only rarely, and studies considering the role of macrophytes in whole microbial loop communities seem to be missing. Moreover, to our knowledge, there have been no studies on the cascading effects of both macrophytes and fish together on whole microbial loop communities.

The present thesis focuses mainly on some of the gaps in lake food web studies mentioned above. The core of the thesis emphasizes planktonic ciliates, which have been considered the central link of a microbial loop. Furthermore, with increasing primary productivity, ciliates can become the most important members of the microbial loop in eutrophic lakes when bacterial densities are sufficient to maintain their communities (Agasild et al., 2012; Sherr & Sherr, 2002). This was also the case in our study because one of the main study lakes in the present thesis (Lake Võrtsjärv) is characterized by a rich and abundant (up to 191 cells mL$^{-1)}$ community and a high (more than 60%) ciliate contribution to the total zooplankton biomass (Zingel & Nõges, 2010). As dominating species play a significant role in the functioning of aquatic ecosystems we were interested in how these highly abundant ciliated protozoans are influenced by aquatic macrophytes (paper **V**), which are known for their key role in structuring aquatic communities. As macrophytes in Lake Võrtsjärv are also known for their strong spatial polarization in relation to abiotic factors (Feldmann & Nõges, 2007), we considered how polarized macrovegetation influences the taxonomic composition, abundance and distribution of planktonic ciliates. Our study seems justified because little is known about ciliate communities in various types of macrophyte beds (Mieczan, 2007, 2008).

Moreover, we tried to identify the main food source of planktivorous fish in the shallow eutrophic Lake Võrtsjärv, where the zooplankton community is dominated by ciliates (paper **III**). Although protozoa can form an important part of the planktonic communities in eutrophic lakes, very few studies have considered direct protist consumption by fish larvae. Most planktonic protozoa are too fragile and have no indigestible hard parts and are therefore easily degraded in the fish guts. Hence, protist ingestion may have been neglected in the past because of rapid degradation of food objects in the fish guts (Fukami et al., 1999), which could also explain seeming paradoxes such as the empty guts of field-caught larvae (Van der Meeren & Næss, 1993). Therefore, one possible food source that

larval fish can ingest and digest quickly is protozoa. If this is the case, the focus on metazoan prey has resulted in fisheries scientists overlooking a key trophic linkage (Montagnes et al., 2010).

In addition to the studies considering the direct influence of planktivorous fish and polarized macrovegetation on ciliated protozoans we also studied the cascading effects of planktivorous fish on whole trophic levels. Despite the growing number of publications on the planktonic food web structure there is still a lack of studies that consider the direct and indirect consequences of planktivorous fish feeding on whole microbial loop communities. The role of fish is often neglected because of difficulties in assessing their abundance and feeding rate. Therefore we conducted food web experiments in shallow europhic ponds to study how the feeding of planktivorous fish on zooplankters influences zooplankters feeding on bacteria in large shallow eutrophic ponds (paper **I**).

Attention was also paid to the regulating role of the metazooplankton community on microbial loop communities, e.g. how changes in metazooplankton community structure influence the community structure and abundance of planktonic ciliates in the large shallow Lake Võrtsjärv and whether the changes cascade down to the abundance of bacteria and phytoplankton (papers **II & IV**).

2. OBJECTIVES OF THE STUDY

The main objective of this thesis was to demonstrate how different organism groups in a microbial loop are regulated by the upper trophic levels. The aim of the project was to address the following major questions (Q1-Q4) and hypotheses (H1-H4):

Q1. How do different macrophyte stands influence the taxonomic composition and abundance of planktonic ciliates in a large shallow eutrophic lake?
H1. Macrophyte composition must have a strong positive influence on the dispersal of ecologically different ciliate groups in this lake, and on their diversity and abundance (paper **V**).

Q2. How does the feeding of planktivorous fish on zooplankters influence zooplankters feeding on bacteria in large shallow eutrophic ponds? What happens in lower trophic levels if the fish community is absent?
H2. In the presence of planktivorous fish, there would be fewer metazooplankters and more protozoans and bacterial numbers would be suppressed. On the other hand, in the absence of fish, protozoan communites would be suppressed by metazoan grazing and this would enable bacterial communities to develop (paper **I**).

Q3. What is the main food source of planktivorous fish in a large shallow eutrophic lake where the zooplankton community is dominated by ciliates?
H3. Ciliates must be an important part of larval fish diet, and the food requirement of fish larvae during the first feeding stages probably cannot be met without consumption of ciliates (paper **III**).

Q4. How do different metazooplankton removal (paper **II**) and enrichment (paper **IV**) treatments influence the community structure and abundance of ciliates in a large shallow eutrophic lake compared to the natural metazooplankton community and abundance? Do changes in the metazooplankton community influence the abundance of bacteria and phytoplankton?
H4. Increase and decrease in metazooplankton abundance affect ciliate community composition but do not directly affect their total numbers. Changes in the metazooplankton community do not cascade down to phytoplankton and bacteria (papers **II & IV**).

3. REVIEW OF THE LITERATURE

3.1. Brief history of protozoology

The first reports of protozoa originate from the year 1565 when Conrad Gessner (1516-1565) described foraminiferans (amoeboids) in fossils. The first living microorganisms, namely microscopic protozoa and bacteria, were discovered during the years 1674 (protozoa) and 1676 (bacteria) by 'a Fellow of The Royal Society', Antonie Philips van Leeuwenhoek (1632-1732), who is now known as 'the Father of Microbiology' (Finlay & Esteban, 2001; Gest, 2004). The discovery of microorganisms was made possible by the fabrication and use of simple microscopes, which magnified objects up to 300-fold (Gest, 2004; Sateesh, 2007). These handcrafted microscopes allowed the first single-celled organisms to be observed; van Leeuwenhoek originally called them 'animalcules' or 'little eels', meaning little animals, and they are now referred to as microorganisms (Gest, 2004). He was the first person in the world to observe protozoa (mainly ciliates), algae, bacteria and rotifers (Sateesh, 2007). In his letters to the Royal Society in 1674 he described protozoa as follows (Gest, 2004): "The fourth sort of creatures, which moved through the three former sorts, were incredibly small, and so small in my eye, that I judged, that if 100 of them lay one by another, they would not equal the length of a grain of coarse sand and according to this estimate, ten hundred thousand of them could not equal the dimension of a grain of such coarse sand." Although van Leeuwenhoek discovered protozoa and bacteria, he did not coin these terms because he differentiated the organisms only by their sizes and different parts. Subsequently, in the years 1714, 1763, 1765, 1805, 1818, 1826, 1832, 1852, 1861, 1861, 1866, 1874, the terms monaden, animalcula infusoria, infusoria, urthiere, protozoa, animalia microscopica, oozoa, archezoa, acrita, protoctista, protista and microzoaires were coined by Gottfried Wilhelm Leibniz, Martin Frobenius Ledermüller, Heinrich August Wrisberg, Lorenz von Oken, Georg August Goldfuss, Jean Baptiste Bory de Saint-Vincent, Carl Gustav Carus, Perty, Richard Owen, John Hogg, Ernst Heinrich Philipp August Haeckel and Edouard De Fromentel, respectively (Wolf & Hausmann, 2001). Nowadays, the most widely-used terms are protozoa, protoctista and protista (Rotschild, 1989; protozoologists tend to use protista and protoctista (Corliss, 1994; Margulis & Corliss, 1990) and parasitologists prefer protozoa (Cox, 1992).

In his twelfth edition of Systema Naturae (1767), Carl von Linné (1707-1778) divided all animalcules then known into four genera: Volvox, Vorticella, Furia and Chaos (Fokin, 2004). At this time van Leeuwenhoek's term 'animalcules' was replaced with 'infusoria' because the organisms were observed in infusions. Linné, known as the father of modern taxonomy, merged all the infusoria into a single species, Chaos infusorium (Bulloch, 1979). Otto Friedrich Müller (1730-1784) arranged the infusoria into genera and species, described many previously unknown minute animal species and created illustrations for the infusoria, which were published in 1786 in the treatise 'Animalcula infusoria luviatilia et marina' (Müller, 1786).

The term 'protozoa', which means first animals, was first introduced by Georg August Goldfuss (1782-1848) in 1818. Goldfuss also established the concept of three great groups of protozoa – amoebae, flagellates and ciliates – on the basis of their methods of movement. Karl Theodor Ernst von Siebold (1796-1866) recognized protozoa as single-celled organisms (1845) but divided them into two groups – the Infusoria (ciliates) and Rhizopoda (amoebae) (1848). Otto Bütschli (1883) added a fourth group to the protozoans, sporozoans (Imam, 2009).

The first observations of plant tissues (1665) by Robert Hooke and of animalcules (1674) by Antonie van Leeuwenhoek led to the formulation of the cell theory by Matthias Jakob Schleiden (1838) and Theodor Schwann (1839), which was given its modern form by Max Schultze (1861). After the cell doctrine was formulated, stating that all living organisms are composed of structural units called 'cells', Felix Dujardin demonstrated that protozoa were composed of 'sarcode' (protoplasm) in which no constituent cells were discernible (Lee, 1973). A futher extension of the doctrine to include protozoans was adopted by von Siebold, who stated that each individual protozoan is itself a cell. He added that protozoans are unicellular animals and all other animals are multicellular. His assertions found world-wide acceptance and have prevailed to this day (Bunch & Hellemans, 2004).

After Linné divided all living organisms into the kingdoms Plantae and Animalia in his 'Systema Naturae' (1758), several 19th century scientists (e.g. Hogg, Haeckel) argued for a third kingdom to encompass the single-celled protozoa, which had no obvious affinities with animals or plants. In 1861,

John Hogg introduced the term 'Protoctista' and established the third kingdom Protoctista for 'the first beings', so that the status of protozoa, formerly a phylum, was raised to that of a kingdom. Haeckel (1866) used the term 'Protista' to include all protozoa, eukaryotic algae and lower fungi. He is credited with dividing the animal kingdom into single-celled (protozoa) and multicellular (metazoa) organisms. Haeckel's classification system has remained almost unchanged in subsequent zoology books and his basic concepts were clearly set out by Craig (1926) (Imam, 2009).

The development of protozoologically-oriented parasitology originated from van Leeuwenhoek's time, when he discovered animalcules inhabiting other animals. During the years 1681-1683 he described animalcules from his own and a frog's intestines, but he did not associate these animalcules with parasitic protozoans (Dobell, 1932). The studies of parasitic protozoans followed with Dufour (1828), who described numerous parasitic species (named *Gregarina*) inhabiting arthropods, especially insects (Leidy, 1853; Wolff & Hausmann, 2001). Again he did not associate these animals with protozoans and considered them to be worms. Scientifically, knowledge of protozoan diseases began with the account by Louis Pasteur (1865) on a disease of silkworms named pebrine. He found that pebrine was caused by a parasitic protozoan, *Nosema bombycis*, and deduced methods for its prevention. His research was of fundamental importance for protozoology because it threw light on methods that allowed such diseases to be studied and described (Sateesh, 2007). Subsequently, many protozoan-caused diseases such as malaria (sporozoon genus *Plasmodium*), babesiosis (sporozoon genus *Babesia*), toxoplasmosis (sporozoon *Toxoplasma gondii*), isosporiasis (sporozoon *Isospora belli*), cyclosporiasis (sporozoon *Cyclospora cayetanensis*), cryptosporidiosis (sporozoon *Cryptosporidium parvum*), amoebiasis (amoeba *Entamoeba histolytica*), giardiasis (flagellate *Giardia lamblia*), African sleeping sickness/nagana (flagellate *Trypanosoma brucei*) and leishmania (genus *Leishmania*) were identified. The increasing number of parasitic protozoans identified within living organisms led to the creation of taxonomic and other groupings at the subgenus and subspecies levels (Collier et al., 1998).

The period from 1878 to 1901 may be considered a constitution phase, during which different institutions were founded: the first three protozoological institutes were established in Germany in 1878, 1884 and 1885 by Bütschli, Schultze and Hertwig, respectively. The years

from 1902 to 1961 may be considered an establishing phase for journals, textbooks, societies, congresses, educational institutions and research departments (Wolf & Hausmann, 2001). The first protozoological journal 'Archiv für Protistenkunde (1902)' (renamed 'Protist' in 1998) was founded by Fritz Schaudin in Germany. Several journals were established subsequently – 'Journal of Protozoology' in 1954 by the Society of Protozoologists (renamed 'Journal of Eukaryotic Microbiology' in 1993), 'Acta Protozoologica' in 1963 (Sikora & Kuźnicki, 2006) and 'Protistologica' in 1968 (renamed European Journal of Protistology in 1987) (Wolf & Hausmann, 2001). 'The Society of Protozoologists' was established in 1947 in Chicago, USA. The first International Congress of Protozoology took place in Prague (1961), the second in London (1965), the third (1969) in Leningrad, etc. (Kuźnicki & Honigberg, 1984; Wolf & Hausmann, 2001). The consolidation phase began from the year 1969 and included integration, technical terminology, secondary disciplines (Wolf & Hausmann, 2001) and specific methods (electron microscopy, DNA, RNA technology, molecular karyotyping) (Wolf & Hausmann, 2001; Imam, 2009).

3.2. Main groups of protozoa

Protozoa, animal-like organisms, are single-celled eukaryotes found world-wide in almost every aquatic environment. They are the most abundant (20,000 free-living species) phagotrophs in the biosphere (Finlay, 1990) and are classified into four major groups – ciliates (Infusoria), flagellates (Mastigophora), amoebae (Sarcodina) and sporozoa (Sporozoa) – according to their methods of movement and different kinds and numbers of locomotory structures (flagella, cilia, pseudopodia). Each group is described more precisely below.

3.2.1. Ciliates

Ciliates (known also as Infusoria or Heterokaryota), both free-living and parasitic species, form the most diverse and homogenous group of protozoans, comprising 4700 free-living and 2500 parasitic species (Levine et al., 1980) and inhabiting various aquatic (lakes, rivers, ponds, oceans and their sediments) and terrestrial (soils) habitats. Ciliates belong to the kingdom Protista and phylum Ciliophora, which in turn consists of 8-10 classes and many orders (Corliss, 1994).

They are usually larger than flagellates, in the size range 15-200 μm (Finlay & Esteban, 1998). They are characterized by cilia or compound ciliary organelles, which cover all or part of their bodies and are used for both movement and feeding. More precisely, these cilia act like oars to move the protozoans through the water and the ciliary structures associated with the cytostome (the so-called cell mouth) produce water currents to bring food particles to the site of ingestion (Laybourn-Parry, 1984). Thanks to the ciliary structures they are the fastest-moving protozoans, certainly faster than flagellates (400–2000 μm/s for ciliates and 20–200 μm/s for flagellates) (McNeill, 1979). Most ciliates have a well-developed cell mouth; their taxonomic status and feeding biology are determined by the complexity and ciliary organelles of the cell mouth (Laybourn-Parry, 1992). They are strictly, or mainly, phagotrophs (Sherr & Sherr, 2002), characterized by a broad diversity of feeding types – filter-feeders (e.g. *Cyclidium, Frontonia, Disematostoma*), raptorial feeders (e.g. *Prorodon*, *Loxodes*, *Askenasia*, *Dileptus*, *Lacrymaria*, *Monodinium*), hoover feeders and diffusion feeders (e.g. *Podophrya* and other suctorians). The smallest ones tend to prey upon bacterial-sized particles (0.2-2 μm), the larger ones on larger single-celled algae, filamentous cyanobacteria, other protozoa and occasionally rotifers and other microzooplankton (Finlay & Esteban, 1998). They possess two types of nucleus, the macro- and micro-nucleus; the macronucleus (only one per cell) regulates the day-to-day functioning of the cell and the micronucleus (more than one) contains genetic material and is involved in sexual processes (Laybourn-Parry, 1984, 1992). Asexual reproduction is achieved by tranverse binary or multiple fission and endogenous/exogenous budding (suctorians). Sexual reproduction is common among ciliates and is achieved by conjugation, regarded as a process involving the exchange of genetic material between two individuals (Laybourn-Parry, 1984; 1992).

3.2.2. Flagellates

Flagellates (known also as Mastigophora), including 5100 free-living and 1800 parasitic species, are an abundant and widely distributed group in diverse aquatic habitats (Levine et al., 1980). They form a heteregenous group, both physiologically and structurally, and exhibit the largest number of species living mixotrophically (Sanders, 1991). Flagellates belong to the kingdom Protista, phylum Sarcomastigophora and sub-phylum Mastigophora, which is divided into two classes – phytoflagellates resembling plants (Phytomastigophorea) and zooflagellates resembling

animals (Zoomastigophorea). The former group consists of flagellates that are wholly or partially autotrophs and the latter group are heterotrophs. Most of them live solitary but colonial species also occur among both groups (Laybourn-Parry, 1992). In this thesis we will consider mainly heterotrophic nanoflagellates, so they are described in more detail here.

Heterotrophic nanoflagellates comprise seven categories – bodonids, euglenids, choanoflagellates, cryptomonads, dinoflagellates, heterokonts and "invertae sedis" (Paterson & Larsen, 1991). As their name implies they are heterotrophs; the term 'heterotrophic' refers to their mode of feeding and the term 'nano' refers to their size, which is from 2 to 20 μm. They are the smallest protozoans, characterized mostly by colourless, ovoid to pyriform cells, which are bounded by a coating called theca. They possess one or two (more) whip-like organelles called flagella, which they use for movement, sensation and feeding (Laybourn-Parry, 1984). Flagellates can move at speeds of 20 - 200 μm/s (McNeill, 1979). Flagellates in pelagic aquatic systems are mainly phagotrophs, i.e. bacterivores, cyanobacterivores, herbivores and carnivores, and in organically enriched systems also osmotrophs (Sanders, 1991). They are regarded as important raptorial feeders (e.g. chrysomonads), filter-feeders (e.g. choanoflagellates) and diffusion feeders (e.g. *Ciliophrys*). As filter-feeding flagellates have relatively large filter areas and the highest volume-specific clearance rates, they can be competitively superior to filter-feeding ciliates (Sherr & Sherr, 2002). Flagellates usually have only one type of nucleus and reproduce mainly asexually through binary fission in a longitudinal plane. Sexual reproduction is not widely reported, but when it occurs it is essentially syngamy (Laybourn-Parry, 1992).

3.2.3. Amoebae

Amoebae (also known as Sarcodina) constitute a diverse group of heterotrophic organisms (Laybourn-Parry, 1992) including 11,300 free-living (of which 4600 are foraminiferans) and 250 parasitic species (Levine et al., 1980) and inhabiting various aquatic and terrestrial habitats. There are free-living species, but most sarcodines (expect heliozoans) are usually associated with surfaces and especially sediments (Finlay & Esteban, 1998). They belong to the kingdom Protista, phylum Sarcomastigophora and sub-phylum Sarcodina, which in turn includes foraminiferans, radiolarians, heliozoans and naked amoebae. Morphologically, amoebae can be divided into two broad groups – the naked amoebae and the testate

or shelled amoebae. The former lack skeletal structures or tests and the later possess tests, which can be proteinaceous, agglutinate, siliceous or calcareous. The most conspicuous are species among the foraminiferans and radiolarians, which have skeletal structures (Laybourn-Parry, 1984). Generally, compared with ciliated and flagellated protozoans, planktonic amoebae are not very common in fresh (Davis et al., 1978) or marine (Laybourn-Parry et al., 1990) waters and occur in quite low numbers. Most of them are generally smaller (<50 µm) than ciliates (Finlay & Esteban, 1998) but they can range from 1 to 500 µm in length (Laybourn-Parry, 1992). These slow-moving protozoans (crawling at speeds up to about 5 µm/s (McNeill, 1979)) are characterized by pseudopodial structures (finger-like protrusions of the cells called pseudopodia), which are used for both movement and feeding. Movement is achieved by cytoplasmic flow (extension and contraction of cytoplasm), which pushes the cell membrane. Sarcodines do not have cell mouths. They (e.g. naked amoebae, *Amoeba proteus* Pal.) engulf food particles by a process in which cytoplasm flows around the particle as soon as it makes contact and encloses it in a vacuole with some of the external liquid medium (Laybourn-Parry, 1984). They are strictly phagotrophs (Sherr & Sherr, 2002); smaller individuals prey upon bacteria and unicellular phytoplanktons and larger ones on other protozoans, filamentous cyanobacteria and micrometazoans (Laybourn-Parry, 1992). A great variety of non-living organic particles and aggregates are also often ingested (e.g. Pelomyxa) (Finlay & Esteban, 1998). Most amoebae have one type of nucleus although multinucleate species do occur. Reproduction is usually achieved asexually; sexual reproduction is not very common. The mode of asexual reproduction of shelled amoebae depends on species and varies from binary and multiple fission in the longitudinal or tranverse plane to budding. Naked amoebae reproduce mainly by binary fission (Laybourn-Parry, 1984).

3.2.4. Sporozoa

Sporozoa (also known as Apicomplexa), as their name implies, are spore-forming protozoans and form an exclusively parasitic but diverse protozoan group including 5600 species (comprising Apicomplexa, Microspora, Myxospora, and Aseetospora) (Levine et al., 1980). They belong to the kingdom Chromalveolata and phylum Apicomplexa (Sporozoa), which in turn consists of two classes (Aconoidasida, Conoidasida) and subclasses.

Sporozoans are characterized by a cellular structure, also called an apical complex or anapicoplast (a type of plastid), which is present during the infective stage of their life cycle and enables them to invade and anchor to the host cells (Sleigh, 1989). These structural features provide the name for this phylum (Levine et al., 1980). Their size ranges from 1 μm (for the smallest intracellular parasites) to 10 mm (Raven, 2000). Sporozoans have no motile structures (flagella, cilia, pseudopods) expect in certain male gamete stages when they possess locomotory organs such as flagella (Sleigh, 1989). These heterotrophic organisms lack any organs for ingesting and digesting solid food, so they obtain nutrients osmotically by absoring organic molecules from host organisms (Mara & Horan, 2003). These unicellular parasites have a complex life cycle involving both asexual and sexual reproduction and infecting more than one host (Hogg, 2013). The asexual phase (generation through division) is referred to as schizogony and starts with members of the infective stage (sporozoites) penetrating into a host cell, where they multiply repeatedly by binary or multiple fission to form numerous daughter cells (merozoites). The sexual phase is termed sporogony (generation of spores) and is followed by gametogony, the production of sexually reproductive cells called gamonts. Gamonts join together in pairs to form a gamontocyst, in which both gamonts divide to form numerous gametes. Gametes fuse in pairs to form zygotes, which give rise by cell division to new sporozoites. When sporozoites invade new host cells, the cycle starts again (Sleigh, 1989). Parasitizing sporozoans cause diseases in a wide variety of living organisms throughout the animal kingdom.

3.3. Concept of microbial loop and its formation

Bacterioplankton and other micro-organisms are considered to be components of aquatic ecosystems (Sorokin, 1981) but their role has long been underestimated because they are difficult to assess quantitatively by traditional methods such as plate counts, serial dilutions or phase-contrast microscopy. The development of modern techniques (epifluorescence, scanning and transmission electron microscopy) and the use of fluorescent dyes to assess microbial standing stocks have revealed bacterial abundances 10-100 times greater than earlier estimates (Hobbie et al., 1977). This finding indicates that heterotrophic bacterial production can reach the same level as autotrophic algal production (Overbeck, 1972), and heterotrophic bacteria, known as remineralisers, could play a role

comparable to phytoplankton in the transfer of energy and nutrients in aquatic ecosystems (Williams, 1981; Kirchman et al., 1982). After Pomeroy (1974), Williams (1981) and Sieburth & Davis (1982) revealed the importance of microbial organisms in the transfer of energy and matter in marine pelagic systems, Azam et al. (1983) synthesized these initial statements into a conceptual framework – microbial loop theory. This theory describes a trophic pathway in a microbial food web, where phytoplankton-derived dissolved organic matter (DOM) is absorbed by heterotrophic bacteria, their production is grazed by ciliates and heterotrophic nanoflagellates and this "DOM-bacteria-protozoa" loop is then coupled with a classical grazing food web by metazoan grazing on the protozoans (Figure 1). Since the microbial loop food web concept was formulated, studies of marine and freshwater systems of different trophic status and in geographical areas have this trophic pathway (e.g. Amblard et al., 1995; Gobler et al., 2008; Peštová et al., 2008; Zingel et al., 2006).

Traditionally, the most important pathway for nutrient and energy fluxes is considered to act through a conventional or classical grazing food web (phytoplankton-metazooplankton-fish), where phytoplankton production is transported to upper trophic levels via metazooplankton and planktivorous fish (Steele, 1974; Figure 1). In fact, this food web is relatively simple and consists mostly of large (>2 μm) phytoplankton, as most zooplankton species cannot prey directly upon small phytoplankton. The microbial loop (DOM-bacteria-protozoa-metazoa-fish) describes an alternative pathway, where the key organisms are heterotrophic bacterioplankton, viruses, small picophytoplankton (<2 μm), heterotrophic nanoflagellates, ciliates and metazooplankton. In particular, free-living heterotrophic bacteria utilize DOM as an energy and nutrient source, which is mainly of phytoplankton origin and is released by algae via passive leakage, lysis or exudation of carbon-rich material (Anderson & Williams, 1998; Sundh, 1991). DOM is also introduced into the water from other sources, which include senescence and decomposition of cells, mortality through viral and bacterial infections (Sundh, 1991), leakage from broken cells during sloppy feeding, incomplete digestion by aquatic organisms (Jumars et al., 1989), excretion from fecal pellets (Lampert, 1978) and enzymatic hydrolysis of particulate material by bacteria (Smith et al., 1992). Furthermore, as organisms tend to utilize particles one order of magnitude smaller than themselves (Sheldon, 1972), heterotrophic bacteria are the most suitable

and principal consumers because of their small size and large surface-to-volume ratio for absorption of nutrients, giving them a competitive advantage over phytoplankton (Azam et al., 1983).
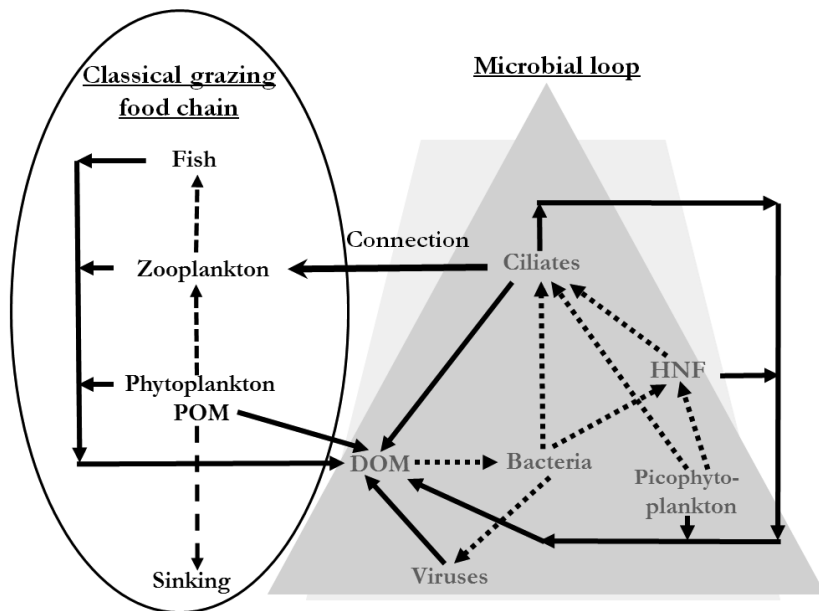
Figure 1. Structure of the pelagic food web in water bodies highlighting the classical grazing food web and the microbial loop (modified from Azam, 1998). Phytoplankton primary production is channelled through a classical grazing food web via zooplankton to fish (dashed arrows). Microorganisms form the microbial loop (on the grey triangle background), in which the production of heterotrophic bacteria and picophytoplankton is transported via protozoans to metazoans and finally to fish (dotted arrows). The supply of dissolved organic matter is introduced into the water by various processes (sources are indicated by solid arrows), which include extracellular release of photosynthetically produced dissolved organic matter by phytoplankton, viral lysis, sloppy feeding, excretion and exudation, and is used by bacteria.

Thus, a large part of the dissolved and particulate organic matter, which is mostly inaccessible by other aquatic organisms, is converted into living biomass via bacteria. Ciliates (Šimek et al., 1995) and heterotrophic nanoflagellates (Güde, 1986; Riemann, 1985) are known to prey on bacteria and these protozoans in turn (being in the same size range as phytoplankton) are a significant food source for metazoans (rotifers, cladocerans, copepods). As mentioned above, small picophytoplankton (<2 µm) form part of the microbial loop because they are too small to be ingested by the members of the classical food web, but are successfully

eaten by protozoans within the microbial loop (Figure 1). Therefore, it follows that protozoans form a trophic nexus in the transformation of bacterial (and picophytoplankton) production to upper trophic levels and energy released as DOM by aquatic organisms. This would otherwise remain inaccessible at higher trophic levels, and is rather inefficiently returned to the main food web via the microbial loop (Azam et al., 1983). Nevertheless, as the microbial loop consists of more trophic levels and energy loss is higher owing to respiration at each level, this kind of food web is considered a less efficient pathway for energy and nutrients than the classical grazing food web (Pomeroy & Wiebe, 1988).

3.4. Microbial loop as sink or link in pelagic food webs

Until the 1980s, or more precisely before the formulation of microbial loop theory, it was thought that all primary production is channelled through a classical food web (Steele, 1974). However, several decades of studies have demonstrated that this simple idea of orderly trophic level transfers of nutrients from phytoplankton to zooplankton to fish is inadequate and misleading because approximately half of algal production can bypass the traditional food web and channel directly through a microbial loop (Berman, 1990; Fenchel, 1988; Pomeroy & Wiebe, 1988; Sherr & Sherr, 1991). In a microbial loop, a crucial part of the primary production is not directly consumed by zooplankton but is channelled through a supply of organic matter before it becomes available to protozoans via bacterial production.

This loop theory has long been a debating issue because it implies another system that can be separated from the classical grazing food web. However, several authors have emphasized that the microbial loop should be considered an integral part of pelagic food webs as there is no separate loop or appendage beside the classical food web, and there is no firm border between them as they are connected to each other in many direct and indirect ways (Porter et al., 1988; Riemann & Christoffersen, 1993). Coupling or uncoupling of the microbial loop from the grazing food web has led to another debate – the 'sink or link' controversy - which focuses on carbon flow in pelagic food webs and asks to what extent the microbial loop represents a loss of fixed carbon from the system (sink) and to what extent it primarily passes fixed carbon to higher trophic levels (link) (Banse, 1984; Ducklow et al., 1986; Sherr et al., 1987; Fenchel, 1988).

Despite a long-term debate it seems now that the microbial loop primarily acts as a sink (Williams, 2000). This follows from the circumstance that a microbial loop basically comprises more trophic levels than a classical food web and a major part of the organically bound energy is respired at each trophic level, only 5-40% of the production of a given level being transferred to the next (Banse, 1990; Gaedke et al., 1995; Wylie & Currie, 1991). Therefore, the number of trophic levels between bacteria and metazooplankton indicates energy losses within the microbial loop. For instance, if DOM-absorbing bacteria are consumed by heterotrophic nanoflagellates, and they in turn are grazed by ciliates and finally by metazoans, only a small fraction of bacterial production will be incorporated into the metazoans; although there are some 'shortcuts' where the energy losses are smaller, for instance some crustaceans prey directly upon bacteria. In particular, cladocerans (especially *Daphnia* spp.) and to some extent copepods are regarded as major bacterial consumers in freshwater lakes (Jürgens, 1994; Pace et al., 1990) while rotifers affect their production only modestly (Pace et al., 1990; Sanders et al., 1989). Thus, the energy incorporated into bacterial biomass is directly transferred to higher trophic levels with smaller energy losses. However, even a two-step microbial food web is a major sink of energy (Pomeroy & Wiebe, 1988). In addition to bacteria, heterotrophic nanoflagellates are also able to absorb DOM with a higher molecular mass (Sherr, 1988). Direct ingestion of DOM by protozooplankton is an alternative and efficient pathway for returning nutrients to higher trophic levels.

However, some scientists (Sherr & Sherr, 1987) believe that a microbial loop can also act as a link because bacteria are an additional food source for metazoans, made available via the protozoan link. Moreover, it has been assumed that because the microbial loop is an integral component of pelagic food webs, which cannot be separated from the classical grazing food web (Porter et al., 1988; Weisse, 1989), the 'sink or link' debate is a non-issue (Sherr & Sherr, 1988).

3.5. Top-down versus bottom-up control and microbial loops

Whether the control of the pelagic food webs is exerted by top-down (predator-based regulation of lower trophic levels) or bottom-up (resource-based regulation of upper trophic levels) forces has also long been debated (Carpenter et al., 1985; Hairston et al., 1960). Power (1992) stated that

according to the bottom-up view, living organisms on each trophic level are controlled by food sources, while the top-down view holds that organisms on upper trophic levels are food-limited, and at successively lower levels they are in turn predator then food-limited. Some authors suggest that bottom-up forces mostly influence lower trophic levels and top-down forces dominate at the top of the food web (McQueen et al., 1986; Sanders et al., 1992), whilst these forces can also alternate between adjacent trophic levels (Hairston et al., 1960, Oksanen et al., 1981). Despite the different ideas, both controlling mechanisms of pelagic food webs are known to occur in water bodies, but they can differ in magnitude and operate simultaneously rather than separately (Thingstad et al., 1997). Moreover, these opposite interactions are primarily responsible for population structure and diversity and include processes such as competition for nutrients and light, commensalism between autotrophs and heterotrophic bacteria, recycling of material, cell lysis, and predation among viruses, bacteria, micro-algae and their predators (Thingstad et al., 1997). It has been suggested that the trophic status of the water bodies is a central determinant of the predominant forces (bottom-up versus top-down) and structuring pelagic food webs. It is well known that the abundance and biomass of all organisms in pelagic food webs generally increase with increasing nutrient concentrations (Berninger et al., 1991; Pace, 1986), but each group can react differently (Christoffersen et al., 1993; Gasol & Vaque, 1993; Jansson et al., 1996). Consequently, nutrient enrichment in water bodies can also greatly influence the structure, diversity and functioning of pelagic communities by changing the interactions among community members. In acknowledgement of that, some studies have demonstrated that structural changes in pelagic communities can cause a shift from bottom-up to top-down regulation of some groups of organisms (e.g. heterotrophic flagellates) (Weisse, 1991).

With reference to bottom-up forces, both phytoplankton and heterotrophic bacteria comprise the lower trophic levels of the food webs producing nutrients by autotrophy and heterotrophy. More precisely, phytoplankton uses inorganic compounds and light energy to produce organic compounds by photosynthesis, whereas photosynthesizing algae lose a substantial part of the organic carbon they produce by a process known as extracellular release of photosynthetically-produced dissolved organic carbon (Sundh, 1989). Some studies have demonstrated that gross release of extracellular organic carbon can range from 5 to 46% of daily primary production

(Fogg, 1983; Sondergaard et al., 1985). On the other hand, heterotrophic bacteria utilize most of these phytoplankton-released compounds (amino acids, peptides, glycolate, monosaccharides, carbohydrates) to recycle inorganic compounds, which in turn are essential for phytoplankters (Sundh, 1991). It appears that algal and bacterial growth depend on each other, the latter generally being dependent on the former; for instance, several studies have demonstrated that bacterial abundance increases with increasing chlorphyll a concentrations (Bird & Kalff, 1984; Linley et al., 1983) and a 1000-fold increase in algal biomass results in a 100-fold increase in bacterial abundance (Fenchel et al., 1990). However, besides phytoplankton, bacteria also utilize mineral nutrients. In environments where algal production is limited by the availability of mineral nutrients, this would lead to competition between phytoplankton and bacteria. For instance, a freshwater bacterium whose carbon is derived from a phosphorus-limited alga can outcompete that alga for phosphorus (Currie & Kalff, 1984). Bacteria are considered more efficient competitors for mineral nutrients and they score an advantage over phytoplankton because of their small size and large surface-to-volume ratio for absorption of nutrients (Azam et al., 1983). Therefore, nutrient pools constitute the base of the classical grazing food web and the microbial loop and the dominance of phytoplankton or heterotrophic bacteria determines the food web structure, functioning, nutrient cycling and sinking flux (Cho & Azam, 1990).

Depending on the trophic status of the water body, it has been suggested that when nutrient concentrations decline, bacterial abundances do not decrease as quickly as phytoplankton biomass (Bird & Kalff, 1984). In support of that, several other studies have confirmed that planktonic communities in oligotrophic waters are commonly dominated by bacterial biomass, and in eutrophic systems by phytoplankton biomass (Cho & Azam, 1990; Simon et al., 1992); the ratio of heterotrophic to autotrophic biomass in freshwater systems declines with increasing nutrient concentrations and phytoplankton biomass (Del Giorgio & Gasol, 1995; Gasol et al., 1997). It appears that as bacteria are more important under oligotrophic conditions than in eutrophic systems, primary production is used to a greater extent by microbial communities in such systems (Bird & Kalff, 1984), and the relative importance of the microbial food web declines with increasing trophic condition. Nutrient recycling within the microbial web is of less significance at high nutrient concentrations in eutrophic systems (Porter et al., 1988), though eutrophic lakes with large

populations of filamentous algae reduce the efficiency of the classical grazing food web and the microbial loop becomes more crucial (Gliwicz, 1969; Hillbricht-Ilkowska & Havens, 1977).

Focusing on top-down forces, the most important groups ingesting bacteria (and small picophytoplankters) in freshwater and marine systems are protozoans – heterotrophic nanoflagellates and ciliates (Sanders et al., 1989). Among protozoans, heterotrophic nanoflagellates (and phagotrophic phytoflagellates to a lesser extent (Bird & Kalff, 1986)) are considered the principal bacterivores (Güde, 1986; Riemann, 1985) because they are in the most suitable size range (2-20 μm). In addition to heterotrophic flagellates, ciliates (15-200 μm) are known to prey upon bacteria (Šimek et al., 1995), but their importance in bacterivory is more significant in such eutrophic waters, where their densities are sufficient to maintain ciliate communities (Agasild et al., 2012; Sherr & Sherr, 2002). In addition, heterotrophic nanoflagellates and ciliates can prey upon a sizeable proportion of the total primary production (Sanders & Wickham, 1993) and have a greater grazing impact on phytoplankton communities than meta- and macro-zooplankton (Weisse et al., 1990). Although protozoans are considered the main consumers of bacteria, some metazoans are nevertheless significant in controlling bacterial communities (Jürgens et al., 1994); for instance, cladocerans (*Daphnia* spp.) and rotifers, though not copepods, are thought to be effective grazers of bacteria (Güde, 1988; Pace et al., 1983; Pace et al., 1990; Sanders et al., 1989). Moreover, *Daphnia* species can have a strong influence on the whole microbial food web structure both by preying upon bacteria, heterotrophic nanoflagellates and ciliates (Christoffersen et al., 1993; Jürgens, 1994) and by suppressing phytoplankton biomass (Vakkilainen et al., 2004). It appears that zooplankton can affect bacteria directly by grazing them, but also indirectly, mediating the resource supply for bacteria via phytoplankton dynamics and accelerated DOM release due to the grazing (Jumars et al., 1989). Although heterotrophic bacteria form a crucial part of planktonic biomass and their generation times are in the order of a day (or less) in temperate regions (Pace, 1988), their numbers in aquatic systems are quite constant, which indicates effective regulatory mechanisms by protozoans and metazoans. Most experiments have revealed that changes in the grazer community can alter cell morphology and community composition rather than changes in the abundance and biomass of bacteria (Jürgens et al., 1994; 1999; Zöllner et al., 2003). Moreover, some studies have revealed that

viruses could also play a key role in structuring heterotrophic bacterial communities. However, viral abundances are not connected with the trophic status of a water body but more likely with the structure of the bacterial community (Riemann & Middelboe, 2002), spreading abundantly in denser bacterial communities (Vanucci et al., 2005) and therefore decreasing the dominance of single species (Wommack & Colwell, 2000).

Ciliates in turn are significant consumers of small heterotrophic nanoflagellates (2-20 μm) and pico- (<2 μm) and nano- (2-20 μm) plankters (Sanders et al., 1989; Zingel et al., 2007). Therefore, ciliates prey upon particles that can also be consumed by metazoans (mainly rotifers and cladocerans), whereas larger metazoans (e.g. copepods) do not effectively consume small pico- (<2 μm) and nano- (2-5 μm) plankton but utilize food objects in the <5-20 μm size range efficiently (Stoecker & Capuzzo, 1990). This in turn leads to potential competition between ciliates and metazoans for the pico- and nano-sized (<20 μm) food objects (Sanders et al., 1989; Weisse & Frahm, 2002). Several earlier studies demonstrated that ciliates are themselves an important food source for larger metazooplankters (Stoecker & Capuzzo, 1990), thus forming a link between pico- and nano-plankton and metazoans. Also, heterotrophic nanoflagellates are suppressed by metazooplankter predation, mainly by cladocerans and rotifers (Jürgerns et al., 1996; Sanders et al., 1994), because copepods are focused on larger particles (ciliates). Thus, the grazing pressure of copepods on ciliate communities can be advantageous for the development of heterotrophic nanoflagellates (Sommer et al., 2003).

Metazooplankton are known to graze on a wide range of food particles from bacteria, detrital particles and unicellular algae to proto- and other meta-zooplankters (Jeppesen et al., 1999; Sanders & Wickham, 1993). Changes in phytoplankton community structure are strongly dependent on the shifts in the zooplankton size range caused by fish predation, which in turn alters the size distribution of phytoplankton community (Berquist et al., 1985; Carpenter et al., 1985). It appears that the size range of phytoplankters ingested depends on zooplankton size: larger zooplankters can ingest larger phytoplankters (Burns, 1968). In macrophyte-dominated shallow eutrophic lakes, where the occurrence of macrophytes and refuges for zooplankton from fish predation

supports the development of large-sized zooplankton communities (e.g. *Daphnia* spp), these large-sized zooplankters are considered key to the development of top-down regulation of phytoplankton populations (Jeppesen et al., 1999). Moreover, some large calanoid copepods (*Eudiaptomus*) and cladocerans (*Daphnia*) can control even large bloom-forming cyanobacteria (Wilson & Chislock, 2013), but these algae are thought to be of poorer food quality for herbivorous zooplankton (rotifers, cladocerans and copepods) (Porter & Orcutt, 1980) than others (flagellates and chlorophytes) (Tillmanns et al., 2008). More generally, in such eutrophic lakes, which are dominated by large cyanobacterial communities, zooplankton cannot usually control their biomass (Jeppesen et al., 1996; Ventälä et al., 2002) because their large forms mechanically interfere with zooplankton grazing on them (Lynch, 1980). In addition, cyanobacteria are known to produce secondary metabolites, which have been demonstrated to inhibit the growth of zooplankton communities, e.g. the fecundity of cladocerans (Gilbert, 1990). On the other hand, in turbid, plankton-dominated, macrophyte-poor lakes with planktivorous fish, small-bodied zooplankters (*Bosmina longirostris*, small cyclopoid copepods) often dominate (Jeppesen et al., 1999). At the same time, a zooplankton community dominated by small-sized rotifers, cladocerans and cyclopoid copepods can lead to a situation where these small zooplankers cannot control ciliates and prey upon small nanoplankton, which in turn increases the biomass of larger phytoplankton (Jürgens & Jeppesen, 2000; Ventälä et al., 2002).

Standing fish stocks are important for structuring the pelagic communities of water bodies (Jeppesen et al., 1996; Jeppesen et al., 2000 a,b). Planktivorous and piscivorous fish as top predators can influence whole aquatic communities by direct (via predation) and indirect (via trophic cascade) forces, triggering changes in community composition, structure and even ecosystem conditions (Carpenter et al., 1985; Lynch, 1979; Søndergaard et al., 1997). The general indirect effect expected of an abundant piscivorous fish community is a decrease in planktivorous fish abundance, a subsequent rise in zooplankton size distribution and biomass, and a reduction in phytoplankton biomass via a trophic cascade (Mills et al., 1987). Therefore, zooplankton plays a central role, serving as food source for planktivorous fish and as consumers of phytoplankton. The general top-down effect of planktivorous fish on a zooplankton community is not suppression of the whole of that community, but only

reduction of preferred prey items; less-consumed prey items gain an advantage (Crowder et al., 1988). For instance, adult planktivorous fish generally prey upon large zooplankton, and this increases the proportion of small zooplankton. Therefore, owing to the grazing pressure of planktivorous fish, larger metazoans (*Daphnia magna* Straus*, Daphnia pulex* L., *Daphnia galeata* G. O. Sars, *Diaptomus* spp., *Mesocyclops edax* Claus, *Leptodora kindtii* Focke) disappear and smaller species (*Daphnia cucullata* G. O. Sars, *Bosmina longirostris* O. F. Müller, *Chydorus sphaericus* O. F. Müller*, Ceriodaphnia lacustris* Birge, and nauplii) become more abundant. This compositional shift can lead to a lower grazing efficiency and impact on phytoplankton (Brooks & Dodson, 1965). On the other hand, smaller planktivorous fish (e.g fish larvae) mostly influence the small and intermediate-sized zooplankton, which again can increase the share of large zooplankton (Crowder et al., 1987). The effect of larval fish feeding on zooplankton depends on the abundance of fish larvae and the duration of planktivory (Mills & Forney, 1988); the last-mentioned phase can range from a very short time period to months or years, and some species may feed on zooplankton throughout their lives (Mittelbach & Persson, 1998; Olson et al., 1995; Persson, 1988). Moreover, feeding type can influence the community structure, for instance visual feeding fish focus on large and active prey items, hence increasing the abundance of smaller and more cryptic zooplankton forms (Crowder & Cooper, 1982). Filter feeding fish in turn concentrate on slow zooplankton or large algae, thereby enhancing the abundance of fast zooplankton and small algae (Drenner et al., 1986). Thus, selective predation by larval and adult zooplanktivorous fish is one of the most important factors in structuring zooplankton communities (Brooks & Dodson, 1965). Despite numerous studies focusing on fish cascading effects on phyto- and zoo-plankton communities (Lynch, 1979; Pace & Funke, 1991), only a few studies have been perfomed in respect of microbial communities (pico-, nano-plankton, protozoans) (Christoffersen et al., 1993; Jeppesen et al., 1996; Müller-Solger et al., 1997; Riemann, 1985; Tzaras et al., 1999; Vaque & Pace, 1992).

4. MATERIALS AND METHODS

4.1. Description of the study areas

Studies included in this thesis were undertaken on a large, shallow, eutrophic lake, Lake Võrtsjärv, known to be plankton-dominated with a rich ciliated protozoa community (**II, III, IV, V**), and on two large, natural ponds on Härjanurme fishfarm (**I**), Estonia. The material used was collected during the years 1997-2009 from Lake Võrtsjärv and during 2005-2006 from the Härjanurme fishfarm ponds.

Lake Võrtsjärv, the second largest lake in Estonia, is situated in the South-Estonian preglacial basin and centred at 58°15'7''N/ 26°1'47''E. It is elongated from north to south, with surface and catchment areas of 270 km^2 and 3374 km2, respectively. Despite its large surface area, the lake is shallow with a mean depth of 2.8 m and maximum depth of 6 m. Owing to its shallowness the lake water is well aerated, non-stratified and turbid because of the surface waves and currents. The water is yellowish-green or greenish-yellow in colour and the mean transparency seldom exceeds 1 m. The lake is ice-covered on an average of 131 days a year (Nõges & Nõges, 2013) and the water temperature usually reaches its maximum in July (on average, 23°C) (Järvet, 2004). The lake water is slightly alkaline (pH ~ 8). The average concentrations of total phosphorus (0.05 mg P L^{-1}) and nitrogen (2 mg N L^{-1}) characterise the lake mostly as an eutrophic to hypertrophic water body. The main factors determining the lake's ecosystem are its shallowness, exposure to westerly and south-westerly winds and large fluctuations of water level (Haberman et al., 1998).

The two studied ponds (denoted subsequently as ponds F (fishpond) and nF (fishless pond)) used for aquaculture are located in Härjanurme fishfarm, Central Estonia (58°40 N/ 26° 22 E). The ponds had been left to dry during the winter months and were refilled with water in the middle of April and emptied in October. Whitefish larvae (*Coregonus lavaretus* L.) were introduced into pond F. Pond nF had no planktivorous fish at all. The surface areas of ponds F and nF were 6 and 7.2 ha, respectively. The mean depth of both ponds was 1.6 m. The concentrations of total phosphorus and nitrogen (average values over the years studied in July) were 48 µg L^{-1} and 0.9 mg L^{-1} respectively in pond F and 47 µg L^{-1} and 1.0 mg L^{-1} respectively in pond nF, characterizing them as eutrophic water

bodies. Water transparency fluctuated over the ranges 0.5-1.8 and 0.6-1.7 m in ponds F and nF, respectively. More detailed descriptions of the study sites are given in papers **II, III, IV, V** for Lake Võrtsjärv and in paper **I** for the Härjanurme ponds.

4.2. Experimental design, sampling and sample analysis

Experiments with introduced planktivorous whitefish larvae (pond F) and without larval fish (pond nF) were conducted in the Härjanurme fishfarm ponds to assess the role of juvenile fish (presence/absence) on the whole trophic structure. Surveys were carried out over two successive years (2005, 2006) to ensure replicability over time. Plankton samples were collected every 10 (2005) and 20 (2006) days, while juvenile fish were sampled every 20 days (2005, 2006) (**I**). The fish larvae were introduced into pond F shortly after hatching in both years. The stocking density was approximately 6500 larvae per hectare.

Special attention was paid to the analyses of planktivorous fish gut contents to determine the importance of ciliates as a crucial food source for larval fish in Lake Võrtsjärv. To determine the diet of planktivorous fish larvae, the most common fish species were collected from the central part of the lake in 2005. Samples were collected on two occasions by several horizontal tows using a conical net (**III**).

Enclosure experiments were conducted with the natural and screened (145 μm) zooplankton communities of Lake Võrtsjärv in 2008 to investigate the effect of small-sized crustacean zooplankton on the ciliate, phyto- and bacterio-plankton communities. Half of the integrated lake water collected from the regular sampling station was used to fill the control enclosures; the other half, for zooplankton removal treatments, was first filtered through a 145 μm mesh plankton net to eliminate the larger fraction of crustaceans. The water was divided into six 20 L plastic containers and incubated in the lake for eight days. Both treatments were carried out in triplicate. During the experiments, bacteria and ciliates were sampled five times, phytoplankton four times and metazooplankton twice (**II**).

Mesocosm experiments (**IV**) with control enclosures (natural metazooplankton concentration), and with cyclopoid copepod enrichment treatments, were carried out in 2009 in Lake Võrtsjärv. Six transparent

plastic enclosures (polyethylene bags with glass-fibre reinforcement; volume 5 m^3) were filled with natural lake water and manipulated with two different crustacean concentrations in the treatment enclosures. Cyclopoid copepods, dominating the metazooplankton community and biomass, were collected for enrichment treatments with a 145 μm mesh plankton net from the same lake. To evaluate different metazooplankton predation effects in experimental treatments with respect to custacean abundance and composition, the collected zooplankton was divided into (a) enclosures 3 and 4 (further referred to as treatment M2), yielding an enrichment of approximately 2.5x of natural copepod biomass over the control media (further referred to as M1), and (b) enclosures 5 and 6 (further referred to as treatment M3) yielding an enrichment of approximately 5x of natural copepod biomass. All the different treatments were performed in duplicate. The experiment lasted 22 days, during which the different plankton communities (bacteria, phytoplankton, ciliates, metazooplankton) were sampled eight times (**IV**).To identify any cascading effects on the planktonic communities, ciliate abundances were counted in two (**IV**) or four (**II**) and phytoplankton abundances in three (**II**, **IV**) size classes.

To investigate the role of macrophytes in the dispersal of different ecological groups of ciliates, samples were collected from eight differently vegetated lake sites. Macrovegetation was studied in summer 1997, and ciliate samples were collected during the annual ciliate peak abundances in July (2002-2004) and August (1997, 1999-2004) from the same sampling points (**V**). A short overview of the experiments conducted is given in Table 1.

Table 1. Summary of fieldwork in studied water bodies (BP – bacterioplankton, PZP – protozooplankton, CILI – ciliates, MZP – metazooplankton, PP – phytoplankton, FI – fish, MP – macrophytes).

Study site/ Index	Year	Study time	Duration (in days)	Samples
Härjanurme ponds (I)	2005 2006	25.04. - 29.09. 25.04. - 28.09.	127 126	BP, PZP, MZP, PP, FI
Lake Võrtsjärv (II)	2008	27.08. - 03.09.	8	BP, PZP, MZP, PP
Lake Võrtsjärv (III)	2005	24.05 & 08.06.	2	CILI, MZP, FI
Lake Võrtsjärv (IV)	2009	20.08. - 11.09.	22	BP, PZP, MZP, PP
Lake Võrtsjärv (V)	1997, 1999-2004	July, August	-	CILI, MP

Plankton was analyzed in the collected integrated lake water using a Ruttner water sampler. For metazooplankters, samples of 5-10 L were poured through a 48 μm mesh net (**I-IV**). For ciliates and phytoplankton, 100-250 mL samples were used, while 20 mL samples were collected for bacteria. Metazooplankton, phytoplankton and ciliate samples were fixed with acidified Lugol's solution (0.5-2% final concentration), and bacterioplankton samples with formaldehyde or glutaraldehyde (1% final concentration). Heterotrophic nanoflagellate samples were fixed with buffered formalin (**I**). As the methods used are well described in the papers, only the principal methods are mentioned here.

- The Utermöhl technique (1958) was used to determine the taxonomic composition, abundance and biomass of phytoplankton (**I, II, IV**) and ciliates (**I-V**).
- A DAPI (4´,6´-diamidino-2-phenylindole) direct count (Porter & Feig, 1980) was used to determine the abundance of heterotrophic bacteria (**I, II, IV**) and nanoflagellates (**I**).
- Bogorov's chambers were used to determine the taxonomic composition, abundance and biomass of metazooplankton (**I-IV**).
- A labelling technique based on leucine (Leu) incorporation (Kirchman et al., 1985) with a modified cold trichloroacetic (TCA) treatment (Wicks & Robarts, 1988) was used to measure bacterial production (**IV**).
- The 14C-assimilation technique (Steeman-Nielsen, 1952) was used to measure primary production (**IV**).

- Grazing by proto- and meta-zooplankton on bacteria was assessed by in situ feeding experiments using fluorescent microspheres (**II, IV**).
- Fish gut contents were analyzed according to Fukami et al. (1999) and Sutela & Husko (2000) (**III**).
- The numerical method (Hyslop, 1980) was used to analyze the fish alimentary tracts (**III**).
- To study macrovegetation, a combined transect and sampling quadrat method (Feldmann & Nõges, 2007) was used (**V**).

4.3. Statistical analysis

Data were analyzed using the nonparametric Spearman's correlation method, factor analysis, Mann-Whitney U-test and Wilcoxon matched pairs test offered by STATISTICA 6.0/8.0 (StatSoft, Inc. 2007).

5. RESULTS

5.1. Aquatic biota in study areas

In the present study, three water bodies differing in abiotic and biotic indices were investigated with particular emphasis on the shallow, large and highly eutrophic Lake Võrtsjärv. Lake Võrtsjärv can be divided into two parts, the large wind-exposed northern and central part (plankton-dominated) and the small, narrow and sheltered southern part (macrophyte-dominated). The phytoplankton community in the plankton-dominated segment of the lake was mostly represented by the fraction of inedible filamentous cyanobacteria (*Aphanizomenon skujae* Kom.-Legn. and Cronb., *Limnothrix planktonica* L., *redekei* (van Goor) Meffert, *Planktolyngbya* spp.) and diatoms (*Synedra* spp., *Navicula* sp., *Nitszchia* spp., *Aulacoseira ambigua* (Grunow) Simonsen), which were numerically dominant (**II, IV**). The dense macrovegetation is agglomerated in the sheltered and narrower southern part of the lake, where *Phragmites australis* (Cav.) Trin. ex Steud. and *Myriophyllum spicatum* L. dominated among the emergent and submerged vegetation (**V**). The standing fish stock was characteristic of other lakes in the European temperate zone and was represented by the most common fish species, *Rutilus rutilus* L., *Esox lucius* L., *Perca fluviatilis* L., *Osmerus eperlanus* L., *Sander lucioperca* L., *Abramis brama* L., *Alburnus alburnus* L. and *Gymnocephalus cernuus* L. (**III**). The metazooplankton community was represented by eutrophic species, among which copepods (mostly *Mesocyclops leuckartii* Claus), rotifers (*Anuraeopsis fissa* Gosse, *Polyarthra* spp., *Keratella* spp.) (**II-IV**) and cladocerans (*Chydorus sphaericus, Bosmina longirostris*) (**III**) prevailed. Protozoans were dominated by ciliates in Lake Võrtsjärv, contributing up to 95% of the total available zooplankton carbon (**III**). Ciliate assemblages mostly comprised oligotrichs (**II-V**), but scuticociliates (**III**), prostomatids (**III**), haptorids (**II, V**) and peritrichs (**V**) were also frequent.

The taxonomic compositions and dominants of the plankton communities differed greatly between the two studied ponds (**I**). Pond F contained planktivorous whitefish, while pond nF had no planktivorous fish. The most important metazooplankton group in both ponds was cladocerans. The metazooplankton community of pond nF was dominated by the large cladoceran *Leptodora kindtii*, which remained numerous throughout the study, while pond F was dominated by smaller species such as *Bosmina*

longirostris and *Daphnia cucullata*. The ciliate community consisted mostly of small bacterivorous oligotrichs (*Halteria grandinella* O. F. Müller) and peritrichs (*Hastatella radians* Erlanger) in pond F, while in pond nF the community was dominated by bacterivorous ciliates (oligotrichs, peritrichs, scuticociliates) in spring and herbivorous ciliates (oligotrichs, gymnostomatids) in summer. In contrast to other plankton groups the taxonomic composition of phytoplankton was quite similar between the studied ponds and was dominated by small (<35 μm) chlorophytes (*Monoraphidium, Chlorella),* diatoms (*Rhizosolenia* spp.) and chrysophytes (*Dinobryon sociale* Ehrenberg).

5.2. The influence of polarized macrovegetation on the central link of the microbial loop (V)

In Lake Võrtsjärv, macrophytes exhibited strong spatial polarization in relation to abiotic factors in both the west-east and north-south directions. In particular, the northern and eastern shores are most wind-exposed, while the most sheltered sites are located in the south. The northern shore was characterized by the coarsest sediments, and the shores at the southernmost end of the lake were dominated by fine sapropel sediments and the highest nutrient loadings. To study the influence of polarized macrovegetation on ecologically different ciliate (euplanktonic, epiplanktonic, benthic) groups, the lake area was divided into eight sites on the basis of homogeneity of macrophyte communities within the shore sites. The number of macrophyte species in these sites ranged from 2 to 21 and was highest in the southern part of the lake. Among dominants, *Phragmites australis* was several times more abundant on average than *Myriophyllum spicatum.* Ciliate communities were most similar between adjacent sites and differed most between remote sites. The total number of ciliates ranged from 36 to 47 in different sites, with no clear trend in the number of ciliate taxa unique to different numbers of lake sites. The number of euplanktonic (13-20) taxa was more stable than the epiplanktonic (3-16) or benthic (12-23) taxa (**V**).

A strong polarization of environmental conditions was demonstrated by factor analysis of the geographical co-ordinates. Factor analysis revealed that the number of euplanktonic ciliate taxa decreased and the number of epiplanktonic ciliate taxa (also the total number of ciliate taxa) increased in the east-west direction. The number of benthic ciliates

taxa decreased in the north-south direction (Table 3, paper **V**). The correlations among environmental, macrophyte and planktonic ciliate variables supported the same spatial gradients. Moreover, the nitrogen loading index had a negative influence on euplanktonic and a positive influence on epiplanktonic ciliate taxa (Table 4, paper **V**).

Further analyses demonstrated that macrophyte diversity had a positive effect on ciliate diversity (all taxa included) and a negative effect on total ciliate abundance (Figure 2). In particular, diverse macrophyte stands supported a high species richness and abundance of the epiplanktonic ciliate community, being most diverse in the macrophyte-rich southern sites. Euplanktonic ciliates correlated negatively with macrophyte diversity and were most diverse and abundant in the most wind-exposed northern sites, where only a few macrophyte species (e.g. *Phragmites australis*) could tolerate the exposed conditions. The trend of distribution of benthic ciliates was somewhat similar to the euplanktonic ciliate taxa, being most abundant in the sites where the Shannon-Weaver index for macrophytes was low.
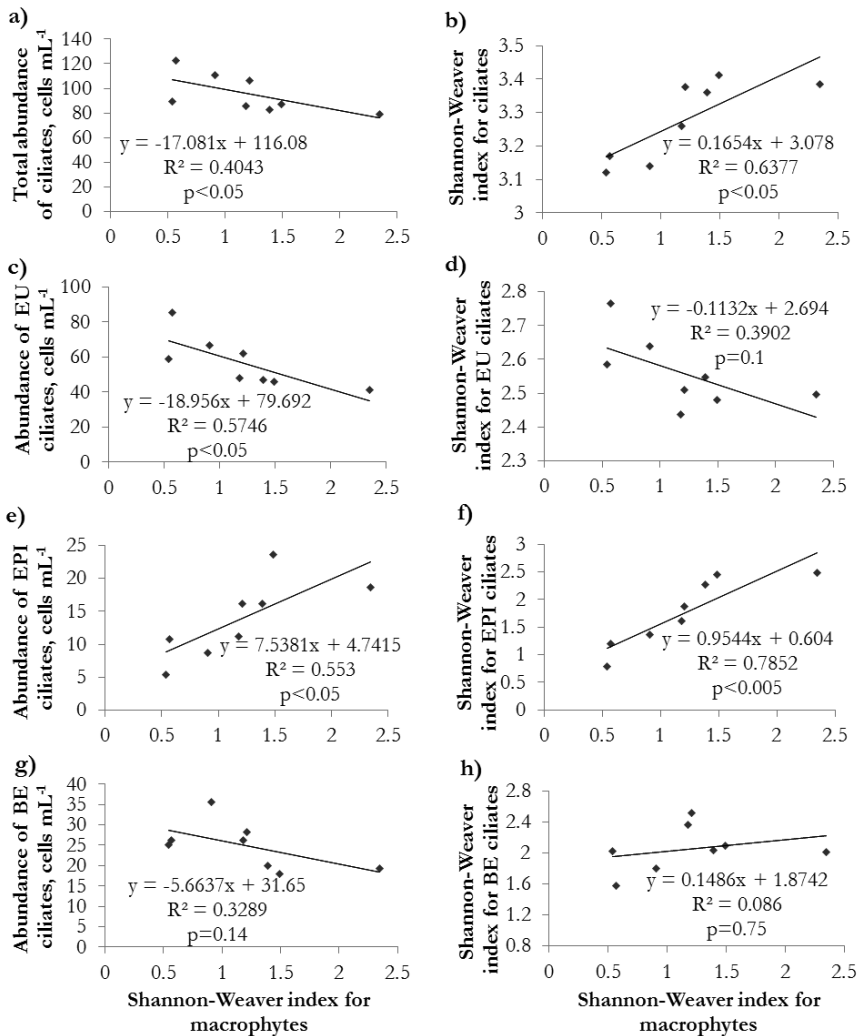
Figure 2. Trends between abundance and species diversity of ciliates (total (a-b), euplanktonic (c-d), epiplanktonic (e-f), benthic (g-h)) and macrophyte diversity.

5.3. Trophic cascade from fish to bacteria (I)

In the presence of fish, different plankton groups were tied to each other and fluctuated greatly, providing strong evidence of cascading interactions from fish to bacterioplankton (Figure 3). More precisely, whitefish larvae had a strong influence on the metazooplankton community composition and structure. Owing to the grazing pressure, the community of zooplankton in fishpond F was dominated by smaller filter-feeding cladocerans (*Bosmina longirostris*, *Daphnia cucullata*), while

large predaceous cladocerans dominated in the fishless pond nF. In the presence of fish, *Leptodora kindtii* occurred only ocassionally and in very low numbers. Differences in metazooplankton community composition were also reflected in the biomass values: the biomass was significantly lower in the presence of larval fish (Figure 2, paper **I**).
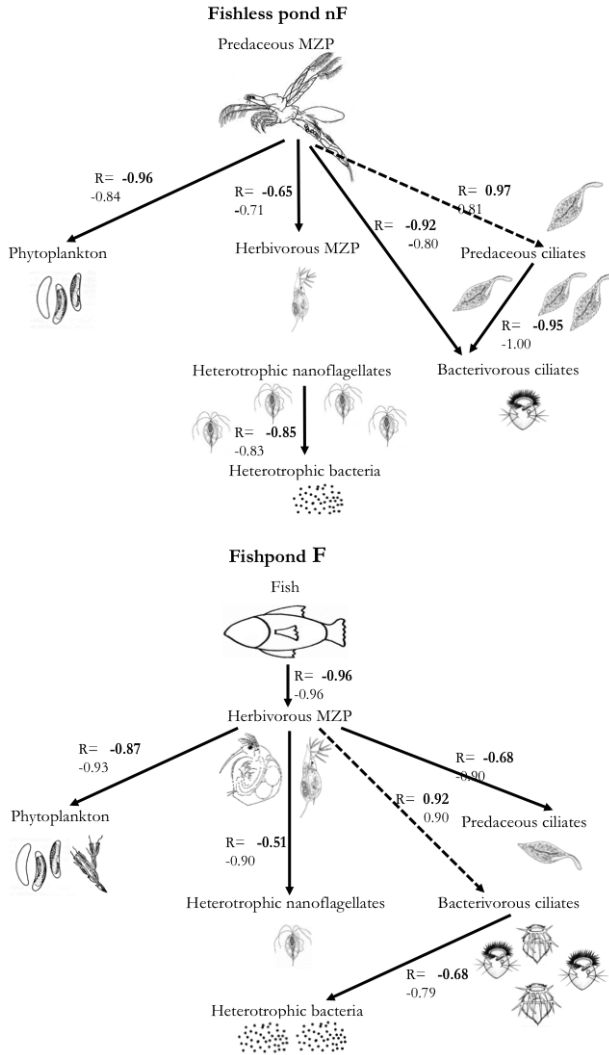
Figure 3. Illustration of the generalized food webs and main trophic interactions in a stocked (F) and a fishless (nF) pond in 2005 (numbers in bold) and 2006. Arrows indicate the main trophic interactions – dashed arrows present positive, solid arrows negative correlations (Spearman correlation coefficients R; $p < 0.05$). The correlations were calculated from the following variables: heterotrophic nanoflagellates, predaceous ciliates, bacterivorous ciliates and bacteria – total abundances; metazooplankton and phytoplankton – WW biomass; fish – feeding on zooplankton biomass.

Our results demonstrated that in fishpond F the amount of MZP eaten by the whitefish in both years correlated negatively with MZP biomass (R = -0.96, p<0.05; Table 1, paper **I**) and with the abundance of bacterivorous ciliates. The impact of fish on the MZP community was considerable; they consumed up to 51% and 27% (in 2005 and 2006, respectively) of the total MZP biomass daily (Figure 3, paper **I**). The total share of ciliates in the whitefish food was high initially (62% and 65% of the consumed zooplankton biomass on May 5 in 2005 and 2006, respectively) but dropped sharply (to 2% and 1%, respectively) and remained at zero throughout the remaining study period. The effect of fish on the standing ciliate stock was minor; they consumed <0.05% of the total ciliate biomass daily.

The amount of zooplankton eaten by the fish was positively correlated (p<0.05) with the abundance of heterotrophic nanoflagellates and predaceous ciliates, and with the biomass of phytoplankton (Table 1, paper **I**). In particular, as the lowest flagellate numbers coincided with the peaks in metazooplankton biomass (Figure 1-2, paper **I**), we can asume that in pond F metazooplankters controlled the abundance of flagellates despite the high fish grazing pressure on them. Moreover, a significant negative relationship was found between the abundance of flagellates and metazooplankton biomass (R= -0.51 and -0.90 in 2005 and 2006, respectively, p<0.05; Table 2, paper **I**). Metazooplankton community, low in biomass but high in abundance, was also able to control the abundance of predaceous and bacterivorous ciliates, expect for the abundance of two species, *Halteria grandinell*a and *Hastatella radians*, which probably had defensive mechanisms against predation. Contrary to our expectations the grazing pressure on bacterial abundances was quite low in the presence of planktivorous fish (Figure 5, paper **I**). However, our results demonstrated that the abundance of bacteria was negatively correlated with the amount of bacteria eaten by ciliates in pond F (R = -0.71 and -0.79, respectively, p<0.05; Table 3, paper **I**). Bacterivorous ciliates were the main grazers of bacterioplankton, especially in late July (Figure 6, paper **I**), while the abundance of HNF dropped (Figure 1, paper **I**) owing to the peak in metazooplankton biomass. Correlation analysis also revealed negative correlations between the biomass of MZP and the amount of bacteria eaten by HNF (R = -0.78, p<0.05; Table 3).

In the fishless pond nF, the seasonal dynamics of plankton communities was very stable throughout the study. The role of the top predator

shifted to the predaceous zooplankter *Leptodora kindtii* (Figure 3), which probably controlled the abundance of smaller metazooplankters, as we found a negative correlation between the abundances of the former and of herbivorous cladocerans (R = -0.65 and -0.71 in 2005 and 2006, respectively, p<0.05). At the same time, metazooplankters had a strong negative influence on the phytoplankton communities in both studied ponds (Table 2, paper **I**). In contrast to our expectations, the predaceous metazooplankton community had no influence on protozoans in the fishless pond: the number of flagellates remained quite high throughout the study period (Figure 1, paper **I**), and as the abundance of predaceous (*Paradileptu*s sp., *Pelagodileptus* sp.) ciliates correlated negatively with the abundance of smaller bacterivorous ciliates (R= -0.95 and -1.0, respectively, p<0.05, Table 2, paper **I**), we can assume that predaceous ciliates rather than metazooplankton controlled the abundance of ciliates, leading to a decrease in total ciliate numbers. As the total number of bacterivorous ciliates was low and a negative correlation was found only between the abundance of bacteria and flagellates (R= -0.85 and -0.83, respectively, p<0.05, Table 2, paper **I**), we can assume that heterotrophic nanoflagellates were the main grazers of bacteria in the fishless pond. Moreover, the total number of bacteria was lower and the grazing pressure on bacteria more active in the fishless pond than in pond F (Figure 3).

5.4. The feeding influence of the fish stock on ciliated protozoans (III)

Studies of the feeding of planktivorous fish larvae revealed that ciliates were the only protozoans consumed by all larval fish species examined in Lake Võrtsjärv. Considering the BULK-diet (based on analysis of the whole alimentary tract), the average ingestion rate for all fish taxa was 161 ciliates per larva^{-1} h^{-1} in May and 272 ciliates per larva^{-1} h^{-1} in June, 2005. Although the number of ciliates ingested in May was significantly lower (Mann-Whitney U-test, p<0.05) we found no significant relationship between the numbers of ingested ciliates and the total length of the larval fish species examined (p>0.05) (Figure 2, paper **III**).

Gut segmentation analysis demonstrated clear differences in food consumption among gut quarters. Hard-bodied cladocerans and rotifers were quite evenly distributed through all four quarters, while soft-bodied species were present only in the first gut segments (**III**).

Ciliates were a very important food source for larval fish, accounting on average for 61% and 62% (FRESH-diet) of the total carbon biomass consumed by fish larvae in May and June, respectively. The ciliate biomass contribution was highest in the diet of smelt and lowest in that of pike (Figure 4). The percentage carbon-specific ingestion rates of fish larvae (µg C of prey µg C larvae-1 d^{-1}) revealed that the metazooplankters examined contributed only an average of 9.2% of larval body C d^{-1} in May and 8.7% in June (**III**).
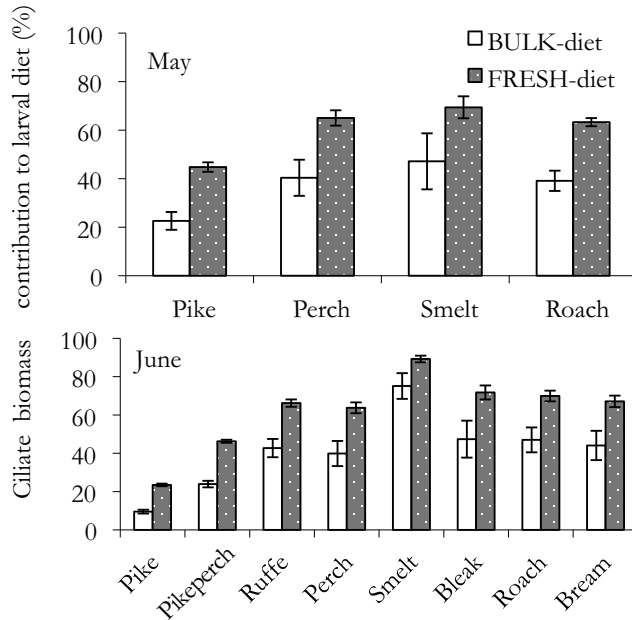

Figure 4. Contribution of ciliate biomass to the total larval diet in Lake Võrtsjärv in May and June, 2005. The diets reconstructed using the content of the first gut quarter (FRESH-diet) and of the whole alimentary tract (BULK-diet) are compared. Error bars indicate standard deviation.

In the pond experiments, the total share of ciliates in the whitefish food was high initially (62% and 65% of the consumed zooplankton biomass on May 5 in 2005 and 2006, respectively) but dropped sharply thereafter to 2% and 1%, respectively and remained at zero throughout the remaining study period. The effect of fish on the ciliate standing stock was minor; they consumed <0.05% of the total ciliate biomass daily (**I**).

5.5. The influence of zp-removal and zp-enrichment treatments on microbial communities (II & IV)

Enclosure experiments with natural [mainly cyclopoid copepods (e.g. *Mesocyclops leuckarti*)] and screened [copepod nauplii, rotifers (e.g. *Polyarthra luminosa* Kutikova, *Anuraeopsis fissa*)] metazooplankton communities revealed that removal of the larger fraction of crustaceans initiated a substantial decrease in total ciliate abundance in zp-removal treatment (Mann-Whitney U-test, $p<0.05$; the ciliate abundance of in control media remained stable. During the incubation, a slight increase in total ciliate biomass was observed in zp-removal, while their biomass in the control decreased (**II**).
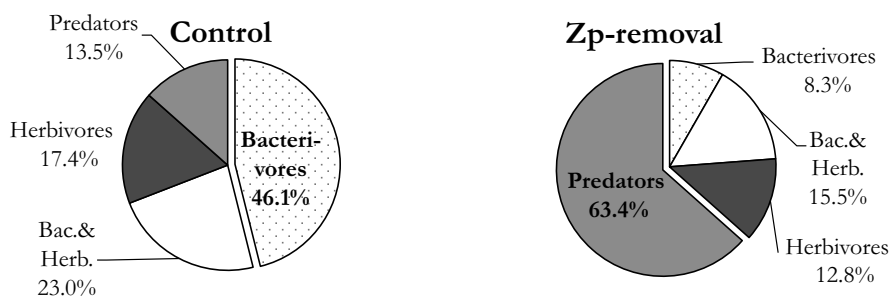
Figure 5. Proportion of functional groups in ciliate biomass at the end of the experiment in the control and zp-removal treatments. Abbreviations: Bac. & Herb. – bacterio-herbivorores.

Ciliate size structure and taxonomic composition differed significantly between the zp-removal treatment and control media (**II**). Among all the ciliate size classes (<20, 20-40, 40-100, >100 µm), we observed a 10-fold increase in the large predaceous ciliates (>100 µm, mainly haptorids) and a parallel reduction in the abundance of the smaller bacterivorous ones (<20, <20-40 µm, mainly oligotrichs and peritrichs). The differences in ciliate size class abundances (<20, 20-40, >100 µm) between the treatments were statistically significant (Mann-Whitney U-test, $p<.0.05$). Considering taxonomic composition, haptorids contributed most to the largest size class of ciliates. In the zp-removal treatment, their smaller cells decreased while larger raptorial species (>300 µm, e.g. *Paradileptus elephantius* Kahl) increased, so the haptorid biomass was significantly greater than in the control (Mann-Whitney U-test, $p<0.05$). Oligotrichs, comprising mainly small bacterivorous (e.g. *Rimostrombidium* spp. and *Pelagostrombidium* spp.)

cells, responded to zp-removal treatment with a substantial reduction in biomass. By the end of the experiment, a significant compositional difference in ciliate communities between the control and zp-removal media was observed. Bacterivores (mainly oligotrichs), the dominant functional group in ciliate biomass in the control media (46.1% of the total ciliate biomass), declined to a very low level in the zp-removal treatment, while predaceous forms (mainly haptorids) contributed 63.4% of the total ciliate biomass (Figure 5). However, the compositional shift in the ciliate community did not cascade down to bacteria and edible phytoplankton (**II**).

Mesocosm experiments with natural (M1) and enriched (M2 & M3, paper **IV**) metazooplankton treatments were characterized by quite similar taxonomic compositions and the same dominants as observed in our previous zp-removal experiments (**II**). As a result of copepod enrichment the biomass of metazooplankton in enriched enclosures (M2 & M3) was initially clearly higher than in the control enclosures (M1) but started to decrease gradually in all treatments and attained the same level as the M1 enclosures at the end of the experiment. Our results (**IV**) revealed that the 2.5- (M2) and 5-fold (M3) increases in crustacean zooplankton biomass initiated an increase in small-sized bacterivorous ciliates because of the simultaneous decline of predaceous ciliate abundance.

Our study demonstrated that crustacean zooplankton had a generally negative effect on predaceous ciliate abundance and an overall positive effect on total ciliate abundances (Figure 4, paper **IV**). Therefore the ciliate community was dominated by the smallest size class (bacterivorous species) in all treatments, while the abundance of predaceous ciliates was low (Figure 2, paper **IV**). However, owing to the decline in copepod biomass at the end of the experiment, the biomass of predaceous ciliates increased sharply, leading to great changes in ciliate functional groups: the final community in M1 & M2 was dominated by predaceous species and the proportion of bacterivorous ciliates decreased (**Figure 6**).

Initial ciliate community
M1
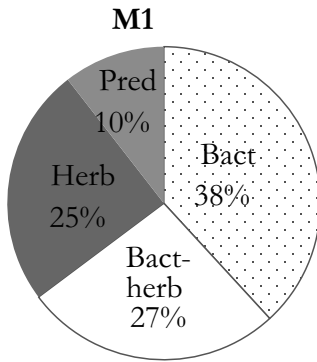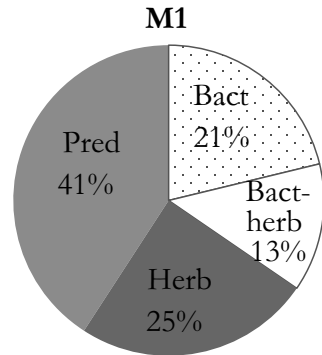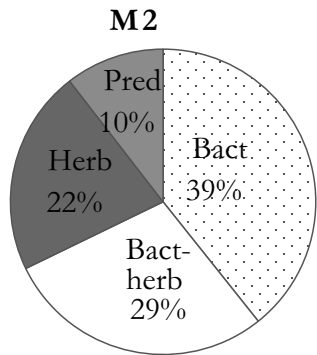
Final ciliate community
M1

Initial ciliate community
M2

Final ciliate community
M2
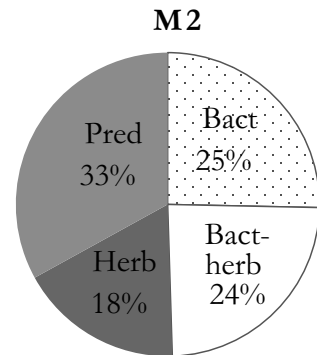
Initial ciliate community
M3
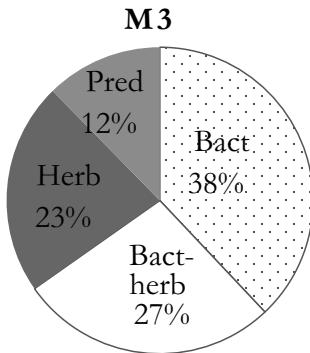
Final ciliate community
M3
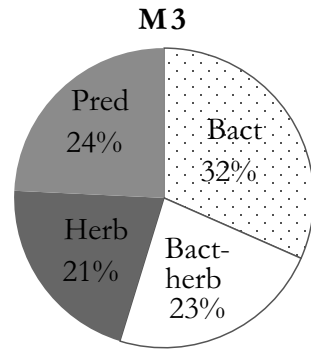
Figure 6. Proportion of functional groups in ciliate biomass at the beginning and end of the experiment in the control (M1) and zp-enrichment M2 (2.5x natural copepod biomass) and M3 (5x natural copepod biomass) treatments. Abbreviations: Pred – predaceous, Bact – bacterivorous, Bact-herb – bacteri-herbivorous, herb – herbivorous ciliates.

We also found negative relationships between the biomass of predaceous and bacterivorous ciliates (Spearman rank R>0.90; p<0.05 in M2 & M3), which indicated that predaceous ciliates generally controlled the abundance of smaller bacterivorous ciliates (**Figure 7**). However, the compositional shift in the ciliate community did not cascade down to bacteria and edible phytoplankton (**IV**).
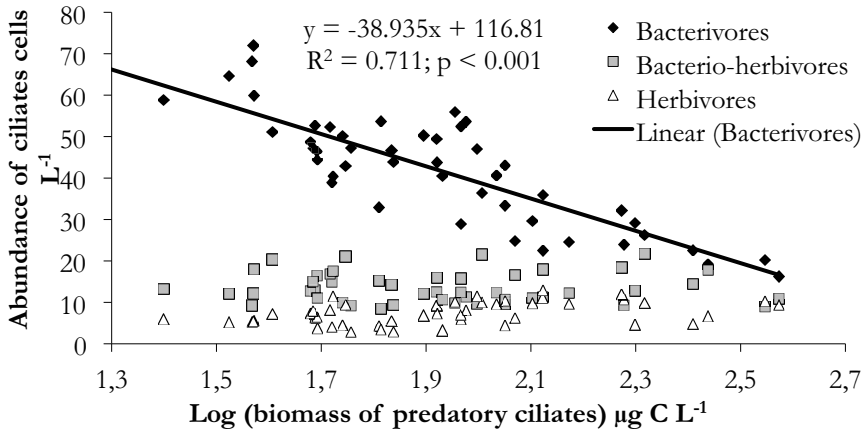
Figure 7. Regression between the total abundance of ciliates and biomass of predatory ciliates using pooled data from all six mesocosms studied.

6. DISCUSSION

6.1. The influence of polarized macrovegetation on the central link of the microbial loop (V)

Although macrophyte and fish communities are known to play a key role in structuring aquatic food webs, most studies have focused only on interactions among selected organism groups, e.g. fish-zooplankton-phytoplankton (Olrik et al., 2007), fish-zooplankton-macrophyte (Jeppesen et al., 1997; Lauridsen & Lodge, 1996; Manatunge et al., 2000; Perrow et al., 1999; Schou et al., 2009; Stansfield et al., 1997), fish-zooplankton-phytoplankton-macrophytes (Schriver et al., 1995; Timms & Moss, 1984) and macrophyte-zooplankton-(phytoplankton) (Lauridsen et al., 1996; Maia-Barbosa et al., 2008; van Donk & van De Bund, 2002). Studies concerning the influence of both (macrophytes and fish) on whole microbial loop communities seem to be absent. Despite the lack of such studies, macrophytes are important for a wide variety of functions. They act as habitats for other aquatic organisms – refuges for zooplankton and fish against predation by predators, planktivorous and piscivorous fish (Burks et al., 2002; Heck & Crowder, 1991; Lauridsen et al., 1996; Persson, 1993; Timms & Moss, 1984), structures for epiphytic and plant-associated invertebrate communities (Heck & Crowder, 1991; Lauridsen et al., 1996; Warfe & Barmuta, 2006; Paterson, 1993), spawning and nesting habitats for some fish (Persson & Eklöv, 1995) and their living or dying forms serve as food sources for many aquatic animals (Cowx & Welcomme, 1998). Several earlier studies have demonstrated the importance of macrophytes as habitats and refuges for a far greater abundance of macroinvertebrates (Heck & Crowder, 1991), zooplankton (Lauridsen et al., 1996; Schou et al., 2009) and fish (Heck & Crowder, 1991) communities than unvegetated areas. The increase in aquatic animal abundances appears to be attributable to the density or biomass of aquatic macrophytes (Crowder & Cooper, 1982; Schriver et al., 1995; Stoner & Lewis, 1985), and the greater environmental heterogeneity and wider spectrum of ecological niches provided by macrophytes in the littoral zone compared to open water areas without macrophytes (Heck & Crowder, 1991; Warfe & Barmuta, 2006). However, fish, particularly piscivorous fish, tend to prefer littoral areas of intermediate structural complexity (Killgore et al., 1991), and futher increase in macrophyte density beyond that point reduces their feeding rate significantly (Heck & Crowder, 1991; Savino et al., 1992).

Therefore, it appears that macrophytes, being themselves components of aquatic food webs, act simultaneously as habitats/refuges and food sources for other aquatic organisms. According to their total share of the water body surface area they can alter predator-prey interactions, for example by reducing the grazing pressure of planktivorous fish on large zooplankton. Many studies have illustrated this by confirming that during daytime, large zooplankton in shallow lakes tend to leave the open water and aggregate near or within submerged plant beds (Lauridsen et al., 1998), where dense macrophyte communities provide shelter from fish grazing by hindering fish movement and sight and therefore reducing foraging efficiency (Manatunge et al., 2000). The resulting increase in large-sized zooplankton can suppress the biomass of phyto- and proto-zooplankton (Jürgens & Jeppesen, 1998), which in turn indicates that the influence of macrophytes (presence/absence, composition, diversity, density, abundance) can indirectly cascade down to lower trophic levels (Schriver et al., 1995). However, the efficiency of macrophytes as a refuge for zooplankton species depends on macrophyte density (Winfield, 1986; Diehl, 1988) and type (Irvine et al., 1990), and on the density and composition of the planktivorous fish present in the lake (Persson, 1991). For example, some studies have revealed that the refuge effect of macrophytes is poor even at low densities of planktivorous fish if the plant volume infested (PVI) is less than 10-15% (Schriver et al., 1995). On the other hand, Perrow et al. (1999) demonstrated that a PVI of <40% was sufficient to allow cladocerans to persist. In addition, planktivorous fish grazing on zooplankton could be reduced if alternative prey are available such as periphyton and macrophyte-associated macroinvertebrates, which are generally more numerous and more diverse than in open water areas (Werner et al., 1981). However, several studies (Perrow et al., 1999; Schriver et al., 1995) suggest that at high densities (1-2 m$^{2)}$) of a suite of fish species, any macrophyte refuge effect for zooplankton is nullified. In addition, we must bear in mind that piscivorous fish hunting prey communities can leave the open water and seek food objects from macrophytes, and this influences the interactions between lower trophic levels and enhances the refuge effect of macrophytes (Perrow et al., 1999).

On the other hand, macrophytes, being components of aquatic environments and known for their structuring role, also influence the abiotic factors of lakes. For instance, they can play a crucial role in regulating water quality in shallow lakes by inhibiting sediment

resuspension and erosion (Blom et al., 1994), maintaining the clear water state by competing with phytoplankton and epiphytes for nutrients (Jeppesen et al., 1997), changing the underwater light climate (Binzer & Sand-Jensen, 2006; Coops & Doef, 1996), etc. In addition, macrophytes themselves are under different abiotic (light, temperature, water movements, sediments, nutrients) and biotic influences, which express themselves via different distribution patterns.

Earlier studies revealed that shifts in the species composition of littoral macrophyte communities are likely to have a significant effect on the abundance and distribution of phytophilous macroinvertebrates (Basu et al., 2000; Colon-Gaud et al., 2004). Although some studies have considered the distribution and abundance of zooplankton between vegetated and unvegetated areas (e.g. Stansfield et al., 1997), knowledge of the diversity and biology of macrophyte-associated zooplankters is still sparse compared to open water areas. Protozoa have significant effects in freshwater lakes; their communities can vary greatly among lakes (Zingel et al., 2002; Zingel, 2005), and only 180 out of 679 freshwater species found in the plankton are truly euplanktonic (Foissner et al., 1999). Nevertheless, most studies have focused on open-water habitats rather than the littoral zones of lakes, and little is known about the ciliate communities in various types of macrophyte beds (Mieczan, 2007; 2008).

It is obvious that different macrophyte stands alter both the prey and the predators of ciliates indirectly. Warfe & Barmuta (2006) found that macrophyte architecture had strong, consistent effects on the macroinvertebrate and periphyton communities; both were most abundant and diverse on the most structurally complex plant analogue. In our study (**V**), there was a strong positive correlation between epiplanktonic ciliates and the Shannon-Weaver index of macrophytes, which was most diverse in the southern sites of Lake Võrtsjärv. This clearly demonstrates that epiplanktonic ciliates are favoured by a high diversity of the macrophytes that provide them with shelter and necessary substrates. The southern sites of the lake are also the most protected from the wind. Plainly, wind-induced disturbance is not favorable for this ciliate group. Our study demonstrated that the more diverse the macrophyte stands, the more numerous the ciliate taxa found in the surrounding pelagial. In contrast, the euplanktonic taxa correlated negatively with the Shannon-Weaver index for macrophytes and were most diverse in the most wind-exposed northern sites of the lake. Only a few macrophyte species (e.g.

Phragmites australis) could tolerate the exposed conditions at the northern shore. Lacking the shelter of diverse macrophyte beds the community of epiplanktonic ciliates declined, giving way to better-adapted euplanktonic taxa. In these sites, most of the taxa found belonged to oligotrichs. The planktonic ciliate community is in general characterized by a high percentage of oligotrichs and is classified as "Oligotrichetea" (Foissner et al., 1991). So the greater abundance of oligotrichs was not unexpected in the more "planktonic" conditions. The trend of distribution of benthic ciliates was somewhat similar to that of the euplanktonic taxa, being most abundant in sites where the Shannon-Weaver index for macrophytes was low. As these sites were characterized by poor macrovegetation, more intense resuspension of the sediments can be expected because rich aquatic macrophyte beds are known to inhibit sediment erosion in shallow lakes (Blom et al., 1994). In our study, the resuspension of sediments most probably increased the abundance of benthic ciliates washed to the pelagial (**V**).

A strong polarization of environmental conditions was demonstrated by factor analysis of the geographical co-ordinates. These co-ordinates were highly loaded on the principal components, so the first factor mainly described the gradients of variables in the east-west direction and the second factor in the north-south direction. In the east-west direction of Lake Võrtsjärv, the number of euplanktonic ciliates decreased while epiplanktonic ciliates increased (the total number of ciliate taxa also increased). In the north-south direction the number of benthic ciliate taxa decreased. These polarized patterns of ciliate diversity follow the macrophyte distribution and have three main causes: lake shape, dominant wind direction and nutrient load. Lake Võrtsjärv is elongated in the north–south direction with the width increasing towards the north; the morphology of the lake, coupled with its considerable size and prevailing wind directions, generates a distinct contrast between the sheltered southwestern and exposed northeastern shores. The southern basin is narrow and sheltered from the winds. The main nutrient loads enter the lake from the south and west. It has been demonstrated that all these factors together explain the species richness and abundance of the predominant emergent and submerged species (Feldmann & Nõges, 2007).

Positive correlations between abiotic indices and the number of euplanktonic taxa again indicated an east-west polarization pattern (Feldmann & Nõges, 2007): there were more euplanktonic taxa in the

eastern area, where the distribution of other ecological groups was limited owing to water movement caused by the dominant wind direction. The correlation between the numbers of eu- and epi-planktonic taxa and the nitrogen loading index also pointed to a polarized pattern of macrophyte distribution. Large inflows into the southern part of the lake, coupled with a narrow lake area and weaker wind influence, generate favorable conditions for macrophyte diversity (Feldmann & Nõges, 2007) and accordingly beneficial conditions for epiplanktonic ciliates. This is further confirmed by the higher macrophyte species numbers and Shannon-Weaver index in the southern part of Lake Võrtsjärv (Feldmann & Nõges, 2007). The negative correlation between epiplanktonic ciliate taxa number and slope index can be explained by mechanical stress, attached taxa being washed away because the littoral zone of Lake Võrtsjärv slopes more steeply in northern areas (Feldmann & Nõges, 2007). Here, wind influence and lake basin shape generate very rough conditions for attached ciliates. Such interactions make conditions more suitable for euplanktonic and less suitable for epiplanktonic ciliates.

Our study demonstrated that a diversity of substrates increased the diversity of ciliates. Generally, habitat characteristics, physical and chemical parameters of the water body, the quality and quantity of available food, and predation are among the factors regulating planktonic protozoan communities (Biyu, 2000; Pierce & Turner, 1992). Macrophytes probably influence protozoan density by increasing spatial heterogeneity and modifying food availability. Additionally, more complex macrophyte communities increase the diversity of microhabitats and provide numerous micro-niches and a greater variety of potential shelters from predators (e.g. Jeppesen et al., 2000b; Kuczyńska-Kippen, 2005; Walsh, 1995). Mieczan (2007, 2008, 2010) found that the total number of ciliate taxa and their abundance increased together with the abundance and the complexity of the spatial and morphological structure of macrophytes. In contrast to the former study, we found that total ciliate abundance was lower in the more diverse and complex macrophyte stands. We found the highest ciliate abundances in the more wind-exposed sites where the community was dominated by euplanktonic ciliate species. In these sites, most of the taxa found belonged to oligotrichs, which are known to be most characteristic of the lake pelagial (Foissner et al., 1991) and are often reported to reach very high abundances (Zingel & Nõges, 2008). Euplanktonic species are most adapted to life in the pelagial, being the

only "true" planktonic ciliates. Therefore it could be anticipated that they were much more abundant than the epiplanktonic and benthic species. Our study demonstrated that our first hypothesis was only partly true: the diversity of macrophyte substrates increased the diversity of ciliates but decreased their total abundances (**V**).

6.2. Trophic cascade from fish to bacteria (I)

Fish as top predators play a major role in structuring zooplankton communities (Hrbacek, 1962; Brooks & Dodson, 1965) because in many aquatic systems zooplankton is the main food of fish larvae and fries (Cushing, 1983; Mehner, 1996; Post & Kitchell, 1997). Most fish have a stage in which they more or less feed on zooplankton (Post & Kitchell, 1997). The planktivory stage can range from a very short time period to months or years, and some species may feed on zooplankton throughout their lives (Mittelbach & Persson, 1998; Olson et al., 1995; Persson, 1988). Therefore, fish feeding on zooplankton can cause substantial shifts in zooplankton community composition, size distribution and abundance owing to size-selective feeding, which can in turn cascade down to lower trophic levels (Carpenter et al., 1985). However, some studies have demonstrated that the refuge effect of macrophytes when the volume of plants infested exceeds 15% of the total can strongly inhibit fish predation on zooplankton (Schriver et al., 1995). Although the top-down control of fish on zooplankton communities is well documented (Hrbacek, 1962; Brooks & Dodson, 1965), few studies have focused on the cascading effect on the whole microbial food web (Pace & Funke, 1991; Riemann, 1985) and the available information is often controversial. Therefore, we conducted pond experiments to assess how the presence and absence of planktivorous fish affected whole plankton communities. We hypothesized that in the presence of fish, there would be fewer metazooplankton and more protozoans and that bacterial numbers would be suppressed (**I**).

Our study demonstrated that feeding by planktivorous fish had remarkable indirect effects on the whole microbial food web (Figure 3). Planktivorous fish had a strong negative influence on metazooplankton biomass, which in turn correlated negatively with HNF in the presence of planktivorous fish and positively in their absence. In our fishless pond the MZP biomass consisted largely of the predaceous *Leptodora kindtii*, which is known to prey mainly on small herbivorous cladocerans (Chang & Hanazato,

2004). Our study also revealed that *Leptodora kindtii* preferred herbivorous metazooplankton to protozoans and were unable to control HNF abundances (Figure 1 c,g, paper **I**). Consumption of bacterivorous ciliates by predaceous ciliates did not suppress HNF either. It has generally been thought that ciliates are not effective grazers of bacteria at concentrations found in the field; rather, they are likely to be consumers of relatively large cyanobacteria and nanoplankters (Epstein & Shiaris, 1992). However, in our study, the abundances of bacterivorous ciliates were tightly coupled to bacterial numbers in the presence of fish. In the fishless pond the controlling role shifted to HNF. There has been increasing evidence that ciliates are capable of significant grazing on bacteria in some water bodies (Kisand & Zingel, 2000; Šimek et al., 1995; Šimek et al., 1998; Zingel et al., 2007) and they can probably outcompete HNF. Bacterivorous ciliates were negatively correlated with HNF only in the presence of fish (R = -0.58 and -1.0 in 2005 and 2006, respectively, p< 0.05; Supplementary Table 2, paper **I**) when their numbers were high. In the fishless pond, where the abundance of bacterivorous ciliates was suppressed by predaceous ciliates, they did not affect HNF (Figure 6, paper **I**). It is evident that when HNF abundances are kept low they cannot control bacterioplankton dynamics. We found that when HNF were numerous they were much more effective grazers on bacterioplankton (Figure 5, paper **I**), and the bacterial numbers were also much lower in this pond. Therefore, our study revealed that the microbial loop was more effective in the presence of planktivorous fish because bacterial numbers were much higher in pond F, and the efficiency of the microbial loop is usually determined by the density of bacteria within it (Taylor & Joint, 1990). Nevertheless, we could not measure the efficiency of the microbial loop because we did not measure bacterial production. Some earlier studies (Nõges & Zingel, 2008) have demonstrated that the microbial loop is stronger in plankton-dominated than macrophyte-dominated lakes because microbial loop organisms (especially bacteria) depend directly or indirectly on exudates produced by phytoplankton. Our pond experiment also demonstrated that the microbial loop was stronger in pond F where the phytoplankton biomass was double that in pond nF.

Planktivorous fish larvae also influenced the food web structure through direct interactions (Figure 3). The amount of MZP eaten by the whitefish was negatively correlated with MZP biomass (R = -0.96, p<0.05; Supplementary Table 1, paper **I**), and fish consumed up to 51%

and 27% (in 2005 and 2006, respectively) of the total MZP biomass daily (Figure 3, paper **I**). Planktivorous fish are known to switch from one prey type to another according to its share of the total prey biomass (Gliwicz, 2002), causing continual fluctuations in different prey type abundances. Owing to fish predation, zooplankton dominance often switches from large-sized (*Daphnia* spp., *Daphnia magna*, *Daphnia pulex, Daphnia galeata, Diaptomus* spp., *Mesocyclops edax, Leptodora kindtii*) to small-sized (*Daphnia cucullata, Bosmina longirostris, Chydorus sphaericus, Ceriodaphnia lacustris* and *nauplii*) zooplankton (Brooks & Dodsdon, 1965; Christoffersen et al., 1993; Jeppesen et al., 1996, 2000; Ohtaka et al., 1996; Riemann, 1985). This compositional shift can lead to a lower grazing efficiency and impact on phytoplankton (Brooks & Dodson, 1965). It was also the case in our study the presence of planktivorous fish led to the development of small MZP species (*Bosmina longirostris, Daphnia cucullata*) with more phytoplankton, whilst in the absence of fish large predaceous *Leptodora kindtii* dominated. In the presence of fish, *Leptodora kindtii* was found only occasionally and in very low numbers. The prey of whitefish consisted mainly of larger dominant crustaceans, which are often recognized as the preferred prey for fish fry in lakes. In turn, these crustaceans are known to feed actively on ciliates, so alterations in the crustacean community caused by fish feeding can ultimately affect the protozoan assemblage by shifting both their size and composition (Wickham, 1995; Zöllner et al., 2003). Moreover, our study demonstrated that in the presence of fish, the small-sized zooplankton community favoured phytoplankton development. We found that the phytoplankton biomass in the fishpond was double that in the fishless pond (Figure 1d, h, paper **I**). There was also a strong positive correlation between the numbers of MZP consumed by fish and phytoplankton biomass (R = 0.96, p<0.05; Supplementary Table 1, paper **I**), indicating that planktivorous fish feeding on zooplankton can be a very important influence on phytoplankton development. These results also support the trophic cascade hypothesis (Carpenter et al., 1985), which is based on size-selective feeding and states that an increase in the standing stock of planktivorous fish leads to domination of the zooplankton community by small and inefficient consumers that cannot control phytoplankton growth.

It appears that when a water body lacks planktivorous fish the potential food base supply for the first feeding larvae is very poor, consisting of unsuitable prey types, among which MZP (e.g. *Leptodora kindtii*) is too

large and PZP (e.g. HNF) too small for larval consumption. Therefore, the food requirement of fish larvae during their first feeding stages would probably not be met and their initial mortality could be very high in ponds (or lakes) where zooplankton communities have developed without planktivorous fish.

Regarding the influence of MZP on lower trophic levels, our study demonstrated that MZP positively influenced bacterivorous ciliates in the fishpond (Figure 3). We predicted that the presence of fish (which would lead to lower MZP densities) should also have a positive effect on the abundance of small bacterivorous ciliates as MZP are known to prey on small ciliates (Agasild et al., 2012; Jürgens & Jeppesen, 2000). However, our results were completely different: the correlation between bacterivorous ciliate abundance and MZP was positive (R = 0.92 and 0.90 in 2005 and 2006, respectively, p< 0.05; Supplementary Table 2, paper **I**). Pond F was dominated by the bacterivorous species *Hastatella radians* and *Halteria grandinella*. There were strong negative correlations between other bacterivorous ciliates (mainly from the genera *Cyclidium* and *Uronema*) and MZP (R = -0.85 and -0.79 in 2005 and 2006, respectively, p< 0.05). *Halteria grandinella* has a jumping escape response against predation (Gilbert, 1994; Tamar, 1979), though it is unclear whether this escape behaviour is an effective reaction to all metazoan predators. The results of some laboratory (Gilbert, 1994; Jack & Gilbert, 1997) and field (Havens & Beaver, 1997; Jürgens et al., 1999) studies imply that the jumping escape response of *Halteria* is probably most effective against predation by rotifers (Gilbert, 1994; Jack & Gilbert, 1997; Jürgens et al., 1999). In any case, our study revealed that *Halteria grandinella* withstood cladoceran predation successfully. The body of *Hastatella radians* has many spines, which could also offer some protection against MZP predation. Both *Hastatella* and *Halteria* exhibited strong negative correlations with fish feeding. It is possible that when MZP numbers were decreased by fish, other ciliates recovered quickly and the abundances of *Hastatella* and *Halteria* fell.

In the fishless pond nF, MZP influenced bacterivorous ciliates negatively. Various studies conducted in enclosures have demonstrated that copepods and cladocerans can control the abundance of ciliates (Gismervik, 2006; Jürgens & Jeppesen, 2000). We had predicted that in the absence of planktivorous fish the MZP density would be much higher and controlled bottom-up, but our study revealed this to be only partly true. In our fishless

pond, the MZP biomass was largely made up of the predaceous *Leptodora kindtii*, which is known to prey mainly on small herbivorous cladocerans (Chang & Hanazato, 2004). The MZP biomass was indeed much higher (Figure 2, paper **I**), but we had predicted lower ciliate biomasses as a consequence. In our fishless pond, *Leptodora kindtii* occupied the role of top predator and seemed to control the herbivorous MZP, hence MZP grazing pressure on the PZP was probably much lower. Nevertheless, the total abundance of ciliates in pond nF was still lower than in pond F. In pond nF the abundance of large predaceous ciliates (*Pelagodileptus trachelioides*, *Paradileptus elephantinus*) increased because they were clearly favoured by the presence of *Leptodora kindtii*; we found a positive correlation between this species and predaceous ciliates (R = 0.97 and 0.81 in 2005 and 2006, respectively, p< 0.05; Figure 3). In earlier studies (Agasild et al., 2013) we likewise found that the removal of herbivorous crustaceans initiated a decrease in total ciliate abundance. These experiments demonstrated that the removal of MZP led to an increased abundance of large predaceous ciliates and a simultaneous decrease in the abundance of smaller bacterivorous ciliate species. Crustaceans can affect large predaceous ciliates through several potential mechanisms, predominantly direct predation, potential effects of mechanical interference and exploitative competition (Agasild et al., 2013).

Our study demonstrated that the second hypothesis was only partly true: fish had a remarkable influence on the zooplankton community leading to a much lower metazooplankton biomass. The presence of planktivorous fish led to the development of small-sized MZP species with more phyto- and bacterio-plankton, whilst in the absence of fish the MZP species composition itself (especially large predaceous species such as *Leptodora kindtii*) determined the food web structure. However, the influence of fish feeding on metazooplankters did not cascade down as we expected: protozooplankton abundance was still suppressed and bacterial numbers were higher in the presence of fish than in the fishless pond. In the fishpond, metazooplankters controlled the abundance of flagellates but favoured the development of bacterivorous ciliates, which had some defensive mechanisms against predation. In contrast, large predaceous *Leptodora kindtii* favoured the development of flagellates in the fishless pond, whereas bacterivorous ciliates were suppressed by predaceous ciliates. It appears that we must also know the species composition of MZP and PZP: are predaceous species present or have

some species developed defences against predation? Only by knowing the species composition of both MZP and PZP can we predict changes in food web dynamics. As there were more bacteria in the presence of fish we can conclude that the microbial loop was more effective in the presence of planktivorous fish, because the efficiency of the microbial loop is usually determined by the density of bacteria within it (Taylor & Joint, 1990).

6.3. The feeding influence of the fish stock on ciliated protozoans (III)

Several studies have recognized metazoans as the main food source for planktivorous fish larvae at their first feeding (Last, 1978a,b; Pierce & Turner, 1992). However, ciliates (except hard-bodied tintinnid ciliates) seem to be missing from the diet of larval fish, though they are comparable in size with small metazoans (copepod nauplii, copepodites, rotifers). Since protozoa move more slowly than most metazoans they can be caught easily by fish larvae, and as they grow much more quickly than most metazoans the contribution of protists to the diet of fish larvae should be much greater (Stoecker & Govoni, 1984). Therefore, we posed a question: what is the main food source of planktivorous fish in the shallow eutrophic Lake Võrtsjärv, where the zooplankton community is largely dominated by soft-bodied ciliates? The ciliate abundances (up to 191 cells mL^{-1}) and their contribution to the total zooplankton biomass (more than 60%) in Lake Võrtsjärv (Zingel & Nõges, 2010) were generally higher than in most eutrophic temperate lakes studied but were still not exceptional. Although protozoa form an important component of planktonic communities, very few studies have considered direct protist consumption by fish larvae. Most planktonic protozoa are fragile and do not have indigestible hard parts (e.g. Lorica) so they are easily degraded in the fish guts. Hence, protist ingestion could have been neglected in the past because food objects are rapidly degraded in the fish guts (Fukami et al., 1999). This could also explain apparent paradoxes such as the empty guts of field-caught larvae (Van der Meeren & Næss, 1993).

In most cases, the standard method for investigating the diet of fish larvae is to dissect the entire alimentary tract under a microscope and count all identifiable prey specimens. We found that when the diet of fish larvae was assessed on the basis of the whole alimentary tract (bulk-

diet) content we obtained biased results (Figures 3, 4; paper **III**). This applied both to the diet composition and to the total amount of food consumed, whereas the content of the first gut quarter (fresh-diet) revealed that more ciliates had been consumed. Sutela & Huusko (2000) stated that much more reliable diet estimates can be achieved from the first quarter of the alimentary tract. They studied metazooplankton, but their results strongly suggested that soft-bodied zooplankton species also are underestimated in the diet if the analysis is based on the whole alimentary tract contents. As soft-bodied species are known to be degraded rapidly (Sutela & Huusko, 2000), neglecting different prey digestion rates evidently leads to diet underestimation and especially to underestimation of the role of protists. Although there are no data on protozoan digestion rate by larval fish, Ohman et al. (1991) indicated that ciliates were digested within 2 h (Ohman et al., 1991). In our study the average gut passage time was estimated to be 4 h, so ingested food objects passed each gut quarter in an hour. The findings of Ohman et al. (1991) are consistent with ours because we were able to detect ciliates only in the first two (out of four) gut quarters, which again means they were digested within 2 h. Therefore, we can conclude that the diet of fish larvae should be determined on the basis of analysis of the foregut content. Nevertheless it must be recognized that even when the foregut contents are used, it is possible to underestimate the contribution of ciliates to the fish diet because many ciliates are so delicate that they can burst on contact with any surface.

As our study indicated, ciliates were present in the guts of all fish larvae and they accounted for approximately ~60% of the total carbon biomass consumed by those larvae. Similar dependencies were apparent in our pond experiments (**II**), where ciliates were a very important food for whitefish larvae, but only in their initial feeding phase, forming 62-65% of the total zooplankton biomass consumed at the beginning of May. Despite their importance in larval fish feeding, quantitative studies of the role of ciliates as larval food in freshwaters seem to be missing. In marine ecosystems, several field studies have emphasized the role of protozoa (mostly hard-bodied ciliates) as food for larval fish (Watson & Davis, 1989), but only a few researchers have attempted to quantify the role of dominant soft-bodied protozoan species (Figueiredo et al., 2005; Fukami et al., 1999; Nagano et al., 2000). Fukami et al. (1999), who studied the predation of fish larvae on naked protozoan microzooplankton in Tosa

Bay, found that some larvae had ingested 60 protozoa and most larvae contained 30. They also found that in some fish species the maximum contribution of protozoa to the gut contents in terms of biomass was roughly equivalent to the other items of the diet. Figueiredo et al. (2005), who studied the larval fish diets in the Irish Sea, found low numbers of cells in larval fish guts (average for all taxa ∼ 0.8 cells larva^{-1}). The prey densities in the Irish Sea remained below 1 cell mL^{-1} during the study period. Nagano et al. (2000) revealed that some fish larvae at first feeding consumed mainly hard-bodied tintinnid ciliates and subsequently switched to a diet of smaller metazoans. The few other studies of larval fish feeding only on ciliates (Ohman et al., 1991) indicated that at high prey densities fish larvae can ingest large numbers of ciliates (i.e., up to 1500 ciliates larva^{-1} h^{-1}). All these experiments indicated a clear increase in ciliate ingestion at higher prey concentration and concluded that the protozoan biomass available in the water column can supplement, if not fully provide for, larval fish survival. Our estimates of ingestion rates were much higher (up to 604 ciliates larva^{-1} h^{-1}) than those detected in the marine environment, but lower than in laboratory experiments (**III)**.

Considering our third hypothesis, we admit that ciliates in Lake Võrtsjärv are crucial food source for fish larvae because they are very abundant and formed ∼ 60% of the total carbon biomass consumed by fish larvae, whereas metazooplankton contributed only an average of 9.2% of larval body C d^{-1} in May and 8.7% in June. Therefore, our study demonstrated that without ciliates the daily food requirement for larval fish estimated to sustain growth (20% of larval body C d^{-1}) would not be met in Võrtsjärv, and this is probably the case in many other freshwater lakes.

6.4. The influence of zp-removal and zp-enrichments on microbial communities (II & IV)

In general, the zooplankton community of Lake Võrtsjärv consists mostly of small forms, small cyclopoids (mainly *Mesocyclops leuckarti*, *Thermocyclops oithonides*) and small-bodied cladocerans (*Chydorussphaericus*, *Daphnia cucullata*, *Bosmina longirostris*), but their numbers and biomasses are relatively low, being obviously affected by strong fish predation pressure (Haberman, 1998; Haberman et al., 2007) and by unfavourable feeding conditions owing to high abundances of filamentous algae and frequent sediment resuspension (Chow-Fraser & Sprules, 1986; Levine et al., 2005). Several

earlier studies (Zingel, 1999; Zingel & Haberman, 2008) in Lake Võrtsjärv revealed a positive correlation between metazooplankton and ciliate biomasses, indicating that planktonic ciliates may not be controlled top-down by the metazooplankton in Võrtsjärv, but rather controlled bottom-up by food availability. Although planktonic microciliates (15–40 µm) are ingested by all dominant cladoceran and copepod taxa in Võrtsjärv, both specific ingestion rates and the effect of the crustacean community on the standing ciliate stock were generally low (Agasild et al., 2012). Therefore, we posed a question: how do different metazooplankton removal (paper **II**) and enrichment (paper **IV**) treatments influence the community structure and abundance of ciliates in a large shallow lake compared to the natural metazooplankton community and abundance, and do changes in them cascade down to phytoplankton and bacteria? We hypothesized that increases and decreases in metazooplankton abundance affect ciliate community composition but do not directly affect their total numbers; and changes in the metazooplankton community do not cascade down to phytoplankton and bacteria (papers **II & IV**).

Our results clearly demonstrated that ciliate abundances were not directly controlled by crustacean grazing in Lake Võrtsjärv (**II & IV**). Total ciliate abundances decreased in response to the removal of crustaceans (**II**), contradicting most previous reports on similar experiments (Adrian & Schneider-Olt, 1999; Ventelä et al., 2002). However, much higher crustacean abundances and biomasses were used in those earlier experiments, where top-down control was found to be important for ciliate community regulation (Adrian & Schneider-Olt, 1999; Ventelä et al., 2002; Zöllner et al., 2003). In our study, the removal of crustaceans indirectly initiated an increase in the abundance of large predaceous ciliates (>100 µm, mainly haptorids) and a simultaneous decrease in the abundance of smaller ciliates (<20–40 µm, bacterivorous and bacterio-herbivorous oligotrichs and peritrichs). These results suggest that predaceous ciliates preyed upon smaller ones, leading to a decrease in total ciliate abundance in zp-removal treatments (**II**). A similar decline in small ciliates, parallel to the increase in large predatory forms (*Lagynophrya* sp. and *Monodinium* sp.) after the removal of mesozooplankton, was reported by Jürgens et al. (1999) for enclosure experiments in the hypertrophic Lake Søbygård. Therefore, there seems to be a common pattern of ciliate community regulation under conditions of very low crustacean abundance and in the presence of large ciliate predators. Our futher mesocosm

experiment (**IV**) with natural (M1) and enriched (M2 & M3) cyclopoid copepod treatments confirmed these earlier findings. At the beginning of the experiment the community of ciliates was dominated by small bacterivorous forms (*Rimostrombidium* spp., *Cyclidium* spp., *Uronema* spp.) in all the treatments. As the biomass of crustacean zooplankton was high at this moment the abundance and biomass of predaceous ciliates (mostly *Paradileptus elephantinus*) was low. However, during the ongoing experiment, the biomass of copepods started to decrease and achieved the same level in the control enclosure as in the enriched enclosures (M2 & M3). The decrease in copepod abundance and biomass in treatments M2 and M3 after the onset of the experiment was apparently related to high predation pressure on their juvenile stages of nauplii and of food limitation caused by substantial copepod enrichment compared to natural conditions. *Mesocyclops leuckarti*, the dominant cyclopoid in the experiment, is mostly predatorous from its early copepodite stages (Hansen & Santer, 1995). Predation by this species can cause substantial mortality of nauplii, thereby affecting the copepod population size. In M2 and M3 the biomass of nauplii fell substantially by experimental days 4 and 7. Owing to this decline in the crustacean biomass the abundance and biomass of predaceous ciliates started to increase with simultaneous reduction in bacterivorous ciliate abundance and biomass. At the end of the experiment there was a shift from domination by bacterivorous ciliates to predaceous ciliates in both the control enclosure M1 and the enriched (2.5x of natural copepod biomass) enclosure M2 (**IV**). This study therefore suggested that predaceous ciliates can control the abundance of bacterivorous ciliates when crustacean numbers decline. If crustaceans achieve sufficient abundance and biomass, they gain control over the large predaceous ciliates. In the zp-removal experiment (**II**) in Lake Võrtsjärv, predatory ciliates were suppressed in control media at about 30 cyclopoid individuals L^{-1} (adult and copepodid stages) and at 20 cladocerans L^{-1}. In different zp-enrichments (**IV**), the development of predaceous ciliates was suppressed at the beginning of the experiment when the biomass and abundance of copepods were high (e.g. up to 79 copepods L^{-1} in M2 and up to 179 copepods L^{-1} in M3) (Figure 1, paper **IV**). In addition, as the concentration of copepods was much higher in M3 (5x natural zooplankton biomass), the biomass of ciliates was still dominated by bacterivorous forms at the end of the experiment, but the predaceous ciliate biomass also doubled in this enclosure (Figure 6).

Although large carnivorous ciliates (>300 µm; *Paradileptus* sp., *Dileptus* sp.) temporarily peak during summer in Lake Võrtsjärv, their abundances are generally not high (Zingel, 1999) and no substantial reductions in small-sized ciliate abundances have been reported in the lake. Peaks in abundance of large carnivorous ciliates mostly follow the peaks of smaller bacterivorous and bacterio-herbivorous ciliates, which again indicates that small ciliates are their major food source in Lake Võrtsjärv (Zingel, 1999). The question remains as to precisely how large carnivorous ciliates are affected by crustaceans. Besides the potential effects of mechanical interference and exploitative competition, direct predation by crustaceans seems the most likely factor in suppressing ciliate numbers. Most probably there is a trophic link between cyclopoid copepods and the large predaceous ciliates. Cyclopoid copepod ingestion of ciliates and a notable effect on ciliate community structure have been demonstrated in several studies (e.g. Wickham, 1995; Hansen, 2000; Zöllner et al., 2003). As raptorial feeders, cyclopoids have also been reported to ingest rotifers, copepod nauplii and copepodites, and small cladocerans (Adrian, 1991). Therefore, attacks on large (>300 µm) and relatively slow-moving ciliates can also be expected. Although there appeared to be an inverse relationship between crustacean zooplankton and large predaceous ciliates, the increase in abundance and biomass of cyclopoid copepods in the experimental treatments (M2 & M3) did not suppress the total ciliate abundances (**IV**). Moreover, crustacean zooplankton in the zp-erichment studies had a clear positive effect on total ciliate abundance, especially on the biomass of small bacterivorous ciliates (Figure 4, paper **IV**). As the dominant species in the Võrtsjärv crustacean community are small cyclopoids and small-bodied cladocerans, they are obviously affected by the unfavourable feeding conditions in Võrtsjärv resulting from the high abundances of filamentous algae (Chow-Fraser & Sprules, 1986; Levine et al., 2005). This probably also reflected the weak top-down control seen in our experiment.

Also, our earlier short-term experiment in Võrtsjärv, in which we removed larger crustaceans instead of augmenting their numbers, did not cause an increase in ciliate abundances, which would be the expected reaction to a weakened top-down control by metazoans. Instead, ciliate abundance decreased steadily and predator-prey relationships regulated the community structure (**II**). Therefore, both our experiments with zp-removal (**II**) and zp-enrichment (**IV**) seemed to demonstrate a clear

pattern of ciliate community regulation under conditions of low crustacean abundance and in the presence of large ciliate predators. These predaceous species most probably reduced the abundances of small ciliates and shifted the size structure and species composition of the ciliate community dramatically. Dolan & Coats (1991) estimated the feeding of predaceous 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 extremely high abundances, ciliates have been found to be the dominant predators of both bacteria and small algae, consuming about 20% of the standing stock of nanoplankton and nearly 100% of the biomass production of bacteria during the growing season, compared to metazoan zooplankton in Võrtsjärv (Zingel et al., 2007). Despite the remarkable shift in the ciliate community and especially among bacterivores, predatory interactions mediated by crustacean zooplankton did not cascade down to affect bacterial abundance (**II & IV**). However, this could have resulted from high bacterial production rates compensating predation effects. Similarly, modest or zero effects on bacterial numbers have previously been reported in metazooplankton manipulation experiments (e.g. Ventelä et al., 2002). Experiments have demonstrated that a change in grazer community composition can cause taxonomic shifts in the bacterial assemblage rather than changes in abundance and biomass (Zöllner et al., 2003).

We also saw no clear effects at the phytoplankton level (**II & IV**). The small phytoplankton (<30 µm), presumed edible by zooplankton, constitutes an average of only 10% of the total phytoplankton biomass in Lake Võrtsjärv and is obviously under strong grazing pressure, especially by herbivorous ciliates (Agasild et al., 2007; Zingel et al., 2007). As the herbivores were the least affected grazer group among ciliates in the experiment, their feeding probably caused no significant alteration in the edible phytoplankton assemblage.

Both studies indicated that the assemblage of small crustaceans in Lake Võrtsjärv did not directly control the abundance of ciliates but could significantly alter ciliate community structure. The removal of metazooplankton or a decline in their biomass initiated an increase in the abundance of large predaceous ciliates and a simultaneous decrease in the abundance of small ciliates, and the zp-enrichments favoured

the development of small ciliates with simultaneous inhibition of larger predaceous ones. This appears to indicate internal ciliate community regulation under conditions of low crustacean abundance. However, the compositional shift in the ciliate community did not cascade down to the level of bacteria and edible phytoplankton (**II & IV**).

7. CONCLUSIONS

1. The distribution of macrophytes in Lake Võrtsjärv exhibited strong spatial polarization in relation to abiotic factors in both the west-east and north-south directions, demonstrating that macrophytes are under different abiotic influences that express themselves via differences in distribution pattern. The strong polarization of the lake's vegetation was also reflected in the planktonic ciliate diversity: geographical co-ordinates correlated with the total number of ciliate taxa and with the numbers of eu- and epi-planktonic taxa. More specifically, diverse macrophyte stands supported a rich epiplanktonic ciliate community but influenced the number of euplanktonic ciliate taxa negatively. Also, the abundance of epiplanktonic ciliates increased and euplanktonic ciliates decreased as the macrophyte communities became more diverse. The distribution pattern of benthic ciliates was similar to that of euplanktonic taxa, being most abundant in sites where the Shannon-Weaver index for macrophytes was low. Therefore, we consider that our first hypothesis was only partly true: macrophyte substrate diversity increased the diversity of ciliates but decreased their total abundances (**V**).

2. Planktivorous fish had remarkable effects on the zooplankton community. In the presence of fish, small-sized metazooplankton dominated, while in the absence of fish large forms prevailed. The shift from a small to a large metazooplankton community cascaded to the protozoan level, which in turn was a crucial link in structuring the microbial food web. However, the influence of fish feeding on metazooplankters did not cascade down as we expected. Protozooplankton abundance was still suppressed and bacterial numbers were higher in the presence of fish than in the fishless pond. Our study demonstrated that we must also know the species composition of MZP and PZP: are predaceous species present or have some species developed defensive responses against predation? Only by knowing the species composition of both MZP and PZP can we predict changes in food web dynamics. For instance, we assumed that in the fishless pond metazooplankton would control protozoan communities, enabling bacteria to proliferate in turn. However, our study revealed that

large predaceous cladocerans became abundant in this pond and larger herbivorous metazooplankton were favoured over protozoans. Therefore, protozoan abundances were still high and bacterial numbers were suppressed. In the fishpond we found that the small-sized metazooplankton community controlled the abundance of flagellates but favoured the development of bacterivorous ciliates, which had some defences against metazoan predation. As bacterial numbers were higher in the presence of fish we can conclude that the microbial loop was more effective in the presence of planktivorous fish. Also, the phytoplankton biomass was double that in this pond, suggesting that phytoplankton produced exudates favouring the development of bacteria. Therefore our second hypothesis was only partly true: the metazooplankton community was suppressed in the presence of fish, but this did not cascade down as we expected (**I**).

3. Protozooplankton as well as metazoans could be a crucial food source for larval fish, especially in eutrophic lakes where the biomass of zooplankton is dominated by small forms, e.g. by ciliates. Our study revealed that ciliates contributed more than half of the total carbon biomass consumed by fish larvae, whereas the metazooplankton made only a minor contribution to the total carbon biomass consumed by fish in Lake Võrtsjärv (**III**). In the pond experiments, the total share of ciliates in fish food was high initially (more than half of the consumed zooplankton biomass) but dropped sharply and remained at zero throughout the remaining study period (**I**). Therefore, we consider our third hypothesis to have been supported: ciliates are a crucial food source for fish larvae, and without ciliates the daily food requirement for larval fish would not be met in Lake Võrtsjärv.

4. Our study demonstrated that in the eutrophic Lake Võrtsjärv, which is characterized by relatively small metazooplankton, the metazoans did not control ciliate abundances directly. Ciliate abundances were predominantly regulated by their internal community structure. In support of that we found that the removal of metazooplankton (**II**) initiated a decrease in total ciliate abundances, because in the absence of metazoan predators large predaceous ciliates became dominant and suppressed the

small bacterivorous ciliate communities. As a result, a shift in ciliate community occurred from the domination of bacterivorous ciliates to predaceous ciliates. Our zp-erichment study (**IV**) confirmed these findings: abundant metazoan communities led to a decreasing number of large predaceous ciliates, which in turn favoured the development of small bacterivorous ciliates. Towards the end of the zp-enrichment experiment (**IV**), the overall biomass of crustaceans decreased owing to the shortage of food sources, and there was a concurrent increase in the predaceous ciliate biomass and a simultaneous reduction in bacterivorous ciliate species. Most probably there is a trophic link between cyclopoid copepods and large predacious ciliates. However, the compositional shift in the ciliate community did not cascade down to the level of bacteria and edible phytoplankton (**II & IV**).

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SUMMARY IN ESTONIAN

Käesoleva töö peamiseks eesmärgiks oli uurida kuidas toiduahela tipus olevad organismid reguleerivad madalamaid toiduahelate tasemeid madalates, eutroofsetes veekogudes – Võrtsjärves ja Härjanurme kalakasvatuse tiikides. Töös mängivad kesket rolli planktilised ripsloomad, kuna neid väikeseid üherakulisi eukarüootseid organisme peetakse ühenduslüliks mikroobikoosluste ja klassikalise toiduahela erinevate tasemete vahel. Olenemata sellest, et mikroobne ling moodustab lahutamatu osa klassikalisest toiduahelast ning seda mööda liigub oluline osa energiast kõrgematele troofilistele tasemetele, on mikroobse lingu tähtsust veekogudes alahinnatud.

Klassikalise toiduahela puhul on uuritud kalade ja suurtaimestiku mõju zooplanktoni ja fütoplanktoni kooslustele, kuid mikroobse lingu puhul pole kõrgemate troofiliste tasemete otsest ja kaudset mõju mikroobikooslustele hinnatud ning kui ongi, siis on saadud tulemused tihti vastandlikud. Samas puudub info suuresti ka selle kohta kas planktontoidulised kalad toituvad lisaks metazooplanktonile ka protozooplanktonist. See on aga eriti oluline neis eutroofsetes veekogudes, kus zooplanktonis domineerivad rohketoitelisi tingimusi eelistavad tsiliaadid. Kuna ühele antud töös käsitletud veekogule, Võrtsjärvele, on iseloomulik äärmiselt liigirikas ja arvukas tsiliaatide kooslus, mis moodustab kogu zooplanktoni biomassist rohkem kui 60% (Zingel & Nõges, 2010), siis uuritigi, mida planktontoidulised kalad söövad järves, kus domineerivad planktilised tsiliaadid.

Varasemad uuringud (Feldmann & Nõges, 2007) on näidanud, et makrofüütide levik sõltub Võrtsjärves tugevalt järve abiootilistest näitajatest. Kuna makrofüüte peetakse veekogudes muude bioloogiliste koosluste kujundajateks, siis uuriti kas muutused makrofüütide koosseisus, ohtrustes ja levikumustrites mõjutavad tsiliaatide liigilist koosseisu, arvukust ja levikut. Kuna varasemad teadustööd antud vallas praktiliselt puuduvad, siis on antud uuringute teostamine igati põhjendatud.

Uuriti eraldi ka metazooplanktoni mõju mikroobsele lingule, täpsemalt seda, kuidas muutused metazooplanktoni koosluse struktuuris (kõrvutades looduslikku metazooplanktoni koosluste selliste kooslustega, kus suuremõõtmeliste metazooplankterite hulka on kas suurendatud või

vähendatud) kutsuvad esile muutusi madalamatel tasemetel, iseäranis tsiliaatide arvukuses ja koosluste ülesehituses.

Meie uuringud keskendusid seega peamistele lünkadele toiduahelate uuringuis, mille käigus esitati järgmised uurimisküsimused (K1-K4) ning püstitati vastavad hüpoteesid (H1-H4):

K1. Kuidas muutused makrofüütide levikumustrites (liigiline koosseis, ohtrused) mõjutavad tsiliaatide (eu- ja epiplanktilised, bentilised) levikut, nende liigilist koosseisu ja arvukust?
H1. Makrofüütide levik ning muutused nende koosluses mõjutavad tugevalt erinevate tsiliaadigruppide levikut, liigirikkust ja arvukust. Makrofüütide liigirikkus mõjutab positiivselt tsiliaatide liigirikkust ja nende arvukust (**V**).

K2. Kuidas planktontoiduliste kalavastsete toitumine metazooplanktonist mõjutab zooplanktoni toitumist mikroobse lingu kooslustest? Mis juhtub madalamatel tasemetel siis kui kalad puuduvad?
H2. Kalade olemasolul on metazooplanktoni biomass alla surutud, see annab eelise protozooplanktoni arengule ning nemad omakorda pärsivad bakterplanktoni arvukust. Seevastu kaladeta tiigis on toitumissurve protozooplanktonile suur ning seeläbi bakterplanktoni kooslus arvukas (**I**).

K3. Millest toituvad planktontoidulised kalad madalas eutroofses järves, kus zooplanktoni koosluses domineerivad tsiliaadid?
H3. Tsiliaadid moodustavad olulise osa kalavastsete toidust, kusjuures esmasel toitumisetapil ei tule kalavastsed ilmselt toime ilma tsiliaatidest toitumata (**III**).

K4. Kuidas suuremõõtmeliste metazooplankterite eemaldamine (**II**) või lisamine (**IV**) mõjutab tsiliaatide koosluse struktuuri ja arvukust suures madalas eutroofses järves võrreldes loodusliku metazooplanktoni kooslusee struktuuri ja arvukusega? Kas muutused metazooplanktoni koosluses mõjutavad füto- ja bakterplanktoni arvukust?
H4. Madalas eutroofses järves mõjutab suuremõõtmeliste metazooplankterite eemaldamine ja lisamine tsiliaatide kooslusee koosseisu, kuid ei avalda mõju nende arvukusele. Muutused metazooplanktoni koosluses ei avalda olulist mõju füto- ja bakterplanktonile (**II & IV**).

Erinevate uuringute tulemusena leiti, et:

Suurtaimestiku levik sõltub Võrtsjärve ida-lääne ja põhja-lõuna suunas kulgevate abiootiliste näitajate gradientidest, kusjuures peamisteks teguriteks, mis mõjutasid taimestiku levikut, olid järve kuju, valitsevad tuuled ja toiteainete koormus. Tsiliaatide koosseisus asetleidnud muutused järgisid muutusi makrofüütide levikumustrites – geograafilised pikkus- ja laiuskraadid korreleerusid tsiliaatide üldarvu ja eu- ning epiplanktiliste ripsloomaliikide arvuga. Nimelt soodustasid mitmekesise suurtaimestikuga piirkonnad liigirikka epiplanktoni arengut, kuid mõjutasid negatiivselt euplanktiliste tsiliaatide liikide arvu. Makrofüüdikoosluste liigirikkuse kasvamisel epiplanktiliste tsiliaatide arvukus suurenes, euplanktiliste tsiliaatide arvukus aga vähenes suuresti. Bentilised tsiliaadid järgisid euplanktiliste tsiliaatide levikumustreid olles arvukaimad piirkondades, kus makrofüütide Shannon-Weaver'i indeks oli madal. Seega peab tunnistama, et esimene hüpotees leidis ainult osaliselt kinnitust – makrofüütide liigirikkus suurendas küll tsiliaatide liigirikkust, kuid mõjus negatiivselt nende üldarvukusele (**V**).

Planktontoiduliste kalade toitumine avaldas tugevat mõju zooplanktoni koosluse struktuurile – kalade olemasolul domineerisid väikesemõõtmelised zooplankterid ning nende puudumisel suuremõõtmelised vormid. Zooplanktoni strukturaalses koosseisus asetleidnud nihe, suuremõõtmeliste zooplankterite koosluse asendumine väikesemõõtmeliste zooplankteritega, kutsus esile muutusi ka protozooplanktonis, kusjuures viimast peetakse omakorda oluliseks lüliks mikroobikoosluste kujundamisel. Hoolimata sellest, et kalade toitumissurve oli metazooplanktonile suur, ei avaldanud see protozooplanktoni kooslusele sellist mõju nagu me algselt eeldasime – protozooplankterite arvukus oli kalade olemasolul ikkagi madal ning bakterite arvukus suurem kaladega tiigis. Meie tulemused näitasid, et taoliste toiduahela uuringute läbiviimisel on väga oluline uurida mitte ainult meta- ja protozooplanktoni arvukust ja biomassi, vaid ka nende liigilist koosseisu – kas koosluses leidub röövtoidulisi liike või kas mõnedel liikidel on välja arenenud ärasöömist takistavad kaitsemehhanismid? Liigilisele koosseisule pilku heitmata on raske ette ennustada toiduahela dünaamikas asetleidvaid muutusi. Näiteks algselt eeldasime, et kaladeta tiigis kontrollivad metazooplankterid protozooplanktoni arvukust, mis omakorda võimaldab bakterite arengut. Meie uuringud näitasid aga, et kaladeta tiigis hakkasid domineerima suuremõõtmelised röövtoidulised

vesikirbulised, kes eelistasid protozooplanktoni asemel toiduobjektidena hoopiski herbivoorset metazooplanktonit. Seega oli protozooplanktoni arvukus ikkagi kõrge ning bakterite areng kaladeta tiigis pärsitud. Lisaks leidsime, et kalade olemasolul kontrollis metazooplankton heterotroofsete nanoflagellaatide arvukust, kuid soodustas selliste bakterivoorsete tsiliaatide arengut, kes omasid mõningaid kaitsemehhanisme ärasöömise vastu. Kuna bakterite arvukus oli kõrgem kaladega tiigis, siis võib järeldada, et mikroobne ling oli effektiivsem kalade olemasolul. Ka fütoplanktoni biomass oli kaks korda suurem just kalade olemasolul, mis viitab sellele, et fütoplanktoni poolt eritatavad fotosünteesi jääkproduktid soodustasid bakterplanktoni arengut. Seega teine hüpotees leidis vaid osaliselt kinnitust – kalade toitumine avaldas tugevat mõju metazooplanktonile, mistõttu oli nende biomass madalam kaladega tiigis. Hoolimata sellest ei avaldunud kalade toitumissurve metazooplanktonist protozooplanktoni kooslusele sellist mõju nagu me algselt eeldasime – protozooplankterite arvukus oli kalade olemasolul ikkagi madal ning bakterite arvukus kõrge. Kaladeta tiikide puhul eeldati protozooplanktonile suurt toitumissurvet ning seega kõrgemaid bakterplanktoni arvukusi, kuid uuringud näitasid vastupidist – kalade puudumisel ei suutnud metazooplankterid protozooplankterite arvukust kontrollida ning seega oli ärasöömissurve bakteritele kõrge (**I**).

Tsiliaadid moodustasid Võrtsjärves rohkem kui poole kalavastsete toidus leiduvast süsiniku biomassist, kusjuures metazooplanktoni panus toiduks tarbitavasse süsiniku biomassi oli väike (**III**). Tsiliaadid moodustasid ka Härjanurme tiikides peamise osa kalavastsete toidus leiduvast süsiniku biomassist, kuid seda vaid varakevadisel perioodil, kalade esmasel toitumisetapil, seejärel aga tsiliaatide osakaal kalavastsete toidus langes järsult ning hilisema uuringu vältel kalad tsiliaatidest ei toitunud (**I**). Seega leidis kinnitust hüpotees, et tsiliaadid olid oluliseks toiduallikaks planktontoidulistele kaladele, eriti nende esmastel toitumisettapidel, kus metazooplankton on ilmselt liiga suur, et neist toituda.

Olgugi, et eutroofsele Võrtsjärvele on iseloomulikud väikesemõõtmelised metazooplankterid, mis võiksid pärssida tsiliaatide arengut, ei leitud, et metazooplankterid kontrolliksid Võrtsjärves nende arvukust. Tsiliaatide arvukus sõltus eelkõige nende endi koosluse struktuurist. Leiti, et metazooplankterite eemaldamisel (**II**) vähenes ka tsiliaatide üldarvukus, kuna röövtoiduliste metazooplankterite puudumisel hakkasid domineerima röövtoidulised tsiliaadid, kes ise pärssisid

väikesemõõtmeliste bakterivoorsete tsiliaatide arengut. Selle tulemusena hakkasid tsiliaatide koosluses bakterivoorsete tsiliaatide asemel domineerima hoopiski röövtoidulised tsiliaadid. Katsed rikastatud metazooplanktoni kooslustega (**IV**) kinnitasid samuti varasemaid oletusi – arvukas metazooplanktoni kooslus mõjus pärssivalt suuremõõtmeliste röövtoiduliste tsiliaatide arengule, mistõttu nende arvukus vähenes, see soodustas aga omakorda väikesemõõtmeliste bakterivoorsete tsiliaatide arengut. Kuna metazooplankteritega rikastatud eksperimendi lõpus zooplanktoni üldbiomass vähenes (ilmselt toiduobjektide nappuse tõttu), siis leidis aset samaaegne langus bakterivoorsete tsiliaatide arvukuses. Kõige tõenäolisemalt eksisteerib troofiline link sõudikuliste aerjalgsete ja suurte röövtoiduliste tsiliaatide vahel. Hoolimata sellest, et tsiliaatide koosluse struktuuris leidsid aset suured muutused ei avaldanud see olulist mõju madalamatele tasemetele – bakteritele ja söödavatele fütoplankteritele (**II & IV**).

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Last but not least I would like to tell you about a dream I had many months ago. I was in a big hall, nervous, almost speechless as usual and not at all ready to defend my thesis. Still, I tried to start with the first slide of my presentation, but suddenly someone from the hall stood up and asked: "Do fish eat figs?"

I

ORIGINAL PUBLICATIONS

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The effects of predation by planktivorous juvenile fish on the microbial food web

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Abstract

The feeding impact of planktivorous fish on microbial organisms is still poorly understood. We followed the seasonal dynamics of the food web in two natural fishponds for two years: one was stocked with planktivorous whitefish while the other had no planktivorous fish. The aim of the study was the simultaneous assessment of the feeding behaviours of planktivorous fish and of bacterivorous meta-/protozooplankters. We hypothesized that in the presence of planktivorous fish there would be fewer metazooplankton, more protozoans and decreased numbers of bacteria. Our results showed that the amount of metazooplankton eaten by the fish was indeed negatively correlated with metazooplankton biomass. The feeding impact of planktivorous fish in shaping the microbial loop was remarkable. The main grazers of bacteria in the fishpond were ciliates, whereas in the pond without fish these were heterotrophic nanoflagellates. In the fishless pond the role of the top predator shifted to the predaceous metazooplankter *Leptodora kindtii* which controlled the abundance of herbivorous metazooplankters. We found a negative relationship between the number of bacteria and flagellates in the fishless pond, while the number of bacterivorous ciliates was suppressed by predaceous ciliates. Therefore the bacteria-grazing activity was higher in the absence of planktivorous fish.
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Keywords: Bacteria; Ciliates; Heterotrophic nanoflagellates; Microbial loop; Planktivorous fish; Predation; Zooplankton

Introduction

Zooplankton is the main food of fish larvae and fry in many aquatic systems (Cushing 1983; Mehner et al. 1996; Post and Kitchell 1997). Predation by adult planktivorous fish and the juveniles of all fish species is a very important factor determining the structure and abundance of the zooplankton community in lakes (Brooks and Dodson 1965; Hrbácek 1962). As a result of fish predation the density of large cladocerans (e.g. *Daphnia galeata* Sars, *Leptodora kindtii* Focke) usually decreases while the proportion of smaller species (e.g. *Bosmina longirostris* O. Müller, *Ceriodaphnia* sp.) increases (Jeppesen et al. 2001; Ohtaka et al. 1996). The predation effects cascade through the food web and can be considered to be a major factor determining the dynamics of the planktonic community (Huston 1979). Usually predation by planktivorous fish mainly influences cladocerans, as the copepods are better adapted to avoid predation (Bergman 1990). Alterations in the metazooplankton (MZP) community structure can in turn influence the algal (Persson 1997) and protozooplankton (PZP) communities (Gilbert and Jack 1993; Jürgens and Jeppesen 2000; Wickham 1995). PZP

abundance and species composition can directly influence bacterioplankton (Beaver and Crisman 1982; Gonzalez et al. 1990; Güde 1989; Hall et al. 1993) and the mediation of dissolved organic matter (DOM) to the higher food web levels. In addition to bacteria, heterotrophic nanoflagellates (HNF) are also able to consume DOM with a higher molecular mass (Sherr 1988). Direct ingestion of DOM by PZP is an alternative and efficient pathway for returning nutrients to higher trophic levels.

Despite the growing number of publications on the planktonic food web structure there is still a lack of studies that consider the cascading effect of planktivorous fish on the microbial loop. The role of fish is often neglected because of difficulties of assessing their abundance and feeding rate. Zingel et al. (2012) revealed that larval fish actively consume planktonic ciliates in a eutrophic lake. Riemann (1985) showed experimentally that increases in the number of planktivorous fish caused significant increases in HNF abundance. He attributed this increase to reduced predation from large cladoceran species. Subsequent enclosure experiments confirmed these early results (Christoffersen et al. 1993; Markošova and Ježek 1993). Fish treatments generally tend to increase the importance of small zooplankton species (Mazumder et al. 1988; Proulx et al. 1996; Vanni 1987). In comparing two lakes with contrasting food web structures, Vaque and Pace (1992) found that the lake dominated by planktivorous fish also contained the highest HNF abundance compared to the lake dominated by piscivorous fish. An enclosure experiment carried out in a small oligotrophic lake on the Canadian Shield (Tzaras et al. 1999) showed that fish alone had no significant effect on the abundance of bacteria and HNF. Müller-Solger et al. (1997) found in a mesocosm experiment conducted in a mesotrophic lake that in the presence of planktivorous fish the total ciliate biovolume increased. However, the impact on ciliates was species specific. Several studies have demonstrated that the addition of planktivorous fish increase rotifer abundance (e.g. Lazzaro et al. 1992; Mazumder et al. 1990).

We can conclude that there are numerous experiments on the food web effects of fish but much less attention has been paid to elucidating the direct and indirect consequences of fish feeding on microbial loop communities. Most data available have usually been obtained by short-term approaches. Without knowing the mechanisms driving the microbial loop community structure and functioning we cannot predict the possible response of the lake food web to changes in the environment. Our goal was therefore to simultaneously study planktivorous fish feeding on zooplankton and zooplankton feeding on bacteria, providing at the same time background data of the two-year seasonal dynamics of bacteria, protozoans, phytoplankton and MZP. We selected two large natural ponds where the actual food composition and abundance of planktivorous fish could be estimated. We hypothesized that in the pond in which planktivorous fish were present there would be less MZP, more PZP and consequently fewer bacteria than in the pond without planktivorous fish. We predicted

that the lowered MZP densities would have a positive effect on small bacterivorous ciliates since the MZP are known to prey on small ciliates (Agasild et al. 2012). Additionally, we assumed that in the absence of planktivorous fish the MZP and bacterial numbers would be much higher.

Material and Methods

To follow the impact of planktivorous fish on the planktonic food chain, we used two different model situations. We selected two large natural ponds (further described as F and nF) in the Härjanurme fish farm (Estonia). To the pond F the larvae of whitefish (*Coregonus lavaretus* Linnaeus) were introduced. The pond nF had no planktivorous fish at all. As the number of the ponds available for the study was limited we could not carry out any replication. Therefore we decided to repeat the study by extending it over a second year. The study was carried out from April 25 to September 29 in 2005 and from April 25 to September 28 in 2006. In the previous year (2004) both ponds were stocked with juvenile pikeperch (*Sander lucioperca* Linnaeus), which were removed at the end of the year. The plankton communities were checked in 2004 in both ponds and found to be generally similar.

The surface area of the ponds F and nF was 6 and 7.2 ha, respectively. The mean depth of both ponds was 1.6 m. Over the years studied, the average values of total phosphorus and nitrogen concentrations in July were 48 μg L^{-1}, 0.9 mg L^{-1} and 47 μg L^{-1}, 1.0 mg L^{-1}, respectively. The values of total phosphorus and total nitrogen in both ponds F and nF characterize them as eutrophic water bodies. The fish larvae were introduced to the pond F shortly after hatching (April 19–April 25) in both years. The ponds were emptied in late autumn and the fish were removed. The stocking density was approximately 6500 larvae per hectare. In both years the plankton sampling started on April 25. In 2005 the samples were collected every 10 days, while fish larvae were sampled every 20 days. In 2006 both plankton and fish larvae were sampled every 20 days. Water samples were collected using a Ruttner water sampler. All plankton analyses were made from pooled water samples collected at half-meter intervals (0, 0.5, 1 and 1.5 m). Each time, two subsamples were taken from the same places – one near the shore and one from the midpoint of the pond.

The total number of bacteria was determined by DAPI (4′,6′-diamidino-2-phenylindole; Polysciences Inc.) direct count (Porter and Feig 1980). Formaldehyde-preserved subsamples were incubated with DAPI (final concentration 10 μg mL^{-1}) for 5 min in the dark and filtered onto black 0.22-μm pore-size polycarbonate filters (Osmonics Inc.). The bacterial abundance was estimated by autofluorescence using an epifluorescence Zeiss Axiovert S100 microscope (Carl Zeiss MicroImaging GmbH, Jena, Germany). To count HNF taxa the samples were fixed with buffered formalin. 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. HNF were examined with an Olympus BX60 fluorescence microscope.

For the phytoplankton and ciliate counts the subsamples were preserved and fixed with acidified Lugol's solution (0.5% of final concentration) and analyzed using the standard Utermöhl (1958) technique. Ciliates were counted and identified using an inverted microscope (Nikon Eclipse Ti-U; Nicon Instruments Europe B.V., Amstelveen, the Netherlands). The entire content of each Utermöhl chamber was surveyed. The first 20 measurable specimens encountered for each taxon were measured. Biovolumes for each taxa were estimated by assuming geometric shapes. Specific gravity was assumed to be $1.0 \, g \, mL^{-1}$ (Finlay 1982), so the biomass was expressed as wet weight (WW). The phytoplankton samples were counted using the same inverted microscope. The mean volume of each species was estimated in all samples by approximating the shape of species to the nearest simple geometric form and the biomass was expressed as wet weight.

MZP was collected by filtering 10 L of depth-integrated pond water (pooled sample collected at 0, 0.5, 1 and 1.5 m) through the plankton net (48 μm), fixed with Lugol's solution and counted in three subsamples, which formed 10–20% of the whole sample volume. The samples were counted and enumerated under a stereomicroscope (Olympus SZ40; Olympus Deutschland GmbH, Hamburg, Germany). The lengths of 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 the wet weights according to Balushkina and Winberg (1979).

The fish larvae and fry were sampled using a seine net. The fish were measured (total length) and weighed. Fish samples and their digestive tracts were preserved in ethanol. The mortality of newborn fish larvae introduced to a pond is usually high. Therefore we used in calculations the fish density (ind. m^{-3}) at the time of seining. The swept water volume was calculated from the seine dimensions and the length of transects seined. This allowed us to roughly estimate the fish densities. The fish diet was evaluated using the numerical method (Hyslop 1980). Daily food consumption was calculated according to an 18-h daily feeding time. The food gut passage rates are known to be temperature dependent. As we lacked suitable temperature-related regression coefficients which could be applied to our conditions we estimated the gut passage time as described in Sutela and Huusko (2000) comparing the degradation gradient of soft-bodied food objects in the whitefish guts from oesophagus to anus. We did not find any noticeable seasonal fluctuations in gut passage time (ranging between 3.8 and 4.2 h), therefore the daily food consumption was calculated according to a mean 4 h. The average reconstructed gut content was calculated based on the Eldridge et al. (1981) method: $C = RH/T$, where C is the daily food consumption (MZP numbers or biomass day^{-1}), R is the average reconstructed gut content (MZP numbers or biomass), H is the hours of active feeding (h day^{-1}) and T is the hours of gut passage time of actively feeding fish (h). The daily MZP consumption by fish is further referred to as fish feeding impact.

To study the consumption of ciliates the gut contents were additionally analyzed according to Fukami et al. (1999) and Sutela and Huusko (2000). At least 20 larvae were analyzed on each sampling occasion. We reconstructed the larval diet based on the content of the first gut quarter Q1 as suggested by Sutela and Huusko (2000) and Zingel et al. (2012). After several rinsings with distilled water on the glass slide, the first gut quarter was opened with a needle, and all its contents were removed and preserved in 1% glutaraldehyde. The gut contents were double-stained using fluorescein isothiocyanate (FITC) and 4′,6-diamidino-2-phenylindole (DAPI) according to Sherr and Sherr (1983). Samples were gently filtered through 0.8-μm pore-size black isopore filters (Poretics 129 Inc., Livermore, CA, USA). Ciliates with DAPI-stained clear nuclei in the gut were counted on a Leica DMBR fluorescence microscope at 1000× magnification using blue (470/40 nm, OG515) and UV light (360/40 nm, GG420). Where possible, the dominant taxa of fluorescently stained ciliates were identified based on the composition of parallel Lugol-fixed samples. We differentiated ciliates and cells detached from trunks of fish intestine as suggested by Fukami et al. (1999).

Grazing of proto- and metazooplankton on bacteria was measured by *in situ* feeding experiments with fluorescent microspheres of diameters of 0.5 μm (Fluoresbrite, Polysciences Inc.). There are major concerns about the use of fluorescently labeled microspheres in grazing experiments, which relate to the knowledge that many predators select their potential prey based on its size (Shannon et al. 2007), food quality (Shannon et al. 2007), surface texture (Mohr and Adrian 2000), and swimming/escaping behaviour (Gilbert and Jack 1993). Although the use of microsphere tracers determines only the grazing rates of unselective filter feeders, several of our earlier protozoans feeding experiments in similar shallow and eutrophic environments have shown that the fluorescent microparticles were effectively consumed by the HNF and the ciliate species that dominated in our study ponds (Agasild et al. 2012; Zingel et al. 2007; Zingel and Nõges 2008). Therefore the method was assumed to be adequate to measure PZP grazing.

The tracer amount that did not exceed 10% of the density of natural picoplankton in pond water was added to integrated pond water. In experiments with metazoans a 3-litre incubation vessel was used. Incubations lasted 7 min, after which the water was filtered through a plankton net of 48 μm mesh size and fixed in formaldehyde (final concentration 4%). For microsphere counting, individual metazooplankters were collected from the samples and filtered onto a polycarbonate filter of 10-μm pore size (Poretics Inc.). The number of microspheres in animal guts was counted at 1000× magnification using an epifluorescence Zeiss Axiovert S 100

microscope. The ingestion (IR; cells ind.$^{-1}$ h^{-1}) rates for each taxon and community ingestion (CIR; cells L^{-1} h^{-1}) rates were calculated as follows:

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

$$CIR = IR \times N;$$

where M_t is the number of microspheres ingested per one individual during the incubation (microspheres ind.$^{-1}$); T is the incubation time (h^{-1}); M is the concentration of tracer cells (microspheres) in incubation vessel (cells mL^{-1}); P is the concentration of food particles (bacteria, picophytoplankton) in incubation vessel (cells mL^{-1}), N is the abundance of the grazer taxon (ind. L^{-1}) (Zingel and Nõges 2008). Community ingestion rates were determined as the sum of species-specific ingestion rates for all representing 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 the samples were fixed with buffered formalin (1% final concentration). 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 an Olympus BX60 fluorescence microscope under 1000× magnification using blue light (470/505 nm, OG 515). As much as possible the dominant taxa of fluorescently stained ciliates were identified based on the knowledge on the composition of parallel Lugol-fixed samples. To estimate the total ciliate grazing rate, their uptake rates were multiplied by their total *in situ* abundances as in the case of the MZP.

The data collected using the above methods were analyzed using nonparametric Spearman rank correlation analysis offered by STATISTICA 8.0 (StatSoft Inc. 2007).

Results

Bacterioplankton and protozooplankton

Both bacterio- (Fig. 1A, E) and protozooplankton (Fig. 1B, C, F, G) showed very similar dynamics in both years studied. The total number of bacteria was high in fishpond F in April, dropped at the end of May and July, and increased again at the end of the study (Fig. 1A, E), ranging from 3.6 to 8.6×10^6 cells mL^{-1} in 2005 and from 4.2 to 7.5×10^6 cells mL^{-1} in 2006. In pond nF the bacterial dynamics were more stable and the abundances much lower (2.1–4.0 and 2.5–4.2×10^6 cells mL^{-1}, respectively) compared with the fishpond F.

In the community of ciliates four orders were most abundant: Oligotrichida, Peritrichida, Scuticociliatida and Gymnostomatida. In pond F the community was dominated by oligotrichs and peritrichs having their lowest abundances

in the beginning of the study (on April 25) and maximum values at the end of May (total abundance 84.5 and 66.8 individuals mL^{-1} in 2005 and 2006, respectively) and July (149.3 and 128.7 individuals mL^{-1}, respectively) (Fig. 1B, F). The oligotrichs were dominated by *Halteria grandinella* O. Müller and the peritrichs by *Hastatella radians* de Laubenfels. The total number of scuticociliates, gymnostomatids and other ciliates remained below 4 individuals mL^{-1}. In the pond nF the total number of ciliates was highest (33.4 and 24.7 individuals mL^{-1}, respectively) in the beginning of the study (on April 25), while the community was dominated by bacterivorous oligotrichs (*Halteria grandinella*, *Rimostrombidium* sp.), peritrichs (*Hastatella radians*) and scuticociliates (*Uronema* sp., *Cyclidium glaucoma* O. Müller). In both years the seasonal dynamics were similar – during the spring period the abundance of peritrichs, scuticociliates and bacterivorous oligotrichs decreased and the herbivorous oligotrichs (*Pelagostrombidium* spp., *Limnostrombidium* spp.,) and gymnostomatids (*Pelagodileptus trachelioides* Zacharias, *Paradileptus elephantinus* Svec) became the most abundant groups (at the end of May). After that the community dynamics and abundances (∼20 individuals mL^{-1}) remained very stable. In pond F the ciliate numbers were dominated by bacterivorous species that made up an average of 86.6% and 86.4% of the total ciliate abundances (in 2005 and 2006, respectively). In pond nF these numbers were much lower: 50.1% and 48.9%, respectively. The same applied to the absolute numbers: in pond F the average abundances of bacterivorous ciliates were much higher (44.2 and 47.3 individuals mL^{-1} in 2005 and 2006, respectively) than in pond nF (11.4 and 10.1 individuals mL^{-1}, respectively). Due to the dominance of smaller bacterivorous species the total ciliate biomass was also smaller in pond F than in pond nF (average values being 4.2 and 4.5 mg WW L^{-1} and 12.8 and 12.4 mg WW L^{-1}, respectively in 2005 and 2006).

The abundance of HNF in the pond F was rather unstable, ranging from 36 to 1210 individuals mL^{-1} in 2005 and from 117 to 1497 individuals mL^{-1} in 2006 (Fig. 1C, G). In pond nF the HNF numbers rose already in spring and were thereafter constantly much higher (up to 5429 and 5519 individuals mL^{-1} in 2005 and 2006, respectively) than in pond F. The same trend applied to HNF biomass ranging in pond F from 1 to 46 μg WW L^{-1} in 2005 and from 5 to 47 μg WW L^{-1} in 2006. In pond nF the HNF biomass was almost an order of magnitude higher (up to 200 μg WW L^{-1} in both 2005 and 2006).

Phytoplankton, metazooplankton and fish

The studied ponds showed very similar dynamics in both years of exploration in respect to phytoplankton and MZP (Figs 1D, H, 2). Chlorophytes predominated in the phytoplankton in the studied ponds throughout the investigation period. Most common species were of the genera
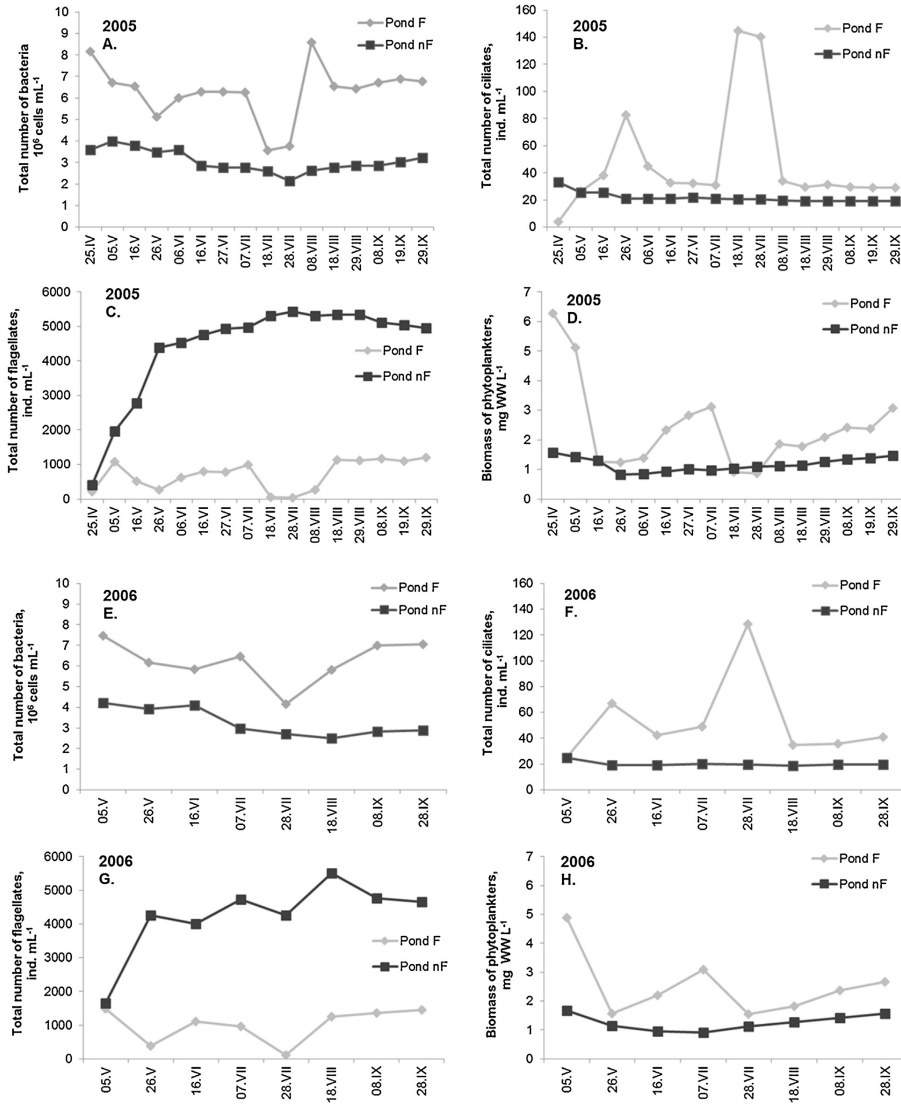
Fig. 1. Seasonal dynamics of different groups of organisms in a stocked (F) and in a fishless pond (nF) in 2005 and 2006.

Monoraphidium and *Chlorella*. Other important groups were diatoms (*Rhizosolenia* spp.) and chrysophytes (*Dinobryon sociale* Ehrenberg). Other phytoplankters were less abundant. The phytoplankton community in the ponds was dominated by small sized species (<35 μm), filamentous or colonial ones were rare. Phytoplankton biomass was highest in both years in the fishpond F in April (6.3 and 4.9 mg WW L^{-1}, respectively), showing small boosts also in July and September (Fig. 1D, H). In the fishless pond nF the biomass was much more stable ranging from 0.8 to 1.6 mg WW L^{-1} and 0.9–1.7 mg WW L^{-1}, respectively.
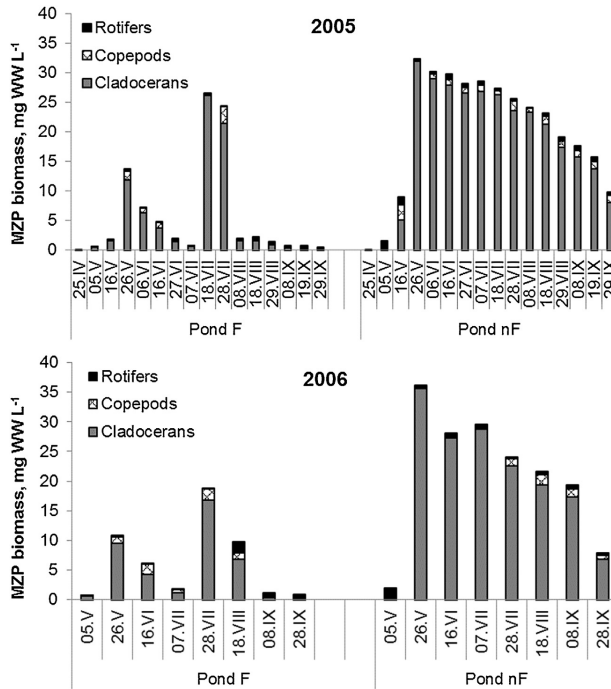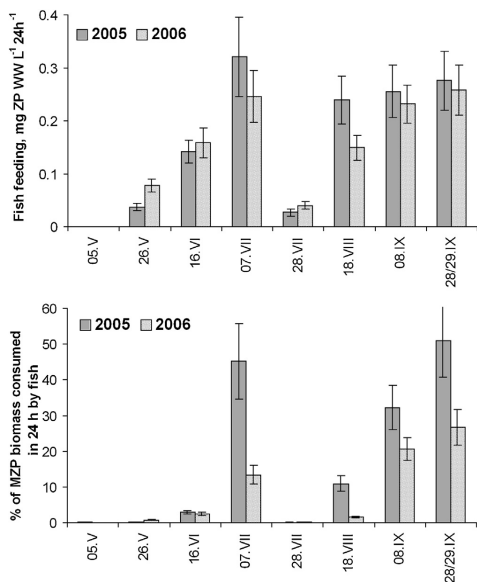
Fig. 2. Metazooplankton biomass (WW) in a stocked (F) and in a fishless pond (nF) in 2005 and 2006.

In both ponds and over both years the MZP biomass started to rise at the beginning of May (Fig. 2). Cladocerans (in pond F mostly *Bosmina longirostris*, *Daphnia cucullata* Sars and in pond nF *Leptodora kindtii*) were the most important zooplankton group in these ponds. In the case of pond F the MZP biomass was relatively stable until strong clado-ceran (*Bosmina longirostris* and *Daphnia cucullata*) peaks occurred in May and July. These peaks were observable in both years but their magnitude was higher in 2005. The MZP biomass of the pond nF peaked in May and decreased steadily until autumn. When comparing the total MZP biomass in the two ponds the highest biomass values were always found in the fishless pond nF. In this pond the most important species was a large-sized cladoceran (*Leptodora kindtii*) which showed high numbers throughout our study period. In the fishpond F this species was found only occasionally and in very low numbers. Abundance of copepods (mostly *Mesocyclops leuckarti* Claus and *Eudiaptomus gracilis* Sars) was quite stable and low throughout the study period in both ponds. In pond F rotifers (mostly *Keratella cochlearis* Gosse and *Keratella quadrata* O. Müller) had some importance in spring and autumn, while in summer cladocerans were more abundant.

The total number of whitefish decreased throughout the study period in both years from ∼32,000 to ∼16,000 individuals per pond. The sharpest decrease in whitefish abundance occurred in July, coinciding with the highest water temperatures. Whitefish consumed the highest amount of zooplankton at the beginning of July (Fig. 3), followed by a very sharp decline at the end of July. This was the warmest period of the study when water temperatures rose above 25 °C in both years.

Linkages between trophic levels

We found strong evidence of cascading interactions from fish to bacterioplankton (Fig. 4). In the fishpond F the amount of MZP eaten by the whitefish in both years was negatively correlated with MZP biomass ($R = -0.96$, $p < 0.05$; Supplementary Table 1) and with the abundance of bacterivorous ciliates ($R = -0.93$ and -0.75 in 2005 and 2006, respectively, $p < 0.05$; Supplementary Table 1). Fish impact on the MZP community was considerable; they consumed up to 51% and 27% (in 2005 and 2006, respectively) of the total MZP biomass daily (Fig. 3). The total share of ciliates in the

Fig. 3. Whitefish feeding on metazooplankton in a stocked pond (F) in 2005 and 2006. Error bars indicate standard deviation.

whitefish food was initially high (62% and 65% of the consumed zooplankton biomass on May 5 in 2005 and 2006, respectively) but sharply dropped to 2% and 1%, respectively, and remained at zero throughout the remaining study period. Fish impact on the ciliate standing stock was minor; they consumed <0.05% of the total ciliate biomass daily. The amount of zooplankton eaten by fish was positively correlated ($p < 0.05$) with the abundance of HNF and predaceous ciliates and with the phytoplankton biomass (Supplementary Table 1).

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejop.2014.01.006.

In the fishless pond nF the role of the top predator shifted to the predaceous zooplankter *Leptodora kindtii* (Fig. 4), which probably controlled the abundance of smaller metazooplankters, as we found a negative correlation between the abundance of the former and herbivorous cladocerans ($R = -0.65$ and -0.71 in 2005 and 2006, respectively, $p < 0.05$). The biomass of MZP had a strong negative relationship also with phytoplankton biomass in both studied ponds (Supplementary Table 2).

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejop.2014.01.006.
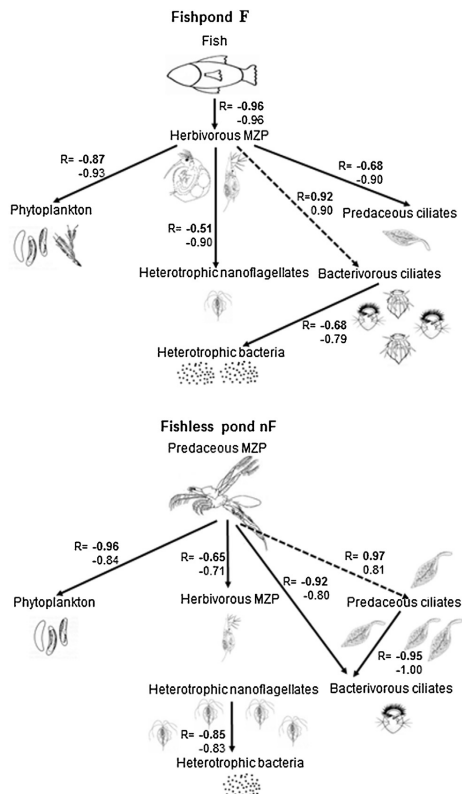
Fig. 4. Illustration of the generalized food webs and main trophic interactions in a stocked (F) and in a fishless pond (nF) in 2005 (numbers in bold) and 2006. Arrows indicate the main trophic interactions – dashed arrows present positive, solid arrows negative correlations (Spearman correlation coefficients R; $p < 0.05$). The correlations are calculated based on the following variables: heterotrophic nanoflagellates, predaceous ciliates, bacterivorous ciliates and bacteria – total abundances; metazooplankton and phytoplankton – WW biomass; fish – feeding on zooplankton biomass.

Data analysis revealed a strong negative relationship between the abundance of bacteria and HNF in pond nF ($R = -0.85$ and -0.83 in 2005 and 2006, respectively, $p < 0.05$; Supplementary Table 2). The abundance of bacterivorous ciliates was low in this pond and we did not find any significant correlations between the abundances of bacteria and ciliates. Instead, the abundance of small bacterivorous ciliates correlated negatively with the abundance of predaceous ciliates ($R = -0.95$ and -1.0, respectively, $p < 0.05$; Supplementary Table 2), indicating a possible grazing pressure. Inversely, we found a negative correlation between the total number

of bacteria and bacterivorous ciliates in the pond F ($R =$ -0.68 and -0.79, respectively, $p < 0.05$; Supplementary Table 2) where the abundance of HNF was low.

Contrary to our expectations the grazing pressure on bacterial abundances was quite low in the presence of planktivorous fish (Fig. 5). Our results showed that the abundance of bacteria was negatively correlated with the amount of bacteria eaten by ciliates in the pond F ($R =$ -0.71 and -0.79, respectively, $p < 0.05$; Supplementary Table 3). Bacterivorous ciliates were the main grazers of bacterioplankton, especially in late July (Fig. 6), while the abundance of HNF dropped significantly (Fig. 1C, G). At that time, there was also a peak in MZP biomass (Fig. 2), which may indicate the cascading effect to the lower trophic levels. Correlation analysis also showed negative correlations between the biomass of MZP and the amount of bacteria eaten by HNF ($R =$ -0.78, $p < 0.05$; Supplementary Table 3).

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejop.2014.01.006.

In contrast to the results for pond F, the total number of bacteria in the pond nF was constantly low and the grazing pressure on bacteria was more active (Fig. 5). Data analysis in both years showed a significant negative relationship ($R =$ -0.97 and -0.79, respectively, $p < 0.05$; Supplementary Table 3) between the total number of bacteria and the amount of bacteria eaten by HNF. No correlation was found between the total number of bacteria and the amount of bacteria eaten by ciliates. HNF were the main grazers of bacterioplankton throughout the study period (Fig. 6).

Discussion

Cascading effects of planktivorous fish on the microbial loop

Our study showed that planktivorous fish had a strong indirect influence on the microbial loop (Fig. 4). MZP biomass was in a negative correlation with HNF in the presence of planktivorous fish and in a positive correlation without fish. In the fishless pond the predaceous *Leptodora kindtii* and predaceous ciliates were not able to control HNF abundances, which were much higher (Fig. 1C, G). It has been generally thought that ciliates are not effective grazers of bacteria at concentrations found in the field; rather they are likely to be consumers of relatively large cyanobacteria and nanoplankters (e.g. Epstein and Shiaris 1992). However, in our study the bacterivorous ciliate abundances were tightly coupled with the bacterial numbers in the presence of fish. In the fishless pond the controlling role shifted to HNF. To date there is increasing evidence that in some water bodies ciliates are capable of significant grazing on bacteria (Kisand and Zingel 2000; Šimek et al. 1995; Šimek et al. 1998; Zingel et al. 2007) and can probably outcompete HNF. Bacterivorous ciliates were negatively correlated with HNF only in

the presence of fish ($R =$ -0.58 and -1.0 in 2005 and 2006, respectively, $p < 0.05$; Supplementary Table 2) when their numbers were high. In the fishless pond where the abundance of bacterivorous ciliates was suppressed they were not able to affect HNF (Fig. 6). It is evident that when HNF abundances are kept low they cannot control bacterioplankton dynamics. We found that when HNF were numerous they were much more effective grazers on bacterioplankton (Fig. 5) and also the bacterial numbers were much lower in the corresponding pond. One possible explanation for the lower bacterial numbers can also be the lack of nutrients released by planktivorous fish. It can play a fundamental role in situations where microorganisms are limited by nutrients (e.g. Vanni et al. 1997).

The microbial loop was more effective in the presence of planktivorous fish. We can draw this conclusion because the bacterial numbers were much higher in pond F and the efficiency of the microbial loop is usually determined by the density of bacteria within it (Taylor and Joint 1990). We cannot determine the magnitude of the microbial loop efficiency, as we did not measure the bacterial production. Beside bacteria, HNF are also able to consume DOM (Sherr 1988) and it is suggested that direct ingestion of DOM by PZP is an alternative and efficient way to make organic matter more efficiently available to higher trophic levels. In the fishless pond the HNF were much more numerous, their biomass made up 14.5 and 12.4% of the phytoplankton biomass in 2005 and 2006, respectively. In pond F these numbers were 1.1 and 1.2%, respectively. The result is that in the absence of fish the HNF may have contributed to the food web efficiency through direct DOM ingestion. As this variable was not measured quantitatively we cannot speculate how important this kind of direct link can be for the food web functioning.

The effect of planktivorous fish on the microbial loop is likely to be dependent on the trophic state of the water body. It has been shown that the number of ciliates increases in more eutrophic systems and that there is an 8-fold difference between ciliate biomasses in oligotrophic and hypereutrophic lakes (Zingel et al. 2002). Beaver and Crisman (1982) related ciliate community structure to trophic status, so that large algivorous oligotrichs are progressively replaced by small bacterivorous ciliates with increasing eutrophication. It has been shown that small bacterivorous ciliates are rather scarce in oligotrophic lakes (Zingel et al. 2002). Tzaras et al. (1999) studied the impact of planktivorous fish on the microbial loop and found no effect on the bacteria and HNF. Their study was carried out under oligotrophic conditions and it may be possible that bacterivorous ciliates were missing in their system. Our study showed that these ciliates are a crucial link in mediating the effect of planktivorous fish to the microbial loop. As Tzaras et al. (1999) did not analyze bacterivorous ciliates this topic can only be speculated. Based on our study we can suggest that the effect of planktivorous fish on the microbial loop probably increases in more eutrophic systems but this topic needs further evaluation.
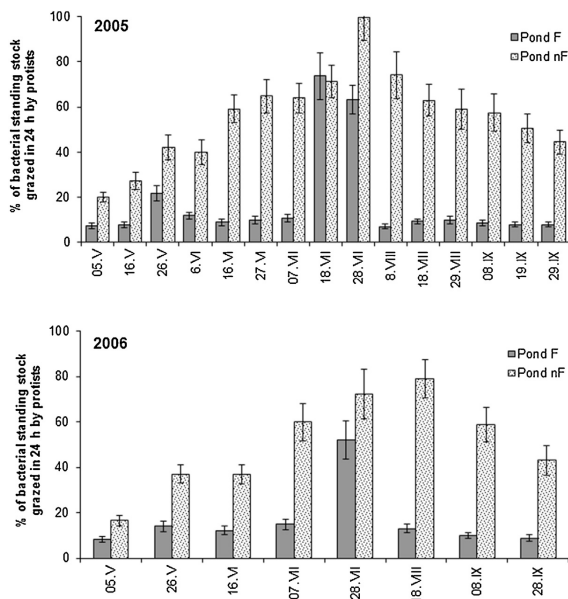
Fig. 5. Protists grazing pressure on bacterial standing stock in a stocked (F) and in a fishless pond (nF) in 2005 and 2006. Error bars indicate standard deviation.

Fish predation on zooplankton

Whitefish were not able to control planktonic ciliates directly. Fish juveniles fed on ciliates only in spring and consumed <0.05% of the total ciliate biomass daily. Nevertheless, ciliates were a very important food for whitefish in their initial feeding phase – ciliates formed 62–65% of the total zooplankton biomass consumed at the beginning of May. The food requirement of fish larvae during the first feeding stages is probably unable to be met without consumption of ciliates. Similar dependencies have been shown in shallow lakes (Zingel et al. 2012).

Fish juveniles influenced the food web structure through direct interactions (Fig. 4). The amount of MZP eaten by the whitefish was negatively correlated with MZP biomass ($R = -0.96$, $p < 0.05$; Supplementary Table 1) and fish consumed up to 51% and 27% (in 2005 and 2006, respectively) of the total MZP biomass daily (Fig. 3). Planktivorous fish are known to switch from one prey type to another according to its share to the total prey biomass (e.g. Gliwicz 2002) causing constant fluctuations in the different prey type abundances. Fish predation is also known to decrease the density of large cladocerans (Jeppesen et al. 2001). We found the same effect in our study – in the pond without planktivorous fish the most important MZP species was the large-sized cladoceran *Leptodora kindtii*, which showed high numbers throughout

both years. In the fishpond F this species was found only occasionally and in very low numbers. The prey of whitefish consisted mainly of larger dominant crustaceans, which are often recognized as the preferred prey for fish fries in lakes. In turn these crustaceans are known to feed actively on ciliates, so the alterations in the crustacean community by fish feeding can ultimately affect the protozoan assemblage by shifting both the their size and composition (Wickham 1995; Zöllner et al. 2003). Our study showed that different plankton groups were tied to each other and fluctuated greatly in the pond where the planktivorous fish were present. We found that in the fishpond the phytoplankton biomass was double that in the fishless pond (Fig. 1D, H). There was a strong positive correlation between numbers of MZP consumed by fish and phytoplankton biomass ($R = 0.96$, $p < 0.05$; Supplementary Table 1), indicating that planktivorous fish feeding may be a very important factor controlling phytoplankton development. We are fully aware that correlations do not automatically mean any causality. Nevertheless, our conclusions do not rely only on the correlation analysis of bulk planktonic parameters but also integrate the results of the two level feeding analysis (fish feeding on zooplankton; zooplankton feeding on bacteria). We believe that relationships we found are mostly causative, reflecting the food chain linkages.

Fish juveniles' influence on the food web structure was not constant but changed seasonally. Whitefish consumed
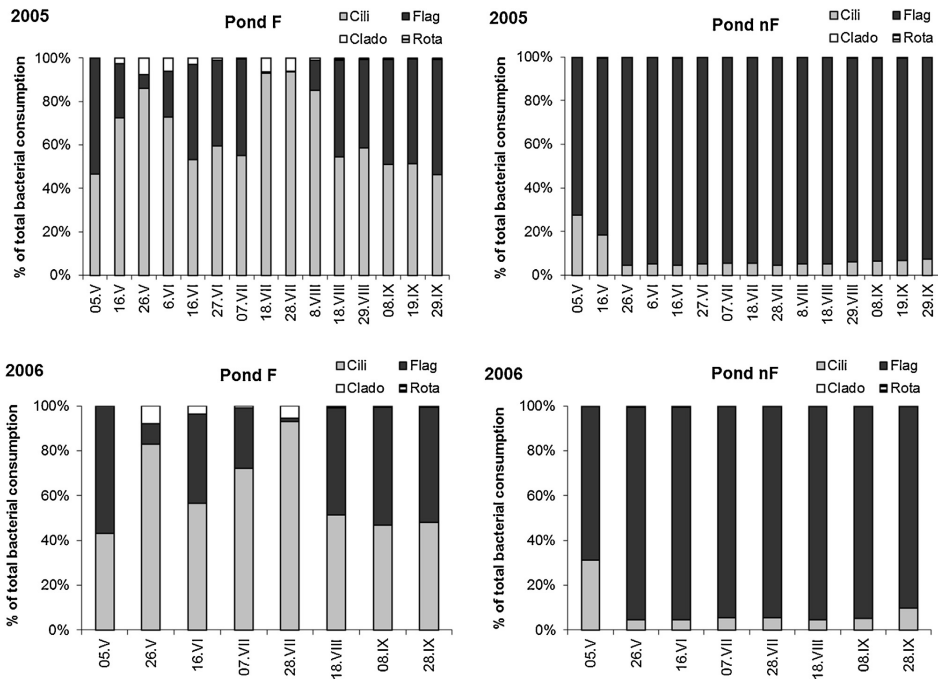
Fig. 6. Percentage of total bacterial consumption by different groups of organisms in a stocked (N) and fishless pond (nF) in 2005 and 2006.

the highest amount of zooplankton at the beginning of July, followed by a very sharp decline at the end of July. This was the warmest period of the year when water temperatures rose above 25 °C. These temperatures were too high for whitefish, which are known to prefer cold water, and their feeding rate dropped. Due to the decline in fish feeding the whole plankton community changed rapidly – the abundance of metazooplankters and ciliates increased, whereas that of phytoplankton, HNF and bacteria decreased due to stronger grazing pressure (Figs 1, 2). Consequently, as a result of changes in the top-down control strength the seasonal dynamics of the food web were very variable in pond F compared to very stable plankton dynamics in the pond nF. It is notable that water temperatures can indirectly influence the whole ecosystem through fish feeding activity. High summer water temperatures are known to greatly influence the dynamics of different plankton organisms (e.g. Wetzel 1983). However, the importance of temperature-dependent changes in 0+ fish feeding activity may easily be underestimated in these processes. The reduced feeding activity may already lead to the remarkable changes in plankton dynamics. Assessing these changes in the field is troublesome as there may be many different species present having different temperature optima.

It appears that when a water body lacks any planktivorous fish the potential food base supply for the first feeding larvae is very poor, consisting of unsuitable prey types where MZP (e.g. *Leptodora kindtii*) is too large and PZP (e.g. HNF) too small for larval consumption. Therefore, it is probable that the food requirement of fish larvae during their first feeding stages would not be met and their initial mortality may be very high in the ponds (or lakes) where zooplankton communities have developed without planktivorous fish. As a consequence, it is essential to know the species composition of both MZP and PZP prior to larval fish stocking.

Metazooplankton effect on ciliates

In the fishpond F MZP positively influenced bacterivorous ciliates (Fig. 4). We predicted that the presence of fish (that would lead to lower MZP densities) should also have a positive effect on small bacterivorous ciliates abundance as the MZP are known to prey on small ciliates (Agasild et al. 2012; Jürgens and Jeppesen 2000). Our results were completely different, though – the correlation between bacterivorous ciliate abundance and MZP was positive ($R = 0.92$ and 0.90 in 2005 and 2006, respectively, $p < 0.05$; Supplementary

Table 2). Pond F was dominated by the bacterivorous species *Hastatella radians* and *Halteria grandinella*. The other bacterivorous ciliates (mainly from genera *Cyclidium* and *Uronema*) showed strong negative correlation with MZP ($R = -0.85$ and -0.79 in 2005 and 2006, respectively, $p < 0.05$). *Halteria grandinella* has a jumping escape response against predation (e.g. Gilbert 1994; Tamar 1979), though it is somewhat unclear whether the escaping behaviour is an effective reaction to all metazoan predators. Results of some laboratory (e.g. Gilbert 1994; Jack and Gilbert 1997) and field studies (Havens and Beaver 1997; Jürgens et al. 1999) imply that the jumping escape response of *Halteria* is probably the most effective against predation by rotifers (Gilbert 1994; Jack and Gilbert 1997; Jürgens et al. 1999). In any case our study revealed that *Halteria grandinella* successfully withstood the cladoceran predation. The body of *Hastatella radians* has many spines, which may also offer some kind of protection against the MZP predation. Both *Hastatella* and *Halteria* were in a strong negative correlation with fish feeding – it is possible that when MZP numbers were brought down by fish, the other ciliates recovered quickly and the abundance of *Hastatella* and *Halteria* decreased.

In the fishless pond nF MZP negatively influenced bacterivorous ciliates. Various studies conducted in enclosures have demonstrated that copepods and cladocerans are able to control the abundance of ciliates (Gismervik 2006; Jürgens and Jeppesen 2000). We had predicted that in the absence of planktivorous fish the MZP density would be much higher, and controlled bottom-up but it was only partly true in our study. In our fishless pond the MZP biomass was largely made up by the predaceous *Leptodora kindtii*, which is known to prey mainly on small herbivorous cladocerans (Chang and Hanazato 2004). The MZP biomass was indeed much higher (Fig. 2), but consequently we had predicted lower ciliate biomasses. In our fishless pond *Leptodora kindtii* occupied the role of the top predator and seemed to control the herbivorous MZP, hence the MZP grazing pressure on the PZP was probably much lower. Despite this, however, the total abundance of ciliates in pond nF was still lower than in pond F. In pond nF the abundance of large predaceous ciliates (*Pelagodileptus trachelioides*, *Paradileptus elephantinus*) increased because they were clearly favoured by the presences of *Leptodora kindtii* – we found a positive correlation between this species and predaceous ciliates ($R = 0.97$ and 0.81 in 2005 and 2006, respectively, $p < 0.05$; Fig. 4). In earlier studies (Agasild et al. 2013) we have found likewise that the removal of the herbivorous crustaceans initiated a decrease in total ciliate abundance. These experiments showed that the removal of MZP led to the increase in large-sized predaceous ciliate abundance and a simultaneous decrease in the abundance of smaller bacterivorous ciliate species. Crustaceans can affect large predaceous ciliates through several potential mechanisms, most likely direct predation, potential effects of mechanical interference and exploitative competition (Agasild et al. 2013).

Conclusions

Our study showed that the feeding impact of planktivorous fish had a remarkable effect in shaping the microbial food web. In the presence of planktivorous fish the main grazers of bacteria were ciliates while in the absence of such fish the main grazers were HNF. The number of bacteria was also much smaller in the absence of fish. In assessing the effect of fish feeding we must know the amount of zooplankton eaten by fish. In situations where the planktivorous fish are not present, the MZP species composition (especially large predaceous species like *Leptodora kindtii*) determines the food web structure. We must also know the species composition of ciliates – are predaceous species present or have the smaller bacterivorous species developed some defensive responses against predation? Only by knowing the species composition of both MZP and PZP can we predict the changes in food web dynamics. Based on our study we may conclude that the pond where planktivorous fish were present had less MZP and higher amounts of phyto- and bacterioplankton. The presence of planktivorous fish led to the development of small-sized MZP species. Alterations in MZP composition cascaded to the protozoan level that in turn was a crucial link in structuring the microbial food web.

Acknowledgements

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Does metazooplankton regulate the ciliate community in a shallow eutrophic lake?

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SUMMARY

1. As grazers on picoplankton and nanoplankton, planktonic ciliates form an important link in pelagic food webs. Ciliate communities may be controlled by predation by metazooplankton. In eutrophic systems, however, where the number of large crustaceans is often low, the mechanisms that regulate ciliate dynamics have rarely been described.

2. We conducted an enclosure experiment with natural and screened (145 μm) summer plankton communities to investigate the effect of the small-sized crustacean zooplankton on ciliate community structure and the microbial loop in a shallow eutrophic lake.

3. The removal of the larger fraction of crustaceans initiated a decrease in total ciliate abundance. At the community level, we observed a substantial increase in large-sized predacious ciliates (>100 μm) and a simultaneous decrease in the abundance of smaller ciliates (<20–40 μm) that were mostly bacterivores and bacterio-herbivores. The compositional shift in the ciliate community, however, did not cascade down to the level of bacteria and edible phytoplankton.

Keywords: ciliates, metazooplankton, microbial loop, predation, trophic link

Introduction

Zooplankton forms a central link in the food web of freshwater lakes, consuming organisms at lower trophic levels and transferring carbon up the food chain. The capacity of zooplankton to utilise phytoplankton production and the suitability of zooplankton as food for fish determine the efficiency of carbon transformation in the food chain. When phytoplankton cannot be grazed by metazooplankton (crustaceans and rotifers) in a classical linear food chain, they enter the less efficient microbial loop that is mediated by bacteria and protists. As a result, planktonic protists are currently being subject to greater scrutiny in aquatic food-web studies.

The microbial loop is widely studied in lakes of contrasting trophic status and different geographical areas (e.g. Amblard *et al.*, 1995; Zingel *et al.*, 2006; Gobler *et al.*, 2008; Peštová, Macek & Martínez-Pérez, 2008). It has been shown that with increasing primary productivity, ciliates may become the most important members of the microbial loop (Sherr & Sherr, 2002). They can be the major bacterivores, the most important early spring algivores and the main consumers of heterotrophic nanoflagellates in lakes (Šimek *et al.*, 1990; Weisse *et al.*, 1990). In turn, ciliates serve as food for various metazooplankters (Wiackowski, Brett & Goldman, 1994; Adrian & Schneider-Olt, 1999; Hansen, 2000), thus forming an important link in the transformation of microbial production to higher trophic levels.

Planktonic ciliates form a particularly important component in the food web of shallow eutrophic Lake Võrtsjärv in Central Estonia (Northern Europe). Ciliates there have exceptionally high abundances (reaching values up to 191 cells ml^{-1}), and their biomasses contribute more than half of the total zooplankton biomass (Zingel & Nõges, 2010). The positive correlation between ciliate and

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metazooplankton biomasses (Zingel, 1999; Zingel & Haberman, 2008) suggests that planktonic ciliates may not be controlled top-down by the metazooplankton in Võrtsjärv, but rather controlled bottom-up by food availability.

We experimentally manipulated the metazooplankton community (i) to investigate the effect of small-sized crustaceans on ciliate abundances and community structure and (ii) to detect any potential cascading effect on bacterial abundance and the biomass and abundance of edible phytoplankton. Our hypothesis was that in a shallow eutrophic lake, ciliate abundance is not directly controlled by metazooplankton grazing.

Methods

Study site

Lake Võrtsjärv is a large (270 km^2) shallow (mean depth 2.8 m, maximum depth 6 m) and highly eutrophic lake in Central Estonia (58°05′–58°25′N and 25°55′–26°10′E). The average annual total phosphorus (dissolved + particulate) concentration is 54 µg L^{-1} and total nitrogen (dissolved + particulate) 1.60 mg L^{-1}. 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. During the growing season, Secchi depth usually does not exceed 1 m in central parts of the lake (Nõges *et al.*, 1998). The phytoplankton community is represented mainly by an association of filamentous algae: species of the diatom genus *Aulacoseira* in spring and cyanobacteria *Planktolyngbya limnetica* (Lemm.) Kom.-Legn., *Limnothrix planktonica* (Wołosz.) Meffert and *Aphanizomenon skujae* Kom.-Legn. et Cronb. in summer and autumn. The mean annual chlorophyll *a* concentration is 27 µg L^{-1} (Nõges *et al.*, 2008). The ciliate community is extremely rich and abundant (average abundance 70–140 cells ml^{-1} during the growing season). The spring peak is usually dominated by large-sized herbivorous oligotrichs. The annual maximum abundance usually occurs in late July or early August, when the community is mostly dominated by small bacterivorous scuticociliates and oligotrich species. The metazoan zooplankton reaches its biomass maximum usually in June when it is dominated by small-bodied cladocerans (mainly *Chydorus sphaericus* (O.F. Müller), *Bosmina longirostris* (O.F. Müller) and *Daphnia cucullata* Sars) and cyclopoid copepods of the genera *Mesocyclops* and *Thermocyclops*. For more details about ciliates and metazooplankton communities in Võrtsjärv, see Haberman (1998), Zingel (1999) and Zingel *et al.* (2007).

Experimental design

The enclosure experiment with zooplankton removal and control treatments was conducted from 27 August to 3 September 2008. Integrated lake water for the experiment was collected from the regular sampling station (depth 4 m) with a Ruttner sampler at 1-m intervals. Half of the integrated lake water was used to fill the control enclosures. The other half was used for the zooplankton removal treatment (zp-removal) and was first filtered through a 145-µm-mesh plankton net to remove the larger fraction of crustaceans, leaving the phytoplankton and ciliates as intact as possible. The water for zp-removal and control treatments was divided into six 20-L plastic containers and incubated in the lake for 8 days. Both treatments had three replicates. During the incubation, the ciliates and bacteria were sampled five times (on days 1, 2, 3, 6 and 8) and phytoplankton four times (on days 1, 3, 6 and 8). Metazooplankton was sampled at the beginning and end of the experiment.

Sampling and counting of plankton communities

From the collected integrated lake water that was used to fill the control enclosures, samples were taken to assess composition, abundance and biomass of ciliates, phytoplankton, metazooplankton and bacterial abundance as estimates for the control enclosures on day 1. The 145-µm-filtered lake water prepared for zp-removal treatments was sampled separately prior to filling the containers to estimate their plankton communities on day 1. For metazooplankton in the zp-removal treatments, samples of 5 L were poured through a 48-µm net. For ciliates and phytoplankton, 100-mL samples were used, while 20-mL samples were collected for bacteria. The experiment was designed so that, after initial sampling, all enclosures (both control and zp-removal) contained 20 L of lake water. On the last day, each container was sampled separately and 10-L samples were used for metazooplankton. Samples for metazooplankton, ciliates and phytoplankton were preserved with acidified Lugol's solution (0.5% final concentration).

The Utermöhl (1958) technique was used for the determination of ciliate and phytoplankton abundance, biomass and community composition. 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; Nicon Instruments Europe B.V., Amstelveen, the Netherlands) at 400–1000× magnification. The entire contents of each Utermöhl chamber were surveyed. Abundances

were counted in four size classes of <20, 20–40, 40–100 and >100 μm. Ciliate carbon content was determined from the measured volumes by using a conversion factor of 190 fg C μm^{-3} (Putt & Stoecker, 1989).

Phytoplankton cells were enumerated with an inverted microscope (Ceti Versus, Ceti Belgium, Kontich-Antwerp, Belgium) at 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. To 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 biovolume conversion factor of 100 fg C μm^{-3} (Nixdorf & Arndt, 1993).

The abundance of heterotrophic bacteria was determined by DAPI (4′,6′-diamidino-2-phenylindole) direct count (Porter & Feig, 1980). Bacterioplankton samples were fixed with glutaraldehyde at a final concentration of 1%. Subsamples (3 mL) were filtered through 0.2-μm pore-size black polycarbonate membrane filters (Poretics Inc., Livermore, CA, U.S.A.) 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.

Metazooplankton samples were counted in triplicate subsamples (2.5 or 5 mL) under a dissecting microscope (Olympus SZ40; Olympus Deutschland GmbH, Hamburg, Germany) at ×60 magnification. Crustacean and rotifer carbon weights were estimated using published length–dry weight relationships (Dumont, Van de Velde & Dumont, 1975; Bottrell *et al.*, 1976; Telesh, Rahkola & Viljanen, 1998) and a carbon : dry weight ratio of 0.5.

The program STATISTICA 8.0 for Windows (StatSoft, Inc. 2007) was used for statistical analyses. The comparison between treatments was made using the Mann–Whitney U-test.

Results

Metazooplankton

In control enclosures, the intact crustacean community was mainly composed of cyclopoid copepods (mainly *Mesocyclops leuckarti* Claus), which contributed 50% to the total metazooplankton biomass at the start and increased

to 60% by the end of the experiment. Among cladocerans, *Daphnia cucullata* dominated; their numbers were relatively high during the course of the experiment. In the zp-removal treatment, mainly copepod nauplii and rotifers [mainly *Polyarthra luminosa* Kutikova and *Anuraeopsis fissa* (Gosse)] were present (Table 1, Fig. 1). The biomasses of nauplii and rotifers were similar in both treatments and were therefore assumed to have similar predation impact on ciliates. The differences in development of ciliate communities between control and removal treatments were thus expected to be a result of presence of the larger crustaceans, including the effect of direct predation on planktonic ciliates, but also a possible indirect effect via crustacean feeding on bacteria and phytoplankton, as well as an impact of nutrient recycling. During incubation, a slight decrease in crustacean abundance and biomass occurred in the control treatment (Table 1, Fig. 1). In the rotifer community, similar changes occurred in composition and abundance in both control and zp-removal treatments.

Ciliate development

Total ciliate abundance was stable in the control treatment (Fig. 2), while a substantial decrease occurred in the zp-removal treatment (Mann–Whitney U-test, $P < 0.05$). Total ciliate biomass at the beginning of the experiment was slightly lower in the zp-removal treatment due to the effects of filtration. During incubation, a decrease in total ciliate biomass was observed in the control, while the biomass in zp-removal increased slightly (Fig. 2).

In the zp-removal treatment, a significant change in ciliate size structure and taxonomic composition occurred compared to the control. Among the ciliate size classes, a 10-fold increase was observed in larger (>100 μm), mostly predacious ciliates with a parallel reduction in the

Table 1 Crustacean and rotifer abundances in control and Zp-removal treatments at the beginning and end of the experiment

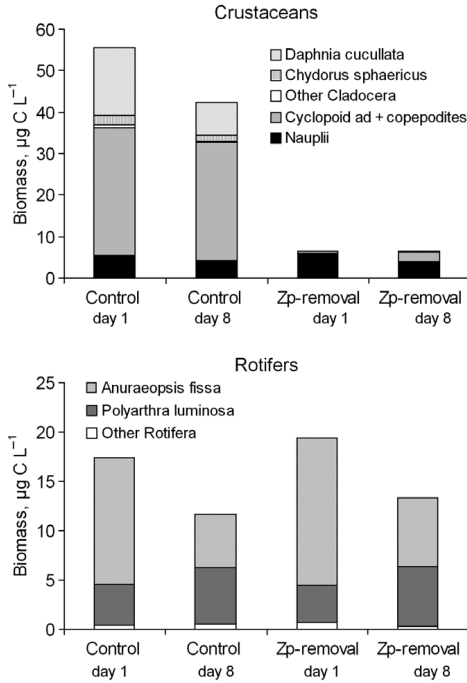| Species	Control (Ind. L^{-1})		Zp-removal (Ind. L^{-1})	
	Day 1	Day 8	Day 1	Day 8
Daphnia cucullata	12.1	10.1	0.0	0.0
Chydorus sphaericus	5.7	7.8	0.0	0.0
Other Cladocera	0.4	0.3	0.0	0.9
Mesocyclops leuckarti	14.3	16.3	0.0	0.6
Copepodites	20.0	6.8	0.5	2.2
Nauplii	80.0	69.4	88.4	64.4
Polyarthra luminosa	189	259	167	273
Anuraeopsis fissa	366	156	422	200
Other Rotifera	25.0	21.6	33.0	18.9

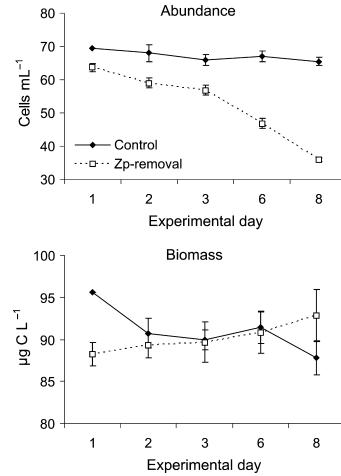Fig. 1 The initial and final biomass of crustaceans and rotifers in the control and zp-removal treatments.

Fig. 2 Ciliate abundance and biomass during the course of the experiment in the control and zp-removal treatments. Error bars refer to standard deviation.

abundance of smaller size classes (<20 and 20–40 μm), which consisted mainly of bacterivorous ciliates (Fig. 3). The differences in abundances of ciliate size classes (<20, 20–40 and >100 μm) between the treatments were statistically significant (Mann–Whitney U-test, $P < 0.05$). Only the size class 40–100 μm showed no statistically significant difference.

Haptorida was the group that contributed most to the largest size class of ciliates. In the zp-removal treatment, their smaller cells decreased, while larger raptorial species (>300 μm, mainly *Paradileptus elephantinus* Kahl) increased, causing a substantial growth in Haptorida biomass compared to their control counterpart (Fig. 4; Mann–Whitney U-test, $P < 0.05$). Oligotrichida, comprising mainly small bacterivorous cells, was the group that responded with substantial reduction in biomass in the zp-removal treatment. The most numerous oligotrichs were *Rimostrombidium humile* Penard., *Rimostrombidium* sp. and *Pelagostrombidium* spp. As the abundance of small

bacterivorous species declined, the relative importance of larger oligotrichs (*Codonella cratera* Leidy, *Limnostrombidium viride* Stein) increased. Also peritrichs (made up mainly by *Vorticella natans* Fauré-Fremiet and *Vorticella* spp.) showed a statistically significant decline (Mann–Whitney U-test, $P < 0.05$) in the zp-removal treatment (Fig. 4). Scuticociliates and prostomatids showed no statistically significant difference between treatments, with relatively stable but low biomasses. The most common scuticociliate species were *Cyclidium* sp., *Uronema* sp. and *Calyptotricha lanuginosa* Penard, and the most abundant prostomatids were *Coleps hirtus* Müller and *Urotricha* spp.

By the end of the experiment, a significant compositional change between ciliate communities in control and zp-removal media was observed (Fig. 5). Bacterivores (mainly Oligotrichida), the dominant functional group in ciliate biomass in the control treatment, declined to a very low level in the zp-removal treatment where predacious forms (mainly Haptorida) contributed over 60% of total ciliate biomass. Bacterio-herbivores and herbivores were less affected by the zp-removal treatment (Fig. 5).

Effects on bacteria and edible phytoplankton

Bacterial abundance followed similar dynamics in both treatments (Mann–Whitney U-test, $P > 0.05$). The total num-
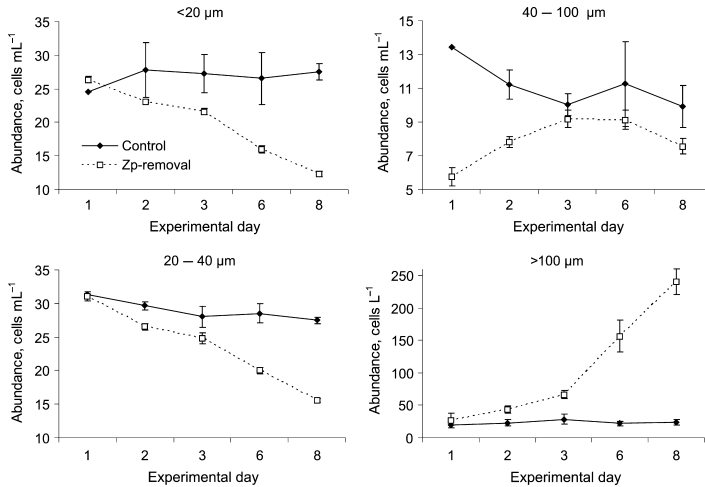
Fig. 3 Abundances of ciliate size classes of <20, 20–40, 40–100 and >100 μm in the control and zp-removal treatments. Error bars refer to standard deviation.

ber of bacteria ranged from 2.08×10^6 to 9.16×10^6 cells mL^{-1}, decreasing marginally during the first six experimental days followed by a slight increase at the end of the experiment (Fig. 6).

The phytoplankton community was dominated by cyanobacteria, comprising 86–92% of total biomass in both control and zp-removal treatments. The fraction of inedible phytoplankton was dominated by filamentous cyanobacteria (e.g. *Aphanizomenon* sp., *Limnothrix plank-tonica* and *L. redekei*). Phytoplankton biomass was highest in the middle of the exposure period (5.1 and 5.3 mg C L^{-1} in control and zp-removal treatments, respectively) and decreased towards the end of the experiment. The biomass of edible phytoplankton (mainly Chlorococcales, but also chrysophytes and cryptophytes) was slightly higher in the zp-removal treatment (0.681 mg C L^{-1} compared to 0.510 mg C L^{-1} in control), but the difference was not statistically significant (Mann–Whitney *U*-test, $P > 0.05$). Also, no clear treatment effect was observed among the edible phytoplankton size classes (Fig. 7).

Discussion

Our results clearly show that summer ciliate abundances were not directly controlled by crustacean grazing in the shallow, eutrophic Lake Võrtsjärv. A decrease occurred in total ciliate abundances in response to the removal of

crustaceans, the opposite of what has been most commonly reported previously in similar experiments (Adrian & Schneider-Olt, 1999; Ventelä *et al.*, 2002). However, much higher crustacean abundances and biomasses were used in these earlier experiments where top-down control was found to be important for ciliate community regulation (Adrian & Schneider-Olt, 1999; Ventelä *et al.*, 2002; Zöllner *et al.*, 2003). The crustacean community of Võrtsjärv is mostly composed of small cyclopoids (mainly *Mesocyclops leuckarti* and *Thermocyclops oithonides* Sars) and small-bodied cladocerans such as *Chydorus sphaericus, Daphnia cucullata* and *Bosmina longirostris*; their numbers and biomasses are relatively low, being obviously affected by strong fish predation pressure (Haberman, 1998; Haberman, Laugaste & Nõges, 2007) and by unfavourable feeding conditions due to high abundances of filamentous algae and frequent sediment resuspension (Chow-Fraser & Sprules, 1986; Levine, Zehrer & Burns, 2005). Furthermore, estimates of crustacean feeding conducted at the same time, using labelled natural ciliates, showed that planktonic microciliates (15–40 μm) are ingested by all dominant cladoceran and copepod taxa in Võrtsjärv, while both specific ingestion rates and the effect of the crustacean community on the ciliate standing stock were generally low (Agasild, Zingel & Nõges, 2012). From July to September, which is the period of intensive development of filamentous cyanobacteria and dominance of small bac-
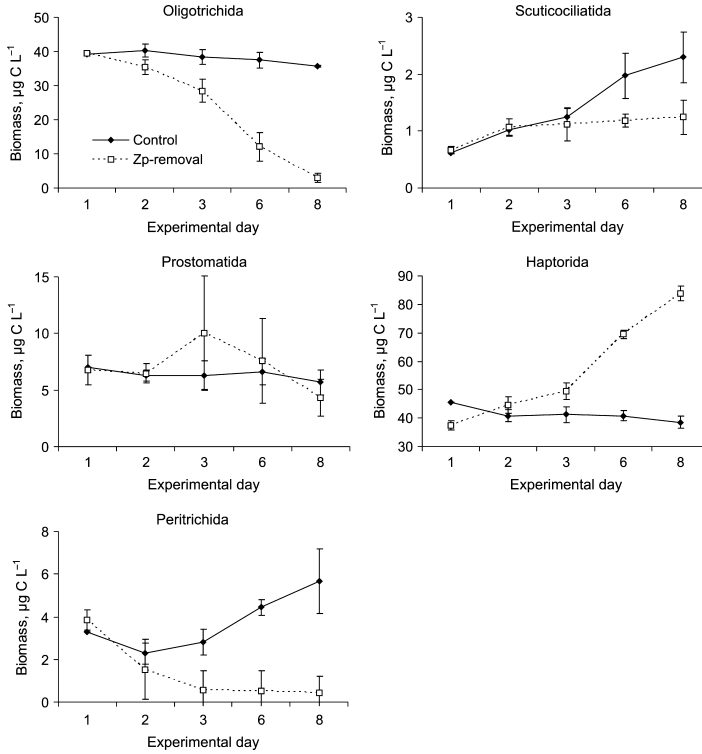
Fig. 4 Biomasses of ciliate groups of Oligotrichida, Scuticociliatida, Prostomatida, Peritrichida and Haptorida in the control and zp-removal treatments. Error bars refer to standard deviation.
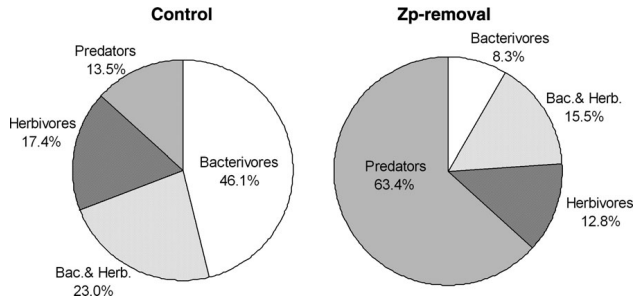

Fig. 5 Proportion of functional groups in ciliate biomass at the end of the experiment in the control and zp-removal treatments.

terivorous ciliates (<30 μm), the daily ingestion of crustaceans comprised only 3.9–6.8% of the ciliate standing stock.

The removal of crustaceans in our study, however, indirectly initiated a significant change in the ciliate community. The removal of the larger fraction of crusta-
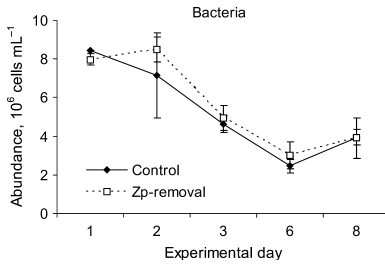
Fig. 6 Bacterial abundance in the control and zp-removal treatments. Error bars refer to standard deviation.
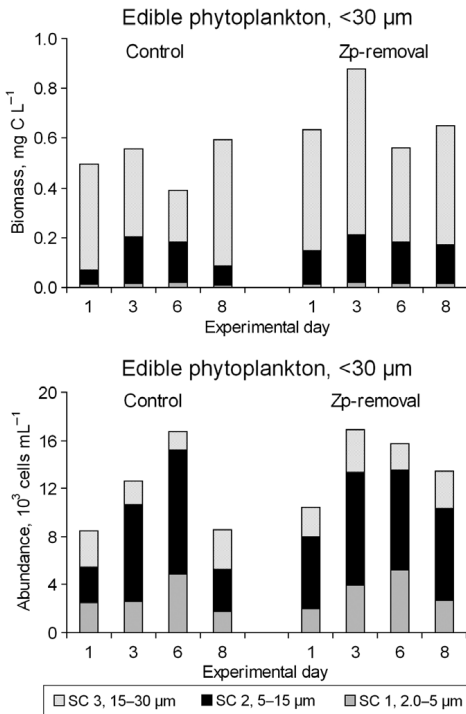

Fig. 7 Biomass and abundance of presumed edible phytoplankton (<30 μm) size classes (2.0–5.0, 5.0–15.0, 15.0–30.0 μm) in the control and zp-removal treatments.

ceans led to an increasing number of large predacious ciliates, which are known to actively feed on small-sized ciliates. Dolan & Coats (1991) estimated the feeding of

predacious ciliates (*Mesodinium pulex* Claparéde & Lachmann, *Euplotes vannus* O. F. Müller and *E. woodruffi* Gaw) on small bacterivorous ciliates at prey abundances similar to those in Lake Võrtsjärv and found ingestion rates (4.5 and 3.4 cells h^{-1} at prey abundances of 75 and 172 cells ml^{-1}, respectively) comparable to those measured for crustaceans in Võrtsjärv (Agasild, Zingel & Nõges, 2012). Owing to their substantially increased abundance towards the end of the experiment (over 200 ind L^{-1}), the predacious ciliates most probably declined to abundances similar to those of small-sized ciliates and thereby shifted dramatically the size structure and species composition of the ciliate community. A similar decline in small-sized ciliates, parallel to the increase in large predatory forms (*Lagynophrya* sp. and *Monodinium* sp.) after the removal of mesozooplankton, was reported by Jürgens, Skibbe & Jeppesen (1999) for enclosure experiments in the hypertrophic Lake Søbygård. There thus seems to be a common pattern of ciliate community regulation in conditions of very low abundance of crustaceans and in the presence of large ciliate predators. If crustaceans achieve sufficient abundance and biomass, they gain control over the large predacious ciliates. In the present experiment in Võrtsjärv, the suppression of predatory ciliates occurred at about 30 individuals of cyclopoids L^{-1} (adult and copepodid stages) and at 20 cladocerans L^{-1} (the crustacean concentration in the control media). Still, one must bear in mind that short-term manipulation experiments may overestimate the effects of predation occurring in lakes over the longer turn. Although large-sized carnivorous ciliates (>300 μm; *Paradileptus* sp., *Dileptus* sp.) temporarily peak during summer in Võrtsjärv, their abundances are generally not high (Zingel, 1999) and no substantial reductions in small-sized ciliate abundances have been reported. Peaks in abundance of large-sized carnivorous ciliates follow mostly the peaks of smaller bacterivorous and bacterio-herbivorous ciliates, which indicates that small-sized ciliates are their major food source in Võrtsjärv (Zingel, 1999).

The question remains as to precisely how large carnivorous ciliates are affected by crustaceans. Besides the potential effects of mechanical interference and exploitative competition, direct predation by crustaceans seems the most likely factor suppressing ciliate numbers. Most probably a trophic link exists between cyclopoid copepods and the large predacious ciliates. Cyclopoid copepod ingestion of ciliates and a notable effect on ciliate community structure have been shown in several studies (e.g. Wickham, 1995; Hansen, 2000; Zöllner *et al.*, 2003). As raptorial feeders, cyclopoids have been also reported to ingest rotifers, copepod nauplii and copepodites, and small

cladocerans (Adrian, 1991). Therefore, attacking large-sized (>300 μm) and relatively slow-moving ciliates can also be expected. Recently, Reiss & Schmid-Araya (2011), in their experiments with the epibenthic cyclopoid *Eucyclops serrulatus* (Fischer), showed direct predation on *Paramecium caudatum* Ehrenberg (mean length 220 μm), a ciliate about 1/3 of the predator's body size. A strong inverse relationship between a cyclopoid copepod (*Apocyclops dimorphus* Kiefer) and large ciliates has been observed, for example, in the Salton Sea (Tiffany *et al.*, 2007). In Võrtsjärv, this kind of relationship has not yet been described and may be masked by other factors driving seasonal changes in ciliate community composition.

Cascading effect on bacteria and phytoplankton

Owning to their extremely high abundances, ciliates have been found to be the dominant predators of both bacteria and small algae, consuming about 20% of the standing stock of nanoplankton and nearly 100% of biomass production of bacteria during the growing season, compared to metazoan zooplankton in Võrtsjärv (Zingel *et al.*, 2007). Despite the remarkable shift in the ciliate community and especially among bacterivores, predatory interactions mediated by crustacean zooplankton did not cascade down to affect bacterial abundance. This, however, might be a result of high bacterial production rates compensating predation effects. Similarly, only modest or no effects on bacterial numbers have been previously reported in metazooplankton manipulation experiments (e.g. Ventelä *et al.*, 2002). Experiments have shown that a change in grazer community composition might cause taxonomic shifts in the bacterial assemblage rather than changes in abundance and biomass (Zöllner *et al.*, 2003). As we did not conduct specific measurements (bacterial biovolume, activity, production or diversity), the structural and compositional changes in the bacterial assemblage potentially resulting from cascading effects remain untested.

We also did not see any clear effects at the phytoplankton level. The small phytoplankton (<30 μm), presumed edible for zooplankton, constitutes an average of only 10% of total phytoplankton biomass in Lake Võrtsjärv and is obviously under strong grazing pressure, especially by herbivorous ciliates (Agasild *et al.*, 2007; Zingel *et al.*, 2007). As the herbivores were the least affected grazer group among ciliates in the experiment, their feeding probably did not cause any significant alterations in the edible phytoplankton assemblage.

In conclusion, our results indicate that the small-sized crustacean assemblage in shallow eutrophic lakes can significantly alter ciliate community structure. However,

this effect is triggered by crustacean grazing especially upon large-sized predacious ciliates, rather than by the ingestion of the bulk of ciliates. The results also suggest that an important energy transfer between metazooplankton and the ciliate community could occur via large-sized predacious ciliates.

Acknowledgments

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III

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Ciliates as the crucial food source of larval fish in a shallow eutrophic lake

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Abstract

We assessed feeding of planktivorous fish larvae on ciliate protozooplankton in shallow eutrophic Lake Võrtsjärv, Estonia to determine whether ciliates are an important part of larval fish diet. We collected larvae of the most common fish species and examined their gut contents by gut segmentation and epifluorescence microscopy. Ciliates were present in the gut of all fish larvae. Gut segmentation analysis showed clear differences in food composition between gut quarters. The hard-bodied food items were quite evenly distributed in the gut, but the soft-bodied ciliates and rotifers were present only in the first gut quarters. Neglecting differences in prey digestion rate leads to underestimation of diet amount and composition and, especially, to underestimation of the role of protists in food. In Võrtsjärv, ciliates account for ~ 60% of the total carbon biomass consumed by fish larvae. The food requirement of fish larvae during first feeding stages (May–Jun) would not be met without consumption of ciliates.

Hjort (1914) was first to put forth the so-called 'critical period' hypothesis: the availability of suitable prey at the time when larval fish switch from endogenous to exogenous feeding determine their survival and, thus, the fish year-class strength. This hypothesis and the further refined theories that derive from it, are all based primarily on the consumption of crustacean prey. Up to date, there are very few studies that have considered the direct protist consumption by fish larvae. Most planktonic protozoa are fragile and therefore get easily degraded in the fish gut. Thus, protist ingestion may have been neglected in the past because of difficulties describing the gut contents of fish larvae, due to a high degree of degradation of the food and the use of normal techniques of microscopy for species identification (Fukami et al. 1999).

It has been suggested that most of the coastal waters do not contain enough (metazoan) prey to support larval growth and survival (MacKenzie et al. 1990). Also, some studies indicate that the guts of field-caught larvae often appear empty (van der Meeren and Næss 1993). One explanation for these paradoxes (i.e., apparent low prey levels and empty guts) is that food is sufficiently abundant, but easily digested and therefore not detected by researchers (Figueiredo et al. 2005; Montagnes et al. 2010). One such possible food source that larval fish can ingest and quickly digest is protozoa. If this is the case, the focus on metazoan prey has resulted in fisheries scientists overlooking a key trophic linkage (Montagnes et al. 2010).

There have been only sparse field studies concerning protozoa as food of the fish larvae, and most of them have focused only on the hard-bodied protists (Watson and Davis 1989). Only few have tried to estimate the importance of soft-bodied protozoans in the larval diet (Fukami et al. 1999; Nagano et al. 2000; Figueiredo et al. 2005). Modern fluorescence microscopy approaches have been rarely used in this specific research field. Lessard et al. (1996) and Fukami et al. (1999) were the first to use the epifluorescence of 4′,6-diamidino-2-phenylindole (DAPI) stained gut contents to identify protists in the diets of marine fish larvae. To our knowledge, there are no corresponding studies from the freshwater environment.

The present study was conducted in Lake Võrtsjärv, Estonia. Our previous investigations have shown that in Võrtsjärv the ciliate community is extremely rich, abundant, and usually > 50% of the whole zooplankton biomass consists of ciliates (Zingel and Nõges 2010). Ciliates move more slowly than most of the metazooplankters and should be easily caught by young larval fish. Our hypothesis was that ciliates must be an important part of larval fish diet. In order to test this hypothesis, we collected larvae of the most common fish species in Võrtsjärv and examined their gut contents by using epifluorescence microscopy. We believe that Võrtsjärv can be treated as an example lake and our findings apply in broad scale to many water bodies.

Methods

Lake Võrtsjärv is a large (270 km²) and shallow (mean depth 2.8 m, maximum depth 6 m) eutrophic lake situated in the Central Estonia. The water temperature reaches its maximum in July (20.1°C, on average). The ice cover lasts from November to April (135 d on average). Homothermy prevails in Võrtsjärv throughout the year (Zingel and Nõges 2010). The water is slightly alkaline; the monthly mean pH ranged from 7.6 to 8.5, salinity ranged from 227 mg L^{-1} to 370 mg L^{-1}, and the concentrations of chlorides and sulphates ranged from 9 mg L^{-1} to 14 mg L^{-1} and from 17 mg L^{-1} to 21 mg L^{-1}, respectively. The average total phosphorus concentration is 54 μg L^{-1}, total nitrogen concentration is 1.6 mg L^{-1}, and the mean Secchi depth is 1.1 m (Zingel and Nõges 2010). During the growing season, Secchi depth does not usually exceed 1 m.

Samples of fish larvae were collected by several horizontal tows (each at 1-m depth, 10 min, ~ 2 m s^{-1}) using a conical net with a mouth diameter of 50 cm and

mesh size of 0.5 mm. Sampling was carried out on two occasions: 24 May and 08 June 2005. Larval samples were fixed immediately with buffered formalin (2% final concentration) and stored at 4°C until analysis. The larval species were identified by using the textbook of Koblitskaya (1981). We used in analyses only larvae, which could be identified. The total length (TL) of each larva was measured and larvae were weighed.

The cut contents were analyzed according to Fukami et al. (1999) and Sutela and Huusko (2000). At least 20 larvae from each species were analyzed. The whole alimentary tract of each individual (ind.) larva was removed by using a sharp operating needle under stereomicroscope, cut into four equal quarters (Q1, Q2, Q3, Q4), and analyzed separately (Sutela and Huusko 2000). After several rinsings with distilled water on the glass slide, the gut quarters were opened with a needle, and all their contents were removed and preserved in 1% glutaraldehyde. The gut contents were double-stained using fluorescein isothiocyanate and DAPI according to Fukami et al. (1999). Samples were gently filtered through 0.8-μm pore-size black isopore filters (Poretics). Ciliates with DAPI-stained clearly visible nuclei in the gut were counted on a Leica DMBR fluorescence microscope at 1000× magnification using blue (470/40 nm, OG515) and ultraviolet light (360/40 nm, GG420). Where possible, the dominant taxa of fluorescently stained ciliates were identified based on the composition of parallel samples preserved in 2% acid Lugol's solution. We differentiated ciliates and cells detached from trunks of fish intestine as suggested by Fukami et al. (1999). Also all ingested metazooplankters were counted and identified as far as possible. All found food objects were measured using calibrated oculars. Biovolumes of ciliates were estimated by assuming simple geometric shapes. Biovolumes were converted to carbon (C) weight using a factor of 190 fg C μm^{-3}. Carbon weight biomass of metazooplankton was estimated by length–carbon relationship according to Manca and Comoli (2000), and by length–dry weight relationships according to Dumont et al. (1975) using the carbon conversion factor of 0.48 μg C per μg dry weight. Fish carbon biomass was calculated as suggested by McGurk (1986) assuming conversion factors of dry weight–carbon for larval fish of 36%.

The composition of diet was evaluated using the numerical method (Hyslop 1980). Daily food consumption was calculated according to an 18-h daily feeding time and a 4-h gut passage time (Sutela and Huusko 2000). We reconstructed the larval diet using two different methods: (1) based on the all four gut quarters (BULK-diet), and (2) based only on the content of the first gut quarter Q1 (FRESH-diet), as suggested by Sutela and Huusko (2000). The calculations were based on the following formula: C = RH × T^{-1}, where C is the daily food consumption (zooplankters ingested fish^{-1} 24 h^{-1}), R is the average reconstructed gut contents (zooplankters ingested fish^{-1}), H the hours of active feeding (h), and T the gut passage time (h) of actively feeding fish. Reconstructed gut content (R) equals the amount of zooplankters found on the whole alimentary tract (BULK-diet) or the amount of zooplankters found on the first gut quarter Q1 multiplied by 4 (FRESH-diet).

Ciliate samples were collected using the Ruttner sampler at the same stations as the fish larvae. Samples were preserved and fixed with 2% acidified Lugol's solution. The samples were stored at 4°C in the dark. Ciliate biomass and community composition were determined using the Utermöhl technique. Volumes of 10–20 mL were allowed to settle for $\geq$ 24 h in plankton chambers. Ciliates were enumerated and identified with an inverted microscope (Nikon diaphot biological inverted microscope) at 200–600× magnification. The entire content of each Utermöhl chamber was surveyed; if the total tally in the counting chamber was < 150 organisms, an additional subsample was counted. Ciliates were identified by consulting Foissner et al. (1999).

For metazooplankton samples, 10 liters of integrated lake water was filtrated through a 48-μm plankton net and concentrated to about 100 mL. The samples were fixed with acidified Lugol's solution (2% final concentration). Triplicate subsamples (2.5 mL or 5 mL) of each metazooplankton sample were counted under an Olympus SZ60 stereomicroscope in a chamber (dimensions 13 × 6 cm, capacity 8 mL) at 60× magnification. Carbon weight biomass of ciliates and metazooplankton was estimated using the above mentioned methods.

The program STATISTICA for Windows version 6.0 was used for statistical analyses.

Results

During the study period the ciliate community was mostly made up by bacterio-herbivorous and herbivorous species, some of them being mixotrophic (e.g., *Limnostrombidium viride*). Most species fell in the size between 20 μm and 100 μm. Oligotrichs, prostomatids, and scuticociliates dominated the community, constituting over 90% of the total abundance. The other ciliate orders present were haptorids and peritrichs. Oligotrichs were the most numerous, accounting for 42% and 38% of the total ciliate abundance (in May and Jun, respectively). The most common oligotrichs were *Rimostrombidium lacustris, R. humile, Limnostrombidium viride,* and *Pelagostrombidium* spp. The most common prostomatids were *Urotricha furcata, U. pelagica, Balanion planktonicum,* and most important scuticociliates were *Uronema* sp. and *Cyclidium* sp. Other protists (including dinoflagellates) are rather scarce in Võrtsjärv and were not present in our samples. Ciliates contributed 95% and 70% of the total available zooplankton carbon (in May and Jun, respectively). The metazooplankton community was dominated by small cladocerans (*Chydorus sphaericus, Bosmina longirostris*), rotifers (*Keratella cochlearis, Polyarthra dolichoptera*), and copepod nauplii (Table 1).

Altogether 8 fish species were studied. In May, we caught roach (*Rutilus rutilus*), pike (*Esox lucius*), perch (*Perca fluviatilis*), and smelt (*Osmerus eperlanus*). In June, pikeperch (*Sander lucioperca*), bream (*Abramis brama*), bleak (*Alburnus alburnus*), and ruffe (*Gymnocephalus cernuus*) were added. These fish are the most common in Lake Võrtsjärv as in the other lakes of the European temperate zone.

Our results revealed that ciliates were present in the guts of all larval fish species examined. We could not find any

Table 1. Physico–chemical and biological parameters of Lake Võrtsjärv on 24 May and 08 June, 2005 (Temp = water temperature, O_2 = oxygen concentration, Sec = Secchi depth, ZP A = metazooplankton abundance, ZP B = metazooplankton carbon biomass, Cili A = abundance of ciliates, Cili B = carbon biomass of ciliates).

	Temp (°C)	O_2 (mg L^{-1})	Sec (m)	ZP A (ind. L^{-1})	ZP B (mg C L^{-1})	Cili A (ind. L^{-1})	Cili B (mg C L^{-1})
May	14.2	12.2	0.9	786	0.05	138,440	1.08
Jun	16.4	11.7	1.0	792	0.18	67,540	0.43

other protists or phytoplankters in larval guts. Average number of ciliates found in larval guts based on bulk measurements of the whole gut is given in Fig. 1. The average ingestion rate for all taxa was 161 ciliates larva^{-1} h^{-1} in May and 272 ciliates larva^{-1} h^{-1} in June (BULK-diet). The correlation between the numbers of ingested ciliates and the TL of the examined fish-species larvae collected at the same date was not significant ($p > 0.05$). However, the amount of ciliates ingested in May was significantly lower (Mann–Whitney U-test, $p < 0.05$) than the ingestion in June (Fig. 2).

The results of gut segmentation analysis showed that there were very clear differences between gut quarters. Hard-bodied cladocerans and rotifers were quite evenly distributed in all gut segments, while the soft-bodied species were present only in first gut quarters (Fig. 3). The distribution of food objects was statistically not different between studied species (Mann–Whitney U-test, $p > 0.05$); therefore, the pooled distribution data were used.

Ciliates were a very important food source for fish larvae. Ciliates formed, on average, 61% (FRESH-diet) of the total carbon biomass consumed by fish larvae in May. In June this value was 62%. Ciliate biomass contribution was highest in the diet of smelt, forming 69% and 89% of the consumed carbon in May and June, respectively (Fig. 4). These values were lowest in the diet of pike—45% and 23% in May and June, respectively.

Percentage of the carbon-specific ingestion rates of fish larvae (μg C of prey μg C larvae^{-1} d^{-1}) showed that without ciliates their daily food requirement to sustain

growth (20% of larval body C d^{-1} by MacKenzie et al. [1990]) would not be met in Võrtsjärv (Fig. 5). Metazooplankton contributed only an average 9.2% of larval body C d^{-1} in May (ranging from 6% to 15% between different fish species) and 8.7% in June (ranging from 5% to 17%).

Discussion

It has been acknowledged and documented by several authors that different food categories of fish vary in their resistance to digestion. Especially soft-bodied species are known for their rapid degradation (Sutela and Huusko 2000). Still, in most cases, the standard method for investigating the diet of fish larvae is to dissect the entire alimentary tract under a microscope and count all identifiable prey specimens. We found that when the diet of fish larvae was assessed based on the whole alimentary tract content we got biased results (Figs. 3, 4). This applied both to the diet composition and total amount of food consumed.

Much more reliable diet estimates could be achieved from the first quarter of the alimentary tract as supposed by Sutela and Huusko (2000). They studied metazooplankton, but also their results strongly suggested that the soft-bodied zooplankton species are underestimated in the diet if the analysis is based on the whole alimentary tract contents. Early feeding larvae have a tubiform alimentary tract and ingested food particles usually pass through the gut without interchanging their position. Foregut method is hard to apply to juveniles and adults, because newly ingested material is
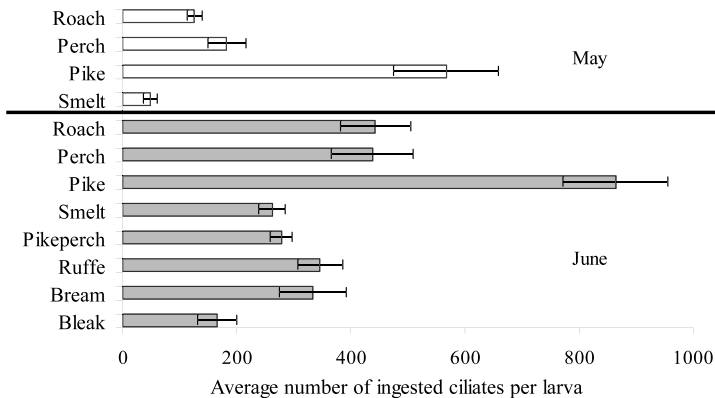

Fig. 1. Average number of ciliates found in guts of different larval species in Lake Võrtsjärv in May and June 2005 based on bulk measurements of the whole gut. Error bars indicate standard deviation.
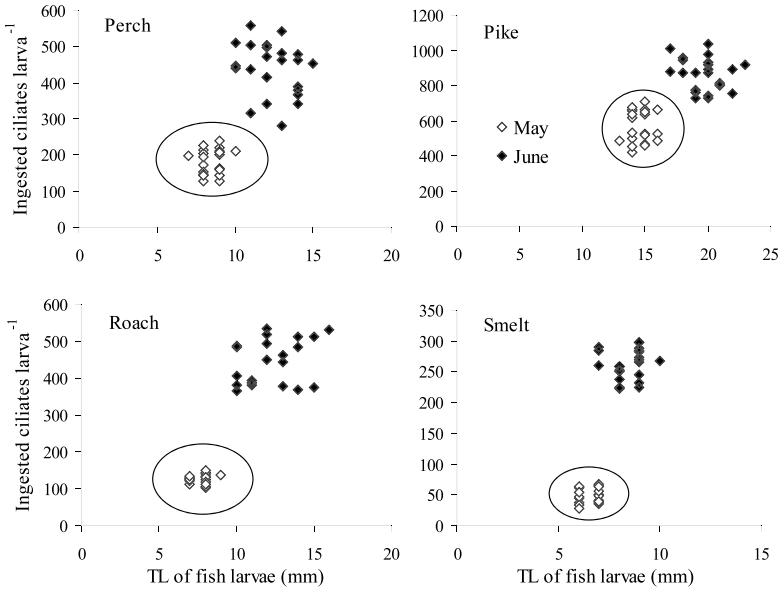
Fig. 2.   Total length (TL) of four species of fish larvae and number of ciliates found in their guts (BULK-diet) in Lake Võrtsjärv in May (circled) and in June 2005.

easily mixed with older material in the sac-like stomach (Sutela and Huusko 2000). It is evident that neglecting different prey digestion rates leads to diet underestimation and especially to underestimation of the role of protists.

To our knowledge, there are no data on protozoan digestion rate by larval fish. However, Ohman et al. (1991), using immunochemical detection of predation on ciliates, indicated that after 30 min antibodies of assimilated ciliate could be identified, and that after 2 h the antibodies decreased. In our study the average gut passage time was estimated to be 4 h, so that the ingested food objects pass each gut quarter in an hour. Ohman et al.'s (1991) findings fit well with ours, because we were able to detect ciliates only in the first two gut quarters. It is evident that they were digested during 2 h. From these data an important conclusion can be derived: that the diet of fish larvae
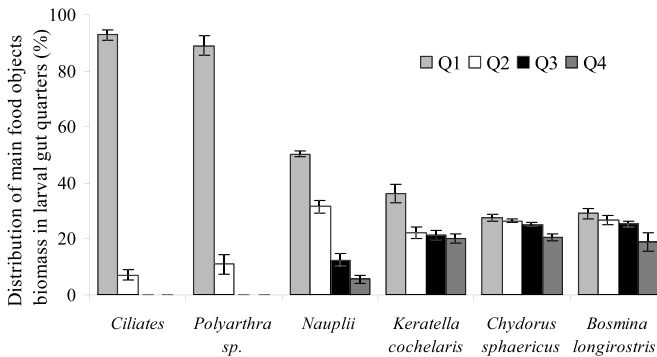

Fig. 3.   Average distribution of main-food-objects biomass in four gut quarters (Q1–Q4) of fish larvae in Lake Võrtsjärv. Error bars indicate standard deviation.
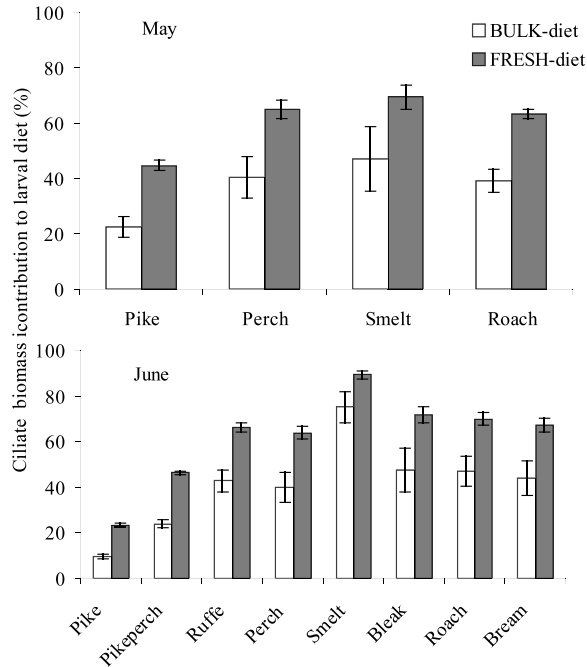
Fig. 4. Contribution of ciliate biomass to the total larval diet in Lake Võrtsjärv in May and June 2005. Compared are the diets reconstructed using the content of the first gut quarter (FRESH-diet) and of the whole alimentary tract (BULK-diet). Error bars indicate standard deviation.

should be determined on the basis of analysis of the foregut content. Still it must be recognized that even when using the foregut contents, it is possible to underestimate the ciliate contribution to fish diet because many ciliates are so delicate that they can burst on contact with any surfaces.

Quantitative studies on the role of the ciliates as larval food in freshwaters seem to be missing. Kamjunke et al. (2009) examined the role of large ciliate *Stentor* spp. as fish food in Patagonian lakes and found feeding rates up to 53 ciliates $fish^{-1}$ d^{-1}. Other ciliate species were not examined, however, so the overall contribution of ciliates to the fish diet could not be estimated.

In marine ecosystems, there have been several field studies emphasizing the role of protozoa as food for larval fish (Watson and Davis 1989) but only few researchers have attempted to quantify the role of dominating soft-bodied protozoan species (Fukami et al. 1999; Nagano et al. 2000; Figueiredo et al. 2005). Fukami et al. (1999), who studied the predation of fish larvae on naked protozoan microzoo-plankton in Tosa Bay, found that some larvae had ingested > 60 protozoa and most larvae contained > 30. They also found that in some fish species the maximum contribution of protozoa to the gut contents in terms of biomass was roughly equivalent to the other items of the diet.

Figueiredo et al. (2007), who studied the larval fish diets in the Irish Sea, found low numbers of cells in larval fish guts (average for all taxa of $\sim$ 0.8 cells $larva^{-1}$). The prey densities in the Irish Sea remained below 1 cell mL^{-1} during the study period. They also conducted experiments feeding flounder larvae (*Platichthys flesus*) with the ciliate *Euplotes* sp. and calculated ingestion rates as the difference of initial and final prey densities over the incubation period. They used ciliate densities up to 60 cells mL^{-1} and found maximum ingestion rates > 1000 ciliates $larva^{-1}$ h^{-1}.

Also, the other few studies of larval fish feeding only on ciliates (Ohman et al. 1991) indicated that, at high prey densities, fish larvae are able to ingest large numbers of ciliates (i.e., up to 1500 ciliates $larva^{-1}$ h^{-1}). Hunt von Herbing and Gallager (2000) demonstrated the ability of Atlantic cod (*Gadus morhua*) larvae to catch protozoa (prostomatid *Balanion*), and that they required protozoan food for survival in their early life stages.

All these experiments indicated that there was a clear increase in ciliate ingestion with higher prey concentration and concluded that protozoan biomass available in the water column can supplement, if not fully provide for, larval fish survival. A maximum average ciliate biomass in marine pelagic systems is estimated to be 0.04 μg C mL^{-1}
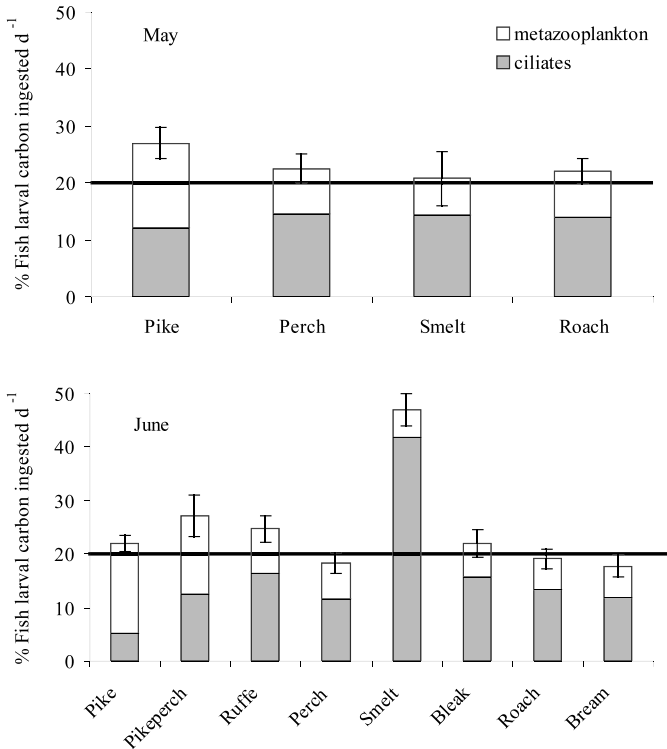
Fig. 5.   Percentage of the carbon-specific ingestion rates (μg C of prey μg C larvae^{-1} d^{-1}) of fish larvae in Lake Võrtsjärv in May and June 2005, and estimated metazooplankton and ciliate contribution to the larval diet. Horizontal lines in the figures indicate the estimated daily food requirement for larval fish to sustain growth (20% of larval body C d^{-1}). Error bars indicate standard deviation.

(Figueiredo et al. 2007). In Võrtsjärv, we found ciliate densities that are much higher compared with marine ecosystems (in May, 1.08 μg C mL^{-1}; 138 cell mL^{-1}) and close to, or even higher than, those used in above-mentioned feeding experiments. Our estimations of ingestion rates were much higher (up to 604 ciliates larva^{-1} h^{-1}) than those detected from the marine environment, but were lower than in laboratory experiments. The latter was expected, because larvae in the lake did not feed exclusively on ciliates but also on metazooplankton.

In Võrtsjärv, the ciliate community was dominated by oligotrichs, prostomatids, and scuticociliates. The most numerous ciliate species found were all fragile, without any hard parts and should therefore be very easily digested and consumed by larval fish. This makes ciliates excellent food for fish larvae in Võrtsjärv. Oligotrichs, prostomatids, and scuticociliates are often reported as a customary component of pelagic protozooplankton (Zingel et al. 2002). All the common ciliate species found in Võrtsjärv are also

frequently reported from other freshwater lakes (Foissner et al. 1999). Recent investigations show that most protists appear to have a cosmopolitan distribution. Therefore, it is reasonable to believe that ciliates form a readily available and solid food base in many lakes.

The ciliate abundances found in Võrtsjärv were generally higher than in most of the studied eutrophic temperate lakes but were still not exceptional (Table 2). In deep meso-eutrophic Lake Constance, spring abundances higher than 80 cells mL^{-1} were described (Müller et al. 1991), and in mesotrophic Ruster Poschen, which is a part of large and shallow Neusiedler See, even higher numbers were detected (annual mean = 51 cells mL^{-1}, maximum = 203 cells mL^{-1}). In 14 Mediterranean shallow lakes with varying trophic states, the mean ciliate abundance was 43 cells mL^{-1} and maximum 229 cells mL^{-1} (Conty et al. 2007). In North American Great Lakes, mean abundances up to 14 mL^{-1} were detected (Munawar and Lynn 2002). Taylor and Heynen (1987) reported ciliate abundances up to 40 cells mL^{-1} in Lake Ontario.

Table 2. Literature values of abundances (cells mL^{-1}) of pelagic ciliates in some eutrophic lakes from Zingel et al. (2002).

Lake	Range	Mean	Latitude
Valencia	—	218	tropical
Floridan lakes	—	55.5	subtropical
Võrtsjärv	0.6–191	45.7	temperate
Finnish lakes	0.6–35.6	9.8	temperate
Beloe	≤11	—	temperate
Esthwaite	≤9.2	—	temperate
Okaro	0.3–10.4	4.0	temperate
Dalnee	0–230	—	temperate

The high proportion of ciliates in total zooplankton biomass is not a unique feature of Võrtsjärv, but this has also been recorded in other lakes. Carrick and Fahnenstiel (1990), who studied the protozoan plankton of the Great Lakes Huron and Michigan, found that the ciliate biomass was equal to ~ 30% of the crustacean zooplankton biomass. Taylor and Heynen (1987) showed that ciliate biomass in Lake Ontario was of the same order of magnitude as crustacean zooplankton.

All fish species in our survey are very common in temperate freshwater lakes. Some species, such as pike, are found in freshwater throughout the northern hemisphere. Other studied species are found throughout Europe and Asia and have very close counterparts in North America (for example, yellow perch [*Perca flavescens*], walleye [*Sander vitreus*], and rainbow smelt [*Osmerus mordax*]). We conclude that the plankton and fish community of Võrtsjärv is not something unique among lakes of the temperate zone, and our findings can be applied in broad scale to many water bodies. Our conclusion is further supported by the fact that the few existing studies that consider the protozoa in larval fish guts have indicated their occurrence to be the rule rather than the exception (Montagnes et al. 2010).

We found that ciliates formed ~ 60% of the total carbon biomass consumed by fish larvae. Ciliates are very abundant in many lakes, they move more slowly than most of the metazooplankters, and they could be easily caught and quickly digested by young larval fish. Therefore, ciliates are crucial food for fish larvae. Our study showed that without ciliates the daily food requirement for larval fish that is estimated to sustain growth (20% of larval body C d^{-1}) would not be met in Võrtsjärv, and this is probably the case in many other freshwater lakes. Without studying ciliates in fish diets, we would easily come to the false conclusion that there are not enough prey to support larval growth and survival.

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IV

Zingel, P., Agasild, H., **Karus, K.,** Kangro, K., Tammert, H., Tõnno, I., Feldmann, T. & Nõges, T. The influence of zooplankton enrichment on the microbial loop in a shallow, eutrophic lake. Submitted to Freshwater Biology.

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

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SUMMARY

1. 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.

2. 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.

3. Our results indicate that 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.

4. The inverse relationship between a crustacean zooplankton and large predaceous ciliates is an important feature not adjusting only the structure of 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 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 factor shaping the zooplankton communities, affecting the structure, abundance and biomass of different organism groups (Mills & Forney, 1983; Jeppesen et al., 1992). Alterations in metazooplankton community structure can in turn influence algal and protozoan community (Gilbert & Jack, 1993; Wickham, 1995a; Jürgens & Jeppesen, 2000). 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 classical grazing food chain and microbial loop, which are connected to each other in many indirect and direct ways (Riemann & 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 being subject to greater scrutiny in aquatic food web studies.

The microbial loop is widely studied in lakes of contrasting trophic status and different geographical areas (Amblard *et al*., 1995; Kisand, Nõges & Zingel, 1998; Zingel, 2005; Zingel *et al*., 2006; Gobler *et al*., 2008; Peštova *et al*., 2008). With increasing primary productivity, ciliates may become the most important members of the microbial loop (Sherr & Sherr, 2002). They can be a significant food source for metazooplankters (Wiackowski, Brett & Goldman, 1994; Adrian & Schneider-Olt, 1999; Hansen, 2000) 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 & Nõges, 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, Arndt & Rothhaupt, 1994; Jürgens & 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 impact on protozoans as well as phytoplankton and large sized bacterial communities

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(Porter *et al*., 1988; Jürgens, 1994). Still, there are few studies (Jürgens *et al*., 1994; Jürgens & 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 (Pace *et al*., 1990; Jürgens, 1994). 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*., 1994; Jürgens, Skibbe & Jeppesen, 1999; Zöllner *et al*., 2003).

   Our recent works have shown the importance of planktonic ciliates in the food web of 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 & Nõges, 2010). The positive correlation between the biomass of ciliates and metazooplankters implies that the small-sized protozooplankters are rather regulated by bottom-up (food availability) than top-down factors (Zingel & Haberman, 2008; Zingel, 1999). Live labeling experiments conducted in Võrtsjärv (Agasild, Zingel & Nõges, 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 large fraction of crustaceans initiated a decrease also in total abundance of ciliates. In 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 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) to detect any cascading effects on bacterioplankton and on edible phytoplankton. Our hypothesis was that in a shallow eutrophic

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lake, ciliate community structure is controlled by copepods. Copepods can suppress predaceous ciliates, leading to increase in small-sized ciliate abundance.

Material and Methods

Study site

Võrtsjärv, the second largest lake in Estonia, is situated in South-Estonian pre-glacial basin, centred at 58°15'7''N and 26°1'47''E. The lake has an elongated shape, with surface area of 270 km^2, length of 34.8 km and width of 14.8 km. Despite of large surface area, the lake is shallow with a mean depth of 2.8 m and maximum depth of 6 m. Lake water is well mixed and turbid by the surface waves and currents. During the growing season Secchi depth usually does not exceed 1 m in 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 (Tuvikene *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 cyanobacteria four filamentous species *Planktolyngbya limnetica* [(Lemm.) Kom.-Legn.], *Limnothrix planktonica* [(Wołosz.) 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 dominates 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, exceeding rarely 100 μg C L^{-1}; biomass maximum usually occurs in June.

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Sampling design and collection of samples

The mesocosm experiment with control enclosures (natural zooplankton concentration) and with cyclopoid copepod enrichment treatments was carried out from August 20th to September 11th, 2009. Six transparent plastic enclosures (polyethylene bags with glass-fibre reinforcement; diameter 1.5 m; depth 4 m, volume 5 m^3), closed at the bottom and fixed in the wooden frame were filled with natural lake water. Enclosures 1 and 2 (further referred as M1) were left intact containing zooplankton of natural concentration and serving as control media. Rest of the enclosures were manipulated with two different copepod concentrations in treatment enclosures. Zooplankton for enrichment treatments was collected with 145-μm-mesh plankton net from the same lake 1 day prior the experiment. During months July, August and September the crustacean community and the total metazooplankton biomass of Võrtsjärv is usually dominated by cyclopoid copepods. The collected fraction of metazooplankton community contained overwhelmingly of copepods (adult and copepodite stages); the rotifer assemblage composed of small-sized species at that time, mainly *Anuraeopsis fissa* and *Keratella tecta* was not caught into this fraction. To obtain different cyclopoid copepod predation impacts in experimental treatments the collected zooplankton was divided to enclosures 3 and 4 (further referred as treatment M2) yielding the enrichment of approximately 2.5× of natural copepod biomass compared to control media (M1), and to enclosures 5 and 6 (further referred as treatment M3) yielding the enrichment of approximately 5× of natural copepod biomass. All the different treatments were performed in two replicates. The enrichment of 2.5× in the onset of experiment is observed as yearly highest abundances and biomasses of cyclopoid copepodites and their adult stages in Võrtsjärv; the enrichment of 5× was chosen to follow the response of ciliate assemblages to the contrasting change in predator biomass.

Experiment lasted 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 followed on 7 dates (on days 4, 7, 9, 13, 16, 19, 22) and phytoplankton primary production in 4 mesocosms (1 M1, 1 M2 and 2 M3) on 5 dates (on days 9, 13, 16, 19, 22). To analyse the plankton dynamics the integrated water sample was 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 analysis were preserved with acidified Lugol's solution (0.5% final

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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 was 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 400–1000x 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 & Stoecker, 1989).

Phytoplankton cells were enumerated and measured with an inverted microscope (Ceti Versus, Kontich-Antwerp, Belgium) at 100x or 400x 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. To 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 biovolume conversion factor of 0.22 mgC mm^{-3} (Reynolds, 1984).

Phytoplankton primary production (PP) was measured 5 times in situ with ^{14}C-assimilation technique (Steeman-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 look Nõges et al. (2011).

The abundance of heterotrophic bacteria was determined by DAPI (4´,6´-diamidino-2-phenylindole) direct count (Porter & Feig, 1980). Samples (3 mL) were filtered through 0.2-µm pore-size black polycarbonate membrane filters (Poretics Inc., Livermore, CA, U.S.A.) 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

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Zeiss MicroImaging GmbH, Jena, Germany) at 1000x 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 for1 h with ^{14}C-Leucine (306 mCi mmol^{-1}, Amersham Ltd.). The incubation was terminated with 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 60x magnification. Crustacean and rotifer carbon weight biomasses were estimated by length-carbon relationships (Telesh, Rahkola & Viljanen, 1998; Manca & Comoli, 2000), and by length dry weight relationships (Dumont, Van de Velde & Dumont, 1975; Bottrell *et al*., 1976) using the carbon conversion factor of 0.48 mg C per mg dry weight (Andersen & Hessen, 1991).

Statistical analysis

The program STATISTICA 8.0 for Windows (StatSoft,Inc. 2007) was used for statistical analyses. The comparison between control enclosures (M1) and copepod-enriched treatments (M2 & M3) was made using the Wilcoxon matched pairs test. 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.

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Results

Metazooplankton

Metazooplankton (MZP) community composition and dominants were in all mesocosms taxonomically quite similar. During the whole experiment copepods (mostly *Mesocyclops leuckarti*) dominated with respect to biomass, constituting 17.7-87.7% of the total MZP biomass (Figure 1a). Rotifers (mainly *Anuraeopsis fissa* Gosse, *Keratella tecta* Gosse, *Polyarthra luminosa* Kutikova,) were numerically dominant, accounting 68.1-95.0% of the total MZP abundance (Figure 1b). Cladoceran community was dominated by *Daphnia cucullata*, occurring generally in low numbers and being on some investigation days the only cladoceran in studied mesocosms. As a result of copepod enrichments the biomass of MZP in the treatment enclosures was initially clearly higher compared to control enclosure. Copepods formed 71% and 85% of total metazooplankton biomass in treatments M2 and M3, respectively, compared to 54% in control media M1. Thereupon 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. Copepod biomass values fluctuated between 35.2 to 7.1, 81.2 to 14.6 and 133.6 to 15.7 μg C L^{-1} in M1-M3, respectively.

Statistically, we found clear differences between all different treatments in the total biomass of MZP (Wilcoxon test, $p<0.05$), in the total biomass of copepods ($p<0.05$) and in the biomass and abundance of adult copepods+copepodites ($p<0.05$). On an average, the abundance and biomass of cladocerans were slightly higher in M2 (8.9 ind. L^{-1}; 12.2 μg C L^{-1}) and M3 (7.5 ind. L^{-1}; 8.8 μg C L^{-1}) compared to M1 (6.7 ind. L^{-1}; 7.5 μg C L^{-1}), however this was statistically not significant ($p>0.05$). As the rotifer community remained intact in the enrichment process their abundance and biomass followed quite similar trend in all treatments ($p>0.05$). Among rotifer species the abundance of *Polyarthra* spp. differed between M1 & M3 ($p<0.05$) and the biomass between M1 & M3 and M2 & M3 ($p<0.05$).

Ciliates

The total abundance of ciliates followed decreasing trend in all the studied treatments (Figure 2b), fluctuating in a range of 41.2–87.3 ind. mL^{-1} and being highest in M3. During the whole experiment, ciliate community was dominated mostly by bacterivorous species (*Rimostrombidium* spp., *Cyclidium* spp., *Uronema* spp.), accounting on an average 64.1 % of the total ciliate abundance (Figure 2b). The less abundant (on an average 1.42 ind. mL^{-1}) species were predaceous ciliates (mainly *Paradileptus elephantinus* Kahl) but their abundance

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and biomass increased at the end of experiment. This lead to the small increase also in the total ciliate biomass (Figure 2a), which generally fluctuated during the experiment only on a small scale (from 709 to 889 µg C L^{-1}). Almost during the whole experiment bacterivorous ciliates dominated with respect to biomass, constituting on an average 40.5 % of the total protozooplankton biomass. However, at the end of experiment the biomass of predaceous ciliates increased sharply, leading to great changes in ciliate functional groups – the final community in M1 & M2 was dominated by predaceous species and the proportion of bacterivorous ciliates decreased (Figure 3). We found also significant differences in the biomass of predaceous ciliates, between the treatments M1 & M3 ($p<0.05$) and M2 & M3 ($p<0.05$); and abundances between the treatments M1 & M2 ($p<0.05$) and M1 & M3 ($p<0.05$). There appeared no statistically significant differences ($p>0.05$) between the treatments in the abundance and biomass of herbivorous ciliates.

Considering different size classes (15-40 µm; >40 µm) the community was dominated by the ciliates in size class of 15-40 µm, constituting on an average 80.5 % of the total ciliate abundance. The abundance of larger species was quite low (on the average 12 ind. mL^{-1}) during the whole experiment. Statistically, the total abundance of ciliates as well the total abundance of smaller ciliates (15-40 µm) differed between M1 & M3 and M2 & M3 ($p<0.05$). In case of larger ciliate abundances we found statistically significant difference between the treatments M1 & M2 and M2 & M3 ($p<0.05$). The abundance of bacterivorous ciliates differed between the treatments M1 & M3 and M2 & M3 ($p<0.05$).

Predaceous ciliates influenced negatively the smaller size class – negative relationships were found between the biomass of predaceous and bacterivorous ciliates (Spearman rank order $R=-0.90$; $p<0.05$ in M2 & M3). In M1 the biomass of predaceous ciliates was also negatively correlated with bacterio-herbivorous ciliates ($R=-0.81$; $p<0.05$). Pooled data from all mesocosms and treatments showed clearly that predaceous ciliates controlled generally the abundance of smaller bacterivorous ciliates (Figure 4a). The biomass of MZP was positively correlated with the abundance of smaller (15-40 µm) ciliates in all the different treatments, especially with the biomass of bacterivorous ciliates (Spearman $R=0.71$; $p<0.05$ in M1 and M2). Pooled data from all mesocosms and treatments showed that copepods had a generally negative effect on the predaceous ciliate abundance and overall positive effect on the total ciliate abundance (Figure 4c & 4b, respectively).

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Bacterioplankton

The total number of bacteria ranged from 3.12–4.82 x 10^6 cells mL^{-1} in control mesocosm, similar range was observed also in treatments M2 (3.08–4.43 x 10^6 cells mL^{-1}) and M3 (3.35–4.87 x 10^6 cells mL^{-1}), decreasing slightly during the whole experiment in all different treatments (Figure 5a). Bacterial abundances were slightly higher in the M3 treatment and control mesocosms but the difference between all the treatments was not statistically significant ($p>0.05$). The biomass of total ciliate community was in a negative correlation 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 the treatments but again the correlations were significant only in M3. Considering bacterial production the lowest values occurred in M2 (Figure 5b), whereas statistically significant differences ($p<0.05$) were found only between M2 & M3.

Phytoplankton

Phytoplankton biomass fluctuated in a similar range between all the treatments (Figure 6a) being on average 5.8±1.0 (st.dev.) mg C L^{-1}. Differences in zooplankton amount did not reflect in total biomass and abundance of phytoplankton, no statistically significant differences were found between the treatments ($p>0.05$). In all treatments the majority of phytoplankton biomass (87.7±3.7%; 4.9±0.8 mg C L^{-1}) was formed by filamentous cyanobacteria (*Limnothrix planktonica* (Wołosz.) Meffert, *L. redekei* Van Goor (Meffert*)*, *Aphanizomenon skujae* Kom.-Legn. et Cronb., *Planktolyngbya* spp.) (Figure 6a). Diatoms (*Synedra* spp., *Navicula* sp., *Nitszchia* spp., *Aulacoseira ambigua* (Grunow) Simonsen) were numerically dominant (Figure 6b). 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-, chrysophytes, euglenophytes and cyanobacteria, forming altogether 14.4-36.3% of phytoplankton biomass and 4.6-13.5 % of abundance. There appeared no statistically significant differences ($p>0.05$) between the treatments in the abundance and biomass of all the different phytoplankton size-classes, also no differences were found in comparing of total edible size fraction (<30 μm). Significant differences were found only in case of chrysophytes, with higher values in M3, both in SC1 and SC2. Chrysophytes were present in rather constant amount (1200 cells mL^{-1}) in M1 during the entire experiment; there was a gradual increase in abundance towards the end of the experiment in M2 and largest

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fluctuations in abundance in M3. In respect of integral primary production the lowest values occurred in M2 and highest value in M1 (Figure 7). Statistical analyses showed significant differences in primary production between M1 & M2 and M2 & M3 ($p<0.05$).

Discussion

In Võrtsjärv copepods have two seasonal peaks, first in spring in May and the second usually in July or August co-occurring the highest abundances of ciliates 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 relatively broad feeding spectrum and they can have a strong direct top-down effect on ciliate abundances (Wiackowski *et al*., 1994; Wickham, 1995a; Hansen, 2000). As 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.

In present study we had clear differences between all the 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 predaceous ciliate numbers (Figure 4b & 4c, 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 of magnifying their number in 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 the effectiveness of microbial food web (Wickham, 1995a; Jürgens *et al*., 1999). Dominant species in Võrtsjärv crustacean community are relatively small cyclopoids (e.g. *Mesocyclops leuckarti* and *Thermocyclops oithonoides* G. O. Sars) and small-bodied cladocerans (*Chydorus sphaericus*, *Daphnia cucullata* and *Bosmina longirostris*). We have formerly carried out crustacean feeding studies in Võrtsjärv, using labelled natural ciliates (Agasild *et al.,* 2012), which 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

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ciliate total numbers was low. From July to September, the crustaceans feeding comprised only 3.9–6.8% of the ciliate standing stock.

In 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 & 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 & Santer, 1993). In our treatment enclosures the biomass of nauplii was substantially decreased on the experimental days of 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 top-down predation. Similar dynamics was not evident among the rotifer dominants with thick lorica, such as *Anuraeopsis fissa* and *Keratella tecta*. Soft-bodied rotifers are known to suffer strongly from copepod predation and their population dynamics are tightly coupled (Dieguez & Gilber, 2001; Devetter & Seda, 2006). As the *M. leuckarti* copepodites and females are strongly dependent on animal food (Hansen & 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 copepods 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 underlying reason probably lies in the ciliate composition. From June onward, the planktonic ciliate assemblage in Võrtsjärv 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 (Wiackowski *et al*., 1994; Wickham, 1995b; Adrian & Schneider-Olt, 1999; Hansen, 2000). 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 larger herbivorous species. Copepod preference to larger prey types among ciliates over small ones is often described, which implies a better detection and

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capture of larger motile prey compared to small ones (Wickham, 1995a; Adrian & Schneider-Olt, 1999; Vincent & Hartmann, 2001; Zöllner *et al*., 2003; Nishibe, Kobari & Ota, 2010). 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 (Nõges *et al*., 1998; Zingel & Nõges, 2010) is partly an adaption to increased cyclopoid predation. This trade-off is resulting also in relatively week transfer of microbial loop carbon through the food web during that phase of plankton seasonal succession.

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 genuses *Mesocyclops* and *Thermocyclops* have been reported to ingest various metazooplankters and insect larvae (Blumenshine & Hambright, 2003; Panogadia-Reyes, Crus & Bautista, 2004). Therefore, attacking large-sized (>300 µm) and relatively slow-moving ciliates is also likely as it is recently showed for epibenthic cyclopoid copepod (Reiss & Schmid-Araya, 2011). A strong inverse relationship between a 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 & 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.

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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. Contrary, 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 simultaneously declined (Figure 5b). 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 ciliates, not metazooplankters are the major consumers of edible algae (Zingel *et al*., 2007), consuming about 20% of the standing stock of nanoplankton. The small-size edible phytoplankton, which is under strong grazing pressure by herbivorous ciliates constitutes on an average only 10% of total phytoplankton biomass in Võrtsjärv (Agasild *et al*., 2007). As the herbivores were the least affected grazers in our experiment (Figure 2), their changed feeding could not cause any significant alterations in the edible phytoplankton biomass. The decline in primary production (Figure 7) in the middle of the experiment was not due to increased grazing, but because of poor light conditions during that day.

In conclusion, our results indicate that an increase in copepod zooplankton biomass in shallow eutrophic lake seems to favour 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 a crustacean zooplankton and large predaceous ciliates is an important feature not adjusting only the structure of ciliate community but also the energy transfer between meta- and protozooplankton.

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Figure captions

Figure 1. The average biomass (a) and abundance (b) of different metazooplankton (MZP) groups in studied mesocosms (M1 = natural copepod biomass concentration, M2 = 2.5× of natural copepod biomass, M3 = 5× of natural copepod biomass).

Figure 2. The average biomass (a) and abundance (b) of ciliates in different functional groups in studied mesocosms (M1 = natural copepod biomass concentration, M2 = 2.5× of natural copepod biomass, M3 = 5× of natural copepod biomass).

Figure 3. Average proportion of functional groups in ciliate biomass in the beginning and at the end of experiment between studied mesocosms (M1 = natural copepod biomass concentration, M2 = 2.5× of natural copepod biomass, M3 = 5× of natural copepod biomass).

Figure 4. Regressions between ciliates total abundance and biomass of predatory ciliates (a), ciliates total abundance and copepod biomass (b) and abundance of predatory ciliates and copepod biomass (c) using pooled data from all 6 studied mesocosms.

Figure 5. Bacterial abundance (a) and production (b) in studied mesocosms (M1 = natural copepod biomass concentration, M2 = 2.5× of natural copepod biomass, M3 = 5× of natural copepod biomass).

Figure 6. The biomass (a) and abundance (b) of presumably edible phytoplankton (PP) groups 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 between studied mesocosms (M1 = natural copepod biomass concentration, M2 = 2.5× of natural copepod biomass, M3 = 5× of natural copepod biomass).

Figure 7. Integral primary production according to treatments (M1 = natural copepod biomass concentration, M2 = 2.5× of natural copepod biomass, M3 = 5× of natural copepod biomass).

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Figures

Figure 1. The average biomass (A) and abundance (B) of different metazooplankton (MZP) groups in studied mesocosms (M1 = natural copepod biomass concentration, M2 = 2.5× of natural copepod biomass, M3 = 5× of natural copepod biomass).

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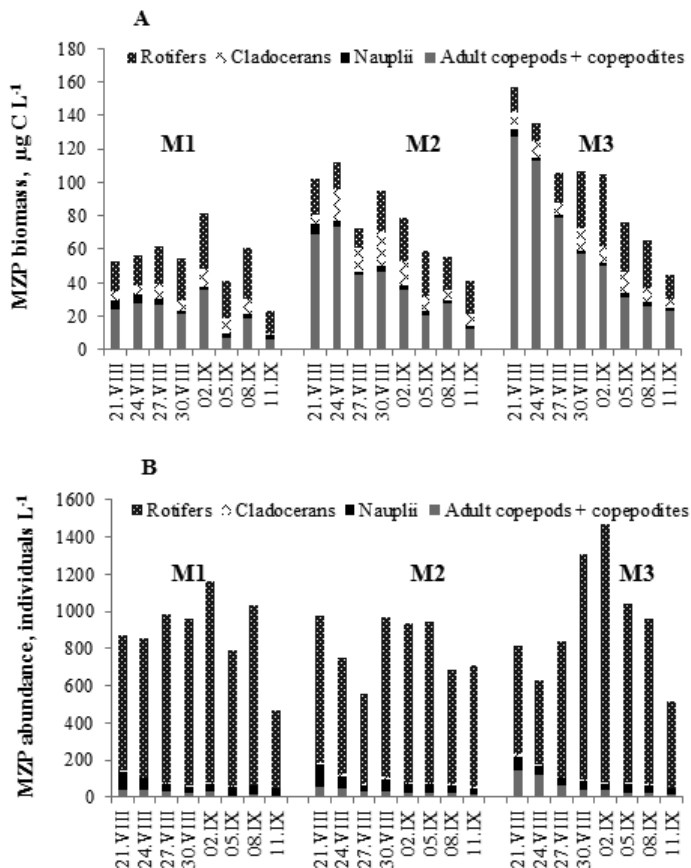Figure 4. Regressions between ciliates total abundance and biomass of predatory ciliates (A),

ciliates total abundance and copepod biomass (B) and abundance of predatory ciliates and

copepod biomass (C) using pooled data from all 6 studied mesocosms.

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Figure 5. Bacterial abundance (A) and production (B) in studied mesocosms (M1 = natural copepod biomass concentration, M2 = 2.5× of natural copepod biomass, M3 = 5× of natural copepod biomass).

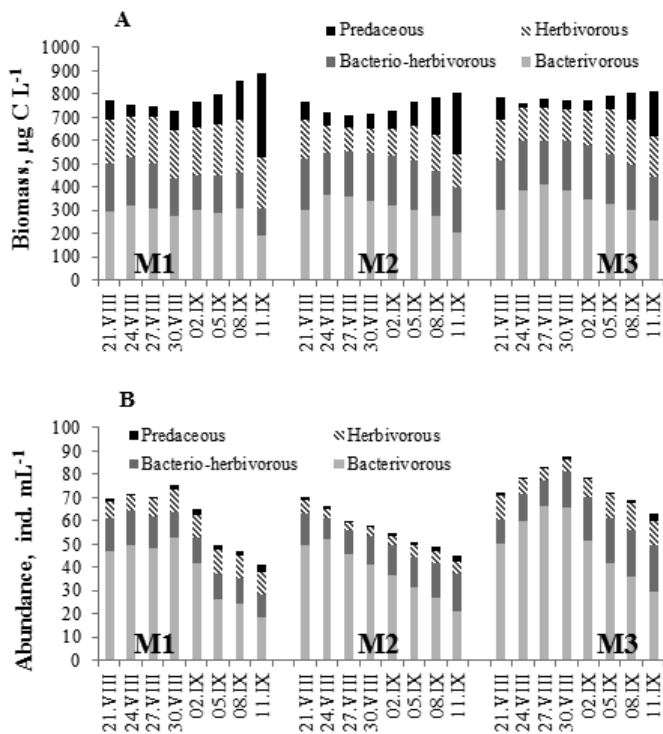Figure 6. The biomass (A) and abundance (B) of presumably edible phytoplankton (PP) groups size classes (SC1 2.0–5.0 µm, SC2 5.0–15.0 µm, SC3 15.0–30.0 µm), and total phytoplankton biomass and abundance between studied mesocosms (M1 = natural copepod biomass concentration, M2 = 2.5× of natural copepod biomass, M3 = 5× of natural copepod biomass).

Figure 7. Integral primary production according to treatments (M1 = natural copepod biomass concentration, M2 = 2.5× of natural copepod biomass, M3 = 5× of natural copepod biomass).

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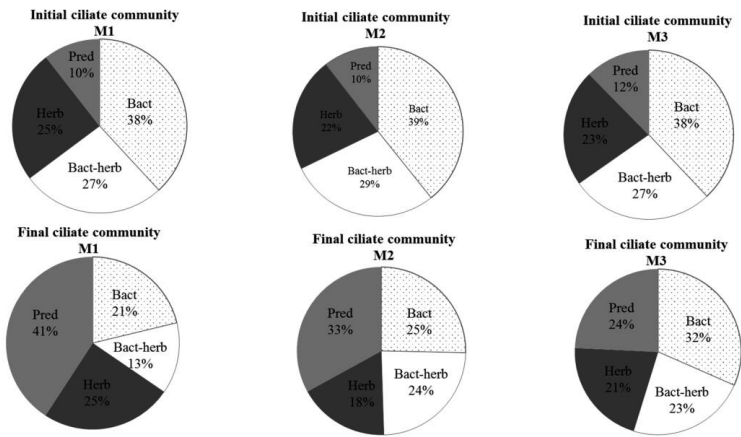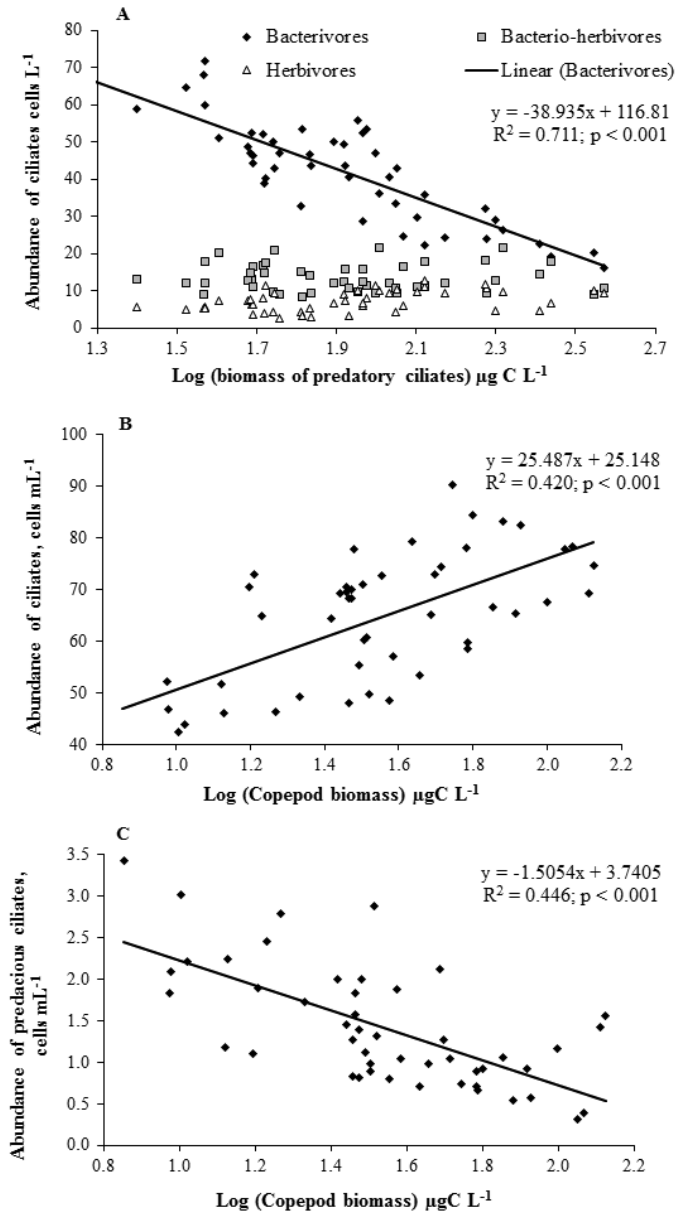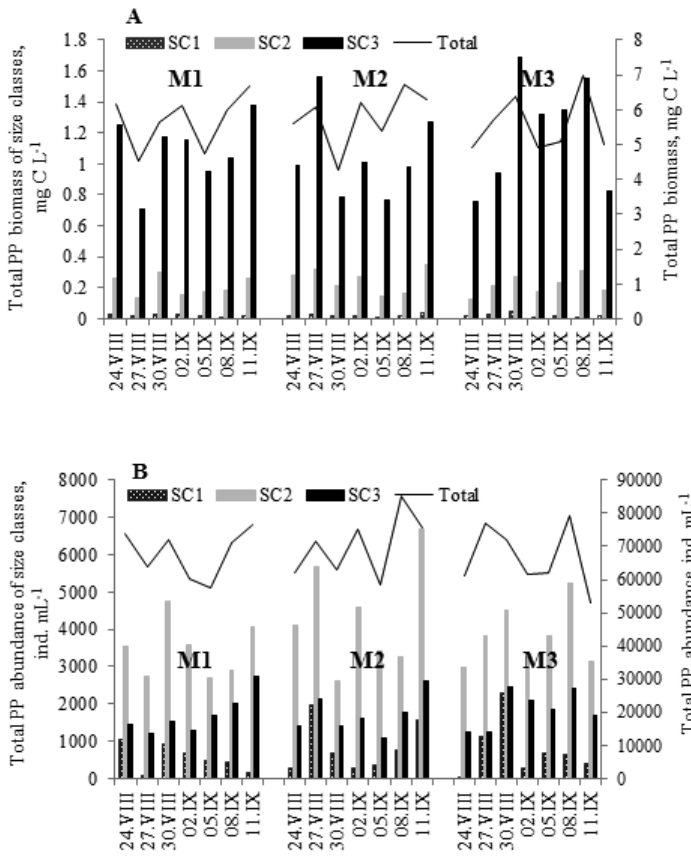Adrian R. & Schneider-Olt B. (1999) Top-down effects of crustacean zooplankton in a mesotrophic lake. *Journal of Plankton Research*, **21**, 2175–2190.

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Karus, K., Feldmann, T., Nõges, P. & Zingel, P. 2014. Ciliate communities of a large shallow lake: association with macrophyte beds. European Journal of Protistology, 50: 382–394.

Ciliate communities of a large shallow lake: Association with macrophyte beds

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Abstract

We investigated the influence of macrophyte composition on ciliate community structure in a large, shallow, eutrophic Lake Võrtsjärv. We hypothesized that macrophyte composition must have strong influence on the dispersal of ecologically different ciliate groups in a shallow lake and that more diverse macrophyte stands cause also a greater diversity in the ciliate community. In Võrtsjärv macrophyte distribution is spatially strongly polarized both in east–west and north–south directions in relation to abiotic factors. *Phragmites australis* and *Myriophyllum spicatum* were the most widespread species occurring in most parts of the lake. Correlation of environmental, macrophyte and planktonic ciliate variables confirmed the suggested spatial gradients. More diverse macrophyte stands supported a high species richness and abundance of epiplanktonic community but showed negative influence on the number and abundance of euplanktonic ciliate taxa. Opposite trends were found relative to the abundance of *P. australis*. Benthic ciliates showed a similar distribution pattern to euplanktonic taxa being most abundant in sites were the Shannon–Weaver index for macrophytes was low. Strong polarizing effect of the lake's vegetation on planktonic ciliate diversity was reflected in correlations of the number of ciliate taxa as well as the numbers of eu- and epiplanktonic taxa with geographic co-ordinates.
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Introduction

The presence of aquatic submerged vegetation is a very important factor in food webs of shallow lakes. Aquatic macrophytes can play a crucial role in regulating water quality in shallow lakes by inhibiting sediment resuspension and erosion (Blom et al. 1994), which may otherwise negatively influence the fitness of cladocerans (van Donk and van de Bund 2002). Macrophyte-associated cladoceran species can

be abundant in macrophyte beds (e.g. Lauridsen et al. 1996) as in colder climate regions they are less vulnerable to fish predation than pelagic cladocerans and have a potentially high grazing impact on algae (Stansfield et al. 1997). Many studies have confirmed that during daytime, large zooplankton in shallow lakes tend to leave the open water aggregating near submerged plant beds (Lauridsen et al. 1998). The resulting increase in large zooplankton density may consequently suppress the biomass of phyto- and protozooplankton (Jürgens and Jeppesen 1998).

Earlier studies have revealed that shifts in the species composition of littoral macrophyte communities will likely have a significant effect on the abundance and distribution of

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phytophilous macroinvertebrates (Basu et al. 2000; Colon-Gaud et al. 2004). Despite the fact that protozoa play a significant role in freshwater lakes and their communities can vary greatly between different lakes (Zingel et al. 2002; Zingel 2005), most studies have focused on open-water habitats, rather than on the littoral zone of lakes, and little is known about ciliate communities in various types of macrophyte beds (Mieczan 2007, 2008). Moreover, many ciliate taxa found in the pelagial of lakes are not euplanktonic but primarily benthic or sessile, actually less than one third of the known freshwater species are truly euplanktonic (Foissner et al. 1999). Many benthic species are temporarily washed out of the sediments or colonize the drifting aggregates in the lakes pelagial. In eutrophic lakes that undergo thermal stratification, members of the benthic community unable to tolerate anoxia, migrate upwards and become established on the oxycline as anoxia develops in the hypolimnion (Laybourn-Parry et al. 1990). Numerous ciliate species found in the pelagial of lakes are actually primarily sessile (Foissner et al. 1999). Sessile ciliates exhibit usually two distinct life stages: an attached stage for feeding, and a free-swimming stage for dispersal. Therefore, the free-swimming stages can regularly be found in the water column of lakes.

It is obvious that the complicated spatial and morphological structure offered by different macrophyte stands must have a strong impact to the ciliate species composition. Moreover, macrophytes themselves are under different hydrodynamic pressure depending on lake size, which results in different distribution patterns. Still there is very little information available on the effect of specific macrophyte communities on the species composition and abundance of planktonic protozoans. Studies on protists can yield valuable information about interactions in lake ecosystems as protists are (i) a biotic group with very large species diversity, (ii) occupy a large variety of niches in the pelagial, benthal, and littoral, and (iii) respond very quickly to changes in the environment. Therefore studying protists may offer a valuable insight into the structuring role of macrophytes in lake food webs. In shallow lakes planktonic communities are especially strongly influenced by macrophyte-associated and benthic fauna due to proximity of these habitats that enhances both accidental and migrational fauna exchange among them. It is acknowledged that climate change can alter remarkably the macrophyte stands in shallow lakes (Feuchtmayr et al. 2009; Kosten et al. 2009; Netten et al. 2010, 2011; Rooney and Kalff 2000), but as we do not know how plants influence the protozooplankton, we cannot predict the effect on food webs.

The aim of our study was to test how different macrophyte stands influence the species composition of planktonic ciliates in a shallow lake. The ciliate community in Võrtsjärv, a large and shallow lake in Estonia, is rich and abundant with an average abundance of 70–140 cells mL^{-1} during the vegetation period (Agasild et al. 2007; Zingel and Haberman 2008; Zingel and Nõges 2010). The ciliate spring peak in the pelagial is usually dominated by large herbivorous oligotrichs; the annual maximum abundance occurs in late July or early August when the pelagic community is dominated by small bacterivorous scuticociliates and oligotrichs (Zingel and Nõges 2010).

We hypothesized that macrophyte composition in a shallow lake must have a strong influence on the dispersal, diversity and abundance of ecologically different ciliate groups. We supposed that more diverse macrophyte stands lead also to a greater ciliate diversity. Therefore we studied the spatial variability in the ciliate community in relation to different macrophyte stands and other potential causal factors in Võrtsjärv.

## Material and Methods

Site description

Võrtsjärv is situated in Central-Estonia in a shallow preglacial basin and has an elongated shape in the north–south direction narrowing down southwards. The lake has an area of 270 km^2, length of 34.8 km and the largest width of 14.8 km. Despite the large area, the lake is shallow with a mean depth of 2.8 m and maximum depth of 6 m. The lake water is turbid, the water column non-stratified and well aerated by wave action and currents. During the growing season, Secchi depth usually does not exceed 1 m. The approximate volume of water at the mean water level is 750×10^6 m^3 (Jaani 1973). On average, the lake's water level fluctuates by 1.4 m per year, the maximum annual fluctuation has reached 2.2 m and the difference between the registered highest and lowest water level is 3.2 m (Nõges and Nõges 1998). The shoreline is 96 km long and rather simple characterized by a shoreline development index of 1.65. The southern and western shores are mainly flat, swampy and overgrown, the northern shores are sandy, and only in the eastern erosional areas a mixture of boulders, till, gravelly sand and sand dominate (Tavast 2004). The predominating westerly and south-westerly winds affect most the northern and eastern shores. The lake has a catchment area of 3374 km^2 (including the lake itself) of which 45.3% is covered by arable land and grassland and 46.1% by forests (Toming et al. 2009). Six larger and a dozen of smaller streams drain the catchment. The outflowing River Emajõgi flows into Lake Peipsi. The retention time of water is on average one year (Jaani 1990). Võrtsjärv has received annually 1300–4400 tons (4.81–16.3 g N m^{-2} year^{-1}) of total nitrogen and 35–100 tons (0.13–0.37 g P m^{-2} year^{-1}) of total phosphorus from the catchment (Järvet 1997). The average total nitrogen concentration in Võrtsjärv is 1.4 mg L^{-1} and that of total phosphorus 0.05 mg L^{-1} that characterize the lake as eutrophic to hypertrophic (Nõges and Nõges 2012). Till areas in the catchment rich in limestone contribute to the high alkalinity of water (on average, 3.3 mequiv. L^{-1}). The average pH is 8.1. The lake is ice-covered on average for 131 days a year (Nõges and Nõges 2013).

Macrophytes cover 18.8% (50.7 km^2) of the lake's surface area, whereby the area is colonized by well-developed

continuous belts of submerged (35.2 km^2; main taxon *Myriophyllum spicatum* L.) and emergent species (12.3 km^2; mostly *Phragmites australis* (Cav.) Trin. ex Steud.). The distribution of floating-leaved plants (3.2 km^2; *Nuphar lutea* (L.) Smith) is mainly restricted to the southern part of the lake (Feldmann and Mäemets 2004).

Sampling design and collection of data

The lake area covered with macrophytes was divided into eight lake sites (Fig. 1): seven shore sites and one site around
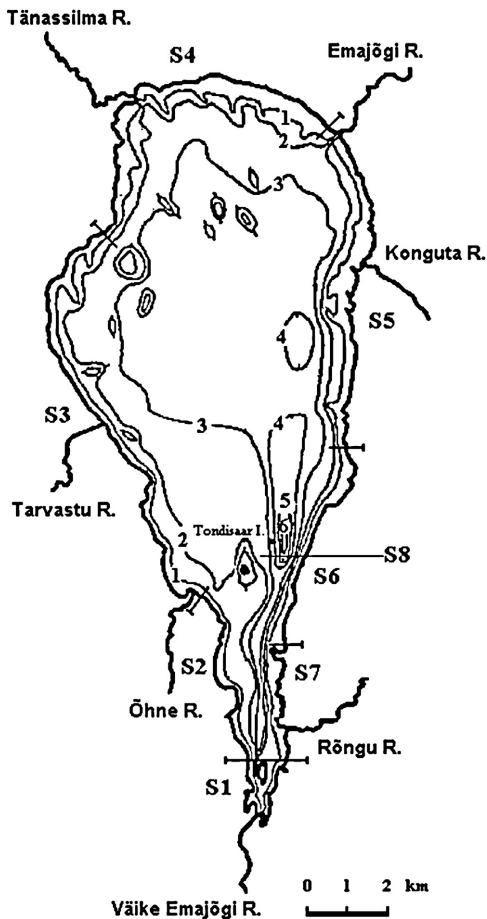
Fig. 1. Location of geobotanical sites in Võrtsjärv used in this study and based on earlier studies (von zur Mühlen and Schneider 1920; Mäemets, 1973); *Abbreviations*: S1–S8, studied lake sites; R, river; I, island.

the island (modified after Feldmann and Nõges 2007). This division followed the traditional approach of earlier investigators (von zur Mühlen and Schneider 1920; Mäemets 1973) and was based on the homogeneity of macrophyte communities within these sites.

Ciliate samples were collected from sampling points located in the afore-mentioned eight sites of the littoral zone (Fig. 1). Samples were collected during annual abundance peaks of ciliates in July (2002–2004) and August (1997, 1999–2004) from the same sampling points. Each sampling location was sampled 10 times and altogether 80 ciliate samples were analyzed. At each location the entire water column was sampled at half-meter intervals using a Ruttner water sampler. Samples were pooled and from each site one 250-ml subsample was preserved and fixed with acidified Lugol's iodine solution for analysis. Additional live subsamples were collected to adjust ciliate identification as suggested by Foissner et al. (1999). Ciliate abundance and community composition were determined using the Utermöhl (1958) technique. Samples were stored at 4 °C in the dark. Volumes of 50–100 mL were settled for at least 24 h in plankton chambers. Ciliates were enumerated and identified with an inverted microscope (Wild Heerbrug M40 and Nikon diaphot TMD) at 200–600× magnifications. The entire Utermöhl chamber was surveyed and if the total number of cells was below 150, an additional subsample was counted. For identification of ciliates several works were consulted (Foissner et al. 1991, 1992, 1994, 1995; Kahl 1930–1935; Kutikova and Starobogatov 1977). The taxonomy followed mainly the scheme of Corliss (1979). During further analyses, the taxa found were divided into three ecological groups depending on their preferred habitat: euplanktonic, epiplanktonic, and benthic (Foissner et al. 1991, 1992, 1994, 1995) (Table 1). We are fully aware that this division is not perfect and not entirely comprehensive. Epiplanktonic ciliates are usually attached to the substrate but have some free-swimming stadium in their lives serving for dispersal and, in this way, are quite often found also in plankton. Some epiplanktonic species may attach to filamentous diatoms and so occur in the plankton. Many basically benthic species migrate occasionally to the water column and may also be washed out from the sediment by resuspension. Still the most common ciliate species forming most of the biomass and abundance in Võrtsjärv could be divided quite reasonably in that way. For further ciliate diversity analysis we used the Shannon–Weaver (1949) index (H_S):

$$H_S = - \sum (N_i \times \ln N_i) \tag{1}$$

where N_i is the relative abundance of species i.

To compare ciliate species diversity between different lake sites we used Jaccard's Similarity Index (IS_J) (Kent and Coker, 1992):

$$IS_J = \frac{a}{a + b + c} \tag{2}$$

Table 1. Ciliate species found in Lake Võrtsjärv (1997, 1999–2004), their preferred habitats and number of lake sites where the species was present (in parenthesis). *Abbreviations:* EU, euplanktonic; EPI, epiplanktonic; BE, benthic.

Colpodida	
Bursaridium pseudobursaria Fauré-Fremiet, 1924	EU (3)
Colpoda steini Maupas, 1883	EU (2)
Cyrtolophosis mucicola Stokes, 1885	BE (1)
Haptorida	
Cyclotrichium viride Gajewskaja, 1933	EU (3)
Didinium nasutum (Müller, 1773) Stein, 1859	EU (3)
Monodinium balbiani Fabre-Domergue, 1888	EU (4)
Mesodinium pulex Claparéde & Lachmann, 1858	EU (3)
Askenasia volvox Claparéde & Lachmann, 1859	EU (4)
Lacrymaria olor Müller, 1786	BE (5)
Lagynophrya acuminata Kahl, 1935	EU (2)
Dileptus margaritifer Ehrenberg, 1838	BE (7)
Dileptus anser Müller, 1773	BE (7)
Paradileptus elephantinus Kahl, 1931	EU (1)
Pelagodileptus trachelioides Zacharias, 1984	EU (2)
Actinobolina radians Stein, 1852	EU (3)
Actinobolina smalli Holt, Lynn & Corliss, 1973	EU (4)
Phialna sp.	BE (7)
Lagynus elegans (Engelmann, 1862) Quennersted, 1867	BE (1)
Homalozoon vermiculare Stokes, 1887	BE (7)
Trachelius ovum (Ehrenberg, 1831) Ehrenberg, 1838	BE (5)
Plagiopyla nasuta Stein, 1860	EU (1)
Monilicaryon monilatus (Stokes, 1886) Jankowski, 1967	BE (3)
Heterotrichida	
Stentor roeselii Ehrenberg, 1835	BE (5)
Spirostomum sp.	BE (5)
Hymenostomatida	
Frontonia leucas (Ehrenberg, 1833) Ehrenberg, 1838	BE (5)
Lembadion magnum (Stokes, 1887) Kahl,1931	BE (6)
Paramecium aurelia complex	BE (6)
Disematostoma tetraedricum Fauré-Fremiet, 1924	EU (1)
Scuticociliatida	
Cyclidium sp. 1	EU (4)
Calyptotricha lanuginosa Penard, 1922	BE (6)
Uronema nigricans (Müller, 1786) Florentin, 1901	BE (7)
Cyclidium glaucoma Müller, 1773	BE (6)
Hypotrichida	
Oxytricha sp.	BE (5)
Euplotes sp.	BE (6)
Stylonychia mytilus Ehrenberg, 1838	BE (6)
Uroleptus piscis Müller, 1773	BE (7)
Stichotricha aculeata Wrzesniowski, 1866	BE (7)
Oligotrichida	
Rimostrombidium lacustris Foissner, Skogstad & Pratt, 1988	EU (2)
Rimostrombidium sp. 1	EU (4)
Rimostrombidium sp. 2	EU (3)
Rimostrombidium sp. 3	EU (5)
Limnostrombidium sp. 1	EU (6)
Limnostrombidium sp. 2	EU (4)
Halteria bifurcata Tamar, 1968	EU (3)
Halteria grandinella (Müller, 1773) Dujardin, 1841	EU (5)
Tintinnidium fluviatile Stein, 1833	EU (6)
Tintinnopsis cylindrata Koffoid & Campbell, 1929	EU (5)
Tintinnopsis tubulosa Levander, 1894	EU (5)
Tintinnopsis sp.	EU (5)
Codonella cratera Leidy, 1877	EU (7)

Table 1 (*Continued*)

Colpodida	
Peritrichida	
Pelagovorticella natans (Fauré-Fremiet, 1924) Jankowski, 1985	EU (1)
Vorticella convallaria complex	BE (2)
Vorticella aquadulcis complex	EPI (3)
Vorticella chlorellata Stiller, 1940	EPI (3)
Vorticella chlorostigma (Ehrenberg, 1831) Ehrenberg, 1838	EPI (6)
Opercularia sp.	EPI (6)
Scyphidia sp.	EPI (6)
Carchesium pectinatum (Zacharias, 1897) Kahl, 1935	EU (1)
Carchesium polypinum (Linnaeus, 1758) Ehrenberg, 1830	EPI (6)
Epistylis anastatica (Linnaeus, 1767) Ehrenberg, 1830	EPI (4)
Epistylis procumbens Zacharias, 1897	EU (1)
Ophrydium versatile Müller, 1786	EPI (6)
Pseudohaplocaulus anabaenae (Stiller, 1940) Warren, 1988	EPI (4)
Pseudohaplocaulus infrvacuolatus Foissner & Brozek, 1997	EPI (4)
Pseudovorticella monilata (Tatem, 1870) Foissner & Schiffman, 1974	EPI (5)
Cothurnia annulata Stokes, 1885	EPI (4)
Pleurostomatida	
Litonotus cygnus (Müller, 1773) Foissner, Berger, Blatterer & Kohmann, 1995	BE (6)
Loxophyllum utriculariae (Penard, 1922) Kahl, 1926	EPI (2)
Prostomatida	
Urotricha furcata Schewiakoff, 1892	EU (6)
Urotricha sp. 1	EU (4)
Urotricha sp. 2	EU (6)
Balanion planctonicum Foissner, Berger & Kohmann, 1994	EU (6)
Coleps hirtus Müller, 1786	EU (2)
Coleps spetai Foissner, 1984	EU (4)
Holophrya ovum Ehrenberg, 1831	BE (4)
Placus luciae (Kahl, 1926) Kahl, 1930	BE (4)
Suctorida	
Podophrya sp.	EPI (3)
Sphaerophrya magna Maupas, 1881	EPI (3)
Acineta grandis Kent, 1882	EPI (3)

where *a* is the number of common taxa; *b* is the number of taxa unique to the first sample; *c* is the number of taxa unique to the second sample.

To analyze the influence of macrophyte composition on the diversity of planktonic ciliates, in August 1997 the macrophyte data were collected with a combined transect and sampling quadrat method and summed up for different lake sites. The vegetation was described in 45 transects, which reached from the water line to the maximum colonization depth of macrophytes. In transects we registered all species met, the dominants, and the depth boundaries of species and ecological groups (helophytes, floating-leaved, submerged plants). A more detailed analysis was made in 112 sampling quadrats of $1\,m \times 1\,m$ (or $2\,m \times 2\,m$ in case of sparse vegetation) where, besides analyzing the species composition, we measured the shoot density and shoot length of dominant species. More detailed descriptions of the study methods are given in Feldmann and Nõges (2007). As the abundances of dominating macrophyte species have not changed over a rather long period of time (1995–2001) (Feldmann and

Mäemets 2004), using the macrophyte data from the year 1997 seemed justified.

To analyze the effect of macrophyte diversity on ciliates dispersal, the Shannon–Weaver (1949) index (H_S) was calculated as described above (1). Additionally we calculated the average macrophyte abundance (*A*):

$$A(\%) = \frac{n_i \times 100}{n} \qquad (3)$$

where n_i is the total shoot density of species *i* in all quadrats of one site; *n* is the total shoot density of all species (m^{-1}) in all quadrats in the site.

For further analysis the maximum distribution depth and average shoot density of two dominant species *Phragmites australis* and *Myriophyllum spicatum* were used.

To characterize the environment, four simple indices were used: the wind exposure index, the sediment index, the nutrient load index and the slope index (Feldmann and Nõges 2007). The wind exposure index (*W*) considers the frequency distribution of wind directions and the fetch of a shore. The

calculations were made for the eight cardinal points of the compass using the following equation:

$$W = \sum_{i=1}^{8} \frac{S_i \times T_i}{8} \qquad (4)$$

where S_i is the frequency (%) of wind in time from direction i; T_i is the fetch of wind (km) from the direction i, as length along that direction across the lake surface.

The sediment index (G) was based on the map of granulometric sediment fractions (Raukas 1995).

$$G = \sum_{i=1}^{9} \frac{f_i \cdot c_i}{100} \qquad (5)$$

Nine sediment fractions delimited on the map were given ascending numbers (f_i) from 1 to 9 (the bigger the number the finer the sediment) and the sediment index G for each shore site was calculated as the average sediment fraction number weighed by the percent cover of each fraction in that shore site (c_i).

In order to get an estimate of the eutrophication stress, the loads of nitrogen (K_N) and phosphorus (K_P) from rivers to the whole lake (kg ha^{-1} year^{-1}) discharging to each shore site, were summed up. If a river entered the lake on the border between two delimited shore sites, its loading was divided between the sites. The riverine nutrient loads were taken from the balance calculated by Nõges and Järvet (1998).

The slope index (L) index (measured from the 1:100,000 map) describes distance (m) between the 0-m and the 1-m isobaths. So the slope index is the reciprocal of the percental slope.

Data were analyzed using factor and correlation analyses offered by STATISTICA 8.0 (StatSoft, Inc. 2007). Factor analysis of biotic and abiotic characteristics was performed using the Principal Component Extraction method to describe the variability among observed indices. A non-parametric Spearman's correlation analysis was used to determine the relationships between the number of ciliates and other biotic and abiotic indices. Linear regression analysis was used to determine the relationships between the Shannon–Weaver index for macrophytes and the number of different ciliate (eu-, epiplanktonic and benthic) groups.

Results

Ciliate distribution

The differences between years in the same lake sites were checked using one-way ANOVA. As the differences were non-significant (in all cases $p > 0.05$), we pooled data from different years for every studied lake site. Also Jaccard's Similarity Index showed that differences between years were very small (IS_J always greater than 0.8), therefore the pooling of data seemed justified.
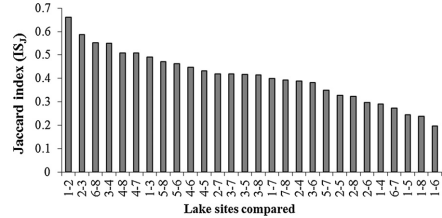

Fig. 2. Similarity of ciliate fauna (Jaccard index) at the studied geobotanical sites of Võrtsjärv (for explanation of lake site numbering see Fig. 1).

We found clear differences between lake sites. In general, most similar were the adjacent sites and most different the remote ones (Fig. 2). Highest similarity was found between the most sheltered southern sites 1–2 and 2–3 ($IS_J = 0.66$ and 0.59, respectively). The similarity was smallest between the most wind protected and exposed sites 1–6 and 1–8 ($IS_J = 0.19$ and 0.24, respectively).

The total number of ciliate taxa found was 79 (Table 1), ranging from 36 to 47 in different sites (Table 2). There was no significant difference in the number of ciliate species along the sampled sites. Despite that, the species present in different sites were far from being similar. Characteristic of the site-specific occurrence of taxa was that none of them was present at every studied site and there were 8 taxa, which occurred only at one single site. Such unique links were found randomly for several sites and none of the sites could be revealed for being inhabited by significantly more of these rare taxa. The average ciliate abundance at different sites ranged from 79.3 to 122.5 cells mL^{-1}; being significantly higher at site 6 (Fig. 3) where the number of ciliate species found was the smallest.

Most diverse ciliate groups were the haptorids (19 taxa), peritrichs (16), oligotrichs (13), prostomatids (8), and hypotrichs (5). Other groups present were the colpodids, heterotrichs, hymenostomatids, pleurostomatids,
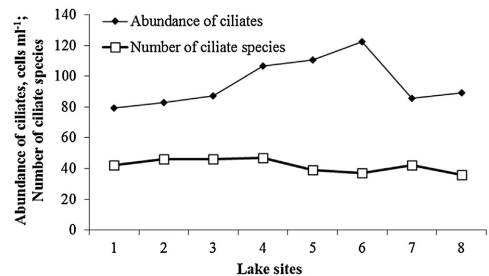
Fig. 3. Number of taxa and total abundance of ciliates at different lake sites (error bars represent standard error; for explanation of lake site numbering see Fig. 1).

Table 2. Wind (*W*), sediment (*G*) and slope index of the littoral zone (*L*); loading indices of nitrogen and phosphorus (K_N and K_P); number of macrophyte species, Shannon–Weaver index; average shoot density, maximum distribution depth, average abundance of *Phragmites australis* and *Myriophyllum spicatum* (modified after Feldmann and Nõges, 2007). Number of euplanktonic, epiplanktonic and benthic ciliates and total number of ciliates for the delimited sites in Lake Võrtsjärv. Numbering of shore sites according to Fig.1.

Indices/site no.	1	2	3	4	5	6	7	8
Abiotic indices:								
W	18.8	16.5	45.9	94.3	99	55.5	25.1	53.6
G	7	3	4.3	1.9	3.1	4.4	2.5	2.5
L (m)	160	161	152	322	316	171	250	350
K_N (kg ha^{-1} year^{-1})	61.3	61.3	20.9	19.9	6.5	0	61.3	0
K_P (kg ha^{-1} year^{-1})	1.3	1.3	0.2	0.6	0.2	0	1.3	0
Biotic indices:								
Total number of species	21	5	9	5	6	3	9	2
Shannon–Weaver index	2.35	1.39	1.49	1.21	0.91	0.57	1.18	0.54
Phragmites australis:								
Shoot density (m^{-2})	22	34	36	33	40	31	28	34
Distribution depth (m)	0.9	1.2	1.3	1.5	0.9	1.9	1.1	1.4
Abundance (%)	6.8	21	32.2	41.3	73.9	83.2	12.1	77.3
Myriophyllum spicatum:								
Shoot density (m^{-2})	1.9	3.4	6.1	7.6	10.2	4.2	1.7	5
Distribution depth (m)	1.3	3	2.1	2.3	2.6	2.5	3	2.5
Abundance (%)	0.3	4.2	1.4	7.1	2.3	7.5	1.5	22.7
Ciliates:								
Total number of taxa	42	46	46	47	39	37	42	36
Euplanktonic taxa	14	15	13	15	18	20	15	17
Epiplanktonic taxa	16	14	16	9	4	4	6	3
Benthic taxa	12	17	17	23	17	13	21	16

scuticociliates, and suctorids. Most common taxa were *Codonella cratera* Leidy (oligotrichs), *Dileptus margaritifer* (Ehrenberg) Dujardin (haptorids), *Uronema nigricans* (Müller) Florentin (scuticociliates) and *Stichotricha aculeata* Wrzesniowski (hypotrichs). Peritrichs and haptorids were the most diverse groups in three lake sites and oligotrichs in two sites. The number of euplanktonic taxa ranged from 13 to 20, being most stable compared to epiplanktonic (3–16) or benthic (12–23) taxa (Table 2).

Macrophyte distribution

The number of macrophyte species in sites varied from 2 to 21 and was highest in the southern part of the lake (Table 2). Also the Shannon–Weaver diversity index was higher in the southern and western sites (see the location in Fig. 1). The dominant species of the emergent plant zone, *Phragmites australis*, had a higher average shoot density compared to *Myriophyllum spicatum* dominating in the submerged plant zone. Maximum distribution depths of both species were quite variable and at all sites *Myriophyllum spicatum* reached deeper than *Phragmites australis*. The average relative abundance of *Myriophyllum spicatum* was several times smaller than the abundance of *Phragmites australis*.

Distribution of forcing factors

According to the wind index, the northern and eastern shores were most wind exposed while the most sheltered sites were located in the south (Table 2). The northern shore was characterized by coarsest sediments while the southern shores as well as the open water site in the southernmost end of the lake were dominated by fine sapropel sediments. The biggest nutrient loading index was found for sites 1, 2, and 7 (Table 2; Fig. 1) located in the southern part of the lake. At sites 6 and 8 the loading index was zero because of the absence of significant inflows in these shore reaches.

Ecological relationships

In the factor analyses carried out among the numbers of ciliate taxa belonging to various ecological groups and geographic co-ordinates, the two factors (hereafter referred as F1 and F2) described 79% of the total variability (Table 3). With F1 associated the geographic longitude, the number of euplanktonic and epiplanktonic taxa and the total number of ciliate taxa. In F2 higher factor loadings were accounted for by geographic latitude and the number of benthic taxa.

In general, the results of correlation analysis (Table 4) showed that abiotic and biotic indices of Võrtsjärv have a

Table 3. Factor loadings (loadings >0.70 marked bold) of the geographic co-ordinates and number of taxa in ecological groups of ciliates in Lake Võrtsjärv and the percentage of total variance explained.

Characteristic	F1	F2
Latitude	−0.06	**−0.87**
Longitude	**−0.71**	+0.08
Euplanktic ciliate taxa	**−0.93**	+0.03
Epiplanktic ciliate taxa	**+0.92**	+0.28
Benthic ciliate taxa	+0.18	**−0.88**
Total number of ciliate	**+0.86**	−0.38
Percent of total variance explained	50	29

stronger effect on the number of eu- and epiplanktonic ciliate taxa than on benthic taxa. Correlations between abiotic variables and the number of ciliate taxa in various ecological groups showed an increase of euplanktonic taxa from west to east. Also the nitrogen loading index had a negative influence on the number of eupanktonic taxa and a positive influence on the number of epiplanktonic taxa.

Further analyses with biotic variables showed clear trends between macrophyte and ciliate diversity. The Shannon–Weaver diversity index for macrophytes had a positive effect on the Shannon–Weaver diversity index for ciliates (all taxa included, Fig. 4b) and a negative effect on total ciliate abundance (Fig. 4a). Accordingly, the number of euplanktonic ciliate taxa and their abundance showed a negative (Fig. 4c, d) and those of epiplanktonic ciliates a positive

(Fig. 4e, f) relationship with the macrophyte diversity index. The abundance of benthic ciliates was negatively related to the macrophyte diversity index but this regression was statistically non-significant (Fig. 4g, $p > 0.05$). Among the dominating macrophyte species the relative abundances of *Phragmites australis* and *Myriophyllum spicatum* had a similarly positive influence on the number of euplanktonic and a negative influence on the number of epiplanktonic ciliate taxa (Table 4).

Discussion

Ciliate communities can well adapt to changing environment (Zingel and Paaver 2005). Many ciliate species are known to be highly adaptable and can be found in a range of habitats (e.g. Finlay et al. 1996). Altogether 679 freshwater species have been found in the plankton but only 180 are truly euplanktonic (Foissner et al. 1999). Alterations in ciliate species composition can be explained by differences in the available prey and/or the presence of different predators, but in many cases we lack the exact knowledge on both factors.

It is obvious that different macrophyte stands alter indirectly both the prey and the predators of ciliates. Warfe and Barmuta (2006) found that macrophyte architecture had strong, consistent effects on macroinvertebrate and periphyton communities: both were most abundant and diverse on the most structurally complex *Myriophyllum variifolium* J. Hooker and less abundant on the simplest one, *Eleocharis sphacelata* R. Br. The species composition of

Table 4. Spearman rank order correlations for number of ciliates taxa (in different ecological groups and total), environmental (G, sediment index; W, wind index; K_N, nitrogen loading index; K_P, phosphorus loading index; L, slope index) and macrophyte variables in Lake Võrtsjärv, correlations marked bold are significant at $p < 0.05$.

Characteristic	Euplanktonic	Epiplanktonic	Benthic	Total taxa
Abiotic variables				
Latitude	+0.27	−0.25	+0.49	+0.19
Longitude	**+0.71**	−0.57	−0.32	−0.69
G	−0.29	+0.51	−0.66	−0.10
W	+0.41	−0.42	+0.12	−0.09
K_N	**−0.73**	**+0.76**	+0.21	+0.61
K_P	−0.65	+0.70	+0.29	+0.65
L	+0.61	**−0.81**	+0.32	−0.35
Biotic variables				
Number of macrophyte species	−0.70	**+0.73**	+0.04	+0.40
Shannon–Weaver index for macrophytes	**−0.88**	**+0.99**	+0.20	**+0.73**
Phragmites australis:				
Shoot density	+0.15	−0.19	+0.25	+0.01
Distribution depth	+0.24	−0.24	+0.02	+0.00
Abundance	**+0.78**	**−0.76**	−0.12	−0.53
Myriophyllum spicatum:				
Shoot density	+0.24	−0.24	+0.27	+0.07
Distribution depth	+0.49	−0.44	+0.37	−0.15
Abundance	**+0.73**	**−0.77**	+0.24	−0.40
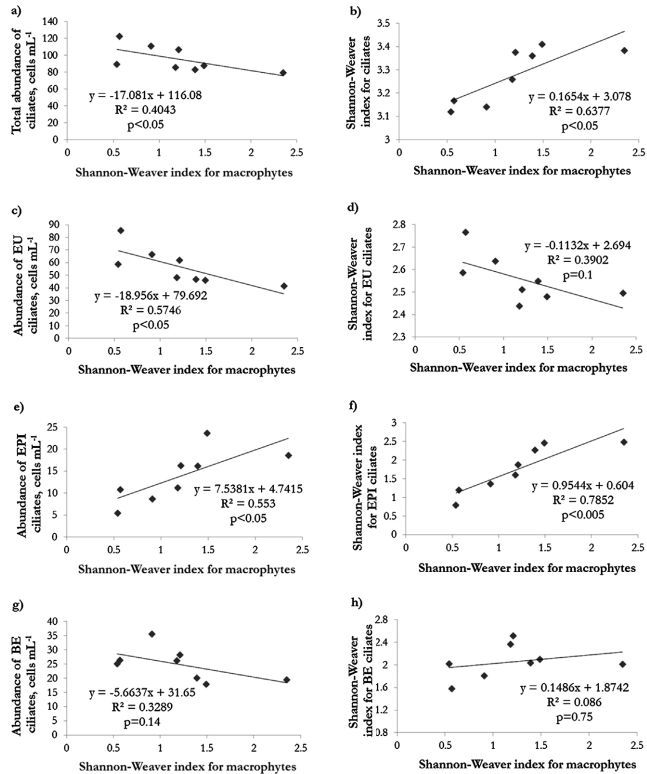
Fig. 4. Relationship between abundance and species diversity of ciliates (total (a and b), euplanktonic (c and d), epiplanktonic (e and f), benthic (g and h)) and macrophyte diversity.

sessile animals is known to differ depending on substrate structure (e.g., Stammer 1961), but still no radical differences have been found between species attaching to reed and other plants (Roos and Trueba 1977). In our study, epiplanktonic ciliates showed a strong positive correlation with the Shannon–Weaver index of macrophytes, both being most diverse in the southern sites. This shows clearly that epiplanktonic ciliates are favored by high diversity of macrophytes that provide them shelter and necessary substrates. The southern sites are also the most protected from the wind. Plainly the wind-induced disturbance is not favorable for this ciliate group. Our study showed that the more diverse are the macrophyte stands, the more numerous are also ciliate taxa found in the surrounding pelagial.

Contrarily, the euplanktonic taxa correlated negatively with the Shannon–Weaver index for macrophytes and were most diverse in most wind exposed northern sites. Only a few macrophyte species (e.g. *Phragmites australis*) were able to

tolerate the exposed conditions at the northern shore. Lacking the shelter of diverse macrophyte beds, the community of epiplanktonic ciliates declined, giving way to the more adapted euplanktonic taxa. At these sites the majority of taxa found belonged to oligotrichs. The planktonic ciliate community is in general characterized by a high percentage of oligotrichs and is classified as "Oligotrichetea" (Foissner et al. 1991). So the higher number of oligotrichs was not unexpected under the more "planktonic" conditions.

Benthic ciliates showed a somewhat similar distribution trend to euplanktonic taxa being most abundant at the sites were the Shannon–Weaver index for macrophytes was low. As these sites were characterized by a poor macrovegetation, a more intense resuspension of the sediments can be expected there. Rich aquatic macrophyte beds are known to inhibit the sediment erosion in shallow lakes (Blom et al. 1994). In our study the resuspension of sediments very likely increased the abundance of benthic ciliates washed to the pelagial. In proof

of that, the factor analysis (Table 2) revealed a decrease in the number of benthic ciliates from north to south, i.e. from the more exposed macrophyte poor sites with higher rates of sediment resuspension to more sheltered macrophyte rich sites.

A strong polarization of environmental conditions was demonstrated through the factor analysis of geographic co-ordinates. Geographic co-ordinates showed high loadings on the principal components, the first factor (F1) describing mainly the gradients of variables in the east–west direction and the second factor (F2) in the north–south direction. In the east–west direction the number of euplanktonic ciliates decreased and that of epiplanktonic ciliates increased (together with an increasing total number of ciliate taxa). In the north–south direction the number of benthic ciliate taxa decreased. These polarized patterns of ciliate diversity follow macrophyte distribution and have three main reasons – lake shape, dominating wind direction and nutrient load. The shape of Võrtsjärv is elongated in the north–south direction with the width growing larger toward north; the morphology of the lake coupled with its considerable size and prevailing wind directions generates a distinct contrast between the sheltered southwestern and the exposed northeastern shore. The southern basin is narrow and sheltered from the winds. The main nutrient loads enter to the lake from south and west. These factors together explain the species richness and abundance of the predominant emergent and submerged macrophytes (Feldmann and Nõges 2007).

Correlations with lake abiotic and macrophytes indices (Table 4) show that eu- and epiplanktonic ciliates are the most impacted ecological groups by the spatial variability of conditions in L. Võrstjärv. The positive correlation of the number of euplanktonic taxa with longitudes showed that the east–west polarization pattern described by Feldmann and Nõges (2007) existed also for ciliates: there were more euplanktonic taxa in eastern part of the lake where other ecological groups were suppressed by high water turbulence caused by dominating winds. Also the correlation of the nitrogen loading index with numbers of eu- and epiplanktonic taxa pointed to the polarized pattern in macrophyte distribution. Large inflows entering the lake from the south coupled with wind shelter in the narrow part of the lake generate favorable conditions not only for diverse macrophyte vegetation (Feldmann and Nõges 2007) but also for epiplanktonic ciliates. While higher nutrient loading favors epiplanktonic ciliate diversity that is explainable by the high nutrient affinity of macrophytes, it has an opposite effect on euplanktonic species, which prefer more pelagic conditions. We can also not rule out the possibility that epiplanktonic species, which are more adapted to the life in the littoral, are more effective in finding food or avoiding predation. The negative correlation between the number of epiplanktonic ciliate taxa and the slope index can be explained by mechanical stress: attached taxa are washed away in northern areas of Võrtsjärv because of the steeper slope of the littoral zone (Feldmann and Nõges 2007) causing a weaker attenuation of wave energy. In this area the wind influence and lake basin shape generate very rough conditions for attached ciliates. Such interactions make conditions more suitable for euplanktonic and less suitable for epiplanktonic ciliates.

Our study showed that diversity of substrates increased the diversity of ciliates. Generally, habitat characteristics, physical and chemical parameters of the water body, the quality and quantity of food available, and predation are among the factors regulating planktonic protozoan communities (Biyu 2000; Pierce and Turner 1992). Macrophytes may influence protozoan density probably by increasing the spatial heterogeneity and modifying food availability. Additionally, increasing complexity of macrophyte communities creates higher diversity of microhabitats and provides numerous micro-niches and a variety of potential shelters from predators (e.g., Jeppesen et al. 2000; Kuczyńska-Kippen 2005; Walsh 1995). The results reported in the literature demonstrate strong effects of habitat structure and plant architecture on the spatial distribution and taxonomic composition of aquatic organisms (Duggan 2001; Mieczan 2007; Pals et al. 2006). Mieczan (2007, 2008, 2010) found that the total number of ciliate taxa and their abundance increased together with the abundance and morphological complexity of macrophytes. Contrarily to that study, we found that the total abundance of ciliates decreased in the more diverse and complex macrophyte stands. It is seen in many communities that higher diversity leads also to higher abundances because more diverse communities appear to be more productive. We found that higher macrophyte diversity was accompanied by higher ciliate diversity (Fig. 4b) but led to lower ciliate abundance (Fig. 4a). The same pattern would emerge if to use ciliate biomass instead of abundance, as the main epiplanktonic species present are not larger than the euplanktonic species (Table 1). It is known that the metazooplankton in the littoral zone of lakes is more diverse than in the pelagic and contributes most to the metazooplankton biodiversity in lakes (e.g. Walseng et al. 2006). In shallow lakes pelagic zooplankters are known to migrate into vegetated littoral zones during the day to avoid fish predation (Burks et al. 2002) and in this way can further increase the overall zooplankton biomass found in the littoral. Similarly, our former studies in Võrtsjärv have shown generally higher metazooplankton abundances in macrophyte zones (Zingel et al. 2006). Therefore it seems likely that protozooplankton is under considerable grazing pressure in more diverse macrophyte stands leading to decreased abundances. This topic needs further studies as currently there is a lack of data concerning proto- and metazooplankton relationships in the littoral. We found highest ciliate abundances in the more wind-exposed sites where the community was dominated by the euplanktonic ciliate species. At site 6, where we found highest abundances, we concurrently recorded the lowest total number of ciliate species (Table 2) but the highest number of euplanktonic ciliate species. The majority of taxa found at that site belonged to oligotrichs, which are known to be most characteristic of the lake pelagial (Foissner et al. 1991) where they often reach

very high abundances (e.g. Zingel and Nõges 2008). Euplanktonic species are most adapted to the life in the pelagial being the only "true" planktonic ciliates. Therefore it was expected and not at all surprising that their abundances were much higher compared to the epiplanktonic and benthic species.

Conclusions

We found that diverse macrophyte stands supported a rich epiplanktonic ciliate community but showed a negative influence on the number of euplanktonic ciliate taxa. Also the abundance of epiplanktonic ciliates increased and that of euplanktonic ciliates decreased with increasing macrophyte diversity. Strong polarization of the lake's vegetation was clearly reflected in the planktonic ciliate diversity as expressed by the correlations of the total number of ciliate taxa and the numbers of eu- and epiplanktonic taxa with geographic co-ordinates.

Acknowledgements

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Publications:

1.1. publications

Karus, K., Paaver, T., Agasild, H. & Zingel, P. 2014. The effects of predation by planktivorous juvenile fish on the microbial food web. European Journal of Protistology, 50: 109–121.

Karus, K., Feldmann, T., Nõges, P. & Zingel, P. 2014. Ciliate communities of a large shallow lake: association with macrophyte beds. European Journal of Protistology, 50: 382–394.

Kolada, A., Willby, N., Dudley, B., Nõges, P., Søndergaard, M., Hellsten, S., Mjelde, M., Penning, E., van Geest, G., Bertrin, V., Ecke, F., Mäemets, H. & Karus, K. 2014. The applicability of macrophyte compositional metrics for assessing eutrophication in European lakes. Ecological Indicators, 45: 407–415.

Karus, K. & Feldmann, T. 2013. Factors influencing macrophyte metrics in Estonian coastal lakes in the light of ecological status assessment. Hydrobiologia, 704: 153–163.

Agasild, H., Zingel, P., Karus, K., Kangro, K., Salujõe, J. & Nõges, T. 2013. Does metazooplankton regulate the ciliate community in a shallow eutrophic lake? Freshwater Biology, 58: 183–191.

Zingel, P., Paaver, T., Karus, K., Agasild, H. & Nõges, T. 2012. Ciliates as the crucial food source of larval fish in a shallow eutrophic lake. Limnology and Oceanography, 57: 1049–1056.

3.2. publications

Feldmann, T. & Karus, K. 2013. Macrophyte flora and ecological status of the lakes Mõisalaht and Kasselaht. In: Kalamees, R., Kukk, T., Sammul, M. (Eds.). Estonian Maritima. Laelatu ajalugu ja loodus (15–29). Tartu: Greif.

6.4. publications

Karus, K. & Feldmann, T. 2012. Results of the studies of coastal lagoons. In: Kose, M., Lotman, K., Ott, I. & Übner, M. (Eds.), Coastal lagoons in Estonia and in the Central Baltic Sea region. Development history, geology and hydrology, biodiversity and nature conservation value. University of Tartu, pp. 57-97.

Karus, K. & Feldmann, T. 2012. Macrophytes of coastal lagoons. In: Kose, M., Lotman, K., Ott, I. & Übner, M. (Eds.), Coastal lagoons in Estonia and in the Central Baltic Sea region. Development history, geology and hydrology, biodiversity and nature conservation value.University of Tartu, pp. 52-53.

Presentations at international conferences:

Karus, K. & Feldmann, T. 2012. Factors influencing macrophyte metrics in Estonian coastal lakes in the light of ecological status assesment. In: WISER (Water bodies in Europe - Integrative Systems to assess Ecological Status and Recovery) Final Conference, 23-26 January, 2012, Tallinn, Estonia. pp. 88-90.

Kolada, A., Dudley, B., Willby, N., Nõges, P., Sondergaard, M., Hellsten, S., Mjelde, M., Penning, E., van Geest, G., Bertrin, V., Ecke, F., Mäemets, H. & Karus, K. 2012. Evaluating taxonomic composition macrophyte metrics for assessment of eutrophication in Europe - searching for the best responding common metric. In: WISER (Water bodies in Europe - Integrative Systems to assess Ecological Status and Recovery) Final Conference, 23-26 January, 2012, Tallinn, Estonia. pp. 92-94.

Karus, K. & Feldmann, T. 2012. Problems of macrophytes indicativity in Estonian coastal lakes. In: International Symposium on Aquatic Plants, 27-31 August, 2012, Poznan, Poland. p. 87.

Karus, K., Agasild, H., Zingel, P., Kangro, K., Salujõe, J. & Nõges, T. 2012. Does planktonic ciliate community regulate bacterioplankton in a shallow eutrophic lake? In: 3rd European Large Lakes Symposium, 08-12 October, 2012, Konstanz, Germany. p. 38.

Karus, K., Zingel, P., Paaver, T. & Agasild, H. 2013. The cascading effects of predation by planktivorous juvenile fish on the microbial loop. In: The 32nd Meeting of the German Society for Protozoology, 27. February - 02. March, 2013, Kartause Ittingen, Warth-Weiningen, Switzerland. p. 33.

Karus, K., Zingel, P. & Agasild, H. 2014. The influence of macrophytes on the feeding of larval fish on ciliates in a shallow Väinameri Sea. In: The Ocean Sciences Meeting (OSM), 23-28 February, 2014, Honolulu, Hawaii, USA.

ELULOOKIRJELDUS

I. Üldine informatsioon

Nimi:	Katrit Karus
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E-mail:	katrit.karus@emu.ee
Aadress:	Limnoloogiakeskus, Põllumajandus- ja keskkonnainstituut, Eesti Maaülikool, Rannu vald 61117, Tartumaa

Haridustee:	1992-2004 Tabivere Gümnaasium
	2004-2007 Eesti Maaülikool, BSc hüdrobioloogias
	2007-2009 Eesti Maaülikool, MSc *cum laude* hüdrobioloogias
	2010-2014 Eesti Maaülikool, PhD hüdrobioloogias

Töökogemus:	2008 - ... Eesti Maaülikool; Põllumajandus- ja keskkonnainstituut, Limnoloogiakeskus, laborant/spetsialist
2008-2009	Eesti Keskkonnauuringute keskus OÜ, Tartu filiaal, laborant

II. Teadustegevus

Peamised uurimisvaldkonnad: mikroobne ling, kõrgemate toiduahela lülide mõju mikroobikooslustele.

Grandid ja projektid:

01.01.14 - 31.12.19	Institutsionaalne uurimistoetuse teema IUT21-2, Järvede toiduahelad ja süsiniku metabolism valgala karbonaatsuse ja kliima gradiendis, uurija.
27.04.12 - 01.02.14	Riiklik keskkonnaseire programm 8-2/T12070PKLJ, Väikejärvede hüdrobioloogiline seire ja uuringud, uurija.

01.01.11 - 31.12.14	Eesti Teadusfondi grant ETF8969, Algloomade tähtsus ja roll kalavastsete toiduna, põhitäitja.
01.01.10 - 31.12.13	Eesti Teadusfondi grant ETF8511, Planktliste ripsloomade ja metazooplanktoni vahelised toitumisseosed eutroofses madalas ja hägusas järves, põhitäitja.
01.01.08 - 31.12.13	Sihtfinantseeritav teema SF0170011s08, Kas kliimamuutus muudab valgala- ja järvesiseste protsesside osakaalu madala järve süsinikubilansis?, doktorant.
18.10.13 - 30.11.13	Siseriiklik leping 8-2/T13148PKLJ, Narva veehoidla Eesti poole kallaste uurimine ujuvsaarte tekkimisvõimaluste avastamiseks ja meetmete väljatöötamiseks nende tekkimise ärahoidmiseks või vähendamiseks, uurija.
01.07.11 - 30.06.13	Estonia-Läti programm 2007-2013, 8-2/T11112PKLJ, Piiriülese Gauja/Koiva vesikonna parema ühise haldamise tegevused, uurija.
12.04.12 - 01.02.13	Siseriiklik leping 8-2/T12064PKLJ, Põlvamaa, Valgamaa ja Võrumaa järvede kompleksuuringu teostamine ja kaitsekorralduslike soovituste andmine, uurija.
04.09.12 - 20.09.12	Siseriiklik leping 8-2/T12132PKLJ, Koorküla looduskaitsealal asuvate Valgjärve, Udsu järve ja Kadastiku järve Natura 2000 vee-elupaikade määratlusele vastavuse kontrollimine ja hindamine „Loodusdirektiivi järve-elupaigatüüpide inventeerimise juhiste" alusel, uurija.
03.05.12 - 31.05.12	Siseriiklik leping 8-2/T12113PKLJ Muti maastikukaitseala kaitse-eeskirja muutmise eelnõu ekspertiis, uurija.

07.05.08 - 01.02.12	Riiklik keskkonnaseire programm 8-2/T8079PKPK, Väikejärvede hüdrobioloogiline seire, uurija.
13.04.11 - 17.12.11	Siseriiklik leping 8-2/T11049PKLJ, Lääne maakonna rannikujärvede uuringud, uurija.
28.02.11 - 30.11.11	Siseriiklik leping 8-2/T11039PKLJ, Jõe- ja järvetüüpide ökoloogilise seisundi klassipiiride interkalibreerimine (2011), uurija.
08.06.10 - 30.09.11	Siseriiklik leping 8-2/T10096PKPK, Viisjaagu, Lavatsi ja Valguta Mustjärve hüdrobioloogilised uuringud ja Viisjaagu, Lavatsi ja Valguta Mustjärve hoiualade kaitsekorralduskavade koostamine, uurija.
27.05.11 - 30.09.11	Siseriiklik leping 8-2/T11103PKLJ, Võnnu kahe paisjärve tervendamise eeltööd - limnoloogiline hinnang, uurija.
30.06.10 - 30.11.10	Siseriiklik leping 8-2/T10121PKPK, Jõe- ja järvetüüpide ökoloogilise kvaliteedi klassipiiride interkalibreerimine (2010), uurija.
10.06.10 - 19.11.10	Siseriiklik leping 8-2/T10104PKPK, Rubina looduskaitsealal paikneva Veisjärve elustiku inventeerimine ning Veisjäve veerežiimi muutmisega kaasnevate mõjutuste hindamine, uurija.
06.07.10 - 19.10.10	Siseriiklik leping 8-2/T10123PKPK, Põlva paisjärve limnoloogilise hinnangu/uuringu koostamine, uurija.

Publikatsioonide loetelu:
1.1. publikatsioonid

Karus, K., Paaver, T., Agasild, H. & Zingel, P. 2014. The effects of predation by planktivorous juvenile fish on the microbial food web. European Journal of Protistology, 50: 109–121.

Karus, K., Feldmann, T., Nõges, P. & Zingel, P. 2014. Ciliate communities of a large shallow lake: association with macrophyte beds. European Journal of Protistology, 50: 382–394.

Kolada, A., Willby, N., Dudley, B., Nõges, P., Søndergaard, M., Hellsten, S., Mjelde, M., Penning, E., van Geest, G., Bertrin, V., Ecke, F., Mäemets, H. & Karus, K. 2014. The applicability of macrophyte compositional metrics for assessing eutrophication in European lakes. Ecological Indicators, 45: 407–415.

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3.2. publikatsioonid

Feldmann, T. & Karus, K. 2013. Mõisa- ja Kasselahe veetaimestik ja ökoloogiline seisund. Kalamees, R., Kukk, T., Sammul, M. (Eds.). Estonian Maritima. Laelatu ajalugu ja loodus (15–29). Tartu: Greif.

6.4. publikatsioonid

Karus, K. & Feldmann, T. 2012. Results of the studies of coastal lagoons. In: Kose, M., Lotman, K., Ott, I. & Übner, M. (Eds.), Coastal lagoons in Estonia and in the Central Baltic Sea region. Development history, geology and hydrology, biodiversity and nature conservation value. pp. 57-97. University of Tartu.

Karus, K. & Feldmann, T. 2012. Macrophytes of coastal lagoons. In: Kose, M., Lotman, K., Ott, I. & Übner, M. (Eds.), Coastal lagoons in Estonia and in the Central Baltic Sea region. Development history, geology and hydrology, biodiversity and nature conservation value. pp. 52-53. University of Tartu.

Rahvusvaheliste konverentside teesid:

Karus, K. & Feldmann, T. 2012. Factors influencing macrophyte metrics in Estonian coastal lakes in the light of ecological status assesment. In: WISER (Water bodies in Europe - Integrative Systems to assess Ecological Status and Recovery) Final Conference, 23-26 January, 2012, Tallinn, Estonia. pp. 88-90.

Kolada, A., Dudley, B., Willby, N., Nõges, P., Sondergaard, M., Hellsten, S., Mjelde, M., Penning, E., van Geest, G., Bertrin, V., Ecke, F., Mäemets, H. & Karus, K. 2012. Evaluating taxonomic composition macrophyte metrics for assessment of eutrophication in Europe - searching for the best responding common metric. In: WISER (Water bodies in Europe - Integrative Systems to assess Ecological Status and Recovery) Final Conference, 23-26 January, 2012, Tallinn, Estonia. pp. 92-94.

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Karus, K., Agasild, H., Zingel, P., Kangro, K., Salujõe, J. & Nõges, T. 2012. Does planktonic ciliate community regulate bacterioplankton in a shallow eutrophic lake? In: 3rd European Large Lakes Symposium, 08-12 October, 2012, Konstanz, Germany. p. 38.

VIIS VIIMAST KAITSMIST

ESTA NAHKUR

COMPARATIVE MORPHOLOGY OF EUROPEAN ELK AND
CATTLE PELVES FROM THE PERSPECTIVE OF CALVING
EUROOPA PÕDRA JA VEISE VAAGNA VÕRDLEV
MORFOLOOGIA SÜNNITUSE SEISUKOHAST
Peaspetsialist **Mihkel Jalakas**, dotsent **Enn Ernits**
15. mai 2014

ANTS TAMMEPUU

EMERGENCY RISK ASSESSMENT IN ESTONIA
HÄDAOLUKORRA RISKIANALÜÜS EESTIS
Professor **Kalev Sepp**
26. mai 2014

ANTS-HANNES VIIRA

STRUCTURAL ADJUSTMENT OF ESTONIAN AGRICULTURE –
THE ROLE OF INSTITUTIONAL CHANGES AND SOCIOECONOMIC
FACTORS OF FARM GROWTH, DECLINE AND EXIT
EESTI PÕLLUMAJANDUSE STRUKTURAALNE KOHANEMINE –
INSTITUTSIONAALSETE MUUTUSTE JA PÕLLUMAJANDUSETTEVÕTETE KASVU,
KAHANEMIST NING TEGEVUSE LÕPETAMIST MÕJUTAVATE
SOTSIAALMAJANDUSLIKE TEGURITE OSA
Professor **Rando Värnik**, dotsent **Reet Põldaru**
5. juuni 2014

MONIKA SUŠKEVIČS

KNOWLEDGE, LEARNING AND LEGITIMACY IN MULTI-LEVEL
PARTICIPATORY ECOLOGICAL NETWORK GOVERNANCE
KAASAMISPROTSESSIDE TÕHUSUS JA TULEMUSLIKKUS ÖKOLOOGILISE VÕRGUSTIKU
PLANEERIMISEL NING RAKENDAMISEL MITMETASANDILISES VALITSEMISMUDELIS
Professor **Mart Külvik**, vanemteadur **Aivar Leito**
16. juuni 2014

MARKO KASS

EFFECT OF CRUDE GLYCEROL FEEDING ON FEED INTAKE, LACTATIONAL
PERFORMANCE AND METABOLIC STATUS OF DAIRY COWS
TOORGLÜTSEROOLI SÖÖTMISE MÕJU LÜPSILEHMADE SÖÖMUSELE,
PIIMAJÕUDLUSELE JA AINEVAHETUSLIKULE SEISUNDILE
Prof. Emer. **Olav Kärt**, Dr. **David Richard Arney**
26. juuni 2014

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