DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
67

THE ROLE OF PLANKTONIC CILIATES IN LAKE ECOSYSTEMS

PRIIT ZINGEL

TARTU 2001

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
67

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
67

THE ROLE OF PLANKTONIC CILIATES IN LAKE ECOSYSTEMS

PRIIT ZINGEL

TARTU UNIVERSITY
PRESS

Institute of Zoology and Hydrobiology, University of Tartu, Estonia

The dissertation is accepted for the commencement of the degree of *Doctor philosophiae* in hydrobiology on May 14, 2001 by the Doctoral Committee of the Faculty of Biology and Geography of the University of Tartu

Opponent: Dr. Pirjo Kuuppo (Plankton Ecology Group, Finnish Environment Institute, Kesäkatu 6, P.O. Box 140, FIN-00251 Helsinki, Finland)

Commencement: June 20, 2001, Room 310, Vanemuise 46

The publication of this dissertation is granted by the University of Tartu

Tartu Ülikooli Kirjastuse trükikoda
Tiigi 78, Tartu 50410
Tellimus nr. 342

"He called me in and asked me what I did,
exactly. Have you ever heard of such a thing?
What sort of question is that? This is a *university*!"

Terry Pratchett

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

Present thesis is based on the following papers, which are referred to in the text with respective Roman numerals

I Nõges, T., Kisand, V., Nõges, P., Põllumäe, A., Tuvikene, L. & Zingel, P. 1998. Plankton seasonal dynamics and its controlling factors in shallow polymictic eutrophic lake Võrtsjärv, Estonia. Int. Rev. ges. Hydrobiol. 83: 279–296.

II Zingel, P. 1999. Pelagic ciliated protozoa in a shallow eutrophic lake: community structure and seasonal dynamics. Arch. Hydrobiol. 146: 495–511.

III Kisand, V. & Zingel, P. 2000. Dominance of ciliate grazing on bacteria during spring in a shallow eutrophic lake. Aquat. Microb. Ecol. 22(2): 135–142.

IV Zingel, P. & Ott, I. 2000. Vertical distribution of planktonic ciliates in strongly stratified temperate lakes. Hydrobiologia 435: 19–26.

V Zingel, P., Huitu, E., Mäkelä, S. & Arvola, L. 2001. The abundance and diversity of planktonic ciliates in 12 boreal lakes of varying trophic state. (submitted to Arch. Hydrobiol.).

1. INTRODUCTION

There are about 20,000 species of the single-celled eukaryotic organisms we call protozoa and they are the most abundant phagotrophs in the biosphere (Finlay, 1990). Ciliates (*Ciliophora*) are one of the largest group of protozoans — over 7,000 species of ciliates are described and they can be found in almost every aquatic environment.

Feeding mechanisms vary widely in planktonic ciliates. A few, such as *Dileptus*, *Lacrymaria* and *Didinium*, are raptorial feeders, preying on other ciliates. They use direct interception to catch their prey. More typically, planktonic ciliates are considered to be suspension feeders. They use ciliary membranelles, which remove suspended particles of food from water (Fenchel, 1980). The size of the particles removed appears to be a function of the spacing of the membranelles, which varies with species (Fenchel, 1980). It is suggested (Stoecker, 1988) that many planktonic ciliates are not indiscriminate suspension feeders, but rather selective grazers, which can discriminate prey type based on variety of mechanisms including chemosensory ones. In strictly suspension-feeding ciliates, discrimination is based on mechanical properties of the particles, particularly size. It has been demonstrated, that certain ciliates can detect preferred prey through chemosensory stimuli over a distance, and can alter their swimming behavior to increase their encounter rates with prey cells (Verity 1988, Buskey and Stoecker 1989).

Recently, it has been recognized that microbial components of aquatic food webs are much more important than previously thought and that they significantly contribute to the functioning of pelagic ecosystems. Parallel to "classical" linear grazing food chain (phytoplankton — metazooplankton — fish) there exists a longer route; the microbial loop: dissolved organic matter — bacteria — protozooplankton – metazooplankton — fish (Azam *et al.*, 1983). The major effect of the microbial loop is that more trophic levels are involved in food chain than has been assumed in the conventional paradigm. Elongation of the food chain implies additional energy loss due to its increased dissipation. However, this pathway allows the channelling of a significant proportion of primary production, which would otherwise remain inaccessible at higher trophic levels, back to the grazing food chain. Ciliates are the major consumers of pico- and nanoalgae (Fenchel, 1987), which may often dominate in phytoplankton biomass and productivity and at the same time are not efficiently grazed by larger metazooplankters. Ciliates are also known to prey upon bacteria (Šimek *et al.*, 1998a, 1998b). It is believed that among protozoans heterotrophic nanoflagellates (HNF) are the most important grazers of bacteria (Sommaruga & Conde, 1997). But up to date, there are available some examples where the studied water ecosystem is characterized by extremely low HNF abundance and grazing on bacteria (e.g. Wieltschnig *et al.*, 1999). It is

reasonable to think that in cases like that ciliates become much more important as bacterivores. There is plenty of evidence that planktonic ciliates are an important food resource for large metazoan plankton (Stoecker & Capuzzo, 1990, Dolan & Coats, 1991, Gifford, 1991). Thus, planktonic ciliates may be a critical link between microbial and macroscopic components of pelagic food webs and play considerable role in the functioning of the microbial loop.

As the microbial loop consists of many trophic levels and carbon is lost through respiration at each level, the microbial food web is often regarded as an inefficient pathway of energy compared with the classic grazing food chain (Pomeroy & Wiebe, 1988). Still there exist many "shortcuts" in the microbial food web which make possible to reduce carbon losses. And in many cases these are ciliates that play the key role in functioning of these shortcuts.

The number of papers dealing with freshwater protozooplankton has increased recently. Nevertheless, concerning planktonic ciliates there is still a lack of detailed studies in many fields. For example up to date, comparatively few reports are available in which bacterivory of freshwater pelagic ciliates has been well documented *in situ*. Also the number of papers describing in detail the seasonal succession and vertical distribution of ciliates in freshwater ecosystems is not great.

The main aim of present study was to establish the role of planktonic ciliates in lake ecosystems, i.e.: to describe in detail ciliates seasonal dynamics and changes in community composition, to represent the changes in ciliates abundance and community structure along a lakes trophic gradient, to describe the pattern of ciliates vertical distribution and to test the hypothesis that bacterioplankton can be controlled by protistan grazing.

2. STUDY AREA

The studies I, II and III incorporated into present thesis were carried out in Lake Võrtsjärv, Estonia. Lake Võrtsjärv is a large (270 km^2) and shallow (mean depth 2.8 m, maximum depth 6 m) eutrophic (mean total phosphorus concentration is 54 $\mu g\ l^{-1}$, total nitrogen concentration 1.6 mg l^{-1}) lake situated in the Central Estonian depression of preglacial origin. During the vegetation period, Secchi depth usually does not exceed 1 m. The water temperature reaches its maximum in July (20.1°C as an average) and ice cover lasts from November to April (135 days on an average). Water samples from L. Võrtsjärv were taken only from one sampling station situated near the deepest part of the lake, close to the Võrtsjärv Limnological Station. Although, this point does not certainly represent all variation occurring in lake plankton, however, a transect study in 1981 (Nõges, 1987) and one-day sampling cruise covering 14 different points in 1996 (unpublished data) revealed no significant spatial differences. Moreover, flow model simulations have shown that the lake water is rapidly mixed (Kivimaa *et al.*, 1998), thus the macropatchiness should not play an important role. Study IV was carried out in eight small (1.7–17.3 ha) and deep (maximum depth reached 11.5–29.7 m) strongly stratified lakes in Southern Estonia. All these lakes are with very small water exchange or closed. They belong to the different lake types and are mostly dimictic, two of these are considered being meromictic. All studied lakes were characterized by the anoxic hypolimnion. Paper V focuses on twelve lakes situated in a relatively small area in Southern Finland. These studied lakes are classified into the following trophic categories on the basis of Forsberg & Ryding (1980): oligotrophic (two lakes), mesotrophic (four), eutrophic (five), and hypereutrophic (one). Clear-water, i.e. oligohumic (water colour $\leq$ 40 mg Pt l^{-1}), mesohumic (>40, $\leq$100 mg Pt l^{-1}) and humic lakes (>100 mg Pt l^{-1}) occurred among studied waterbodies.

3. METHODS

Sampling

The ciliate samples were collected using either Moltchanov's sampler (Papers I and II), Ruttner sampler (Paper III), Limnos sampler (Paper V) or a special vacuum probe (see similar to Guerrero *et al.*, 1985) (Paper IV). In case of Lake Võrtsjärv (Papers I, II and III) the integrated samples were used (water taken from the whole water column with an interval of 0.5 m was mixed in one bigger tank). In paper I weekly sampling was performed for one year (1995) and in paper II weekly (1995) and beweekly (1996) for two years. In paper III samples were collected weekly during spring 1998 (March 31 to May 12).

In paper IV the sampling was carried out once in summer 1998. Altogether eight subsamples from each lake were collected for the ciliate counts: two from the epi- and the hypolimnion and four from the metalimnion.

In the case of paper V the samples were collected on four occasions during 1997: in early spring (April), when lakes were still ice-covered; in spring (late May-early June), after the ice-break and complete overturn of the water column; in summer (August) during the period of steep thermal stratification; and in autumn (October) after breakdown of the summer stratification. Integrated samples were taken from the whole water column in lakes where the maximum depth was < 2 m. In deeper lakes, the epi- and hypolimnion were sampled separately; for the epilimnion an integrated sample from the uppermost 2 m was taken, and for the hypolimnion one sample from the depth of double depth was obtained.

Sample fixation and microscopy

Ciliate samples were fixed with acidified Lugol's solution. Ciliate biomass and community composition were determined using the Utermöhl (1958) technique. Volumes of 10–100 ml were settled for at least 24 hours in plankton chambers. Ciliates were enumerated and identified with an inverted microscope at 100–600X magnifications. The entire content of each Utermöhl chamber was surveyed. Ciliates were usually identified to genus by consulting several works (e.g. Kahl, 1930–1935, Patterson & Hedley, 1992, Foissner & Berger, 1996). The taxonomy followed mainly the scheme of Corliss (1979). The first 20 measurable specimens encountered for each taxon were measured. Biovolumes of each taxa were estimated by assuming geometric shapes. Specific gravity was assumed to be 1.0 g ml^{-1} (Finlay, 1982) and biomass was expressed as wet weight (Papers I, II) or converted to carbon weight (Papers III, IV, V) using a factor of 190 fg C μm^{-3} (Putt & Stoecker, 1989).

Ciliates grazing on planktonic bacteria was measured from fluorescently labeled bacteria (FLB) uptake rates. FLB (heat killed and DTAF stained) were obtained according to the protocol of Sherr & Sherr (1993). Samples were incubated for 45–360 min, but usually no longer than 60 min at the ambient temperature of the lake water. Subsamples for FLB counts in food vacuoles of ciliates were taken with 10–20 min intervals and averaged uptake rate was calculated on the bases of linear range of FLB uptake. Samples were fixed with buffered formalin (1% final concentration). Preserved samples were stained for 1–2 min. with DAPI at a final concentration 2 $\mu g\ ml^{-1}$ and 5–15 ml of water was gently filtered through 0.8-µm pore-size black isopore (Poretics Inc.) filters. Ciliates and the contents of their food vacuoles were examined with a Leica DMBR fluorescence microscope under 1250x magnification using blue light (470/505 nm, OG 515). The results of staining with both DTAF and DAPI were examined on the same microscopic field by switching filter sets without disturbing the position of the slide and between 200–400 fields were examined. As much as possible the dominant taxa of fluorescently stained ciliates were identified based on knowledge on the composition of parallel Lugol fixed samples.

4. SUMMARY AND DISCUSSION OF PAPERS

Paper I focuses on plankton seasonal dynamics and its controlling factors in Lake Võrtsjärv. Weekly samples for one year were analysed using the PEG model approach. The aim of the study was to follow the seasonal succession of phyto-, zoo- and bacterioplankton in conditions where the microbial loop is supposed to be important.

Paper II represents more detail description of ciliates seasonal succession and community structure over two year period in Lake Võrtsjärv. The study was initiated because Paper I showed that the ciliates have very important role in Lake Võrtsjärv, making up over 50% of the whole zooplankton biomass.

The aim of Paper III was to test the hypothesis that bacterioplankton is controlled by protistan grazing during the spring and to compare the importance of ciliates and heterotrophic nanoflagellates (HNF) in planktonic bacterivory.

Paper IV focuses to the vertical distribution and community structure of ciliates in eight small strongly stratified temperate lakes. Its main purpose was to describe the differences between ciliates communities in lake's epi-, meta- and hypolimnion and to analyze the factors causing these differences.

The aim of Paper V was to evaluate the ciliate communities along a wide trophic gradient in 12 boreal lakes ranging from oligotrophic to hypereutrophic.

The seasonal dynamics of planktonic ciliates

In Lake Võrtsjärv the abundance (1995 mean = 39 cells ml^{-1}, 1996 mean = 60.1 cells ml^{-1}) and biomass (1995 mean = 0.68 mg l^{-1}, 1996 mean = 2.91 mg l^{-1}) of ciliates was high and similar to the range reported for eutrophic subtropical lakes (Beaver & Crisman, 1982). Ciliates accounted for 50 to 64% of the total zooplankton biomass (in 1995 and 1996, respectively). Oligotrichs, haptorids, scuticociliates, prostomatids and peritrichs dominated the community of ciliates. Greatest peaks in ciliate abundance occurred in spring (May) and in late summer (July, August) reaching values up to 191.1 cells ml^{-1} (Figure 1). The spring peak was made up by large sized herbivorous oligotrichs (mostly *Strombidium* and *Tintinnidium*). In late summer when the annual maximum abundance was described, the community of ciliates was dominated by small bacterivorous species as *Strobilidium* sp., *Halteria* sp., *Uronema* sp. and *Cyclidium* sp. In autumn abundance of ciliates peaked in October. In this period the community was dominated by herbivorous oligotrichs (1995) or by haptorids and prostomatids (1996). In winter the dominant species were *Tintinnidium fluviatile, Strobilidium* sp. (Ø > 50 µm) and *Coleps* sp. The biomass maxima did not coincide with maximum abundances and were tied up with the mass occurrence of large-sized carnivorous species. The abundance

and biomass of planktonic ciliates were significantly positively ($p<0.0001$) correlated with temperature, chlorophyll a, bacterial abundance and metazooplankton biomass.

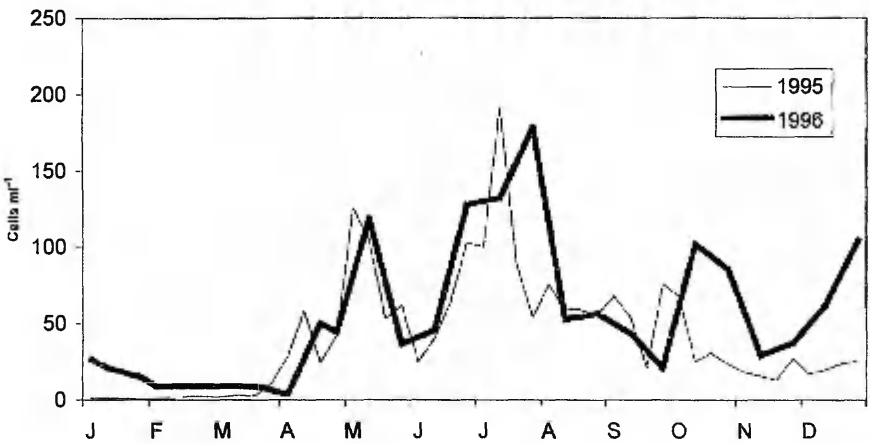

Figure 1. Ciliate abundance in Lake Võrtsjärv in 1995 and 1996

Changes in planktonic ciliates community structure along a trophic gradient of lakes

The ciliate abundance and biomass showed a trend of increasing toward eutrophication, and both were strongly correlated ($p<0.05$) with the abundance of bacteria and phytoplankton. The mean cell densities for ciliates were: 2.9, 5.0, 9.8 and 20.3 cells ml^{-1} for oligotrophic, mesotrophic, eutrophic and hypereutrophic lakes, respectively (Figure 2). The ciliate communities were dominated by four orders: oligotrichs, haptorids, scuticociliates and prostomatids. The relative importance of oligotrichs and haptorids decreased along a trophic gradient from oligotrophy to eutrophy. In more eutrophic conditions these orders were progressively replaced by small scuticociliates and prostomatids. The diversity of ciliates in lakes was positively correlated ($R^2 = 0.84$) with the concentration of chlorophyll a. The peak abundances, in terms of both cell numbers and biomasses, were recorded in spring and early summer (most eutrophic lakes) or autumn (oligotrophic and most mesotrophic lakes). There were only very small differences between the abundance and biomass of planktonic ciliates in coloured and clearwater lakes for a given trophic state. Compared with clearwater lakes, the coloured lakes displayed greater

importance of prostomatids and smaller importance of oligotrichs in the eutrophic range and greater representation of oligotrichs in the mesotrophic range.

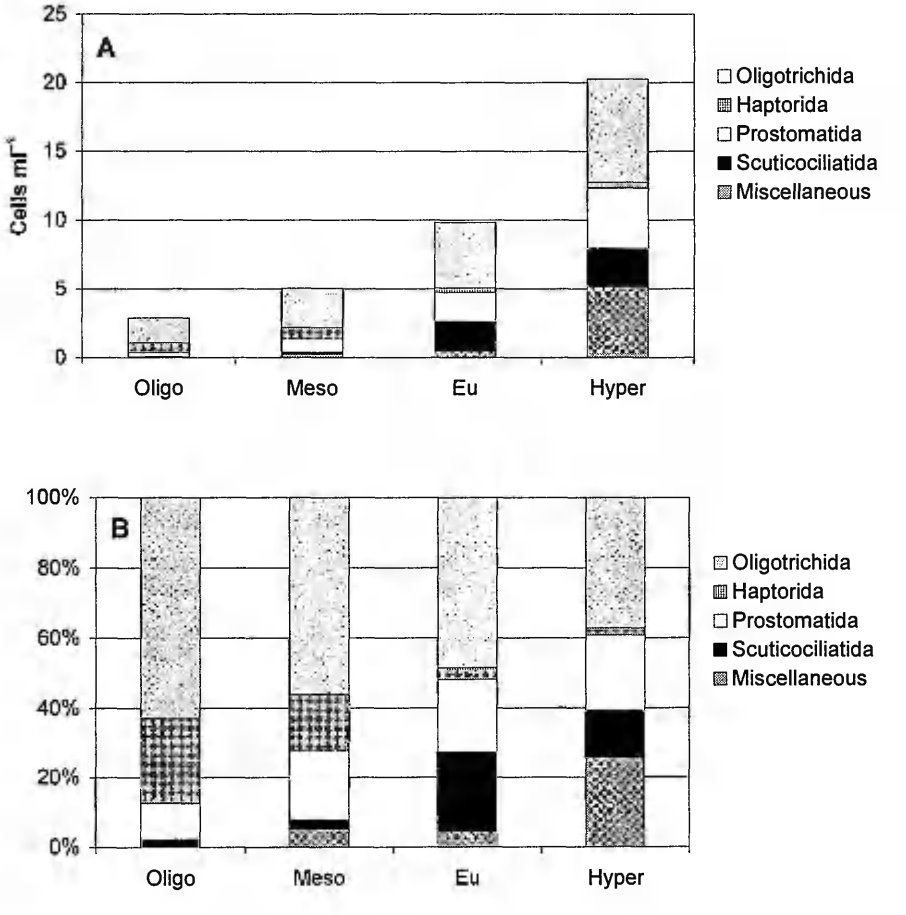

Figure 2. The abundance (A) and relative importance (B) of different groups of ciliates in study lakes grouped according to trophic state

Vertical distribution of planktonic ciliates

In our study lakes ciliate abundance and biomass were usually the highest (mean 39.9 cells ml^{-1} and 181.9 µg C l^{-1}) in the epi-, and the lowest (mean 8.2 cells ml^{-1} and 97.6 µg C l^{-1}) in the hypolimnion. In some lakes the greatest abundance and biomass were found in metalimnion. The community of ciliates

was dominated by five orders: *Oligotrichida, Haptorida, Prostomatida, Scuticociliatida* and *Peritrichida*. The community composition varied greatly with depth. In the epilimnion the ciliate numbers were dominated by oligotrichs but small algivorous prostomatids, peritrichs and haptorids were also numerous. In the metalimnion these groups were replaced by scuticociliates and mixotrophic prostomatids. In the hypolimnion species known as benthic migrants appeared. Also scuticociliates stayed important in the hypolimnion. We found a positive significant correlation ($p < 0.05$) between ciliate numbers and Chl a and bacterial densities. The correlation was strongest in epilimnion. Only in the hypolimnion the correlation between ciliates numbers and Chl a was not significant. In majority of lakes we found positive correlation between oxygen concentrations and abundance of peritrichs and oligotrichs. Negative correlation between the scuticociliate numbers and oxygen concentrations was found.

Planktonic ciliates grazing on bacteria

Bacterivorous ciliates were extremely important grazers of bacteria during spring in the shallow and eutrophic L. Võrtsjärv. Ciliate numbers (55 to 180 cells ml ^{1}) dominated clearly over the heterotrophic nanoflagellate's (HNF) numbers (8.4 to 27.1 cells ml^{-1}). There is no literature reference of so low numerical ratio of HNF/ciliates (~0.15). The population of ciliated protozoans was dominated by scuticociliates (*Uronema sp.*, *Cyclidium spp.*, one unidentified scuticociliate) and oligotrichs (*Strobilidium spp.* (Ø > 50 µm), *Strobilidium spp.* (Ø < 50 µm), *Strombidium sp.*). Ciliates were predominant grazers of bacteria (Figure 3), they showed higher clearance rates (14–65 nl $ciliate^{-1}$ h^{-1}) than HNF (1–11 nl HNF^{-1} h^{-1}), the total grazing rate of ciliates (on average 2398 bacteria h^{-1} ml^{-1}) was 28 times higher than total grazing of HNF (87 bacteria h^{-1} ml^{-1}). Among protozoans, HNF are usually believed to be the most important grazers of bacteria. But this belief is mainly based on knowledge that HNF abundance is usually 1–2 orders higher (Sommaruga & Conde, 1997; Šimek *et al.*, 1997). In our study oligotrichs and small scuticociliates were responsible for >97% of total mortality of bacteria due to grazing. In general terms protozoan grazing balanced the bacterial production but was not sufficient to support ciliate growth: on the basis of conversion factors the calculated generation time of bacterivorous ciliates was long, on average 121 days (ranging from 43 to 198 days). At the measured grazing rates bacterivorous ciliates need to have some other food source beside bacteria.

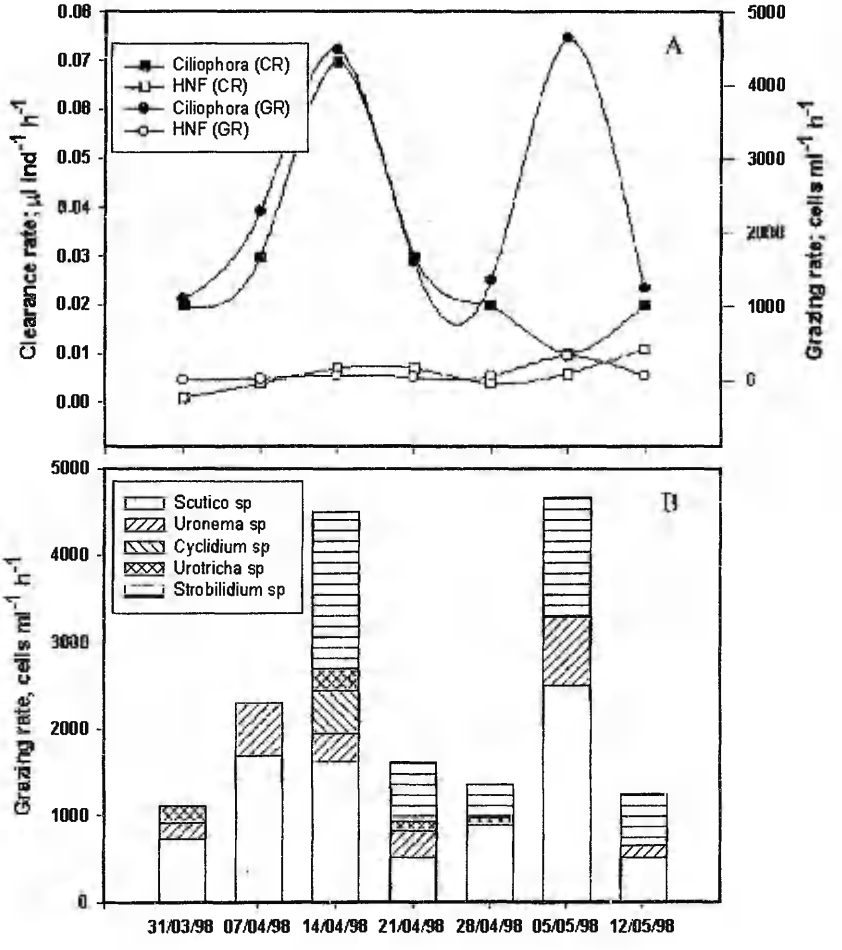

Figure 3. Clearance rate (CR) µl protozoa (individual cell)$^{-1}$ h^{-1}; total grazing rate (GR) cells h^{-1} ml^{-1} of protists in L. Võrtsjärv in 1998. A: CR and GR of ciliates and HNF; B: GR of the main bacterivorous ciliates

5. CONCLUSIONS

1. Community structure of ciliates is highly dynamic and responds rapidly to the changes in the environment and ecosystem. In case of Lake Võrtsjärv the greatest peaks in ciliate abundance occur in spring (mostly herbivores) and in summer (mostly bacterivores).
2. Ciliates are distributed throughout the lake's water column and can be found even in the anoxic conditions. Ciliate abundance and biomass are usually the highest in the epi-, and the lowest in the hypolimnion but in some cases the maximum values are found in metalimnion.
3. Most common ciliate groups found in lakes are: oligotrichs, prostomatids, peritrichs, scuticociliates and haptorids. In anoxic hypolimnion the dominants are mostly so called benthic migrants (belonging to many groups e.g. heterotrichs, hypotrichs, odontostomatids and hymenostomatids).
4. Ciliate abundance and biomass show a trend of increasing along a trophic gradient from oligotrophy to eutrophy. The relative importance of oligotrichs and haptorids decreased in more eutrophic lakes and they were progressively replaced by small scuticociliates and prostomatids.
5. Ciliates can in some cases be the predominant grazers on bacteria and be responsible for > 97% of total mortality of bacteria due to grazing. But even then they should have also some other food source besides bacteria to support sufficient growth.
6. Ciliates can in some cases be the predominant group in zooplankton (in terms of biomass and abundance). Considering their ability to graze effectively on picoplankton (and also on nanoplankton) it is obvious that they are very important trophic link in lake ecosystems.

REFERENCES

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-ReilL, L. A. & Thingstad, F. (1983). The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10: 257–263.

Beaver, J. R. & Crisman, T. L. (1982). The trophic response of ciliated protozoans in freshwater lakes. Limnol. Oceanogr. 27: 246–253.

Buskey, E. J. & Stoecker, D. K. (1989). Behavior responses of the marine tintinnid *Favella* sp. to phytoplankton: influence of chemical, mechanical, and photic stimuli. J. Exp. Mar. Biol. Ecol. 132: 1–16.

Corliss, J. O. (1979). The ciliated protozoa: Characterization, classification and guide to the literature. 2nd Edition. Pergamon press, London, 455p.

Dolan, J. R. & Coats, D. W. (1991). Preliminary prey digestion in a predacious estuarine ciliate and the use of digestion data to estimate digestion. Limnol. Oceanogr. 36: 558–565.

Fenchel, T. (1980). Relationship between particle size selection and clearance in suspension feeding ciliates. Limnol. Oceanogr. 25: 415–422.

Fenchel, T. (1987). Ecology of Protozoa. The biology of free-living fagotrophic protists. *Springer-Verlag*, Berlin, 197 p.

Finlay, B. J. (1982). Procedures for the isolation, cultivation and identification of protozoa. In: Burns, R. G. & Slater, J. H. (eds.): Experimental Microbial Ecology. Blackwell Scientific Publications, Oxford, pp. 44–65.

Finlay, B. J. (1990). Physiological ecology of free-living protozoa. In: Marshall, K. C. (eds.): Advances in Microbial Ecology, vol. 11. Plenum Publishing Corporation, pp. 1–35.

Foissner, W. & Berger, H. (1996). A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. Freshwater Biol. 35: 375–482.

Forsberg, C. & Ryding, S. O. (1980). Eutrophication parameters and trophic state indicies in 30 Swedish waste-receiving lakes. Arch. Hydrobiol. 89: 189–207.

Gifford, D. J. (1991). The protozoan-metazoan trophic link in pelagic ecosystems. J. Protozool. 38: 81–86.

Guerrero, R., Pedros-Alio, C., Schmidt, T. M. & Mas, J. (1985). Phototrophic sulphur bacteria in two Spanish lakes: Vertical distribution and limiting factors. Limnol. Oceanogr. 30: 807–819.

Kahl, A. (1930). Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 1. Allgemeiner Teil und Prostomata. Tierwelt Deutschlands 18: 1–180.

Kahl, A. (1931). Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 2. Holotricha außer den im 1. Teil behandelten Prostomata. Tierwelt Deutschlands 21: 181–398.

Kahl, A. (1932). Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. Tierwelt Deutschlands 25: 399–650.

Kahl, A. (1935). Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 4. Peritricha und Chonotricha. Tierwelt Deutschlands 30: 651–886.

Kivimaa, R., Huttula, T. & Podsetchine, V. (1998). Hydrodynamical studies. (Huttula, T. & Nõges, T., eds.) pp. 60–78. Pirkanmaa Regional Environment Centre.

Nõges, P. (1987). Produktsionnaya ekologiya fitoplanktona ozera Võrtsjärv. Cand. Biol. Autoreferaat, Tartu. 20 p.
Patterson, D. J. & Hedley, S. (1992). Free-living freshwater protozoa. A color guide. Wolfe Publishing Ltd., England, 223 p.
Pomeroy, L. R. & Wiebe, W. J. (1988). Energetics of microbial food webs. Hydrobiologia 159: 7–18.
Putt, M. & Stoecker, D. K. (1989). An experimentally determined carbon:volume ratio for marine 'oligotrichous' ciliates from estuarine and coastal waters. Limnol. Oceanogr. 34: 1097–1103.
Sherr, E. B. & Sherr, B. F. (1993). Protistian grazing rates via uptake of fluorescently labeled prey. In: Kemp, P. F., Sherr, B. F., Sherr, E. B. & Cole, J. J. (eds.): Microbial Ecology. Lewis Publishers, Boca Raton Ann Arbor London Tokyo.
Sommaruga, R. & Conde, D. (1997). Seasonal variability of metabolically active bacterioplankton in the euphotic zone of a hypertrophic lake. Microbial. Ecol. 13: 241–248.
Stoecker, D. K. (1988). Are marine planktonic ciliates suspension feeders? J. Protozzol. 35: 252–255.
Stoecker, D. K. & Capuzzo J. M. (1990). Predation on protozoa: its importans to zooplankton. J. Plankton Res. 12: 891–908.
Šimek, K., Hartman, P., Nedoma, J., Pernthaler, J., Springmann, D., Vrba, J. & Psenner, R. (1997). Community structure, picoplankton grazing and zooplankton control of heterotrophic nanoflagellates in a eutrophic reservoir during the summer phytoplankton maximum. Microbial. Ecol. 12: 49–63.
Šimek, K., Armengol, J., Comerma, M., Garcia, J.-C., Chrzanowski, T. H., Macek, M., Nedoma, J. & Straskrabova, V. (1998a). Charactristics of protistan control of bacterial production in three reservoirs of different trophy. Inter. Rev. Hydrobiol. 83: 485–494.
Šimek, K., Babenzien, D., Bitl, T., Koschel, R., Macek, M., Nedoma, J. & Vrba, J. (1998b). Microbial food webs in an artificially divided acidic bog lake. International Revue Hydrobiology 83: 3–18.
Utermöhl, H. (1958). Zur Vervollkommnung der quantitativen Phytoplankton Methodik. Mitt. Int. Ver. Theor. Angew. Limnol. 9: 1–38.
Verity, P. G. (1988). Chemosensory behavior in marine planktonic ciliates. Bull. Mar. Sci. 43: 772–782.
Wieltschnig, C., Wihlidal, P., Ulbricht, T., Kirschner, A. & Velimirov, B. (1999). Low control of bacterial production by heterotrophic nanoflagellates in eutrophic backwater environment. Aquat. Microb. Ecol. 17: 77–89.

PLANKTILISTE TSILIAATIDE OSA JÄRVEDE ÖKOSÜSTEEMIS

Kokkuvõte

Aastatel 1995–1998 uuriti planktiliste tsiliaatide osa järvede planktonikooslustes ja toiduahelates. Töö eesmärgiks oli vaadelda planktiliste tsiliaatide sesoonset dünaamikat (Võrtsjärves), tsiliaadikoosluste erinevust erisuguse troofsusega järvedes (12 Lõuna-Soome järves), koosluse vertikaalset jaotumist veesambas (8 Lõuna-Eesti järves) ja tsiliaatide osa bakterplanktoni tarbijana (Võrtsjärves). Leiti järgmist:

1. Planktiliste tsiliaatide kooslused on väga dünaamilised ja reageerivad kiiresti keskkonna ja ökosüsteemi muutustele. Võrtsjärves langevad suurimad tsiliaatide arvukused kevadele (domineerivad herbivoorid) ja hilissuvele (domineerivad bakterivoorid).
2. Tsiliaadid on levinud kogu veesambas ja neid võib leida ka hapnikuvabas hüpolimnionis. Tsiliaatide arvukus ja biomass on tavaliselt suurimad epi- ja väikseimad hüpolimnionis. Mõningatel juhtudel võib maksimumväärtusi leida ka metalimnionis.
3. Tavalisemad järvedes levinud tsiliaatidest kuuluvad rühmadesse oligotrihhid, prostomatiidid, peritrihhid, skutikotsiliaadid ja haptoriidid. Tingimustes, kus hüpolimnionis puudub hapnik, on põhilisteks dominantideks niinimetatud bentilised migrandid (kuuluvad paljudesse rühmadesse, nagu heterotrihhid, hüpotrihhid, odontostomatiidid ja hümenostomatiidid).
4. Tsiliaatide arvukus ja biomass olid suuremad kõrgema troofsustasemega järvedes. Eutroofsemates järvedes vähenes oligotrihhide ja haptoriidide suhteline osatähtsus ja nad asendusid järk-järgult väikeste skutikotsiliaatide ja prostomatiididega.
5. Tsiliaadid võivad mõningatel juhtudel olla peamised bakterplanktoni tarbijad ja süüa > 97% kõigist bakteritest. Kuid isegi sellisel juhul peab neil olema veel mõni teine toiduallikas tagamaks piisavalt kiiret kasvu.
6. Tsiliaadid võivad mõningatel juhtudel olla kogu zooplanktoni tähtsaim rühm (nii arvukuse kui ka biomassi poolest). Arvestades tsiliaatide võimet toituda efektiivselt pikoplanktonist (ja ka nanoplanktonist), on ilmne, et sellisel juhul on planktilised tsiliaadid veekogude toitumisahelates äärmiselt oluliseks lüliks.

ACKNOWLEDGEMENTS

First, I want to express my gratitude to my family, who has supported me in many ways during my studies.

I want to give my thanks to Tiina Nõges, who has been my supervisor in the word's best meaning.

I want to thank my friend and coworker Veljo Kisand, who carries the responsibility that I am now studying planktonic protozoans at all.

Most part of the work was carried out in the Võrtsjärv Limnological Station and the whole staff deserves my warmest regards for their support and patience.

I am grateful to Dr. Lauri Arvola for support and advice from the very beginning of my studies in protozoology. Best regards also to the whole staff of Lammi Biological Station.

Many thanks to my friends, especially to old classmates from Lilleküla, who were always present when I needed them.

PUBLICATIONS

I

Nõges, T., Kisand, V., Nõges, P., Põllumäe, A.,
Tuvikene, L. & Zingel, P. 1998.
Plankton seasonal dynamics and
its controlling factors in shallow polymictic
eutrophic lake Võrtsjärv, Estonia. Int. Rev. ges.
Hydrobiol. 83: 279–296.

Internat. Rev. Hydrobiol.	83	1998	4	279–296

TIINA NÕGES, VELJO KISAND, PEETER NÕGES, ARNO PÕLLUMÄE, LEA TUVIKENE and PRIIT ZINGEL

Estonian Agricultural University, Institute of Zoology and Botany,
Võrtsjärv Limnological Station, Rannu, Tartu County, Estonia, 61101
University of Tartu, Institute of Zoology and Hydrobiology, 46 Vanemuise St., Tartu, Estonia, 51014

Plankton Seasonal Dynamics and Its Controlling Factors in Shallow Polymictic Eutrophic Lake Võrtsjärv, Estonia

key words: primary production, bacterioplankton, protozooplankton, metazooplankton, PEG-model

Abstract

An analysis of plankton seasonal succession in large shallow eutrophic lake Võrtsjärv (270 km^2, mean depth 2.8 m, max. depth 6 m) is presented. Weekly samples for 1995 have been analysed using the PEG model approach. In winter, light was the main factor controlling phytoplankton growth. In early spring phytoplankton was mainly resource-controlled, competition for phosphorus being the main driving force. Ciliates (*Paradileptus* sp., *Strobilidium* sp. and *Vorticella* sp.) were the first herbivores which started to increase in April causing a twofold decline of phytoplankton biomass. The annual maximum of primary production (PP) in early May was probably caused by soluble reactive phosphorus (SRP), regenerated by herbivores, and stirred up from bottom sediments as a result of strong wind stress. This primary production peak provided substrate for further increase of bacterial biomass. The more abundant food supply supported the development of the second spring peak of herbivores (ciliates, rotifers, juvenile copepods) which was followed by the second modest "clear water phase" in late May. Silicon was depleted by the end of May causing a strong decrease in primary production of the diatom-dominated community, whereas the biomass of the cyanophytes increased under the improved nutrient conditions. Some weeks later, inorganic N was depleted and the period of N limitation with the appearance of N-fixing cyanophytes began. The ciliate collapse at the beginning of June coincided with the start of the cladoceran development and with the increase of other metazooplankton groups. This explains the further decrease of the biomass of phytoplankton and bacteria in spite of their high production. Beginning from late June, silicon appeared again and SRP started to occur periodically, while inorganic N remained close to zero until November. During this period, phytoplankton development relied to a great extent on the N-fixation and N-regeneration potential. The collapse of the ciliate community in September removed the top-down control from bacteria and their biomass increased, while the development of cladocerans still suppressed phytoplankton biomass in spite of a quite high PP. In October phytoplankton biomass and chlorophyll *a* (Chl*a*) increased, SRP was completely depleted by the middle of October reflecting a slow regeneration due to the declined activity of zooplankton in cold water. In November nitrates appeared again, and silicon reached the same level as in spring. The biomass of N-fixing *Aphanizomenon skujae* decreased while *Limnothrix redekei* and *L. planctonica* were quite abundant together with diatoms.

1. Introduction

The investigation of plankton seasonal dynamics has a long history (HUTCHINSON, 1967). Although the patterns of seasonality within individual bodies of water can be regular, comparison between water bodies often leaves the impression of chaos. The question of the primacy of bottom-up *vs.* top-down forces in the controlling of plankton succession has been acute during last decades (SOMMER, 1989). There is evidence for both forces acting in a community but they probably do not act at the same time and scale, the importance of both may vary during seasonal succession (LAMPERT *et al.*, 1992). From 1976 a group of leading

plankton ecologists (PEG) started discussions aimed at the development of a comprehensive explanation of plankton seasonal succession. Their effort resulted in establishing a 24 step word model describing step by step the seasonal events occurring in the phytoplankton and zooplankton of an idealized 'standard' lake. The PEG-model was based upon the well studied Lake Constance while the statements were confronted with the real situation in 24 lakes, reservoirs and fish ponds representing different trophic, climatological and stratification types (SOMMER *et al.*, 1986). For each step a mechanistic explanation was provided. The composers of the PEG model succeeded to overcome the division between the top-down and bottom-up views and reached the synthesis of both forces controlling the seasonal succession of plankton community. As microbial loop was not initially included into the PEG-model, and only few attempts have been made to consider bacterial succession in that framework (PEDRÓS-ALIÓ, 1989; GÜDE, 1989) we decided to provide an example. The aim of the present paper is to follow the seasonal succession of phyto-, zoo- and bacterioplankton in a large shallow eutrophic temperate lake, to provide explanations and to analyse the controlling factors of that development. We tried to test the PEG-model approach in conditions where the microbial loop is supposed to be important and the detrital food chain seems to be prevalent (NÕGES *et al.*, 1998).

Descriptlon of the Lake

Lake Võrtsjärv (270 km^2) in Central Estonia is the second largest lake in Baltic countries. It is a shallow and turbid eutrophic lake with a mean depth of 2.7 m and maximum depth of 6 m. The large amplitude of water level fluctuations (the annual mean 1.38 m, annual maximum 2.20 m and the absolute range 3.20 m) is the leading physical factor determining the ecological status of the lake (NÕGES and NÕGES, 1998). Increased sediment resuspension during low-level periods enriches water with phosphorus causing cyanophyte blooms and contributes to the formation of a high seston concentration and high turbidity during summer. The average total phosphorus concentration (P_{tot}) is 54 µg l^{-1}, total nitrogen concentration (N_{tot}) 1.6 mg l^{-1}, and the mean Secchi depth (S) 1.1 m. During the vegetation period, S usually does not exceed 1 m. Most of the organic compounds in the lake are of autochthonous character. The average chemical oxygen demand (by K_2MnO_4 method) is 11 mg O l^{-1}, and biochemical oxygen demand (BOD_7) 3.8 mg O l^{-1}.

The mean water temperature in the warmest month July is 20.1 °C. The ice cover lasts from November to April, 135 days as an average. Homothermy prevails in L. Võrtsjärv throughout the year. The water belongs to the calcium carbonate group with a salinity ranging from 227 to 370 mg l^{-1}. The monthly mean pH range is between 7.6 and 8.5 (HABERMAN *et al.*, 1998).

2. Material and Methods

Weekly sampling was performed at a station near to the deepest part of L. Võrtsjärv. The sampling period lasted from December 1994 to the end of 1995.

Particulate primary production (PP_{part}) of phytoplankton was estimated using the $^{14}CO_2$ assimilation technique introduced first by STEEMAN-NIELSEN (1952). Water from the surface and from the depths equal to 0.25 S, 0.5 S, 1 S, 2 S, and 3 S (S = Secchi depth) was poured into 36 ml glass scintillation vials, 100 µl of sterile $NaH^{14}CO_3$ solution (1.3 µCi per 1 vial) was added to reach a final activity of 0.06 µCi/ml. After that the vials were exposed for 2 hours at mid day (usually from 11 a.m. to 1 p.m.) at the same depths where the water was sampled. Non-photosynthetic carbon fixation was measured in darkness using water from the surface layer and from the depth of 3 S. In 1995 PP_{part} was measured in 2 size fractions: total and <40 µm (achieved by 40 µm mesh sieving). Six ml of water of both fractions were filtered, in 2 replicates, through 0.45 µm pore size membranes (Millipore HA). Filters were treat-

ed with HCl vapours for 5 min to remove the excess of the inorganic ^{14}C. Radioactivity of the filters was measured in toluene-PPO-POPOP cocktail using the LSC RackBeta (LKB-Wallac). PP_{part} values were calculated according to the standard formula (Anonymous, 1984). The trapeze integration over depth and time was applied for calculating values per m^2 and per year, accordingly. Daily PP values were calculated using the equation relating daily PP (PP_{day}; $mgC/(m^2 \cdot day)$) with PP at mid day (PP_{hour}; $mg\ C/(m^2 \cdot h)$) and the length of the light day (DL; h):

$$PP_{day} = PP_{hour}/(0.230 - 890 \cdot 10^{-5} \cdot DL)\ ; \qquad R^2 = 0.66\ , \qquad p < 0.01\ . \tag{2}$$

This regression was found on the basis of 14 series of seasonal measurements of PP_{day} by 2-hour values during the whole day, performed in L. Võrtsjärv in 1989 (unpublished).

Water transparency (S) was measured using a Secchi disc. Concentration of chlorophyll *a* (Chl*a*) was determined in 2 size fractions: total and <40 µm (after 40 µm sieving) to achieve the estimate of the share of "edible" phytoplankton. Seston was collected on Whatman glass fibre filters (GF/C). Pigments were extracted with 90% acetone and analyzed spectrophotometrically (Anonymous, 1979). The equation of JEFFREY-HUMPHREY (1975) was applied in calculation of Chl*a*.

Chemical analyses were performed in depth integrated water samples, using the methods described by GRASSHOFF *et al.* (1982). Dissolved reactive phosphorus (SRP) was measured by the molybdate blue method using ascorbic acid as reductant. Nitrate and nitrite were determined by reduction with a cadmium column. Ammonium was measured by the indophenol-blue method. In order to determine total nitrogen and total phosphorus, organic compounds were mineralized into nitrite and phosphate, using persulfate. For the estimation of dissolved inorganic silicate, molybdosilicate method was used. Standard photometric analysis was applied to complete each named estimation (GRASSHOFF *et al.*, 1982). Conductivity of the water was measured using a HACH Conductivity/TDS Meter (model 44600), and pH with HACH ONE pH-meter (model 43800-00). Oxygen was measured by oxygen meter Aqua-Oxy (ELKE MS Ltd., Tallinn, Estonia). For alkalinity measurements water samples were titrated with hydrochloric acid in the presence of methyl red indicator, and results were given as mg HCO_3^- per litre, using the equivalent of 61 (CZENSNY, 1960). Suspended solid concentration was measured as 105 °C dry weight of suspended material filtered on Whatman GF/C filters. Biochemical oxygen demand (BOD_7) was determined during 7 days at 20 °C in darkness. Chemical oxygen demand (COD_{Cr}) was determined by means of dichromate oxidation.

The abundance of bacteria was determined as direct count by acriflavine staining (AFDC) under epifluorescence microscope, magnification ×1000 (TULONEN, 1993). The protein biomass of bacteria was calculated on the basis of mean cell volume using the power function $m = CV^a$ (NORLAND, 1993), where m is the protein biomass (fg/cell), V is the biovolume of cells (μm^3/cell), C is the conversion factor relating protein biomass to volume (88.6 from SIMON and AZAM, 1989), and a is the scaling factor (0.72 from NORLAND, 1993). The protein biomass was converted to carbon biomass by multiplying it by 0.86 (SIMON and AZAM, 1989). The number of saprophytic bacteria (plate count, PC) was determined as a plate count on fish peptone agar (0.5–1 ml water to Petri dishes with growth medium). Production of heterotrophic bacteria was measured by the tritiated leucine incorporation (TLI) method. Hot acid extraction procedure (SIMON and AZAM, 1989), followed by cold ethanol (80%) wash was used to extract proteins. Incorporation activity was determined by LSC RackBeta 1211 (LKB Wallac) using external quenching method. Incorporation activity of leucine was transformed to the production of proteins and carbon using theoretical assumptions (SIMON and AZAM, 1989).

Phytoplankton samples were preserved and fixed with Lugol's iodine, species were identified and the biomass was determined at ×400 magnification (UTERMÖHL, 1958) technique. The mean length of each filamentous species dominating in the lake was determined separately in each sample by measuring about 60 trichomes. Samples were counted until reaching at least 400 counting units (filaments, cells, colonies), which gives a counting error of ±10 % for the total biomass.

The ciliated protozoan samples were preserved and fixed with Lugol's iodine, recommended by HOBRO and WILLEN (1977). Samples were stored at 4 °C in the dark. Volumes of 10–100 ml were settled for at least 24 hours in plankton chambers. Ciliates were enumerated and identified with an inverted microscope (Wild Heerbrug M40 and Nikon diaphot-TMD) using the UTERMÖHL (1958) technique. At least 150 small protozoans (approximately less than 40 µm in length) were counted and identified at ×400–600 magnification. To count large species, the entire chamber was surveyed at ×100 magnification. Ciliates were usually identified to genus by consulting several works (KAHL, 1930, 1931, 1932, 1935; KUTIKOVA and STAROBOGATOV, 1977; PATTERSON and HEDLEY, 1992; FOISSNER *et al.*, 1991, 1992, 1994, 1995). The first 20 measurable specimens encountered for each taxon in each sample were

measured. Biovolumes of each taxon were estimated by assuming geometric shapes. For tintinnids the cell volume was calculated assuming a cell occupancy of 30% of the lorica volume (GILRON and LYNN, 1989; LINS DA SILVA, 1991). The wet weight biomass for each taxon was obtained by multiplying the estimated volume by abundance and a conversion factor of 0.1 pg/μm^3.

For metazooplankton counting two samples were taken from depth integrated lake water: 20 l was filtered through a net of 80 µm mesh size and 1 l was filtered through a net of 10 µm mesh size (for counting of small rotifers). Samples were fixed with formalin (4% final concentration). Metazooplankton was usually counted in four 1–2 ml subsamples (8 subsamples in wintertime). Individuals of *Leptodora kindtii* were counted separately in the whole sample. At least 20 individuals of each species were measured in every sample for biomass calculation. The individual weights of rotifers were estimated from average lengths according to RUTTNER-KOLISKO (1977). The lengths of crustaceans were converted to wet weights according to STUDENIKINA and CHEREPAKHINA (1969) for nauplii and BALUSHKINA and WINBERG (1979) for other groups. If less than 20 individuals of a species were found in a sample, the mean length of this species was applied in wet weight calculations.

3. Results

3.1. Physical Environment and Water Chemistry

Water temperature achieved its annual maximum (24 °C) on June 5 (Fig. 3b). At the same time, a temporary thermal stratification formed with surface temperature exceeding the near bottom temperature by 4 degrees. The second short stratification (4.1 °C temperature difference) was observed on June 26. The formation of such vertical gradients, rather strong for polymictic L. Võrtsjärv, was conditioned by permanent calm and sunny weather and high water level in June.

The Secchi depth varied from 2.4 m in the middle of January to 0.6 m on several occasions from July to September (Fig. 3b). The annual mean Secchi depth was 1.05 m and the median value 0.9 m.

Mean conductivity of the water was 324 ± 31 (±SE anywhere in this paper) µS/cm, being more stable in the second half of the year. pH varied from 7.55 to 8.84, being lower in wintertime and showing one clear period of increase in March–April. Alkalinity (HCO_3^-) was 191.1 ± 4.2 mg/l on average, being lower during the ice-free period (Figs 1a and 6c); the yearly maximum, 290 mg/l, occurred as a peak on September 18.

The total phosphorus concentration (P_{tot}) ranged from 0.017 to 0.11 mg/l, (mean value 0.050 ± 0.003), and SRP from 0 to 0.052 mg/l (0.010 ± 0.002). Rapid fluctuations of SRP took place from the beginning of August till the beginning of October. From October to February the value was rather stable and close to zero (Fig. 1b). The most stable period of high SRP concentration lasted from February till mid-March when, on the average, 19% of P_{tot} was in inorganic form.

The content of all nitrogen compounds was higher in winter months (from December till March), when the lake was ice-covered (Fig. 1c). Inorganic nitrogen (N_{min}) dropped to the lowest level by July, when it made up only 0.02–0.2% of N_{tot}. The ratio N_{min}/N_{tot} in the first half of the year was higher than in late summer and autumn. Mean N_{tot}/P_{tot} in L. Võrtsjärv was 32 (6.6–76.5).

Dissolved inorganic silicon ranged from 0.09 to 4.74 mg Si/l (average 2.09 ± 0.16). It decreased from January to the end of May and began to rise again from the middle of June (Fig. 1d).

3.2. Phytoplankton, Chlorophyll and Primary Production

Diatoms (mostly *Melosira* spp.) predominated in the phytoplankton from the end of February, achieving the first noticeable biomass (>5 mg WW/l) at the beginning of April, and keeping the dominating position until the end of May (Fig. 2a). Cyanophytes

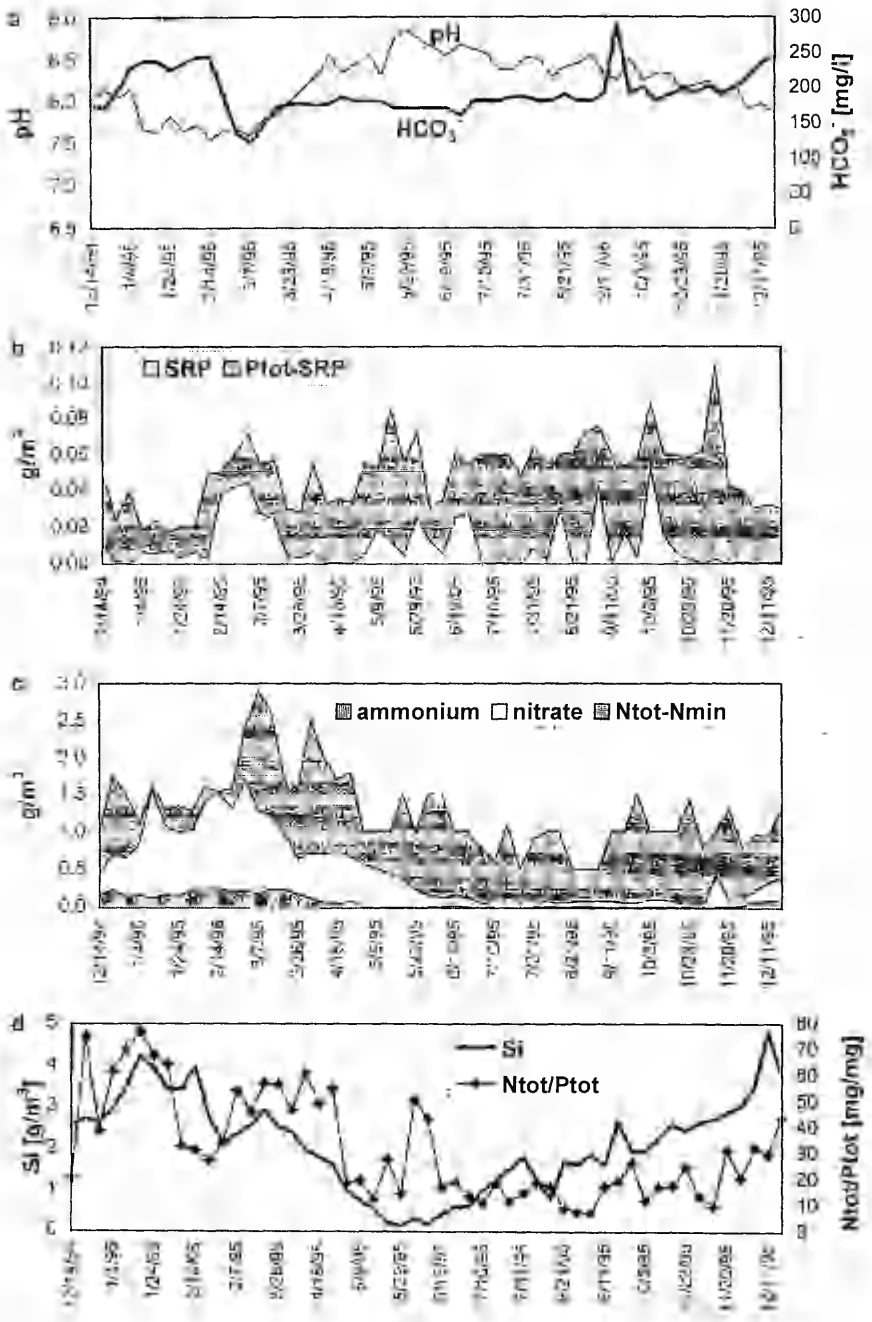

Figure 1. Seasonal course of alkalinity (HCO_3), pH, mineral and total nitrogen and phosphorus, the ratio of total N and P, and dissolved silicon in L. Võrtsjärv.

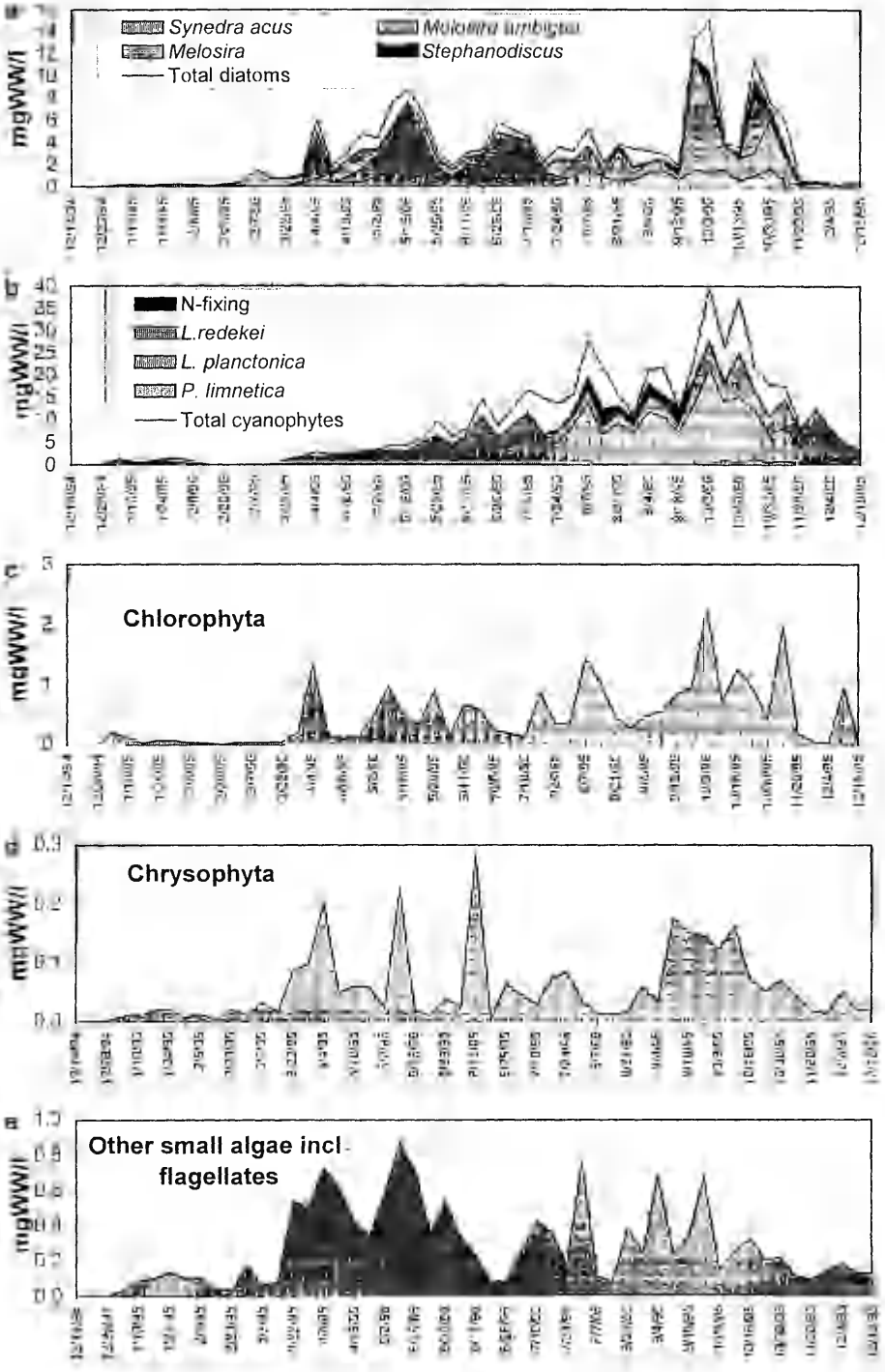

Figure 2. Seasonal course of the development of phytoplankton structure in L. Võrtsjärv.

started in mid-March, first with *Limnothrix redekei,* accompanied by *L. planctonica* (in our earlier papers referred as *Oscillatoria amphibia* f. *tenuis*) from mid-May, and N-fixing *Aphanizomenon skujae* (in our earlier papers referred as *A. gracile*) from July to September (Fig. 2b). Other phytoplankton groups were of much smaller importance (Fig. 2c, d, e). The average phytoplankton biomass in 1995 was 15 ± 2 mg WW/l. The maximum value (57 mg WW/l) was measured in the beginning of October.

The average Chl*a* in 1995 was 25 ± 2 μg/l (median 24 μg/l). Chl*a* reached its maximum (64 μg/l) on October 23 (Fig. 3a).

Chl*a* in the <40 μm fraction formed 77 ± 2% of the total chlorophyll concentration. The average difference between the total Chl*a* and Chl*a* in the <40 μm fraction was 5.2 μg/l. The proportion of the <40 μm fraction was smaller in the diatom dominated phytoplankton in spring, and increased in late summer and autumn when cyanophytes were prevalent (Fig. 3a). The total Chl*a* correlated well (all significant at $p < 0.001$ level) with the total biomass of phytoplankton (B_{phyto}), and with the biomass of diatoms and green algae ($r = 0.9$, 0.79 and 0.79, respectively). Chl*a* in the <40 μm fraction gave even stronger correlations ($r = 0.93$, 0.9 and 0.81, respectively).

Integral particulate primary production achieved the highest values (226 mg C/($m^2 \cdot$ h)) on May 9 (Fig. 3c). Average PP_{part} in 1995 was 50 ± 7 mg C/($m^2 \cdot$ h), the annual value being 200 g C/m^2.

The assimilation number of phytoplankton (AN) as the ratio of the vertical maximum of PP_{part} (mg C/($m^3 \cdot$ h)) to Chl*a* concentration, reflecting the photosynthetic potential of a Chl*a* unit, ranged from 0.1 to 5.4 (mean 1.76 ± 0.2) mg C/(mg Chl*a* · h). The algal production to biomass ratio (P/B) ranged from 0.014 to 0.77 (mean 0.18 ± 0.03) 1/day (Fig. 3d).

3.3. Bacterioplankton

Bacterial abundance (AFDC) and biomass (B_{bact}) were low from the end of January to the middle of April developing peaks (>60 mg C/m^3) in May, July, October, November and late December. Bacterial production was extremely high (22 mg C/($m^3 \cdot$ h) on August 7, remaining lower than 4 mg C/($m^3 \cdot$ h) during the rest of the year (Fig. 4a). The number of saprophytic bacteria (PC) had the peak at the end of February (1200 cells/ml), remaining through of year rather low (200–300 cells/ml). The only maximum in summer was observed in July 18 (600 cells/ml). PC increased again up to 920 cells/ml at the beginning of December, after the formation of ice.

B_{bact} was in significant ($p < 0.001$) positive correlation (Spearman $r = 0.7$) with Chl*a*, with phytoplankton biomass (B_{phyto}; $r = 0.77$), with the concentration of suspended solids (SS, $r = 0.7$), with biochemical oxygen demand (BOD_7, $r = 0.6$), with chemical oxygen demand (COD_{Cr}, $r = 0.6$), with water temperature (Temp.; $r = 0.55$) and with pH ($r = 0.6$). The correlation was negative with Secchi depth ($r = -0.68$), with the concentration of nitrate ($r = -0.8$), total nitrogen (N_{tot}; $r = -0.6$) and with the N_{tot}/P_{tot} ratio ($r = -0.66$). Bacterial production (P_{bact}) correlated ($p < 0.01$) positively with B_{bact} ($r = 0.43$) and biochemical oxygen demand (BOD_7, $r = 0.41$), and negatively with Secchi depth ($r = -0.45$).

3.4. Protozooplankton

Twenty nine taxa were identified in the planktonic ciliate community. The highest species diversity occurred in June and July. Peaks in the total ciliate abundance were observed in May (125 · 10^3 ind./l), July (191 · 10^3 ind./l) and October (75.5 · 10^3 ind./l). The lowest

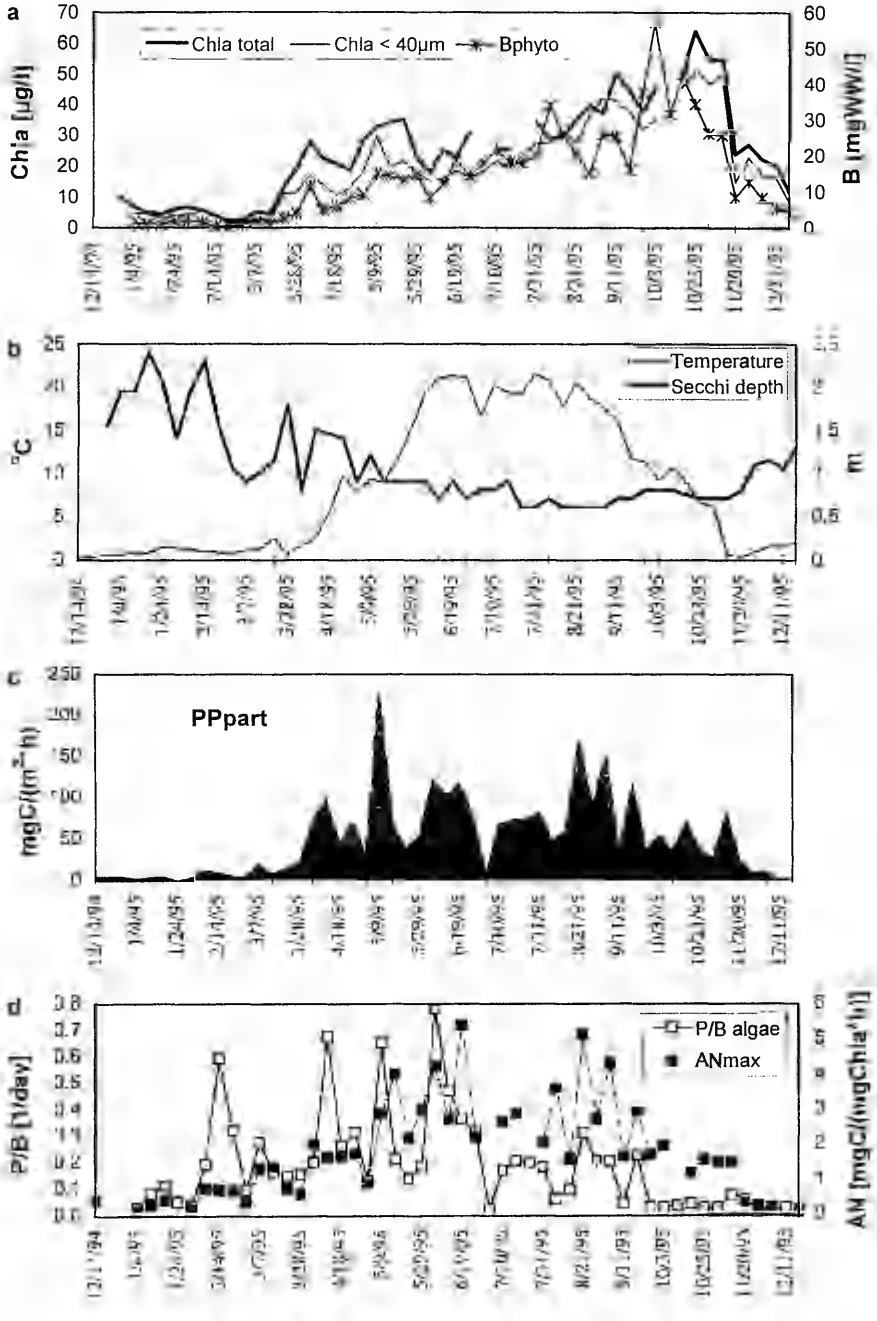

Figure 3. Seasonal course of particulate primary production (PPpart), chlorophyll *a* (Chl*a*), phytoplankton biomass (Bphyto), water temperature, Secchi depth, maximum assimilation number (ANmax) and P/B coefficient of phytoplankton in L. Võrtsjärv.

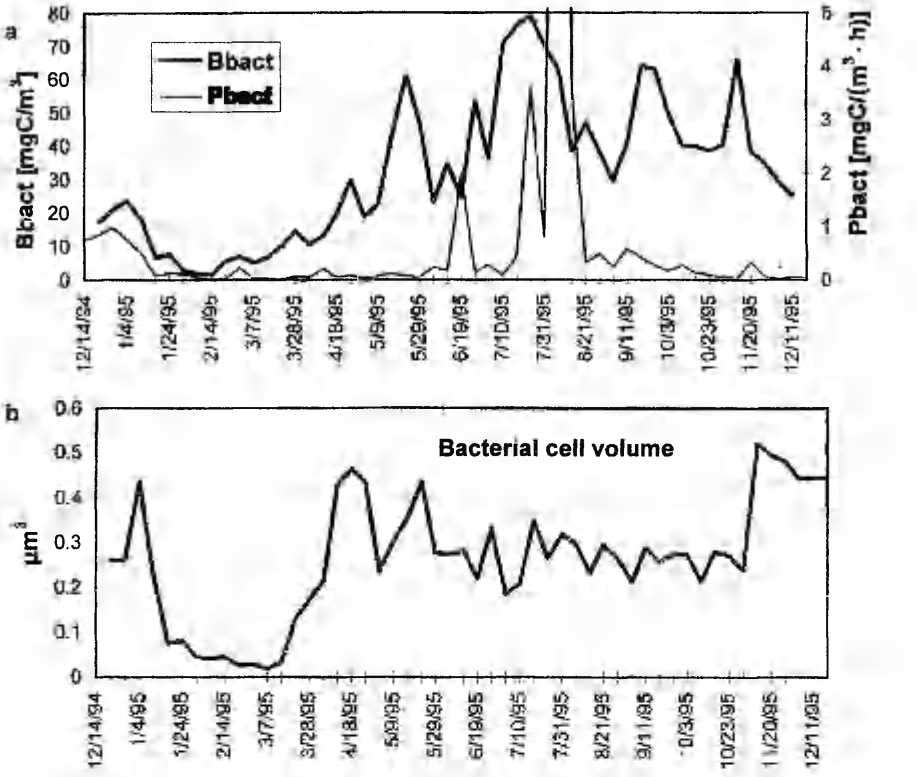

Figure 4. Seasonal course of bacterial biomass (Bbact), production (Pbact) and cell volume in L. Võrtsjärv.

abundance (600 ind./l) was in January and February. The mean ciliate abundance was $39 \cdot 10^3$ ind./ l. The ciliate biomass was high in April (3.7 mg WW/l), May (2.3 mg WW/l) and August (5.6 mg WW/l), and low in January and February (0.01 mg WW/l). The mean ciliate biomass was 0.68 ± 0.14 mg WW/l (Fig. 5a).

The main ciliate groups were oligotrichs, haptorids and peritrichs. On some occasions the scuticociliates, hymenostomatids and prostomatids were also numerous. The most common oligotrichs were *Strobilidium* and *Strombidium*. In April their biomass started to increase. In that period the large *Strombidium* and *Strobilidium* species (*Strobilidium lacustris* and *Strombidium viride*) dominated. During summer and early autumn smaller species of the genus *Strobilidium* (25 µm) dominated. During summer small (15 µm) *Strobilidium* were also abundant, but the contribution of small individuals to biomass was less significant. Among tintinnids *Tintinnidium fluviatile* and *Codonella cratera* were most common, the former being the winter dominant. In summer *Tintinnidium fluviatile* occurred only occasionally and in low densities. *Codonella cratera* was found only in the summer (June–August) samples. The most common haptorid was *Mesodinium* sp. which was abundant throughout the ice-free period. In April and May *Paradileptus caducus* appeared. Its abundance was insignificant but, due to the large size (350 µm), its contribution to the total ciliate biomass was remarkable. In April and May also peritrichs (vorticellid ciliates) peaked.

Other species were more sporadic in appearance, occurring for short periods, but often reaching relatively high densities. For example *Stentor amethystinus* occurred in the water

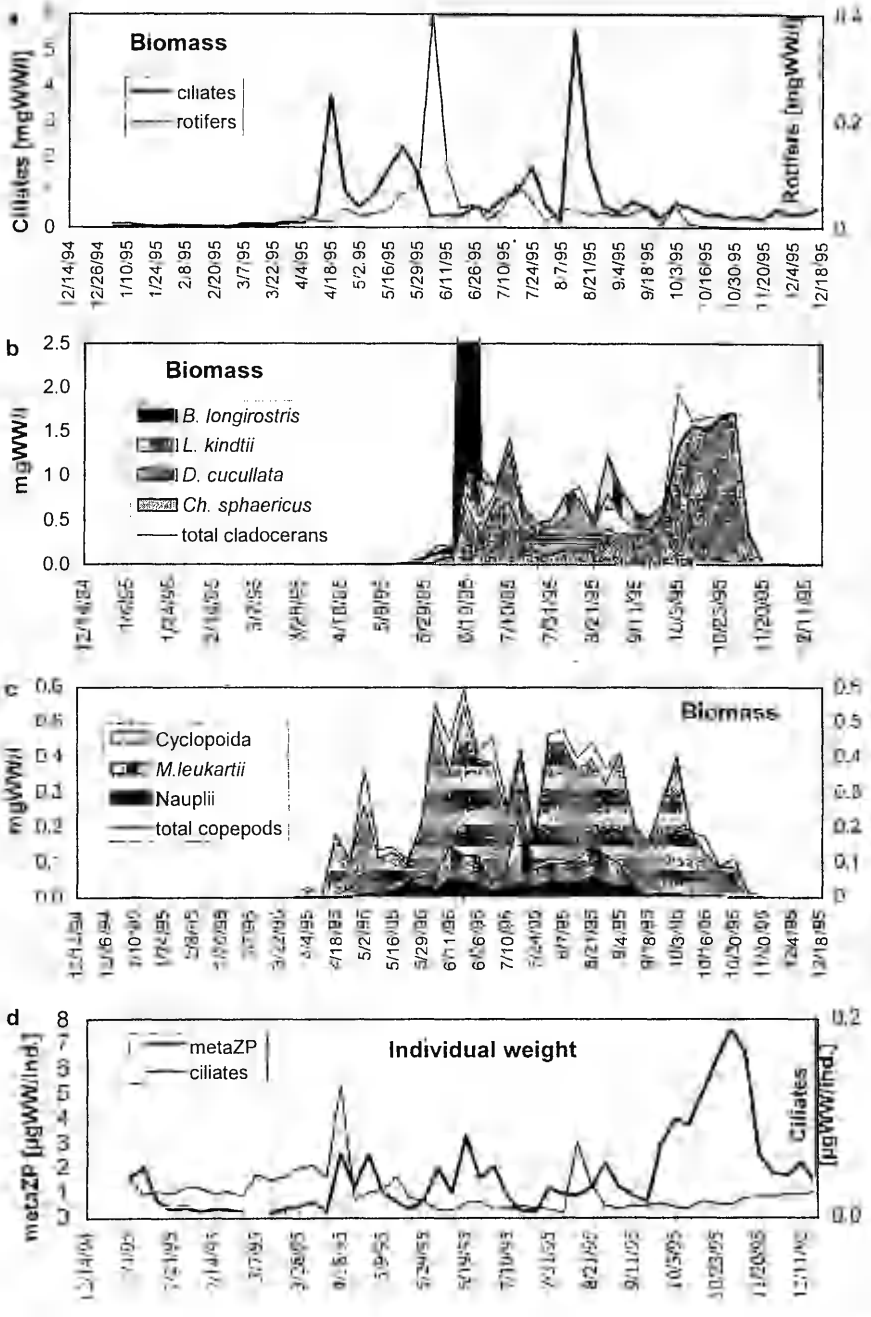

Figure 5. Seasonal course of proto- and metazooplankton biomass and individual weight L. Võrtsjärv.

column only in the middle of August but reached biomass values of 5 mg WW/l. On some occasions *Diflugia limnetica* was present in relatively high densities (maximum 6107 ind./l in the beginning of August). In spring a heliozoan *Chlamydaster* sp. occurred (maximum 22 536 ind./l on April 25).

3.5. Metazooplankton

In wintertime the biomass and abundance of metazooplankton were low, starting to increase immediately after ice melting.

Rotifers started to grow first (Fig. 5a), the population of *Polyarthra dolichoptera* reached an abundance of 300 ind./l and a biomass of 0.02 mg WW/l at the end of April. Some weeks later, the population of *Keratella cochlearis* started to grow, reaching its maximum on May 22 (470 ind./l, 0.04 mg WW/l) and remaining on a high level (300 ind./l, 0.01 mg WW/l) until the end of June. At the end of May *P. dolichoptera* started to be replaced by *Polyarthra luminosa* that had its first maximum in June (130 ind./l, 0.04 mg WW/l). After that it disappeared but reappeared again in August to be a dominating rotifer by biomass (max. 0.02 mg WW/l) until the formation of the icecover. Simultaneously with *P. luminosa*, the first individuals of *Trichocerca rousseleti* and *Anuraeopsis fissa* appeared. The rapid growth of *Trichocerca* population started in the middle of June and reached soon the abundance of 150–200 ind./l (maximum on July 18, 1000 ind./l). On July 18 the maximum of *Anuraeopsis fissa* occurred (4800 ind./l, 0.03 mg WW/l). This indicator of hypertrophic state started to grow at the beginning of July keeping very high abundance (400–900 ind./l) until the end of September, when the whole rotifer community declined. The highest rotifer biomass (0.4 mg WW/l), accounted mostly for by the big predator *Asplanchna priodonta* (20 ind./l), was estimated in the first week of June.

Among cladocerans, *Chydorus sphaericus* was the dominating species throughout the whole ice-free period (Fig. 5b). First animals appeared after ice melting but the population started to grow in the middle of May. The first peak was reached at the end of June (150 ind./l, 0.6 mg WW/l). After a few weeks the population decreased to 40 ind./l and started a rapid increase again at the end of September reaching 350 ind./l and 1.6 mg WW/l. The average individual weight of *Chydorus sphaericus* increased towards autumn. Among other cladoceran species *Daphnia cucullata* occurred abundantly (30–40 ind./l, 0.2 mg WW/l) from June to September, and *Alona quadrangularis* (max. 50 ind./l and 0.2 mg WW/l) from September until the formation of the ice-cover. In summertime a remarkable biomass was also developed by predatory *Leptodora kindtii* (up to 0.5 mg WW/l). The sample taken on June 19 must be mentioned separately. Extremely high concentration of *Bosmina longirostris* (2600 ind./l, 8.9 mg WW/l) was estimated in the integrated water sample (Fig. 5b) while, according to the unpublished data of J. HABERMAN, the number of *B. longirostris* was neglible (1.3 ind./l) in the sample taken by Juday plankton net as a vertical howl. This big difference could be explained by assuming either a relatively small and very dense shoal of *Bosmina* or a high near-bottom concentration of this species accessible for the water sampler, but not for the net. The comparison of two different zooplankton sampling methods will be published elsewhere.

Among copepods *Mesocyclops leuckarti* was the dominating species (Fig. 5c). From April to September its abundance was 10–15 ind./l and biomass 0.05–0.1 mg WW/l (maximum in June). The numbers of juvenile stages (copepodids and nauplii) were much higher. The first peak was reached by both groups by the second half of June (abundance 80 ind./l and 65 ind./l, biomass 0.35 and 0.05 mg WW/l, respectively). After a subsequent decrease, both groups reached their lowest level in the middle of July coinciding with the minima in the individual body weight of both groups. A new peak was reached by the end of August. Nauplii disappeared in October and copepodids in November.

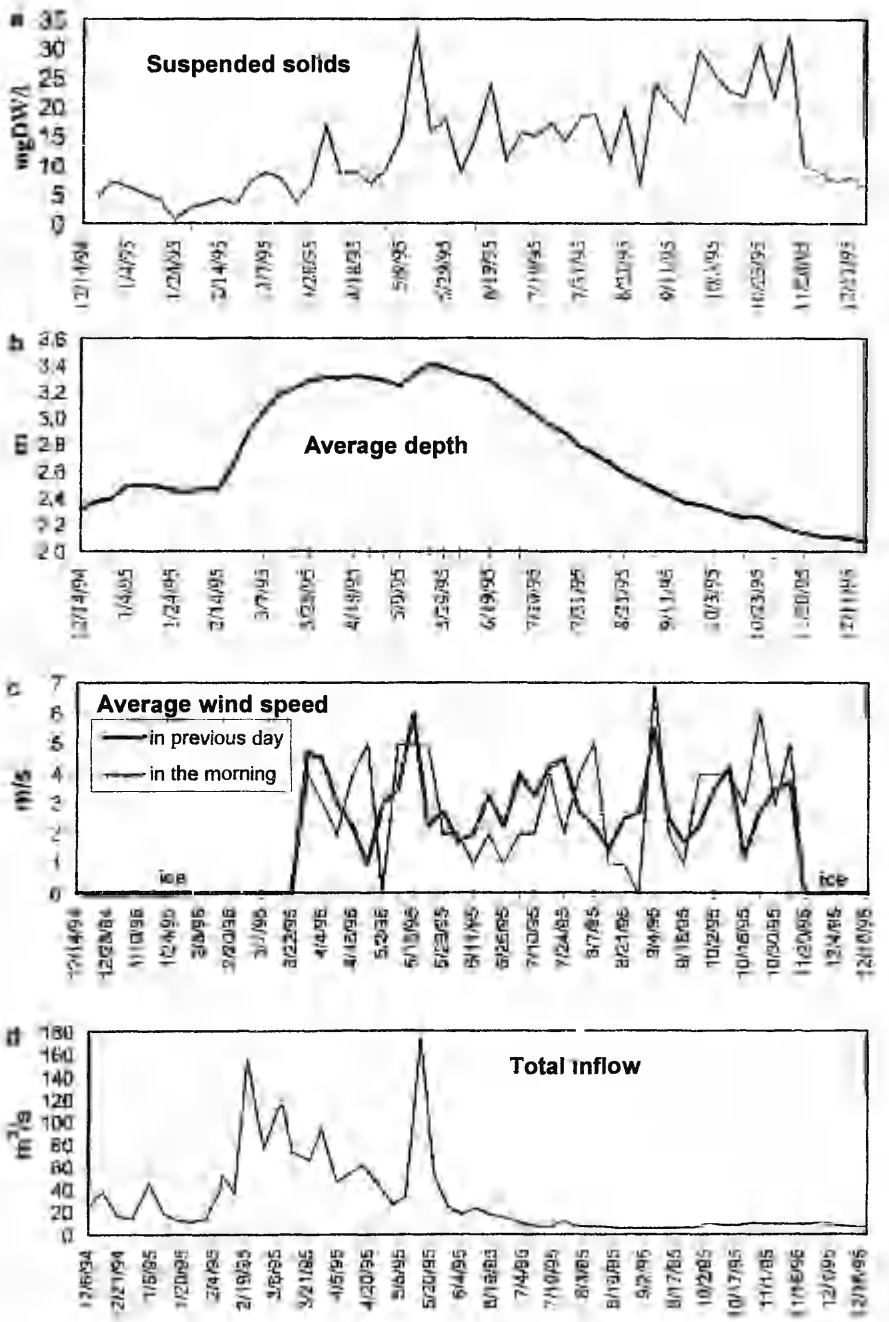

Figure 6. Seasonal course of suspended solid (SS) concentration, average depth, wind speed and total inflow in L. Võrtsjärv.

The biomass of the whole metazooplankton (B_{zp}), as well as the biomasses of their different groups (cladocerans, copepods, rotifers) and ciliates were positively correlated ($p < 0.01$) with temperature ($r = 0.63–0.9$), B_{phyto} ($r = 0.4–0.9$), PP_{part} ($r = 0.5–0.7$), B_{bact} ($r = 0.46–0.7$) and P_{bact} ($r = 0.47–0.6$).

4. Discussion

The winter community of the phytoplankton, which was dominated by *Limnothrix redekei* from the beginning of the year until the middle of February, coexisted with a zooplankton population of rotifers (*Keratella quadrata, Synchaeta verrucosa*) and ciliates (*Strobilidium* sp., *Tintinnidium fluviatile*). As grazing of these small microfiltrators on filamentous *L. redekei* is hard to be expected (GLIWICZ, 1969; KUTIKOVA and STAROBOGATOV, 1977; FOISSNER *et al.*, 1991; ANDERSEN, 1997), the winter phytoplankton community was evidently bottom-up controlled (resource limited). As the lake was ice-covered (Fig. 6c), light was the main factor controlling phytoplankton growth.

In winter the bacterial biomass was low and the population consisted of extremely small cells (Fig. 4b), this could be caused by phosphorus limitation as in January–February nitrogen was almost totally mineralized while a considerable amount of phosphorus remained in compounds other than SRP (Fig. 1b). The sharp decrease of bacterial biomass in January could be caused by grazing of ciliates and rotifers, though, the amount of zooplankton was very low and a hard top-down control is not expected.

From the second half of February the flood period started (Fig. 6d). The influx of allochthonous organic matter from the watershed was probably the reason of the first small peak of P_{bact} at the end of February.

Spring phytoplankton seemed to be mainly resource-controlled. The appearance of a considerable amount of SRP under the ice in February–March (Fig. 1b) favoured the development of large diatoms (Fig. 2a). The depletion of SRP and the ice-break at the end of March coincided with the end of diatom dominance and with the appearance of the spring populations of *L. redekei, L. planctonica* and *Planktolyngbya limnetica* as well as with the increase of chlorophytes, pyrrophyte and chrysophyte populations. This could be the result of the competition for phosphorus.

The first increase in primary production after the ice-break at the end of March resulted in a quite considerable increase of B_{phyto} and Chl*a* in early April (Fig. 3a) reflecting low grazing on phytoplankton. In spite of the highest water level in this period (Fig. 6b), wind stress could cause sediment resuspension revealed by temporary peaks of suspended solids, N_{tot}, P_{tot} and SRP (washout from pore water) concentration (Fig. 1b, c). The diatom peak on April 4 could be also caused partly by cells stirred up from the bottom (Fig. 2a).

A rapid phytoplankton growth in spring is common in the majority of lakes. The general PEG model refers to the development of small fast-growing algae as Cryptophyceae and small centric diatoms in spring (step 1), the development of large diatoms was noticed in 8 PEG-lakes (SOMMER *et al.*, 1986). In three of 24 PEG-lakes (Vechten, Tjeukemeer and Ø. Heimdalsv.) both large diatoms and small chlorococcales were abundant in spring as it occurred in L. Võrtsjärv. This feature seems to be common to shallow nonstratified eutrophic lakes.

In the middle of April, Chl*a* and B_{phyto} decreased while PP remained almost at the previous level (Fig. 3). At the same time, a pronounced increase in the biomass of ciliates *Paradileptus* sp., *Strobilidium* sp. and *Vorticella* sp. (up to 3.7 mg WW/l), as well as of juvenile copepods, occurred (Fig. 5). Most probably, small phytoplankton could be consumed by these species (Fig. 2) as the share of the smaller fraction in Chl*a* also decreased by the end of April (Fig. 3a). The increase of herbivore populations after the first peak of small algae was consistent with PEG-model (step 2): the crop of small algae is grazed upon by her-

bivorous zooplanktonic species which become abundant due both to hatching from resting stages and to high fecundity induced by high levels of edible algae (SOMMER *et al.*, 1986). The first herbivores to increase in L. Võrtsjärv were ciliates. It corresponds well to the data of four PEG-lakes, for which information on protozoans was available (Glebokie, Piburger-See, Lunzer U. See and Ø. Heimdalsv.). In shallow eutrophic Lake Müggelsee the first spring peak of herbivores was also formed by protozoa (NIXDORF and ARNDT, 1993).

The decrease of phytoplankton biomass in L. Võrtsjärv in April while the water transparency increased (Fig. 3b) could be regarded as a clear water phase as a consequence of herbivore grazing (step 5 in the PEG model), the occurrence of which is reported by many authors (e.g. LAMPERT *et al.*, 1986; LUECKE *et al.*, 1990; ARNDT and NIXDORF, 1991; VYHNALEK *et al.*, 1991). The about twofold decrease of phytoplankton in L. Võrtsjärv was comparatively smaller than in the majority of lakes referred by SOMMER *et al.* (1986). However, a smaller decrease in phytoplankton was characteristic to the tested nonstratified lakes, while in two of them (Tjeukemeer and Neusiedlersee) a clear water phase did not occur.

The development of non-edible large diatoms *(Melosira)* started in L. Võrtsjärv in April, soon after the decline of small algae which were kept at a low level until the beginning of May (Fig. 2). The food limitation probably caused the sharp decline of the biomass and body weight in herbivores, most of all in ciliates (Fig. 5a and d), as explained in the PEG-model step 6.

The maxima of primary production and algal P/B ratio on May 9 (Fig. 3d) could be attributed, besides diatoms, also to minor groups of phytoplankton (Fig. 2). This PP peak was probably caused by SRP, regenerated by ciliate, copepod and heterotrophic flagellate grazers, and/or washed up from bottom sediments in the result of high wind stress (Fig. 6c).

The pronounced increase in phytoplankton production in early May (Fig. 3c) was assumed to provide substrate for the following increase of bacterial biomass (Fig. 4a). The abundant food supply supported the development of the second spring peak of herbivores (ciliates, rotifers, juvenile copepods) in May (Fig. 5) which was followed by a modest "clear water phase" in late May–early June (Fig. 3). Such double occurrence of herbivore spring maxima and of the "clear water phase" was not described in the PEG-model. The phenomenon can occur also in other lakes where the first peak could have been missed for various reasons, e.g. because of low sampling frequency (biweekly or monthly) in early spring. A decrease of phytoplankton abundance and biomass was evident in Lake Balaton in early May, 1980 (PADISAK, 1992), though, not being considered by the author as the clear water phase caused by grazing.

Silicon was depleted by May 22 (Fig. 1d) causing a strong decrease in primary production of the diatom-dominated community (Figs. 2a and 3c). The biomass of diatoms decreased rapidly due to sedimentation while the biomass of cyanophytes increased in the improved nutrient conditions. Such development is in good agreement with the 12th statement in PEG-model: silica-depletion leads to a replacement of the large diatoms by large dinoflagellates and/or Cyanophyta.

By the beginning of July, inorganic N was completely depleted (Fig. 1c). Nitrogen depletion favours the shift to nitrogen-fixing species of filamentous blue-green algae (PEG-model step 13). The outburst of N-fixing cyanophytes in L. Võrtsjärv in July (Fig. 2b) confirmed the assumption of the starting N-limitation period in the lake. The N_{tot}/P_{tot} ratio remained permanently less than 20 and dropped even below 10 in several cases during the development N-fixing cyanophytes (Fig. 1d) indicating N-limitation (SCHINDLER, 1978; DE HAAN and MOED, 1982). As the lakes where nitrogen depletion occurred were rare in PEG data-set (SOMMER *et al.*, 1996), the data on L. Võrtsjärv provide a confirmation to the above mentioned PEG statement.

The SRP peak on May 29 (Fig. 1a) seemed to be caused mostly by reduced P-uptake. The collapse of the diatom population (Fig. 2a) due to the exhausted pool of silicon (Fig. 1d) resulted in diminishing of primary production (Fig. 3c) and, hence, in low nutrient uptake.

Resuspension could not affect the SRP concentration due to calm weather (Fig. 6c). The density of grazers was quite low in late May but started to grow rapidly after that. The decrease of bacterial biomass at the beginning of June (Fig. 4a) was evidently the result of grazing (Fig. 5).

Ciliate collapse at the beginning of June coincided with the start of cladoceran development and with the increase of other metazooplankton groups (Fig. 5). This explains, probably, the further decrease of B_{phyto} and Chl*a*, especially of the <40 μm fraction, while the rate of PP remained quite high (Fig. 3). The growing abundance of rotifers and copepod nauplii resulted in increased grazing pressure on bacteria which biomass and cell volume decreased in spite of high bacterial production (Fig. 4).

Beginning from late June, silicon appeared again and SRP started to occur periodically, while inorganic N remained close to zero until November (Fig. 1). Consequently, phytoplankton development relied to a great extent on the N-fixation and N-regeneration potential during this period.

The peak of P_{bact} on June 19 brought about the following increase of B_{bact} (Fig. 4a). The low grazing on bacteria could be assumed from the low biomass of ciliates and rotifers (Fig. 5a). During some periods SRP was available, probably due to resuspension (high wind speed, SS, P_{tot}, N_{tot}), and PP was quite high providing substrate for bacterial development. The following peaks of P_{bact} in late July and August (Fig. 4a) coinciding with high PP of the cyanophyte populations (Figs. 2b and 3c) were not followed by an increase of B_{bact}. The bacteria produced were most probably grazed by ciliates, whose maximum followed closely the P_{bact} peak (Figs. 4a and 5a).

The periodical appearance of SRP could be caused by several reasons. If it coincided with an increase in P_{tot}, SS and a high wind speed (as June 19, July 31, September 4, October 10), the resuspension was most probably responsible for it. In the rest of the cases either grazing (ciliate maximum on August 14) or the collapse of the dominating phytoplankton community (September 18) could release remarkable amounts of SRP.

Strong wind and resuspension at the beginning of September (Fig. 6c) could be responsible for the increase of bacterial abundance in spite of quite moderate production (Fig. 4a). Hence, the case here could be interpreted as an event of intermediate disturbance which is defined as a nonbiotic, stochastic event that results in distinct and abrupt changes in the composition and which interferes with internally-driven progress towards selforganization and ecological equilibrium (REYNOLDS *et al.*, 1993).The decline of the biomass and individual weight of ciliates in September (Fig. 5a, d) could be the result of metazooplankton grazing. The collapse of the ciliate community removed the top-down control from bacteria and could be the additional cause of the increase of their biomass.

The development of metazooplankton in September (Fig. 5b) still seemed to prevent the increase of phytoplankton biomass (Fig. 3a) in spite of quite high PP (Fig. 3c). The assumed cladoceran grazing is confirmed also by decreased Chl*a* < 40 μm.

In October and November, phytoplankton biomass and Chl*a* increased in spite of a numerous cladoceran community and a moderate PP. SRP did not occur in measurable amounts after the middle of October reflecting low regeneration, most probably due to a low activity of zooplankton in cold water. Nitrates appeared in the water again, and silicon concentrations reached the spring level. The biomass of N-fixing *Aphanizomenon skujae* decreased while *L. redekei* and *L. planctonica* were quite abundant together with diatoms. Low water level and strong winds (Fig. 6 b, c) caused high resuspension and large amounts of suspended solids in water (Fig. 6a). The increase of diatom biomass in this period was most probably an artefact resulting from the resuspension of sedimented shells. After the formation of ice cover in late November (Fig. 6c), diatoms settled down and disappeared very rapidly while *L. redekei, L. planctonica* and minor algal groups proceeded its development (Fig. 2) and even contributed to the primary production (Fig. 3c) until light was available.

5. Acknowledgements

This work was supported by grants No 1641 and 2017 of the Estonian Science Foundation, by the State monitoring program of Estonian Ministry of Environment and by Finnish Ministry of Environment through the Regional Environmental Agency of Häme. Water level and wind data were kindly provided by the Estonian Institute of Hydrology and Meteorology. The help of Dr. GERTRUD CRONBERG in identification of cyanophyte species as well as the advice given by Dr. JUTA HABERMAN in zooplankton analyses is greatly appreciated. The authors thank HELMI TAMMERT, EVI LILL and KATRIN OTT for technical assistance.

6. References

ANDERSEN, T., 1997: Pelagic nutrient cycles: herbivores as sources and sinks. – Springer-Verlag, Berlin, Heidelberg. 280 pp.

ANONYMOUS, 1979: Recommendations for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll: The Baltic Marine Biologists. Publ. No. 5, 38 pp.

ANONYMOUS, 1984: Guidelines for the Baltic monitoring programme for the third stage: The Baltic Marine Biologists. Publ. No. 1. 2nd Edition.

ARNDT, H. and B. NIXDORF, 1991: Spring clear water phase in a eutrophic lake: Control by herbivorous zooplankton enhanced by grazing on components of the microbial food web. – Verh. Internat. Verein. Limnol. **24**: 879–883.

BALUSHKINA, E. V. and G. G. WINBERG, 1979: Relation between body mass and length in planktonic animals. – *In*: Obshchiye osnovy izucheniya vodnykh ekosistem. G. G. WINBERG (Ed.) Nauka. Leningrad. pp. 169–172. (In Russian).

CZENSNY, R., 1960: Wasser-, Abwasser- und Fischereichemie. VEB Verlag Technik, Berlin: pp. 147–148.

DE HAAN, H. and J. R. MOED, 1984: Phosphorus, nitrogen and chlorophyll *a* concentrations in a typical Dutch polder lake, Tjeukemeer, in relation to its water regime between 1968 and 1982. – Wat. Sci. Tech. **17**: 733–743.

FOISSNER, W., H. BLATTERER, H. BERGER, and F. KOHMANN, 1991: Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems – Band I: Cyrtophorida, Oligotrichida, Hypotrichi, Colpodea. – Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft **1/91:** 1–478.

FOISSNER, W., H. BERGER, and F. KOHMANN, 1992: Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems – Band II: Peritrichia, Heterotrichida, Odontostomatida. – Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft **5/92:** 1–502.

FOISSNER, W., H. BERGER, and F. KOHMANN, 1994: Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems – Band III: Hymenostomata, Prostomatida, Nassulida. – Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft **1/94:** 1–548.

FOISSNER, W., H. BERGER, H. BLATTERER, and F. KOHMANN, 1995: Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems – Band IV: Gymnostomatea, *Loxodes*, Suctoria. – Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft **1/95:** 1–540.

GILRON, G. L. and D. H. LYNN, 1989: Assuming a 50% cell occupancy of the lorica overestimates tintinnine biomass. – Mar. Biol. **103**: 413–416.

GLIWICZ, Z. M., 1969: Studies on pelagic zooplankton in lakes with varying trophy. – Ekol. Polska Ser. A. 17, **36**: 663–708.

GRASSHOFF, K., M. EHRHARDT and K. KREMLING (Eds.), 1982: Methods of Seawater Analysis. Verlag Chemie.

GÜDE, H., 1989: The role of grazing on bacteria in plankton succession. – *In*: U. SOMMER (Ed.): Plankton Ecology. Succession in Plankton Communities. – Springer Verlag. New York, Berlin, Heidelberg: 337–364.

HABERMAN, J., P. NÕGES, T. NÕGES, E. PIHU, K. KANGUR, A. KANGUR, and V. KISAND, 1998: Characterization of Lake Võrtsjärv. – Limnologica **28** (1): 3–11.

HOBRO, R. and E. WILLEN, 1977: Phytoplankton countings. Intercalibration results and recommendations for routine work. – Int. Rev. ges. Hydrobiol. **62**: 805–811.

HUTCHINSON, G. E., 1967: A Treatise on Limnology, Vol. 2, Introduction to Lake Biology and the Limnoplankton. Wiley, New York, 115 pp.

JEFFREY, S. W. and G. F. HUMPHREY, 1975: New spectrophotometric equations for determining chlorophylls *a*, *b*, c_1 and c_2 in higher plants, algae and natural phytoplankton. – Biochem. Physiol. Pflanzen (BPP) **167**: 191–194.

KAHL, A., 1930: Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 1. Allgemeiner Teil und Prostomata. – Tierwelt Deutschlands **18:** 1–180.

KAHL, A., 1931: Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 2. Holotricha außer den im 1. Teil behandelten Prostomata. – Tierwelt Deutschlands **21:** 181–398.

Kahl, A., 1932: Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. – Tierwelt Deutschlands **25:** 399–650.

KAHL, A. 1935: Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 4. Peritricha und Chonotricha. – Tierwelt Deutschlands **30:** 651–886.

KUTIKOVA, L. A. and A. J. STAROBOGATOV, 1977: A key to freshwater invertebrates of European USSR (plankton and benthos). – Gidrometeizdat, Leningrad, 510 p. (in Russian)

LAMPERT, W., W. FLECKNER, H. RAI, and B. E. TAYLOR, 1986: Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase. – Limnol. Oceanogr. **31**: 478–490.

LAMPERT, W., W. GABRIEL, and K. O. ROTHAUPT, 1992: Ecophysiological models: A tool for understanding interactions in freshwater communities. – Verh. Dtsch. Zool. Ges. **85** (2): 5–110.

LINS DA SILVA, N. M., 1991: Etude de la repartition spatio-temporelle des peuplements microbiens planctoniques en Mer Ligure (Mediterranee Nord-Occidentale). – Ph.D. Dissertation, These Doctorat Oceanogr. Biol., Paris. 199 pp.

LYECKE, CH., M. J. VANNI, J. J. MAGNUSON, J. F. KITCHELL, and P. T. JACOBSON, 1990: Seasonal regulation of Daphnia populations by planktivorous fish: Implications for the spring clear-water phase. – Limnol. Oceanogr. **35**: 1718–1733.

NIXDORF, B. and H. ARNDT, 1993: Seasonal changes in the plankton dynamics of a eutrophic lake including the microbial web. – Int. Rev. ges. Hydrobiol. **78**: 403–410.

NORLAND, S., 1993: The relationship between biomass and volume of bacteria. – *In:* Handbook of methods in aquatic microbial ecology. P. F. KEMP, B. F. SHERR, E. B. SHERR, J. J. COLE, (Eds.). Lewis Publishers. Boca Raton, Ann Arbor, London, Tokyo: 303–307.

NÕGES, P. and T. NÕGES, 1998: The effect of fluctuating water level on the ecosystem of Lake Võrtsjärv, Central Estonia. – Proc. Estonian Acad. Sci. Biol. Ecol., **47**(2): 98–113.

NÕGES, T., P. NÕGES, J. HABERMAN, V. KISAND, K. KANGUR, A. KANGUR, and A. JÄRVALT, 1998: Food web structure in shallow eutrophic Lake Võrtsjärv (Estonia). – Limnologica, **28**(1): 115–128.

PADISAK, J., 1992: Seasonal succession of phytoplankton in a large shallow lake (Balaton, Hungary) – a dynamic approach to ecological memory, its possible role and mechanisms. – J. of Ecology, **80**: 217–230.

PATTERSON, D. J. and S. HEDLEY, 1992: Free-living freshwater protozoa. A colour guide. – Wolfe Publishing Ltd., England, 223 p.

PEDRÓS-ALIÓ, C., 1989: Toward an autecology of bacterioplankton. – *In*: U. SOMMER (Ed.): Plankton Ecology. Succession in Plankton Communities. – Springer Verlag. New York, Berlin, Heidelberg: 297–336.

REYNOLDS, C.S., J. PADISAK, and U. SOMMER, 1993: Intermediate disturbance in the ecology of phytoplankton and the maintenance of species diversity: a synthesis. – Hydrobiologia **249**: 183–188.

RUTTNER-KOLISKO, A., 1977: Suggestions for biomass calculation of planktonic rotifers. – Arch. Hydrobiol. **8**: 71–76.

SCHINDLER, D., 1978: Factors regulating phytoplankton production and standing crop in the world's freshwaters. – Limnol. Oceanogr. **23**: 478–486.

SOMMER, U., Z. M. GLIWICZ, W. LAMPERT and A. DUNCAN, 1986: The PEG-model of seasonal succession of planktonic events in fresh waters. – Arch. Hydrobiol. **106**: 433–471.

SOMMER, U., 1989: Toward a Darwinian ecology of plankton. – *In*: U. SOMMER (Ed.): Plankton Ecology. Succession in Plankton Communities. – Springer Verlag. New York, Berlin, Heidelberg: 1–8.

STEEMAN-NIELSEN, E., 1952: The use of radioactive carbon (^{14}C) for measuring primary production in the sea. – Journal du Conseil permanent international pour l'exploration del la mer. **18**: 117–140.

STUDENIKINA, E. I. and M. M. CHEREPAKHINA, 1969: Srednii ves osnovnykh form zooplanktona Azovskogo morya (Mean weight of basic zooplankton forms of the Azov Sea). – Gidrobiol. zhurnal **5**: 89–91. (in Russian).

SIMON, M. and F. AZAM, 1989: Protein content and protein synthesis rates of planktonic marine bacteria. – Mar. Ecol. Prog. Ser. **51**: 201–213.

TULONEN, T., 1993: Bacterial production in a mesohumic lake estimated from [^{14}C]leucine incorporation rate. – Microb. Biol. 26: 210–217.

UTERMÖHL, H., 1958: Zur Vervollkommnung der quantitativen Phytoplanktonmethodik. – Mitt. Int. Ver. Theor. Angew. Limnol. **9**: 1–38.

VYHNALEK, V., J. KOMARKOVA, J. SEDA, Z. BRANDL, K. ĐIMEK, and N. JOHANISOVA, 1991: Clear-water phase in the Rimov Reservoir (South Bohemia): Controlling factors. - Verh. Internat. Verein. Limnol. **24**: 1336–1339.

Manuscript received October 15th, 1997; revised April 14th, 1998; accepted May 20th, 1998

II

Zingel, P. 1999.
Pelagic ciliated protozoa in a shallow eutrophic lake:
community structure and seasonal dynamics.
Arch. Hydrobiol. 146: 495–511.

Arch. Hydrobiol. 146 4 495–511 Stuttgart, November 1999

Pelagic ciliated protozoa in a shallow eutrophic lake: community structure and seasonal dynamics

Priit Zingel[1]

Võrtsjärv Limnological Station, Estonia, University of Tartu, Estonia

With 9 figures and 3 tables

Abstract: Seasonal population dynamics and community composition of planktonic ciliates of shallow eutrophic Lake Võrtsjärv were studied from January 1995 to December 1996. The abundances (1995 mean = 39.0×10^3 cells/l, 1996 mean = 60.1×10^3 cells/l) and biomasses (1995 mean = 0.68 mg/l, 1996 mean = 2.91 mg/l) were high and showed an increasing trend with the decrease in water level. Ciliate abundances peaked in spring (May) and in late summer (July, August) reaching values up to 191.1×10^3 cells/l. The biomass maxima did not coincide with maximum abundances and were tied with the mass occurrences of large-sized carnivorous species. Ciliates accounted for 50 to 64 % of the zooplankton biomass (in 1995 and 1996, respectively). Oligotrichs, haptorids, scuticociliates and prostomatids dominated the community of ciliates.

Introduction

Recent studies have highlighted the importance of heterotrophic flagellates and ciliates in the predation and control of picoplanktonic algae and bacteria (BARK 1981, BEAVER & CRISMAN 1982, SHERR & SHERR 1984, NAGATA 1988, GÜDE 1989, GONZALES et al. 1990). Ciliates also graze particles that can be ingested by rotifers and cladocerans (SANDERS et al. 1989), hence there may be exploitative competition between ciliates and metazooplankton. Clear evidence exists that planktonic ciliates are an important food resource for large metazooplankton (PORTER et al. 1979, DOLAN & COATS 1991, GIFFORD 1991). It has been shown that metazooplankters suppress ciliates through predation and interference (WICKHAM & GILBERT 1991, 1993). While ciliates can consume sizeable proportions of bacterio- and phytoplankton production, metazooplankton predation on ciliates could be an important trophical link be-

[1] **Author's address:** University of Tartu, Institute of Zoology and Hydrobiology, Vanemuise 46, Tartu, Estonia, 51014. E-mail: pzingel@zbi.ee

0003-9136/99/0146-0495 $ 4.25

tween pico- and nanoplankton and metazoans. In addition to their role in energy transfer to higher trophic levels, ciliated protozoa act in bio-geochemical cycling of phosphorus and nitrogen and can increase the availability of nutrients for phytoplankton growth (JOHANNES 1965, BUECHLER & DILLON 1974, BERMAN et al. 1987). The number of papers based on freshwater protozooplankton investigations has increased recently (HECKY & KLING 1981, PACE & ORCUTT 1981, TAYLOR & HEYNEN 1987, BEAVER et al. 1988, CARRICK & FAHNENSTIEL 1990, LAYBOURN-PARRY et al. 1990, CARRIAS et al. 1994, JAMES et al. 1995), but the role of ciliated protozoa in lakes is far from clear. Their preys and their predators are mostly a matter of spculation. Also, detailed studies describing the seasonal succession of ciliates in freshwater ecosystems are rare.

The aim of this sutdy was to describe the community structure, abundance and seasonality of planktonic ciliated protozoa in a shallow eutrophic lake over two consecutive years.

Materials and methods

Lake Võrtsjärv is a large (270 km^2) and shallow (mean depth 2.8 m, maximum depth 6 m) eutrophic lake situated in the Central Estonian depression of preglacial origin. The water temperature reaches its maximum in July (20.1 °C on average). The ice cover lasts from November to April (135 days on average). Homothermy prevails in L. Võrtsjärv throughout the year. The water is slightly alkaline, the monthly mean pH ranging from 7.6 to 8.5, salinity from 227 to 370 mg/l, and the concentrations of chlorides and sulphates from 9 to 14 mg/l and from 17 to 21 mg/l, respectively. The average chemical and biochemical oxygen demand (COD by K_2MnO_4 method and BOD_7) are 11 and 3.8 mg O/l, accordingly. The average total phosphorus concentration (P_{tot} is 54 µg/l, total nitrogen concentration (N_{tot} 1.6 mg/l, and the mean Secchi depth 1.1 m. During the vegetation period, Secchi depth usually does not exceed 1 m. Water blooms are a common phenomenon in L. Võrtsjärv. In addition, the shallowness of the lake and the wave-induced resuspension of bottom sediments contribute to the formation of high seston concentrations and high turbidity during summer. Most of the organic compounds in the lake are of autochthonous character (NÕGES et al. 1998). The hydrobiological and physiochemical characteristics of Lake Võrtsjärv in 1995–1996 are summarised in Table 1. Changes in water level are shown in Fig. 1 and oxygen concentrations in Fig. 2.

Ciliate samples were collected weekly in 1995 and biweekly in 1996 at the station near the deepest area of Lake Võrtsjärv. The sampling period lasted from January 1995 to December 1996. The entire water column was sampled at one-meter intervals using a Moltchanov's water sampler. Samples were integrated and then 250 ml subsamples were preserved and fixed with acidified Lugol's solution. Ciliate biomass and community composition were determined using the UTERMÖHL (1958) technique. Samples were stored at 4 °C in the dark. Volumes of 10–100 ml were settled for at least 24 h in

Table 1. Range of hydrobiological and physicochemical parameters of Lake Võrtsjärv, Estonia during 1995-1996 (Temp = temperature; O_2 = oxygen; Tot N = total nitrogen; Tot P = total phosphorus; Chl-a = Chlorophyll-a; Metazp. = metazooplankton biomass; WW = wet weight). The mean total nitrogen and total phosphorus values are given in parentheses.

Year	Temp (°C)	O_2 (mg l^{-1})	Secchi depth (m)	Tot N (mg l^{-1})	Tot P (mg l^{-1})	Chl-a (µg l^{-1})	Metazp. (mgWW l^{-1})
1995	0.3–21.6	6.9–13.5	0.6–2.4	0.5–2.9 (1.07)	0.017–0.088 (0.051)	2.1–64.0	0.0004–3.13
1996	1.4–24.8	0.0–14.5	0.15–2.7	0.5–2.5 (1.48)	0.014–0.330 (0.076)	0.3–105.3	0.0–7.04

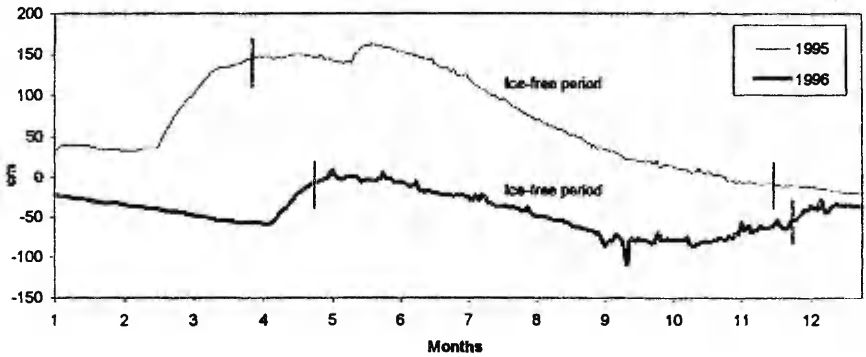

Fig. 1. Water level and ice-free period in Lake Võrtsjärv in 1995 and 1996 (0 = watermark at Jõesuu observation post).

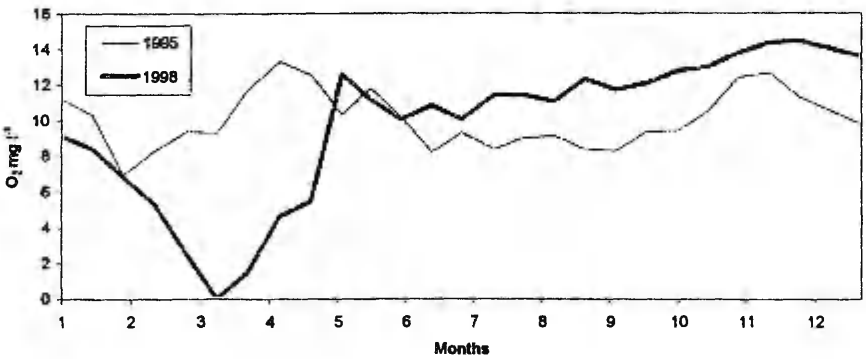

Fig. 2. Oxygen concentrations in Lake Võrtsjärv in 1995 and 1996.

plankton chambers. Ciliates were enumerated and identified with an inverted microscope (Wild Heerbrug M40 and Nikon diaphot-TMD) at 200–600× magnification. The entire content of each Utermöhl chamber was surveyed, if the total tally was <150 organisms, an additional subsample was counted. Ciliates were usually identified to genus by consulting several works (Kahl 1930–1935, Kutikova & Starobogatov 1977, Patterson & Hedley 1992, Foissner & Berger 1996). The taxonomy fol-

lowed mainly the scheme of CORLISS (1979). Additional live subsamples were used to aid identification. The first 20 measurable specimens encountered for each taxon were measured. Biovolumes of each taxa were estimated by assuming geometric shapes. Specific gravity was assumed to be 1.0 g/ml (FINLAY 1982), so the biomass was expressed as wet weight (WW).

The abundance of bacteria (acriflavine direct count) was determined under an epifluorescence microscope (magnification 1,000×) as in TULONEN (1993). For sampling metazooplankton, 20 l of depth-integrated lake water were filtered through a net of 80 µm mesh size. Samples were fixed with formalin (4 % final concentration). The numbers of small rotifers (e.g. *Keratella cochlearis, Anuraeopsis fissa* etc.) were estimated from the samples collected and fixed with the same methods as the ciliate samples. For more details see NÕGES et al. (1998).

Nonparametric methods were used for statistical analysis as all data had neither normal nor lognormal distribution.

Results

The ciliate community composition

During the investigation period 1995/1996 the population of ciliated protozoans was largely dominated by oligotrichs (Fig. 3). The most common oligotrichs were *Strobilidium* spp. *Strombidium* spp., and *Tintinnidium fluviatile*. On some occasions, also haptorids (*Mesodinium* sp., *Dileptus* sp., *Askenasia volvox, Monodinium* sp.), prostomatids (*Urotricha* sp., *Coleps* spp.), peritrichs (*Vorticella* spp., *Epistylis procumbens*) and scuticociliates (*Uronema* sp., *Cyclidium* sp.) were quite abundant. The greatest species diversity in both years was present in summer. A list of species is given in Table 2. The ciliate community consisted in 1995 of 34 and in 1996 of 38 identifiable taxa.

Abundance, biomass and seasonal dynamics of ciliates

In 1995, the mean abundance of ciliated protozoa was 39.0×10^3 cells/l (111×10^6 cells/m^2) and the mean biomass was 0.68 mg/l (1.98 g/m^2). In 1996, these numbers were 60.1×10^3 cells/l (114×10^6 cells/m^2) and 2.91 mg/l (5.36 g/m^2), respectively. The abundance and biomass of planktonic ciliates were significantly positively ($p < 0.0001$) correlated with temperature, chlorophyll-a, bacterial abundance and metazooplankton biomass (Table 3).

In the beginning of both investigated years, the community of planktonic ciliates was dominated by large-sized (∅ > 50 µm) species (Fig. 4). During this period, the number and biomass of ciliates was low (Figs. 5 and 6). This situation lasted until ice break when the abundance of ciliates started to rise rapidly. This peak was made up by large-sized herbivores (mostly *Stromibidium,* and *Tintinnidium*). Spring maximum numbers were in both years achieved in

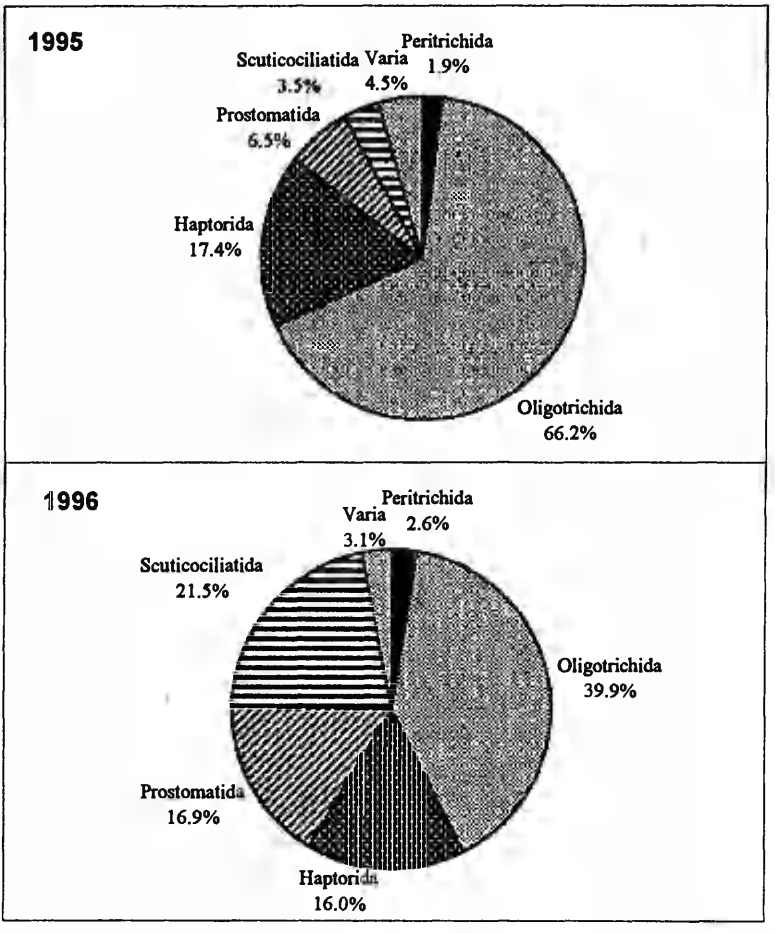

Fig. 3. Relative importance of different groups of ciliates in Lake Võrtsjärv during 1995 and 1996 as a percentage of abundance.

May (125.2×10^3 cells/l and 118.2×10^3 cells/l in 1995 and 1996, respectively) and were followed by a fast decrease.

The second large peak of ciliates occurred in late July, early August when the annual maximum abundance was reached (191.1×10^3 cells/l and 178.5×10^3 cells/l in 1995 and 1996, respectively). During this period, the community of ciliates was dominated by small bacterivorous species like *Strobilidium* sp., *Halteria* sp., *Uronema* sp. and *Cyclidium* sp. In 1995 the summer peak was mostly made up by oligotrichs and in 1996 by scuticociliates (Fig. 7). In summer 1996, the number of herbivores (prostomatids and larger oligotrichs) was also high. Large (∅ > 300 µm) carnivorous ciliates (*Paradileptus* sp., *Dileptus* sp., *Stentor amethystinus*) showed several peaks in both summers.

Table 2. Species list of Ciliophora found in shallow eutrophic Lake Võrtsjärv, Estonia during 1995-1996 (× = present, – = not present).

	1995	1996
Colpodida		
Bursaridium sp.	–	×
Cyrtolophosis sp.	–	×
Haptorida		
Cyclotrichium limneticum KAHL, 1932	×	–
Didinium sp.	×	–
Didinium balbiani BÜTSCHLI, 1874	×	×
Mesodinium pulex CLAPARÉDE & LACHMANN, 1858	×	×
Mesodinium sp.	×	×
Askenasia volvox CLAPARÉDE & LACHMANN, 1859	×	×
Lacrymaria sp.	×	–
Dileptus sp.	×	×
Paradileptus caducus KAHL, 1932	×	×
Actinobolina radians STEIN, 1852	×	–
Actinobolina sp.	×	×
Ilonema sp.	×	×
Heterotrichida		
Stentor amethystinus LEIDY, 1880	×	–
Hymenostomatida		
Paramecium sp.	–	×
Disematostoma tetraedrium FAURÉ-FREMIET, 1924	–	×
Oligotrichida		
Strobilidium lacustris FOISSNER, SKOGSTAD & PRATT, 1988	×	×
Strobilidium sp.	×	×
Strombidium sp.	×	×
Halteria bifurcata TAMAR, 1968	×	–
Halteria sp.	×	–
Tintinnidium fluviatile STEIN, 1833	×	×
Tintinnopsis cylindrata KOFFOID & CAMPBELL, 1929	×	–
Tintinnopsis tubulosa LEVANDER, 1894	×	×
Tintinnopsis sp.	×	×
Codonella cratera LEIDY, 1877	×	×
Peritrichida		
Vorticella natans FAURÉ-FREMIET, 1924	–	×
Vorticella sp.	×	×
Opercularia sp.	×	–
Scyphidia sp.	–	×
Vaginicola ingenita MÜLLER, 1786	–	×
Epistylis procumbens ZACHARIAS, 1897	–	×
Lagenophrys sp.	–	×
Ophrydium versatile MÜLLER, 1786	×	×
Pleurostomatida		
Litonotus sp.	×	×
Loxophyllum sp.	×	×

Table 2. Continued.

	1995	1996		1995	1996
Scuticociliatida			Prostomatida		
Cyclidium sp.	×	×	*Urotricha* sp.	×	×
Calyptotricha lanuginosa PENARD, 1922	–	×	*Coleps spetai* FOISSNER, 1984	–	×
Uronema sp.	×	×	*Coleps* sp.	–	×
Hypotrichida			Suctorida		
Oxytricha sp.	×	–	*Podophrya sp.*	×	×
Stylonychia mytilus EHRENBERG, 1838	×	–	*Sphaerophrya magna* MAUPAS, 1881	–	×
Uroleptus piscis MÜLLER, 1773	–	×			
Stichotricha aculeata WRZESNIOWSKI, 1866	–	×			

Table 3. Correlation coefficients (Spearman R) between abundance and biomass of planktonic ciliates and physicochemical and biological parameters in Lake Võrtsjärv, Estonia during 1995-1996 (Metazp. = metazooplankton; * = $p < 0.0001$; ^{ns} = not significant).

	Temperature	O_2	Chloropyll-a	Bacterial abundance	Metazp. biomass
Ciliate abundance	0.7659*	0.1943^{ns}	0.5870*	0.7387*	0.7026*
Ciliate biomass	0.5628*	0.1896^{ns}	0.5366*	0.7174*	0.5145*

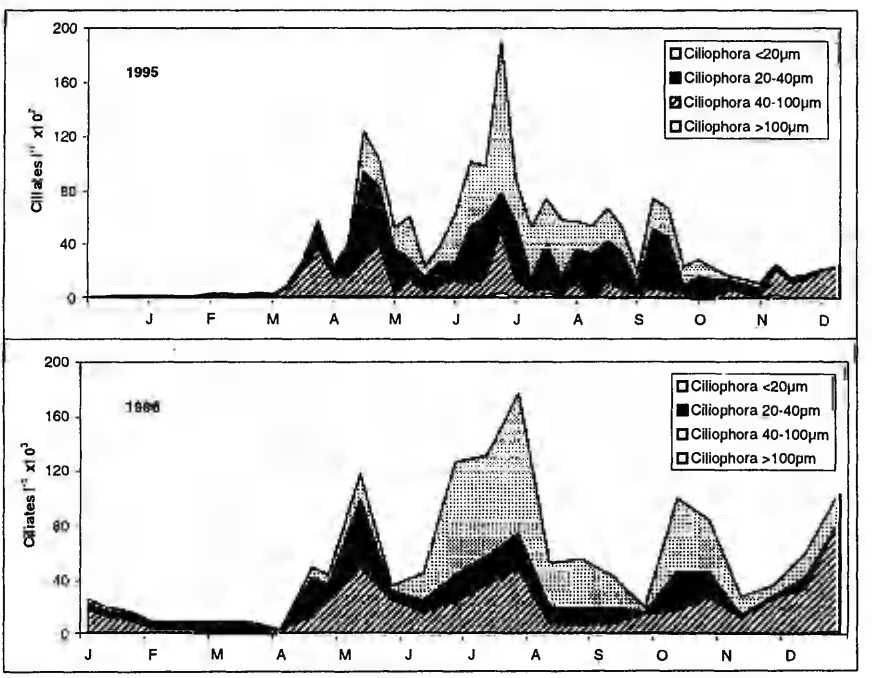

Fig. 4. Seasonal changes in the size spectrum of ciliates in Lake Võrtsjärv during 1995 and 1996.

Their abundance was not high, but due to the large size, their contribution to the total biomass was remarkable. The highest values of biomass coincided always with these peaks (5.6 mg/l and 16.1 mg/l in 1995 and 1996, respectively). The year 1996 showed much higher biomasses than 1995, even though there were no great differences in ciliate abundances, because the abundances of the above-mentioned large-sized carnivorous ciliates were often high, especially in autumn and winter. The large carnivorous species *Dileptus* and *Paradilep-*

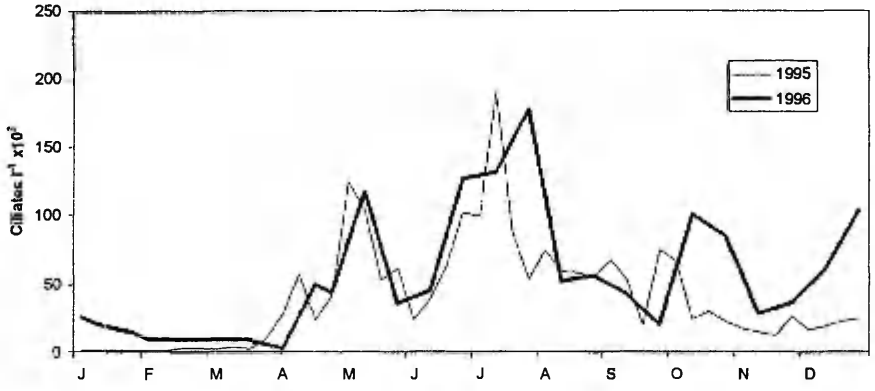

Fig. 5. Ciliate abundance in Lake Võrtsjärv during 1995 and 1996.

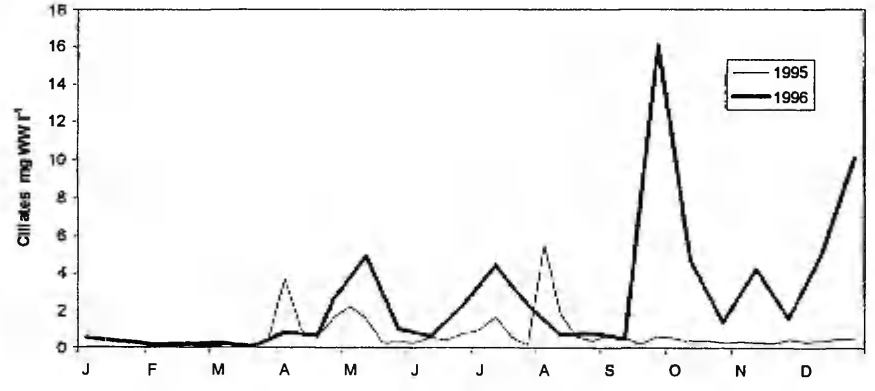

Fig. 6. Ciliate biomass in Lake Võrtsjärv during 1995 and 1996.

tus made up 97 % of the maximum biomass, that was achieved in 1996 on 1st October.

In autumn, ciliate abundances peaked in October when the community was dominated by herbivorous oligotrichs (1995) or by haptorids and prostomatids (1996). The dominant species in winter were *Tintinnidium fluviatile, Strobilidium* sp. (∅ > 50 µm) and *Coleps* sp.

Planktonic ciliates formed up to 50 % of the whole zooplankton biomass of Lake Võrtsjärv in 1995 and up to 64 % in 1996 (Fig. 8).

Bacteria and metazooplankton

The number of bacteria ranged from 0.19×10^6 to 2.69×10^6 cells/ml in 1995 (mean 1.17×10^6 cells/ml) and from 0.54×10^6 to 4.37×10^6 cells/ml in 1996 (mean 1.83×10^6 cells/ml). The annual maxima were reached in July and in

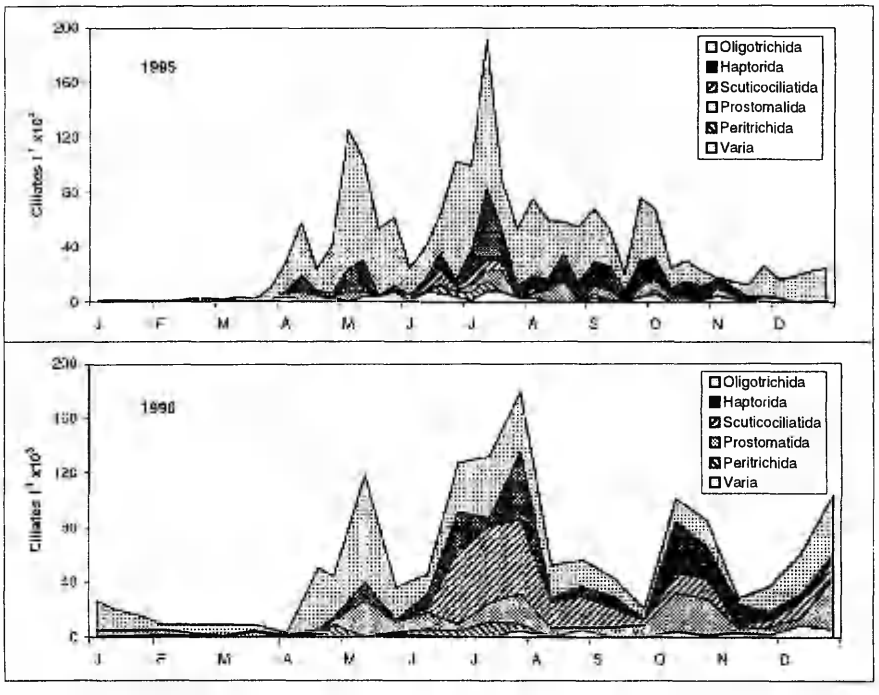

Fig. 7. Seasonal changes in abundance of quantitatively important ciliate groups in Lake Võrtsjärv during 1995 and 1996.

September (in 1995 and 1996, respectively). Smaller peaks occurred also in May, September and November (1995) and in March, June and October (1996).

The biomass of metazooplankton ranged from 0 to 3.13 mg WW/l in 1995 (mean 0.71 mg WW/l) and from 0.005 to 7.04 mg WW/l in 1996 (mean 1.57 mg WW/l). The annual maxima were reached in July (1995) and in June (1996) (Fig. 9). The community was mostly dominated by small cladocerans, mainly *Chydorus sphaericus*. The most abundant rotifers were *Anuraeopsis fissa* and *Trichocerca rousseleti, Mesocyclops leuckarti* was the dominating species among copepods.

Discussion

Abundances of planktonic ciliates recorded in Lake Võrtsjärv correspond to the range reported for eutrophic subtropical lakes (Beaver & Crisman 1982), but are higher than in most reported eutrophic temperature lakes (Laybourn-Parry 1992). They fall within the range determined for the mesotrophic Rus-

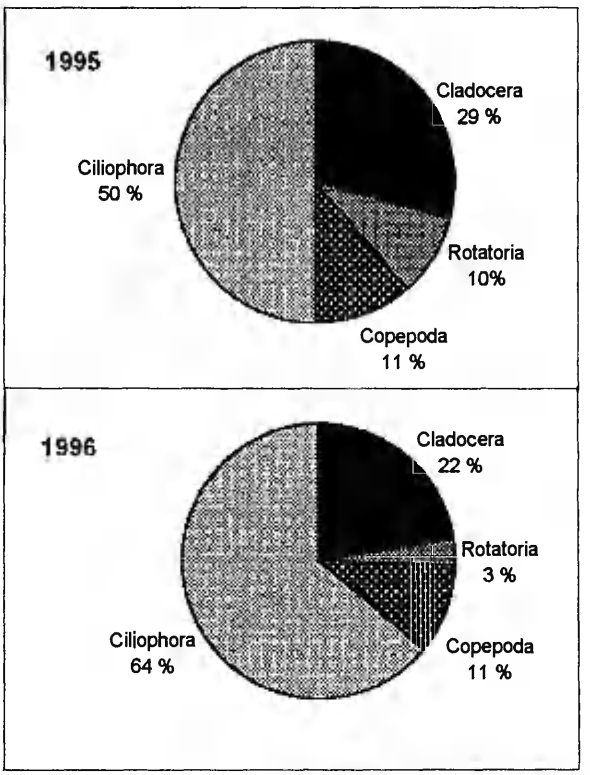

Fig. 8. Relative importance of different zooplankton groups in Lake Võrtsjärv during 1995 and 1996 as a percentage of biomass.

ter Poschen (mean 51×10^3 cells/l, maximum 203×10^3 cells/l) a part of the large and shallow Neusiedler See (SCHÖNBERGER 1994). The ciliate genera found in this study are typical of temperate lakes. In Lake Võrtsjärv, the communities were dominated by oligotrichs, haptorids, scuticociliates and prostomatids. All these groups are often reported as relatively common component of lacustrine protozooplankton (SHCHERBAVOV 1969, MAMAEVA 1976, PACE & ORCUTT 1981, HECKY & KLING 1981, BEAVER & CRISMAN 1982, CARRICK & FAHNENSTIEL 1990, LAYBOURN-PARRY et al. 1990, MÜLLER et al. 1991, JAMES et al. 1995).

BEAVER & CRISMAN (1982) who investigated 20 freshwater lakes along a trophic gradient, found that in eutrophic lakes oligotrichs, haptorids and scuticociliates made up 28, 11 and 33 % of total the abundance, respectively, and that the large, algivorous species were progressively replaced by small bacterivorous ciliates with increasing Chl-a concentrations. In the eutrophic Lake Võrtsjärv, bacterivorous scuticociliates were less important and made up only

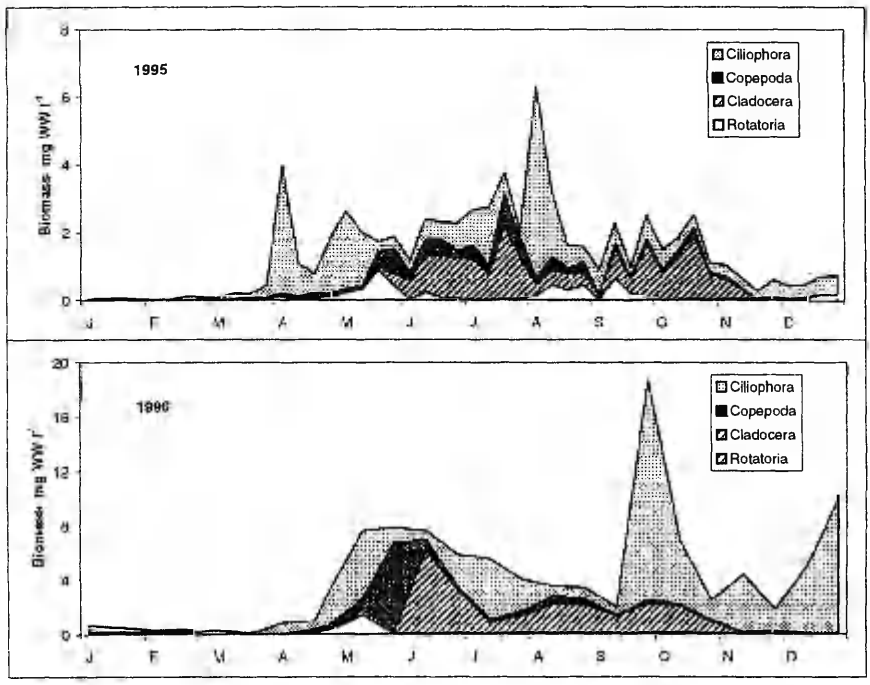

Fig. 9. Seasonal changes in biomass of main zooplankton groups in Lake Võrtsjärv during 1995 and 1996. Note that the scales are different.

4 and 22 % of total the abundance in 1995 and 1996, respectively. MÜLLER et al. (1991) reported that small scuticociliates accounted for less than 2 % of the ciliate numbers in the upper 20 m of meso-eutrophic Lake Constance. They suggest that scuticociliates tend to concentrate in the oxycline where bacterial productivity is high. Hence, the relationship between Chl-a and scuticociliate concentrations may largely be due to the build-up of oxygen gradients in more productive systems (MÜLLER et al. 1991). The shallow Lake Võrtsjärv is constantly mixed by wind resulting in a fully oxygenated water column (anoxia is rare), which may partly explain the relatively small importance of scuticociliates. According to BERK et al. (1976) and FENCHEL (1980), the bacterivorous ciliates are mostly excluded from lakes with less then 5×10^6 bacterial cells/ml. In Lake Võrtsjärv, the abundance of bacteria never reached that level in 1995–1996. However, in 1996 when the mean bacterial abundance was higher than in 1995, also the importance of scuticociliates increased while oligotrichs and haptorids were less important (Fig. 3). Peaks in abundance of large-sized carnivorous ciliates followed mostly the peaks of smaller bacterio- and bacterio-herbivorous ciliates, which are very likely the major food source for carnivores.

In 1996 the mean abundance of ciliates increased (c.f. 1995) more than 1.5 mes, while the mean biomass increased more than 4.5 times. Resuspension f sediments was more intensive due to very low water level in 1996 and a ırge amount of nutrients could be released from the sediment pore-water. his leads to a rapid increase of phytoplankton biomass (NÕGES & NÕGES 998). The algal community of Lake Võrtsjärv is mostly dominated by fila- ıentous species like *Aulacoseira* spp., *Limnothrix* spp., *Planktolyngbya lim- etica* and *Aphanizomenon skujae,* accompanied by small chlorophytes and hrysophytes (NÕGES et al. 1998). In 1996, the filamentous species were tem- orally replaced by fast growing, small-sized chroococcal cyanobacteria NÕGES & NÕGES 1998), which are easily consumed by herbivorous ciliates. he abundance of ciliates was significantly ($p<0.0001$) correlated with chloro- hyll-a. Such correlation has been demonstrated in several studies (PACE 1982, EAVER et al. 1988, JAMES et al. 1995) and seems to be a common feature for ıany temperate lakes.

Peaks in planktonic ciliate number described in Lake Võrtsjärv in spring nd late summer are in good accordance with the data on temporal occurrence f ciliates (BEAVER & CRISMAN 1989). The formation of a spring peak domi- ated by larger herbivorous ciliates and a second peak during summer domi- ated mostly by smaller bacterivores has been described frequently (e.g. CAR- ICK & FAHNENSTIEL 1990, ŠIMEK & STAŠKRABOVÁ 1992). MÜLLER et al. 1991) described a seasonal pattern in abundance of planktonic ciliates in the ıeso-eutrophic Lake Constance over a three-year period. They distinguished ɔur seasonal phases: spring, clear-water phase, summer/autumn and winter. ıbundance peaks occurred in spring and summer/autumn, while ciliate num- ers were low during the clear-water phase (late spring) and in winter. This orresponds well with the results of the present study. However, in Lake 'õrtsjärv the highest ciliate numbers were recorded in late summer and not in ɔring. In most temperate lakes across the trophic spectrum the maximum bundance of ciliates is achieved in late spring or early summer (LAYBOURN- ARRY 1992), although there are exceptions to the trend. In the shallow Ruster oschen, the maximum abundance of ciliates was recorded in late summer, not ı spring (SCHÖNBERGER 1994). Also, GATES & LEWG (1984) described late ummer peaks in several oligotrophic lakes in Ontario.

Ciliate collapses at the beginning of June coincided with the start of the ladoceran development and with the increase of other metazooplankton roups (Fig. 9). Metazooplankton are known to prey intensively on ciliates SOROKIN & PAVELIEVA 1972, MALY 1975, BERK et al. 1977, PORTIER et al. 979, HEINBOKEL & BEERS 1979) and can affect their numbers. Various studies onducted in enclosures have demonstrated the limiting effects of copepods on ıe abundance of ciliates (CARRICK et al. 1991, TAYLOR & JOHANNSSON 1991). VICKHAM & GILBERT (1991, 1993) showed in their experiments that both

large and small cladocerans could suppress ciliates through predation and interference, rather than exploitative competition. On the other hand, according to LAYBOURN-PARRY (1992), competition for food resources may be one of the main aspects controlling the temporal patterns of protozoan occurrence and abundance. Ciliates have the advantage of rapid division rates, which enable them to establish large populations relatively quickly. When the populations of metazooplankton are built up, ciliates are often largely excluded because the grazing rates of metazooplankters are higher than those of ciliates (LAYBOURN 1992). BEAVER & CRISMAN (1982) suggested that limiting food resources control the ciliate community composition and abundance rather than metazoan grazing. This may also be the case in Lake Võrtsjärv where ciliates are clearly bottom-up controlled, as suggested by the fact that metazooplankton and ciliates in Lake Võrtsjärv were positively correlated, not negatively.

The patterns of ciliate abundance and species composition seen in this study are unlikely to be controlled by any single factor. This paper demonstrated that the ciliates are an important trophic link in a eutrophic freshwater ecosystem and seem to be the most important group of zooplankton in Lake Võrtsjärv as they accounted for 50 to 64 % of total zooplankton biomass (in 1995 and 1996, respectively). Further studies on the distribution and feeding modes of ciliated protozoans in shallow eutrophic lakes are needed to get more detailed information about the described patterns.

Acknowledgements

I want to express my gratitude to TIINA NÕGES, PEETER NÕGES, VELJO KISAND, JUTA HABERMAN, LEA TUVIKENE, KATRIN OTT and HELEN TAMMERT for allowing to use the Lake Võrtsjärv hydrobiological and physicochemical data and to Lammi Biological Station for allowing to use the microscopes for processing of ciliate samples. I also thank TIINA NÕGES and two anonymous reviewers who offered constructive suggestions on an earlier version of the manuscript. This research was supported by the grant nr. 2017 of the Estonian Science Foundation and by the Finnish Ministry of Environment through the Regional Environmental Agency of Häme.

References

BARK, A. W. (1981): The temporal and spatial distribution of planktonic and benthic protozoan communities in a small productive lake. – Hydrobiologia **85:** 239–255.

BEAVER, J. R. & CRISMAN, T. L. (1982): The trophic response of ciliated protozoans in freshwater lakes. – Limnol. Oceanogr. **27:** 246–253.

– – (1989): The role of ciliated protozoa in pelagic freshwater ecosystems. – Microb. Ecol. **17:** 111–136.

BEAVER, J. R., CRISMAN, T. L. & BIERNERT, R. W. (1988): Distribution of planktonic ciliates in highly colored subtropical lakes: Comparison with clearwater ciliate communities and the contribution of mixotrophic taxa to total autotrophic biomass. – Freshwat. Biol. **20:** 51–60.

BERK, S. G., BROWNLEE, D. C., HEINLE, D. R., KLING, H. J. & COLWELL, R. R. (1977): Ciliates as a food source for marine planktonic copepods. – Microb. Ecol. **4:** 27–40.

BERK, S. G., COLWELL, R. R. & SMALL, E. B. (1976): A study of feeding responses to bacterial prey by estuarine ciliates. – Trans. Amer. Microsc. Soc. **95:** 514–520.

BERMAN, T., NAWROCKI, M., TAYLOR, G. T. & KARL, D. M. (1987): Nutrient flux between bacteria, bacterivorous nanoplanctonic protists and algae. – Mar. Microb. Food Webs **2:** 69–82.

BUECHLER, D. G. & DILLON, R. D. (1974): Phosphorus regeneration in freshwater *Paramecia*. – J. Protozool. **21:** 339–343.

CARRIAS, J. F., AMBLARD, C. & BOURDIER, G. (1994): Vertical and temporal heterogeneity of planktonic ciliated protozoa in a humic lake. – J. Plankton Res. **16:** 471–485.

CARRICK, H. J. & FAHNENSTIEL, G. L. (1990): Planktonic protozoa in Lakes Huron and Michigan: seasonal abundance and composition of ciliates and dinoflagellates. – J. Great Lakes Res. **16:** 319–329.

CARRICK, H. J., FAHNENSTIEL, G. L., STORMER, E. F. & WETZEL, R. G. (1991): The importance of zooplankton-protozoan trophic couplings in Lake Michigan. – Limnol. Oceanogr. **36:** 1335–1345.

CORLISS, J. O. (1979): The ciliated protozoa: Characterization, classification and guide to the literature. 2nd Edition. – Pergamon Press, London, 455 p.

DOLAN, J. R. & COATS, D. W. (1991): Preliminary prey digestion in a predacious estuarine ciliate and the use of digestion data to estimate digestion. – Limnol. Oceanogr. **36:** 558–565.

FENCHEL, T. (1980): Suspension feeding in ciliated protozoa: Functional response and particle size selection. Feeding rates and their ecological significance.-Microb. Ecol. **6:** 13–25.

FINLAY, B. J. (1982): Procedures for the isolation, cultivation and identification of protozoa. – In: BURNS, R. G. & SLATER, J. H. (eds.): Experimental Microbial Ecology. – Blackwell Scientific Publications, Oxford, pp. 44–65.

FOISSNER, W. & BERGER, H. (1996): A user-friendly guide to the ciliates (Protozoa, Ciliphora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. – Freshwat. Biol. **35:** 375–482.

GATES, M. A. & LEWG, U. T. (1984): Contribution of ciliated protozoa to the planktonic biomass in a series of Ontario lakes, quantitative estimates and dynamical relationships. – J. Plankton Res. **6:** 443–456.

GIFFORD, D. J. (1991): The protozoan-metazoan trophic link in pelagic ecosystems. – J. Protozool. **38:** 81–86.

GONZALES, J. M., SHERR, E. B. & SHERR, B. F. (1990): Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. – Appl. Environ. Microbiol. **56:** 583–589.

GÜDE, H. (1989): The role of grazing on bacteria in plankton succession. – In: SOMMER, U. (ed.): Plankton ecology: Succession in Planktonic Communities. – Brock/Springer, Berlin, pp. 337–364.

HECKLY, R. E. & KLING, H. J. (1981): The phytoplankton and proto-zooplankton of Lake Tanganyika: species composition, biomass, chlorophyll content and spatio-temporal distribution. – Limnol. Oceanogr. **26:** 548–564.

HEINBOKEL, J. F. & BEERS, J. R. (1979): Studies on the functional role of tintinnids in the Southern California Bight. 3. Grazing impact of natural assemblages. – Mar. Biol. **52:** 23–32.

JAMES, M. R., BURNS, C. W. & FORSYTH, D. J. (1995): Pelagic ciliated protozoa in two monomictic, southern temperate lakes of contrasting trophic state: seasonal distribution and abundance. – J. Plankton Res. **17:** 1479–1500.

JOHANNES, R. E. (1965): Influence of marine Protozoa on nutrient regeneration. – Limnol. Oceanogr. **10:** 434–442.

KAHL, A. (1930): Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 1. Allgemeiner Teil und Prostomata. – Tierwelt Deutschlands **18:** 1–180.

– (1931): Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 2. Holotricha außer den im 1. Teil behandelten Prostomata. – Tierwelt Deutschlands **21:** 181–398.

– (1932): Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. – Tierwelt Deutschlands **25:** 399–650.

– (1935): Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 4. Peritricha und Chonotricha. – Tierwelt Deutschlands **30:** 651–886.

KUTIKOVA, L. A. & STAROBOGATOV, A. J. (1977): A key to freshwater invertebrates of European USSR (plankton and benthos). – Gidrometeizdat, Leningrad, 510 p. (in Russian).

LAYBOURN-PARRY, J. (1992): Protozoan plankton ecology. – Chapmann & Hall Ltd., London, 231 p.

LAYBOURN-PARRY, J., OLIVER, J., ROGERSON, A. & DUVERGÉ, P. L. (1990): The temporal and spatial patterns of protozooplankton abundance in a eutrophic temperate lake. – Hydrobiologia **203:** 99–110.

MALY, E. J. (1975): Interactions among the predatory rotifer *Asplancha* and two prey, *Paramecium* and *Euglena*. – Ecology **56:** 346–358.

MAMAEVA, N. V. (1976): Planktonic ciliates in the Ivan'kovsky water reservoir. – Zool. Zhur. Uk. **55:** 657–664.

MÜLLER, H., SCHÖNE, A., PINTO-COELHO, R. M., SCHWEIZER, A. & WEISSE, T. (1991): Seasonal succession of ciliates in Lake Constance. – Microb. Ecol. **21:** 119–138.

NAGATA, T. (1988): The microflagellate-picoplankton food linkage in the water column of lake Biwa. – Limnol. Oceanogr. **33:** 504–517.

NÕGES, T., KISAND, V., NÕGES, P., PÕLLUMÄE, A., TUVIKENE, L. & ZINGEL, P. (1998): Plankton seasonal dynamics and its controlling factors in shallow polymictic eutrophic lake Võrtsjärv, Estonia. – Int. Rev. ges. Hydrobiol. **83:** 279–296.

NÕGES, T. & NÕGES, P. (1998): The effect of fluctuating water level on the ecosystem of Lake Võrtsjärv, central Estonia. – Proc. Estonian Acad. Sci. Biol. Ecol. **47:** 98–113.

PACE, M. L. (1982): Planktonic ciliates: their distribution, abundance and relationship to microbial resources in a monomictic lake. – Can. J. Fish. Aquat. Sci. **39:** 1106–1116.

PACE, M. L. & ORCUTT, J. D. (1981): The relative importance of protozoans, rotifers, and crustaceans in a freshwater zooplankton community. – Limnol. Oceanogr. **26:** 822–830.

PATTERSON, D. J. & HEDLEY, S. (1992): Free-living freshwater protozoa. A color guide. – Wolfe Publishing Ltd., England, 223 p.

PORTER, K. G., PACE, M. L. & BATTEY, J. F. (1979): Ciliate protozoans as links in freshwater planctonic food chains. – Nature **277:** 563–565.

SANDERS, R. W., PORTER, K. G., BENNETT, S. J. & DEBIASE, A. E. (1989): Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. – Limnol. Oceanogr. **34:** 673–687.

SCHÖNBERGER, M. (1994): Plankton ciliated protozoa of Neusiedler See (Austria/Hungary) – a comparison between the turbid open lake and a reedles brown-water pond. – Mar. Microbial Food Webs. **8:** 251–263.

SHCHERBAVOV, A. D. (1969): Quantity and biomass of Protozoa in the plankton of a eutrophic lake. – Hydrobiol. J. **5:** 9–15.

SHERR, E. B. & SHERR, B. F. (1984): Role of heterotrophic protozoa in carbon and energy flow in aquatic ecosystems. – In: KLUG, M. J. & REDDY, C. A. (eds.): Current Perspectives in Microbial Ecology. – Amer. Soc. Microbiol., Washington, pp. 412–423.

ŠIMEK, K. & STRAŠKRABOVÁ, V. (1992): Bacterioplankton production and protozoan bacterivory in a mesotrophic reservoir. – J. Plankton Res. **14:** 773–787.

SOROKIN, Y. I. & PAVELJEVA, E. B. (1972): On the quantitative characteristics of the pelagic ecosystem of Dalnee Lake (Kamchatka). – Hydrobiologia **40:** 519–552.

TAYLOR, W. D. & HEYNEN, M. L. (1987): Seasonal and vertical distribution of Ciliophora in Lake Ontario. – Can. J. Fish. Aquat. Sci. **44:** 2185–2191.

TAYLOR, W. D. & JOHANSSON, O. E. (1991): A comparison of estimates of productivity and consumption by zooplankton for planktonic ciliates in Lake Ontario. – J. Plankton Res. **13:** 363–372.

TULONEN, T. (1993): Bacterial production in a mesohumic lake estimated from [^{14}C]leucine incorporation rate. – Microb. Ecol. **26:** 210–217.

UTERMÖHL, H. (1958): Zur Vervollkommnung der quantitativen Phytoplankton Methodik. – Mitt. Int. Verein. Limnol. **9:** 1–38.

WICKHAM, S. A. & GILBERT, J. J. (1991): Relative vulnerabilities of natural rotifer and ciliate communities to cladocerans: laboratory and field experiments. – Freshwat. Biol. **26:** 77–86.

– – (1993): The comparative importance of competition and predation by *Daphnia* on ciliated protists. – Arch. Hydrobiol. **126:** 289–313.

Submitted: 10 December 1997; accepted: 10 February 1999.

III

Kisand, V. & Zingel, P. 2000.
Dominance of ciliate grazing on bacteria during spring
in a shallow eutrophic lake.
Aquat. Microb. Ecol. 22(2): 135–142.

Vol. 22: 135–142, 2000 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published September 8

Dominance of ciliate grazing on bacteria during spring in a shallow eutrophic lake

Veljo Kisand[1,2,*], Priit Zingel[1,2]

[1]Võrtsjärv Limnological Station Rannu, Tartumaa 61101, Estonia
[2]University of Tartu, Institute of Zoology and Hydrobiology, Vanemuise 46, Tartu 51014, Estonia

ABSTRACT: The objective of this study was to investigate the relationships between bacterioplankton biomass and activity and protistan grazing during the spring in shallow Lake Võrtsjärv, Estonia. Bacterial and heterotrophic protistan (flagellate and ciliate) abundance was determined by fluorescence direct counts, protistan grazing on planktonic bacteria was measured from fluorescently labeled bacteria uptake rates, and the estimate of bacterial heterotrophic activity was obtained from leucine and thymidine incorporation rates. The abundance of heterotrophic nanoflagellates (HNF) was low, ranging from 8.4 to 27.1 cells ml^{-1}, while ciliate numbers dominated, ranging from 55 to 180 cells ml^{-1}. The population of ciliated protozoans was dominated by scuticociliates (*Uronema* sp., *Cyclidium* spp., and 1 unidentified scuticociliate) and oligotrichs (*Strobilidium* spp. (diameter > 50 µm), *Strobilidium* spp. (diameter < 50 µm), and *Strombidium* sp.). Ciliates were predominant grazers of bacteria, showing higher clearance rates (14 to 65 nl $ciliate^{-1}$ h^{-1}) than HNF (1 to 11 nl HNF^{-1} h^{-1}); the total grazing rate of ciliates (on average 2398 bacteria h^{-1} ml^{-1}) was 28 times higher than total grazing of HNF (87 bacteria h^{-1} ml^{-1}). In general terms protozoan grazing balanced with bacterial production but was not sufficient to support ciliate growth: on the basis of conversion factors the calculated generation time of bacterivorous ciliates was long, on average 121 d (ranging from 43 to 198 d).

KEY WORDS: Bacterioplankton · Protist grazing · Ciliates · Shallow lake

INTRODUCTION

Previously, ciliates were only considered to be primarily bacterivores in isolated cases (Hall et al. 1993 and references therein). It is generally thought that ciliates are not effective grazers at concentrations of bacteria found in the field; rather, they are likely consumers of relatively large cyanobacteria and nanoplankters (Epstein & Shiaris 1992 and references therein). To date, several reports are available in which bacterivory by freshwater pelagic ciliates has been well documented *in situ* (Šimek et al. 1990, 1995, 1998b, Šimek & Straskrabova 1992, Stabell 1996).

Previous studies on bacterioplankton in Lake Võrtsjärv, Estonia, have shown that bacterial numbers are low during spring; despite considerable bacterioplankton productivity the abundance of bacteria did not increase. Sufficient organic substrates were produced by the spring bloom of diatoms (Kisand & Nõges 1998) and no nutrient deficiency could be expected (Kisand et al. unpubl.) during this season. The aim of the present study was to test the hypothesis that bacterioplankton is controlled by protistan grazing during the spring, and to compare the importance of ciliates and heterotrophic nanoflagellates in planktonic bacterivory. Also, we attempted to determine the relative importance of different ciliate taxa as bacterivores in Lake Võrtsjärv. In order to address to these questions, weekly samples were taken from the lake for the 6 wk period from March 31 to May 12, 1998; the grazing rate of protists was estimated along with the abundance of bacteria and protists, and the activity of bacteria (thymidine and leucine incorporation rates).

*E-mail: kisand@ut.ee

MATERIAL AND METHODS

Samples were collected weekly from eutrophic (1.6 g N m^{-3} mean total nitrogen concentration and 54 mg P m^{-3} total phosphorus) and shallow (mean depth 2.8 m) Lake Võrtsjärv during spring 1998 (March 31 to May 12, 1998). The sampling station was situated in the deepest area of the lake (max. depth 6 m), near to Võrtsjärv Limnological Station. Samples were taken at 0.5 m intervals throughout the water column with a 2 l Ruttner sampler and mixed in a barrel to make a 20 l pooled sample, which was transported to the onshore laboratory. Several previous studies (Nõges et al. 1997, 1998a,b) have shown that pooled samples represent the whole water column of the lake fairly well.

The abundance of heterotrophic bacteria was determined by staining with DAPI (Porter & Feig 1980). Water (20 ml) was fixed with buffered formalin (1% final conc.) and 1 to 5 ml of subsample was filtered through 0.2 µm membrane filters (Poretics) immediately after sampling. Filters were frozen at –20°C until counting on a Leica DMBR fluorescence microscope at 1250× magnification using violet light (425/460 nm, GG475). The abundance of heterotrophic nanoflagellates (HNF) and small ciliates (Ø < 50 µm) was counted on the same filters which were used in short-term grazing experiments (see below). Auto- and mixotrophy of flagellates was checked on the basis of autofluorescence of chlorophyll under green light (546/565 nm, OG 590). A total of 250 (if >30 cells were encountered) to 400 (if <15 cells were encountered) fields of view were counted on each filter. The total biomass and taxonomic groups of ciliates (usually to the genus level) were determined from Lugol's fixed samples by inverted microscopy (Olympus IX50 or Nikon diaphot-TMD) at 200 to 600× magnification using the Utermöhl (1958) technique. Volumes of 50 ml were settled and the entire content of each Utermöhl chamber was surveyed. Ciliate identification was based on Patterson & Hedley (1992) and Foissner & Berger (1996) and taxonomy followed Corliss (1979). The dimensions of the first 20 measurable specimens encountered for each taxon were measured. Biovolumes of each taxon were estimated by assuming standard geometric shapes (Finlay 1977).

^{3}H-leucine and -thymidine incorporation was used to estimate bacterial heterotrophic activity. Saturation curves were always used to determine the rate of incorporation: ^{3}H-leucine (Amersham International, SA [specific activity] = 62.0 Ci $mmol^{-1}$) or ^{3}H-thymidine (Amersham International, SA = 26 Ci $mmol^{-1}$) was added to 5 ml samples (2 samples + 2 formalin-killed controls; 2% final conc.) at a concentration range of 0.5 to 54 nM. The vials were incubated at *in situ* temperature for 60 or 30 min for leucine or thymidine incorporation, respectively. The incubation was stopped by adding 40% formalin (2% final conc.). Cold TCA-ethanol extraction was applied after filtering of samples on cellulose acetate (Millipore) 0.2 µm filters. The filters were dried and placed in a scintillation vial with 5 ml Quickscint (Zinssler Analytic Inc.) scintillation cocktail. Samples were measured by a Rackbeta 1211 liquid scintillation counter; the external standard ratio method was applied for quenching correction. Incorporation rate of leucine (TLI) was used to calculate biomass production of bacteria (BPB) and incorporation rate of thymidine (TTI) for cell production (BPC). Empirically determined conversion factors (ECF) from earlier studies (Kisand & Nõges 1998) were used in our calculations: ECF_{TLI} was 4.25×10^9 µg C mol^{-1} leucine and ECF_{TTI} was 3.8×10^8 cells mol^{-1} thymidine.

Preparation of fluorescently labelled bacteria (FLB) and grazing experiments. Bacterioplankton from the lake was concentrated on 0.2 µm pore-size filters after prefiltration through GF/C filters. Bacteria were grown in 0.2 µm filtered lake water batch cultures and harvested by centrifugation (Šimek et al. 1995, Dr K. Šimek pers. comm.). FLB (heat killed and DTAF stained) were obtained according to the protocol of Sherr & Sherr (1993). The size of FLB was comparable to cell size in natural populations of bacteria (although no real measuring was performed). In each FLB uptake experiment, bacterial density in the lake water was measured, and FLB were added to give a final concentration of 4 to 22% of the native bacterial counts. Samples were incubated for 45 to 360 min, but usually no longer than 60 min at the ambient temperature of the lake water; in calculations, only incubation times up to 75 min, which corresponded to the linear phase of uptake rate, were used. Subsamples for FLB counts in food vacuoles of protists were taken at 10 to 20 min intervals and the average uptake rate was caclulated on the basis of linear range of FLB uptake (usually the shortest and the longest incubations resulted in lower rates but at least 3 time points had similar rates in the middle). Samples were fixed with buffered formalin (1% final conc.). Preserved samples were stained for 1 to 2 min with DAPI at a final concentration of 2 µg ml^{-1} and 5 to 15 ml of water was gently filtered through 0.8 µm pore-size black isopore (Poretics Inc.) filters. Protists and the contents of their food vacuoles were examined with a Leica DMBR fluorescence microscope at 1250× magnification using blue light (470/505 nm, OG 515). The results of staining with both DTAF and DAPI were examined on the same microscopic field by switching filter sets without disturbing the position of the slide, and between 200 and 400 fields were examined. The dominant taxa of fluorescently stained ciliates were identified as much as possible based on knowledge of the composition of parallel Lugol's fixed samples.

Calculations. Biomass of different organisms was calcluated on the basis of published conversion factors: bacteria, 20 fg C cell^{-1}; heterotrophic flagellates, 220 fg C µm^{-3} (Børsheim & Bratbak 1987); ciliates 190 fg C µm^{-3} (Putt & Stoecker 1989). Grazing rates of protists were calculated directly from FLB uptake values considering total number of cells in incubation bottles (FLB + natural concentration). To estimate total protistan grazing rate, average uptake rates of HNF and ciliates were multiplied by their total *in situ* abundance. The doubling time of bacteria was calculated from TTI and TLI; the doubling time of protists assuming bacteria as the only food was estimated taking into account the biomass of protists, carbon content of bacteria, growth efficiency 40% (Fenchel 1987) and specific grazing rate of protists (bacterial cells ind.$^{-1}$ h^{-1}).

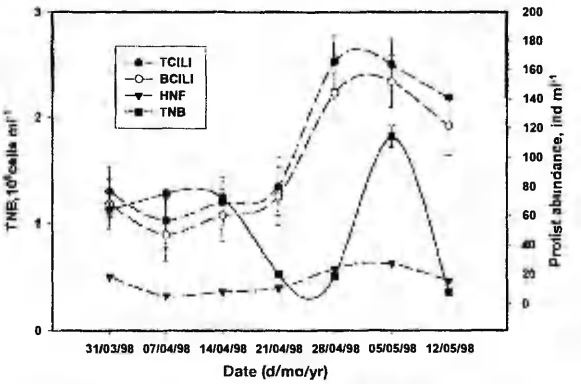

Fig. 1. Total number of bacteria (TNB), heterotrophic nanoflagellates (HNF) and ciliates (TCILI: total number of ciliates; BCILI: bacterivorous ciliates). Error bars represent ±SE

RESULTS

Physical-chemical parameters and phytoplankton

The investigated period (from March 31 to May 12, 1998) represented the period between last weeks of ice cover on the lake, ice break (in April 11) and warming up of water column. The period was characterized by relatively low temperatures and a well-mixed water column. Under the ice the water temperature was around 1.5°C and the water had warmed up to 12°C by May 12 (temperature increased almost linearly). Water transparency decreased to 1.3 m from the initial values of 1.8 to 2.0 m. The total phytoplankton biomass measured as chlorophyll *a* (chl *a*) concentration increased about 3 times (from 15 to 46 µg chl *a* l^{-1}) in a similar manner to the temperature. Total nitrogen (N_{tot}) and inorganic forms of nitrogen (N_{in}) decreased during the study period (N_{tot}: from 2.1 to 1.4 mg N l^{-1} and N_{in}: from 1.31 to 0.45 mg N l^{-1}), whilst total phosphorus (P_{tot}) almost doubled (35 µg P l^{-1} at the beginning and 66 µg P l^{-1} at the end). The phosphate concentration was quite stable at a level around 4 to 10 µg P l^{-1}.

Bacterioplankton

Bacterial numbers were relatively stable and low during the whole sampling period: 0.36 to 1.82 × 10^6 cells ml^{-1} (Fig. 1). The activity of heterotrophic bacteria (measured as TLI and TTI) were also at a low level; only at the end of investigated period did both parameters increase significantly (Fig. 2). Biomass production of bacteria calculated on the basis of TLI and cell production calculated from TTI ranged from 0.045 to 0.80 µg C l^{-1} h^{-1} and 2.5 to 12.1 × 10^6 cells l^{-1} h^{-1}, respectively. In these calculations ECFs from earlier studies (Kisand & Nõges 1998, Kisand et al. unpubl.) were used: ECF_{TLI} was 4.25 × 10^9 µg C mol^{-1} leucine and ECF_{TTI} was 3.8 × 10^{18} cells mol^{-1} thymidine. Median generation time of bacteria was 7.8 (range from 1.2 to 30) and 8.9 (range from 0.4 to 15) d^{-1}, calculated from TTI and TLI, respectively.

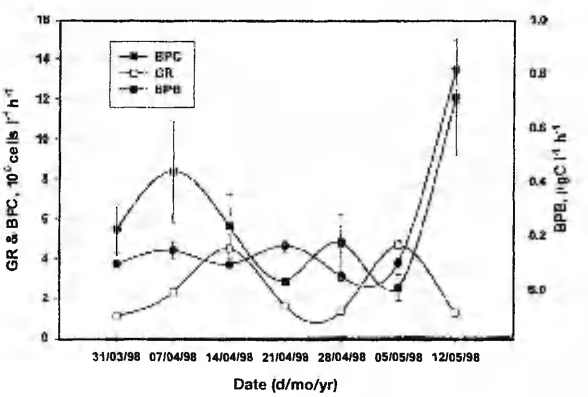

Fig. 2. Bacterial biomass (BPB, µg C l^{-1} h^{-1}), cell production (BPC, cells l^{-1} h^{-1}) estimated by TLI and TTI, respectively, and protistan grazing rate on bacteria (GR, bacterial cells l^{-1} h^{-1}) during investigated period. Error bars represent ±SE

Protozooplankton

In protozooplankton HNF abundance was low, ranging from 8.4 to 27.1 cells ml^{-1} (Fig. 1), while ciliate numbers dominated, ranging from 55 to 180 cells ml^{-1}. HNF cell volume ranged from 2.4 to 170 μm^{-3} (median 25). Abundance of these 2 main groups of protists gave an exceptionally low numerical HNF/ciliate ratio of 0.1 to 0.24 (average 0.15).

During the investigation period the population of ciliated protozoans was dominated by scuticociliates and oligotrichs (Fig. 3). The most common scuticociliates were *Uronema* sp. (8 to 25 ind. ml^{-1}), *Cyclidium* spp. (1 to 4 ind. ml^{-1}) and 1 unidentified scuticociliate (Scutico., 16 to 49 ind. ml^{-1}). Oligotrichs were dominated by *Strobilidium* spp. (Ø > 50 µm, 5 to 68 ind. ml^{-1}; Ø < 50 µm 13 to 24 ind. ml^{-1}), and *Strombidium* sp. (2 to 30 ind. ml^{-1}). Also, haptorids (*Mesodinium* sp., 0.1 to 12 ind. ml^{-1}) and prostomatids (*Urotricha* sp., 4 to 10 ind. ml^{-1}; *Coleps* spp., 0.7 to 2.3 ind. ml^{-1}) were always present. At the beginning of the investigation period the community was dominated by smaller species like *Uronema* sp., *Cyclidium* sp. and *Urotricha* sp. In the middle of April the abundance of large (Ø > 50 µm) *Strobilidium* and *Strombidium* species rose quickly, as well as the abundance of some large haptorids (*Dileptus* sp., ~0.1 ind. ml^{-1}; and *Monodinium* sp., ~0.12 ind. ml^{-1}). Altogether 12 identifiable taxa were recorded during the investigation period.

Protist grazing of FLB

The uptake rate of FLB by protists was usually linear during incubations of 15 to 75 min; these values were used to calculate the average rate of FLB uptake. Ciliates were predominant grazers of bacteria, and showed higher clearance rates (median 23; range: 14 to 65 nl $ciliate^{-1}$ h^{-1}) than HNF (median 6; range: 1 to 11 nl HNF^{-1} h^{-1}, Fig. 4A). The total grazing rate of ciliates was 28 times higher than the total grazing of HNF: ciliates grazed, on average, 2398 bacteria h^{-1} ml^{-1}, HNF only 87 bacteria h^{-1} ml^{-1} (Fig. 4A).

The main grazers of bacteria among quite small ciliate species (see also Table 1) were: *Uronema* sp., *Urotricha* sp., *Strobilidium* sp. (Ø < 50 µm) and Scutico., with average clearance rates of about 38 to 53 nl $ciliate^{-1}$ h^{-1}. Species-specific grazing rate was the highest for *Cyclidium* sp. (123 bacterial cells $ind.^{-1}$ h^{-1}) but its abundance was low during the period investigated. Other taxa showed specific grazing rates in the range of 25 to 62 bacterial cells $ind.^{-1}$ h^{-1} (Table 1). The unidentified scuticociliate (Scutico.) was responsible for the highest proportion of the total grazing (on average 1205 cells ml^{-1} h^{-1}), small *Strobilidium* sp. (Ø < 50 µm) had a lower grazing rate (970 cells ml^{-1} h^{-1}), and *Uronema* sp. and *Urotricha* sp. grazed 390 and 160 cells ml^{-1} h^{-1}, respectively (Fig. 4b).

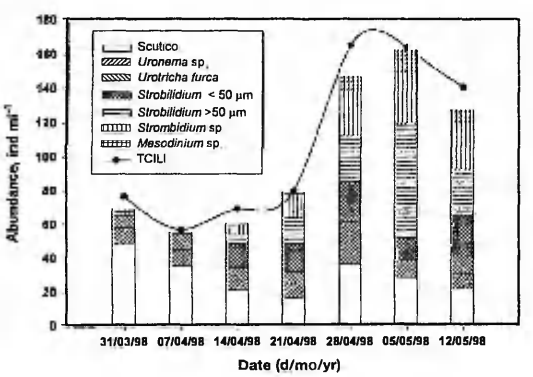

Fig. 3. Abundance of identified taxa of ciliates ind. ml^{-1}. TCILI: total number of ciliates

Protistan grazing and bacterial production

In general terms protozoan grazing balanced with the production of bacteria, being on average 61% (Fig. 2). In these calculations an ECF for calculating the cell production was used (ECF_{TTI} was 3.8×10^{18} cells mol^{-1} thymidine). If the ECF_{TTI} was actually lower (literature average is ~2×10^{18}), protists could consume about 100% of bacterial production. On the basis of published conversion factors and specific grazing rate the mean generation time of bacterivorous ciliates was rather long (121 d^{-1}). That calculation assumes that bacteria are the only food source for these ciliates. The median of HNF generation time was 6.7 d^{-1} (range from 2.8 to 98), assuming that bacteria are the only food source.

DISCUSSION

Ciliates versus HNF as grazers of bacteria in Lake Võrtsjärv

Among protozoans, HNF, and not ciliates, are known to be the most important grazers of bacteria. This knowledge is based on 2 basic factors: HNF abundance is 1 to 2 orders higher than that of ciliates (Šimek et al. 1997, Sommaruga & Conde 1997) and ciliates are considered to graze mostly on nano-sized organisms (Sherr et al. 1990). There is clear evidence that ciliates, especially smaller ones, are able to graze effectively on

pico-sized particles (including bacteria), and specific grazing rates of ciliates are higher than those of HNF (Epstein & Shiaris 1992, Iriberri et al. 1993). Earlier studies have suggested that ciliates cannot survive exclusively on bacteria in pelagic systems because they require high bacterial concentrations. Still, data from coastal waters indicate that 10^6 bacteria ml^{-1} might be sufficient to allow ciliates Ø < 15 µm to grow at a rate of 1 doubling per 48 h (Sherr et al. 1989). Small species (mostly Ø < 30 µm) often numerically dominate ciliate communities in most of the meso- to eutrophic lakes and they can exploit a variety of food resources ranging from picoplankton to nanophytoplankton (Beaver & Crisman 1989, Sherr et al. 1991). Recent laboratory experiments (Zubkov & Sleigh 1995) have shown that *Uronema* sp. reduced the bacterial concentration to a limiting level of less than 8×10^5 cells ml^{-1} during 2 h. In Lake Võrtsjärv, bacterivorous ciliates showed moderate clearance (average 30 nl $ciliate^{-1}$ h^{-1}); the clearance rate of HNF was about 6 nl HNF^{-1} h^{-1}. The main reason for these rather low clearance rates was probably environmental conditions, mainly low temperature.

The abundance of HNF was very low, indeed there is no literature reference of such a low numerical ratio of HNF/ciliates (~0.15). In the naturally acidic bog lake (Große Fuchskuhle) in northeastern Germany, the populations of protists were also characterized by a low numerical proportion of HNF to ciliates (~1.5 to 3.5; Šimek et al. 1998b). However, these 2 lakes are different, as Lake Võrtsjärv is slightly alkaline (pH 7.6 to 8.5) and is not considered to be a humic lake. It is noteworthy, however, that average organic carbon concentrations are quite high (total [TOC] ~ 10 mg C ml^{-1}, particulate [POC] ~ 5.6 mg C ml^{-1}, and dissolved humic [DHC] ~3.9 mg C l^{-1}). To date, 1 other example of a eutrophic environment (backwater system of the Alte Donau) in which HNF are present at a very low abundance (2 to 400 HNF ml^{-1}) and graze at a low rate on bacteria, is known (Wieltschnig et al. 1999). These waterbodies conform to the rule established mostly from marine systems that

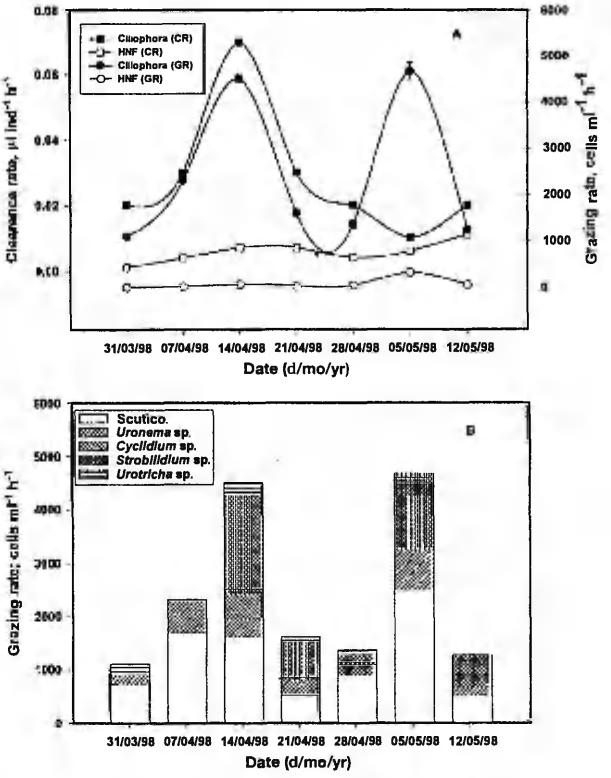

Fig. 4. Clearance rate (CR, µl protozoa^{-1} h^{-1}); total grazing rate (GR, bacterial cells h^{-1} ml^{-1} of protist). (A) CR and GR of total ciliates and HNF. Error bars represent ±SE. (B) GR of the main bacterivorous ciliates

Table 1. Species-specific characteristics of main bacterivorous ciliates

Taxon	Biovolume µm^{-3}	SE	Clearance rate (ml ind.$^{-1}$ h^{-1})			Specific grazing rate (bacterial cells ind.$^{-1}$ h^{-1})		
			Average	Min.	Max.	Average	Min.	Max.
Scutico.	3812	28	0.044	0.014	0.062	44	15	87
Uronema sp.	4530	60	0.047	0.022	0.091	46	17	79
Cyclidium sp.	3195	42	0.099[a]	–	–	123[a]	–	–
Urotricha furcata	4469	37	0.038	0.034	0.045	25	17	33
Strobilidium Ø < 50 mm	3323	62	0.053	0.027	0.084	62	20	125

[a]*Cyclidium* sp. was encountered only in 1 sample

in most cases HNF dominate both in abundance and in the control of bacterial populations, but there can be exceptions, e.g., eutrophic lakes. According to unpublished data from 1997 in Lake Võrtsjärv, HNF abundance is low throughout the whole season. At the same time abundance of ciliates found in Lake Võrtsjärv is in the range found in other eutrophic waterbodies (Sanders et al. 1989, Šimek et al. 1995). Studies from 1995 to 1997 (Kisand et al. 1998, Zingel 1999) indicated high biomass of ciliates in this lake. The species composition of ciliates in Lake Võrtsjärv is highly variable. Oligotrichs, haptorids, scuticociliates and prostomatids dominate the community of ciliates. The most common oligotrichs are *Strobilidium* spp., *Strombidium* spp., and *Tintinnidium fluviatile*. On some occasions scuticociliates (*Uronema* sp., *Cyclidium* sp.), haptorids (*Mesodinium* sp., *Dileptus* sp., *Askenasia volvox*, *Monodinium* sp.), prostomatids (*Urotricha* sp., *Coleps* spp.) and peritrichs (*Vorticella* spp., *Epistylis procumbens*) are also quite abundant. The annual maximum abundance is usually found during late July or early August, when the community of ciliates is mostly dominated by small (Ø < 30 µm) bacterivorous species. One might speculate that the ciliates could keep the numbers HNF low (Šimek et al. 1990, Weisse et al. 1990, Jürgens et al. 1996). For example, *Urotricha* spp. and omnivorous oligotrichs could efficiently control HNF dynamics. The ciliate community is extremely rich in this lake. In 1995 the mean abundance of ciliated protozoa was 39 cells ml^{-1} and mean biomass was 0.68 mg WW (wet weight) l^{-1}. In 1996 these numbers were 60 cells ml^{-1} and 2.91 mg WW ml^{-1}, respectively. Of the whole zooplankton biomass of Lake Võrtsjärv the planktonic ciliates formed up to 50% in 1995 and up to 64% in 1996 (Zingel, 1999). Thus, the high grazing pressure of ciliates on HNF could be the main reason why HNF population is kept at such a low level. There is no serious reason to believe that the counting methodology used (which is also routinly used in other waterbodies) missed most HNF or that extremely delicate species should dominate in this lake. The volumes of 5 to 10 ml should be sufficient for counting HNF and a filtration pressure below 80 mm Hg (in this case a pressure of 20 mm Hg was used) has been reported to avoid cell breakage (Gasol & Moran 1999 and references therein).

Although, to date, comparatively few reports are available in which bacterivory of freshwater pelagic ciliates has been well documented *in situ*, the importance or even prevalence in grazing on bacteria is stressed in several works from various types of lakes and reservoirs (Sanders et al. 1989, Christoffersen et al. 1990, Šimek & Straskrabova 1992, Šimek et al. 1995). In most cases ciliates appeared to become important grazers at the end of summer or even in autumn. However, with increasing trophy of the system, ciliates become more important as bacterivores (Šimek et al. 1998a). The minor effect of HNF on the bacterial population (on average 5% of cell production) was also observed in the eutrophic backwater system of the Alte Donau (Wieltschnig et al. 1999). Unfortunately, however, ciliate grazing was not measured in this case.

Due to high abundance and more efficient grazing rate (clearance rates of ciliates are almost always found to be higher than those of HNF), grazing of bacterivorous nanociliates on bacteria predominated. In a similar study of Lake Frederiksborg Slotssø, Denmark (Hansen & Christoffersen 1995), HNF grazing on bacteria was assumed to prevail, while ciliates were assumed to graze preferentially on primary producers and only marginally on bacteria. Bacterial abundance was much higher in this Danish lake, but the abundance and species composition of ciliates were similar to Lake Võrtsjärv. Hansen & Christoffersen (1995) assumed that HNF grazed preferentailly on bigger bacterial cells, while at the same time bacterial size distribution was skewed toward small cells. If this population of small and inactive cells could escape from predation by both HNF and ciliates, this could account for the huge standing stock of bacteria. At the same time, the productive part of the population, which is often found to have larger cells, could be quickly grazed by HNF and ciliates. In Lake Võrtsjärv, the standing stock of bacteria was much lower and the proportion of grazed bacteria was higher and more or less equal to the production of bacteria. Thus, the prevalence of small cells was not observed and ciliates played the most important role in bacterial grazing.

According to the literature, oligotrichs dominate (Šimek et al. 1998a) the total ciliate bacterivory (65 to 70%), and the other important bacterivorous ciliate groups are scuticociliates, accounting for 8 to 15% of the total ciliate bacterivory (Šimek et al. 1998a), and peritrichs (Carrias et al. 1996, Stabell 1996, Šimek et al. 1998a). In Lake Võrtsjärv, bacterivorous ciliates were mostly fine suspension-feeding ciliates such as scuticociliates and small oligotrichs. By virtue of their elaborate oral membranelles, scuticociliates seem especially well adapted to concentrate very small food particles from the environment (Fenchel 1986). The only bacterivorous ciliate whose feeding apparatus was not equipped for filter feeding was the prostomatid *Urotricha* sp. This species is considered to be a raptorial feeder. In addition, in Lake Võrtsjärv, *Urotricha* sp. showed the lowest total grazing rates. The other ciliates such as haptorids (*Dileptus* sp., *Monodinium* sp., *Mesodinium* sp.) large oligotrichs (*Tintinnidium fluviatile*, *Codonella cratera*, *Strombidium* sp.) and some prostomatids (*Coleps* spp.) did not show any ability to take up bacterioplankton.

In conclusion bacterivorous ciliates were extremely important grazers of bacteria during spring in the shallow and eutrophic Lake Võrtsjärv. They were more abundant than HNF and had a higher specific grazing rate on bacteria. Oligotrichs and small scuticociliates were responsible for >97% of total mortality of bacteria due to grazing. At the observed gazing rates, bacterivorous ciliates must also have a food source other than bacteria.

Acknowledgements. This work was supported by grant no. 2017 of the Estonian Science Foundation (to Dr Tiina Nõges). The authors are thankful to Dr Karel Šimek for his kind introduction to methods for grazing measurements of protists. We also appreciate Dr Kalle Olli's (University of Tartu) and Lauri Arvola's (University of Helsinki) good will in providing microscope equipment.

LITERATURE CITED

Beaver JR, Crisman TL (1989) The role of ciliated protozoa in pelagic freshwater ecosytems. Microb Ecol 17:111–136

Borsheim KY, Bratbak G (1987) Cell volume to cell carbon conversion factors for bacteriovorous *Monas* sp. enriched from sea water. Mar Ecol Prog Ser 36:171–175

Carrias JF, Amblard C, Bourdier G (1996) Protistan bacteriovory in an oligomesotrophic lake: importance of attached ciliates and flagellates. Microb Ecol 31:249–268

Christoffersen K, Riemann B, Hansen LR, Klysner A, Sorensen HB (1990) Qualitative importance of the microbial loop and plankton community structure in a eutrophic lake during a bloom of cyanobacteria. Microb Ecol 20: 253–272

Corliss J (1979) The ciliated protozoa: characterization, classification and guide to the literature. Pergamon Press, London

Epstein SS, Shiaris MP (1992) Size-selective grazing of coastal bacterioplankton by natural assemblages of pigmented flagellates, colorless flagellates, and ciliates. Microb Ecol 23:211–225

Fenchel T (1986) Protozoan filter feeding. Progr Protisol 1: 65–113

Fenchel T (1987) The ecology of heterotrophic microflagellates. Microb Ecol 9:57–97

Finlay BJ (1977) The dependence of reproductive rate on cell size and temperature in freshwater ciliated Protozoa. Oecologia 30:75–81

Foissner W, Berger H (1996) A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. Freshw Biol 35:375–482

Gasol J, Moran X (1999) Effects of filtration on bacterial activity and picoplankton community structure as assessed by flow cytometry. Aquat. Microb Ecol 16:251–264

Hall JA, Barrett DP, James MR (1993) The importance of phytoflagellate, heterotrophic flagellate and ciliate grazing on bacteria and picophytoplankton sized prey in a coastal marine environment. J Plankton Res 15:1075–1086

Hansen B, Christoffersen K (1995) Specific growth rates of heterotrophic plankton organisms in a eutrophic lake during a spring bloom. J Plankton Res 17:413–430

Iriberri J, Ayo B, Unanue M, Barcina I, Egea L (1993) Channeling of bacterioplanktonic production toward phagotrophic flagellates and ciliates under different seasonal conditions in a river. Microb Ecol 26:111–124

Jürgens K, Wickham S, Rathhaupt KO, Santer B (1996) Feeding rates of macro- and microzooplankton on heterotrophic nanoflagellates. Limnol Oceanogr 42:1833–1839

Kisand V, Nõges T (1998) Seasonal dynamics of bacterio- and phytoplankton in large and shallow eutropic Lake Võrtsjärv, Estonia. Int Rev Hydrobiol 83:205–216

Kisand V, Nõges T, Zingel V (1998) Diel dynamics of bacterioplankton activity in eutrophic shallow Lake Võrtsjärv, Estonia. Hydrobiologia 380:93–102

Nõges P, Nõges T, Laugaste R, Haberman J, Kisand V (1997) Tendencies and relationships in the pelagic environment and plankton community of Lake Võrtsjärv in 1964–93. Proc Estonian Acad Sci Biol Ecol 46:40–57

Nõges T, Kisand V, Nõges P, Põllumäe A, Tuvikene L, Zingel P (1998a) Plankton seasonal dynamics and its controlling factors in shallow polymictic eutropic Lake Võrtsjärv, Estonia. Int Rev Hydrobiol 83:279–296

Nõges T, Nõges P, Haberman J, Kisand V, Kangur K, Kangur A, Järvalt A (1998b) Food web structure in the shallow eutrophic Lake Võrtsjärv. Limnologica 28:115–128

Patterson DJ, Hedley S (1992) Free-living freshwater protozoa. A color guide. Wolfe Publishing Ltd, Aylesbury

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–948

Putt M, Stoecker DK (1989) An experimentally determined carbon:volume ratio for marine 'oligotrichous' ciliates from estuarine and coastal waters. Limnol Oceanogr 34: 1097–1103

Sanders RW, Porter KG, Bennett SJ, DeBiase AE (1989) Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. Limnol Oceanogr 34:673–687

Sherr BF, McDaniels J, Gonzales J, Hanson R (1990) Size selective grazing by bacterivorous protozoa: implications for bacterial production. EOS 71:235–240

Sherr EB, Sherr BF (1993) Protistian grazing rates via uptake of fluorescently labeled prey. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Microbial ecology. Lewis Publishers, Boca Raton, p 695–701

Sherr EB, Rassoulzadegan F, Sherr BF (1989) Bacterivory by pelagic choreotrichous ciliates in coastal waters of the NW Mediterranean Sea. Mar Ecol Prog Ser 55:235–240

Sherr EB, Berman T, Hadas O (1991) High abundance of picoplankton-ingesting ciliates during late fall in Lake Kinneret. J Plankton Res 13:789–799

Šimek K, Straskrabova V (1992) Bacterioplankton production and protozoan bacteriovory in a mesotrophic reservoir. J Plankton Res 14:773–787

Šimek K, Macek J, Seda J, Vyhnalek V (1990) Possible food chain relationships between bacterioplankton, protozoans, and cladocerans in a reservoir. Int Rev Ges Hydrobiol 75: 583–596

Šimek K, Bobkova J, Macek M, Nedoma J, Psenner R (1995) Ciliate grazing on picoplankton in a eutrophic reservoir during the summer phytoplankton maximum: a study at the species and community level. Limnol Oceanogr 40: 1077–1090

Šimek K, Hartman P, Nedoma J, Pernthaler J, Springmann D, Vrba J, Psenner R (1997) Community structure, picoplankton grazing and zooplankton control of heterotrophic nanoflagellates in a eutrophic reservoir during the summer phytoplankton maximum. Microb Ecol 12:49–63

Šimek K, Armengol J, Comerma M, Garcia JC, Chrzanowski TH, Macek M, Nedoma J, Straskrabova V (1998a) Characteristics of protistan control of bacterial production in

three reservoirs of different trophy. Int Rev Hydrobiol 83: 485–494

Šimek K, Babenzien D, Bitl T, Koschel R, Macek M, Nedoma J, Vrba J (1998b) Microbial food webs in an artificially divided acidic bog lake. Int Rev Hydrobiol 83:3–18

Sommaruga R, Conde D (1997) Seasonal variability of metabolically active bacterioplankton in the euphotic zone of a hypertrophic lake. Microbial Ecol 13:241–248

Stabell T (1996) Ciliate bacterivory in epilimnetic waters. Aquat Microb Ecol 10:265–272

Utermohl H (1958) Zur Vervollkommung der quantitativen Phytoplanktonmethodik. Mitt Int Ver Theor Angew Limnol 9:1–38

Weisse T, Müller H, Pinto-Coelho RM, Schweizer A, Springman D, Baldringer G (1990) Response of the microbial loop to the phytoplankton spring bloom in a large prealpine lake. Limnol Oceanogr 35:781–794

Wieltschnig C, Wihlidal P, Ulbricht T, Kirschner A, Velimirov B (1999) Low control of bacterial production by heterotrophic nanoflagellates in a eutrophic backwater environment. Aquat Microb Ecol 17:77–89

Zingel P (1999) Pelagic ciliated protozoa in shallow eutrophic lake: comunity structure and seasonal dynamics. Arch Hydrobiol 146:495–511

Zubkov MV, Sleigh MA (1995) Ingestion and assimilation by marine protists fed on bacteria labeled with radioactive thymidine and leucine estimated without separating predator and prey. Microb Ecol 30:157–170

Editorial responsibility: Fereidoun Rassoulzadegan, Villefranche-sur-Mer, France

Submitted: July 12, 1999; Accepted: May 26, 2000
Proofs received from author(s): July 19, 2000

IV

Zingel, P. & Ott, I. 2000.
Vertical distribution of planktonic ciliates
in strongly stratified temperate lakes.
Hydrobiologia 435: 19–26.

Hydrobiologia 435: 19–26, 2000.

Vertical distribution of planktonic ciliates in strongly stratified temperate lakes

Priit Zingel[1,2] & Ingmar Ott[2]
[1]*Institute of Zoology and Hydrobiology, University of Tartu, 46 Vanemuise St, 51014 Tartu, Estonia*
[2]*Võrtsjärv Limnological Station, Institute of Zoology and Botany, Estonian Agricultural University, Rannu, 61101 Tartu County, Estonia*

Received 30 July 1999; in revised form 2 March 2000; accepted 21 April 2000

Key words: ciliates, protozooplankton, vertical distribution, metalimnion

Abstract

The vertical distribution of planktonic ciliates in eight strongly stratified temperate lakes was studied in summer 1998. Ciliate abundance and biomass were highest (mean 39.9 cells ml^{-1} and 181.9 $\mu g\ C\ l^{-1}$) in the epi-, and lowest (mean 8.2 cells ml^{-1} and 97.6 $\mu g\ C\ l^{-1}$) in the hypolimnion. The community of ciliates was dominated by five orders: *Oligotrichida, Haptorida, Prostomatida, Scuticociliatida* and *Peritrichida*. The community composition varied greatly with depth. In the epilimnion, the ciliate numbers were dominated by oligotrichs but small algivorous prostomatids, peritrichs and haptorids were also numerous. In the metalimnion, these groups were replaced by scuticociliates and mixotrophic prostomatids. In the hypolimnion species known as benthic migrants appeared. We found a positive significant correlation ($p < 0.05$) between ciliate numbers and Chl *a* and bacterial densities. Only in the hypolimnion, the correlation between ciliates numbers and Chl *a* was not significant.

Introduction

Studies in the last two decades have highlighted the importance of planktonic ciliates in freshwater ecosystems. Ciliates are important in predation and control of picoplanktonic algae and bacteria (Bark, 1981; Beaver & Crisman, 1982; Sherr & Sherr, 1984; Nagata, 1988; Güde, 1989; Gonzales et al., 1990) but they also graze particles that can be ingested by metazooplankton (Sanders et al., 1989). Clear evidence exists that planktonic ciliates are an important food resource for large metazooplankton (Porter et al., 1979; Dolan & Coats, 1991; Gifford, 1991). It is understood that metazooplankton predation on ciliates is an important trophic link between pico- and nanoplankton and metazoans. Planktonic ciliates at times constitute over 50% of zooplankton biomass in lakes (Zingel, 1999).

The number of papers on freshwater protozooplankton has increased recently (Hecky & Kling, 1981; Pace & Orcutt, 1981; Taylor & Heynen, 1987; Beaver et al., 1988; Carrick & Fahnenstiel, 1990; Laybourn-Parry et al., 1990; Carrias et al., 1994; James et al., 1995), but there still are few detailed studies about ciliates vertical distribution in strongly stratified lakes, especially in the metalimnion. During the formation of the metalimnion, an adaptation of organisms to changeable conditions will take place. In summer stagnation, the vertical distribution of organisms differs essentially from that in the moment of circulation. In the relatively narrow metalimnetic layer, a temporary microbial loop will form, in which aerobic, microaerobic and anaerobic conditions occur. Its nutrient supply is guaranteed by gravitation and by a diffusion gradient. Lack of turbulence will prevent the dispersion of community and keep the chemical gradients intact. In addition, vertical gradients of temperature, oxygen and radiation will form inside the metalimnion, and cause a succession of microniches and environments in time and space. A great variety of microbes will allow for the functioning of a microbial loop. This metalimnetic circulation of matter consists of microalgae, bacteria, heterotrophic nanoflagellates, ciliates and other microzooplankton; the organic matter produced by this loop can in principle

return into classical matter of circulation (Steenbergen et al., 1993). Little is know about the role of ciliates in the described pattern.

The aim of our study was to describe the vertical distribution and community structure of planktonic ciliates in eight temperate lakes during strong stratification, focusing in the metalimnion.

Materials and methods

The studied lakes were situated in Southern Estonia. The sampling was carried out once in summer 1998. Investigated lakes were all small (1.7–17.3 ha), deep (maximum depth reached 11.5–29.7 m) and with very small water exchange or closed. They belonged to the different lake types and were mostly dimictic, two of these considered being meromictic. All studied lakes were strongly stratified and with the anoxic hypolimnion. Data about their physical and chemical characteristics are given in Table 1.

Altogether eight subsamples (at 11–14 am) from each lake were collected for the ciliate counts: two from the epi- and the hypolimnion and four from the metalimnion. The samples from the surface were gathered directly to the bottle. The other samples were gathered using special vacuum probe (see similar to Guerrero et al., 1985). A masterflex pump (model N 7533-60) with 'easy-load' pump-head (model 7518-12) was used for pumping water to the surface. Hose with inner Ø 8 mm was allocated vertically into the water. Lower tip of the vertically allocated hose was closed and water was sucked through horizontal tube (length 7 cm) in order to get the water from horizontal layers as precisely as possible. Capacity of the complex device is app. 2 l per min. Samples were preserved and fixed with acidified Lugols solution. Ciliate biomass and community composition were determined using the Utermöhl (1958) technique. Samples were stored at 4 °C in the dark. Volumes of 50 ml were settled for at least 24 h in plankton chambers. Ciliates were enumerated and identified with an inverted microscope (Olympus IX50) at 400–1000× magnification. The entire content of each Utermöhl chamber was surveyed. Ciliates were usually identified to genus by consulting several works (Kahl, 1930–1935; Kutikova & Starobogatov, 1977; Patterson & Hedley, 1992; Foissner & Berger, 1996). The taxonomy followed mainly the scheme of Corliss (1979). The first 20 measurable specimens encountered for each taxon were measured. Biovolumes of each taxa were estimated by assuming geometric shapes and converted to carbon weight using a factor of 190 fg C μm^{-3}(Putt & Stoecker, 1989). Physical and chemical analyses were made by using standard methods used in earlier studies (cf. Ott et al., 1997). For statistical analysis, nonparametric methods were used.

Results

The ciliate abundance and biomass were usually highest in the epilimnion (mean 39.9 cells ml^{-1} and 181.9 $\mu g\ C\ l^{-1}$) and lowest in the hypolimnion (mean 8.2 cells ml^{-1} and 97.6 $\mu g\ C\ l^{-1}$) (Figures 1 and 2). In the metalimnion, the mean abundance and biomass were 31.1 cells ml^{-1} and 118.3 $\mu g\ C\ l^{-1}$, respectively. Maximum abundance (97.9 cells ml^{-1}) was recorded in the metalimnion of Lake Verevi. The ciliate numbers were lowest (1.3 cells ml^{-1}) in the hypolimnion of Lake Peta. We found in studied lakes positive significant correlation ($p < 0.05$) between ciliate numbers and Chl *a* and bacterial densities (Figure 3). Only in the hypolimnion, the correlation between ciliates numbers and Chl *a* was not significant. The correlation was strongest in epilimnion. Five orders dominated the community of ciliates: *Oligotrichida, Haptorida, Prostomatida, Scuticociliatida* and *Peritrichida* (Table 2). The community composition varied greatly with depth. In most lakes oligotrichs dominated in the epilimnion. The numbers of oligotrichs were made up mostly by large *Strombidium* and *Strobilidium* species and by tintinnids (*Tintinnidium fluviatile, Codonella cratera*). Also prostomatids (*Urotricha* spp., *Coleps hirtus*) and peritrichs (*Vorticella* spp.) were important in the epilimnion. The number of oligotrichs and peritrichs decreased in most lakes in the metalimnion and they were almost absent in the hypolimnion. In majority of lakes we found positive correlation between oxygen concentrations and abundance of peritrichs and oligotrichs (Table 3). In the metalimnion the most important groups were prostomatids (*Coleps hirtus, Coleps spetai*) and scuticociliates (*Cyclidium* spp., *Uronema* spp., *Calyptotricha lanuginosa*). Negative correlation between the scuticociliate numbers and oxygen concentrations was found (Table 3).

In the hypolimnion, the community of ciliates was dominated by scuticociliates (mostly *Uronema nigricans* and *Pseudocohnilembus* sp.) and 'varia' which was made up mostly by heterotrichs (*Caenomorpha* spp., *Metopus* spp.) and hymenostomatids (*Frontonia* spp.). Greatest species diversity was found in the

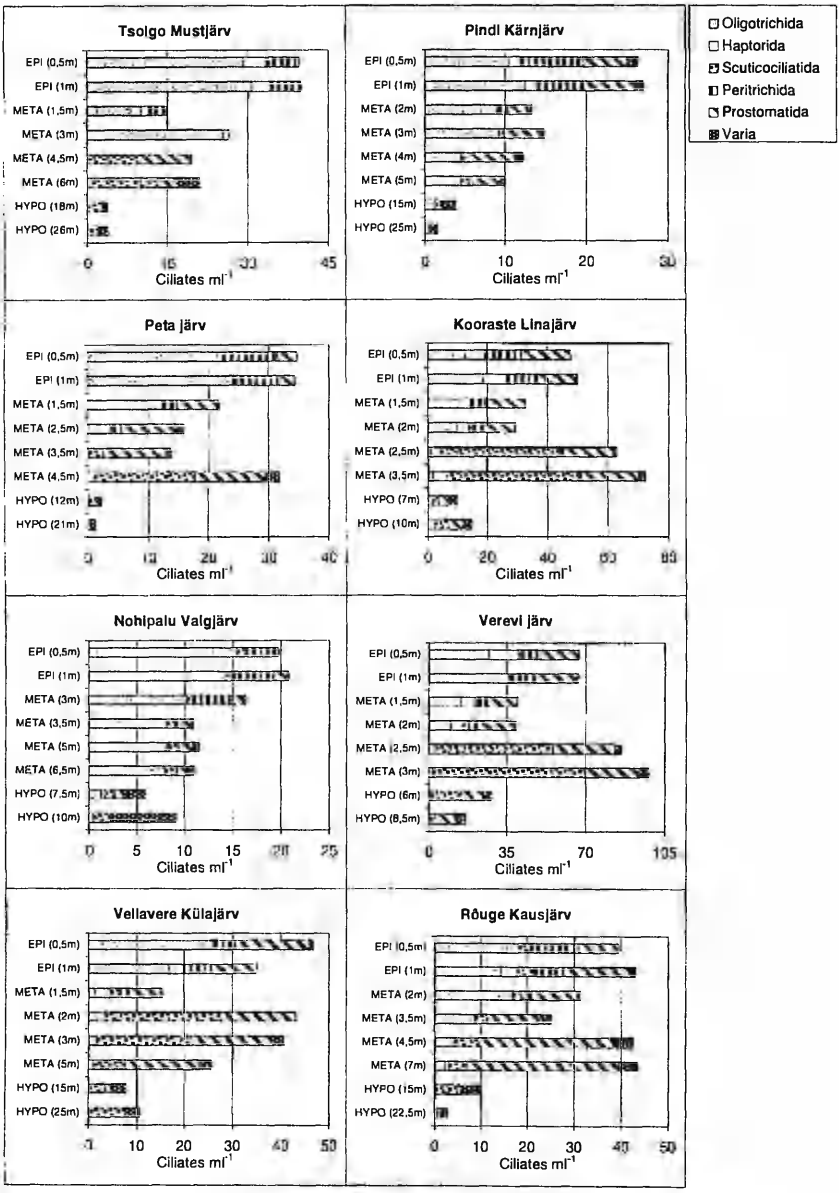

Figure 1. Abundance of different groups of ciliates in the water column of study lakes.

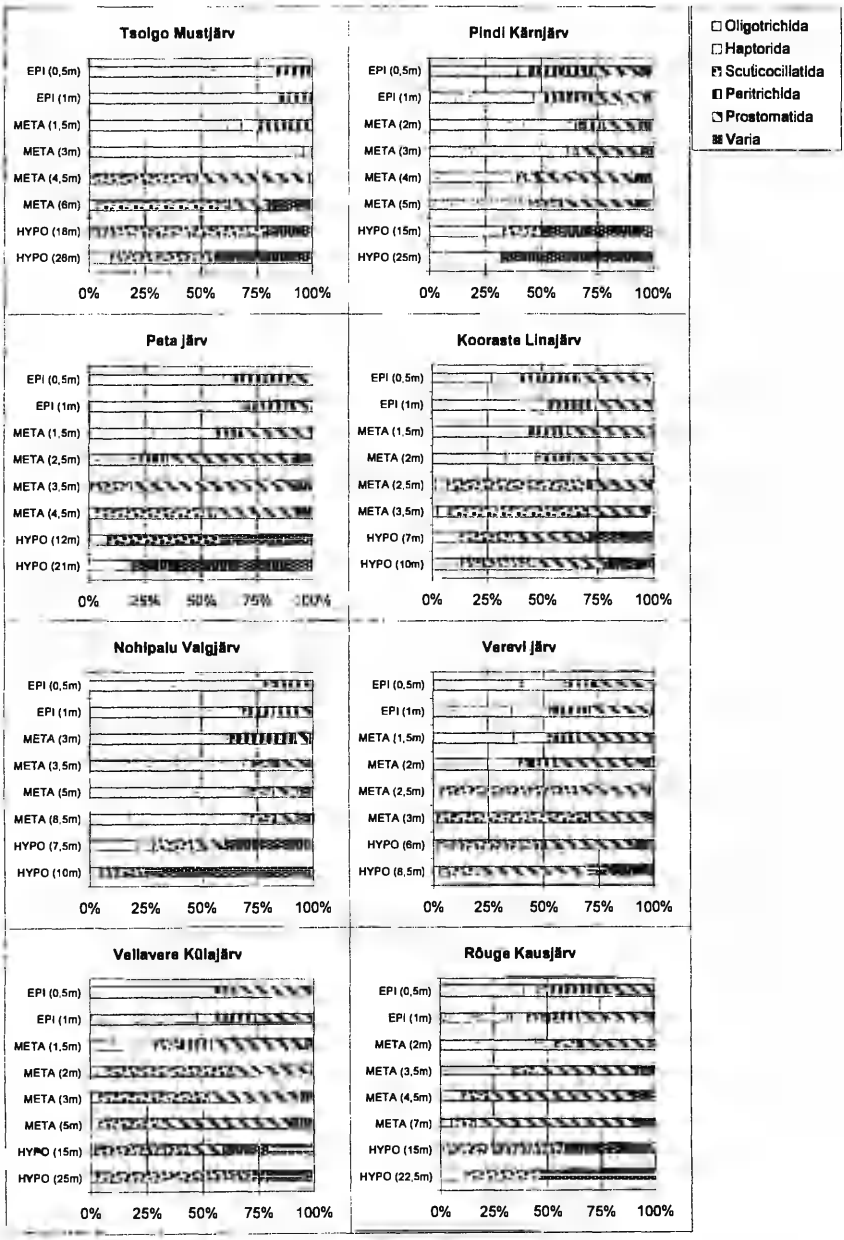

Figure 2. Relative importance of different groups of ciliates in the water column of study lakes as a percentage of abundance.

Table 1. General limnological characteristics of the investigated lakes in 1998

Lake	Surface	Max. depth	Trasparency[a]	Yellow matter[b]	COD_{Cr}[c]	Tot-P	tot-N	HCO_3[d]	pH in surface
Unit	Ha	m	m	$mg \cdot l^{-1}$	$mgO \cdot l^{-1}$	$mg \cdot m^{-3}$	$mg \cdot m^{-3}$	$mg \cdot l^{-1}$	
Tsolgo	6	29.7	0.7	11.3	41.9	55	1141	28.3	9.1
Mustjärv									
Pindi	8.3	26	1.6	22.2	52.8	30	950	113	7.7
Kärnjärv									
Petajärv	3.6	25	1.9	8.9	37.3	63	1054	30.2	7.6
Kooraste	2.7	12.7	1.2	6.1	42.9	3899	57709	136.9	
Linajärv avg.									
Kooraste						68	1244	3	6.8
Linajärv surface									
Nohipalu	6.3	12.5	4.5	6.6	16.1	21.3	366	4	5.6
Valgjärv									
Verevi avg.	12.6	11	0.7	13.6	53.2	454	367	312	
Verevi surface						152	2430	228	8.3
Vellavere	4.6	25	1.1	1.3	50.2	74.8	1341	223	8
Külajärv									
Kaussjärv	1.9	22	1.5	19.2	42.1	73	1563	223	7.5

[a] Transparency – Secchi disc transpareny.
[b] Yellow matter – Optically active substances.
[c] COD_{Cr} – Chemical oxygen demand (dichromate oxygen consumption).
[d] HCO_3 – total alkalinity.

Table 2. Relative importance of different groups of ciliates in epi-, meta-, and hypolimnion of 8 strongly stratified temperate Estonian lakes as a percentage of abundance

	Epi	Meta	Hypo
Oligotrichida	49.3%	19.8%	0.9%
Scuticociliatida	1.7%	32.7%	38.1%
Prostomatida	20.4%	34.8%	23.2%
Haptorida	9.0%	4.5%	6.4%
Peritrichida	18.6%	3.6%	0%
Varia	1.0%	4.6%	31.4%

epilimnion of Lake Kooraste Linajärv (31 taxa) and scarcest in the metalimnion of Lake Nohipalu Valgjärv (6 taxa).

Discussion

In our study, we found that both ciliate numbers and community composition varied greatly with the depth. In the epilimnion, the community was dominated by oligotrihs. These ciliates were mainly composed of large sized taxa (>40 μm ∅) including both heterotrophic (*Strobilidium* spp., *Tintinnidium fluviatile*, *Tintinnopsis tubulosa*, *Codonella cratera*) and mixotrophic species (*Strombidium viride*) feeding mainly on nanoplanktonic algae. Oligotrichs are often reported as relatively common component of lacustrine protozooplankton especially in the epilimnion (Laybourn-Parry et al., 1990; Müller et al., 1991). Abundance of oligotrichs decreased quickly with the depth and in most lakes they were already absent in the deeper layers of the metalimnion. Small-sized prostomatid *Urotricha* showed similar distribution pattern as oligotrichs. Presence of this algivorous species is related to the occurrence of abundant nanoplalanktonic prey (Müller et al., 1991). So, it is reasonable to speculate that their vertical distribution is bottom-up controlled.

The abundance of haptorids (e.g. *Askenasia volvox*, *Dileptus* sp., *Didinium* sp.) was highest in the epilimnion. The majority of species representing that group are known as predators. So our results are in good accordance with the opinion that predatory species are associated with the surface because they require abundant and highly diversified food for their development (Müller et al., 1991).

Table 3. Correlation coefficients (Spearman R) between abundance of planktonic ciliates and oxygen concentration in 8 strongly stratified temperate Estonian lakes (* = $p < 0.05$, ** = $p < 0.01$, ^{ns} = not significant)

	Oligotrichida	Haptorida	Scuticociliatida	Peritrichida	Prostomatida
Tsolgo Mustjärv	0.870*	0.943**	−0.941**	0.941**	−0.778^{ns}
Pindi Kärnjärv	0.943*	0.543^{ns}	−0.986*	0.880*	0.429^{ns}
Peta järv	0.638^{ns}	−0.664^{ns}	−0.770^{ns}	0.529^{ns}	−0.638^{ns}
Kooraste Linajärv	0.543^{ns}	0.257^{ns}	−0.657^{ns}	0.486^{ns}	−0.943**
Nohipalu Valgjärv	0.679^{ns}	−0.408^{ns}	−0.357^{ns}	0.334^{ns}	0.429^{ns}
Verevi järv	1.000**	1.000**	−0.861*	0.870*	−0.486^{ns}
Vellavere Külajärv	0.714^{ns}	0.429^{ns}	−0.754^{ns}	0.941**	−0.771^{ns}
Rõuge Kaussjärv	0.829*	0.371^{ns}	−0.638^{ns}	0.941**	−0.664^{ns}

In the meta- and the hypolimnion were very abundant small-sized scuticociliates (*Cyclidium* spp., *Uronema nigricans* and *Pseudocohnilembus* sp.), which are known to be effective filter-feeding bacterivores. Also, Pace (1982) reported from high numbers of scuticociliates near the metalimnion of eutrophic Lake Oglethorpe. It is suggested (Müller et al., 1991) that scuticociliates tend to concentrate in the oxycline, where bacterial productivity is high. Usually in the meta- and the hypolimnion, the abundance of metazooplankton that potentially prey on these small-sized ciliates decreases. This may explain the higher abundances of scuticociliates in the deeper layers. In the epilimnion scuticociliates were replaced by small-sized oligotrichs (*Strobilidium* spp., *Halteria grandiella*) that feed also on planktonic bacteria and are so competitors against scuticociliates. In deeper layers, small-sized oligotrichs were almost absent. It is suggested that scuticociliates are more effective grazers than small oligotrichs in lower temperatures (Schönberger, 1994). This may be one reason for scuticociliate dominance in the deeper layers as well. In addition, the epilimnion contributed a various community of peritrichs, which are known to having high specific ingestion rates (Simek et al., 1995) and feeding mainly on planktonic bacteria. Peritrichs were abundant only in the epilimnion and similarly to small oligotrichs almost absent in the deep layers. Their association with the surface waters has been usually explained with distribution of colonial microcyanobacterium (e.g. *Anabaena flos-aquae*), which act as a support (Müller et al., 1991; Carrias et al., 1998), but we speculate that also the competition for food resources may have a strong impact.

In many lakes, we observed high densities of prostomatids *Coleps hirtus* and *Coleps spetai* in the metalimnion. Both species contain symbiotic algae. It has been suggested that these ciliates could be benefiting from oxygen evolution by the algal symbionts, and can so cope with a low-oxygen environment (Esteve et al., 1989). We speculate that this is also the case in our study lakes.

In the hypolimnion, we found many species, which are so called benthic migrants (*Loxodes* spp., *Metopus* sp, *Caenomorpha* spp., *Frontonia* spp., *Plagiopyla* sp.). When the hypolimnion becomes anoxic, these species are known to migrate into the previously vacated water column from the sediment and form distinct planktonic community (Laybourn-Parry et al., 1990). In our study, they were never found in the epilimnion or in the upper layers of metalimnion and were most abundant just under the metalimnion. There is not much knowledge about respiratory biochemistry of these benthic migrants. Still it is shown that oxygen is toxic to *Loxodes* and its effects are exacerbated by light (Finlay et al., 1986) and that this species can respire nitrate as well as oxygen (Finlay, 1985). It is suggested that the distribution patterns of this species and also other benthic migrants can be explained as a compromise between avoiding high oxygen concentrations and meeting the need for aerobic metabolism in a zone where predation from metazooplankton will be minimal and where at the same time food is readily available. As the metalimnetic processes can contribute remarkably to the lakes productivity (Konopka, 1989), it is logical that the benthic migrants tend to concentrate near the metalimnion. Laybourn-Parry et al. (1990) suggests that the development of two distinct communities of planktonic protozoa (an epi-

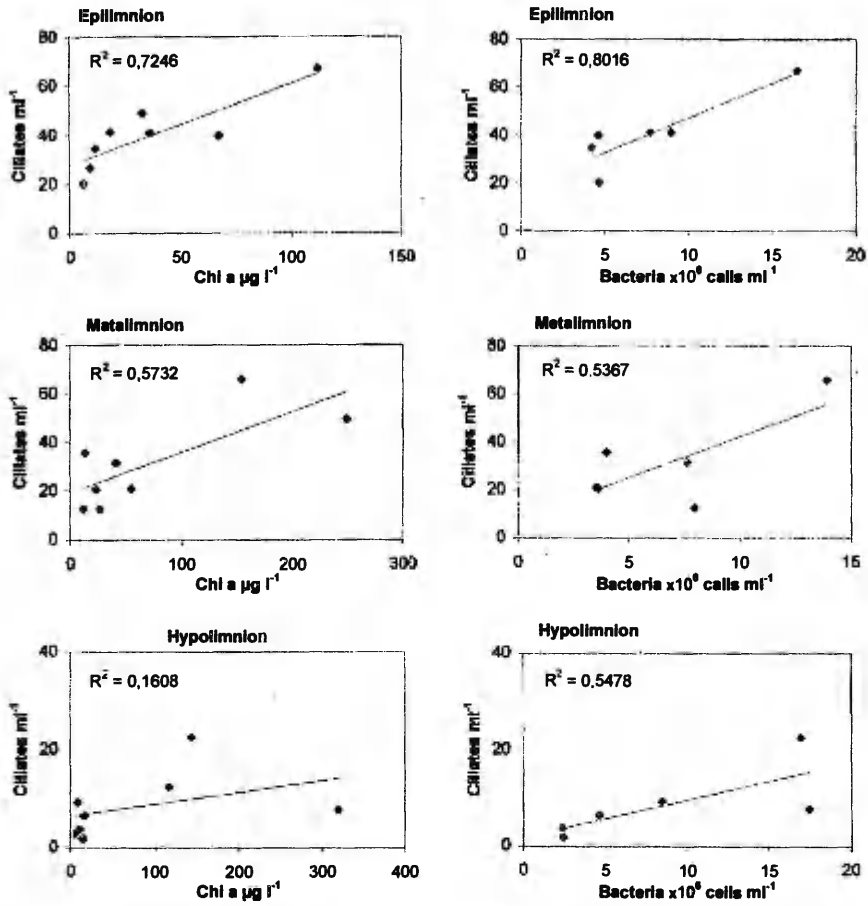

Figure 3. Relationship between mean ciliate abundance and chlorophyll *a* concentration and bacterial numbers in study lakes epi- , meta- and hypolimnion.

limnetic community of obligate planktonic ciliates and a hypolimnetic community of benthic migrants) is a characteristic for lakes with the anoxic hypolimnion.

In conclusion, this study showed clear distribution patterns of planktonic ciliates. In the epilimnion, the ciliate community was dominated by oligotrichs but small algivorous prostomatids, peritrichs and haptorids were also numerous. Abundance of these groups decreased in the metalimnion and for new dominants became scuticociliates and mixotrophic prostomatids as *Coleps* spp. In the hypolimnion species known as benthic migrants appeared. Also, scuticociliates stayed important in the hypolimnion.

Acknowledgements

We want to express our gratitude to Veljo Kisand, Toomas Kõiv, Peeter Nõges, Tiina Nõges, Katrin Ott, Kristjan Piirimäe and Helen Tammert. This research was partly supported by ESF grant N 3579, target financed research project N 0370208 and Nessling foundation grant N 99084.

References

Bark, A. W., 1981. The temporal and spatial distribution of planktonic and benthic protozoan communities in a small productive lake. Hydrobiologia 85: 239–255.

Beaver, J. R. & T. L. Crisman, 1982. The trophic response of ciliated protozoans in freshwater lakes. Limnol. Oceanogr. 27: 246–253.

Beaver, J. R., T. L. Chrishan & R. W. Biernert, 1988. Distribution of planktonic ciliates in highly colored subtropical lakes: comparison with clearwater ciliate communities and the contribution of mixotrophic taxa to total autotrophic biomass. Freshwat. Biol. 20: 51–60.

Carrias, J. F., C. Amblard & G. Bourdier, 1994. Vertical and temporal heterogeneity of planktonic ciliated protozoa in a humic lake. J. Plankton Res. 16: 471–485.

Carrias, J. F., C. Amblard & G. Bourdier, 1998. Seasonal dynamics and vertical distribution of planktonic ciliates and their relationships to microbial food resources in the oligomesotrophic Lake Pavin. Arch. Hydrobiol. 143: 227–255.

Carrick, H. J. & G. L. Fahnenstiel, 1990. Planktonic protozoa in Lakes Huron and Michigan: seasonal abundance and composition of ciliates and dinoflagellates. J. Great Lakes Res. 16: 319–329.

Corliss, J. O., 1979. The Ciliated Protozoa: Characterization, Classification and Guide to the Literature. 2nd edn. Pergamon Press, London: 455 pp.

Coats, Dolan, J. R. & D. W. Coats, 1991. Preliminary prey digestion in a predacious estuarine ciliate and the use of digestion data to estimate digestion. Limnol. Oceanogr. 36: 558–565.

Esteve, I., J. M. N. Gaju, H. McKhann & L. Margulis, 1989. Green endosymbiont of Coleps from Lake Cisco identified as Chlorella vulgaris. Symbiosis. Balaban Publ., Rehovoth, Israel.

Finlay, B. J., 1985. Nitrate respiration by protozoa (*Loxodes* spp.) in the hypolimnetic nitrate maximum of productive freshwater pond. Freshwater Biol. 15: 333–346.

Finlay, B. J., T. Fenchel & S. Garfdiner, 1986. Oxygen perception and oxygen toxicity in the freshwater ciliated protozoon Loxodes. J. Protozool. 33: 157–165.

Foissner, W. & H. Berger, 1996. A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes and waste waters, with notes on their ecology. Freshwat. Biol. 35: 375–482.

Gifford, D. J., 1991. The protozoan-metazoan trophic link in pelagic ecosystems. J. Protozool. 38: 81–86.

Gonzales, J. M., E. B. Sherr & B. F. Sherr, 1990. Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. Appl. Environ. Microbiol. 56: 583–589.

Guerrero, R., C. Pedros-Alio, T. M. Schmidt & J. Mas, 1985. Phototrophic sulphur bacteria in two Spanish lakes: vertical distribution and limiting factors. Limnol. Oceanogr. 30: 807–819.

Güde, H., 1989. The role of grazing on bacteria in plankton succession. In Sommer, U. (ed.) Plankton Ecology: Succession in Planktonic Communities. Brock/Springer, Berlin: 337–364.

Hecky, R. E. & H. J. Kling, 1981. The phytoplankton and protozooplankton of Lake Tanganyika: species composition, biomass, chlorophyll content and spatio-temporal distribution. Limnol. Oceanogr. 26: 548–564.

James, M. R., C. W. Burns, & D. J. Forsyth, 1995. Pelagic ciliated protozoa in two monomictic, southern temperate lakes of contrasting trophic state: seasonal distribution and abundance. J. Plankton Res. 17: 1479–1500.

Kahl, A., 1930. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 1. Allgemeiner Teil und Prostomata. Tierwelt Deutschlands 18: 1–180.

Kahl. A., 1931. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 2. Holotricha auβer den im 1. Teil behandelten Prostomata. Tierwelt Deutschlands 21: 181–398.

Kahl, A., 1932. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. Tierwelt Deutschlands 25: 399–650.

Kahl, A., 1935. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 4. Peritricha und Chonotricha. Tierwelt Deutschlands 30: 651–886.

Konopka, A., 1989. Metalimnetic cyanobacteria in hard-water lakes: Buoyoncy regulation and physiological state. Limnol. Oceanogr. 34: 1174–1184.

Kutikova, L. A. & A. J. Starobogatov, 1977. A key to freshwater invertebrates of European USSR (plankton and benthos). Gidrometeizdat, Leningrad; 510 pp. (in Russian).

Laybourn-Parry, J., J. Olver, A. Rogerson & P. L. Duverge, 1990. The temporal and spatial patterns of protozooplankton abundance in a eutrophic temperate lake. Hydrobiologia 203: 99–110.

Müller, H., A. Schöne, R. M. Pinto-Coelho, A. Schweizer & T. Weisse, 1991. Seasonal succession of ciliates in Lake Constance. Microb. Ecol. 21: 119–138.

Nagata, T., 1988. The microflagellate-picoplankton food linkage in the water column of lake Biwa. Limnol. Oceanogr. 33: 504–517.

Ott, I., R. Laugaste, S. Lokk & A. Mäemets, 1997. Plankton changes in Estonian small lakes in 1951–93. Proc. Est. Sci. Biol. Ecol. 46: 58–79.

Pace, M. L. & J. D. Orcutt, 1981. The relative importance of protozoans, rotifers, and crustaceans in a freshwater zooplankton community. Limnol. Oceanogr. 26: 822–830.

Pace, M. L., 1982. Planktonic ciliates: their distribution, abundance and relationship to microbial resources in a monomictic lake. Can. J. Fish. aquat. Sci. 39: 1106–1116.

Patterson, D. J. & S. Hedley, 1992. Free-living Freshwater Protozoa. A Color Guide. Wolfe Publishing Ltd., England; 223 pp.

Porter, K. G., M. L. Pace & J. F. Battey, 1979. Ciliate protozoans as links in freshwater planctonic food chains. Nature 277: 563–565.

Putt, M. & D. K. Stoecker, 1989. An experimentally determined carbon:volume ratio for marine 'oligotrichous' ciliates from estuarine and coastal waters. Limnol. Oceanogr. 34: 1097–1103.

Sanders, R. W., K. G., Porter, S. J. Bennett & A. E., DeBiase, 1989. Seasonal patterns of bacterivory by flagellates, ciliates, rotifers and cladocerans in a freshwater planktonic community. Limnol. Oceanogr. 34: 673–687.

Schönberger, M., 1994. Planktonic ciliated protozoa of Neusiedler See (Austria/Hungary) – a comparison between the turbid open lake and a reedless brown-water pond. Mar. Microbial Food Webs. 8: 251–263.

Sherr, E. B. & B. F., Sherr, 1984. Role of heterotrophic protozoa in carbon and energy flow in aquatic ecosystems. In Klug, M. J. & C. A. Reddy, (eds) Current Perspectives in Microbial Ecology. Amer. Soc. Microbiol., Washington: 412–423.

Simek, K., J. Bobkova, M. Macek, J. Nemoda & R. Psenner, 1995. Ciliates grazing on picoplankton in a eutrophic reservoir during summer phytoplankton maximum: a study at the species and community level. Limnol. Oceanogr. 40: 1077–1090.

Steenbergen, C. L. M., J.-P. Sweerts & T. E. Cappenberg, 1993. Microbial Biogeochemical Activities in Lakes: Stratification and Eutrophication. 4. Aquatic Microbiology. An Ecological Approach. Blackwell Science Publications: 69–101.

Taylor, W. D. & M. L. Heynen, 1987. Seasonal and vertical distribution of Ciliophora in Lake Ontario. Can. J. Fish. aquat. Sci. 44: 2185–2191.

Utermöhl, H., 1958. Zur Vervollkommung der quantitativen Phytoplankton Methodik. Mitt. int. Ver. Theor. Angew. Limnol. 9: 1–38.

Zingel, P., 1999. Pelagic ciliated protozoa in a shallow eutrophic lake: community structure and seasonal dynamics. Arch. Hydrobiol. 146: 495–511.

V

Zingel, P., Huitu, E., Mäkelä, S. & Arvola, L. 2001.
The abundance and diversity of planktonic ciliates
in 12 boreal lakes of varying trophic state.
(submitted to Arch. Hydrobiol.).

The abundance and diversity of planktonic ciliates in 12 boreal lakes of varying trophic state

Priit Zingel[1,2], Eeva Huitu[3], Suvi Mäkelä[3] and Lauri Arvola[3]

[1]Institute of Zoology and Hydrobiology,
University of Tartu, 46 Vanemuise St, 51014 Tartu, Estonia
[2]Võrtsjärv Limnological Station, Institute of Zoology and Botany,
Estonian Agricultural University, Rannu, 61101 Tartu County, Estonia
[3]Lammi Biological Station, University of Helsinki, FIN-16900 Lammi, Finland

Keywords: Ciliates, lake trophy, boreal lakes

Abstract

The cell density, biomass and community structure of planktonic ciliates in 12 Finnish boreal lakes ranging from oligotrophic to hypereutrophic were studied in 1997. Ciliate numbers and biomasses showed a trend of increasing toward eutrophication, and both were strongly correlated with the abundance of bacteria and phytoplankton. The mean cell densities for ciliates were: 2.9, 5.0, 9.8 and 20.3 cells ml^{-1} for oligotrophic, mesotrophic, eutrophic and hypereutrophic lakes, respectively. The ciliate communities were dominated by four orders: oligotrichs, haptorids, scuticociliates and prostomatids. The relative importance of oligotrichs and haptorids decreased along a trophic gradient from oligotrophy to eutrophy. In more eutrophic conditions these orders were progressively replaced by small scuticociliates and prostomatids. The diversity of ciliates in lakes was positively correlated ($R^2 = 0.84$) with the concentration of chlorophyll *a*. The peak abundances, in terms of both cell numbers and biomasses, were recorded in spring and early summer (most eutrophic lakes) or autumn (oligotrophic and most mesotrophic lakes).

Introduction

Ciliates are unicellular eukaryotes, which can be found in almost every aquatic environment. They have an important role in both freshwater and marine food webs, although their significance in pelagic food chains has been fully recognized only during the last decade. Clear evidence exists that planktonic ciliates are an important food resource for large metazooplankton (e.g. PORTER *et al.* 1979, DOLAN & COATS 1991, GIFFORD 1991). It has been shown that metazooplankters suppress ciliates through predation and interference competition (WICKHAM & GILBERT 1991, 1993), while ciliates can consume sizeable proportions of bacterio- and phytoplankton production. Therefore metazooplankton predation on ciliates can be an important trophic link between pico- and nanoplankton and metazoans. In addition to their role in energy transfer to higher trophic levels, ciliated protozoa act in bio-geochemical cycling of

phosphorus and nitrogen and can increase the availability of nutrients for phytoplankton growth (JOHANNES 1965, BUECHLER & DILLON 1974, BERMAN et al. 1987).

The number of papers dealing with freshwater protozooplankton has increased recently (Hecky & Kling 1981, Pace & Orcutt 1981, Taylor & Heynen 1987, Beaver *et al.* 1988, Carrick & Fahnenstiel 1990, Laybourn-Parry *et al.* 1990, Carrias *et al.* 1994, James *et al.* 1995). It is well known that ciliate communities are very dynamic, and their community structure may quickly respond to changing physical, chemical and biological conditions in the environment. Nevertheless there is a lack of detailed studies describing the response of planktonic ciliates to increasing eutrophication and this is particularly true for boreal lakes. The aim of this study was, therefore, to evaluate the ciliate communities along a wide trophic gradient in 12 boreal lakes in southern Finland.

Method

The studied lakes are situated in the same region in Southern Finland (Figure 1). Therefore the lakes are influenced by similar weather conditions. More data about their physical and chemical characteristics are given in Table I. Some basic information about the lakes and their catchment areas are also given by Huitu and Mäkelä (1999).

Samples were collected on four occasions during 1997: in late winter (April), when lakes were still ice-covered; in spring (late May-early June), after the ice-break and complete overturn (except in a few lakes) of the water column; in summer (August) during the period of steep thermal and chemical (only in some lakes) stratification; and in autumn (October) after breakdown of the summer stratification. The studied lakes were classified into the following trophic categories on the base of FORSBERG & RYDING (1980); oligotrophic (two lakes), mesotrophic (four), eutrophic (five), and hypereutrophic (one). Lakes were also classified into clear-water, i.e. oligohumic (water colour ≤ 40), mesohumic (>40, ≤ 100) and humic lakes (>100 mg Pt l^{-1}).

The samples were taken integrally from the whole water column in lakes where the maximum depth was < 2m. In deeper lakes, the epi- and hypolimnion were sampled separately; for the epilimnion an integrated sample from the uppermost 2 m was taken, and for the hypolimnion one sample from the depth of 2-times the Secchi depth. The samples were always collected from the deepest area of lake. 250 ml subsamples were preserved and fixed with acidified Lugols solution for ciliate counts. Samples were stored at 4°C in the dark. Ciliate biomass and community composition were determined using the UTERMÖHL (1958) technique. Volumes of 50 ml were settled for at least 24 hours in plankton chambers. Ciliates were enumerated and identified with an inverted microscope (Olympus 1X50) at 400–1000 X magnification. The entire content of each Utermöhl chamber was surveyed. Ciliates were usually identified to genus by consulting several works (KAHL 1930–1935, KUTIKOVA & STAROBOGATOV 1977, PATTERSON & HEDLEY 1992, FOISSNER & BERGER 1996). The taxonomy followed mainly the scheme of CORLISS (1979). Additional live subsamples were used to aid identification. The first 20 measurable specimens encountered for each taxon were measured. Biovolumes of each taxa were estimated by assuming geometric shapes and converted to carbon weight using a factor of 190 fg C μm^{-3} (PUTT & STOECKER 1989). The abundance of bacteria was determined under epifluorescence microscope (magnification 1000×) as described by TULONEN (1993).

Physical and chemical analyses (colour, chlorophyll *a*, total nitrogen and phosphorus) were made using standard methods (cf. ARVOLA *et al.* 1996).

Results

The community of ciliates was dominated by four orders: Oligotrichida, Haptorida, Prostomatida and Scuticociliatida (Figure 2). Other registered orders were: Peritrichida, Odontostomatida, Heterotrichida, Hypotrichida, Pleutostomatida, Hymenostomatida and Suctorida. Oligotrichs were the most abundant group in all investigated lakes. Their relative importance was greater in oligotrophic conditions and decreased with increasing trophic state. Also haptorids showed this trend (Table II). In contrast, the importance of prostomatids and scuticociliates grew along the trophic gradient. Greatest species diversity was present in hypereutrophic (35 taxa) and scarcest in oligotrophic conditions (10 taxa). We found a positive correlation between Chl a and number of found ciliate taxa (Figure 3). Most common genera were Strombidium, Strobilidium, Tintinidium, Halteria, Urotricha, Coleps, Mesodinium and Askenasia. Odontostomatids (Pelodinium reniforme, Epalxella spp.) and colpodids (Bursaria truncatella, Colpoda spp.) were found only in eutrophic and hypereutrophic lakes.

There were only very small differences between the abundance and biomass of planktonic ciliates in coloured and clearwater lakes for a given trophic state (Table III). Compared with clear water lakes, the coloured lakes displayed greater importance of prostomatids and smaller importance of oligotrichs in the eutrophic range and greater representation of oligotrichs in the mesotrophic range.

The mean cell density of ciliates was strongly correlated with the mean biomass of ciliates in the study lakes (R^2=0.98, $p< 0.05$). Peaks in ciliate abundances were mostly recorded either in spring or in autum. The only exception was the hypereutrophic Lake Takaperänjärvi, which showed the maximum abundance in late winter, before the ice-break. In oligotrophic and in most mesotrophic lakes, the ciliate peak occurred in autumn after summer stratification had broken down and was made up by a haptorid *Mesodinium sp.* which was abundant in the hypolimnion. Also the prostomatid *Urotricha spp.* peaked in oligotrophic lakes in autumn. In eutrophic systems the peaks were recorded mostly in spring after the icebreak, when the water column was already mixed. During this period the dominating orders were oligotrichs, scuticociliates and prostomatids. The maximum cell density (35.6 cells ml^{-1}) and biomass (178 µg C l^{-1}) were observed in eutrophic Lake Taka-Killo in spring. The mean annual cell numbers and biomasses were 2.9 cells ml^{-1} and 12.2 µg C l^{-1} for oligotrophic lakes, 5.0 cells ml^{-1} and 21.3 µg C l^{-1} for mesotrophic lakes, 9.8 cells ml^{-1} and 49.4 µg C l^{-1} for eutrophic lakes, and 20.3 cells ml^{-1} and 98.0 µg C l^{-1} for hypereutrophic lakes. In winter the abundance was lowest.

The cell number and biomass of ciliates were positively related with Chl *a* and bacterial densities (Figure 4), while the latter were positively related with tot N and tot P (Table IV). In spring and summer water colour and ciliate numbers related negatively in the hypolimnion (Figure 5). In the epilimnion this trend was not observed, however.

Discussion

The abundance of planktonic ciliates found in Finnish freshwater lakes was in the same range as those reported for temperate lakes (Table V), but the values still appeared to be at the lower end of the spectrum compared with subtropical lakes (PACE & ORCUTT 1981, BEAVER & CRISMAN 1982, BEAVER *et al.* 1988). This is undoubtedly the result of higher temperatures and longer growing seasons in lower latitudes, which makes available much higher overall annual productivity of phytoplankton and bacterioplankton and the organisms which exploit them (LAYBOURN-PARRY 1992). We found a strong positive correlation between ciliate abundance and Chl *a* and bacterial numbers, which emphasizes that there is a direct relationship between phyto- and bacterioplankton productivity and ciliate numbers. Although Chl *a* does not specify whether food is edible or not for ciliates as it consists of small pico- and nanoalgae as well as large filamentous cyanobacteria and diatoms. However, Chl *a* provides a rough estimate of the amount of food available for cilliates (MÜLLER et al. 1991) and in boreal lakes also bacterial production is often closely correlated with Chl *a* (cf. ARVOLA & KANKAALA 1989, ARVOLA *et al.* 1996) In Finnish lakes the ciliate communities collapse during a long duration of ice-cover but subtropical lakes sustain relatively abundant winter populations (e.g. PACE & ORCUTT 1981), and in spring ciliate communities may there reach high abundances much quicker than in their northern counterparts. In the study lakes there was a 7-fold difference between ciliate numbers in oligotrophic and hypereutrophic lakes, and an 8-fold difference between biomasses. BEAVER & CRISMAN (1982), who investigated 20 subtropical freshwater lakes along a trophic gradient, found a 14-fold difference between ciliate numbers and biomasses in oligotrophic and hypereutrophic systems. As the reasons mentioned above permit higher abundances in subtropical lake systems, it is reasonable to assume that the same reasons also permit greater differences between lakes with differing trophic state.

BEAVER & CRISMAN (1982) related ciliate community structure to trophic status, so that large algivorous oligotrichs are progressively replaced by small bacterivorous ciliates, mainly *Scuticociliatida*, with increasing eutrophication. Also in Finnish boreal lakes the share of scuticociliates increased and share of oligotrichs decreased from oligotrophy to hypereutrophy (Table II). In eutrophic conditions the relative importance of scuticociliates was greater than in hypereutrophic lakes, but only because other small bacterivorous ciliate species (e.g. *Colpoda spp.*) were also abundant in the latter. Our studies do not support the results of MATHES & ARNDT (1994) who found that the importance of oligotrichs did not decrease in the Mecklenburgian temperate lakes along the trophic gradient leading to eutrophy. Also the importance of scuticociliates was not significant in their study, as the prostomatids composed a major part of the nanociliate group. Prostomatids, which were not mentioned by BEAVER & CRISMAN (1982), accounted for 10 to 22% of total ciliate numbers in the studied Finnish lakes. The importance of prostomatids has also been recorded in other studies. In the pelagic zone of mesoeutrophic Lake Constance, small prostomatids were the most abundant ciliate group (MÜLLER *et al.* 1991) and in mesotrophic Lake Vassivière they accounted for 18% of the total abundance in the upper layers (CARRIAS *et al.* 1994). In Lakes Huron and Michigan these ciliates accounted for 10–20% of total ciliate biomass in late summer and autumn (CARRICK & FAHNENSTIEL 1990). MÜLLER et al. (1991) suggested that these nanociliates effectively feed on flagellates, which are respectively known to be more abundant in eutrophic conditions. This accords well with our results, as the

importance of prostomatids grew with increasing eutrophication (Table II). Haptorids were mainly represented by predatory species, feeding on other ciliates. Their share decreased from oligotrophy to hypereutrophy (Table II) but their total abundance remained almost the same. Since the food requirements of predatory ciliates may overlap with those of larger metazooplankton, there may easily occur competition for food resources between haptorids and metazooplankton. We may speculate that this can depress the abundance of haptorids, whereas there is no such depressor for bacteriovorous species, and their relative importance increases with the increasing lake trophy.

It has been shown that in most temperate lakes the abundance and biomass of ciliates have a peak in late spring and early summer, and this coincides or follows the peak of phytoplankton (LAYBOURN-PARRY 1992). This is in good accordance with the eutrophic Finnish lakes we studied. Ciliate populations decreased in most lakes in late summer, which might be a result of competition and predation by metazooplankton. Metazooplankton are known to prey intensively on ciliates (SOROKIN & PAVELJEVA 1972, MALY 1975, BERK *et al.* 1977, PORTER *et al.* 1979, HEINBOKEL & BEERS 1979) and so can affect their numbers. Various studies conducted in enclosures have demonstrated that copepods and cladocerans are able to control (or modify) the abundance of ciliates (CARRICK *et al.* 1991, TAYLOR & JOHANNSSON 1991, SALONEN *et al.* 1992, WICKHAM & GILBERT 1991, 1993). Also competition for food resources may be one of the main factors controlling the temporal patterns of protozoan occurrence and abundance (BEAVER & CRISMAN 1982). In spring ciliates have their chance because their rapid division rate enables them to establish large populations relatively quickly. However, when the dense populations of metazooplankton develop, ciliates are often largely excluded because the grazing rate of metazooplankton is usually much higher than that of ciliates (LAYBOURN-PARRY 1992).

Temperate lakes frequently experience an autumnal secondary peak of primary production associated with mixing. According to LAYBOURN-PARRY (1992) the protozooplankton do not generally appear to respond to this resurgence in food resources. In our study, oligotrophic and some mesotrophic lakes showed clear autumn peak in ciliate abundance. Predation or exclusion may be operating in lakes with still higher productivity as the autumn peaks were not recorded in the studied eutrophic lakes. Most oligotrophic temperate lakes studied to date, show maximum abundance of ciliates in spring and early summer (LAYBOURN-PARRY 1992). In contrast, most subtropical oligotrophic waters show peaks in ciliate abundance in autumn. These peaks are also associated with the recycling of nutrients from lower waters layers during the thermal overturn in autumn which creates a phase of high productivity (BEAVER & CRISMANN 1989). Peaks in studied Finnish oligotrophic lakes were made up mostly by *Mesodinium sp.*, *Urotricha furcata* and *Urotricha farcta* which are known to prey on nanoplankton. During these peaks, *Mesodinium* was always more abundant in the hypolimnion than in the epilimnion. Similarily, CARRIAS *et al.* (1994) and CARRICK and FAHNENSTIEL (1990) reported major development of *Mesodinium sp.* in the hypolimnion.

We recorded only small differences between the abundance and biomass of planktonic ciliates in coloured and clearwater lakes for a given trophic state. The data about ciliate communities in humic lakes is scarce, but BEAVER *et al.* (1988) also reported that the ciliate numbers found in Floridan clearwater and humic lakes were rather similar. In spring and summer we found a negative correlation between lake color and ciliate numbers in the hypolimnion. This might be a direct result of the fact that in lakes rich with humic substances the depth of the euphotic zone is reduced and the algal

prey is much more concentrated in the upper part of the water column. Still in humic lakes there was only very small annual difference between the abundance of ciliates in the hypo- and epilimnion (10.2 cells ml^{-1} and 9.8 cells ml^{-1}, in the epi- and hypolimnion, respectively). So one may assume, that in spite of reduced light, there must be enough food for planktonic ciliates also in the hypolimnion of humic lakes. This seems to be true because in many small humic lakes there are dense populations of bacteria and algae which are living in the metalimnion or upper part of the hypolimnion (e.g. SALONEN *et al.* 1990), and often there are also populations of nanoplanktonic algae which undergo diurnal migrations up and down in the water column (e.g. ARVOLA 1984, ARVOLA *et al.* 1992).

Our study shows that both abundance and community structure of planktonic ciliates are highly dynamic and may change quite remarkably along the trophic gradient. However, further studies are needed to evaluate the role of planktonic ciliates in the food webs of the lakes with different trophy and organic matter content.

Acknowledgements

This study was supported by the Nordic Council. We thank the staff of Lammi Biological Station for making chemical measurements. We thank Dr. Roger I. Jones for his comments on the M.S. and checking the English.

References

Arvola, L. (1984) Vertical distribution of primary production and the vertical distribution of phytoplankton in two small lakes with different humus concentration in southern Finland. *Holarct. Ecol.*, 7, 390–398.

Arvola, L. and Kankaala, P. (1989) Winter and spring variability in phyto- and bacterioplankton in lakes with different water colour. *Aqua Fennica,* **19**, 29–39.

Arvola, L., Salonen, K., Kankaala, P. and Lehtovaara, A. (1992) Vertical distributions of bacteria and algae in a steeply stratified humic lake under high grazing pressure from *Daphnia longispina*. *Hydrobiologia*, **229**, 253–269.

Arvola, L., Kankaala, P., Tulonen, T. and Ojala, A. (1996) Effects of phosphorus and allochthonous humic matter enrichment on the metabolic processes and community structure of plankton in a boreal lake (Lake Pääjärvi). *Can. J. Fish. Aquat. Sci.*, **53**, 1646–1662.

Beaver, J. R. and Crisman, T. L. (1982) The trophic response of ciliated protozoans in freshwater lakes. *Limnol. Oceanogr.*, **27**, 246–253.

Beaver, J. R., Crisman, T. L. and Biernert, R. W. (1988) Distribution of planktonic ciliates in highly colored subtropical lakes: Comparison with clearwater ciliate communities and the contribution of mixotrophic taxa to total autotrophic biomass. *Freshwater Biol.*, **20**, 51–60.

Beaver, J. R. and Crisman, T. L. (1989) The role of ciliated protozoa in pelagic freshwater ecosystems. *Microb. Ecol.*, **17**, 111–136.

Berk, S. G., Brownlee, D. C., Heinle, D. R., Kling, H. J. and Colwell, R. R. (1977) Ciliates as a food source for marine planktonic copepods. *Microb. Ecol.*, **4**, 27–40.

Berman, T., Nawrocki, M., Taylor, G. T. and Karl, D. M. (1987) Nutrient flux between bacteria, bacterivorous nanoplanctonic protists and algae. *Mar. Microb. Food Webs*, **2**, 69–82.

Buechler, D. G. and Dillon, R. D. (1974) Phosphorus regeneration in freshwater *Paramecia. J. Protozool.,* **21**, 339–343.

Carrias, J. F., Amblard, C. and Bourdier, G. (1994) Vertical and temporal heterogeneity of planktonic ciliated protozoa in a humic lake. *J. Plankton Res.,* **16**, 471–485.

Carrick, H. J. and Fahnenstiel, G. L. (1990) Planktonic protozoa in Lakes Huron and Michigan: seasonal abundance and composition of ciliates and dinoflagellates. *J. Great Lakes Res.*, **16**, 319–329.

Carrick, H. J., Fahnenstiel, G. L., Stormer, E. F. and Wetzel, R. G. (1991) The importance of zooplankton-protozoan trophic couplings in Lake Michigan. *Limnol. Oceanogr.,* **36**, 1335–1345.

Corliss, J. O. (1979) The ciliated protozoa: Characterization, classification and guide to the literature. 2nd Edition. — Pergamon press, London, 455p.

Cruz-Pizarro, L., Reche, I. and Carillo, P. (1994) Plankton dynamics in a high mountain lake (Las Yeguas, Sierra Nevada, Spain). Indirect evidence of ciliates as food source for zooplankton. *Hydrobiologia,* **274**, 29–35.

Dolan, J. R. and Coats, D. W. (1991) Preliminary prey digestion in a predacious estuarine ciliate and the use of digestion data to estimate digestion. *Limnol. Oceanogr.,* **36**, 558–565.

Foissner, W. and Berger, H. (1996) A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. *Freshwater Biol.,* **35**, 375–482.

Forsberg, C. and Ryding, S. O. (1980) Eutrophication parameters and trophic state indicies in 30 Swedish waste-receiving lakes. *Arch. Hydrobiol.,* **89**, 189–207.

Gates, M. A. and Lewg, U. T. (1984) Contribution of ciliated protozoa to the planktonic biomass in a series of Ontario lakes, quantitative estimates and dynamical relationships. *J. Plankton Res.,* 6, 443–456.

Gifford, D. J. (1991) The protozoan-metazoan trophic link in pelagic ecosystems. *J. Protozool.,* **38**, 81–86.

Hecky, R. E. and Kling, H. J. (1981) The phytoplankton and proto-zooplankton of Lake Tanganyika: species composition, biomass, chlorophyll content and spatio-temporal distribution. *Limnol. Oceanogr.,* **26**, 548–564.

Heinbokel, J. F. and Beers, J. R. (1979) Studies on the functional role of tintinnids in the Southern California Bight. 3. Graizing impact of natural assemblages. *Mar. Biol.,* **52**, 23–32.

Huitu, E. and Mäkelä, S. (1999) Survey of 40 lakes: from headwaters to lowland lakes. *Lammi Notes,* **26** (in press).

James, M. R., Burns, C. W. and Forsyth, D. J. (1995) Pelagic ciliated protozoa in two monomictic, southern temperate lakes of contrasting trophic state: seasonal distribution and abundance. *J. Plankton Res.,* **17**, 1479–1500.

Johannes, R. E. (1965) Influence of marine Protozoa on nutrient regeneration. *Limnol. Oceanogr.,* **10**, 434–442.

Kahl, A. (1930) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 1. Allgemeiner Teil und Prostomata. *Tierwelt Deutschlands*, **18**, 1–180.

Kahl, A. (1931) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 2. Holotricha auβer den im 1. Teil behandelten Prostomata. *Tierwelt Deutschlands,* **21**, 181–398.

Kahl, A. (1932) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. *Tierwelt Deutschlands,* **25**, 399–650.

Kahl, A. (1935) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 4. Peritricha und Chonotricha. *Tierwelt Deutschlands,* **30**, 651–886.

Kutikova, L. A. and Starobogatov, A. J. (1977) A key to freshwater invertebrates of European USSR (plankton and benthos). Gidrometeizdat, Leningrad, 510 p. (in Russian)

Laybourn-Parry, J., Olver, J., Rogerson, A. and Duvergé, P. L. (1990) The temporal and spatial patterns of protozooplankton abundance in a eutrophic temperate lake. *Hydrobiologia*, **203**, 99–110.

Laybourn-Parry, J. (1992) Protozoan plankton ecology. Chapmann & Hall, Ltd., London, 231 p.

Lewis, W. M. (1985) Protozoan abundance in the plankton of two tropical lakes. *Arch. Hydrobiol.*, **104**, 337–343.

Maly, E. J. (1975) Interactions among the predatory rotifer *Asplancha* and two prey, *Paramecium* and *Euglena*. *Ecology*, **56**, 346–358.

Mamaeva, N. V. (1976) Planktonic ciliates in the Ivan'kovsky water reservoir. *Zool. Zhur. Uk.*, **55**, 657–664.

Mathes, J. and Arndt, H. (1994) Biomass and composition of protozooplankton in relation to lake trophy in north German lakes. *Mar. Microb. Food Webs*, **8**, 357–375.

Müller, H., Schöne, A., Pinto-Coelho, R. M., Schweizer, A. and Weisse T. (1991) Seasonal succession of ciliates in Lake Constance. *Microb. Ecol.*, **21**, 119–138.

Nauwerck, A. (1963) Die Beziehungen Zwischen Zooplankton and Phytoplankton in See Erken. *Symb. Bot. Ups.*, **17**, 1–163.

Pace, M. L. and Orcutt, J. D. (1981) The relative importance of protozoans, rotifers, and crustaceans in a freshwater zooplankton community. *Limnol. Oceanogr.*, **26**, 822–830.

Pace, M. L. (1982) Planktonic ciliates: their distribution, abundance and relationship to microbial resources in a monomictic lake. *Can. J. Fish. Aquat. Sci.*, **39**, 1106–1116.

Patterson, D. J. and Hedley, S. (1992) Free-living freshwater protozoa. A color guide. Wolfe Publishing Ltd., England, 223 p.

Porter, K. G., Pace, M. L. and Battey, J. F. (1979) Ciliate protozoans as links in freshwater planctonic food chains. *Nature*, **277**, 563–565.

Putt, M. and Stoecker, D. K. (1989) An experimentally determined carbon:volume ratio for marine 'oligotrichous' ciliates from estuarine and coastal waters. *Limnol. Oceanogr.*, **34**, 1097–1103.

Rigler, F. H., MacCallum, M. E. and Roff, J. C. (1974) Production of zooplankton in Char lake. *J. Fish. Res. Board Can.*, **31**, 637–646.

Salonen, K., Järvinen, M., Kuoppamäki, K. and Arvola, L. (1990) Effects of liming on the chemistry and biology of a small acid humic lake. In: Acidification in Finland (Eds. P. Kauppi, P. Anttila & K. Kenttämies), Springer-Verlag, Berlin, Heidelberg, pp. 1145–1167.

Salonen, K., Arvola, L., Tulonen, T., Hammar, T., Metsälä, T-R., and Kankaala, P. (1992) Planktonic food chains of a highly humic lake. I. A mesocosm experiment during the spring primary production maximum. *Hydrobiologia*, **229**, 125–142.

Schönberger, M. (1994) Planktonic ciliated protozoa of Neusiedler See (Austria/Hungary) — a comparison between the turbid open lake and a reedless brown-water pond. *Mar. Microbial Food Webs.*, **8**, 251–263.

Shcherbakov, A. D. (1969) Quantity and biomass of Protozoa in the plankton of a eutrophic lake. *Hydrobiol. J.*, **5**, 9–15.

Sorokin, Y. I. and Paveljeva, E. B. (1972) On the quantitative characteristics of the pelagic ecosytem of Dalnee Lake (Kamchatka). *Hydrobiologia*, **40**, 519–552.

Taylor, W. D. and Heynen, M. L. (1987) Seasonal and vertical distribution of Ciliophora in Lake Ontario. *Can. J. Fish. Aquat. Sci.*, **44**, 2185–2191.

Taylor, W. D. and Johansson, O. E. (1991) A comparison of estimates of productivity and consumption by zooplankton for planktonic ciliates in Lake Ontario. *J. Plankton Res.*, **13**, 363–372.

Tulonen, T. (1993) Bacterial production in a mesohumic lake estimated from [^{14}C]leucine incorporation rate. *Microb. Ecol.*, **26**, 210–217.

Utermöhl, H. (1958) Zur Vervollkommnung der quantitativen Phytoplankton Methodik. *Mitt. Int. Ver. Theor. Angew. Limnol.*, **9**, 1–38.

Wickham, S. A. and Gilbert, J. J. (1991) Relative vulnerabilities of natural rotifer and ciliate communities to cladocerans: laboratory and field experiments. *Freshwater Biol.*, **26**, 77–86.

Wickham, S. A. and Gilbert, J. J. (1993) The comparative importance of competition and predation by *Daphnia* on ciliated protists. *Arch. Hydrobiol.*, **126**, 289–313.

Zingel, P. (1999) Pelagic ciliated protozoa in a shallow eutrophic lake: community structure and seasonal dynamics. *Arch. Hydrobiol.*, **146**, 495–511.

Table I. Average annual values for some physico-chemical parameters of the studied Finnish lakes for 1997.

				Epilimnion							
Lake	Area km^2	Max. depth m	Retention Years	pH	Cond. MS m^{-1}	Colour mg Pt l^{-1}	TOC mg C l^{-1}	Tot P mg P m^{-3}	Tot N mg N m^{-3}	Chl *a* µg l^{-1}	
Säynäjärvi	0.42	20	3.7	7.1	5.19	17	6.5	7	311	1,8	
Pyhäjärvi	9.33	35		7.4	11.49	35	8.9	13	550	5,1	
Riikosten Valkjärvi	0.08	8	3.6	6.1	2.47	74	10.1	14	366	4,7	
Mustakatos	0.11	6		6.0	3.04	214	18.1	16	543	4,6	
Harasjärvi	0.42	5	1.4	7.2	6.75	36	10.2	19	589	12,4	
Avusjärvi	1.44	7		6.9	6.43	93	12.2	22	632	6,4	
Lehee	1.05	2	0.8	7.2	12.63	69	10.1	23	719	9,2	
Ormajärvi	6 53	30	3.1	7.9	15.27	30	9.8	25	666	10,6	
Teuronjärvi	1.34	5	0.3	7.0	9.21	87	10.5	29	914	12,1	
Taka-Killo	0.03	16	1.6	6.9	2.22	21	8.7	31	674	15,2	
Lovojärvi	0.05	16	0.2	7.0	12.24	158	14.7	45	953	21,3	
Takaperä	0 19	1	0.5	6.8	4.86	182	29.8	102	2003	73,4	

		Hypolimnion						
Lake	pH	Cond. mS m^{-1}	Colour mg Pt l^{-1}	TOC mg C l^{-1}	Tot P Mg P m^{-3}	Tot N mg N m^{-3}	Chl *a* µg l^{-1}	Oxygen < 1 mg O^2 l^{-1}
Säynäjärvi	6.5	5.54	25	6.8	9	409	1,3	
Pyhäjärvi	7.2	11.62	36	8.3	14	564	4.4	
Riikosten Valkjärvi	5.8	2.65	109	11.0	25	437	5,2	Yes
Mustakatos	5,8	3.13	247	18.8	20	614	2,5	Yes
Harasjärvi	7.1	7.14	41	10.2	21	619	12,3	
Avusjärvi	6 6	7.66	123	16.5	18	853	7,7	Yes
Lehee	7.1	12.67	75	8.9	29	681	9,2	
Ormajärvi	7.1	16.2	31	9.3	29	1016	3,5	
Teuronjärvi	7.0	9.4	89	10.5	24	810	7,8	
Taka-Killo	6.2	4.11	42	9.4	328	2117	23,5	Yes
Lovojärvi	6 7	24,78	959	25.8	638	8373	11,3	Yes
Takaperä	6.8	4,86	182	29.8	102	2003	73,4	

Table II. Relative importance of different groups of ciliates in lakes of various trophic state as a percentage of total ciliate density.

	Oligotrophic	Mesotrophic	Eutrophic	Hypereutrophic
Oligotrichida	62.7	56.3	48.5	37.1
Haptorida	24.8	16.0	3.3	2.1
Prostomatida	10.4	19.9	20.9	21.6
Scuticociliatida	2.0	2.9	22.8	13.6
Miscellaneous	0.2	5.0	4.5	25.5

Table III. Density and biomass of planktonic ciliates in different types of Finnish boreal lakes.

	Clearwater	Mesohumic	Humic
Density, cells ml^{-1}			
Oligotrophic	2.7	3.1	–
Mesotrophic	5.5	–	4.6
Eutrophic/Hypereutrophic	11.8	7.4	10.4
Biomass µg C l^{-1}			
Oligotrophic	13.4	11.1	–
Mesotrophic	25.3	–	19.6
Eutrophic/Hypereutrophic	62.0	38.8	48.5

Table IV. Correlation coefficients (Spearman R, $p < 0.05$) between cell density and biomass of planktonic ciliates, and chlorophyll *a*, bacterial abundance, total nitrogen and total phosphorus. NS = not significant.

	Chl *a*	Bacteria	Tot N	Tot P
Epilimnion				
Ciliates (density)	0.874	0.683	0.797	0.874
Ciliates (biomass)	0.916	0.717	0.832	0.881
Chl *a*	–	0.767	0.874	0.902
Bacteria	0.767	–	0.850	0.850
Hypolimnion				
Ciliates (density)	0.891	0.857	0.842	0.806
Ciliates (biomass)	0.879	0.810	0.806	0.770
Chl *a*	–	0.905	0.624	0.588
Bacteria	0.905	–	0.714	0.429 NS

Table V. Cell densities (cells ml^{-1}) for planktonic ciliates in lakes of varying trophic status and geographical location.

Lake	Range	Mean	Trophic state	Latitude	References
Floridan lakes	≤ 214	155.0	Hypereutrophic	Subtropical	BEAVER & CRISMANN 1982
Finnish lakes	13.4–28.3	20.3	Hypereutrophic	Boreal	This study
Valencia	–	218	Eutrophic	Tropical	LEWIS 1985
Floridan lakes	–	55.5	Eutrophic	Subtropical	BEAVER & CRISMANN 1982
Võrtsjärv	0.6–191	45.7	Eutrophic	Temperate	ZINGEL 1999
Finnish lakes	0.6–35.6	9.8	Eutrophic	Boreal	This study
Beloe	≤ 11	–	Eutrophic	Temperate	SHCHERBAKOV 1969
Esthwaite	≤ 9.2	–	Eutrophic	Temperate	LAYBOURN-PARRY ET AL. 1990
Okaro	0.3–10.4	4.0	Eutrophic	Temperate	JAMES ET AL. 1995
Dalnee	0–230	–	Eutrophic	Temperate	SOROKIN & PAVELJEVA 1972
Oglethorpe	0.1–190	18.0	Mesoeutrophic	Temperate	PACE 1982
Constance	–	15.3	Mesoeutrophic	Temperate	MÜLLER1991
Ruster Poschen	≤ 203	51.0	Mesotrophic	Temperate	SCHÖNBERGER 1994
Floridan lakes	–	27.5	Mesotrophic	Subtropical	BEAVER & CRISMANN 1982
Neusiedler See	≤ 56	17.0	Mesotrophic	Temperate	SCHÖNBERGER 1994
Finnish lakes	0.08–16.5	5.0	Mesotrophic	Boreal	This study
Vassivière	1.4–20.4	4.8	Mesotrophic	Temperate	CARRIAS ET AL. 1994
Erken	0.1–10	–	Mesotrophic	Temperate	NAUWERCK1963
Glubokoe	≤7	–	Mesotrophic	Temperate	SHCHERBAKOV 1969
Las Yeguas	2–10	–	Oligotrophic	Subtropical	CRUZ-PIZARRO ET AL. 1994
Ontario	4.8–7.9	–	Oligotrophic	Temperate	TAYLOR & HEYNEN 1987
Jack	≤7.4	–	Oligotrophic	Temperate	GATES & LEWG 1984
Tanganyika	–	8.8	Oligotrophic	Tropical	HECKY & KLING 1981
Floridan lakes	–	10.8	Oligotrophic	Subtropical	BEAVER & CRISMANN 1982
Finnish lakes	0.6–7.3	2.9	Oligotrophic	Boreal	This study
Ivan'kovsky	≤1.9	–	Oligotrophic	Temperate	MAMAEVA 1976
Taupo	0.2–2.6	0.9	Oligotrophic	Temperate	JAMES ET AL. 1995
Char	0–10.3	2.4	Ultraoligotrophic	Arctic	RIGLER ET AL. 1974

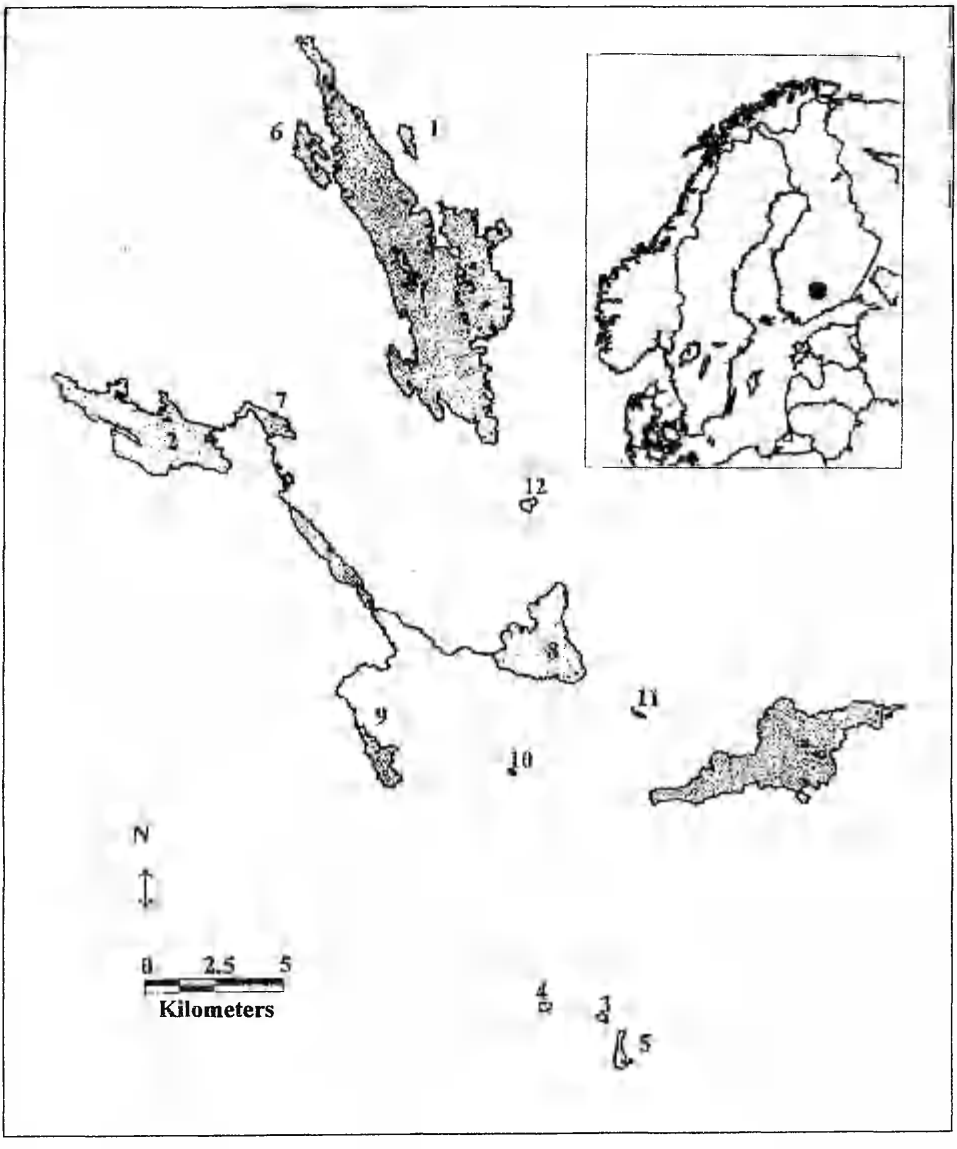

Figure 1. The study lakes and their location in southern Finland. Numbers equal the order of lakes in Table I.

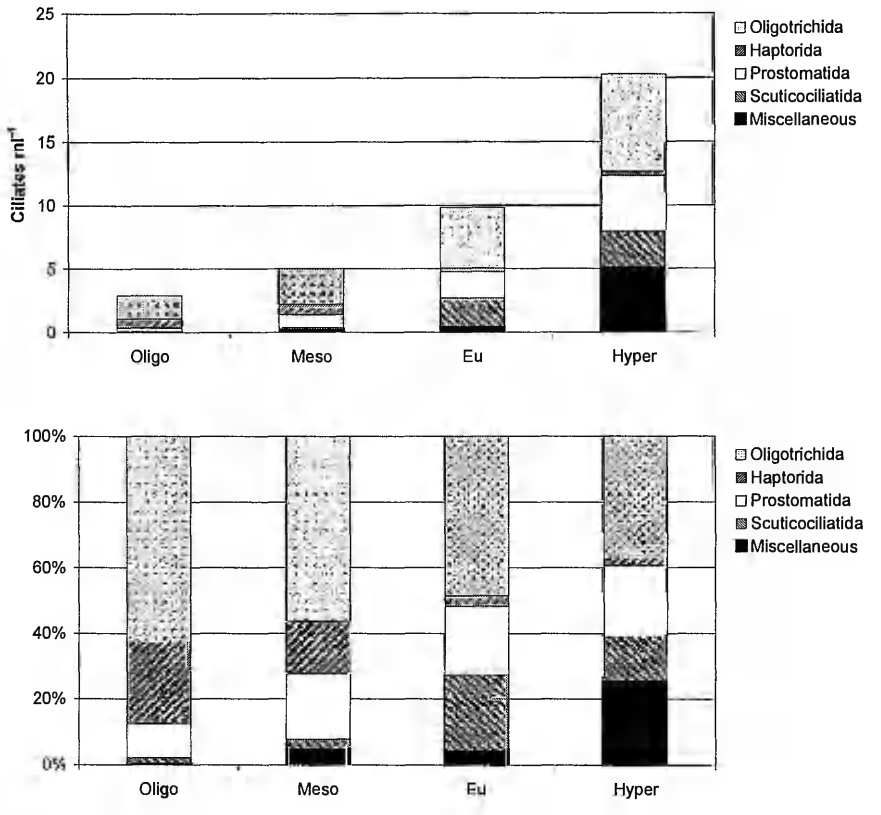

Figure 2. Relative importance of different groups of ciliates in study lakes grouped according to trophic state.

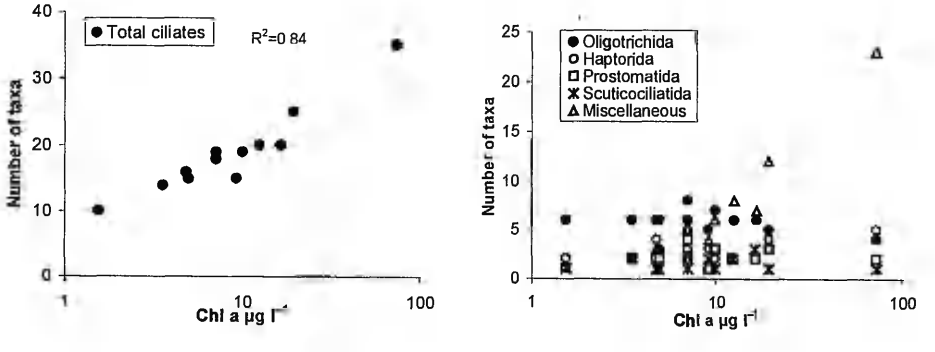

Figure 3. Relationship between number of ciliate taxa and mean annual chlorophyll *a* concentration in study lakes.

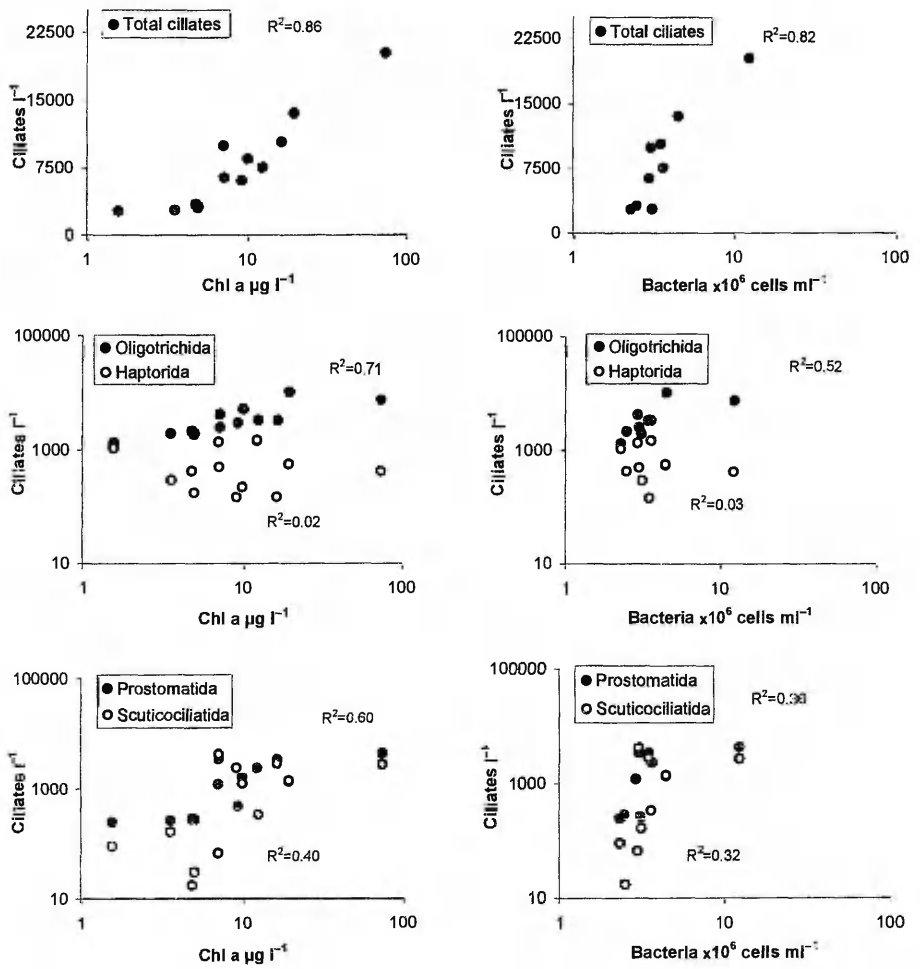

Figure 4. Relationship between ciliate number and mean annual chlorophyll *a* concentration and bacterial abundance in study lakes.

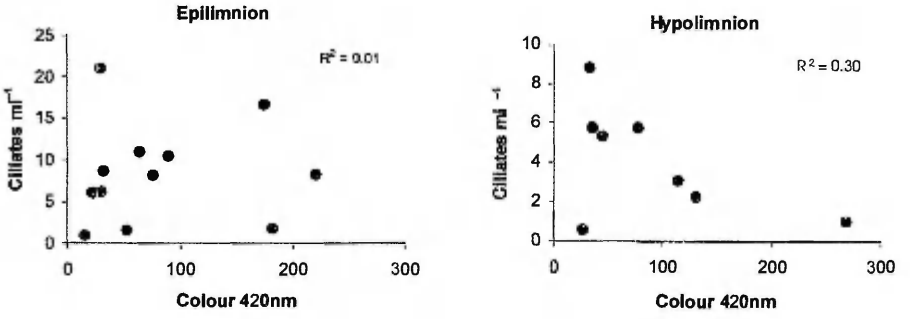

Figure 5. Relationship between ciliate abundance and lake colour in studied lakes epi- and hypolimnion in summer.

CURRICULUM VITAE

General facts

Name: Priit Zingel
Date and place of birth: February 7, 1972, in Tallinn (Estonia)
Nationality: Estonian
Current employment: doctoral student at the Institute of Zoology and Hydrobiology, University of Tartu; stipendiary at the Võrtsjärv Limnological Station of the Institute of Zoology and Botany
Marital status: married
Present address: Marta 3–13, Tartu 50103, Estonia; phone: +37 27 343550;
e-mail: pzingel@zbi.ee

Education:

1979–1987, Tallinn Elementary School No. 27
1987–1990, Tallinn Secondary School No. 3
1990–1995, biology studies at the faculty of Biology and Geography of University of Tartu (graduated in June, 1995; Diploma work on 'Diurnal vertical migration of planktonic ciliates in Lake Võrtsjärv' (in Estonian))
1995–1997, master studies at the Institute of Zoology and Hydrobiology, University of Tartu, Master of Biology (Hydrobiology) of University of Tartu (graduated in June 1997; Master Thesis on 'Planktonic ciliates in Lake Võrtsjärv: comparison of different methods of collecting and fixing, community structure and seasonal dynamics' (in Estonian))

Professional experience:

In 1994 started field works at Võrtsjärv Limnological Station
Since 1995 in various occupations at Võrtsjärv Limnological Station

Foreign languages:

English, Russian, Finnish

Scientific activity

Fields of research: Protozoology, lake ecology, hydrobiology

Related publications

Nõges P., Haberman J., **Zingel P.**, Tammert H. & Nõges T. (1997). Võrtsjärve seisund madalaveelisel 1996. aastal. In: Frey, T. (ed.) Kaasaegse ökoloogia probleemid. Eesti 7. Ökoloogiakonverentsi lühiartiklid. Tartu, pp. 149–154.

Kisand, V., Nõges, T. & **Zingel, P.** (1998). Diel dynamics of bacterioplancton activity in eutrophic shallow Lake Võrtsjärv, Estonia. Hydrobiologia 380: 93–102.

Sarvala, J., Kankaala, P., **Zingel, P.** & Arvola, L. (1999). Zooplankton. In: Keskitalo, J. & Eloranta, P. (eds.) Limnology of Humic waters. Backhuys Publishers, Leiden, pp. 173–191.

Zingel, P. (2000). Pelagic ciliated protozoa in Lake Peipsi: community composition and seasonal dynamics. Proc. Estonian Acad. Sci. Biol. Ecol. 49(1): 62–70.

Zingel, P. (2001). Planktonic ciliates (Ciliophora) of Lake Peipsi. In: Pihu, E. & Haberman, J. (eds.) Lake Peipsi: flora and fauna. Sulemees, Tartu, pp. 69–73.

Grants:

from CIMO for research in Lammi Biological Station, Finland (1997–1998)

CURRICULUM VITAE

Üldandmed

Nimi: Priit Zingel
Sünnikoht ja -aeg: Tallinnas 7. veebruar 1972
Rahvus: eestlane
Töökoht: Tartu Ülikooli zooloogia ja hüdrobioloogia instituudi doktorant; Zooloogia ja Botaanika Instituudi stipendiaat
Perekonnaseis: abielus
Aadress: Marta 3–13, Tartu 50103; telefon: 07 343550;
e-post: pzingel@zbi.ee

Haridus

1979–1987, Tallinna 27. Algkool
1987–1990, Tallinna 3. Keskkool
1990–1995, Tartu Ülikooli bioloogia-geograafiateaduskonna bioloogia osakonna üliõpilane, diplomitöö "Tsiliaatide ööpäevane vertikaalne migratsioon Võrtsjärves"
1995–1997, Tartu Ülikooli zooloogia ja hüdrobioloogia instituudi magistrant, magistrikraad hüdrobioloogia erialal kaitstud 1997. aasta juunis teemal "Planktilised tsiliaadid Võrtsjärves: erinevate kogumis- ja fikseerimismeetodite võrdlus, koosluse struktuur ja sesoonne dünaamika"

Teenistuskäik

1994. a. esimesed välitööd Võrtsjärve Limnoloogiajaamas
Alates 1995. a. mitmetel ametikohtadel Võrtsjärve Limnoloogiajaamas

Võõrkeeled

Inglise, vene, soome

Teadustöö

Uurimisvaldkonnad: protozooloogia, järvede ökoloogia, hüdrobioloogia

Tööga seotud publikatsioonid

Nõges P., Haberman J., **Zingel P.**, Tammert H. & Nõges T. (1997). Võrtsjärve seisund madalaveelisel 1996. aastal. In: Frey, T. (ed.) Kaasaegse ökoloogia probleemid. Eesti 7. Ökoloogiakonverentsi lühiartiklid. Tartu, pp. 149–154.

Kisand, V., Nõges, T. & **Zingel, P.** (1998). Diel dynamics of bacterioplancton activity in eutrophic shallow Lake Võrtsjärv, Estonia. Hydrobiologia 380: 93–102.

Sarvala, J., Kankaala, P., **Zingel, P.** & Arvola, L. (1999). Zooplankton. In: Keskitalo, J. & Eloranta, P. (eds.) Limnology of Humic waters. Backhuys Publishers, Leiden, pp. 173–191.

Zingel, P. (2000). Pelagic ciliated protozoa in Lake Peipsi: community composition and seasonal dynamics. Proc. Estonian Acad. Sci. Biol. Ecol. 49(1): 62–70.

Zingel, P. (2001). Planktonic ciliates (Ciliophora) of Lake Peipsi. In: Pihu, E. & Haberman, J. (eds.) Lake Peipsi: flora and fauna. Sulemees, Tartu, pp. 69–73.

Toetused

CIMO stipendiumid uurimistööks Lammi bioloogiajaamas, Soomes (1997–1998)

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

1. **Toivo Maimets**. Studies of human oncoprotein p53. Tartu, 1991, 96 p.
2. **Enn K. Seppet**. Thyroid state control over energy metabolism, ion transport and contractile functions in rat heart. Tartu, 1991, 135 p.
3. **Kristjan Zobel**. Epifüütsete makrosamblike väärtus õhu saastuse indikaatoritena Hamar-Dobani boreaalsetes mägimetsades. Tartu, 1992, 131 lk.
4. **Andres Mäe**. Conjugal mobilization of catabolic plasmids by transposable elements in helper plasmids. Tartu, 1992, 91 p.
5. **Maia Kivisaar**. Studies on phenol degradation genes of *Pseudomonas* sp. strain EST 1001. Tartu, 1992, 61 p.
6. **Allan Nurk**. Nucleotide sequences of phenol degradative genes from *Pseudomonas sp.* strain EST 1001 and their transcriptional activation in *Pseudomonas putida.* Tartu, 1992, 72 p.
7. **Ülo Tamm**. The genus *Populus* L. in Estonia: variation of the species biology and introduction. Tartu, 1993, 91 p.
8. **Jaanus Remme**. Studies on the peptidyltransferase centre of the *E.coli* ribosome. Tartu, 1993, 68 p.
9. **Ülo Langel**. Galanin and galanin antagonists. Tartu, 1993, 97 p.
10. **Arvo Käärd**. The development of an automatic online dynamic fluorescense-based pH-dependent fiber optic penicillin flowthrought biosensor for the control of the benzylpenicillin hydrolysis. Tartu, 1993, 117 p.
11. **Lilian Järvekülg**. Antigenic analysis and development of sensitive immunoassay for potato viruses. Tartu, 1993, 147 p.
12. **Jaak Palumets**. Analysis of phytomass partition in Norway spruce. Tartu, 1993, 47 p.
13. **Arne Sellin**. Variation in hydraulic architecture of *Picea abies* (L.) Karst. trees grown under different enviromental conditions. Tartu, 1994, 119 p.
13. **Mati Reeben**. Regulation of light neurofilament gene expression. Tartu, 1994, 108 p.
14. **Urmas Tartes**. Respiration rhytms in insects. Tartu, 1995, 109 p.
15. **Ülo Puurand**. The complete nucleotide sequence and infections *in vitro* transcripts from cloned cDNA of a potato A potyvirus. Tartu, 1995, 96 p.
16. **Peeter Hõrak**. Pathways of selection in avian reproduction: a functional framework and its application in the population study of the great tit (*Parus major*). Tartu, 1995, 118 p.
17. **Erkki Truve**. Studies on specific and broad spectrum virus resistance in transgenic plants. Tartu, 1996, 158 p.
18. **Illar Pata**. Cloning and characterization of human and mouse ribosomal protein S6-encoding genes. Tartu, 1996, 60 p.

19. **Ülo Niinemets**. Importance of structural features of leaves and canopy in determining species shade-tolerance in temperature deciduous woody taxa. Tartu, 1996, 150 p.
20. **Ants Kurg**. Bovine leukemia virus: molecular studies on the packaging region and DNA diagnostics in cattle. Tartu, 1996, 104 p.
21. **Ene Ustav**. E2 as the modulator of the BPV1 DNA replication. Tartu, 1996, 100 p.
22. **Aksel Soosaar**. Role of helix-loop-helix and nuclear hormone receptor transcription factors in neurogenesis. Tartu, 1996, 109 p.
23. **Maido Remm**. Human papillomavirus type 18: replication, transformation and gene expression. Tartu, 1997, 117 p.
24. **Tiiu Kull**. Population dynamics in *Cypripedium calceolus* L. Tartu, 1997, 124 p.
25. **Kalle Olli**. Evolutionary life-strategies of autotrophic planktonic microorganisms in the Baltic Sea. Tartu, 1997, 180 p.
26. **Meelis Pärtel**. Species diversity and community dynamics in calcareous grassland communities in Western Estonia. Tartu, 1997, 124 p.
27. **Malle Leht**. The Genus *Potentilla* L. in Estonia, Latvia and Lithuania: distribution, morphology and taxonomy. Tartu, 1997, 186 p.
28. **Tanel Tenson**. Ribosomes, peptides and antibiotic resistance. Tartu, 1997, 80 p.
29. **Arvo Tuvikene**. Assessment of inland water pollution using biomarker responses in fish *in vivo* and *in vitro*. Tartu, 1997, 160 p.
30. **Urmas Saarma**. Tuning ribosomal elongation cycle by mutagenesis of 23S rRNA. Tartu, 1997, 134 p.
31. **Henn Ojaveer**. Composition and dynamics of fish stocks in the gulf of Riga ecosystem. Tartu, 1997, 138 p.
32. **Lembi Lõugas**. Post-glacial development of vertebrate fauna in Estonian water bodies. Tartu, 1997, 138 p.
33. **Margus Pooga**. Cell penetrating peptide, transportan, and its predecessors, galanin-based chimeric peptides. Tartu, 1998, 110 p.
34. **Andres Saag**. Evolutionary relationships in some cetrarioid genera (Lichenized Ascomycota). Tartu, 1998, 196 p.
35. **Aivar Liiv**. Ribosomal large subunit assembly *in vivo*. Tartu, 1998, 158 p.
36. **Tatjana Oja**. Isoenzyme diversity and phylogenetic affinities among the eurasian annual bromes (*Bromus* L., Poaceae). Tartu, 1998, 92 p.
37. **Mari Moora**. The influence of arbuscular mycorrhizal (AM) symbiosis on the competition and coexistence of calcareous crassland plant species. Tartu, 1998, 78 p.
38. **Olavi Kurina**. Fungus gnats in Estonia (*Diptera: Bolitophilidae, Keroplatidae, Macroceridae, Ditomyiidae, Diadocidiidae, Mycetophilidae*). Tartu, 1998, 200 p.
39. **Andrus Tasa**. Biological leaching of shales: black shale and oil shale. Tartu, 1998, 98 p.

40. **Arnold Kristjuhan.** Studies on transcriptional activator properties of tumor suppressor protein p53. Tartu, 1998, 86 p.
41. **Sulev Ingerpuu.** Characterization of some human myeloid cell surface and nuclear differentiation antigens. Tartu, 1998, 163 p.
42. **Veljo Kisand.** Responses of planktonic bacteria to the abiotic and biotic factors in the shallow lake Võrtsjärv. Tartu, 1998, 118 p.
43. **Kadri Põldmaa.** Studies in the systematics of hypomyces and allied genera (Hypocreales, Ascomycota). Tartu, 1998, 178 p.
44. **Markus Vetemaa.** Reproduction parameters of fish as indicators in environmental monitoring. Tartu, 1998, 117 p.
45. **Heli Talvik.** Prepatent periods and species composition of different *Oesophagostomum* spp. populations in Estonia and Denmark. Tartu, 1998, 104 p.
46. **Katrin Heinsoo.** Cuticular and stomatal antechamber conductance to water vapour diffusion in *Picea abies* (L.) karst. Tartu, 1999, 133 p.
47. **Tarmo Annilo.** Studies on mammalian ribosomal protein S7. Tartu, 1998, 77 p.
48. **Indrek Ots.** Health state indicies of reproducing great tits (*Parus major*): sources of variation and connections with life-history traits. Tartu, 1999, 117 p.
49. **Juan Jose Cantero.** Plant community diversity and habitat relationships in central Argentina grasslands. Tartu, 1999, 161 p.
50. **Rein Kalamees.** Seed bank, seed rain and community regeneration in Estonian calcareous grasslands. Tartu, 1999, 107 p.
51. **Sulev Kõks.** Cholecystokinin (CCK) — induced anxiety in rats: influence of environmental stimuli and involvement of endopioid mechanisms and erotonin. Tartu, 1999, 123 p.
52. **Ebe Sild.** Impact of increasing concentrations of O_3 and CO_2 on wheat, clover and pasture. Tartu, 1999, 123 p.
53. **Ljudmilla Timofejeva.** Electron microscopical analysis of the synaptonemal complex formation in cereals. Tartu, 1999, 99 p.
54. **Andres Valkna.** Interactions of galanin receptor with ligands and G-proteins: studies with synthetic peptides. Tartu, 1999, 103 p.
55. **Taavi Virro.** Life cycles of planktonic rotifers in lake Peipsi. Tartu, 1999, 101 p.
56. **Ana Rebane.** Mammalian ribosomal protein S3a genes and intron-encoded small nucleolar RNAs U73 and U82. Tartu, 1999, 85 p.
57. **Tiina Tamm.** Cocksfoot mottle virus: the genome organisation and translational strategies. Tartu, 2000, 101 p.
58. **Reet Kurg.** Structure-function relationship of the bovine papilloma virus E2 protein. Tartu, 2000, 89 p.
59. **Toomas Kivisild.** The origins of Southern and Western Eurasian populations: an mtDNA study. Tartu, 2000, 121 p.

60. **Niilo Kaldalu.** Studies of the TOL plasmid transcription factor XylS. Tartu 2000. 88 p.
61. **Dina Lepik.** Modulation of viral DNA replication by tumor suppressor protein p53. Tartu 2000. 106 p.
62. **Kai Vellak.** Influence of different factors on the diversity of the bryophyte vegetation in forest and wooded meadow communities. Tartu 2000. 122 p.
63. **Jonne Kotta.** Impact of eutrophication and biological invasionas on the structure and functions of benthic macrofauna. Tartu 2000. 160 p.
64. **Georg Martin.** Phytobenthic communities of the Gulf of Riga and the inner sea the West-Estonian archipelago. Tartu, 2000. 139 p.
65. **Silvia Sepp.** Morphological and genetical variation of *Alchemilla L.* in Estonia. Tartu, 2000. 124 p.
66. **Jaan Liira.** On the determinants of structure and diversity in herbaceous plant communities. Tartu, 2000. 96 p.

ISSN 1024–6479
ISBN 9985–56–565–7