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Structure and seasonal dynamics of the protozoan community (heterotrophic flagellates, ciliates, amoeboid protozoa) in the plankton of a large river (River Danube, Hungary)

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This paper is dedicated to the occasion of the 70th birthday of Dr. Magdolna Cs. Bereczky, researcher on ciliates in the Danube

Abstract

Seasonal dynamics of all major protozoan groups were investigated in the plankton of the River Danube, upstream of Budapest (Hungary), by bi-weekly sampling over a 1-year long period. Sixty-one heterotrophic flagellate, 14 naked amoeba, 50 testate amoeba, 4 heliozoan and 83 ciliate morphospecies were identified. The estimated abundance ranges of major groups throughout the year were as follows: heterotrophic flagellates, $0.27–7.8 \times 10^6$ ind. l^{-1}; naked amoebae, max. 3300 ind. l^{-1}; testaceans, max. 1600 ind. l^{-1}; heliozoans, max. 8500 ind. l^{-1}; ciliates, 132–34,000 ind. l^{-1}. In terms of biovolume, heterotrophic flagellates dominated throughout the year (max. 0.58 $mm^3 l^{-1}$), and ciliates only exceeded their biovolume in summer (max. 0.76 $mm^3 l^{-1}$). Naked amoeba and heliozoan biovolume was about one, and testacean biovolume 1–3, orders of magnitude lower than that of ciliates. In winter, flagellates, mainly chrysomonads, had the highest biomass, whilst ciliates were dominated by peritrichs. In 2005 from April to July a long spring/summer peak occurred for all protozoan groups. Beside chrysomonads typical flagellates were choanoflagellates, bicosoecids and abundant microflagellates (large chrysomonads and *Collodictyon*). Most abundant ciliates were oligotrichs, while *Phascolodon*, *Urotricha*, *Vorticella*, haptorids, Suctoria, *Climacostomum* and *Stokesia* also contributed significantly to biovolume during rapid succession processes. In October and November a second high protozoan peak occurred, with flagellate dominance, and slightly different taxonomic composition.

Keywords: Heterotrophic flagellates; Naked amoebae; Testate amoebae; Ciliates; Seasonal succession; River protozoa

Introduction

Heterotrophic protists (protozoa) play an important role in matter and energy flow in most aquatic ecosystems. Their potential function in classical herbivore food webs has been known for a long time, while understanding of their essential role in the microbial loop is more recent (Azam et al. 1983; Pomeroy 1974). Since the recognition of the microbial loop, system model investigations encouraged quantitative studies of microbial loop components, especially heterotrophic

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nanoflagellates (Berninger et al. 1991; Gasol and Vaque 1993) and ciliates (Beaver and Crisman 1989). However, most quantitative surveys only took these two protozoan groups into account (Sanders et al. 1989; Sommargua and Psenner 1995), while other groups were neglected. There have been relatively few investigations which provided quantitative data from every major protozoan group (Garstecki et al. 2000; Kopylov et al. 2002; Mathes and Arndt 1995), thereby allowing direct comparison of the potential role of different groups. Most of the quantitative data from the 1980s and early 1990s lack details of taxonomic composition, especially on heterotrophic flagellates. Since then it has become known that protozoan functional diversity, trophic relations and niches in aquatic habitats are related to different taxonomic levels: from higher taxonomic groups to morphospecies (Arndt et al. 2000), and in some cases to cryptic species (Scheckenbach et al. 2006) and ecotype (Weisse and Montagnes 1998) levels. Thus, a community ecology with a fine taxonomic resolution is sorely needed in protistan ecology to overcome problems that are untreatable by using big black boxes, such as 'bacteria', 'flagellates' and 'ciliates' in ecosystem models. On the other hand, every quantitative study has to make compromises in taxonomic resolution, since it is not practicable to identify as many species as a taxonomic/faunistic survey using enrichment cultures. There are some methodological problems too: fixed-sample direct counting, live counting and MPN-based techniques have different efficiencies in quantitativity and identification possibilities for different protozoan groups (Arndt et al. 2000; Smirnov and Brown 2004).

Planktonic protozoan communities in rivers have received less study than those in lakes and oceans, and there is little information on community structure and the relative quantitative importance of different protozoan groups. It is also difficult to decide what components of the protistan assemblage that can be captured by sampling the water column are euplanktonic, since, at least in smaller rivers, a majority of the protists carried in the water flow have benthic origins. Heterotrophic flagellates (HF) may reach a high abundance in rivers (Basu and Pick 1997; Carlough and Meyer 1989; Jochem 2003; Lair et al. 1999; Sorokin 1990), and a few investigators have given both quantitative data and species composition (Kopylov et al. 2006; Kosolapova 2007). A modern conceptual work revealed the annual quantitative changes of higher taxa in the River Rhine (Weitere and Arndt 2003). Occurrence data on many species are available for the River Volga (Zhukov et al. 1998) and the River Tisza (Danube tributary, e.g. Hamar 1979), but without any quantitative indications. Regarding ciliates, a considerable number of investigations have been performed (Carlough and Meyer 1989; Sorokin 1990), and in many instances community structure was also analysed (Lair et al. 1998; Madoni and Bassanini 1999; Mordukhai-Boltovskoi 1979; Primc-Habdija et al. 1996; Tirjakova 2003). Furthermore, Foissner et al. (1999) list many other papers on ciliates in riverine plankton, most of them containing faunistic and quantitative data. There are several reports about testate amoebae in riverine plankton, most of them containing quantitative data (Bini et al. 2003; Bonecker et al. 1996; Green 1963; Velho et al. 1999). Gál (1966) also provided some semiquantitative data on testate and naked amoebae in the plankton of the River Tisza. In many of the mentioned investigations on testate amoebae, samples were collected with large mesh size plankton nets (up to 70 μm), and the zooplankton community was monitored in parallel in the same samples, indicating a selection for larger testacean forms. Planktonic naked amoebae are still a very neglected protist group, although evidence is increasing about their potential role in planktonic habitats (Murzov and Caron 1996; Rogerson and Gwaltney 2000; Rogerson et al. 2003). There are detailed data on abundance and dynamics of amoebae in estuarine waters (Anderson 2007; Rogerson and Laybourn-Parry 1992; Zimmermann-Timm et al. 1998), while in rivers, data are scarce (plankton and benthos: Ettinger et al. 2003; only benthos: Mrva 2003; periphyton: Baldock et al. 1983). Similarly, there is very little quantitative information on the role of heliozoa: most investigations focus on lakes (Bell and Weithoff 2003; Bell et al. 2006; Biyu 2000; Packroff 2000; Zimmermann et al. 1996), but a few indications on river benthos are in Aguilera et al. (2007).

Protozoological investigations in the River Danube have been made by a number of authors, although there is no detailed comprehensive picture on all constituent groups. Heterotrophic flagellate data comprise nanoflagellate counts (Hoch et al. 1995; Kasimir 1992; Vörös et al. 2000). In contrast, ciliates have been well investigated. In addition to the data on the Slovakian (Matis and Tirjaková 1995), Yugoslavian (Pujin 1994) and Bulgarian (Naidenow 1962) sections, Bereczky has contributed altogether 28 publications on planktonic ciliates in the Hungarian section of the River Danube. Few investigations have been made on testate amoebae in the Danube plankton (Slovakia: Ertl 1954; Hungary: Bereczky 1978, 1979). Sporadic species records without quantitative data are available on naked amoebae and heliozoa, except for the limited abundance data on naked amoebae by Bereczky (1978).

Since quantitative data for different protozoan groups in large rivers are either lacking or very limited, there is now an urgent need for investigations with high taxonomic resolution in protozoan ecology. This work is aimed to obtain a comprehensive overview of the protozoan community in the plankton of the River Danube during a 1-year long period. Major aims

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are: (1) to obtain a comprehensive picture of the absolute and relative numbers and biomass of all protozoan major groups in the plankton during the seasonal succession; (2) to identify the most abundant species (mainly flagellates and ciliates) during seasonal changes to a reasonable taxonomic level by light microscopy; (3) to ascertain the species assemblages present during seasonal succession and search for long-term changes of some protozoa groups in the River Danube by comparison with earlier data.

Material and methods

Samples were taken at Göd, upstream from Budapest (river km 1668, Hungary), from the east bank of the river, at a point where the water depth was 1.5 m and the current averaged between 1 and 1.5 m s^{-1}. Records of the water temperature and the water discharge at Vác at the time of sampling are given in Fig. 1a. Water discharge data were recorded by the Middle-Danube-Valley Environmental Directorate. Sampling was carried out every 2 weeks from 17 November 2004 to 3 November 2005, so that altogether 26 samples were taken (three of which were in each of the calendar months of December and June). Water samples were collected in plastic vessels from about 10 cm under the water surface, and were assumed to be representative for the whole mixed water column.

Protozoa were sampled in two major size fractions. For cells up to 20 μm in diameter (mainly nanoflagellates) one composite dipped sample was taken on each

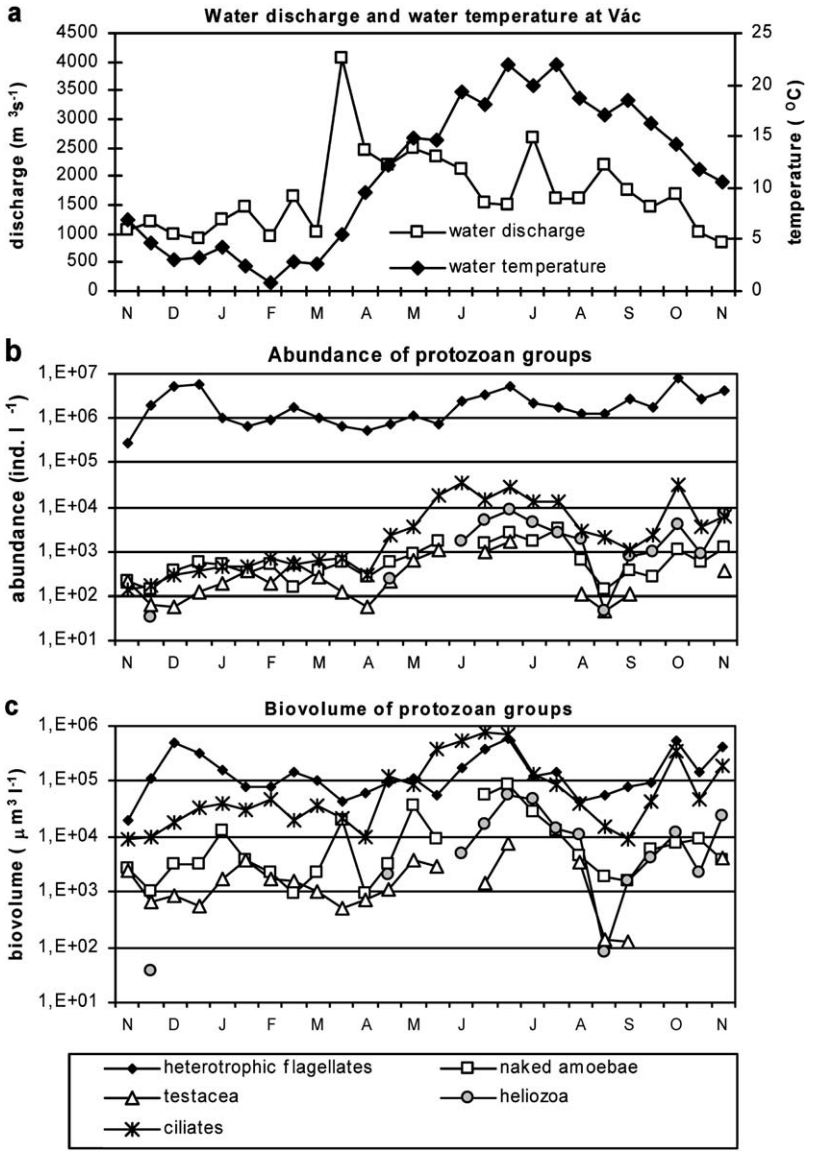

Fig. 1. Seasonal changes in (a) temperature and water discharge of the River Danube at Vác, (b) abundance, and (c) biovolume of the main protozoan groups on logarithmic scales.

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sampling visit; this was made by mixing three dipped samples of 50 ml each, taken from the river at 1 min intervals, with the result that the subsamples were 60–100 m apart along the longitudinal river profile. Organisms were killed by pouring the composite sample into saturated aqueous mercuric chloride (fixative to sample ratio = 1/10). Counting and possible identification were done by the Utermöhl technique with an Olympus IX-70 inverted microscope, with 60× HI objective, using DIC optics. Twenty milliliters of the fully mixed sample was settled in the chamber for 1 day; 50 specimens per sample were counted, and the screened area was measured.

For larger protozoans (>20 μm), a net sample was taken on each sampling visit, 4–8 l of water (depending on seston content) being filtered through a 10 μm mesh size net. Aliquots of 1–2 l water were taken from the river by a plastic jug, and 3–4 of these aliquots, collected at 1 min intervals, were passed through the net. The retained concentrated plankton was killed immediately with mercuric chloride. A subsample corresponding to 100 ml–2 l of the original river water was settled in one chamber, depending on seston content. Counting and identification were done by the same technique, using a 20× objective for searching, and higher magnifications for specimen identification. When it was necessary, a specimen was picked out with a micropipette to a coverslip, turned to the appropriate side with a manipulator and investigated with a 60× HI objective (this treatment was useful for ciliates and testate amoebae). One hundred specimens were counted per sample, and the screened area recorded. In addition, the whole chamber was scanned under a 4× objective looking for big and rare ciliates and testaceans, with knowledge of the screened area.

No replicates were done on the same sampling date, because of the time-consuming analysis and identification procedures. Thus, our quantitative data probably have higher error than those studies counting higher numbers of cells and using replicates, but this hardly affects the observed trends and deduced conclusions. Modular organisms (e.g. *Codonosiga botrytis*) were counted cell by cell. Nano-sized *Spumella* and *Paraphysomonas* taxa were not separated in fixed samples, but referred to as '*Spumella*-like' specimens. Only living specimens were included in quantitative estimations, not empty tests. Biovolume (biomass) was calculated from individual measurements of cell sizes of every specimen, using simple geometric equations. Cell volume shrinkage was assumed to be 70%. In addition to fixed samples, living samples were also taken (both dipped and filtered) and investigated immediately in the laboratory, using 4–100× HI objectives. Satisfactory identification is much improved by combining observation of live and fixed material, and the community structure is revealed by a deeper taxonomic resolution. For the identification of the different protozoan groups more than a hundred papers were used, including many original species descriptions (not listed here). In the taxon list, the sp. notation stands for those taxa that did not fit any of the current species descriptions. Unidentified species were not listed, except for those genera where identification was possible merely to generic level. Species classification within major groups is according to Adl et al. (2005). A short critique on methodology is given in Discussion.

Results

Altogether 249 protozoan taxa were found during the investigation period, 212 of them identified to at least species level (Table 1). Subtracting the three subspecies found, the 246 taxa comprised at least the same number of morphospecies. According to major protozoan groups, 70 heterotrophic flagellate taxa (61 named species), 14 naked amoeba morphotypes, 62 testacean taxa (50 species), 7 heliozoan taxa (4 species) and 94 ciliate taxa (83 species) were found.

When comparing seasonal abundance changes of the major groups (Fig. 1b), heterotrophic flagellates were the most abundant. In wintertime they represented three, and from April about two, orders of magnitude higher abundance than the second most abundant group, the ciliates. Naked amoebae were the third most abundant group, in wintertime approximating, and sometimes exceeding, ciliate abundance; from April being one order of magnitude lower than ciliates. Heliozoa, occurring from April, yielded about half an order of magnitude lower abundance than ciliates. Testaceans were the least abundant, in winter about half an order of magnitude lower, in summer-autumn 1.5–2 orders of magnitude lower compared to ciliates. According to biomass (Fig. 1c), heterotrophic flagellates also dominated throughout the year, ciliates only exceeded their biomass from late May to early July. The biomass of naked amoebae and heliozoa was usually about one order of magnitude lower than ciliate biomass. Testacean biomass was the lowest, 1–3 orders of magnitude lower than that of ciliates. Seasonal dynamics of ciliates and amoeboid organisms followed each other well, while flagellate abundance, and to a lesser extent biomass, showed a different pattern, particularly in winter and spring. Details of the abundance of the more prominent taxa in all samples are shown in a series of histograms as Fig. 5 in the Appendix to the online version of this paper.

Temporal dynamics and taxonomic composition of heterotrophic flagellates

The seasonal dynamics of heterotrophic flagellate abundance and biomass were tightly coupled (Fig. 2a),

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with three salient peaks. Two were more extended in December and June, while a sudden double peak occurred in October and November 2005. Maximal abundance was in early October, with 7.8×10^6 ind. l^{-1}, maximal biovolume in late June reached 0.58 $mm^3 l^{-1}$. Nanoflagellates (<15 μm length) completely dominated numerically with a mean yearly contribution of 99.7% to the total flagellate abundance, microflagellates (>15 μm length) had only a maximal 1.4% contribution. Despite this, microflagellates compose an important fraction of the total flagellate biomass in June and July, and from September to November, reaching maximally 53% in mid-June. The taxonomic contributions of microflagellate groups are shown in the Appendix of the online version as Fig. 7.

Although diverse taxonomic groups were found (Table 1), relatively few taxa contributed remarkably either to abundance (Fig. 2b) or to biomass (Fig. 2c); slightly more detailed versions of these figures are given in Fig. 6a and b in the Appendix of the online version. Most important of these were the chrysomonads, constituting an average yearly 63% (26–88%) of flagellate abundance. *Spumella*-like unicells are numerous during the whole year (Appendix Fig. 5d), with two high peaks in late December (4.7×10^6 ind. l^{-1})

Table 1. List of taxa found in this investigation.

HETEROTROPHIC FLAGELLATES			
Allantion tachyploon **nD**	*Cryptomonas* sp. (*Chilomonas*)	*Ministeria vibrans* **nf, nr, nD**	*Polytoma uvella*
Amastigomonas debruynei **nD, c**	*Desmarella moniliformis*	*Monosiga* cf. *ovata* **nD**	*Protaspis gemmifera* **nf, nr, nD**
Amastigomonas mutabilis **nr, nD, c**	*Diploeca flava*	*Monosiga* cf. *varians* **nD**	*Protaspis simplex* **nD**
Ancyromonas sigmoides **nD, c**	*Diplophrys archerii* **nD**	*Neobodo curvifilus* **nD**	*Pteridomonas pulex* **nD, c**
Anisonema acinus	*Goniomonas truncata*	*Neobodo designis* **nD, c**	*Quadricilia rotundata* **nD**
Aulomonas purdyi **nD**	*Gymnodinium lantzschii* **nr, nD**	*Neobodo saliens* **nr, nD, c**	*Rhyncomonas nasuta* **nD, c**
Bicosoeca campanulata **nd**	*Heteromita globosa* **nD**	*Notosolenus apocamptus* **nD**	*Salpingoeca amphoridium*
Bicosoeca conica **nD**	*Heteromita minima* **nD**	*Paramastix conifera* **nD**	*Salpingoeca frequentissima*
Bicosoeca crystalline	*Heteromita reniformis* **nD**	*Paraphysomonas* spp.	*Salpingoeca globulosa* **nr, nD**
Bicosoeca lacustris	*Histiona* cf. *aroides* **nD**	*Paraphysomonas vestita*	*Salpingoeca urceolata* **nD**
Bicosoeca mitra **nr, nD**	*Histiona* cf. *velifera* **nD**	*Peranema trichophorum*	*Salpingoeca variabilis* **nD**
Bicosoeca planktonica	*Kahtablepharis* sp. **nD**	*Peridinium* sp. (colourless)	*Spumella* spp.
Bodo saltans **c**	*Kathablepharis ovalis* **nD**	*Petalomonas minuta* **nD, c**	*Stelexomonas dichotoma*
Bodo spora	*Kentrosiga thienemannii* **nD**	*Petalomonas poosilla* **c**	*Telonema subtile* **nr, nD**
Cercomonas spp.	*Kiitoksia kaloista* **nr, nD**	*Ploeotia oblonga* **nr, nD**	*Thaumatomonas* sp. **nD**
Codonosiga bohrytis	*Kiitoksia ystava* **nf, nr, nD**	*Polytoma eupapillatum* **nr, nD**	*Trigonomonas* sp. **nD**
Collodictyon cf. *triciliatum*	*Mastigamoeba* spp.	*Polytoma granuliferum*	
Colpodella angusta **nD**	*Menoidium obtusum* **nD**	*Polytoma oligochromatum* **nr, nD**	
NAKED AMOEBAE			
Cochliopodium spp.	cf. *Hartmanella cantabrigiensis*	*Nuclearia radians*	*Thecamoeba* spp. (rugose)
eruptive morphotype	mayorellian morphotype	*Nuclearia simplex*	*Thecamoeba* spp. (striate)
fan shaped morphotype	monotactic hartmanellid morphotype	cf. *Rhizamoeba*	
flamellian morphotype	*Nuclearia delicatula*	cf. *Saccamoeba* spp.	
TESTACEA			
Allelogromia brunneri	*Centropyxis aculeata*	*Cryptodifflugia oviformis*	*Difflugia linearis*
Arcella arenaria	*Centropyxis aërophila*	*Cryptodifflugia sacculus*	*Difflugia manicata*
Arcella excavata	*Centropyxis cassis*	*Cryptodifflugia* sp.	*Difflugia minuta*
Arcella hemisphaerica	*Centropyxis constricta*	*Cryptodifflugia vogiti*	*Difflugia oviformis*
Arcella hemisphaerica intermedia	*Centropyxis ecornis*	*Cyphoderia ampulla*	*Difflugia penardi*
Arcella rotundata	*Centropyxis gibba*	*Cyphoderia laevis*	*Difflugia pristis*
Arcella rotundata aplanata	*Centropyxis plagiostoma*	*Difflugia elegans*	*Difflugia pulex*
Campascus minutus	*Centropyxis platystoma*	*Difflugia globularis*	*Difflugia* sp.
Centopyxis minuta	*Chlamydophrys* sp.	*Difflugia gramen*	*Euglypha acantophora*
Centropyxiella spp. **nf, nr, nD**	*Cryptodifflugia crenulata*	*Difflugia lanceolata*	*Euglypha laevis*
Heleopera sp.	*Microchlamys/Spumochlamys* sp.	*Plagiopyxis intermedia*	*Pseudodifflugia* cf. *gracilis*
Hyalospenia papilio	*Microcorycia flava*	*Plagiopyxis minuta oblonga*	*Pseudodifflugia* spp.
Hyalosphaenia punctata	*Nebela* sp.	*Plagiopyxis* sp.	*Tracheleuglypha dentata*
Hyalosphenia cuneata	*Paramphitrema lemanense*	*Psammonobiotus* sp.	*Triema enchelys*
Lecythium hyalinum	*Paraquadrula irregularis*	*Pseudodifflugia* cf. *fascicularis*	*Trinema lineare*
Lecythium sp.	*Pareuglypha reticulata*	*Pseudodifflugia* cf. *fulva*	
HELIOZOA			
Acanthocystidae	*Actinosphaerium eichhornii*	*Heterophrys myriopoda*	Raphidiophrydae
Actinophrys sol	*Clathurina elegans*	*Pompholyxophrys* sp.	

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Table 1. (*continued*)

CILIATA

Acineria uncinata
Acineta flava
Acineta tuberosa
Aspidisca cicada **pl**
Aspidisca lynceus
Aspidisca turrita
Belonophrya pelagica **nD, e**
Blepharisma sp.
Caenomorpha medusula **nD**
Campanella umbellaria **nD**
Carchesium polypinum **pl**
Chilodonella uncinata **pl**
Cinetochilum margaritaceum **pl**
Climacostomum virens
Codonella cratera **e**
Coleps elongatus **e**
Coleps hirtus hirtus **e**
Coleps hirtus viridis **e**
Coleps nolandi **nD, e**
Colpidium colpoda **pl**
Colpoda inflata
Ctedoctema acanthocryptum **nD**
Cyclidium glaucoma **pl**
Cyclotrichium viride **nD, e**
Dexiostoma campylum
Didinium nasutum **e**
Dileptus sp.
Epenardia myriophylli
Epistylis plicatilis **pl**
Epistylis spp.
Euplotes sp.
Frontonia angusta
Glaucoma scintillans
Lacrymaria sp.
Lembadion lucens
Leptopharynx costatus
Linostomella vorticella **e**
Litonotus alpestris **nD**
Litonotus mononucleatus
Membranicola tamari **nr, nD, e**
Metacystis exigua **nr, nD**
Metacystis sp.
Monodinium balbianii balbianii **e**
Monodinium chlorigellum **nr, nD, e**
Nassula sp.
Opercularia nutans
Opercularia sp.
Oxytricha sp.
Paradileptus elephantinus **e**
Paramecium bursaria **pl**
Paramecium caudatum **pl**
Paramecium putrinum **pl**
Pelagostrombidium mirabile **e**
Pelagovorticella mayeri **e**
Pelagovorticella natans **e**
Phascolodon vorticella **e**
Phialina pupula **nD**
Pleuronema coronatum
Pseudomicrothorax dubius **nD**
Pseudovorticella moniliata
Rimostrombidium brachykinetum **nD, e**
Rimostrombidium humile **e**
Rimostrombidium hyalinum **nD, e**
Rimostrombidium lacustris **nD, e**
Spirostomum minus
Staurophrya elegans **e**
Stentor igneus
Stentor multiformis
Stentor niger
Stentor roeselii pl
Stokesia vernalis **e**
Strobilidium caudatum **pl**
Stylonychia mytilus complex **pl**
Tintinnidium fluviatile **e**
Tintinnidium pusillum / *Tintinnopsis cylindrata* **e**
Tintinnidium semiciliatum **nD**
Tokophrya infusionum **nD**
Trichodina domerguei
Trichophrya sp.
Trimyema compressum
Trithigmostoma cucullulus **pl**
Uronema nigricans
Urotricha castalia **nr, nD, e**
Urotricha cf. *globosa* **nD, e**
Urotricha farcta **e**
Urotricha furcata **nD, e**
Urotricha matthesi **nD, e**
Urotricha platystoma **nD**
Vorticella aquadulcis complex
Vorticella campanula **pl**
Vorticella convallaria complex **pl**
Vorticella infusionum complex
Vorticella microstoma compex **pl**
Vorticella octava complex
Vorticella picta

If at least one species could be identified in a genus, the sp. notation was used if no current species description fits the investigated specimen. Unidentified specimens were not listed as sp., unless only unidentified specimens were found in a genus. Abbreviations: **nf**: likely first freshwater record, **nr**: likely first record in rivers, **nD**: likely new for Danube, **c**: one of the 20 most common reported flagellates (Arndt et al. 2000), **e**: euplanktonic ciliate, **pl**: ciliate reported at least 15 times from planktonic habitats (Foissner et al. 1999). The authors of the species are listed in the Appendix to the online version of this paper (Appendix Table).

and early October (4.8×10^6 ind.l^{-1}). Large chrysomonads, like *Paraphysomonas vestita* had an ascendant summer peak (66,000 ind.l^{-1} in late July), and smaller autumnal peaks (App. Fig. 5e). Besides *P. vestita*, other large (diam. 20–40 μm) chrysomonads were numerous, including highly vacuolated forms, further investigations will be needed to reveal their taxonomic identity. Kinetoplastids also had a whole year contribution, but were never numerous (mean 3.8% abundance contribution). In contrast, choanoflagellates were represented only from April, with two high peaks, first in late April, caused mainly by *Codonosiga botrytis* (App. Fig. 5a, 0.2×10^6 ind.l^{-1}), then in mid-June, by *Monosiga* spp. (App. Fig. 5b, 0.7×10^6 ind.l^{-1}); the latter with a 35% contribution to abundance. Bicosoecids were represented from May, with their highest peak in June (17%, 0.41×10^6 ind.l^{-1}). The microflagellate *Collodictyon triciliatum* was present from late May, with negligible contribution to abundance (App. Fig. 5f), but reaching a remarkable 15% contribution to biomass in late July. Other microflagellate groups had low biomass contribution, like mastigamoebids (max. 4.7%), euglenids (max. 1.7%), large kathablepharids (max. 1.3%), and dinoflagellates (max. 1%). The nanoflagellate *Paramastix conifera* also had negligible contribution to abundance (App. Fig. 5g), but its biomass was comparable with other microflagellates (0.5% in late May, October and November).

Temporal dynamics and taxonomic composition of amoeboid organisms

Naked amoebae

Temporal abundance (Fig. 3a) and biomass (Fig. 3b) dynamics of naked amoebae were not so tightly coupled. Abundance had two elongated peaks, the first in June and July, with a maximal abundance of 3300 ind.l^{-1} at the end of July, and the second moderate one in October and November. Sudden moderate biomass peaks also occurred in January, March and May. The highest biomass peak was in late June, with a maximal biomass of 0.09 $mm^3 l^{-1}$, while the autumnal peak was flat and insignificant. Regarding size (length) distribution of naked amoebae (Fig. 3c), the majority of amoebae were usually smaller than 30 μm. In the winter period the amoebae were a little larger, and an abundant fraction between 31 and 40 μm was present. In spring, most specimens were smaller than 30 μm. In summer and autumn, size distribution was more random. Large (> 50 μm) specimens occurred sporadically, constituting,

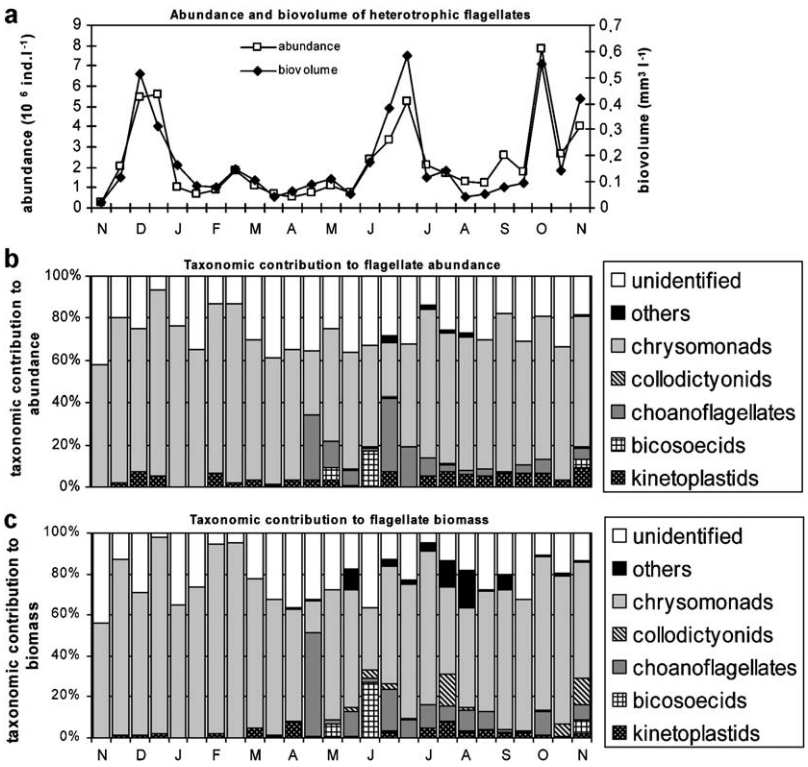

Fig. 2. Seasonal changes in heterotrophic flagellate abundance and biovolume (a), relative contribution of taxonomic groups to total flagellate abundance (b), and total flagellate biomass (c) in each collected sample.

however, a considerable portion in mid- and late July. This corresponded with the highest amoeba biomass peak. *Cochliopodium* spp. occurred in early winter and then in spring (App. Fig. 5h) with an increasing abundance in late spring.

Heliozoa

Appeared markedly only from June and were present during summer and autumn. Abundance and biomass dynamics were similar (Fig. 3a, b). An elongated summer peak from June to August was determinative, with the peak in late June reaching 8500 ind.l^{-1} maximal abundance and 0.057 $mm^3 l^{-1}$ maximal biomass. Lower, but sharp abundance and biomass peaks in early October and November were notable.

Testate amoebae

Annual dynamics of testaceans did not show such pronounced changes as in the other amoeboid organisms; abundance and biomass, however, correlated well. In the winter-spring period their presence was continuous, while from June it became sporadic. However, the highest peak occurred in late July, with 1600 ind.l^{-1} maximal abundance and 0.0076 $mm^3 l^{-1}$ maximal biomass. During the relatively steady winter and spring period, the mean abundance was 300 ind.l^{-1} and the mean biomass was 0.0016 $mm^3 l^{-1}$. *Microchlamys*/*Spumochlamys* sp. occurred in winter and spring, with an increasing abundance from January, culminating in a 300 ind.l^{-1} peak in February (App. Fig. 5i). *Pseudodifflugia* spp. are present in winter and spring, reaching 550 ind.l^{-1} in late May, and probably more in late June (App. Fig. 5j). Maximal abundance values of some other abundant species were: *Arcella rotundata* 16 ind.l^{-1}, *Difflugia* spp. 100 ind.l^{-1}, *Cryptodifflugia* spp. 43 ind.l^{-1}, *Cyphoderia ampulla* 112 ind.l^{-1}, *Euglypha acanthophora* 230 ind.l^{-1} and *Trinema lineare* 230 ind.l^{-1}.

Temporal dynamics and taxonomic composition of ciliates

Temporal dynamics of ciliate abundance and biomass (Fig. 4a) correlated well with each other. The annual values differed in orders of magnitude between the two major periods that were readily distinguishable: winter and early spring to April, and late spring, summer and autumn from April to November. In winter, abundance rose slowly and continuously from early December to early February, then after a drop in March rose again, and fell again during April. The February maximum reached 660 ind.l^{-1} abundance and 0.048 $mm^3 l^{-1}$

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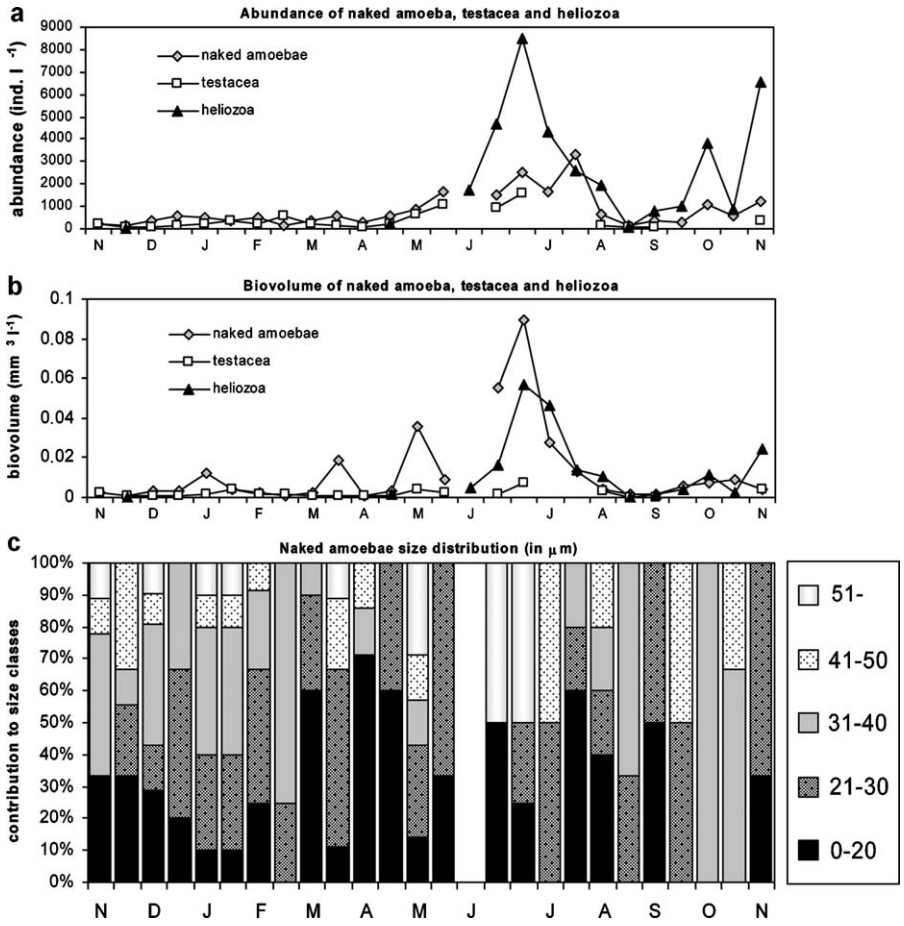

Fig. 3. Seasonal changes in (a) abundance, and (b) biovolume of the three major amoeboid groups; (c) relative contributions of naked amoeba size classes (lengths in μm) to abundance.

biomass. From late April to early August the highest elongated, divided peak occurred, with a maximal abundance of 34,000 ind. l^{-1} in early June and a maximal biomass of 0.76 $mm^3 l^{-1}$ in mid-June; the latter being the highest biomass value among protozoan groups. A sharp high peak followed in early October reaching 31,000 ind. l^{-1} abundance, then a smaller one again in November.

The species were distributed among the major groups as follows: peritrichs 17.7%, peniculids, scuticociliates and hymenostomatids 15.6%, haptorids 13.5%, prostomatids 12.5%, oligotrichs and choreotrichs 11.5%, heterotrichs 8.3%, stichotrichs and hypotrichs 6.3%, suctoria 5.2%.

Taxonomic composition based on abundance and biomass (Fig. 4b, c, with more detailed colour version in Fig. 8 in the online Appendix) showed some clear trends. From November to early April, with decreasing contribution from March, the community was dominated both in abundance and biomass by peritrichs, with a mean contribution to abundance of 59% (37–71%). This community was comprised mainly of *Carchesium polypinum* (App. Fig. 5y), *Pseudovorticella monilata* (App. Fig. 5aa), *Vorticella convallaria* complex (App. Fig. 5ab), *V. octava* complex (App. Fig. 5ad) and *V. picta* (App. Fig. 5ae). Some species, like *Pelagovorticella mayeri* (App. Fig. 5z), *Pseudovorticella monilata* and *V. infusionum* complex (App. Fig. 5ac) have sharp summer peaks of abundance. *V. picta* (App. Fig. 5ae) had a presence throughout the year with two extreme high peaks in early (2600 ind. l^{-1}) and late June (3400 ind. l^{-1}).

Rising from February, choreotrichs became dominant from late April to autumn with an average of 74% (51–90%) relative abundance. *Rimostrombidium* and *Strobilidium* spp. (App. Fig. 5l), *Codonella cratera* (App. Fig. 5n) and small tintinnids (App. Fig. 5q) were present throughout the year having peaks in late May (*Rimostrombidium* + *Strobilidium* 4600 ind. l^{-1}, *Codonella* 330 ind. l^{-1}) or early June (tintinnids 25,000 ind. l^{-1}), and all three also in October. *Tintinnidium fluviatile* (App. Fig. 5p) appeared first in March, but had a similar abundance pattern (late June peak, 2550 ind. l^{-1}). In contrast, *Rimostrombidium lacustris* (App. Fig. 5m,

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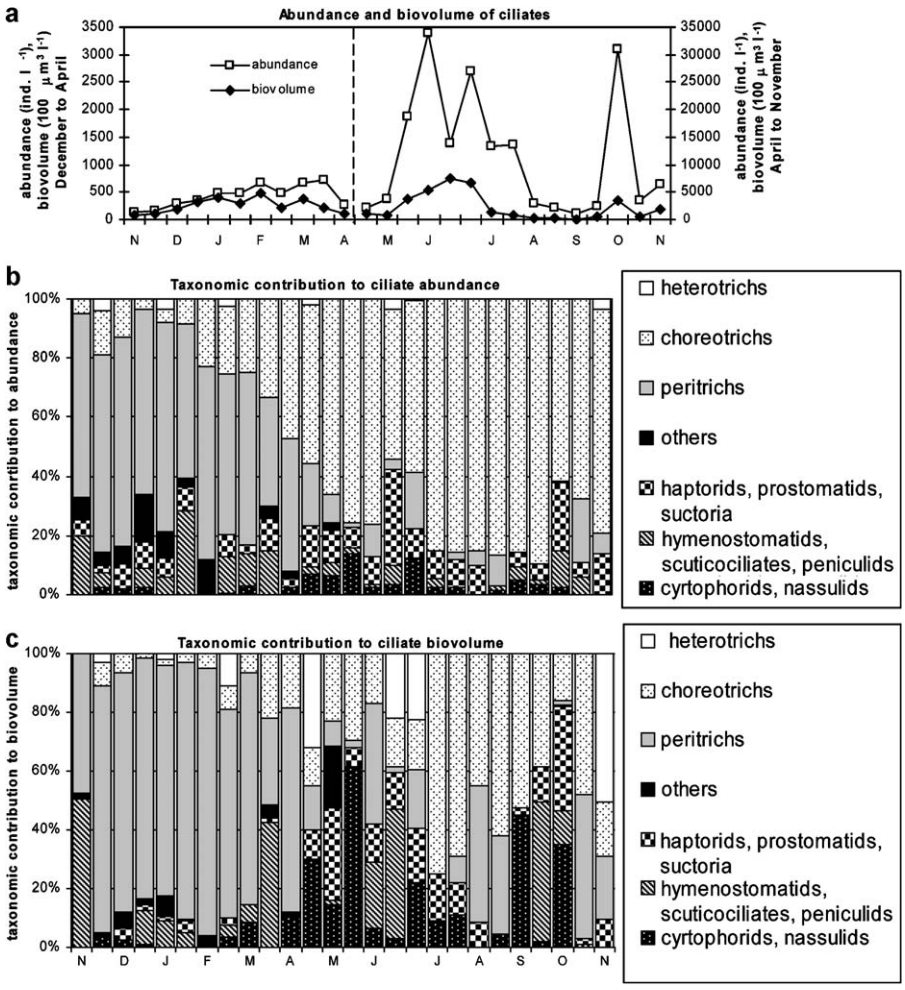

Fig. 4. Seasonal changes in ciliate abundance and biovolume (a), and relative contribution of taxonomic groups to: (b) total ciliate abundance and (c) total ciliate biomass. In (a) the scale for the left part (from November to April) is on the left axis, and right part (from April to November) on the right axis, both of them are in the same units.

middle June peak, 470 ind. l^{-1}), and *Membranicola tamari* (App. Fig. 5o, late July peak, 1300 ind. l^{-1}) had only a restricted short summer appearance.

Examining the maximal contribution to biovolume during the year (see later percentages in brackets), the following groups were also remarkable: *Phascolodon vorticella* (App. Fig. 5s), the most abundant cyrtophorid (max. 61% biomass), had three peaks, first in late May (2600 ind. l^{-1}), then in late June (3400 ind. l^{-1}) and also in early October. Heterotrichs (max. 50%), mainly *Climacostomum virens* and *Linostomella vorticella* had peaks in June and November, 2005. Among the peniculids (max. 48%), the most important species *Stokesia vernalis* had a prominent late June peak (470 ind. l^{-1}). Nassulids (max. 44%) occurred in August and September, though with low abundances. Hymenostomatids (max. 42%) occurred only in wintertime from November to March, with low but increasing number from February (80 ind. l^{-1} in March). Important species were *Colpidium colpoda*, *Dexiostoma campylum* and *Glaucoma scintillans*. The most abundant haptorid (max. 34%) species was *Monodinium chlorigellum* (App. Fig. 5r), that had an increasing abundance from late May to mid-June (max. 950 ind. l^{-1}). Suctoria (App. Fig. 5t, max. 23%) were typical in May and June (max. total abundance 960 ind. l^{-1}). Prostomatids (max. 17%), in contrast to many of the former groups, were present throughout the year; from December to April their total abundance was under 100 ind. l^{-1} (*Coleps* and *Urotricha* spp.), from late April to August an elongated peak occurred reaching a maximal abundance in mid-June (total 2800 ind. l^{-1}), and finally two smaller sharp peaks were seen in early October (750 ind. l^{-1}) and November. Only *Urotricha* spp. were present in summer and autumnal peaks; large *Urotricha* species (>60 µm, App. Fig. 5w) were numerous from late April to early July (470 ind. l^{-1} in mid-June), while *Urotricha* cf. *globosa* appeared later

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(App. Fig. 5u) and was numerous from late May to early August (950 ind. l^{-1} in mid-June). Hypotrichs (max. 3.7%), mainly *Aspidisca cicada* and *A. lynceus*, were present only in winter from December to February, reaching only 80 ind. l^{-1} in February (App. Fig. 5k). Scuticociliates had always a low contribution to biomass (max. 2.9%), though they were present throughout the year; winter and early spring abundances were under 100 ind. l^{-1}, increasing from late April to 470 ind. l^{-1} in June, while there was an extreme high peak in October with 3800 ind. l^{-1}. This pattern was also followed by *Cyclidium* spp. (App. Fig. 5x), which constituted a large fraction of scuticociliates in the growing season. Other groups, like oligotrichs (sensu Adl et al. 2005), stichotrichs, armophorids, colpodids and plagiopylids were never found to be significant either in abundance or in biomass.

Discussion

Methodology

Mercuric chloride causes the least loss of abundance in ciliates, compared to several other fixatives, according to Pace and Orcutt (1981) and Sime-Ngando et al. (1990); disappearance due to the lysis of the cells is negligible. Mercuric chloride seems to cause relatively slight shrinkage in ciliate cell volume after fixation (Sime-Ngando et al. 1992). Few data exist on the effect of mercuric chloride on flagellates, but Rice et al. (1997) reported no lysis and 16% shrinkage for *Paraphysomonas vestita*. The degree of shrinkage is often species specific (e.g. Sime-Ngando et al. 1992, Wiackowski et al. 1994); 70% shrinkage was used here (Carrias et al. 2001). Counting of heterotrophic flagellates was made in Utermöhl chambers, which is an uncommon method for smaller protozoa, although it is a recommended standard method for marine microplankton, including ciliates and dinoflagellates (Burkill et al. 1994). We found that specimens were easily visible down to 1.5 μm, although not as bright as after fluorescent staining. The identification to the lowest taxonomic level is of course not possible in all cases. However, with taxonomic experience and parallel investigation of living material, higher taxonomic groups can be easily recognised, and many typical forms can be identified to morphospecies. Likewise, Brandt and Sleigh (2000) identified flagellates after fluorochrome staining with fairly high success, according to their parallel electron microscopy studies. Without fluorescent staining, the inner structures, especially food vacuoles, are also easily visible. The live counting technique has a better taxonomic resolution, but the smallest specimens are easily overlooked (Arndt et al. 2000).

Rivers are turbid environments with high seston content that makes quantitative investigations troublesome. Large amounts of seston particles render direct counting very difficult, owing to the surface masking of specimens by particles; on the other hand, sufficient dilution of the sample would decrease the efficiency of the quantitative research, because of the rarity of specimens.

We used a 10 μm mesh size plankton net to get rid of numerous mineral particles, when investigating protozoa above 20 μm size. Unquestionably, some specimens larger than 20 μm escape through the 10 μm net, and in spite of gentle washing a few organisms remain attached on the net surface, causing some loss. Scherwass et al. (2002) improved density gradient centrifugation combined with quantitative protargol staining (QPS) for riverine ciliate investigations, and compared their results to those obtained by net hauls. If we average the abundances resulting from the two methods (excluding one data point, where QPS gave 20 times higher abundance than net haul, which seems to be unlikely), centrifugation combined with QPS yielded 1.35 times more cells than the net hauls. In our investigation we made a comparison between slightly concentrated dipped samples and net hauls involving three replicates at a period of low seston content, and found 1.3 times higher abundances in dipped samples. The latter conversion factor was used to refine quantitative data. Using a plankton net seemed to be a reasonable compromise, because of its simplicity and applicability for all larger protozoan groups.

The volumes of samples analysed and the numbers of cells counted per sample are less than desirable. The time taken to analyse samples and identify and measure specimens limited these volumes and numbers. Although it was a compromise decision, it was thought that the analysis of smaller samples at 2-week intervals would be more representative of the annual cycle than larger samples at longer intervals.

Species composition

In order to compare the species diversity in our investigation with that obtained by other researches, we collected many protozoa species records from published riverine studies. We included species records from 36 papers on heterotrophic flagellates and 52 papers on ciliates from around 20 rivers in Europe, Asia, the USA and Australia. Most records involve planktonic forms, but some benthic records are inseparable. There are extensive records from rivers, which list 378 named heterotrophic flagellate species and 653 ciliate species, totals which reduce to 332 and 598, respectively, after removal of synonyms and doubtful records (see Foissner et al. 1999). Valid species include 57 flagellates from the River Danube, 137 from the River Tisza and 169 from the River Volga. Similarly, 292 ciliates have been

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recorded from the River Danube, at least 173 from the River Rhine and 261 from the River Volga.

In this survey three flagellate species (*Kiitoksia ystava*, *Ministeria vibrans* and *Protaspis gemmifera*) are very likely the first records from freshwater, 13 species are new for rivers, and 42 are new for the River Danube (see Table 1). The high proportion of new species for the Danube (68% of species found here) and for investigated rivers (21% of the species) probably indicates the relative lack of modern species level resolution studies in riverine protozooplankton research. This is supported by the following two facts: (1) 18% of species found here were first described in the last two decades; (2) from the 20 most common HF species reported worldwide (Patterson and Lee 2000), 10 were found by us in the Danube (Table 1), and a further 19 species are rather common.

In contrast, some species have been rarely reported (e.g. *Heteromita minima, Kiitoksia ystava, Kiitoksia kaloista, Ministeria vibrans, Paramastix conifera, Polytoma granuliferum, Polytoma oligochromatum, Quadricilia rotundata*). Most of these rare species were found sporadically, although *Heteromita minima* was found in several samples and *Paramastix conifera* had an autumnal peak. Moreover, there is a clear indication for the presence of some species new to science in the Danube; these will be described in forthcoming papers. Taking into account that besides many common morphospecies, there are a few rarely recorded species and some likely new undescribed ones, it seems that freshwater, particularly riverine, HF communities may differ significantly from the well-investigated marine ones.

In this survey almost three quarters of the detected ciliate species are very or moderately common. Three species, however, are probably new for rivers (*Membranicola tamari*, *Monodinium chlorigellum*, *Urotricha castalia*) and 22 are new for the Danube (Table 1). All three species mentioned above are recently described. There are also clear indications for the presence of new ciliate species in the Danube (the 30 μm long truncated *Metacystis* sp.1 has 4–5 body rings, two anterior macronuclear nodules and an open hemispherical lorica, and a large oligotrichid will be described in a forthcoming paper).

The considerably high number of species new to the Danube (27% of species found here are new), is more or less identical with the remaining one quarter of uncommon species. The ciliate fauna of the Danube has been extensively studied both by Tirjakova and Matis, and especially by Bereczky, working with silver impregnation techniques; the last author screened thousands of samples during three decades, since 1969. From the 22 new Danubian species only 7 were described in the last two decades, while 6 others are infrequent in plankton (see Table 1). Some of these ciliates were possibly found earlier but grouped into other species (like *Membranicola tamari*, *Rimostrombidium hyalinum*, *Urotricha castalia*, *U. matthesi*). There is, however, a number of species, which are large enough and/or very distinctive (flagship-like) that surely would have been reported earlier, if found (these are: *Campanella umbellaria*, *Cyclotrichium viride*, *Monodinium chlorigellum*, *Rimostrombidium lacustris*). These species were found here in several samples, sometimes being abundant. It is assumed that these four species appeared only recently in the Danube, confirming restricted geographic distribution of freshwater ciliates, and possibly indicating invasions related to human activity, or significant environmental changes in the river. This conclusion is supported by the observation of Bereczky (Bereczky and Nosek 1994) on the first appearance of *Paradileptus conicus* syn. *Paradileptus elephantinus* in the main arm of the Danube in 1994; this species was previously common in the eutrophic side branches, but had never been found in the main arm before. We also observed *P. elephantinus* on several occasions, sometimes being subdominant. As visible from the former comparisons, our knowledge about the diversity and distribution of either heterotrophic flagellates or ciliates is not complete, and high-resolution taxonomic works are needed.

Seasonal dynamics of protozoan groups

Heterotrophic flagellates

When we compare the abundance and biomass ranges of heterotrophic flagellates found in the Danube to other rivers (Table 2), minimal and maximal values fall into the middle range of extremities found elsewhere. The difference between extreme values in the Danube during the annual cycle is 1.4 orders of magnitude in abundance (1.1–2.1 in other rivers) and 1.5 in biovolume (1.6–2.9 in other rivers). These values are moderate and seem to be normal during seasonal succession. Seasonal dynamics of heterotrophic flagellates in rivers are very variable.

When the contribution of higher taxonomic groups is compared to the Rhine, the relative amount and seasonal changes of groups are similar in the two rivers (continuous, high proportion of chrysomonads and low proportion of kinetoplastids, summer and autumnal choanoflagellate and bicosoecid peaks). Euglenids are more frequent in the Rhine. The annual average HF taxonomic composition corresponds well with lakes, and supports the high similarity found in the proportion of large taxonomic groups in freshwater pelagic habitats by Arndt et al. (2000). Microflagellates, which had a summer maximum 53% biomass contribution in the Danube (max. 52% in Rhine), show the important potential role of microflagellates in the flow of pelagic matter.

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Testate amoebae

Species diversity and abundance of testate amoebae in riverine plankton, just like in the plankton of lakes, are generally low (comparision of rivers in Table 2). The communities of investigated rivers are dominated by *Arcella*, *Centropyxis* and *Difflugia* species, the former two being the most numerous, while, in contrast to benthic habitats, *Difflugia* are less frequent. *Cyphoderia ampulla* is also constant and subdominant in the Tisza. Maximal abundances found in this investigation were 1–2 orders of magnitude higher than in previous reports. Living *Arcella*, *Centropyxis* and *Difflugia* specimens were sporadic, and the community was constantly dominated by smaller *Pseudodifflugia*, *Microchlamys*/*Spumochlamys*, *Trinema*, *Lecythium* and *Cryptodifflugia* species. Some rare species were also found, like *Allelogromia brunneri*, *Cryptodifflugia crenulata*, *Cryptodifflugia voigti*, *Paramphitrema lemanense*, *Psammonobiotus* sp. and a *Centropyxiella* species; for the latter genus this is the first freshwater record. This discrepancy may be explained by the fact that previous workers used large (25–68 μm) mesh-sized plankton nets, and because,

Table 2. Comparison of abundance, biomass and number of taxa of major protozoan groups found in this study to literature data from other rivers (in the case of heliozoa to literature data from a mesotrophic lake).

Habitat	Abundance	Biomass	No. of taxa	Reference
Heterotrophic flagellates	**10^6 ind. l^{-1}**	**$mm^3 l^{-1}$**		
River Mississippi	1.4	0.012		Jochem (2003)
River Loire	0.2–2.4	max. 0.23		Lair et al. (1999)
River Yenisei	0.4–4.5	0.003–0.12		Sorokin (1990)
River Rhine	0.1–5.0	0.01–0.4	65	Weitere and Arndt (2003)
River Latka	0.15–6.0	0.036–1.8	29	Kosolapova (2007)
River Danube	0.27–7.8	0.019–0.58	70	This study
Black Creek	0.09–11	0.012–10		Carlough and Meyer (1989)
River Ogechee	0.09–11	0.01–5.0		Carlough and Meyer (1989)
River Danube	2.1–12			Hoch et al. (1995)
31 Canadian rivers	1.2–17			Basu and Pick (1997)
River Selenga and tributaries	0.15–19	0.032–1.0		Kopylov et al. (2006)
River Danube	1–22			Kasimir (1992)
River Danube	3.3–38	0.2-2.8		Vörös et al. (2000)
Testate amoebae	**ind. l^{-1}**	**$mm^3 l^{-1}$**		
River Tisza	0.8–1.2		58	e.g. Gál (1966)
Paraná floodplain rivers	~7		73	Velho et al. (1999)
River Doce			22	Bonecker et al. (1996)
River Danube	max. 26		24	Bereczky (1979)
River Danube	max. 1600	max. 0.0076	62	This study
Naked amoebae	**ind. l^{-1}**	**$mm^3 l^{-1}$**		
James River			5	Ettinger et al. (2003)
River Tisza	0.75		4	e.g. Gál (1966)
River Danube	max. 4		2	Bereczky (1978)
A chalk stream (total rhizopods)	2000			Sleigh et al., 1992
River Danube	3300	0.09	14	This study
Heliozoa	**ind. l^{-1}**	**$mm^3 l^{-1}$**		
Lake Constance (mesotrophic)	max. 6600	max. 0.060	7	Zimmermann et al. 1996
River Danube	max. 8500	max. 0.057	7	This study
Ciliates	**ind. l^{-1}**	**$mm^3 l^{-1}$**		
River Tisza	61–512			Pujin (1994)
A chalk stream	mean 700			Sleigh et al. (1992)
River Rhine	15–1340	0.00016–0.11	66	Scherwass and Arndt (2005)
Black Creek	1500–13000	0.011–0.021		Carlough and Meyer (1989)
River Danube	132–34000	0.009–0.76	94	This study
River Yenisei	300–40000	0.003–1.5		Sorokin (1990)
River Loire	5400–49000	0.49–1.1		Lair et al. (1999)
River Ogechee	~100–150000	~0.00001–0.34		Carlough and Meyer (1989)

Units are bold, habitats are in ascending order according to maximal abundance values, River Danube values found in this study are underlined.

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in focusing on larger testaceans, minute forms were probably overlooked. The relatively high number of *Arcella*, *Centropyxis*, *Cyphoderia* and *Microchlamys* can be explained by their light test and/or flattened shape that facilitates their drift from benthos/periphyton, and hampers sinking back to the bottom. The nearly globular mineral particle-agglutinated tests of *Pseudodifflugia* species and *Difflugia pulex* are presumably kept floating because of their small size. Little is known about the trophic role of testaceans in the plankton. Large lobose forms are probably unable to feed there, while minute filoseans are likely to be capable of feeding and growing in plankton, either by catching particles on extended filopodia, or by attaching to and grazing on aggregates. This is supported by our frequent findings of cells with extended filopodia. In conclusion, the most abundant, and possibly trophically active, community of small-sized planktonic testate amoebae seems to be overlooked till now. As an exception, de Groot (1979) provided a qualitative report about the bulk of small and rare filoseans in small oligotrophic lowland creeks in the Netherlands.

Naked amoebae

There are few data on morphotype number and abundance of naked amoebae in river plankton (Table 2). In the Danube 14 morphotypes were found, the maximal abundance reaching 3300 ind. l^{-1}. All quantitative records were obtained using direct counting, which, because of surface masking, may seriously underestimate abundance of amoebae compared to MPN-based methods, especially in benthic and soil habitats (e.g. Foissner 1983). Quantitative data of Bereczky and Gál seem to be very low, all the other authors found a few thousand ind. l^{-1} amoebae in rivers. Morphotype richness values for many planktonic records seem to be underestimates; culture methods may probably reveal a much higher diversity. In the James River, the two most frequent gymnamoebean genera were *Naegleria* and *Vanella*. These two morphotypes (eruptive and fan shaped) were also frequently found in the Danube; *Cochliopodium* and *Thecamoeba* were also common.

The size distribution of naked amoebae found by this study (Fig. 3c), in which the smallest specimens are still perhaps under-represented, clearly shows the dominance of small-sized amoebae (under 30 μm). These minute forms very likely grow actively on aggregates. Confirming this, we observed mostly locomotive or non-directed forms, floating forms were rare, and some individuals had many filled food vacuoles. In contrast, larger amoebae are probably not able to grow in plankton, but contribute significantly to biomass. *Nuclearia* species, occurring in summer and autumn, were found as voracious phytoplankton feeders, thus their occurrence was probably related to high phytoplankton abundance in the spring and autumn.

Heliozoa

Few data exist on freshwater heliozoan abundance; comparable reports are mostly from lakes (Table 2). Heliozoa in mesotrophic lakes are in the same maximal abundance and biomass range as found in the Danube. In Lake Constance, as in the River Danube, heliozoa have mid-summer peaks and are present in the autumn. Heliozoa are, at least partially, algivorous in the Danube, and their occurrence is possibly related to high phytoplankton abundance.

Ciliates

When abundance and biovolume of ciliates in the plankton of the Danube is compared to other rivers (Table 2), minimal and maximal values fall into the middle range of extremities found elsewhere. The difference between extreme values in the Danube during the annual cycle is 2.4 orders of magnitude in abundance (0.9–3.2 in other rivers) and 1.9 orders in biovolume (1.3–4.5 in rivers). These values exceed the differences between minimal and maximal values of HF, indicating more extreme environment-tolerance relations (biotic and abiotic) for ciliates. Of the 83 species found in the Danube plankton 30 species (36%) are euplanktonic and further 17 species (20%) have been reported at least 15 times from planktonic habitats (Foissner et al. 1999, see Table 1). Other species are mainly benthic. Species distribution among the major ciliate groups (see results) is in rather good accordance with that reported from freshwater pelagic habitats summarised by Foissner et al. (1999). Slightly fewer haptorid, stichotrich and hypotrich species were found here, while the number of choreotrichs is a bit higher in this study.

In the Danube, as in the Loire (Lair et al. 1998) and Tisza (Pujin 1994), maximal ciliate abundance occurs in summer, while the abundances are low in winter. This pattern is certainly caused by the high summer phytoplankton supply. Contrary to this, in the Rhine, abundance is high in winter and spring, while low in summer and autumn, presumably because of low discharge, coupled with benthic grazing (Scherwass and Arndt 2005). In the Danube peritrichs and oligotrichs are the dominant groups, just as reported from the Loire (Lair et al. 1999), and the Rhine (Scherwass and Arndt 2005). Our findings support the conclusion of Scherwass and Arndt (2005) that this feature, and the relatively high proportion of benthic species, are probably characteristic for large rivers.

The summer and often autumnal peaks of choreotrichs, *Phascolodon vorticella*, most *Urotricha* species, *Stokesia vernalis* and *Climacostomum virens* in the Danube are very likely related to high algal abundances. The similar peaks of Suctoria and *Monodinium* spp.

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correspond with high prey ciliate abundances. Other temporal distribution patterns cannot be explained easily based on rough trophic relations (although bacterial concentrations have not yet been taken into account): e.g. the dominance of peritrichs in winter and early spring (as in the Rhine), the winter presence of *Aspidisca* spp., higher winter contribution of hymenostomatids (*Colpidium colpoda*, *Dexiostoma campylum*, *Glaucoma scintillans*), or the summer lack of *Coleps* species.

Long-term changes of protozooplankton in River Danube

Heterotrophic flagellates

The abundance of flagellates in the Danube seems to have fallen in recent years; the maximal numbers of 7.8 million cells l^{-1} in 2005 compare with 38 million in 1991 (Vörös et al. 2000) and 22 million in 1992 (Kasimir 1992). Similarly, biomass fell from 2.8 $mm^3 l^{-1}$ in 1991 to 0.58 $mm^3 l^{-1}$ in 2005. Differences in methodology may also be responsible. The spring peaks in April 1991 and June 2005, and the autumnal peaks in October 1991 and 2005 are very likely equivalent. Both correspond with the highest phytoplankton abundances in these years, and probably represent a general phenomenon in the Danube.

Summary of earlier ciliate data

Changes in the abundance of ciliates at Göd between 1981 and 1995 have been reported by Bereczky (1996, 1998). The minimal and maximal abundances fell between 5–15 and 2280 ind. l^{-1}. Highest mean annual abundance and highest monthly abundance peaks were found in 1982 and 1990, with a gradual decline between these years (see App. Fig. 9). The annual cycle of ciliate numbers was rather similar every year; a spring peak (rising from March or April, falling in June) and an autumnal peak (rising from October, maximum in November) were typical. Autumnal peaks were mostly higher, except in 1989–1990, when the spring peaks that started early were very high. In 1990 the spring peak rose continuously from January to an April maximum. The spring peaks correspond well with the spring bloom in lakes; however, they occur later, possibly because the spring floods prevent growth and succession of plankton. In some years a small peak occurs also in winter.

Species composition changes considerably during seasonal succession and long-term trends can also be observed. From 1974, the number of *Phascolodon vorticella* (autumnal peak), *Stokesia vernalis* (spring peak), *Vorticella* spp. (autumn), *Stentor polymorphus* (whole year) increased. In 1990 *Phascolodon vorticella* already constituted up to 60% of ciliate abundance (spring max. 1220 ind. l^{-1}), presumably because of eutrophication. In the early 1990s typical species composition during an annual cycle was the following. In winter *Paramecium* spp., *Colpidium colpoda*, *Glaucoma scintillans* and *Vorticella* spp. were characteristic. In the spring and autumnal peaks *Phascolodon vorticella* and *Stokesia vernalis* were most abundant, other species also being numerous from spring to autumn (*Stentor polymorphus*, *Rimostrombidium viride*, *Codonella cratera*, *Rimostrombidium humile*, *Coleps hirtus*, *Urotricha farcta*, *Monodinium balbianii*). *Tintinnidium fluviatile* was present only in summer. *Vorticella* spp. were found throughout the year, often in the autumnal peak, and they sometimes had a winter peak (as in 1988).

Long-term changes of ciliate fauna since 1990

When the minimal and maximal abundances (130–34000 ind. l^{-1}) for the year 2005 are compared with those of the early 1990s, an increase of at least one order of magnitude is evident. This increase can be only partially explained by the larger mesh of the net used by Bereczky. Confirmation of this remarkable increase is seen, when maximal abundances of some larger species are compared, e.g. *Phascolodon vorticella* (1220 ind. l^{-1} formerly, 3400 this study) or *Tintinnidium fluviatile* (460 ind. l^{-1} formerly, 2550 this study). Thus, we assume that the number of ciliates has increased during the last 15 years. The difference between minima and maxima has also increased (0.5–2 orders of magnitude in former years vs. 2.4 here). The seasonal dynamics of ciliate numbers agree with those reported formerly (highest peaks in spring and autumn). Exceptionally, the highest peak in 2005 was shifted from spring to June–July, due to the high water discharge persistent until May in that year. The winter peak in 2005, which increased until March, was also found in for example 1987 and 1995. The long-term changes of ciliate abundance do not follow the long-term changes of phytoplankton (the latter in Kiss 1994). The dominant taxa are generally similar to those reported from the early 1990s, but there are notable differences. In addition to the newly appeared species mentioned above, such as abundant *Rimostrombidium lacustris*, *Membranicola tamari*, *Monodinium chlorigellum* and *Cyclotrichium viride*, a very high peak of *Tintinnidium pusillum*/*Tintinnopsis cylindrata*, the first records of *Urotricha castalia* and *U. matthesi*, high abundances of medium and large *Urotricha* species, and a very high early summer peak of *Vorticella picta* represent changes in community structure in the past 10 years.

Summary of seasonal dynamics of the protozoa community

Although still very few data are available, some cautious generalisations might be drawn from the recent

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investigation. The whole year can be divided into two parts: a winter season from November/December to March/April, and a growing season from April/May to November. In winter, the flagellate community is dominated by small *Spumella*-like flagellates, with the sporadic occurrence of some presumed cold-adapted species, such as *Stelexomonas dichotoma*, and a lack of microflagellates. A prominent HNF peak was found in 2005, dominating all protozoan winter biomass. Some testate amoebae species (*Microchlamys*/*Spumochlamys* sp., *Pseudodifflugia* spp., *Trinema* spp.) and naked amoebae (e.g. *Cochliopodium* spp.) may occasionally reach significant biomass contributions. The most important ciliates were peritrichs (*Vorticella* spp., *Carchesium polypinum*), represented mostly by single, torn off, but actively feeding cells. Other typical ciliates were hymenostomatids (*Colpidium colpoda*, *Dexiostoma campylum* and *Glaucoma scintillans*) cyrtophorids (*Chilodonella uncinata* and *Trithigmostoma cucullulus*), hypotrichs (*Aspidisca* spp.) and few scuticociliates (*Cinetochilum margaritaceum*). A small elongated ciliate winter peak may occur. From about April, lasting sometimes until July, a spring protozoan peak emerges. Succession may be so rapid that an abundant species can be outcompeted or overgrown within 2 weeks. The highest flagellate biomass peak occurred at this time in 2005. Typical flagellates, besides minute chrysomonads, are choanoflagellates (*Codonosiga botrytis*, *Monosiga* spp. and *Salpingoeca frequentissima*), bicosoecids (*Bicosoeca lacustris*) and high-biomass microflagellates (large chrysomonads and *Collodictyon triciliatum*). Naked amoebae, testate amoebae and heliozoa (*Actinophrys sol*, Acanthocystidae and Raphidiophrydae) also have their highest peaks, but without significant contribution to biomass. An elongated ciliate spring peak, including the highest protozoan biomass, consists of a rapid succession of different species. Abundant taxa are choreotrichs (*Rimostrombidium* spp., *Strobilidium caudatum*, *Membranicola tamari* and tintinnids), *Phascolodon vorticella*, *Monodinium chlorigellum*, Suctoria, *Urotricha* spp. *Vorticella picta*, *Stokesia vernalis* and *Climacostomum virens*. After an overall decrease, a further high peak occurred in October–November in every major group (autumnal peak). In 2005, the highest abundance peak of flagellates occurred at this time. The abundant flagellate taxa are similar to those in the spring peak, except the autumnal appearance of *Paramastix conifera*. Both for heliozoa (Acanthocystidae, Raphidiophrydae and *Heterophrys myriapoda*) and especially for naked amoebae, the autumnal peak was moderate in 2005. Ciliate abundance in autumn was as high as in the spring peak, but the biomass peak in 2005 was much lower. Frequent taxa were similar to those present in the spring peak, but *Linostomella vorticella* occurred in autumn, and some spring peak species were infrequent or lacking.

Concluding remarks

While in shallower, smaller and more turbulent rivers with high floating seston content, euplanktonic algae and protozoa are generally not present, larger, slower lowland rivers maintain autochthonous protozooplankton production. Many rivers of the latter type show pronounced seasonal succession events, as well known for lakes. In the Danube in winter, because of low primary production, the microbial loop dominates, with high flagellate biomass being important, and functionally determinative within all protist groups. In the ciliate community many tychoplanktonic species are doubtless present, besides this, the abundant peritrichs clearly play a significant part in trophic transfer. In the spring, the classical herbivore web also plays an important role. Herbivory by protozoa (mainly ciliates and microflagellates) has to be particularly emphasized in these rivers, because of their high growth rates and production compared to rotifers, and the generally low quantity of larger crustacean riverine zooplankton (e.g. Kobayashi et al. 1998). Thus, the planktonic food web structure in these rivers may differ significantly from that in lakes, and protozoa might be the most important phytoplankton consumers.

As found in the River Danube, there may be very short successional events, and realignment of dominance relations of abundant groups within a couple of days. Flood events may also alter community structure very quickly. Revealing the ecological constraints of these fast succession events will be the challenge of future works; these unquestionably have an important role in matter flow and water quality aspects.

In spite of these fast succession events, there are a lot of consistent elements in community structure and abundance relations of protozooplankton during annual cycles in the Danube. Data on long-term changes in river plankton communities are unfortunately sparse (for Danube: phytoplankton in Kiss 1994, cladocera in Illyová and Némethova 2005). Some long-term changes can be observed in ciliate abundances and species assemblages in the Danube. A few ciliate species seem to have appeared only recently in the Danube. Causal understanding of long-term changes in abundance and community structure would allow us to find relations with long-term environmental changes and those partially mediated by human activity. Furthermore, detailed investigation of colonization and invasion processes would help us to a better understanding of protistan biogeography and distributional processes in freshwater habitats.

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Appendix A. Supplementary material

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