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STRUCTURAL COMPOSITION OF PROTOZOOPLANKTON COMMUNITIES IN RELATION TO ENVIRONMENTAL FACTORS IN SHALLOW LAKES AND RESERVOIRS OF RĪGA, LATVIA

Linda Buholce[1#], Vita Līcīte[2], Elmīra Boikova[2], and Uldis Botva[2]

[1] University of Latvia, Kronvalda bulv. 4, Rīga, LATVIA;
lindabuholce@gmail.com

[2] Institute of Biology, University of Latvia, Miera iela 3, Salaspils, LATVIA;
vita@ezeri.lv, elmira@hydro.edu.lv

[#] Corresponding author

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Protozooplankton are dominant grazers of phytoplankton and an important component of the microbial food web, as a link between pico and nanoplankton to higher trophic levels. Their fast growing rate, relative abundance, biomass and diversity are used as indicators of organic and toxic pollution. The impact of urbanisation on ecosystems and their sustainability and biodiversity have recently been much studied. We studied the protozooplankton ciliate communities during the vegetation period from April to October in two small lakes (Bābelītis, Gaiļezers) and two reservoirs (Bolderāja, Saurieši). The largest peak of biomass (15.7×10^2 mg/l) was found in Gaiļezers Lake in August and of abundance (60.2×10^3 org/l) in Bābelītis Lake in July. The lowest biomass (0.006 mg/l) and abundance (0.12×10^3 org/l) were found in the Saurieši Reservoir station. The most abundant ciliates were from the order Oligotrichida.

Key words: *urban ecology, ciliated protozoa, plankton, shallow lake, structural composition.*

INTRODUCTION

The 21st century will be the century of urbanisation (Marcel and Wilfried, 2014). Urban areas rely on ecosystems beyond the city limits, and also benefit from internal urban ecosystems (Bolund and Hunhammar, 1999). In the 1920s, a small group of University of Chicago sociologists developed the term "urban ecology", which became part of human ecology (Wu, 2014). Urban ecosystems are fragmented landscapes dominated by buildings, roadways and pollution-tolerant vegetation (Nasell-Flores, 2008). The study of urban areas is important due to the altered urban environment itself and its impacts on ecosystem services for the fast increasing urban population (Lakes and Kim, 2012). Urban freshwater ecosystems are among the most threatened ecosystems in the world (Anonymous, 2005; Yoshioka *et al.*, 2014). Shallow lakes and reservoirs are mostly ignored in most studies of global processes (Downing, 2010), while some have been carried out (Pfister *et al.*, 2002; Auer *et al.*, 2004; Naselli-Flores, 2008; Downing, 2010 etc.; Zou *et al.*, 2013). Urban lakes tend to have a higher trophic state than in non-urban lakes (Naselli-Flores, 2008). In the mentioned previous studies, one of the best water quality indicators had been suggested to be ciliated protozoans.

Protozooplankton is the dominant grazer of phytoplankton and is an important component of the microbial food web as a link between pico and nanoplankton to higher trophic levels (Fenchel, 1987; Laybourn-Parry, 1992; Zou *et al.*, 2013). Their fast growing rate, high abundance, biomass and diversity are used as indicators of organic and toxic pollution (Foissner, 1988; Pfister *et al.*, 2002). There is some evidence that ciliated protozoans can promote the availability of phosphorus and nitrogen for phytoplankton growth (Xu and Cronberg, 2010). The significance of small-sized (<40 μm) individuals tends to increase with trophy (Beaver and Crisman, 1982; Van Wichelena *et al.*, 2013), while large-sized ciliates are characteristic of oligotrophic ecosystems (Velho *et al.*, 2005).

This study was carried out to provide new information on water quality of Rīga city lakes and reservoirs. There was a need to provide new information about planktonic ciliate communities in shallow lakes and reservoirs, as there were only a few studies, including a doctoral thesis (Liepa, 1975), on benthic ciliate ecology in Latvian freshwater rivers. Large studies on ciliate ecology and ecotoxicology have been carried out by E. Boikova in the Gulf of Riga and open Baltic Sea (Boikova, 1989).

MATERIALS AND METHODS

Sampling sites. Selection of sample locations (Table 1) was based on different levels of anthropogenic pressure on waterbodies. The sampling sites were located two small lakes (Bābelītis, Gaiļezers) and two reservoirs (Bolderāja, Saurieši) in Rīga (Fig. 1). The bottom of Bābelītis Lake was sludge, despite sandy coasts. Dominant macrophytes were *Sparganium*, *Carex*, *Scirpus* and *Nuphar*. Gaiļezers Lake is a typical macrophyte lake overgrown with *Phragmites* and *Scirpus*. The largest area of Gaiļezers Lake was covered by *Nymphaea* species. The lakebed was sludgy and slope of the shore was low. The shores of Bolderāja Reservoir were overgrown with *Salix* and *Hippopha* and the dominant macrophyte was *Phragmites*. A pine forest occurred beside the Bolderāja Reservoir. The bottom of the reservoir was mostly sandy, in some places sludgy. The bottom of Saurieši Reservoir was gypsum deposits. *Typha* and *Phragmites* occurred along the shores of Saurieši Reservoir. Dead tree appear at low water levels.

Sampling. Water samples were taken monthly during the vegetation period from April to October 2014 in the two lakes and two reservoirs. Each sample consisted of 250 ml lake water taken from the surface. The water samples were fixed with Lugol's solution (2% final concentration) and stored at 4 $^{\circ}$C. Measured environmental variables were temperature, oxygen, nutrients NO_2–N, NO_3–N, NH_4–N, PO_4–P, tot P, tot N and Chlorophyll a.

Quantitative and qualitative analysis of ciliates. Ciliates were counted in sedimentation chambers (10 and 25 ml) using an inverted microscope Leica at 200-fold magnification following the Utermöhl method (Paxinos and Mitchell, 2000). Sedimentation time was more than 24 h. Some ciliates were identified only to genus level, but all specimens in

Table 1

COORDINATES OF SAMPLE LOCATIONS

	Bābelītis Lake	Gaiļezers Lake	Bolderāja Reservoir	Saurieši Reservoir
Latitude	N56° 99.21'	N56° 97.00'	N57° 01.85'	N56° 91.43'
Longitude	E24° 22.09'	E24° 23.61'	E24° 03.61'	E24° 34.93'

samples were measured. Identification was made using literature using Kahl (1930–1935) and Corliss (2007).

Biomass and biodiversity index of ciliated protozoans. Biomass was estimated using simple geometric shapes (e.g. sphere, ellipsoid, cone with the hemisphere) using the formulas given by Hillebrand *et al.* (1999) as follows,

For ellipsoids - $(\frac{2.14}{6}) \cdot d^2 \cdot h$

For sphere - $(\frac{2.14}{6}) \cdot d^3$

For cones with the hemisphere $(\frac{2.14}{12}) \cdot d^2 \cdot (h + \frac{d}{2})$

in which d is diameter (μm); h is height (μm).

The Shannon-Wiener biodiversity index of the ciliate communities was calculated using the formula given by Shannon and Weaver (1949) and Keylock (2005) as follows,

$$(H) = -\sum_{i=1}^{s} p_i \ln p_i$$

in which p is the proportion (n/N) of individuals of a particular species (n) divided by the total number of individuals (N), and s is the number of species.

Principal components analysis (PCA) was used to summarize ecosystem relations with environmental variables and ciliate species number during the investigation period concentrate.

RESULTS

Ciliated protozoan composition. During the investigation period (from April to October 2014), 9 genera and 4 species from 7 orders were found. Among them, 6 taxons were from Oligotrichida, 2 taxons from Peritrichia, and but 1 from Choreotrichida, Gymnostomatida, Hymenostomata, Cyclotrichida and Prostomatida (Table 2). Common genera were *Strobilidium*, *Strombidium*, *Lohmaniella, Cyclidium*, *Halteria*, and *Vorticella.*

Seasonal change of ciliate abundance and biomass. Seasonal dynamics of mean ciliate abundance had one peak

Fig. 1. Map of sampling sites in Rīga city.

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SPECIES LIST OF CILIATE TAXONS FOUND IN RIGA CITY SHAL-LOW LAKES AND RESERVOIRS DURING THE INVESTIGATION PERIOD (April till October 2014)

Taxon (org/l)	Bolderāja Reservoir	Sauriešu Reservoir	Bābelītis Lake	Gaiļezers Lake
Prostomatida				
Coleps hirtus	-	-	8	-
Peritrichia				
Vorticella sp.	-	4	215	12
Epistilis sp.	44	-	-	-
Hymenostomata				
Cyclidium sp.	96	3	118	44
Cyclotrichida				
Mesodinium pulex	-	12	-	-
Gymnostomatida				
Dileptus sp.	-	-	8	-
Oligotrichida				
Halteria sp.	29	6	92	106
Strobilidium sp.	131	73	2922	139
Strombidium conicum	16	84	88	4
Strombidium sp.	251	53	1058	168
Tintinnidae sp.	-	-	8	6
Tintinnopsis codonella	-	-	28	-
Choreotrichida				
Lohmaniella sp.	57	6	102	28
Ciliata	119	15	39	4

(Fig. 2) (23×10^3 org/l), which occurred in July, and the lowest ciliate density (5.8×10^3 org/l) was observed in April. The highest peak of mean ciliate biomass occurred in August (6.2×10^2 org/l), and the lowest peak in October (1.6×10^2 org/l). Seasonal dynamics of total abundance of

ciliates is shown in Fig. 3. The greatest peak of biomass (15.7×10^2 mg/l) occurred in Gaiļezers Lake in August and abundance (60.2×10^3 org/l) in Bābelītis Lake in July. The lowest biomass (0.006 mg/l) and abundance (0.12×10^3 org/l) were observed in the Saurieši reservoir station. Lakes (Bābelītis and Gaiļezers) had larger biomass and abundance than reservoirs (Bolderāja and Saurieši). Seasonal changes of proportional abundance of major ciliated protozoan groups are demonstrated in Fig. 4. *Oligotrichida* (*Halteria* sp., *Strombidium* sp., *Strobilidium* sp., *Tintinopsis* sp.) dominated in urban hydroecosystems (more than 75%). *Hymenostomata (Ciclydium* sp.) and *Peritrichia (Vorticella* sp.) mostly were found in lakes, and undefined ciliata species mostly in reservoirs.

Size structure of ciliated protozoans assemblages. Small-sized (≤ 30 μm) ciliates dominated in spring, and medium-sized (30–55 μm) ciliates in summer and autumn (Fig. 5). Medium-sized ciliates dominated in all stations during the investigation period. One-third of ciliates were small-sized (≤ 30 μm). Large-sized (≥ 55 μm) ciliates dominated in spring in Bolderāja Reservoir, and small-sized (≤ 30 μm) ciliates in summer and autumn seasons in Saurieši Reservoir.

Seasonal changes of species richness and biodiversity index. The highest taxon richness (9) and biodiversity index (1.4) peaks were found in Bābelītis Lake in August. The lowest Shannon-Wiener biodiversity index was in Bābelītis Lake in June (0.2) and the lowest taxon richness (2) in Saurieši Reservoir in May and June (Fig. 6). The highest taxon richness was generally higher in lakes (5.1) than reservoirs (4.2). The highest average taxon richness (5.6) and Shannon-Wiener biodiversity index (1.0) was in Bābelītis Lake. The lowest mean taxon richness (3.9) and Shannon-Wiener biodiversity index (0.8) was in Saurieši Reservoir.
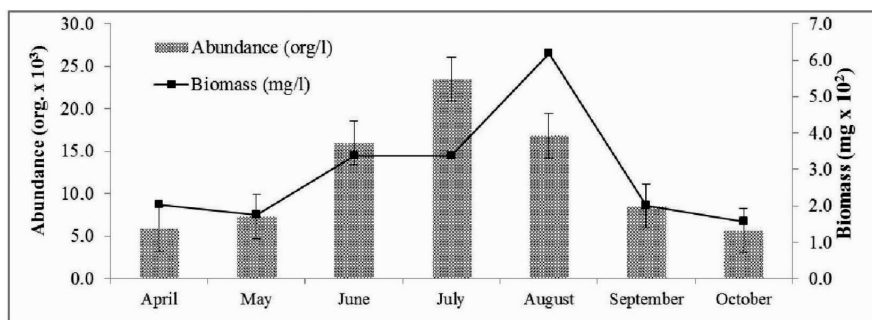

Fig. 2. Seasonal changes of average ciliate abundance and biomass during the investigation period (from April till October).
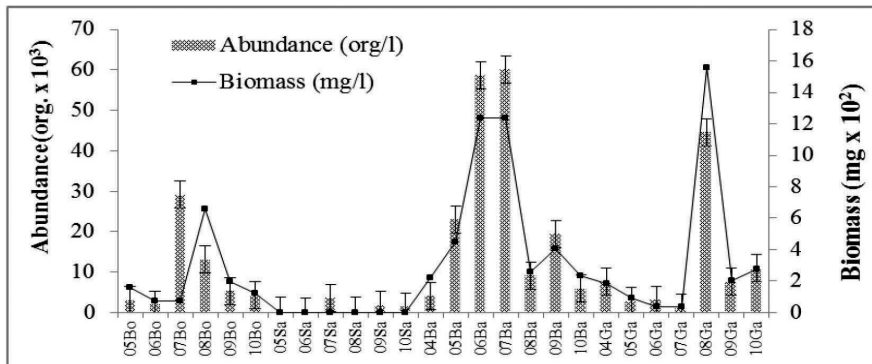

Fig. 3. Seasonal changes of total ciliate abundance and biomass in Rīga city lakes and reservoirs. Bo – Bolderāja Reservoir, Sa – Saurieši Reservoir, Ba – Lake Bābelītis, and Ga – Lake Gaiļezers. Months are marked as numbers (04 is April, etc.).
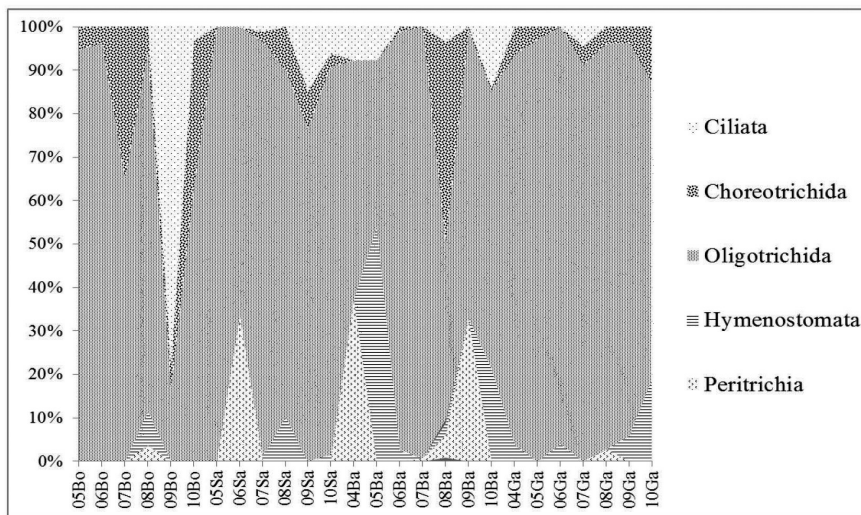
Fig. 4. Seasonal changes of proportional abundance of major ciliated protozoan groups in Rīga city lakes (Bābelītis, Gaiļezers), reservoirs (Bolderāja, Saurieši). For abbreviations see Fig. 3.
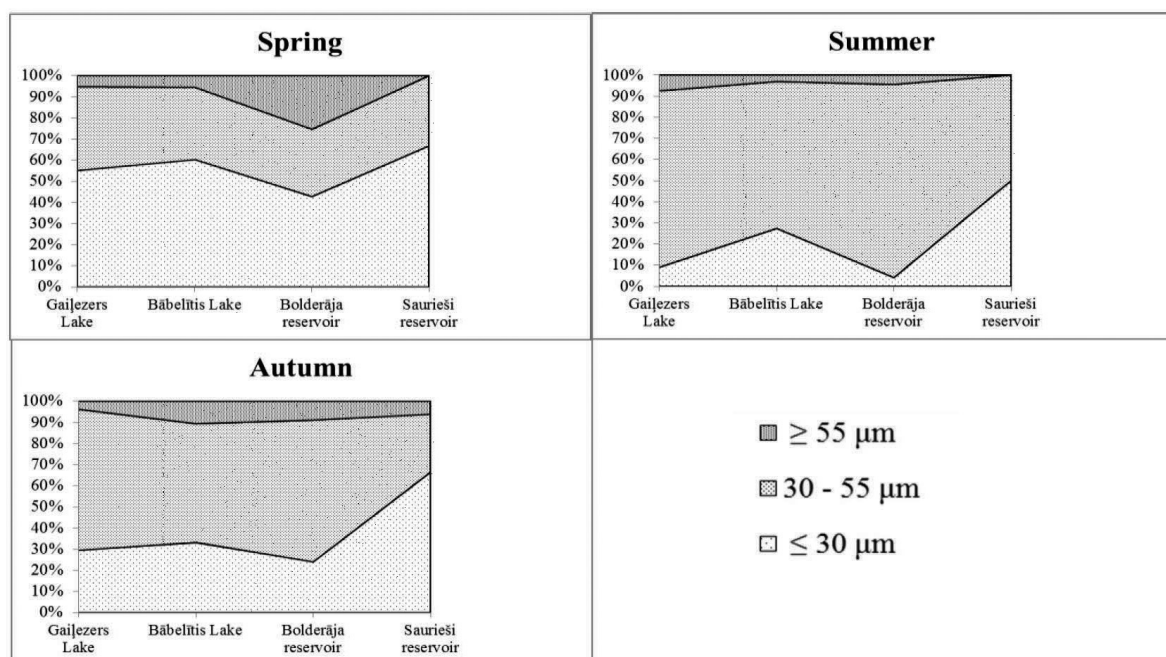

Fig. 5. Size structure of average ciliate assemblages during the investigation period in Rīga city shallow lakes and reservoirs.
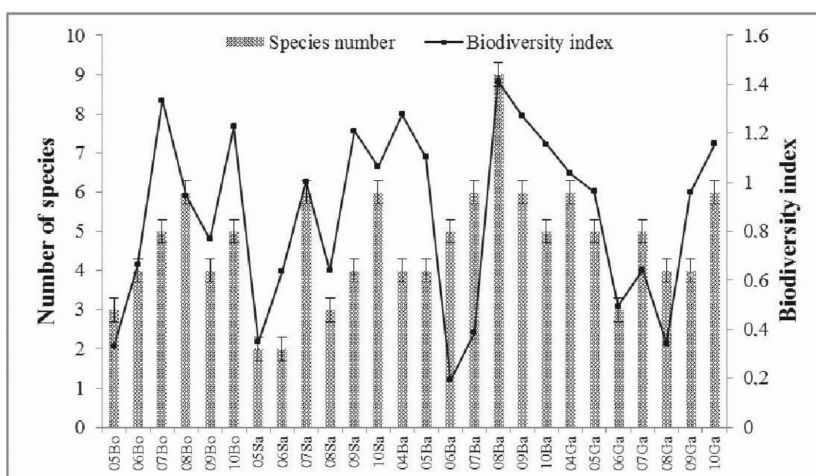

Fig. 6. Seasonal changes of ciliate richness and biodiversity index during the investigation period in Rīga city shallow lakes and reservoirs. For abbreviations see Fig. 3.

Seasonal changes of environmental variables. Figure 7 shows seasonal dynamics of temperature, Nmin, dissolved oxygen and PO4. The highest water temperature occurred in August in Bābelītis Lake (26.0 °C), and the lowest in April in Bābelītis Lake (7.6 °C). Nmin was higher in Saurieši reservoir (28.2 µmol-N/l). The lowest dissolved oxygen values

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occurred in September in Gaiļezers Lake (3.4 mg/l), and the highest in May in Bolderājas Reservoir (8.7 mg/l). The highest PO_4 concentration (0.24 µmol/l) occurred in October in Bābelītis Lake.

Ciliate abundance, biomass and size structure relations with environmental variables. Ecosystem relations with environmental variables and ciliate species number during the investigation period concentrate in Bābelītis Lake, Gaiļezers Lake and Bolderāja Reservoir shown in Fig. 8. Bray–Curtis similarity index (hierarchial clustering) of environmental variables and ciliate species number, average biomass and abundance during the investigation period show similarity between Gaiļezers Lake and Bolderāja Reservoir, as well as Bābelītis Lake (Fig. 9). *Spearman's* rank correlation analysis showed that ciliate abundance, biomass and size structure were generally correlated with biotic and abiotic factors ($p \leq 0.05$).

Gaiļezers Lake. Average ciliate abundance and biomass were significantly correlated positively with Chlorophyll a, and Nmin negatively with ciliate abundance and biomass. pH was correlated negatively and NH4 positively with abundance of small-sized (≤ 30 µm) ciliates. Abundance of ciliates with size ≤ 30 µm and ≥ 55 µm were correlated negatively with dissolved oxygen. Average abundance of large-sized (≥ 55 µm) ciliated protozoans were correlated negatively with pH and Nmin.

Saurieši Reservoir. Ciliate abundance correlated positively with pH, and correlated negatively with total phosphorous. Biomass of ciliates with size ≤ 30 µm and ≥ 55 µm were correlated negatively with PO_4 and dissolved oxygen.

Bolderāja Reservoir. Average ciliate abundance, biomass and abundance of large-bodied ciliates were correlated negatively with nitrogen. Abundance of small-sized ciliates was correlated positively with Chlorophyll a. Abundance of
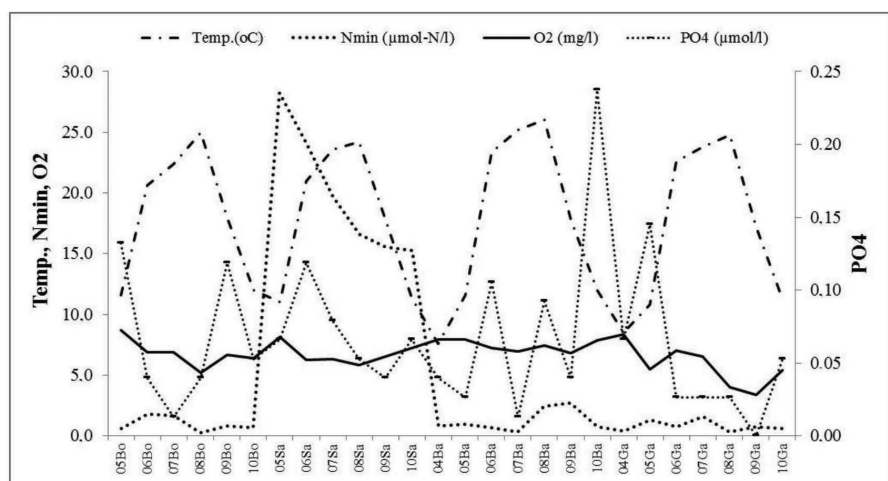

Fig. 7. Environmental variable (temp., Nmin., O_2, PO_4) dynamics during the investigation period in shallow lakes and reservoirs of Rīga. For abbreviations see Fig. 3.
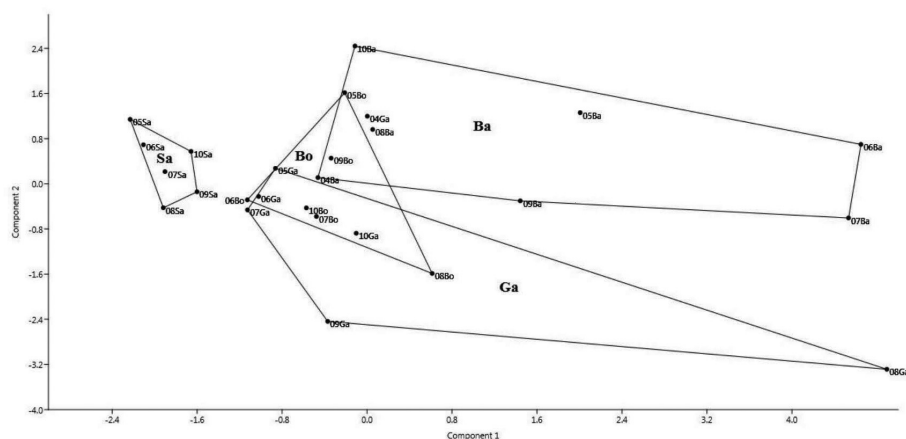

Fig. 8. Principal components analysis (PCA), correlation matrix between-groups of ecosystem relations with environmental variables and ciliate species number during the investigation period in Rīga city shallow lakes and reservoirs. For abbreviations see Fig. 3.
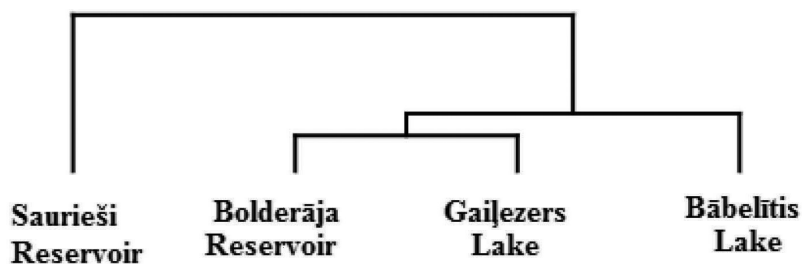

Fig. 9. Hierarchial clustering, paired group (UPGMA) algorithm, Bray–Curtis similarity index of environmental variables and ciliate species number, average biomass and abundance during the investigation period in Rīga city shallow lakes and reservoirs.

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ciliates with size 30–55 μm were correlated negatively with dissolved oxygen.

Bābelītis Lake. There was no correlation between ciliate abundance, biomass, size structure and biotic or abiotic factors ($p \leq 0.05$).

DISCUSSION

Ciliate communities were dominated by *Oligotrichida, Peritrichia, Cyclotrichida, Choreotrichida, Gymnostomatida, Hymenostomata*, and *Prostomatida*. All these groups have been reported as relatively common, for example, in Lake Dongshan (Zou *et al.,* 2013), Lake Ringsjön (Xu and Cronberg, 2010), Lake Võrtsjärv (Zingel, 2001), Lake Mackenzie (Galand *et al.*, 2008), and German lakes (Pfister *et al.,* 2002).

Previous studies have shown that the highest peaks in ciliate abundance and biomass occur in spring (May) and in late summer (July, August) (Zingel, 2001; Xu and Cronberg, 2010; Zou *et al.,* 2013), as also shown for Rīga city shallow lakes and reservoirs. Ciliate biomass increases with trophy level (Zou *et al.* 2013). High nutrient availability promotes phytoplankton and bacterial abundance, which facilitates development of planktonic ciliate populations (Velho *et al.,* 2005). Saurieši Reservoir was characterised by a relatively low population size under conditions of typical increases of nutrient concentrations, which is in contrast to the well known trend of ciliate abundance and biomass increasing along a trophic gradient from oligotrophy to eutrophy (Zingel, 2001).

Although medium-sized (30–55 μm) ciliates dominated in all stations during the investigation period, small-sized ($\leq$ 30 μm) ciliates form almost one third of size structure during the investigation period. Beaver and Crisman (1982) and Van Wichelena *et al.* (2013) found that large-sized ciliates ($\geq$ 55 μm) are progressively replaced by small-sized ciliates as trophic state increased. Ciliate size structure indirectly indicates different trophic roles during the growing season.

The highest number of species was found in summer and spring months, as previously observed by Zou *et al.* (2013). Species richness tends to increase with lake size (Van Wichelena *et al.* 2013). In our study, the Shannon-Wiener biodiversity index was low in spring and higher in summer, despite waterbody size.

Opinions differ on the effect of physical-chemical parameters, including concentrations of nutrients, on ciliate abundance, biomass and size structure. Considering that protozooplankton are the dominant grazers of phytoplankton, it might be expected that their optimum population growth occurs under similar conditions of UV light, high temperature, and N : P ratio = 7.2:1 (Horne, 1994). Relationships between ciliate abundance, biomass, size structure and biotic and abiotic factors ($p \leq 0.05$) differed among the studied lakes and reservoirs. Results from literature also differ.

Abundance of ciliates was not significantly correlated with temperature (Stoecker *et al.,* 1984). In bottom waters, abundance of ciliates appeared to be influenced by oxygen, temperature, and total phosphorus (Kalinowska *et al.,* 2013). The common species were pH-tolerant and the rare species were pH-sensitive (Weisse and Stadler, 2006). Abundance of ciliates was positively correlated with water temperature and Chlorophyll a and negatively correlated with various inorganic nutrients, and was strongly influenced by the food availability (Yang *et al.,* 2014). Ciliate abundance was observed to increase with Chlorophyll a concentration and total phosphorous, while temperature, pH and dissolved oxygen did not have any impact on ciliated organisms (Pfister *et al.,* 2002). Ciliate dynamics need to be studied for several years to determine relationships between population variable and environmental factors in the Rīga city lakes and reservoirs. Bolderāja Reservoir could be described as a similar one to a natural lake, and used as a potential reference for urban ecosystem research. For further evaluation, Gaiļezers Lake and Bābelītis Lake can represent an urban stress level. Future research should include oligotrophic lakes to determine differences in ciliate communities.

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PROTOZOOPLANKTONA SABIEDRĪBAS VIDES FAKTORU IETEKMĒ RĪGAS PILSĒTAS SEKLAJOS EZEROS UN KARJEROS

Ciliāti kā vadošā protozooplanktona grupa ir būtiski mikrobiālās ķēdes komponenti. Pateicoties savai bioloģiskajai organizācijai, ciliāti ir labi zināmi arī kā bioindikatori, jo spēj strauji reaģēt uz apkārtējās vides izmaiņām. Urbānās vides hidroekosistēmas ir relatīvi maz pētītas, tāpēc darba gaitā uzsākti pētījumi četrās Rīgas pilsētas un piepilsētas ūdenstilpēs ar mērķi iegūt informāciju par ciliātu sezonālo sukcesiju, analizējot to bioloģisko daudzveidību, skaitu, biomasu, populāciju izmēra struktūru dinamiku saistībā ar vides abiotiskiem un biotiskiem faktoriem. Rezultāti liecina, ka Gaiļezeru un Bābelīša ezeru var izmantot kā modeļsistēmu urbānā stresa novērtējumam, savukārt Bolderājas

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