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Comparative study of periphytic ciliate communities and succession on natural and artificial substrata in two shallow lakes (Eastern Poland)

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The colonization and succession of periphytic ciliate communities on the natural (*Phragmites australis* and *Typha Iatifolia*) and artificial (glass slides) substrata were studied in 2 shallow lakes (Polesie Lubelskie, Eastern Poland). Sampling was done on a monthly basis from April to November 2003-2004. During each sampling occasion 6 periphton samples were collected from each type of substrata at 3 sites: the land/water contact zone, among emergent macrophytes, and at the emergent macrophytes/open water border. Irrespective of the type of substrate and trophic state, species number and densities of periphytic ciliates reached the highest value in the land/water contact zone and decreased in the direction of the area of emergent macrophytes/open water zone. In land/water contact zone, the biggest factors limiting the ciliate communities were temperature, conductivity, concentrations of total organic carbon and nutrient (P_{tot} and $N\text{-}NH_4$). In turn, in the other 2 zones, the influence of chlorophyll *a* concentration increased.

Keywords: shallow lakes, ciliates community, natural and artificial substrata, colonization

Introduction

The littoral zone in shallow lakes comprises a mosaic of vertical and horizontal microhabitats, provided by macrophytes and open patches (Kiss et al. 2000, Mészános et al. 2003). These habitats generally reflect a very diverse composition with successive development being essential due to emergent and submerged vegetation that comprises a number of life forms. One of the fascinating roles of macrophytes is their potential as a refuge for large zooplankton species, which, in turn, control the phytoplankton, which may also affect the structure and function of the microbial community (Jürgens & Jeppesen 1997). However, macrophytes constitute a vast substrate for the growth of periphytic communities (Messyasz & Kuczyńska-Kippen 2006). Periphyton is a biological layer found in various substrata in natural waters and consists of a mucilage of slime, bacteria, algae, fungi, protozoa and small metazoans (Hameed 2003). Many recent studies have shown that ciliates play a very important trophic role in periphytic communities, and as an indication of the degree of pollution in rivers and lakes (Primc-Habdija et al. 2000, Mieczan 2005a). Natural substrata that maintain periphyton in lakes are different in origin and size; this variability in the nature of substrata, and the corresponding variation of microbial communities, have always made quantitive studies difficult. To facilitate such studies, artificial substrata have been used for several years. Their usage simplifies the natural complexity (uniform colonization time, material, texture and size) and reduces the disruption of the habitat because there is no need to remove large amounts of natural substrata. Furthermore, since the total surface area is known, problems with the measurement of irregular natural substrata are eliminated. Because of their uniform size and inert surface, glass slides are among the most frequently used artificial substrata (Krajl et al. 2006). Some researchers have noted that artificial substrates are biased with respect to characterizing natural communities, while others maintain that natural substrates present problems with sampling design and qualification. Boothroyd & Dickie (1989), Kauer & Mehra (1998) have reported the presence of similar epiphytic communities on macrophytes as well as on natural and artificial substrates.

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In the present study, the colonization of ciliates followed a similar pattern on both types of substrates where Cyrtophorida and Peritrichida dominated. However, even more attention has been paid to the periphytic ciliates in river ecosystems (Slàdečkova 1994, Mehra & Kauer 1998). To date, no research has been carried out on periphytic ciliates on natural and artificial substrates in the transitional zones between the land and water ecosystems, and in relation to the trophic state of lakes. The aim of the present study, therefore, was to establish the following: whether differences exist between periphytic ciliate communities on different substrates; to determine whether colonization time would yield an abundance and taxonomic composition of periphytic ciliates; to assesss the effect of physical and chemical factors on the distribution of periphytic ciliates in two shallow lakes of different trophic state.

Study area

The study area comprised two lakes in the Polesie Lubelskie region of Eastern Poland (51°N, 23°E). These were the eutrophic Lake Rotcze, with small-lake phytolittoral (area: 54.8 ha, max. depth: 4.3 m), and the humic Lake Długie, with atrophic phytolittoral (area: 28.7 ha, max. depth: 1.3 m). The littoral classification was accepted after Bartanowicz and Zachwieja (1966). The authors of the present study noted that the small-lake phytolittoral is a luxurious development of emergent macrophytes located close to the shore, with an absence of floating-leaved plants. The atrophic phytolittoral is adjacent to a floating mat which consists of interlocked roots, rhizomes, and stems of aquatic, marshy, as well as terrestrial vegetation close to the lake shore. In the eutrophic Lake Rotcze, well developed belts of emergent (*Phragmites australis* (Car.) Trin ex Stend. and *Typha latifolia* L.) and submerged macrophytes (*Ceratophyllum demersum* L., *Chara hispida* L., *Chara fragilis* Desvaux and *Elodea canadenis* L.) dominate in the littoral. Vegetation of high peatbog (with a dominance of *Sphagmum*) dominates in Lake Długie. The emergent macrophytes were dominated by *Phragmites australis* (Car.) Trin ex Stend. and *Typha latifolia* L., and submerged macrophytes by *Ceratophyllum demersum* L. and *Elodea canadensis* L.

Materials and methods

The periphyton was collected from the rush vegetation (*Phragmites australis* and *Typha latifolia*) and glass slides at 3 sites: the land/water contact zone (at a depth of 0.1 m), among emergent macrophytes (0.5 m), and at the emergent macrophytes/open water border (1 m). Three perspex frames with 6 microscopic glass-slides (2 cm x 5 cm each) were placed near the *Phragmites* and *Typha* bed. The frames were placed horizontally and the slides vertically. Sampling was done on a monthly basis from April to November 2003-2004. During each sampling occasion 6 periphton samples were collected from each type of substrata at each of the 3 sites. New substrata were then placed for colonization during the following month. One sample consisted of 10 cm^{-2} of periphyton taken from the macrophyte stems and glass-slides by means of a scalpel. In order to determine the density of ciliates, 4 samples were fixed with Lugol's solution (1% v/v) and settled for at least 24 h in plankton chambers. The ciliates were counted and identified with an inverted microscope at magnification x 400-1000. Ciliates are highly perishable, and their type of motility is a species specific feature; for this reason, species determination and measurements were carried out on live material immediately after return to the laboratory and after silver impregnation (Augustin et al. 1984). Taxonomic identication was based primarily on Foissner & Berger (1996), Foissner et al. (1999).

The water samples for chemical analysis were taken simultaneously with the periphyton samples. The following physical and chemical factors were examined: temperature, pH, conductivity, total organic carbon (TOC), chlorophyll *a*, ammonium-nitrogen, nitrate-nitrogen, phosphates and total phosphorus. Temperature, conductivity and pH were recorded (Jenway 3405 or Hydrolab electrode) *in situ*. Total organic carbon (TOC) was determined by using the PASTEL UV; the remaining factors were analysed in the laboratory (Hermanowicz et al. 1976). Chlorophyll a was determined by spectrophotometric analysis of the acetone extract of the algae (Golterman 1969).

The frequency of occurrence of a particular species was calculated as a percentage of collected samples in which the species occurred. All species were found were classified into 4 groups as follows: very constant species (occurring in 61-100% of samples; constant species (occurring in 41-61% of samples); accidental species (occurring in 21-40% of samples; accessory species (occurring in less than 20% of samples).

The similarity of periphytic communities between the substrates was calculated by Jaccard's method:

$$S_{xy} = (c / a+b-c) \times 100\%$$

where: S_{xy} - faunistic similarity between data sets x and y, c - number of taxa common for sets'x and y, a - number of taxa in set x, b - number of taxa in set y.

Table 1. Physical and chemical characteristics of the littoral of investigated lakes (average values for period April-November 2003-2004, ±SD); 1- the land/water contact zone, 2 - emergent macrophytes, 3 - emergent macrophytes/open water border.

Lake	zone	Temp. °C	pH	Conduct. µS cm^{-1}	Chlorophyll *a* µg dm^{-3}	N-NH$_4$ mgN dm^{-3}	N-NO$_3$ mgN dm^{-3}	P$_{tot}$ mgP dm^{-3}	PO$_4$ mgPO$_4$ dm^{-3}	TOC mgC dm^{-3}
Lake Rotcze	1	18.3±6.5	8.8±0.20	320.0±43.02	23±6.6	0.523±0.08	0.125±0.10	0.276±0.53	0.284±0.03	5.1±2.5
	2	15.2±6.2	8.2±0.79	290.0±40.01	48±5.2	0.336±0.08	0.113±0.11	0.266±0.23	0.227±0.01	4.2±1.5
	3	11.4±5.6	7.2±0.69	220.3±30.52	68±7.9	0.299±0.07	0.111±0.11	0.266±0.02	0.027±0.01	3.1±1.1
Lake Długie	1	18.1±6.6.	5.2±0.28	230.6±20.52	15±5.5	0.328±0.04	0.037±0.01	0.138±0.08	0.004±0.01	12.5±2.5
	2	15.4±6.3	5.8±0.94	200.6±20.54	23±5.0	0.280±0.07	0.024±0.01	0.128±0.01	0.013±0.01	9.5±3.0
	3	11.0±6.7	6.5±0.97	180.4±12.32	34±6.2	0.220±0.05	0.039±0.02	0.128±0.01	0.010±0.01	7.7±1.7

All data collected were statistically analysed by means of GLM and CORR procedures of the SAS Programme (SAS Institute Inc. 2001). One-way ANOVAs with *post-hoc* Bonferroni tests were run on abundance data to assess separately the protozoan variability caused by the lakes, and the zones between periphytic communities on natural and artificial substrata (n = 58). The similarity between periphytic communities on natural and artificial substrata of the different lakes was compared by using the Euclidean distance measurement. Correlation between physical and chemical parameters and ciliate density were analysed by calculating Pearson's correlation.

Results

Water chemistry

Statistically significant differences between individual littoral zones in the lakes studied were indicated in water temperature, chlorophyll *a*, conductivity and the concentration of total organic carbon (p = 0.0112-0.0301). The next significantly high concentration was in nutrient (N-NH$_4$) found in the land/water contact zone in both lakes (p = 0.0301), whereas, generally, phosphorus and phosphates concentrations were significantly high only in the eutrophic lake (p = 0.0311). Water reaction in both lakes studied, together with the phosphates content in the humic lake, did not show a statistically significance differences between littoral zones (p > 0.05). In both lakes examined, the water temperature reached the highest value in the land/water contact zone (18.1°C -18.3°C), and decreased in the direction of the emergent macrophyte/open water zone (11.0°C - 15.2°C). In eutrophic lake the concentrations of chlorophyll a reached the highest value in the emergent macrophyte/open water zone (68µg dm^{-3}), whereas in the land/water contact zone it was the lowest and did not exceed 23µg dm^{-3}. In the humic lake, the chlorophyll *a* content reached 34µg dm^{-3} in the emergent macrophyte/open water zone and only 15µg dm^{-3} in the land/water contact zone. Conductivity decreased in the direction of the emergent macrophyte/open water zone and ranged from 220-320 µS cm^{-1} in eutrophic lake to 180-230 µS cm^{-1} in humic lake. Likewise, the TOC content significantly rose in the water/land contact zone (from 5 mgC dm^{-3} in the eutrophic lake to 12 mgC dm^{-3} in the humic lake) and decreased in the direction of the emergent macrophyte/open water zone. Concentrations of P$_{tot}$, P-PO$_4$, N-NO$_3$ and N-NH$_4$ were mostly low in both lakes, and showed an increasing tendency along the transeckt with the maximum in the land/water contact zone (Table 1).

Taxonomic composition and abundance

A total of forty five species were found in the studied lakes 45. The number of ciliate taxa was similar on both natural and artificial substrata but revealed a statistically significant difference between the investigated littoral zones (p = 0.0021). In the Rotcze and Długie lakes the highest richness were found in the land/water contact zone (44 and 32, respectively), and the lowest on the border of emergent macrophytes/open water zone (38 and 25, respectively). In the land/ water contact zone *Chilidonella uncinata* was a very constant species; in the 2 remaining zones *Cinetochilum margaritaceum* (Table 2). Cyrtophorida and Scuticociliatida, irrespective of the type of substrata and littoral zone, reached large numbers in spring and autumn. In summer, however, Oligotrichida increased in the emergent macrophyte/open water zone.

In general, the rates of colonization and patterns of succession of periphytic microfauna on glass-slides were identical to those on natural substrate. The

Table 2. The composition and frequency (% of samples) of majority of periphytic ciliate taxa found on investigated substrata (Phragm. – Phragmites australis, Typh. – Typha latifolia, Glass-sl. – glass-slides), average values for period April-November 2003-2004).

Taxon	Lake Rotcze			Lake Długie		
	Phragm.	Typh.	Glass-sl.	Phragm.	Typh.	Glass-sl.
CYRTOPHORIDA						
Chilodonella uncinata (Ehrenberg, 1838)	92	87	90	86	72	80
Chilodonella sp.	21	9	20	9	8	9
HAPTORIDA						
Askenasia volvox (Kahl, 1930)	6					
Dileptus margaritifer (Ehrenberg, 1838)	8	7	6			
Enchelys gasterosteus (Kahl, 1926)	5	3	4			
Lacrymaria olor (Mueller, 1786)	22	20	19	5	3	3
Spathidium sensu lato	4	7	5			
HETEROTRICHIDA						
Spirostomum ambigum (Mueller, 1786)	4	5	3	5	6	5
Stentor amethystinus (Leidy, 1880)	2	3	2	3	2	2
Stentor coeruleus (Pallas, 1766)	22	20	20	15	13	14
Stentor multiformis (Mueller, 1786)	3			2		
Stentor polymorphus (Mueller, 1773)	10	19	14	1	3	2
HYMENOSTOMATIDA						
Frontonia leucas (Ehrenberg, 1833)	4	5	5	6	4	3
Lembadion sp.	28	22	24	3	4	2
Paramecium bursaria (Ehrenberg, 1831)	16	18	17	6		
Stokesia vernalis (Wenrich, 1929)	2	12	6			
Urocentrum turbo (Mueller, 1786)	3	6	4			
SCUTICOCILIATIDA						
Cinetochilum margaritaceum (Ehrenberg, 1831)	59	61	60	48	41	50
HYPOTRICHIDA						
Aspidisca costata (Mueller, 1786)	41	29	35	18	19	22
Euplotes sp.	23	17	20	2	6	4
Holosticha pullaster (Mueller, 1773)	20	19	20			
Stylonychia mytilus- Komplex	9	8	9	8		
Urostylla grandis (Ehrenberg, 1830)	4					
OLIGOTRICHIDA						
Codonella cratera (Leidy, 1877)	12	17	15	4	7	5
Halteria gradinella (Mueller, 1773)	13	15	12	8	8	10
Strombidium viride (Stein, 1867)	12	35	28	14	23	28
PERITRICHIDA						
Astylozoon sp.	2	2	2			
Carchesium sp. (Zacharias, 1897)	9	5	10	8	10	
Phascolodon vorticella (Stein, 1859)	2			4		
Pseudovorticella monilata (Tatem, 1870)	6	8	7			
Vorticella convallaria- Komplex	30	33	30	2	2	15
Vorticella companula (Ehrenberg, 1831)	32	30	28	28	12	13
Vorticella microstoma- Komplex	10	13	11	2		5

Table 2. (continued)

Taxon	Lake Rotcze			Lake Długie		
	Phragm.	Typh.	Glass-sl.	Phragm.	Typh.	Glass-sl.
PLEUROSTOMATIDA						
Amphileptus cleparedei (Stein, 1867)	9	7	10			
Amphileptus pleurosigma (Stokes, 1884)	4			4	8	
Amphileptus proceus (Penard, 1922)		1	0	1	1	1
Litonotus cygnus (Mueller, 1773)	26	30	28	5	8	17
Litonotus lamella (Mueller, 1773)	31	5	18			
Litonotus varsaviensis (Wrześniowski, 1866)	1	1				
Loxophyllum meleagris (Mueller, 1773)	4			3		
PROSTOMATIDA						
Bursellopsis sp.	1	12	6	1	4	4
Coleps hirtus (Mueller, 1786)	68	60	62	27	33	40
Coleps spetai (Foissner, 1984)	25	28	27		6	21
Holophrya sp.	29	13	18	14	13	14
SUCTORIDA						
Acineta sp.	2	3	2	3	3	2
No. of taxa: 45	44	40	38	32	28	25

Jaccard similarity index reached a mean value of 89-90%. Analysis of the periphytic communities revealed that a greater similarity was found in most cases among particular substrata and littoral zones (Fig. 1A-B). With respect to the trophic state, the mean numbers of periphytic ciliates formed changed in the individual littoral zones. In the eutrophic lake, in all types of substrata, there was a significantly higher abundance of ciliates prevalent in the land/water contact zone (from 70 ind. cm^{-2} to 79 ind. cm^{-2}) and decreased in the direction of the emergent macrophyte/open water zone ($p = 0.012$) (Fig. 2A-B). In every zone, the lowest number of ciliates were found on the *Typha* (48-70 ind. cm^{-2}), whereas the highest on the *Phragmites* (59-79 ind. cm^{-2}) ($p = 0.013$). In the humic lake, the number of ciliates increased signifcantly between the littoral zones studied ($p = 0.011$); however, no significant difference was found between individual types of substrata ($p > 0.05$). The lowest density of ciliates was noted on the border between the macrophyte and open water zone - 22-25 ind. cm^{-2}. In the other two zones, the number of ciliates fluctuated from 40-42 ind. cm^{-2} in the emergent macrophytes to 50 ind. cm^{-2} in the land/water contact zone.

In three littoral zones the highest cell densities of ciliates occurred in spring (April) and autumn (November), together with very high total organic carbon (8-15 mgC dm^{-3}) and nutrient concentrations, especially phosphorus (>0.300 mgP dm^{-3}). Correlation of environmental variables with ciliate density produced a moderate to strong positive relationship with periods of higher productivity (Table 3). In the land/water contact zone, as well as in the emergent macrophytes, the number of ciliates had the strongest correlation with water temperature ($r = 0.70$-0.73, $p < 0.01$), conductivity ($r = 0.63$-0.69, $p < 0.01$), concentrations of TOC ($r = 0.80$-0.88, $p < 0.01$) and nutrient (P_{tot} and N-NH_4) ($r = 0.55$-0.60, $p < 0.01$). In turn, in the emergent macrophyte/open water zone, there was a significant rise in the strong correlation between the number of ciliates and the chlorophyll *a* concentration ($r = 0.68$-0.78, $p < 0.01$) (Table 3).

Table 3. Significant correlations (p<0.01) of physical and chemical parameters with ciliate density. Correlation coefficients are Pearson product moment type (n=58); 1- the land/water contact zone, 2 - emergent macrophytes, 3 - emergent macrophytes/open water border,
n.s. - not significant.

Lake	zone	Temp.	pH	Conduct.	Chlorophyll *a*	N-NH_4	N-NO_3	P_{tot}	PO_4	TOC
Lake	1	0.73	n.s.	0.69	0.50	0.60	n.s.	0.60	n.s.	0.88
Rotcz	2	0.63	n.s.	0.52	0.51	0.50	n.s.	0.45	n.s.	0.70
e	3	0.45	n.s.	0.50	0.78	0.50	n.s.	0.50	n.s.	0.69
Lake	1	0.70	n.s.	0.63	0.50	0.55	n.s.	0.56	n.s.	0.80
Długi	2	0.60	n.s.	0.55	0.52	0.50	n.s.	0.50	n.s.	0.77
e	3	0.55	n.s.	0.53	0.68	0.51	n.s.	0.50	n.s.	0.70

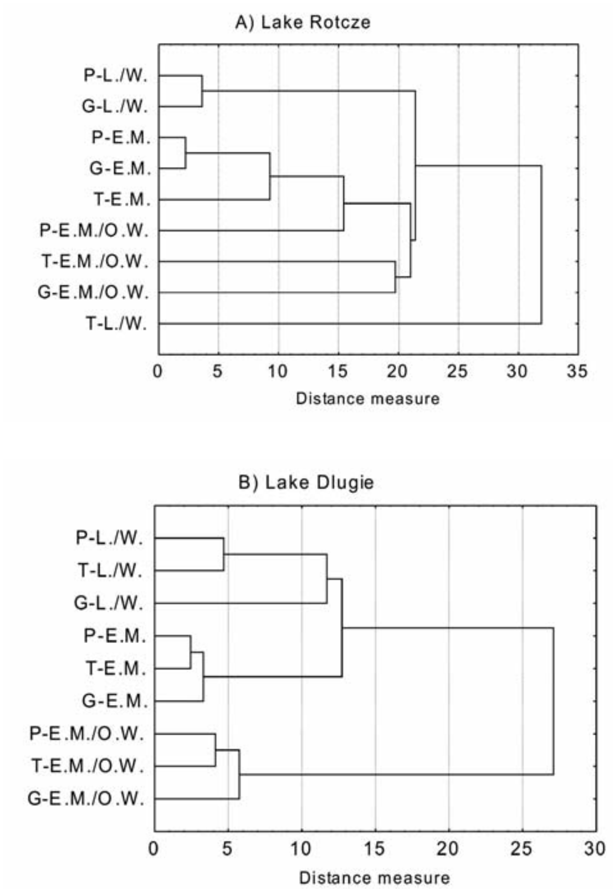

Fig. 1 A-B. The average value of the periphytic ciliates community similarity on natural and artificial substrata in littoral of investigated lakes (P – Phragmites australis, T – Typha latifolia, G – glass-slides, L./W. - the land/water contact zone, E. M. - emergent macrophytes, E. M./O. W. - emergent macrophytes/open water border).

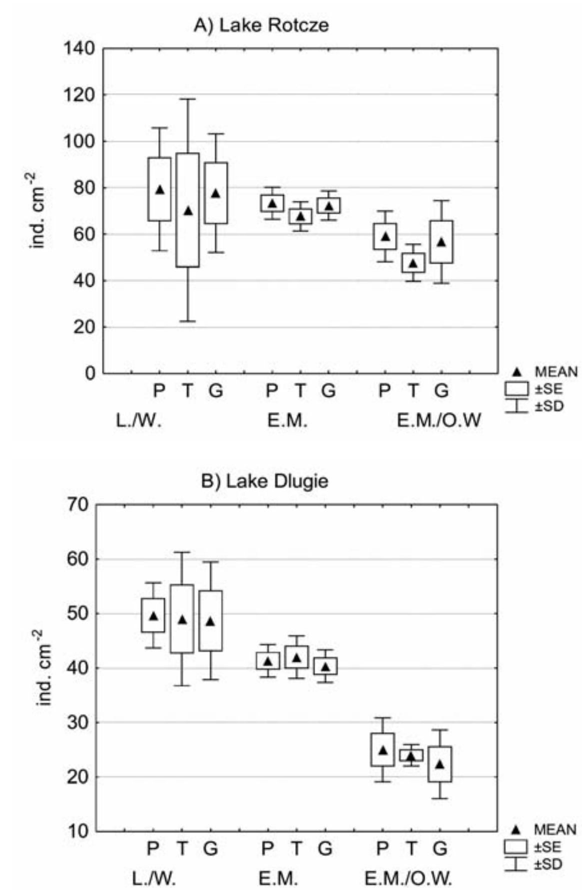

Fig. 2 A-B. Average (April-November 2003-2004) density of periphytic ciliates found on natural and artificial substrata in littoral of investigated lakes (P – Phragmites australis, T – Typha latifolia, G – glass-slides, L./W. - the land/water contact zone, E. M. - emergent macrophytes, E. M./O. W. - emergent macrophytes/open water border).

Discussion

In general, the rates of colonization and succession patterns of periphytic microfauna on glass-slides were almost identical to those on natural substrata. This high degree of similarity was confirmed by the Jaccard index. Several authors (Chadwick & Canton 1983, Boothroyd & Dickie 1989) have reported the presence of similar periphytic communities on macrophytes and artificial substrata. In the lakes studied, regardless of their trophic state and type of substrata, the highest species number were found in the land/water contact zone, while in the remaining zones the number of species was considerably lower. The high richness of ciliate in the land/water zone could be caused by the so-called 'contact effect' which is very often responsible for the increase in species diversity (Naiman & Decamps 1997). Eddison & Ollason (1978) consider that protozoans which inhabit continuously changing habitats are more qualitively diversified than those living on more stable and homogenous substrata. However, the lowest number of species in the macrophytes/open water zone could be associated with the 'rinsing out' of ciliates from that zone by waving.

The polulation density of ciliates ranged 22 ind. cm^{-2} to 50 ind. cm^{-2} in the humic lake and 48 ind. cm^{-2} to 79 ind. cm^{-2} in the eutrophic lake. For comparison, on glass slides that were exposed in vertical profile in Lake Visivac (Croatia), ciliate numbers were from

40-2400 ind. cm^{-2} (Primc-Habdija et al. 1997). On glass-slides in a dystrophic bog lake in northern Germany, a ciliate abundance of 40-580 ind. cm^{-2} was noted (Strüder-Kypke 1999, Strüder-Kypke & Schönborn 1999). In the present study, only in the 3 studied zones in the eutrophic lake were the numbers of ciliates significantly different between the studied substrata, with the lowest numbers on the *Typha* and the highest on the *Phragmites*. The increase in abundance of ciliates on *Phragmites australis* may be the result of profitable feeding conditions. It also seems that the reason for abundant ciliates on precisely this substrate may be partly explained by the situation prevailing in the belts of *Phragmites* during the spring season. The littoral was characterized by the presence of a considerable amount of decaying plant remains. Such a type of environment could enhance a massive development of ciliates. In turn, the lower numbers of ciliates in the humic lake might also result from humus originating from the peat limiting light penetration, and hence limiting autotrophic and mixotrophic microorganisms that constitute a food source for them (Amblard et al. 1996). In the present study, the highest numbers were noted in the land/water contact zone. The higher numbers of ciliates in this zone are most likely a consequence of the high conductivity, concentrations of total organic carbon and nutrient. A significant correlation with organic matter has also been observed in European lakes (Amblard et al. 1995, Mieczan 2005a). Sarvala et al. (1999) and Mieczan (2005a, 2006) have shown a definite correlation between the number of ciliates, conductivity, and trophic parameters in lakes with a different trophic state. Temperature is another factor likely to substantially influence ciliate succession. According to Beaver & Crisman (1990), the growth and reproduction of freshwater ciliates are strongly correlated with temperature. As shown by previous research by Finlay (1980), the water temperature has an additionally significant influence on the occurrence of groups of ciliates in significantly fertile reservoirs. In the two lakes studied, it was ascertained that temperature had a significant influence on the number of ciliates which were significantly higher in the land/water contact zone in comparison with the other zones. Clearly alternating habitat conditions in that zone could influence the highest numbers of ciliates, which concurs with the studies by Beech & Landers (2002). As a rule, most of the Crytophorida and Scuticociliada occurred in the land/water contact zone or the zone of emergent macrophytes. In the emergent macrophytes/open water zone, the existence of large forms could be limited by waving; the small Scuticociliatada and Oligotrichida were frequent and numerous. In this zone, the factor influencing the number of ciliates in a significant way was concentration of chlorophyll *a*. Generally, the high content of chlorophyll *a* during the summer period was accompanied by an increase in the number of species belonging to the moxotrophic Oligotrichida. It is probable that this is a result of advantageous feeding conditions prevailing in both lakes at this time. In turn, the significant influence of chlorophyll *a* on the number of ciliates observed in lakes of different trophic state (Beaver & Crisman 1990). The results presented show ciliate abundances are impacted by chlorophyll *a* in summer, whilst in spring and autumn the total organic carbon and nutrient concentrations are probably the major regulators of abundance.

It seems that the abundance of periphytic ciliates to the highest degree is determined mainly by temperature, concentrations of organic matter, nutrients and chlorophyll *a*. The results presented show ciliate abundances are impacted by temperature, conductivity, TOC, and nutrients in land/water contact zone. In turn, in the other 2 zones, the influence of chlorophyll *a* concentration increased. However, with the aim of clarifying the understanding of the role of factors conditioning the presence of periphytic ciliates, it is necessary in future research to explain the biotic factors such as, among others, the abundance of bacteria and of microalgae.

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