Development of ciliate community on artificial substrates associated with vertical gradients of environmental conditions in a karstic lake

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Abstract: The composition of ciliate communities, their abundance and biomass were studied on glass slides exposed in the epilimnion (1 m), metalimnion (9 m) and hypolimnion (18 m) in the karstic barrage Visovac Lake (SW Dinarid, Croatia). After one-month exposure periods from May 1995 to February 1996, mean ciliate biomass decreased with depth (at 1 m 54 µg cm^{-2}, at 9 m 37 µg cm^{-2} and at 18 m 28 µg cm^{-2}), whereas the mean population density was relatively uniform (675 Ind. cm^{-2} at 1 m, 592 Ind. cm^{-2} at 9 m, and 678 Ind. cm^{-2} at 18 m). Sessile forms (peritrichs and suctorians) were dominant during all exposure periods and at all three depth layers. Omnivorous ciliates were dominant in the epilimnion, whereas in the metalimnion and hypolimnion bacterivores and carnivores were most abundant. Ciliates inhabiting artificial substrates in the epilimnion had two temporal peaks of density and biomass: in June and in October. In the deeper layers these maxima appeared one or two months later. Seasonal changes in ciliate biomass and community and trophic composition were associated with changes in thermal stratification and vertical oxygen gradients as important abiotic parameters, periphyton biomass as food source, and the tufa deposit determining the properties of substrate.

Key words: ciliates, periphyton, tufa deposit, lakes.

Introduction

Important characteristics of the aquatic systems in the Dinaric Karst region are the formation of tufa and lake sediments. The term "tufa" is commonly used for porous, cool, freshwater CaCO$_3$ deposits (FORD & PEDLY 1996). Typically

it contains the remains of micro- and macrophytes and bacteria. Crystallogenesis has multiple causes including the specific conditions of biomineralization (influenced by the organism to different degrees) and the particular environmental conditions (Golubić 1973, Chafetz & Folk 1984, Freytet & Verrecchia 1998, Chen et al. 2004). The precipitation of calcite generally requires the water to be 5–10 times supersaturated with calcite. This is usually achieved by the removal of CO_2, causing a rise of pH and a shift in the carbonate equilibrium. Carbon dioxide removal can be associated with turbulence, evaporation, mixing of different waters and metabolic uptake of CO_2 by photosynthetic organisms such as cyanobacteria, algae, mosses and plants (Chafetz & Folk 1984, Stumm 1985, Herman & Lorah 1987, Viles & Goudie 1990, Chen et al. 2002). The effect of photosynthesis may be especially significant in tufa deposition in standing water, where CO_2 removal by biogenic activity may be equally or even more important than removal by physico-chemical means (Pedley 2000, Chen et al. 2004).

One very frequent result of intense tufa deposition in the Dinaric Karst is the formation of travertine barriers. The development of travertine barriers causes the differentiation of a river profile into a series of lakes and waterfalls (Golubić 1969). One of these barrage lakes is Visovac Lake in the lower course of the Krka River, where we investigated the associations of periphytic ciliates on homogeneous substrates in vertical gradient of the lake.

The planktonic environment in lakes displays considerable heterogeneity in both the vertical and horizontal plane. Temperature, light climate, nutrients, wind-induced movements and hydraulic throughput vary in both the short term and seasonally in relation to depth. Such factors determine the productivity of a given habitat and the nature and makeup of the heterotrophic and autotrophic assemblages of organisms (Laybourn-Parry 1992). The development of the substrate-associated protozoan communities in stratified lakes depends on the vertical gradient of physical and chemical features of lake water, composition of plankton community, and properties of periphytic community. According to Jones et al. (1976) these communities can be grouped into two categories – hypolimnetic and epilimnetic. They found significantly greater number of species on epilimnetic substrates. Similar trends were found by Zingel & Ott (2000) for planktonic ciliates – in strongly stratified temperate lakes ciliate abundance and biomass were highest in epi-, and lowest in the hypolimnion.

Environmental factors affecting ciliate communities are complex and vary with time and space, and each species has a characteristic life style concerning food habits, growth rate, habitat selection and physiological tolerance. Temperature, dissolved oxygen concentration, pH, nutrient status and trophic state are the most important abiotic factors responsible for different patterns of ciliate distribution and abundance in a spatial and temporal context (e. g. Pratt

et al. 1987, PRIMC & HABDIJA 1987, SOLA et al. 1996, COPPELLOTTI & MATA-RAZZO 2000, BHARATI et al. 2001, LANDERS & PHIPPS 2003). In general, ciliate densities and/or biomass increase with increasing trophic state (HENEBRY & CAIRNS 1984, BEAVER & CRISMAN 1982, MATHES & ARNDT 1994, ZINGEL & OTT 2000, PFISTER et al. 2002, WICKHAM et al. 2004). Moreover, ciliate numbers display strong positive correlations with bacterial abundance (e. g. ZINGEL & OTT 2000, LANDERS & PHIPPS 2003) and with heterotrophic flagellates biomass (e. g. AUER & ARNDT 2001).

PRIMC-HABDIJA et al. (2001) reported that in karstic waters recent tufa deposition and periphyton development were also factors significantly influencing the community properties of periphytic ciliates. The deposition of calcium carbonate modifies the physical structure of the substrate by rough and porous tufa providing a new microsubstrate for settlement. In addition, microtopographical features offer refuges from currents and predators. Several authors have indeed pointed out the importance of the morphological (topographical) structure of a substrate for colonization (e. g. HARPER, 1977, STEVENSON 1983, BAKER 1984, DAVIS & BARMUTA 1989, DENICOLA & MCINTIRE 1990). HARMSWORTH & SLEIGH (1993) concluded that surface irregularities appear to enhance attachment. It is suggested that colonization is determined by available settlement sites, available food, and predation.

In the interaction with thermal, chemical and food source stratification, tufa deposition and periphyton biomass are influential ecological determinants in karstic lakes. If ciliate community structure are partially determined by these characteristics, the communities found on artificial substrates at different depths should be different. Therefore, the objective of this study was to examine seasonal changes in the periphytic ciliate composition, and their biomass after a one-month colonization of artificial substrates exposed in the vertical profile of a karstic barrage lake.

Methods

From April 1995 to February 1996 calcite deposits associated with periphyton biomass and community structure of ciliates in periphyton community were examined on glass slides (2.5 cm by 7.5 cm) exposed at three depths: epilimnion (1 m), metalimnion (9 m) and hypolimnion (18 m) of the karstic barrage Lake Visovac. At each depth, 8 glass slides were placed in a PVC frame. The slides were oriented vertically in order to avoid sedimentation. Once a month, after 30 days of exposure, colonized slides were taken for examination, and renewed. Removed slides were taken to the laboratory in small containers (one for each glass slide) of lake water. In the laboratory, periphyton samples were scraped from the slides and suspended in the lake water. Two replicate slides were used for the analysis of community composition and other slides for the analysis of periphyton standing crop biomass and calcite deposition rate.

Using a phase contrast microscope, ciliates were identified in vivo or with the use of various cytological techniques. In the calculation of population density, two replicates of 10×0.1-ml subsamples were used to determine average values. In the colonial forms (*Carchesium* and *Zoothamnium*), each zooid was counted separately. Cell biomass of identified species was estimated according to FOISSNER & BERGER (1996) assuming that 1 pg fresh weight equals $1 \mu m^3$ biovolume (FENCHEL 1967, FINLEY 1982).

Standing crop of periphyton was determined as ash-free dry weight (AFDW) after 24 h drying at 104 °C and 6 h ashing at 400 °C. Tufa deposition was determined by drying two replicate slides at 104 °C, ashed at 400 °C, weighed and then treated with diluted HCl to dissolve $CaCO_3$ and weighed again. The difference was taken as tufa deposition after an exposure of 30 days. Tufa deposition and periphyton growth rate per day were calculated linearly so that each average value of two replicate samples was divided by 30. Seasonal changes of environmental conditions: temperature, oxygen and CO_2 concentrations and pH were measured according to APHA (1985).

Correlations between total ciliate abundance/biomass and environmental factors were determined using the Spearman rank R calculated with Statistica (StatSoft Inc. 2002). The level of similarity among ciliate communities was determined using cluster analysis (StatSoft Inc. 2002) based on population densities (untransformed data of Ind. cm^{-2}). The tree clustering and complete linkage methods were based on 1-Pearson r as a measure of the distance among the ciliate composition patterns. The relationship between ciliate community composition and environmental variables was investigated by means of Canonical Correspondence Analysis (CCA) developed by TER BRAAK (1986) which was done with PC-ORD software (MCCUNE & MEFFORD 1997). Ordination axes in CCA are linear combinations of environmental variables, and this constrained ordination method creates ordination diagrams in which points represent species and sites, and vectors represent environmental variables. CCA models a unimodal response of the species to the environmental variables along a gradient (TER BRAAK 1986) and therefore is preferred for most ecological data sets. Biplot01.xla macro (LIPKOVICH & SMITH 2002) was used to draw ordination plots from PC-ORD results. Prior to analysis ciliate abundances were log $(x + 1)$ transformed.

Study site

Visovac Lake is located in the karstic region SW of the Dinarid Mountains at about 20 km from the Adriatic coast (Croatia). By origin, Visovac Lake is travertine barrage lake developed in the Quaternary by the formation of the Skradin fluvial travertine barrage in the lower course of the Krka River (Fig. 1). The surface area of the lake is 7.9 km^2 with a maximum depth of 30 m. The epilimnetic water flows over the basic travertine barrier Skradinski Buk, forming a waterfall 47.7 m high (commonly known as Krka Falls). The artificial substrates for our investigations were placed in a vertical profile located approximately 500 m upstream of the basic barrier Skradinski Buk (Fig. 1). Depth in this part of the lake is about 20 m.
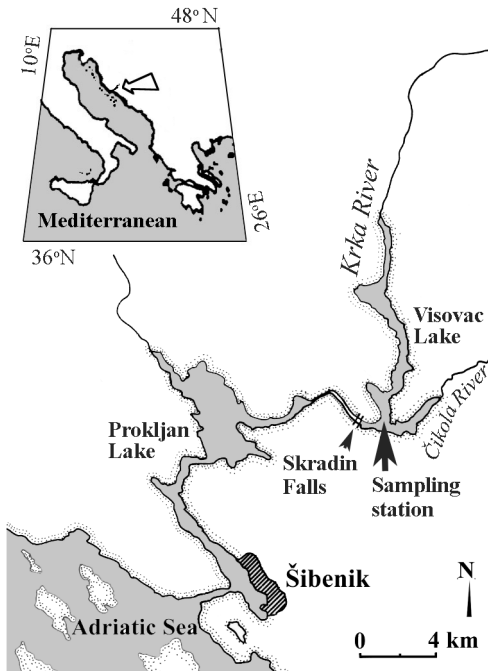
Fig. 1. Visovac Lake is situated in the Krka river in Croatia.

Results

Physical and chemical properties of lake

Lake Visovac is a monomictic lake, the formation of thermal stratification begins in early spring after the winter period of mixing (average temperature in winter 7.3 °C) (Fig. 2). In summer a thermocline can be found at a depth between 4 and 8 m. In 1995, epilimnion temperatures varied from 21 to 23.4 °C, the metalimnion from 15.5 to 18.9 °C and the hypolimnion from 13.7 to 14.5 °C. In the epilimnion the average vertical temperature gradient (ΔT) was out 0.3 °C m^{-1}, in the metalimnion 0.85 °C m^{-1} and in hypolimnion 0.08 °C m^{-1}. Lake Visovac is never ice-covered.

Seasonal changes in the vertical distribution of dissolved oxygen, free carbon dioxide and pH values were affected by thermal stratification and also by the biological activity of plankton community. Oxygen in spring was distributed in a clinograde way, which is characterized by relatively high oxygen content in the epilimnion (Fig. 2). During the study period the maximum concentration was found at a depth of 5 m. Oxygen concentrations declined in the hypolimnetic layer, and in August and September hypoxia were found near the bottom (below 1 mg O_2 l^{-1}). At beginning of mixing in autumn, oxygen con-
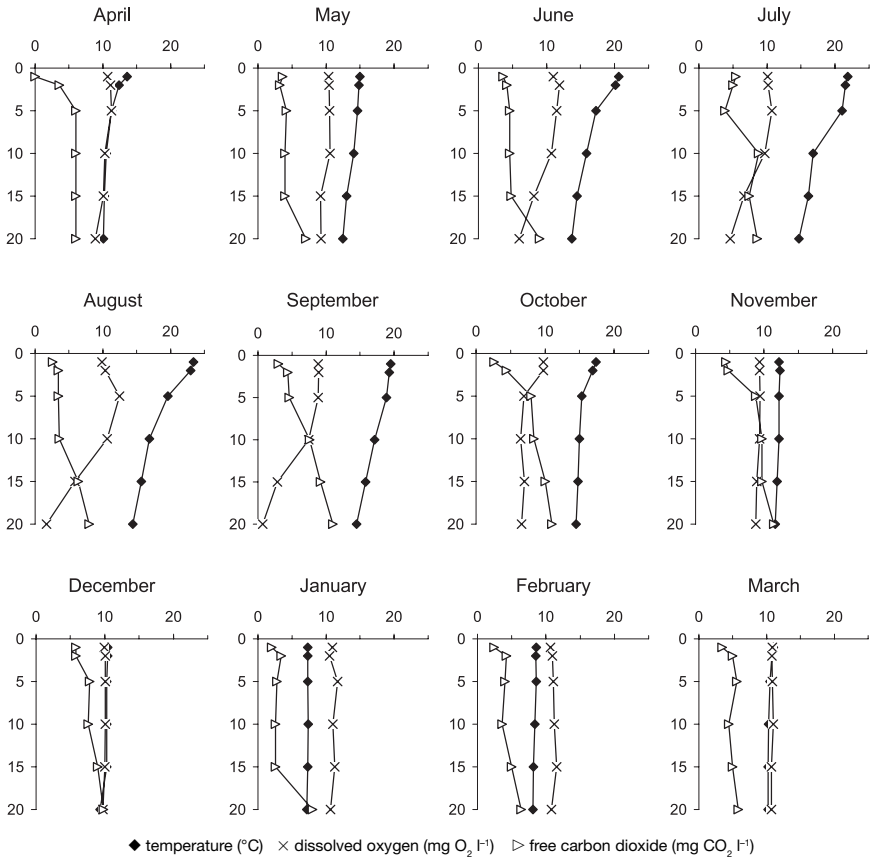
Fig. 2. Seasonal changes in vertical stratification of temperature, dissolved oxygen and free carbon dioxide.

centrations were uniform with depth. Carbon dioxide distribution had an inverse relationship to oxygen. CO_2 concentration decreased in the epilimnion and increased from a depth of 10 m to the hypolimnetic layers. This hypolimnetic increase of CO_2 coincided with a pH decrease (below 8.0). In the epilimnion and metalimnion pH values varied from 7.85 to 8.30 (annual mean value was 8.09), and in the hypolimnion from 7.42 to 8.06 (annual mean 7.73). The Secchi disk transparency of the lake varied from 3 m in June to 8 m in November.

Periphyton growth and tufa deposit rate

Periphyton growth and tufa deposit rate showed a marked decrease with depth (Fig. 3). At a water depth of 1 m the periphyton biomass rate varied in range
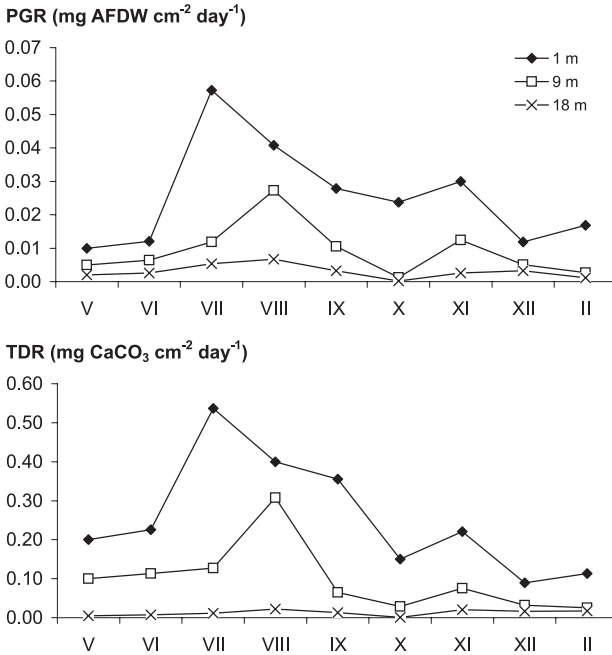
PGR (mg AFDW cm^{-2} day^{-1})

TDR (mg CaCO$_3$ cm^{-2} day^{-1})

Fig. 3. Temporal variations of periphyton growth rate (PGR) and tufa deposition rate (TDR) in the layers of 1 m, 9 m and 18 m. Roman numbers stand for months.

Table 1. Spearman correlation coefficient between biotic and abiotic parameters in Visovac Lake. Significant relationships were defined as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; df = 25.

	Periphyton growth rate	Tufa deposit rate	Temperature	Oxygen	Carbon dioxide	pH
Periphyton growth rate	–		0.515**	0.228	−0.587**	0.725***
Tufa deposit rate	0.868***	–	0.578**	0.484**	−0.708***	0.800***
Ciliate abundance	0.264	0.123	−0.132	0.181	−0.029	0.147
Ciliate biomass	0.518**	0.445*	0.391*	0.070	−0.065	0.407**

from 0.01 to 0.057 mg AFDW cm^{-2} d^{-1} (mean 0.026), at 9 m from 0.0013 to 0.0273 mg AFDW cm^{-2} d^{-1} (mean 0.009), and at 18 m from 0.0012 to 0.0067 mg AFDW cm^{-2} d^{-1} (mean 0.003). In all three depth layers the maximum periphyton growth rate was observed in the summer months. Tufa deposit rate varied in range from 0.09 to 0.54 mg CaCO$_3$ cm^{-2} d^{-1} (mean 0.26) at 1 m, at 9 m from 0.025 to 0.31 mg CaCO$_3$ cm^{-2} d^{-1} (mean 0.097), and at 18 m from 0.001 to 0.022 mg CaCO$_3$ cm^{-2} d^{-1} (mean 0.013). The highest tufa deposition rate was in July at 1 m water depth (Fig. 3). The tufa deposit rate was
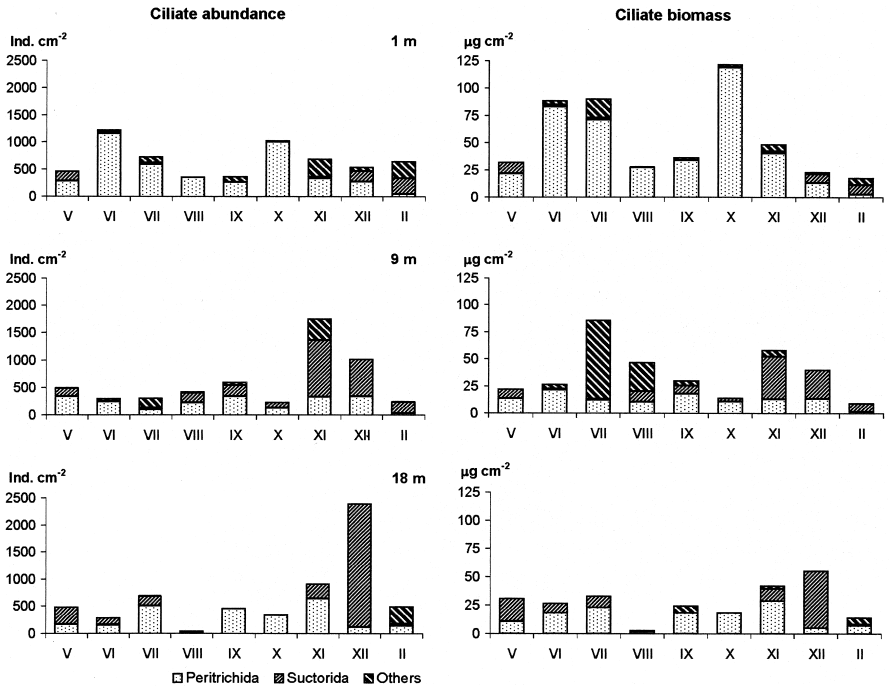
Fig. 4. Ciliate assemblage structure. Left panels: Abundance (Ind. cm^{-2}); Right panels: Wet biomass (μg cm^{-2}).

strongly associated with biotic and abiotic parameters showing positive correlations with periphyton growth rate, temperature, oxygen concentration and pH and negative correlation with the carbon dioxide concentration (Table 1).

Abundance, biomass and community composition of ciliates

The total number of ciliates on the glass plates ranged from 41 Ind. cm^{-2} to 2393 Ind cm^{-2} (Fig. 4). Although average population densities were relatively uniform at all depths (675 Ind. cm^{-2} in 1 m depth, 592 Ind. cm^{-2} in 9 m, and 678 Ind. cm^{-2} in 18 m) the seasonal dynamics of ciliate abundance differed markedly between depths. At 1 m depth the highest populations were observed in June and October, while in the deeper layers the total ciliate abundance was highest in November and December. No significant correlations were found between ciliate abundance and periphyton growth, tufa deposit rate, temperature, pH, oxygen and carbon dioxide concentration (Table 1).

The average biomass of ciliates showed a marked decrease with depth (Fig. 4). In the epilimnion, biomass averaged 54 μg cm^{-2}, in the metalimnion 37 μg cm^{-2} and in the hypolimnion 28 μg cm^{-2}. Two biomass peaks in biomass
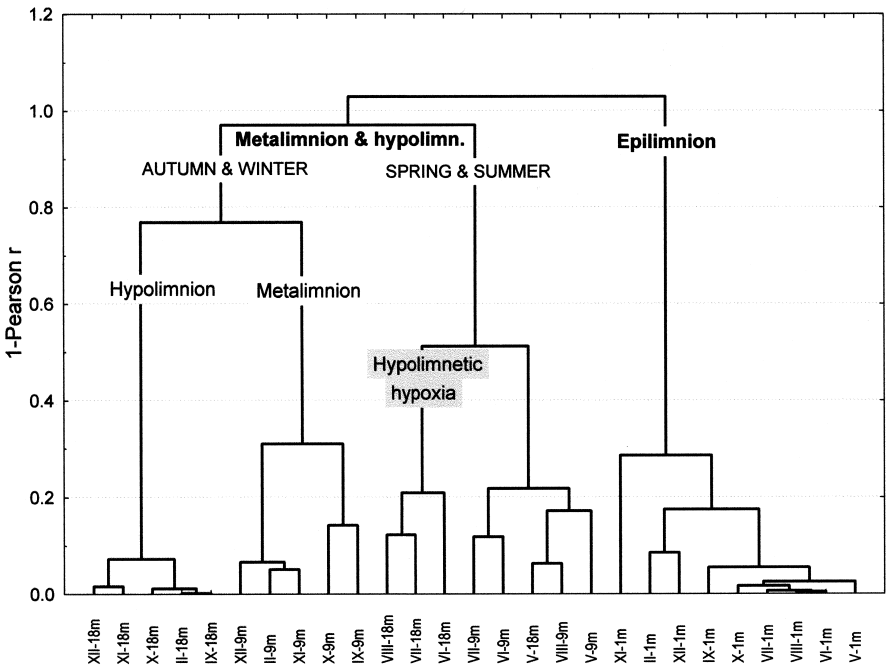
Fig. 5. Cluster analysis of the ciliate associations. Roman numbers stand for months, followed by depth (1 m, 9 m and 18 m).

were observed in the epilimnion, in June and in October. In the deeper layers, these maxima appeared one or two months later. In the metalimnion and hypolimnion the first maximum was in July. In the metalimnion the second maximum was in November and in the hypolimnion in December. Spearman correlation analyses showed that total ciliate biomass significantly correlated with periphyton and tufa deposit rate, pH and temperature (Table 1).

Sessile forms (peritrichs and suctorians) were dominant (Fig. 4). Their overall mean share in total biomass was 87 % (in the epilimnion 90 %, in the metalimnion 80 % and in the hypolimnion 92 %). The most abundant peritrichs were *Vorticella campanula, Vorticella* sp., *Carchesium polypinum* and *Zoothamnium simplex*, and the most abundant suctorians were *Acineta fluviatilis, Metacineta* sp. and *Heliophrya rotunda*.

Ciliates of other groups were rarely present in high numbers or biomass. The exceptions are *Stentor igneus, Paramecium bursaria* and *Uroleptus piscis.* Although they were always observed in low abundances, these relatively large ciliates contributed substantially to the total ciliate biomass in epi- and metalimnion. Other observed ciliates belonged to Prostomatida (*Placus* sp.), Gymnostomatida (*Lacrymaria olor*), Pleurostomatida (*Acineria uncinata, Litonotus* spp., *Hemiophrys* sp.), Nassulida (*Zosterodasys transversa, Leptopha-*
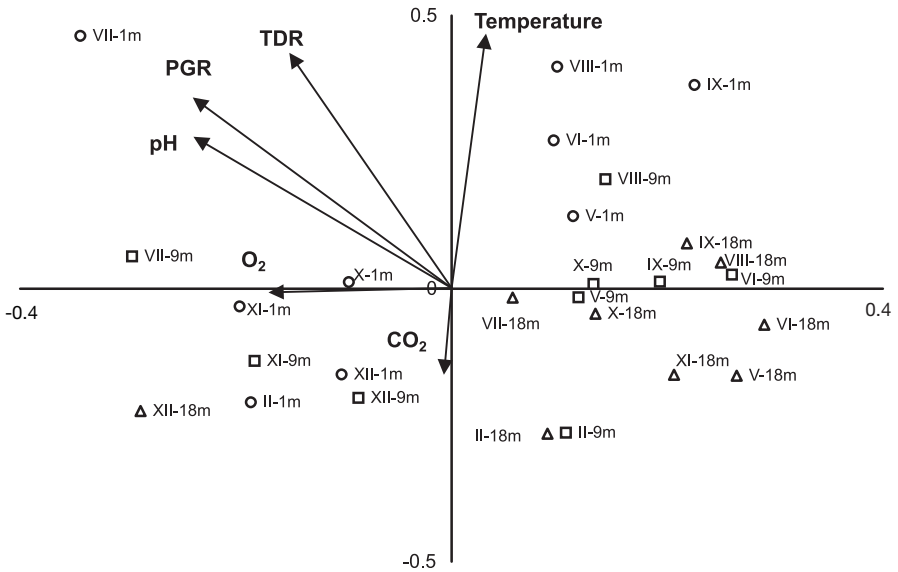
Fig. 6. Canonical correspondence analysis ordination plot for ciliate assemblages and environmental variables. Code for assemblages and environmental variables: ○: epilimnion, □: metalimnion, Δ: hypolimnion, TDR: tufa deposit rate, PGR: periphyton growth rate.

rynx costatus), Cyrthophorida (*Chilodonella* spp., *Trochilia minuta*), Hymenostomata (*Cinetochilum margaritaceum, Frontonia acuminata, Pleuronema* sp., *Uronema nigricans*), Heterotrichida (*Stentor* spp.), and Hypotrichia (*Aspidisca cicada, Euplotes* spp., *Holosticha* spp., *Kerona pediculus, Stylonychia mytilus*-complex*, Tachysoma pellionellum, Uroleptus* sp.).

Omnivorous ciliates represented by *Vorticella campanula* and *Stentor igneus* were dominant in the epilimnion, whereas in the metalimnion and hypolimnion bacterivores (*Vorticella* sp.) and carnivores (*Metacineta* sp. and *Acineta fluviatilis*) were the most abundant.

Cluster analysis, based on complete linkage methods and 1-Pearson r, as a similarity measure, illustrated the clustering of ciliate composition patterns for each of 9 one-month colonization in three depth layers (Fig. 5). At the first level, patterns of ciliates in the epilimnion were separated from the ciliate associations in the periphyton in the metalimnion and hypolimnion. At the second level of clustering the associations of ciliates were separated seasonally. Ciliate assemblages in autumn and winter period represented the first cluster, whereas spring and summer ciliate patterns formed the second cluster. At the third level of clustering there is a separation between ciliate associations colonized in hypolimnetic and metalimnetic layers. Especially worth drawing at-

tention to is the cluster formed by ciliate associations in the hypolimnion characterized by oxygen deficit during the summer months.

The first two axes of the CCA biplot explained 17.2 % of the total variance in the species data (Fig. 6). Species-environmental factor Pearson correlations were CCA1 = 0.89 and CCA2 = 0.90 which indicated high correlation of the environmental variables used in CCA. The most influential variables were periphyton growth rate, tufa deposition rate, pH and temperature. Samples from epilimnion collected during summer months were positioned in upper quadrants, with marked separation of July epiliminon sample characterised by PGR and TDR peaks (Fig. 3). August maximum of the same variables in metalimnion probably influenced ciliate assemblage making it more similar to those in epilimnion (Fig. 6). Samples taken during late autumn and winter were mostly positioned in lower left and right quadrant, even those from 1 m depth, indicating the strong influence of low water temperatures. Metalimnion and hypolimnion samples from summer and early autumn are concentrated along right side of CCA 1, directly opposite to dissolved oxygen vector, suggesting that low oxygen concentration influenced those ciliate communities.

Discussion

Ciliate abundances on exposed artificial substrates ranged from 40 to 2400 Ind. cm^{-2}. For comparison, on glass slides exposed in lotic biotopes (in the same period as these experiments) at the travertine barrier Skradinski Buk downstream of Visovac Lake, ciliate numbers were from 70 up to 879 Ind. cm^{-2} (PRIMC-HABDIJA et al. 2001). Samples from the metalimnion generally had greater species richness than those from the epi- and hypolimnion although, in both lotic and lentic waters, the method of protozoan dispersal to the substrates is assumed to be by water currents, and the major source (or epicentre) is assumed to be the benthos (CAIRNS & HENEBRY 1982). JONES et al. (1976) observed that epilimnetic communities took longer to reach equilibrium, but then achieved higher species numbers. In contrast, BEECH & LANDERS (2002) proposed that the physical environment of sites had an impact on the protozoan's ability to colonize the glass slide; they observed higher total ciliate densities at deeper sites, with less wave action and physical stress.

In our study sessile ciliate genera, peritrichs and suctorians, were dominant, and are commonly found also in other habitats (FENCHEL 1987, HARMSWORTH & SLEIGH 1993, COPPELLOTTI & MATARAZZO 2000, BEECH & LANDERS 2002, LANDERS & PHIPPS 2003). They colonize even micro- and macroaggregates in rivers and estuaries (ZIMMERMANN-TIMM 2002). These stalked ciliates, together with abundant *Stentor* species, can persistently influence the three-dimensional structure of periphyton (MARTÍN-CERECEDA et al. 2001,

ARNDT et al. 2003). In addition to this impact on periphyton architecture, ciliates can enhance the transport of biochemically important solutes into and within the biofilm layer (GLUD & FENCHEL 1999). Modification of physical and chemical properties in periphyton is very important in tufa deposition processes too. Furthermore, trophic interactions between periphyton and surrounding media are also important. Dominant peritrichs are filter feeders (FENCHEL 1987). They are bacterivorous, and some species could also feed on minute suspended algae (FOISSNER & BERGER 1996). Suctoria are carnivorous and feed on other ciliates. WEITERE et al. (2003) demonstrated a tight linkage between plankton and periphyton and pointed to the quantitative importance of the periphyton in shallow lakes and streams with extensive firm substrata where it can reduce the plankton significantly.

The differences in ciliate number, biomass and composition between epilimnetic, metalimnetic and hypolimnetic layer clearly differentiate these areas as distinct environments within the lake basin. Greater periphyton biomass can offer more food for all trophic types of ciliates because of autochthonic development of organic matter and retention of allochthonous detritus. Probably, this is not a linear progression. At higher periphyton biomass an abundant metazoan community could develop, which could lead to competition for food and space, and higher predatory pressure on ciliates. WICKHAM et al. (2004) reported nonsignificant or even positive responses to grazer presence, whereas others found negative effects of macrozoobenthos and meiobenthos on ciliate abundance and biomass (TAYLOR 1980, McCORMICK & CAIRNS 1991, BOTT & BORCHARDT 1999, WICKHAM et al. 2000).

Cluster analysis suggested that temporal and spatial changes in ciliate composition were influenced by temporal changes of thermal and oxygen stratification and also that the gradient of tufa deposit and periphyton overgrow rate could also affect seasonal differences between ciliate associations on slides exposed in the three depth layers. CCA also separated ciliate assemblages and recognized periphyton growth rate, tufa deposition rate, pH and temperature as most important factors structuring ciliate communities (see also STEWART et al. 1986 and LANDERS & PHIPPS 2003). High correlation between those variables (Fig. 6, Table 1) makes indirect effect of specific variables difficult to disentangle. Beside summer epilimnion samples, CCA also separated ciliate assemblages seasonally, with strong clustering of samples collected in metalimnion and hypolimnion during months with lower dissolved oxygen concentrations.

It can be concluded that seasonal changes of ciliate biomass and its community and trophic composition correlated with thermal stratification and vertical gradient of oxygen as the important abiotic parameters, periphyton biomass as food source, and tufa deposition, which determines the properties of substrate.

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