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Driving factors affecting spatial and temporal variations in the structure of phytoplankton functional groups in a temperate reservoir

by

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Abstract

During the twenty-five years of existence, water quality has declined and severe blooms of cyanobacteria have occurred in the Grlište Reservoir. Changes in phytoplankton functional groups over time and along horizontal and vertical gradients were investigated in the course of a one-year study in this water-supply reservoir. We identified 19 dominant taxa, classified into 12 phytoplankton associations. The presence of the codons C, P, D and S1 differentiated the transitional from the lacustrine part of the reservoir. The nitrogen-fixing cyanobacteria *Dolichospermum viguieri* dominated the phytoplankton community in the epilimnion during August and September, when the reservoir showed P-limitation, but the bloom was not observed. The driving factors that accounted for the main variability in phytoplankton functional groups along the seasonal and vertical profile were identified using the direct gradient analysis (RDA). Our results revealed the importance of two bipolar factors. The first factor explained the variability in phytoplankton due to thermal stratification and physical mixing, each process affecting the algal community in contrasting ways. The second factor was interpreted as reduction vs. oxidation processes. Positive correlation between stratification and water pumping by a drinking water plant indicated that human activities were not severe enough to break down the thermal stability of the reservoir and to cause a cyanobacterial bloom.

Key words: cyanobacteria, drinking water plant, functional groups, phytoplankton, reservoir

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Introduction

The distribution and dynamics of phytoplankton in lakes are conditioned by numerous factors, but depend on place and time, some of them are likely to be relatively more important than others (Reynolds 2000). This also applies to man-made lakes or reservoirs that are similar to natural lakes with regard to limnology (Jørgensen et al. 2005), species composition and habitats (Kalff 2002). Nevertheless, reservoirs have their own characteristics that can influence the spatial and temporal distribution of pelagic assemblages. These waterbodies usually have a shorter water residence time, more pronounced hydrological variations and longitudinal heterogeneity, represented by riverine, transition and lacustrine zones, each with different characteristic features (Straškrábová et al. 2005).

The periodicity of phytoplankton in lacustrine parts of reservoirs shows similarities with the PEG-model of seasonal development observed in lakes (Sommer et al. 1986). The authors of this model stress the importance of biological interactions (e.g. competition, grazing and predation) in phytoplankton succession, but they also state that physical factors can be responsible for changes in species composition. The thermal-physical dynamics of a water column are particularly important to pelagic communities in deep lakes and reservoirs. Recently, many studies have referred to the hypothesis that thermal stratification and/or mixing regime plays a crucial role in the phytoplankton dynamics in the pelagic zone via its impact on nutrients and light availability (Becker et al. 2009, Winder et al. 2009, Becker et al. 2010, Xiao et al. 2011). These physical processes are controlled by climatic conditions and there is evidence that recent climate changes are connected with a more intensified stratification and shift toward smaller-sized diatom species (Winder et al. 2009).

The exploitation of water resources affects the phytoplankton succession in reservoirs through different mechanisms. Intensive water consumption is often followed by extreme water level fluctuations which can lead to a breakdown of thermal stratification and a collision between deoxygenated, but nutrient-rich hypolimnetic waters, and upper nutrient-limited oxygenated layers (Naselli-Flores, Barone 2005). In the Liuxihe reservoir, artificial drawdown of water affects the light regime via the diffusion of suspended particles from the shore to the pelagic zone (Xiao et al. 2011). Many studies have shown that hydrological variations play an important role in the seasonal dynamics of phytoplankton; however, only a few of these have analyzed the relationship between human-influenced water discharge and the driving factors of plankton communities in temperate reservoirs (Komárková et al. 2003).

In our study, the structure of phytoplankton was analyzed using the functional associations of species proposed by Reynolds et al. (2002). These groups are based on species that share common physiological, morphological and ecological adaptive features and that contribute >5% to the total phytoplankton biovolume. Numerous applications and higher discriminatory power, compared to traditional taxonomic groups (Kruk et al. 2002), motivated us to apply the functional classification in the multivariate approach to phytoplankton analysis.

The primary objectives of the present study were therefore as follows: (i) to identify the dominant driving forces that account for the main variability in phytoplankton functional groups along the seasonal and vertical profile of the reservoir; (ii) to determine if there is a difference in phytoplankton assemblages along the longitudinal axis of the reservoir; (iii) to analyze the relationship between the water usage by the drinking water plant and the identified driving factors of the phytoplankton community.

Materials and methods

Study site

Grlište Reservoir is a small water body with a maximum volume of 12×10^6 m^3 and a water retention time of 158 days (calculated as the ratio of the storage volume to the total water discharge over the study period; pumped and released daily flow rates were available). The reservoir was created in 1989 by damming the Grliška River. The average depth of the reservoir is 6 m, while the maximum depth is 22 m. It has a surface area of 110 ha at 193 m above sea level (the full storage level). The reservoir receives water from an area of about 178 km^2 and has two major tributaries, the Lenovačka and Lasovačka rivers. The basin is located in an

area with a distinctive continental climate, with an average summer and winter air temperature of 27°C and 4°C, respectively (Stanković 2005). The reservoir is monomictic due to pronounced stratification taking place only during summer months. During the phase of designing, building and filling with water, the main purpose of the reservoir was to act as a water supply source, although over time, it has become polyfunctional. Besides its main function, the reservoir is also used for flood control and sport fishery. The water of the Grlište Reservoir is meso- to eutrophic with occasional occurrence of cyanobacterial blooms (Svirčev et al. 2007).

Sampling

Water samples were collected monthly from April 2012 to March 2013 at two sites: Site 1: the transitional zone (43°48'58"N; 22°13'12"E) and Site 2: the lacustrine zone (43°48'44"N; 22°13'55"E) (Fig. 1). Due to the inaccessibility of the sites – high snow cover and harsh conditions – sampling was not done during December. Samples for physical, chemical and algological analyses were taken using a Ruttner water sampler from the following depths: Site 1: 0 m, 5 m, 10 m and above the bottom (15 m); Site 2: 0 m, 5 m, 10 m and 20 m. Samples for phytoplankton qualitative analysis were collected by pulling a plankton net (mesh size of 22 µm) from the bottom to the water surface. Phytoplankton samples were transferred to plastic bottles and immediately preserved in Lugol's iodine solution for quantitative analysis and in 4% solution of formaldehyde for qualitative analysis.

Field measurements and analysis of physical and chemical water parameters

Measurements of water temperature (T), pH and dissolved oxygen (DO) were conducted in situ using a MULTI 340i/SET (WTW, Weilheim, Germany) water field kit, while turbidity (TURB) was recorded using a HI 93703 turbidity meter (Hanna Instruments, USA). Transparency (TRANS) was measured with a Secchi disc. The euphotic zone (z_{eu}) was calculated as 2.7 times the Secchi depth (Cole 1983). Physical and chemical water parameters were analyzed at the Institute of Chemistry, Technology and Metallurgy (University of Belgrade) and the Public Water Company in Zaječar. The

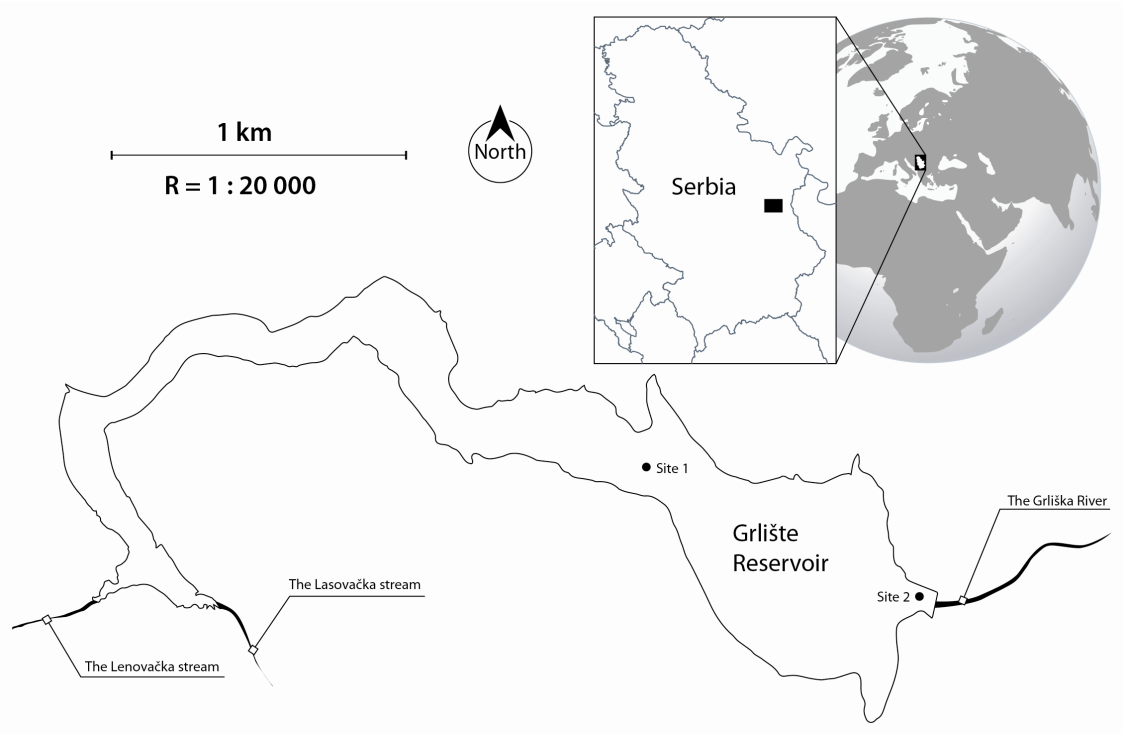

Figure 1
Map and location of the sampling sites for the Grlište Reservoir

concentration of dissolved biodegradable organic matter (COD_{KMnO4}) in the water was determined as $KMnO_4$ consumption by Kübel-Tiemann titration (SZZZ 1990). Total ammonia nitrogen ($N\text{-}NH_4^+$), nitrite nitrogen ($N\text{-}NO_2^-$) and orthophosphate ($P\text{–}PO_4^{3-}$) were analyzed with a UV Vis spectrophotometer (PerkinElmer Inc., Waltham, USA) after direct nesslerization (APHA 1992), after the formation of azo dye (APHA 1998a) and using the ammonium molybdate method (APHA 1998b), respectively. Nitrate nitrogen ($N\text{-}NO_3^-$) and sulfate (SO_4^{2-}) concentrations were measured using a Dionex 2020i ion chromatograph (Dionex Co., Sunnyvale, USA). Chloride anions (Cl^-) were analyzed by silver nitrate titration with a chromate indicator following ISO 9297 (1989). Determination of heavy metals, manganese (Mn) and iron (Fe) was based on flame atomic absorption spectrometry (APHA 1995) using AAnalyst 200 (PerkinElmer Inc., Waltham, USA). Chlorophyll *a* (CHL*a*) was determined according to ISO 10260 (1992). The data about the amount of water taken for the drinking water production (UPTAKE, expressed as $l\ s^{-1}$) were provided by the Public Water Company in Zaječar. Relative water column stability (RWCS) was used to evaluate the strength of water column stratification. RWCS was calculated using the following formula: $RWCS=(D_b\text{-}D_s)/(D_4\text{-}D_5)$, where D_s is the water density at the surface, D_b is the water density at the bottom and D_4 and D_5 are water densities at 4°C and 5°C, respectively (Padisák et al. 2003). Water density was calculated from water temperature values (points: 0, 3, 5, 10, 15 and 20 m) using the DensiCal v. 0.15 software and based on the IAPWS-95 formula, taking into account pressure changes according to depth.

Phytoplankton analyses

Algae and cyanobacteria were identified using a Zeiss Axioimager M1 microscope with a camera system and AxioVision 4.8 software (Carl Zeiss, Jena, Germany) at 400× and 2500× magnifications. Phytoplankton individuals were counted in a chamber (Hydro-Bios, Kiel, Germany) using an inverted Leica DM IL (Wetzlar, Germany) microscope at 400× magnification and following the method of Utermöhl (1958). Biovolume was obtained using geometric approximations of individual algal cells (Hillebrand et al. 1999). Species density was multiplied by the volume of a single cell and considering the mean dimensions of 25 individual specimens of each species. Phytoplankton functional groups were defined according to Reynolds et al. (2002) and Padisák et al. (2009), using the species that contributed at least 5% to the total phytoplankton biovolume in at least one sample.

Data analysis

Prior to statistical analysis, all parameters were tested for normality (Shapiro-Wilk test) using the statistical package STATISTICA 6.0 (Statsoft, Inc., Tulsa, USA). Based on the normality test, RWCS, TURB, $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ and $P\text{-}PO_4^{3-}$ were log transformed, DO was square-root transformed, while Fe and Mn were not included in the analysis. The phytoplankton density was transformed using $\sqrt{(\log X)}$. Indirect and direct gradient analyses with graphical outputs were performed using the program CANOCO 4.5 (ter Braak, Šmilauer 2002). DCA was used to check whether linear or unimodal techniques were more appropriate for data analysis. The length of the gradient along the first DCA axis was always <2, indicating a linear response, therefore, the redundancy analysis (RDA) was used. More precisely, RDA was used to directly explain changes in the biovolume of the identified phytoplankton functional groups according to changes in month and sampling depth. Since measured variables were expressed in different units, RDA based on the correlation matrix was selected. All variables were centered (subtracting the mean) and standardized (divided by standard deviation). The significance of the first ordination axis and all axes together was tested using the Monte Carlo test with 999 permutation. Physical and chemical water parameters were used as supplementary variables.

Results

Physical and chemical water parameters

During the course of this study, the reservoir underwent a seasonal cycle of stratification that was dependent on changes in the water temperature. At the beginning of spring, in March, there were no signs of stratification and mixing was present in the entire water column. Starting from April, mixing

slowed down and the onset of stratification was visible in May (Figs 2, 3). The temperature difference increased from the surface to the bottom, with the biggest changes occurring in the layer up to a 5 m depth. From July to September, the development of two distinct strata was observed: the formation of epilimnion and hypolimnion. Thermocline was formed between a depth of 5 m and 10 m. By November, the stratification disappeared and vertical mixing remained until spring the following year.

The highest water levels in the lake were observed during spring with the maximum recorded in May. Water levels steadily dropped during summer and autumn, and the minimum level was recorded in November. The water level difference between the maximum and minimum values was 2.54 m. The average euphotic zone (z_{eu}) was 4.6 m. In August, the highest z_{eu} reached a depth of 8.4 m at Site 2, while in March the lowest z_{eu} was 2.7 m, recorded at Site 1.

DO levels changed as a result of water stratification, while they remained relatively stable during mixing

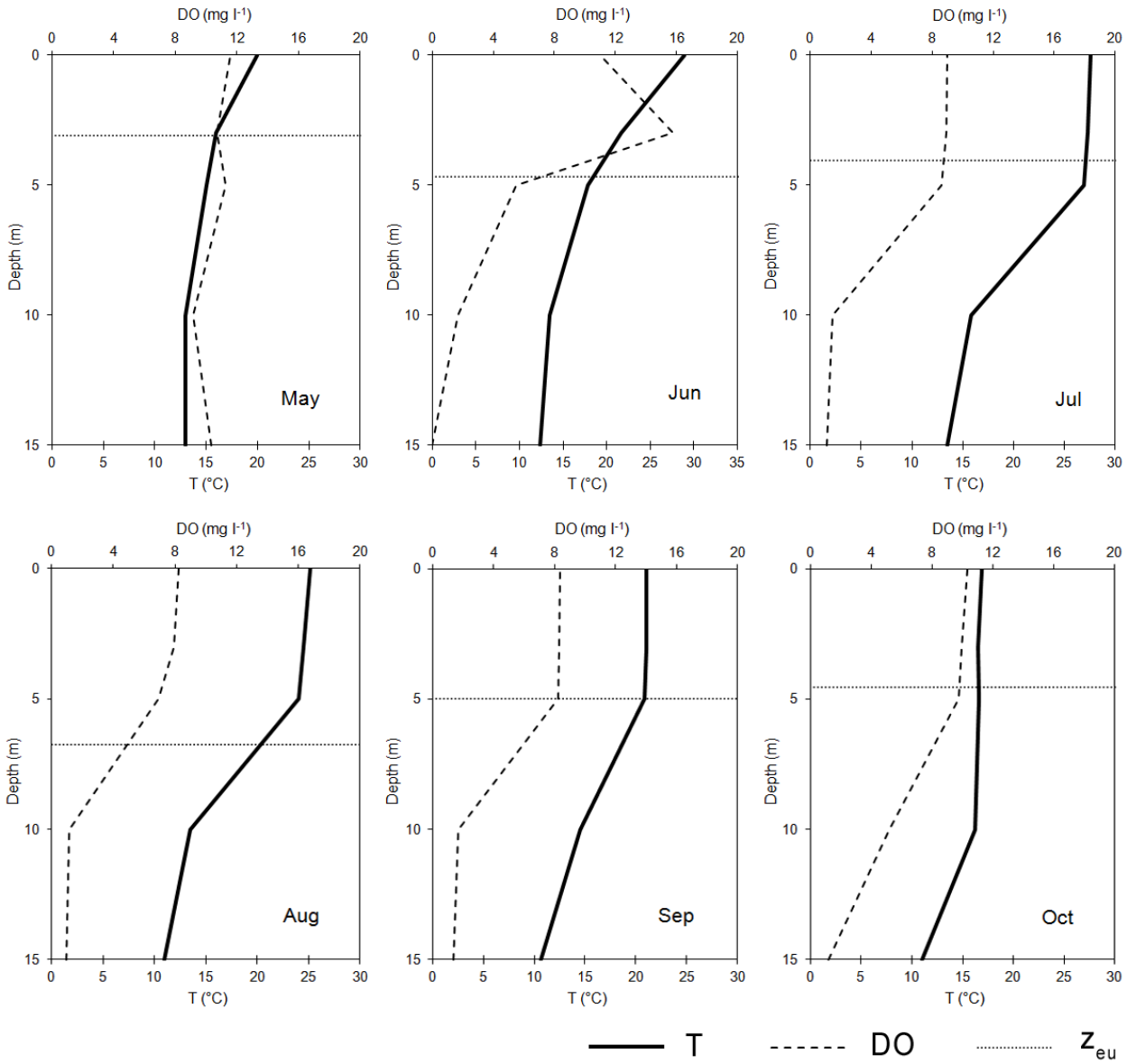

Figure 2

Vertical profiles of water temperature (T) and dissolved oxygen (DO) during the period of stratification (May to October) at Site 1. The euphotic zone (z_{eu}) is represented by the dotted line.

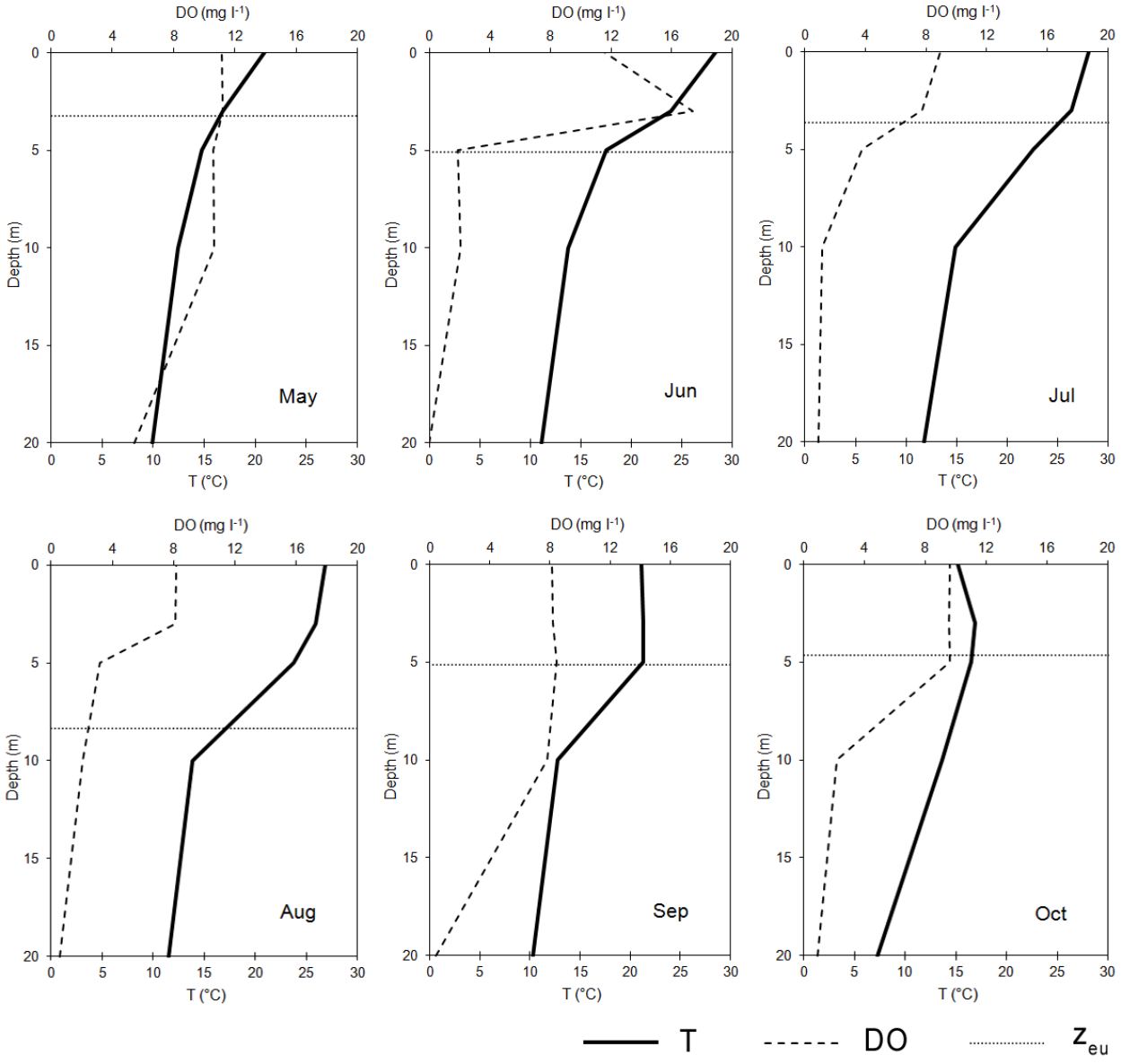

Figure 3

Vertical profiles of water temperature (T) and dissolved oxygen (DO) during the period of stratification (May to October) at Site 2. The euphotic zone (z_{eu}) is represented by the dotted line.

periods. Summer thermal stratification induced the highest DO concentrations at the surface (Table 1 and 2). These concentrations abruptly dropped to a depth of 5 m and then gradually from 5 m to the bottom. The maximum DO concentrations (13.2 mg l^{-1}) occurred at the surface in January, while anoxia was present in June at both sites near the bottom.

$N\text{-}NH_4^+$ and $N\text{-}NO_2^-$ were the lowest at the surface with a gradual increase toward the bottom, where they reached the maximum values. During the period of stratification, $N\text{-}NH_4^+$ was considerably higher at 10 m and near the bottom, while its concentrations were low at the surface and at a depth of 5 m. Within the entire depth range, $N\text{-}NO_3^-$ was higher during the mixing period than during stratification. The maximum $N\text{-}NO_3^-$ values (0.72 to 0.82 mg l^{-1}) occurred in March.

At Site 2, SO_4^{2-} levels were significantly lower at all depths in August. During the same month,

Table 1

Ranges of physical and chemical water parameters at Site 1

Variable		Depth (m)			
	Unit	0	5	10	15
Temperature	°C	6.7–28.9	6.4–26.9	6.4–16.2	6.5–13.5
TRANS	m	1.0–2.5			
TURB	NTU	2.0–5.5	2.3–11.0	2.2–10.5	1.5–12.5
pH		6.9–8.4	7.4–8.3	7.3–8.3	6.8–8.3
COD_{KMnO_4}	mg O_2 l^{-1}	2.37–3.90	2.60–3.71	2.45–3.79	2.31–3.39
DO	mg l^{-1}	8.2–13.0	5.5–12.5	1.1–12.7	0.0–12.5
$N–NH_4^+$		0.07–0.30	0.08–0.31	0.10–0.49	0.09–2.91
$N–NO_2^-$		0.00–0.02	0.00–0.02	0.00–0.02	0.00–0.06
$N–NO_3^-$		0.04–0.72	0.02–0.67	0.00–0.65	0.00–0.66
$P–PO_4^{3-}$		0.00–0.02	0.00–0.02	0.00–0.03	0.01–0.06
Cl^-		4.2–8.0	6.0–9.0	5.6–8.0	6.0–9.0
SO_4^{2-}		1.5–28.7	5.3–26.1	16.4–25.3	17.5–31.5
Fe		0.00–0.23	0.01–0.29	0.02–0.30	0.08–0.38
Mn		0.00–0.02	0.00–0.04	0.00–0.30	0.00–1.34
CHL*a*	µg l^{-1}	2.21–17.66	2.02–16.21	1.27–10.58	1.01–8.24

Table 2

Ranges of physical and chemical water parameters at Site 2

Variable		Depth (m)			
	Unit	0	5	10	20
Temperature	°C	3.5–28.4	4.0–23.8	3.5–14.9	4.0–11.8
TRANS	m	1.1–3.1			
TURB	NTU	1.6–10.0	1.8–12.8	1.2–10.5	4.1–40.7
pH		7.4–8.4	6.9–8.3	6.7–8.3	7.2–8.3
COD_{KMnO_4}	mg O_2 l^{-1}	1.78–4.05	2.09–4.21	2.37–3.30	2.43–3.63
DO	mg l^{-1}	8.1–13.2	1.9–12.9	1.2–12.5	0.0–12.0
$N–NH_4^+$		0.06–0.26	0.06–0.44	0.08–0.60	0.08–3.63
$N–NO_2^-$		0.00–0.02	0.00–0.02	0.01–0.07	0.00–0.10
$N–NO_3^-$		0.03–0.82	0.03–0.67	0.00–0.65	0.00–0.63
$P–PO_4^{3-}$		0.00–0.02	0.00–0.02	0.00–0.02	0.00–0.22
Cl^-		6.0–7.4	4.8–7.2	6.0–8.0	5.6–9.0
SO_4^{2-}		1.6–29.4	3.2–29.0	0.9–29.0	1.0–29.9
Fe		0.01–0.25	0.00–0.30	0.02–0.23	0.05–0.95
Mn		0.00–0.03	0.00–0.03	0.00–0.15	0.02–1.28
CHL*a*	µg l^{-1}	2.02–12.90	2.02–10.32	1.15–6.57	1.01–7.04

SO_4^{2-} was also low at Site 1 at the surface and at a depth of 5 m. The highest values of Fe and Mn were recorded near the bottom in October and November. Chlorophyll *a* concentrations ranged from 1.01 to 17.66 µg l^{-1}. High values were observed at the surface and at a depth of 5 m.

Phytoplankton analysis

Nineteen dominant phytoplankton taxa were recorded in the Grlište Reservoir. They were classified into 12 functional groups: B, C, D, N, P, S1, Y, F, G, J, H1 and Lo (Table 3) based on morphological, physiological and ecological criteria proposed by

Table 3

List of species with >5% contribution to the total phytoplankton biovolume in at least one sample, with their maximum biovolume contribution (%) and functional groups

Codon	Species	Site 1				Site 2			
		Depth (m)							
		0	5	10	15	0	5	10	20
B	*Cyclotella ocellata* Pantocsek	97.1	97.7	95.8	97.6	97.7	96.1	99.2	96.0
C	*Cyclotella ocellata* Pantocsek								
	Asterionella formosa Hassall	0.0	6.8	0.3	0.2	0.1	1.0	0.3	1.5
D	*Ulnaria ulna* (Nitzsch) P.Compère	1.2	2.2	5.5	5.2	4.0	1.8	2.6	4.6
N	*Cosmarium abbreviatum* Raciborski	0.1	3.4	0.3	0.0	25.1	1.3	0.3	0.8
	Cosmarium tenue W.Archer	8.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P	*Closterium acutum* var. *variabile* (Lemmermann) Willi Kreiger	11.6	10.8	15.8	1.2	1.6	3.9	2.0	0.5
	Staurastrum sp.	4.4	5.4	4.9	8.2	0.6	0.2	3.5	3.0
S1	*Phormidium* sp.	0.0	0.9	0.3	16.0	0.0	0.1	0.0	1.6
Y	*Cryptomonas erosa* Ehrenberg	4.5	4.9	54.0	0.0	0.7	31.0	0.2	1.4
	Cryptomonas ovata Ehrenberg	0.1	0.0	0.0	0.0	3.4	0.0	11.7	0.0
F	*Coenococcus planctonicus* Korshikov	1.4	1.4	0.8	7.7	2.7	2.4	1.2	0.2
	Sphaerocystis planctonica (Korshikov) Bourrelly	13.6	6.0	0.1	0.2	17.1	8.9	0.5	0.0
G	*Carteria* sp.	9.8	5.4	5.5	4.9	20.6	2.5	2.3	2.6
	Eudorina elegans Ehrenberg	0.0	0.0	0.0	0.0	0.0	0.1	1.4	11.0
J	*Pediastrum duplex* Meyen	3.6	46.7	69.8	6.5	5.0	7.9	67.6	31.1
H1	*Dolichospermum viguieri* (Denis & Frémy) Wacklin, L.Hoffmann & Komárek	18.1	7.3	0.4	0.3	15.1	11.2	1.5	0.3
Lo	*Ceratium hirundinella* (O.F.Müller) Dujardin	18.0	32.9	17.7	23.0	6.9	32.2	4.0	1.2
	Peridiniopsis cunningtonii Lemmermann	5.7	0.0	2.3	0.0	2.8	0.0	1.6	13.1
	Parvodinium umbonatum (Stein) S.Carty	0.7	0.2	0.0	0.0	8.0	2.0	0.4	0.1

Reynolds et al. (2002) and Padisàk et al. (2009). All mentioned FG were found at Site 1, while the representatives of four codons (C, P, D, S1) did not exceed the 5% threshold in the total phytoplankton biovolume at Site 2.

During most of the study period, a few diatom species (*Cyclotella ocellata* Pantocsek, *Handmannia comta* (Ehrenberg) Kociolek & Khursevich and *C. planctonica* Brunnthaler) dominated the phytoplankton community at both sites in the reservoir. In addition to diatoms of the group B, the codons which showed relative biovolumes >30% in the Grlište Reservoir were within the chlorophytes (J), cryptophytes (Y) and dinoflagellates (Lo). *Pediastrum duplex* Meyen was classified into group J and dominated the phytoplankton community in epilimnion at Site 1 in June with >69% of the total phytoplankton biovolume. Members of group Y, *Cryptomonas erosa* Ehrenberg and *C. ovata* Ehrenberg had the highest abundance and relative biovolume at a depth of 10 m, 54% in July and 11.7% in June, respectively (Table 3). The large dinoflagellates belonging to group Lo were often recorded in summer months in the Grlište Reservoir. *Ceratium hirundinella* (O.F.Müller) Dujardin was the most important with >32% of the total phytoplankton biovolume observed at a depth of 5 m.

In addition to codon J, chlorophytes identified in the Grlište Reservoir included representatives of two groups of differently adapted phytoplankton species. Colonial green algae, observed in clear epilimnion in June (*Coenococcus planctonicus* Korshikov and *Sphaerocystis planctonica* (Korshikov) Bourrelly) were classified into group F. In contrast, group G was built around motile green algae. The highest contribution of *Carteria* sp. was recorded in April at the surface, when it reached 9.8% and 20.6% of the total phytoplankton biovolume at Site 1 and 2, respectively.

Dominant cyanobacteria recorded at Grlište Reservoir were members of two codons occurring in the different water layers. Group H1 included the species *Dolichospermum viguieri* (Denis & Frémy) Wacklin, L.Hoffmann & Komárek, adapted to life

in transparent layers of stratified low-nitrogen lakes. Different shade-adapted cyanobacteria of the order Oscilatoriales were found near the bottom (*Geitlerinema amphibium* (C.Agardh ex Gomont) Anagnostidis, *Jaaginema subtilissimum* (Kützing ex Forti) Anagnostidis et Komárek, *Komvophoron minutum* (Skuja) Anagnostidis & Komárek and *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová & Cronberg); however, because of their low biovolume contribution, the S1 group was built around species of the genus *Phormidium* Kützing ex Gomont (>16% of the total phytoplankton biovolume).

Phytoplankton composition explained by month and depth

The first two RDA axes explained 45.5% of the variance within the functional groups and 66.1% of the functional groups-month and depth relation at Site 1 (Table 4). The first axis was positively correlated with June and negatively correlated with April, and can be interpreted as stratification vs. mixing (Table 5). Among supplementary environmental variables, UPTAKE was positively correlated with the stratification process, while TURB and DO were positively correlated with spring mixing (Fig. 4). Codons J, N and P were located at the right side of the graph (stratification development), while D and G were positioned on the left side and toward mixing conditions. The second axis was positively correlated with the maximum depth of 15 m and can be interpreted as the result of reduction vs. oxidation processes (Table 5). Similar results were obtained

Table 4

Summarized results of redundancy analysis (RDA) of the sampling month and depths explaining the structure of phytoplankton functional groups at Site 1

Axes	1	2	3	4
Eigenvalues	0.301	0.153	0.082	0.053
Species-environment correlations	0.898	0.929	0.902	0.731
Cumulative percentage variance				
of species data:	30.1	45.5	53.7	59.0
of species-environment relation:	43.8	66.1	78.1	85.8
Total variance				
Sum of all eigenvalues	1.000			
Sum of all canonical eigenvalues	0.688			

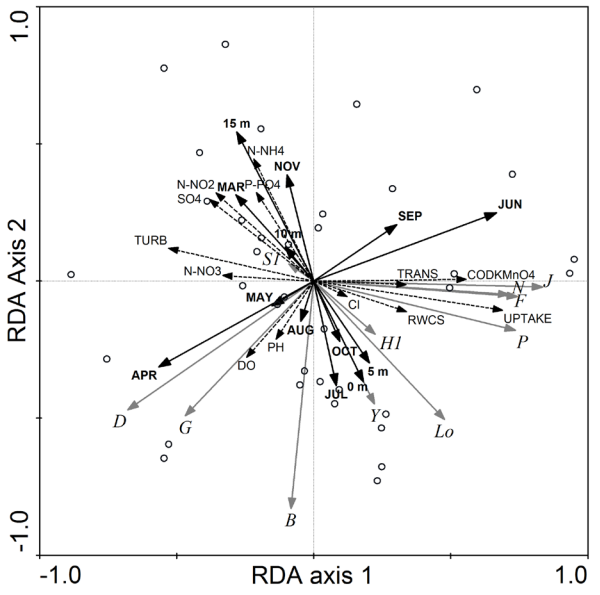

Figure 4

RDA triplot (the first two axes). circles – samples, solid black vectors – environmental variables – month (MAR = March, APR = April, etc.) and depth (0 m, 5 m, 10 m and 15 m), solid grey vectors – response variables – phytoplankton functional groups (B, D, N, P, S1, Y, F, G, J, H1 and Lo), and dashed vectors supplementary variables – physical and chemical parameters.

Table 5

Correlations of environmental variables and first two redundancy axes for Site 1

	Axis 1	Axis 2
0	0.16	-0.34
5	0.18	-0.28
10	-0.09	0.12
15	-0.25	**0.50**
APR	**-0.51**	-0.29
MAY	-0.14	-0.08
JUN	**0.60**	0.23
JUL	0.07	-0.35
AUG	-0.04	-0.14
SEP	0.27	0.19
OCT	0.09	-0.21
NOV	-0.09	0.36
MAR	-0.25	0.29
Interpretation	Stratification vs. mixing	Reduction vs. oxidation

Coefficients in bold were used for interpretation.

when phytoplankton composition was explained by the seasonal and spatial effect at Site 2 (Table 6).

Table 6

Summarized results of redundancy analysis (RDA) of the sampling month and depths explaining the structure of phytoplankton functional groups at Site 2

Axes	1	2	3	4
Eigenvalues	0.269	0.181	0.100	0.067
Species-environment correlations	0.915	0.862	0.857	0.822
Cumulative percentage variance				
of species data:	26.9	45.0	54.9	61.7
of species-environment relation:	38.5	64.4	78.6	88.3
Total variance				
Sum of all eigenvalues	1.000			
Sum of all canonical eigenvalues	0.698			

Discussion

Development of the phytoplankton community in the Grlište Reservoir did not clearly match the distinct phases of seasonal succession proposed by the PEG model (Sommer et al. 1986). Small-sized *Cyclotella* species, belonging to codon B, dominated the community throughout the year, with the highest abundance and phytoplankton biovolume contribution observed in May. After the substantial decrease in cell numbers during the short clear-water phase in June, the number of these diatoms increased again in July, especially at Site 1. During the following months, *Cyclotella* species retained a relatively high contribution in the community, despite their lower absolute abundance. In the present study, other functional groups mostly occurred as subdominants to codon B.

The spring peak of small-sized diatoms was related to the onset of stratification that occurred in May. According to Sommer et al. (1986), the beginning of stratification results in mixing-depth change, allowing algae to remain in the euphotic zone for longer and grow under favorable conditions. The first pronounced shift in species dominance coincided with a five-fold decrease in algal biovolume and a roughly 50% increase in water transparency in June. This clear-water phase lasted one month and was characterized by the highest values of RWCS at both sites. The colonial green alga, *Sphaerocystis planctonica*, classified into group F, was the most abundant in the clear epilimnion in June, followed by codominant *Cryptomonas ovata*, a member of group Y. While the non-motile chlorococcales were evenly distributed in the epilimnion, the *C. ovata* population was abundant at 5 m (euphotic zone depth), particularly at Site 2. The similar peak of the *C. erosa* population (1140 ind. ml^{-1}) was recorded at a depth of 10 m at Site 1, in July. Pedrós-Alió et al. (1987) investigated the ecology of the *Cryptomonas phaseolus* Skuja population forming a metalimnetic bloom in a small monomictic lake in Spain. The authors revealed that sedimentation acted as a major loss factor for cryptomonads directly after stratification and discussed the possibility that many of their cells remained trapped in the hypolimnion, unable to swim upward. Since the habitus of cryptomonad cells sampled from the Grlište Reservoir indicated a good physiological state, it is likely that the occurrence of the dense *Cryptomonas* population in the contact zone of the meta- and hypolimnion, as well as in the upper hypolimnion, was a result of migration, rather than sedimentation. Cryptomonads belonging to codon Y inhabit a wide range of lentic ecosystems where grazing impact is low (Padisák et al. 2009). Our results are in accordance with those of Reynolds (1976), who stated that *Cryptomonas* species can avoid the surface and increase in numbers toward the bottom of the euphotic zone.

In July, the number of *Cyclotella* cells increased again in the epilimnion, followed by the occurrence of numerous colonies of *Dinobryon divergens* O. E. Imhof (Chrysophyta), which did not exceed the threshold of 5% in the total phytoplankton biovolume. According to the PEG-model of phytoplankton seasonal development, the summer diatom phase is usually characterized by the development of large-sized diatoms (Sommer et al. 1986). In the Grlište Reservoir, two changes related to diatoms were observed in summer. Firstly, among the large-sized diatoms, *Fragilaria acus* (Kützing) Lange-Bertalot was the most abundant at this time of the year, but its density and biovolume was below the 5% threshold in the community. Secondly, a shift in *Cyclotella ocellata* cell size toward "larger" cells (>15 µm) was noticed. Despite the above mentioned

tendencies, it is difficult to say whether the large-sized diatoms phase occurred in the Grlište Reservoir. The dominance of small-sized diatoms, e.g. *Cyclotella* species, in summer or even over the entire season, gave rise to some controversial explanations (Padisák, Reynolds 1998; Winder et al. 2009). In Lake Balaton, the contribution of various diatoms, including *Cyclotella ocellata*, to the phytoplankton community was related to wind regimes in summer (Padisák, Reynolds 1998). The authors mentioned that these diatoms can be dominant even during very windy years. Depending on its velocity, wind can cause turbulence in the water column and significantly contribute to an increased depth of the mixed layer (Serra et al. 2007). Contrary to this, Winder et al. (2009) found that the genus *Cyclotella* was favored over other diatom species as a result of strong summer stratification. Increased stratification leads to reduced mixing and consequently to a nutrient-depleted chemical environment that promotes the success of small-sized diatoms as good competitors for nitrogen (Winder et al. 2009). In our study, wind velocity and internal wave oscillations were not monitored; however, based on vertical temperature profiles, it appears that the surface layer was well-mixed in July, above a depth of 5 m at Site 1 and 3 m at Site 2.

As the summer progressed, the presence of *Dolichospermum viguieri*, a member of group H1, together with codominant *Cyclotella ocellata* in the epilimnion, was a noticeable feature of the phytoplankton community in the Grlište Reservoir. However, densities of cyanobacterial cells (1246 cells ml^{-1} at Site 1 and 1164 cells ml^{-1} at Site 2) did not indicate the development of a water bloom. This fact was corroborated by the highest measured water transparency in August, 1.85 m (Site 1) and 1.9 m (Site 2). Members of group H1 are adapted to life in stratified low-nitrogen lakes and are able to maintain themselves in the surface mixed layer (Reynolds et al. 2000, Reynolds et al. 2002). The incidence of nitrogen-fixing cyanobacteria in the Grlište Reservoir coincided with low dissolved inorganic nitrogen concentrations ($N\text{-}NH_4^+ + N\text{-}NO_2^- + N\text{-}NO_3^-$) recorded in August, 0.20 mg N l^{-1} (Site 1) and 0.14 mg N l^{-1} (Site 2). Still, low ambient nitrogen levels did not exceed the critical range for N-limited phytoplankton growth (0.08-0.1 mg N l^{-1}) as proposed by Reynolds (2006). It is more likely that the algal growth in the Grlište Reservoir had been determined by the supply of phosphorus, concentrations of which were below the limit of 3 µg P l^{-1} (Reynolds 2006) in August at both sites.

The end of summer was mainly characterized by the assemblage of diatoms (group B) and desmids in the epilimnion, particularly *Closterium acutum* var. *variabile* (Lemmermann) Willi Kreiger, assigned to codon P. The occurrence of group P is associated with the summer season in temperate lakes, where the surface layer of 2 to 3 m thickness is mixed, e.g. in the epilimnia of stratified lakes (Reynolds et al. 2002). *C. acutum* var. *variabile* is particularly known for occurring in eutrophic waters (Coesel 1993). Although present at both sites, the species *C. acutum* var. *variabile* contributed significantly to the phytoplankton community only at Site 1, which was shallower and had a higher trophic state (discussed below).

We selected two sampling points (Site 1 and Site 2) in the Grlište Reservoir to investigate changes in the phytoplankton assemblage along its longitudinal axis. More taxa with >5% contribution to the total phytoplankton biovolume were found in the transitional part compared to the lacustrine part of the reservoir, represented e.g. by members of codons C, P, D and S1. The presence of codon C was recorded in June when *Asterionella formosa* Hassall co-dominated with centric diatoms (mainly *C. ocellata*) in the epilimnion of Site 1. *Asterionella formosa* (a representative of codon C) and *Closterium acutum* var. *variabile*, together with several species of the genus *Staurastrum*, belonging to codon P, inhabited waters of higher trophic states, e.g. eutrophic small- and medium-sized lakes (Padisák et al. 2009). The other two codons, D represented by *Ulnaria ulna* (Nitzsch) P. Compère and S1 represented by a few species of the genus *Phormidium*, are associated with turbid environments including rivers (Padisák et al. 2009). This tendency of reservoirs to improve the water quality along the longitudinal axis from the river inlet to the dam has also been reported and discussed by Caputo et al. (2008).

According to the results of our study, physical factors played a major role in shaping the phytoplankton community. The first factor, which accounted for the structure of the phytoplankton functional groups in the Grlište Reservoir, reflected the opposite effects of two processes, i.e. thermal

stratification and physical mixing. Phytoplankton organisms are affected by various environmental factors, including light and nutrients, both of which are crucial to their growth. Several studies indicate that water movement greatly affects the availability of these resources (Becker et al. 2009, Becker et al. 2010, Xiao et al. 2011). For example, Becker et al. (2009) used the term "mixing regime" to describe the main factors affecting the resource availability and as a consequence, the seasonal dynamics of the phytoplankton in a monomictic, meso-eutrophic reservoir.

The second factor extracted in the redundancy analysis (reduction vs. oxidation) also had a bipolar nature. N-NH_4^+ and P–PO_4^{3-} were positively correlated with the deepest water layer (15 m at Site 1), indicating the effect of sediment on water quality. Redox conditions near the bottom influenced the phosphorus release from the sediment; one of the mechanisms through which this occurs is the reduction of iron and dissolution of iron-phosphorus complexes (Boström et al. 1988). The elevated concentrations of Fe were detected above the sediment at both sites in the Grlište Reservoir. Additionally, ammonia is one of the first compounds to leave the anaerobic sediment (Beutel et al. 2008). When analyzing the spatial and temporal changes of environmental variables in a reservoir, Becker et al. (2009) described the redox potential gradient as one of the major factors influencing the dynamics of these variables.

The development of thermal stratification in reservoirs depends primarily on their morphometry and natural hydrodynamic and climatic conditions, but at times it can be determined by human-induced impacts. Naselli-Flores and Barone (2005) showed that water drawdown above some threshold values can lead to breaking of the thermocline and transformation of the monomictic water bodies to amictic, polymictic or atelomictic environments. The authors also stressed that bloom-forming cyanobacteria dominated the phytoplankton assemblage in years when reservoirs were characterized by low water levels. The explained mechanism involved the following steps: an increased water usage in summer, a decreased reservoir's depth, a thermocline shifted down, an inclusion of anoxic zones into the circulation, a release of nutrients that support the phytoplankton growth. However, if the water loss from a reservoir is not severe, or if it is compensated by the inflow, the reservoir's depth does not decrease enough to disrupt the thermal stability. We believe that the total water-level fluctuation of 2.5 m in the Grlište Reservoir was not sufficient to shift the reservoir outside its homeostatic plateau and to cause a severe phytoplankton bloom. Our results revealed a positive correlation between stratification and water pumping by the drinking water plant. The months when the water column was stratified coincided with the period of the highest exploitation of water, or with summer months when the demand for drinking water was the highest.

Conclusion

- The one-year study carried out in the Grlište Reservoir revealed that thermal stratification and physical mixing, followed by the redox potential gradient, were the major factors influencing the dynamics of the phytoplankton assemblage in this temperate reservoir.
- The differences in the structure of phytoplankton along the reservoir's longitudinal gradient were evident due to the presence of more functional groups (C, P, D and S1) identified at the transitional compared to the lacustrine sampling site. The differential codons are associated with a higher trophic level.
- It was shown that human-induced water level fluctuations due to the pumping activity by the drinking water plant in summer months did not break down thermal stratification, one of the causes of serious cyanobacterial blooms.

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