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Periphytic diatoms in the presence of a cyanobacterial bloom: A case study of the Vrutci Reservoir in Serbia

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Abstract: Despite their unique ecology and implications for ecological assessment, diatoms in lentic ecosystems are still insufficiently studied in both scientific research and operational monitoring. In particular, the ecology of periphytic diatoms relative to the global expansion of cyanobacterial blooms in lakes has not been described at all. This study aims to describe the diversity and dynamics of a periphytic diatom community in the Vrutci Reservoir during *Planktothrix rubescens* bloom, and to evaluate the adequacy of standard diatom index implementation in lentic ecosystems relative to the diatom index adapted for lakes – the Trophic Diatom Index for Lakes (TDIL). The study was conducted in the Vrutci Reservoir in western Serbia. Periphyton was developed on an artificial glass substrate during the summer of 2015, following a depth gradient of a stratified water column. Diatom diversity and abundance, as well as diatom indices were estimated. Discounting the cyanobacterial bloom, 79 taxa of diatoms were recorded and among them the species *Aneumastus stroesei* as the first representative of the entire genus *Aneumastus* ever to be detected in Serbia. Diatom distribution along the depth gradient was clearly associated with shifts in environmental conditions. TDIL showed an advantage over standard diatom indices in terms of stability during experimental period and uniformity along the depth gradient, indicating the necessity for further testing of this index performance in lakes, and consequently local water-quality legislation update.

Keywords: *Aneumastus stroesei*; diatom diversity; diatom indices; TDIL; Vrutci Reservoir

INTRODUCTION

The importance of periphyton has long been recognized in lotic environments, as described in number of detailed studies, whereas in lentic environments phytoplankton research prevailed [1]. The general ratio of publications dealing with periphyton and phytoplankton in leading hydrobiological journals was 1:20 in the 1990s owing to the opinion that phytoplankton is the leading and dominant primary producer in lakes [2]. The state-of-the-art and accentuated importance of periphyton in primary production, nutrient cycling and food chains in lake ecosystems is evident [3], but even in the second decade of the 20th century, periphyton studies were outnumbered 10-fold by phytoplankton studies in lakes [4].

Diatoms in a lake's periphyton are still not a well-known category, although suggestions have been made that diatoms in a lentic environment have a specific taxonomical composition, diversity and morphology, and that lake-specific aspects of periphyton should be a central point in future research [4,5]. A possible reason for the research deficiency is the lack of an adequate and universal substrate for periphyton development in lakes; the use of artificial substrates is a good alternative when there is no natural (or universal) substrate available for sampling. In Serbia, there are only a few studies dealing with lake periphyton and lake diatom communities [6,7]. The recently updated list of diatoms in Serbia reported findings from several lentic environments [8]. Thus, data on diversity are still scarce, as are reliable data for upgrading the legislative of lake biomonitoring based on the phytobenthos/periphyton to meet the EU Water Framework Directive [9,10].

A phenomenon that accompanies the modern age and anthropogenic pressure on aquatic environ-

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ments is occurrence of more frequent cyanobacterial blooms. Cyanobacterial blooms cause phytoplankton diversity loss, the most probable reason for this being secondary metabolism products including cyanotoxins [11]. It is well known that *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek has a potential to produce hepatotoxic microcystins-LR and -RR, and neurotoxic anatoxin-a, which have different harmful effects on biota [12,13]. It was expected that the cyanobacterial bloom in the Vrutci Reservoir could affect periphyton development and diversity. On the other hand, when *Microcystis aeruginosa* (Kützing) Kützing was considered [14], it was suggested that diatoms/bacteria predominant in the biofilm express allelopathic control of cyanobacterial blooms. Precise characterization of periphyton developed in the presence of cyanobacterial blooms, when various species causing them are considered, is still lacking. Specifically, diatom diversity and community composition in the presence of cyanobacterial blooms could be of interest considering the previously mentioned study [14] and the bioindication capacity of this group of algae [9].

The first objective of this study was to describe the diversity and dynamics of the periphytic diatom community in a very specific environment in the Vrutci Reservoir in the presence of a cyanobacterial bloom of *P. rubescens*. The second objective was to compare standard diatom indices relative to the diatom index adapted for lakes (TDIL), with regard to the applicability and objectivity in lentic ecosystems, based on the Vrutci Reservoir case study.

MATERIALS AND METHODS

Study site

The Vrutci Reservoir is a freshwater reservoir situated in western Serbia (43°50’34” N, 19°41’36” E). It extends along the Đetinja River Canyon (the reservoir was made by the damming the Đetinja River), lying between two mountains, Tara and Zlatibor. This multipurpose water body was primarily built for water supply and flood protection of the city of Užice. In December 2013, the reservoir was not in use due to a constant *P. rubescens* bloom [15].

Experimental design

A floating platform was used for carrying artificial substrates – glass slides, for periphyton development. Substrates were incubated at a 1-m depth in epilimnion, at 5.5- and 8-m depths on the top and bottom of the metalimnetic layer, and at 12 m on a hypolimnetic layer. Glass slides were set up for incubation in June 2015, and sampling was done monthly, in July, August, September and October, in a continuously incubated series (CiS). From July onwards, new slides for incubation were introduced each month, and from August, the slides were incubated only for a one-month period in a monthly incubated series (MiS).

Physical and chemical parameters

Water transparency was measured using a Secchi disk. The temperature (T, °C), conductivity (Cond, µS/cm), total dissolved substances (TDS, g/L), pH, dissolved oxygen (DO, mg/L) and chlorophyll *a* (Chl *a*, µg/L) were measured *in situ* using a YSI 6600 V2 multiparameter water quality probe. Water samples were taken at the same time as periphyton samples and were further analyzed in the laboratory in the Institute for Development of Water Resources “Jaroslav Černi” in Belgrade, Serbia, where all measurements were done according to standard analytical methods [16]. The measured parameters were total nitrogen (TN, mg/L), total phosphorous (TP, mg/L), dissolved organic carbon (DOC, mg/L) and total organic carbon (TOC, mg/L). Trophic status classification was established according to OECD criteria [17].

Periphyton analyses

On each sampling occasion, glass slides with developed periphyton were stored in separate labeled plastic containers (depending on incubation depth and series, the samples were designated as either CiS or MiS), stored in a mobile freezer and transported to the laboratory of the Department for Algology, Mycology and Lichenology, Institute for Botany, Botanical Garden “Jevremovac”, Faculty of Biology, University of Belgrade, where further processing and analyses were performed. In the laboratory, the periphytic biofilm was scraped from the upper surface of each glass slide using a stainless steel razorblade and suspended in 100 mL of distilled water. The suspension was homogenized by a hand blender and separated into subsamples.

Part of the preserved (4% formaldehyde) subsample material) was treated by the standard method as described [18], and mounted with Naphrax® to obtain permanent slides for diatom taxonomic identification and quantification. Taxonomic identification was performed according to the standard procedure. Identification of diatom taxa and quantification (relative abundance) was performed by counting at least 400 valves at each permanent slide using a Carl Zeiss AxioImager M1 microscope and a digital camera AxioCam MRc5 with AxioVision 4.8 software. Diatom indices were calculated using OMNIDIA 6 software [19], and seven indices were considered for water-quality assessment of the Vrutci Reservoir as follows: Biological Diatom Index (IBD), Pollution Sensitivity Index (IPS), Group Index CEE (CEE), Trophic Diatom Index for Lakes (TDIL), Trophic Diatom Index (TDI), Trophic Index (Rott TI), and Saprobic Index (Rott SI). Water-quality classes were determined [20].

According to the Republic of Serbia legislative, only IPS diatom index is recommended for assessment of the ecological potential of the Vrutci Reservoir (accumulation formed on water body type 4) [10,21]. The values of the IPS diatom index below 9 refer to "very bad", between 9 and 12 to "bad", between 12 and 14 to "moderate", and over 14 to "good" and a maximal ecological potential according to the mentioned legislative for the Vrutci Reservoir [10].

Scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS)

For precise identification of the newly recorded taxon, SEM-EDS was carried out using a JEOL JSM-6610LV SEM (with a W-filament as a beam source), coupled with an X-Max EDS. The samples were covered with gold using a BALTEC-SCD-005 sputter coating device. The results were recorded under high vacuum conditions. SEM-EDS and XRPD analyses were performed in the SEMLAB, laboratory at the University of Belgrade, Faculty of Mining and Geology.

Statistical analyses

Principal component analysis (PCA) was performed to summarize the variations in species composition using the data obtained after quantitative analyses of the diatom communities, and was interpreted with the help of environmental variables. Besides certain chemical and physical parameters, the incubation depth, sampling month and incubation series (CiS or MiS) were used as supplementary variables. Statistical analysis was conducted using CANOCO for Windows, ver. 5.0 [22].

RESULTS

The results for parameters that are significant for the trophic status classification of lakes are presented as ranges during the experimental period (Table 1). Each parameter pointed to different levels of trophic status; hypereutrophy could be detected based on the TP, slight eutrophy according to transparency, while phytoplankton primary production in the Vrutci Reservoir was characteristic for mesotrophic status.

Table 1. Trophic status of the Vrutci Reservoir during the summer of 2015 according to OECD criteria.

	TP (mg/m^3)	Secchi depth (m)	Chl *a* (mg/m^3)
MIN.	80	1.20	2.5
AVERAGE	220	2.89	5.5
MAX	470	3.95	13.05
TROPHIC STATUS (OECD 1982)	Hypertrophic	Eutrophic	Mesotrophic

In both sample series, 79 taxa (32 genera) of diatoms were recorded (Supplementary data). The most prominent genera were *Cymbella* and *Nitzschia*. *Achnanthidium caledonicum* and *Pantocsekiella ocellata* taxa were dominant, whereas *Achnanthidium minutissimum* and *Melosira varians* were subdominant. Nineteen taxa recorded in the Vrutci Reservoir were found to have a status in the German Red List of diatoms [23,24] (Supplementary data).

It is important to point out that the species *Aneumastus stroesei* (Fig. 1) is the representative of the entire genus *Aneumastus*, which was detected in Serbia for the first time.

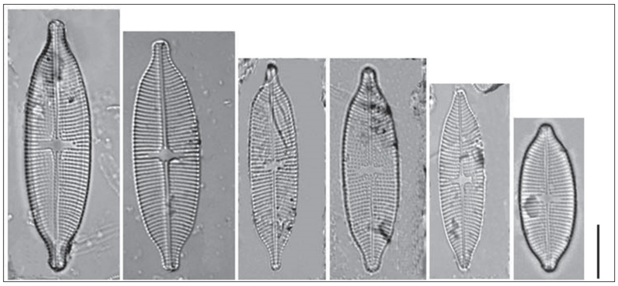

Fig. 1. Light micrographs (LM) of *Aneumastus stroesei,* size diminution series, scale bar 10 µm.

Aneumastus stroesei (Østrup) D. G. Mann (Fig. 1, 2 and 3)

Basionym: *Navicula tuscula* var. *stroesei* Østrup

Homotypic synonym: *Navicula stroesei* (Østrup) A. Cleve, *Navicula tuscula* var. *stroesei* Østrup

Reference: Lange-Bertalot, H. (2001) (p. 119, figs 1-4; p. 120, figs 1,4: 157)

The main morphological characteristics are follows: valves linear-elliptic to lanceolate, narrowing to rostrate or subrostrate ends. Raphe almost filiform, with slight undulations at both raphe branches. Valve length 27.7-50.5 µm, breadth 11.2-15.3 µm. Axial area narrow and linear. Central area stretches to stauroid fascia, surrounded by 1-3 shorten striae. Striae parallel to slightly radiate, (13) 14-16/10 µm. Areolae visible under LM, 15-18/10 µm. SEM external valve view: typical uniseriate areolae arrangement along the edge of the axial area can be distinguished (Fig. 2). Areolae elongated in the proximal valve area, except in the central area. SEM internal valve view: on the inner side of the valve, a specific areolae morphology is visible, as well as valvocopula (Fig. 3).

PCA was used to assess the relationship between diatom abundance and environmental factors in the Vrutci Reservoir with respect to the time and incubation depth gradient (Fig. 4). The first two PCA axes together explained 35.5% (first 18.8%, and second 16.7%) of the variability of the data set. Incubation depths of 1 m and 5.5 m were positioned at the positive side of the first PCA axis (determined by high pH, conductivity and T values), while depths of 8 m and 12 m were positioned in the negative part of this axis (determined by the highest TDS, TN and orthophosphates). As regards the second PCA axes, depths of 5.5 m and 8 m were associated with the positive part, while depths of 1 m and 12 m were associated with the negative part of the axis (determined by the highest transparency and carbon content, TOC and DOC). Three groups of diatoms could be distinguished on Fig. 4, each surrounding one incubation depth, except the deepest (12 m), where the group was not created because of generally low abundance. The group around an incubation depth of 1 m was comprised of the following taxa: *Achnanthidium caledonicum, A. minutissimum, Encyonopsis microcephala, E. subminuta* and *Fragilaria acus.* The group around depth 5.5 m was comprised of *Cymbella cymbiformis, C. neocistula,*

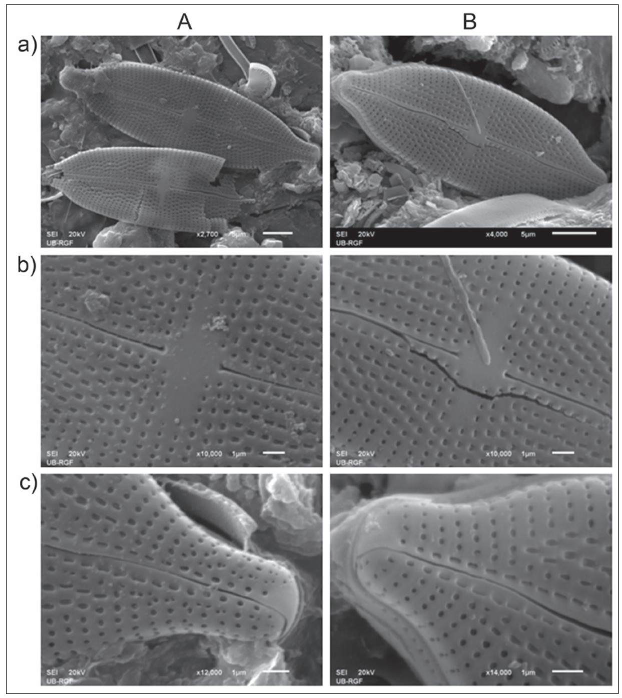

Fig. 2. SEM micrographs of two individuals (columns **A** and **B**) of *A. stroesei* from the Vrutci Reservoir – comparative overview; **a** – valves, **b** – central area; **c** – valve ends.

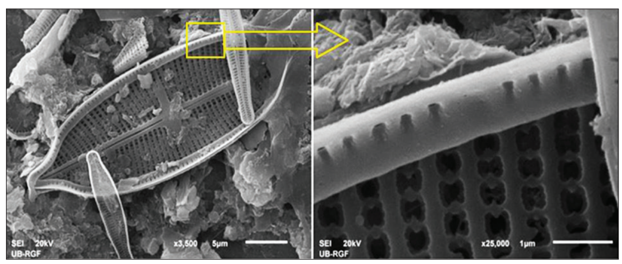

Fig. 3. SEM micrographs of *Aneumastus stroesei* from the Vrutci Reservoir; inner side of the valve and valvocopula.

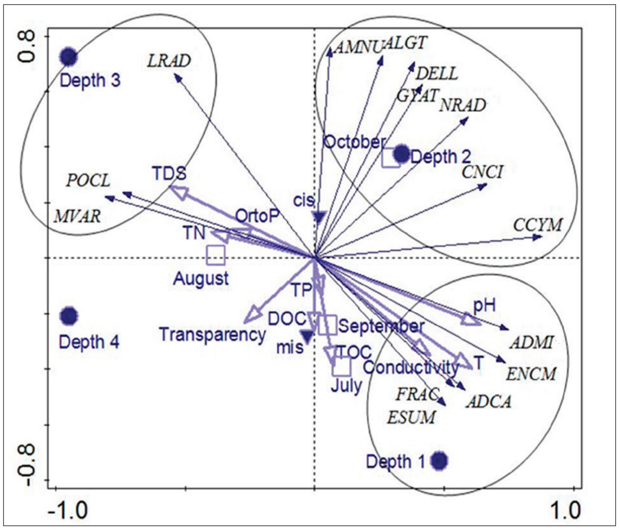

Fig. 4. Relationship between diatom abundance and environmental factors in the Vrutci Reservoir (PCA). Diatom abbreviations: *Achnanthidium caledonicum* (ADCA), *Achnanthidium minutissimum* (ADMI), *Cymbella cymbiformis* (CCYM), *Encyonopsis microcephala* (ENCM), *Encyonopsis subminuta* (ESUM), *Ulnaria acus* (FRAC), *Navicula radiosa* (NRAD), *Pantocsekiella ocellata* (POCL), *Amphora minutissima* (AMNU), *Cymbella neocistula* (CNCI), *Lindavia radiosa* (LRAD), *Melosira varians* (MVAR), *Amphora lange-bertalotii* var. *tenuis* (ALGT), *Diploneis elliptica* (DELL), *Gyrosigma attenuatum* (GYAT).

Amphora lange-bertalotii var. *tenuis, A. minutissima, Diploneis elliptica, Gyrosigma attenuatum* and *Navicula radiosa*. Centric diatoms, *Lindavia radiosa, Melosira varians* and *Pantocsekiella ocellata* formed a group at the incubation depth of 8 m. Generally, *A. caledonicum* was dominant at 1-m depth, and *Pantocsekiella ocellata* was dominant at a depth of 8 m. The PCA ordination diagram shows separation of CiS and MiS along the second PCA axis, indicating that colonization at greater depths was slower and took more than one-month of incubation for the community to become abundant.

According to the results of quantitative analyses of diatoms, OMNIDIA 6 provided values for selected diatom indices, and these are presented in Table 2. The first obvious fact is that the differences between the indices imposed different water-quality classifications that ranged from very good to even bad. When each index (except TDIL) was examined, the decline in water quality could be observed along the depth gradient. Only the TDIL index showed stability and uniformity along the depth gradient and steadily indicated a good water quality of the Vrutci Reservoir. IBD, IPS and Rott SI indices pointed to very good quality in the upper water

Table 2. Selected diatom indices values in the Vrutci reservoir

Series	Date	Depth (m)	IBD	IPS	TDIL	CEE	TDI	Rott TI	Rott SI
CiS	7/2015	1	20	19	14.7	18.2	15.4	15.9	19.7
	8/2015	1	19.9	17.1	14.2	15.8	13.8	13.4	19
	9/2015	1	20	18	13.9	15.3	15.2	14.5	19.4
	10/2015	1	20	18.4	14.4	17.6	15.4	15.1	19.5
	7/2015	5.5	15.3	14.3	14.7	13.4	8.3	10.3	17
	8/2015	5.5	19.9	16.9	14.8	17.1	15.1	13.8	19.4
	9/2015	5.5	19.3	16.1	14.6	16.6	15.4	11.7	17.9
	10/2015	5.5	18.2	16.1	13.9	17.6	16	12.5	19.2
	8/2015	8	13.9	13.5	14.3	12.9	4	9.2	14
	9/2015	8	13.8	13	13.5	12.7	2.1	8.3	12
	10/2015	8	14.2	14.4	15.7	14.6	6.5	9.6	15.7
	9/2015	12	14.4	12.8	14.5	12.6	2.9	9.4	12.8
	10/2015	12	13.8	12.8	13.6	12.5	1.4	8.4	11.8
MiS	7/2015	1	20	19	14.7	18.2	15.4	15.9	19.7
	8/2015	1	19.3	15.9	14.2	13.9	13.3	12.9	18.9
	9/2015	1	19.3	16.6	14.2	15.9	13.7	13.1	18.8
	10/2015	1	20	17.6	14.2	17.3	15.5	14.5	19.5
	7/2015	5.5	15.3	14.3	14.7	13.4	8.3	10.3	17
	8/2015	5.5	16.8	15.1	13.5	13.1	9.8	10.5	17.8
	9/2015	5.5	18.8	15.8	14.9	17.2	15	14.3	19
	10/2015	5.5	18.7	16.9	13.9		12	12.3	18.8
	8/2015	8	13.6	12.2	14.3	10.6	2.3	9.1	12.5

Water quality classes (Prygiel&Coste 2000):

Very bad	Bad	Moderate	Good	Very good
<5	≥5 - <9	≥9 - <13	≥13 -<17	≥17 - 20

layers. CEE values were variable; Rott TI indicated good to moderate quality, while in TDI, poor water quality was reported at incubation depths of 8 m and 12 m. According to our results for IPS, the Vrutci Reservoir prevalently reflected features of a class II ecological potential (IPS>14), which represents a good ecological potential [10]. No substantial differences in diatom indices were observed between series CiS and MiS.

DISCUSSION

Although the concentration of TP in the Vrutci Reservoir during the period of study indicated hypereutrophy, according to primary phytoplankton production (approximated by Chl *a*), this reservoir was mesotrophic [17].

A low diversity in phytoplankton, and also in periphyton as a consequence of the *P. rubescens* bloom, could be expected [25]. Although the low diversity in periphyton that developed in the presence of *P. rubescens* was actually detected at a wider scale when non-diatom groups were considered [26], diatom diversity could not be characterized as either low or scarce. As a phenomenon in the Vrutci Reservoir periphyton, we point out that 19 (24%) out of the 79 detected diatom taxa were found to be on the German Red List of diatoms [23,24]. Not having a referent diatom Red List for Serbia, it is appropriate to discuss our results in a wider context, relying on German Red List data. Generally, scarce data on diatoms in lentic environments in Serbia could be one of the reasons why these remarkable results were obtained in the Vrutci Reservoir using artificial substrates, which indicates that diatom diversity in the lakes and reservoirs in Serbia should be explored in detail. On the other hand, this could be debated in the context of low susceptibility (i.e. resistance) of diatoms to the well-known negative effects of cyanobacterial blooms on diversity.

According to the literature, *Aneumastus stroesei* is a typical inhabitant of oligo- to mesotrophic lentic chalk-rich ecosystems, where they are not very common and their populations are generally scattered [24,27]. According to the data available in Algaebase [28], *A. stroesei* has a worldwide distribution. This species has been detected in Europe, Asia, North America, New Zealand and even on Pacific islands. As regards their distribution in Europe, *A. stroesei* has been confirmed in diatom flora in Belarus, Britain, France, Germany, Ireland, FYR Macedonia, Netherlands, Poland, Romania, and Switzerland [28]. Literature shows that *A. stroesei* was also reported in Albania and Bulgaria [29, 30], neighboring countries of Serbia. Our finding of *A. stroesei* in the Vrutci Reservoir is a significant contribution to the regional distribution of this species, particularly as *A. stroesei* populations are declining due to habitat eutrophication [27].

The genus *Aneumastus* accounts for around 30 species, of which 10 are described in Baikal Lake diatom flora [31]. The authors suggested that ancient lakes such as Baikal, Prespa and Ohrid, due to their long history and species flock phenomenon, express a high diversity of rare genera such as *Aneumastus* [31]; these lakes served as *refugia* for species that are nowadays rare or endangered. Although *A. stroesei* is one of the most frequently found representatives of the *Aneumastus* genus, its detection in the Vrutci Reservoir is a valuable contribution to the biogeographic data of *Aneumastus* spp. in Europe.

Achnanthidium caledonicum, which predominated in the majority of periphyton samples from the Vrutci Reservoir, is characterized as a vulnerable species in the German Red List [23,24], and our data provide a valuable record on the ecology and distribution of this species. *A. caledonicum* is described as common in circumneutral and slightly alkaline waters with low nutrient concentration [32]. This species is expected in chalk-rich and nutrient-poor lentic ecosystems in mountainous regions, and is an indicator of good ecological quality [24], thus their presence and abundance in the Vrutci Reservoir can be discussed. As the Vrutci Reservoir is situated between two mountains, Tara and Zlatibor, in western Serbia, and is moderately chalk-rich, it seems like a perfect habitat for *A. caledonicum*. Still, the Vrutci Reservoir's trophic status as regards phosphorous is higher than expected for the habitat of *A. caledonicum* [32,24]. On the other hand, *Pantocsekiella ocellata*, as the species that shared dominance with *A. caledonicum* in the Vrutci Reservoir, is a typical oligosaprobic species distributed in oligo- to slightly eutrophic ecosystems [24,33]. When subdominant species are considered, *Achnanthidium minutissimum* presence and abundance is typical for a good and excellent ecological status [34], while *Melosira varians* is an α mesosaprobic species characteristic of eutrophic ecosystems [33]. Bearing in mind the ecological features of dominant and subdominant taxa, it can be anticipated that diatom community structure points to a good ecological potential of the Vrutci Reservoir. This can be discussed in the context of relatively recent eutrophication or even re-oligotrophication of the Vrutci Reservoir. Considering that in our study phytoplankton primary production indicated mesotrophic conditions in the Vrutci Reservoir, it seems that neither phytoplankton nor periphyton communities reflected eutrophic conditions. Even the *Planktothrix rubescens* bloom was confirmed to be symptomatic for oligo-mesotrophic conditions, often linked to re-oligotrophication [26].

After analyzing the relationship between diatom abundance and environmental parameters in the Vrutci Reservoir, we found clear groupings of diatom taxa around parameters defining incubation depths. Also, CiS and MiS were arranged along the depth gradient. CiS were associated with the highest diatom abundance at the incubation depth of 5.5 m, and MiS with the incubation depth of 1 m. It is interesting to note that the diatom group formed around an incubation depth of 1 m exclusively consisted of taxa belonging to a low-profile guild [35], characterized by resistance to physical disturbances while being resource-stressed, which makes them decline in thicker biofilms [36]. Around the depth of 5.5 m the majority of taxa also consisted of low-profile representatives, although with increasing proportions (*Cymbella* spp. and *Amphora* spp.); also motile representatives *Gyrosigma attenuatum* and *Navicula radiosa* [35] were present, which points to an environment that was less prone to disturbance (upper metalimnion) and increased biofilm thickness. The diatom group formed around the incubation depth of 8 m consisted of very few centric representatives, with *Lindavia radiosa* and *Pantocsekiella ocellata* that belong to a planktonic guild [35,36] typical for biofilms developed in stabile environments lacking disturbances and competitors among periphytic diatoms [37]. The underdeveloped community and low diversity at 8 m depth could be a consequence of a reduced light regime, considering the dense metalimnetic bloom of *P. rubescens*. Generally, the highest diversity was detected at a 5.5-m incubation depth, while a decrease in diversity could be observed along a depth gradient, implying that most of the diatoms in periphyton in the Vrutci Reservoir were adapted to the moderate light environment [38]. We can conclude that diatom guild distribution along a depth gradient clearly describes environmental conditions in different water-column strata.

Diatom indices pointed to different water-quality classes in the Vrutci Reservoir, and it is interesting that all the indices, except TDIL, pointed to a decline in water quality along the depth gradient. The reason for this could be that the majority of diatom indices were developed for lotic ecosystems, thus centric diatoms are naturally lacking in indicator lists. This is why TDIL, which is adapted for lakes [39], was the only indicator that expressed uniformity and stability in the Vrutci Reservoir. Besides TDIL, similar adaptations to lakes were observed in Great Britain (LTDI) [34] and Germany (DISeen) [40,41]. Our results show that the percentage of centric diatom taxa in periphyton significantly increased along a depth gradient. These taxa are not recognized by TDI and the majority of other diatom indices [39]. In indicator lists for TDI and Rott TI indices, centric taxa are lacking, which usually leads to an overrating of the trophic conditions in lakes [42,39]. Our results suggest that centric taxa underrate water quality along the depth gradient, but this is only because diatom abundance at depths of 8 m and 12 m was generally low, and apart from centric, very few other taxa could be used for the calculation of different indices. Thus, the decrease in water quality along the depth gradient in the Vrutci Reservoir, as implied by IBD, IPS, CEE, TDI, Rott TI and Rott SI indices, was the consequence of the sensitivity of the selected indices rather than of changes in environmental characteristics. As previously stated, according to the Serbian legislative [10], only the IPS diatom index is recommended for the assessment of the ecological potential of water body types as the Vrutci Reservoir. The IPS diatom index is considered to be one of the most precise indices as it includes about 2000 species [43]. Still, IPS, like the majority of diatom indices, was primarily developed for rivers and streams [44]. Data on the ecological potential of the Vrutci Reservoir were provided in 2012, and according to the IPS index, a moderate ecological potential was established [45]. Although, the Vrutci Reservoir has experienced constant cyanobacterial blooms since 2013, our results suggest that the reservoir reflects the features of class II water quality (IPS >14), which corresponds to a good ecological potential [10]. Thus, our results based on

IPS point to a slight improvement in water quality in the Vrutci Reservoir since 2012; however, this index appeared to be depth-sensitive in terms of centric taxa prevalence at deeper depths.

CONCLUSION

According to our results we can assume that diatom diversity was not drastically affected by the permanent *P. rubescens* bloom in the Vrutci Reservoir. In our study, *Aneumastus stroesei* as the entire genus *Aneumastus* was detected for the first time in Serbia. Diatom guild distribution and dynamics clearly reflected shifts in environmental conditions along the depth gradient and in the duration of the incubation period. Only a hint of the negative effect of the *P. rubescens* bloom could be observed as low diversity and abundance in lower metalimnion and hypolimnion, most probably due to a reduced light regime. Our results emphasize the applicability and objectivity of TDIL in water-quality assessment in lentic environments relative to the other selected diatom indices, since only TDIL showed uniformity and stability along the depth gradient and during the experimental period. Nevertheless, further testing of this index in other lakes and reservoirs of various trophic status throughout Serbia should be conducted. The results of this study could be a starting point for further consideration of inserting of the TDIL index in the Serbian legislative for assessing the ecological status/potential of lakes and reservoirs.

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Supplementary Data

Supplementary data are available at: http://serbiosoc.org.rs/NewUploads/Uploads/Trbojevic%20et%20al_3652_Supplementary%20data.pdf