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Diatom diversity and seasonality in two rivers in Serbia

Разновидност и сезонски аспекти на дијатомеите во две реки во Србија

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Abstract

During the four seasons of investigation in the Rača and Studenica rivers (spring 2011, summer 2011, winter 2011 and spring 2012) 171 and 190 epilithic diatoms were recorded, respectively. The most numerous genera in both rivers were *Gomphonema*, *Navicula*, and *Nitzschia*. Of total recorded taxa 11 are new for the diatom flora of Serbia. The highest species diversity was in spring, while the lowest was during the summer and winter periods. In almost all seasons *Cocconeis lineata* occurred with the highest frequencies in the Rača River. In the Studenica River epilithic diatom community was dominated by *Achnanthidium pyrenaicum*, except in winter 2011 and spring 2012 when *Diatoma moniliformis* and *Gomphonema* species were the most abundant diatoms, respectively. The regular pattern of diatom succession was not recorded in two investigated rivers.

Key words: epilithic diatoms, diversity, Rača River, Studenica River

Апстракт

Со спроведените истражувања на реките Рача и Студеница во тек на четири сезони (пролет 2011, лето 2011, зима 2001 и пролет 2012 година) се регистрирани 171 и 190 епилитски дијатомејски алги, соодветно. Најзастапени родови во двете реки беа *Gomphonema, Navicula* и *Nitzschia*. За флората на Србија за првпат се регистрирани 11 таксони. Највисок диверзитет беше забележан во пролетните сезони, а најнизок во тек на летото и зимата. Видот *Cocconeis lineata* беше најфреквентен во реката Рача во скоро сите истражувани сезони. Во епилитските дијатомејски заедници во реката Студеница доминираше *Achnanthidium pyrenaicum*, освен во тек на зимата 2011 и пролетта 2012 година во кои најабудантни беа *Diatoma moniliformis* и видовите од родот *Gomphonema*, соодветно. Не беше утврден правилен образец на промени на дијатомеите во двете истражувани реки.

Клучни зборови: епилитски дијатомеи, река Рача, река Студеница.

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Introduction

Diatoms as microscopic single-celled algae with the opaline silica cell wall are one of the most successful groups of photosynthetic eukaryotic organizams and they colonize various micro-habitats in aquatic ecosystems (Round et al. 1990, Spaulding & Edlund 2008). Diatoms are also bioindicators of environmental change. Each species has a specific tolerance for environmental variables and because of short generation time, they respond rapidly to change (Schönfelder et al. 2002). The use of diatoms as bioindicators is widespread in all water bodies and they are vital for ecological status assessment (Spaulding & Edlund 2008, Bere & Tundisi 2010, Kelly et al. 2012, Schneider et al. 2013, Bennion et al. 2014, Poikane et al. 2016).

During the XX century, numerous algological studies were published in Serbia, including diatoms (Blaženčić et al. 1985, Blaženčić 1986). In the early 2000s, intensive research of different rivers in Serbia began, aiming to collect diatoms and biodiversity data. Still, a large number of habitats remained unexplored including Rača and Studenica rivers. The aims of this paper are to identify and quantify epilithic diatoms in two rivers Rača and Studenica through four seasons and to present diatom diversity through a combination of species richness (the number of species present), Shannon's diversity index and physico-chemical parameters.

Material and methods

The Rača River is a right-bank tributary of the Drina River in the western part of Serbia (Fig. 1). It belongs to the Black Sea drainage basin. It is 19.6 km long, with a catchment area of 59 km². It springs at about 970 m a.s.l. on the slopes of Tara Mountain (Jevtić 1999). The Studenica River is a left-bank tributary of the Ibar River in the western part of Serbia (Fig. 2). It is 60.5 km long, with a catchment area of 582 km². It springs at about 1100 m a.s.l. on the slopes of Golija Mountain (Janković 2009).

Samples were collected during four seasons (spring 2011, summer 2011, winter 2011, and spring 2012) (Fig. 1). In each season the samples were collected from the six points. Epilithic samples were removed by scrubbing the surface of stones with a toothbrush and were fixed with formaldehyde to a final concentration of 4%.

Conductivity, oxygen (DO), pH, and water temperature were measured *in situ* with a PCE-PHD (Germany). Flow velocity was measured using a GEOPACKS Stream Flowmeter (UK). The concentration of nitrates (NO_3^-), total phosphorus (TP), orthophosphate (PO_4^{3-}) and ammonium (NH_4^+) were examined at the Institute of General and Physical Chemistry, University of Belgrade. Analyses of total phosphorus and orthophosphates were preformed according to APHA protocols (APHA 1998, method 4500-P E), the content of ionized ammonia was analyzed according to standard methods for testing of hygienic integrity (P-V--2/B method, Škunca-Milovanović et al. 1990), while for analyses of nitrates EPA method was used (Pfaff 1993).

Diatom samples were treated with concentrated sulfuric acid according to the method given by Krammer and Lange-Bertalot (1986). Permanent slides of the cleaned material were mounted with Naphrax®. Light microscope observations and micrographs were made using a Zeiss AxioImagerM.1 microscope with DIC optics and AxioVision 4.9 software. The abundance was estimated by counting 400 valves

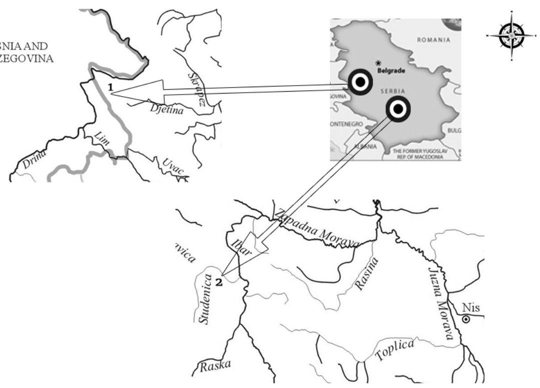

Figure 1. Map of the investigated rivers in Serbia. 1 – Rača River, 2 – Studenica River.

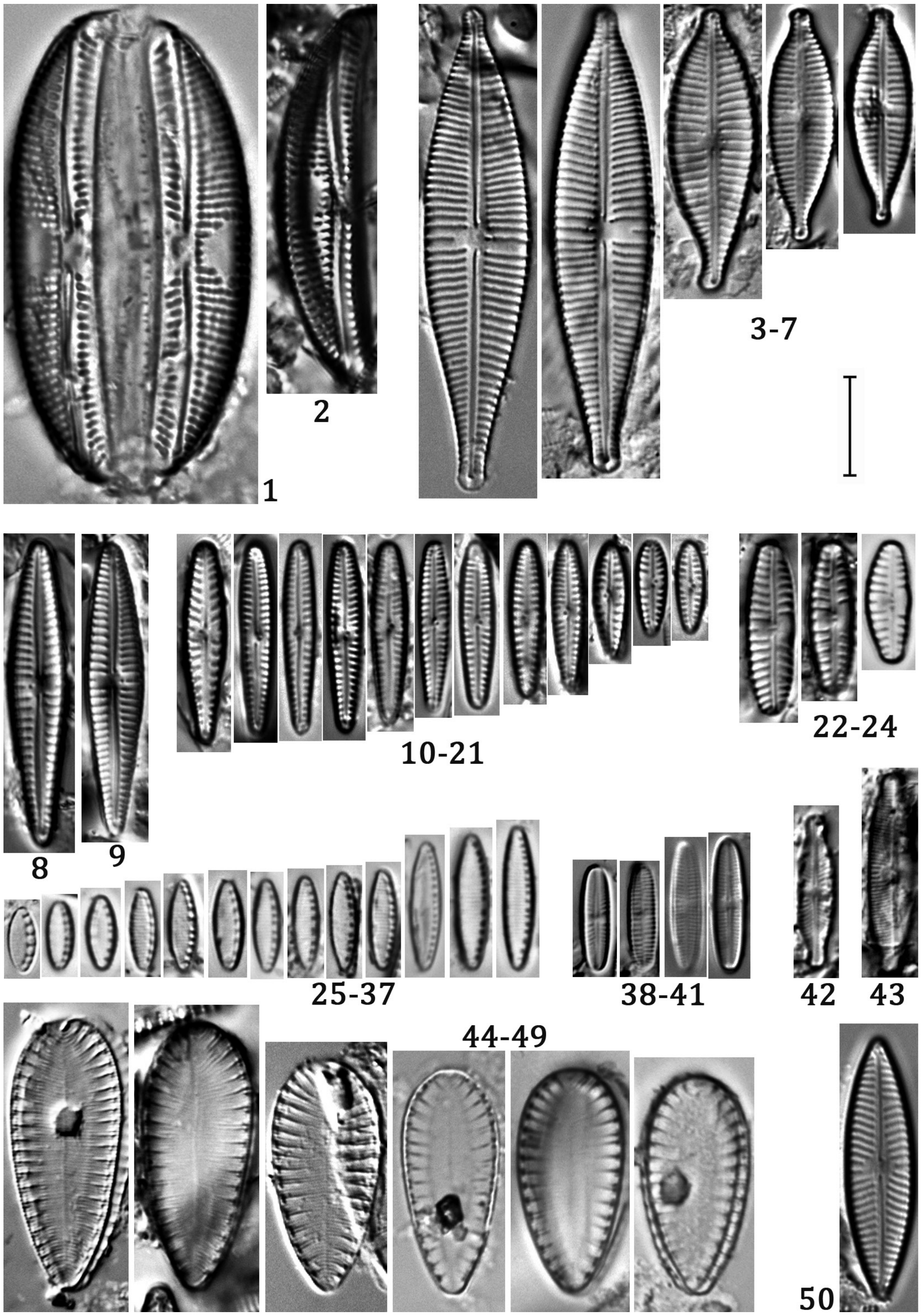

Figure 2. Light microscopy (LM) micrographs. 1, 2 *Amphora lange-bertalotii*; 3-7 *Gomphonema parvuliforme*; 8, 9 *G. ristovskae*; 10-21 *G. angustivalva*; 22-24 *Reimeria ovata*; 25-37 *Nitzschia soratensis*; 38-41 *Achnanthidium atomoides*; 42 *Stauroneis thermicola*; 43 *Adlafia brockmannii*; 44-49 *Surirella lacrimula*; 50 *Navicula moenofranconica*. Scale bar = 10 μm

per slide. Shannon's diversity index (H) was computed with OMNIDIA software.

Before statistical analysis, data on diatom species abundances were transformed using the arcsine of the square root of their relative abundance. Indirect gradient analysis was performed using the program CANOCO 4.5 (Ter Braak & Šmilauer 2002). Principal Component Analysis (PCA) was used to explain seasonal changes in diatom assemblages of two investigated rivers (to avoid repetition of spring season only samples from 2011 were used for indirect gradient analysis). Since each species was weighted by its variance, PCA based on covariance matrix was used (centering by species only).

Results

The physico-chemical analysis of water

According to the mean values of physico-chemical parameters water of Rača and Studenica rivers is alkaline, with moderate electrolyte concentration, with medium to fast velocity. According to the classification given by Dodds et al. (1998), the values of total phosphorus indicate that the water is oligo- to mesotrophic for both rivers (Tab. 1 and Tab. 2).

Epilithic diatom community

In the Rača River, 171 epilithic diatom taxa were identified, while in the Studenica River 190 taxa. The most numerous genera in both rivers were *Gomphonema*, *Navicula,* and *Nitzschia*.

Table 1. Average values of physical and chemical parameters of water from the Rača River.

Seasons	Para-meters	T (°C)	pH	Cond. (µS/cm)	DO (mg/l)	TP (µg/l)	PO_4^{3-} (µg P/l)	NH_4^+ (µg/l)	NO_3^- (mg/l)	Velocity (m/s)
Spring 2011	min	10.00	8.10	296.00	9.75	47.00	21.00	96.00	2.18	0.40
	avg	10.42	8.36	302.50	9.86	67.67	34.50	134.33	2.43	0.49
	max	11.00	8.60	308.00	9.98	78.00	45.00	211.00	2.61	0.62
Summer 2011	min	14.40	8.40	372.00	9.5	13.80	9.60	54.40	2.08	0.16
	avg	15.83	8.52	377.00	10.47	21.28	12.20	207.25	2.30	0.40
	max	17.30	8.57	381.00	12.5	31.10	15.60	639.90	2.66	0.62
Winter 2011	min	5.70	7.90	329.00	9.8	6.70	6.00	32.20	2.05	0.16
	avg	10.10	8.19	342.67	10.78	19.63	6.92	61.28	2.55	0.42
	max	11.90	8.32	365.00	12.4	38.80	8.10	109.40	2.83	0.56
Spring 2012	min	11.70	8.19	347.00	10.03	ND	0.00	38.83	2.46	0.28
	avg	13.22	8.36	355.67	10.39	11.05	4.03	119.40	2.85	0.43
	max	14.80	8.51	362.00	10.66	21.30	14.20	209.83	3.32	0.56

Cond – Conductivity; DO - dissolved oxygen; TP – Total phosphorus; ND – No data

Table 2. Average values of physical and chemical parameters of water from the Studenica River.

Seasons	Para-meters	T (°C)	pH	Cond. (µS/cm)	DO (mg/l)	TP (µg/l)	PO_4^{3-} (µg P/l)	NH_4^+ (µg/l)	NO_3^- (mg/l)	Velocity (m/s)
Spring 2011	min	6.20	7.69	146.00	10.6	39.00	21.00	97.00	1.80	0.41
	avg	7.33	7.93	150.00	11.3	59.17	31.17	140.50	2.03	0.49
	max	8.00	8.23	156.00	12.0	73.00	37.00	176.00	2.21	0.53
Summer 2011	min	15.20	8.56	210.00	9.2	17.50	10.30	0.00	1.29	0.31
	avg	17.18	8.62	226.00	9.7	37.45	12.90	60.78	1.57	0.38
	max	18.50	8.72	233.00	10.4	49.10	15.10	117.10	1.82	0.54
Winter 2011	min	0.70	7.78	153.00	14.4	ND	ND	24.10	1.00	0.31
	avg	1.35	7.88	185.40	15.2	ND	ND	108.98	1.28	0.43
	max	2.20	7.95	243.00	16.4	ND	ND	145.90	1.70	0.55
Spring 2012	min	6.80	7.81	164.40	10.4	ND	ND	181.70	1.95	0.52
	avg	7.20	7.91	166.20	10.6	25.97	13.18	204.72	2.06	0.58
	max	7.60	7.97	168.50	10.8	34.50	17.90	244.60	2.21	0.63

Cond – Conductivity; DO - dissolved oxygen; TP – Total phosphorus; ND – No data

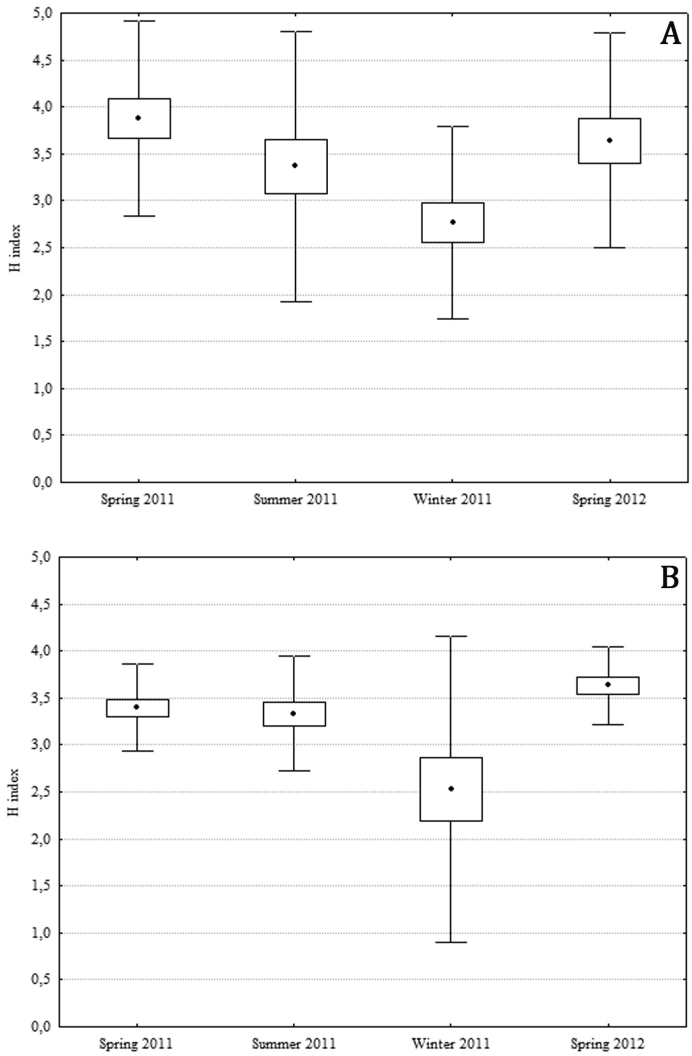

Figure 3. Seasonal changes in Shannon‘s diversity index (H) in two rivers. A - Rača River; B - Studenica River. Middle point refers to mean, box values refer to St. Error and whiskers refer to ± 2 Standard Deviations.

The most common taxa in the Rača River were: *Cocconeis lineata* Ehrenberg, *C. pseudolineata* (Geitler) Lange-Bertalot, *C. pediculus* Ehrenberg, *Achnanthidium minutissimum* (Kützing) Czarnecki, *A. pyrenaicum* (Hustedt) Kobayasi, *Amphora inariensis* Krammer, *A. pediculus* (Kützing) Grunow, *Navicula tripunctata* (O. F. Müller) Bory, *Reimeria uniseriata* S. E. Sala, J. M. Guerrero & M. E. Ferrario C. and *Ulnaria ulna* (Nitzsch) Compère. In the Studenica River, the most common taxa were: *A. minutissimum*, *A. pyrenaicum*, *A. inariensis*, *A. pediculus*, *C. lineata*, *C. pseudolineata*, *Cymbella compacta* Østrup, *Gomphonema olivaceum* (Hornemann) Ehrenberg, *G. pumilum* (Grunow) E. Reichardt & Lange-Bertalot, *G. tergestinum* (Grunow) Fricke, *Navicula cryptotenella* Lange-Bertalot, *N. lanceolata* Ehrenberg, *N. tripunctata*, *Nitzschia dissipata* (Kützing) Rabenhorst, *N. linearis* W. Smith and *Reimeria uniseriata*.

Among the identified taxa in both rivers, 11 are new for the diatom flora of Serbia (Fig. 2). A list of the new taxa with the dimensions of valves is shown in Tab. 3.

Diversity and seasonality of diatoms

In both investigated rivers the values of Shannon's diversity index (H) showed the highest diversity in spring periods (values about 3) (Fig. 3). Whit mean values of 2.5 the lowest diversity was during the winter period in both rivers (Fig. 3). It has been observed that with the value of Shannon‘s diversity index above 3, the diversity of codominant taxa is increased and their relative abundance is uniform.

Table 3. List of the new taxa for the diatom flora of Serbia.

Taxa	Length	Width	Striae per 10 μm	River	Fig. 2
Achnanthidium atomoides Monnier, Lange-Bertalot & Ector	7.3-12.6	2.3-3.4	R-valve 18-26 RL-valve 22-28	Studenica	38-41
Adlafia brockmannii (Hustedt) Bruder & Hinz	20.2	4.5	27	Studenica	43
Amphora lange-bertalotii Levkov & Metzeltin	48.7	24.2	11-12	Studenica	1, 2
Gomphonema angustivalva Reichardt	9.0-28.1	2.6-4.5	14-19	Studenica	10-21
Gomphonema parvuliforme Levkov, Mitic-Kopanja & E.Reichardt	21.4-48.7	6.5-10.9	12-15	Studenica	3-7
Gomphonema ristovskae Levkov & Tofilovska	29.6-31.0	5.7-6.0	13-14	Studenica	8, 9
Navicula moenofranconica Lange-Bertalot	26.6	6.9	14	Rača	50
Nitzschia soratensis E. Morales & Vis	6.9-16.3	2.5-3.5	23-29 9-16 fibulae/10 μm	Rača, Studenica	25-37
Reimeria ovata (Hustedt) Levkov & Ector	12.6-15.3	4.4-4.8	11-12	Studenica	22-24
Stauroneis thermicola (Petersen) Lund	15.8	3.7	25	Studenica	42
Surirella lacrimula J.D.English	22.4-27.7	9.5-11.4	7-9 waves/10 μm	Studenica	44-49

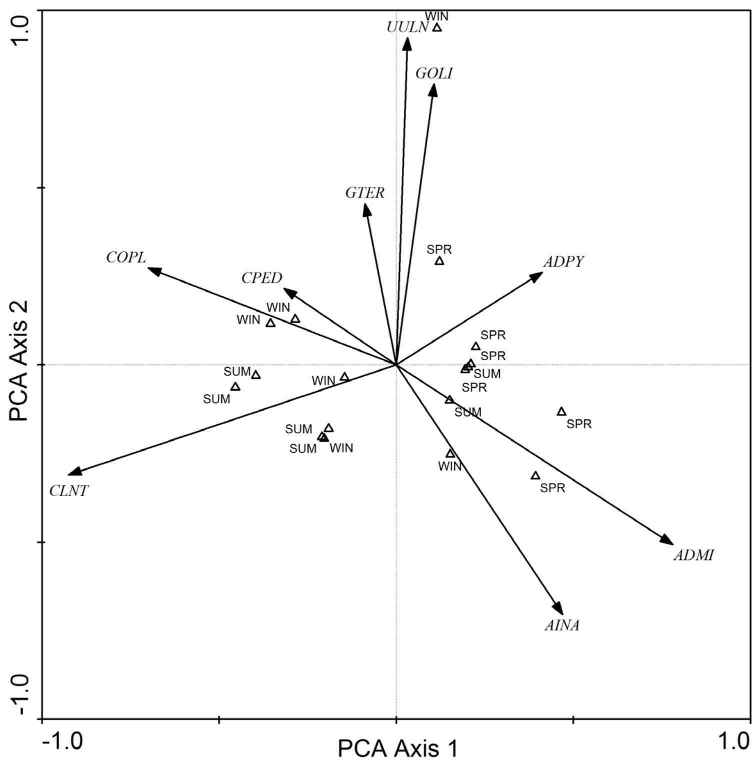

Figure 4. PCA biplot, the first two axes (River Rača). triangles – samples, arrows – dominant diatom species. ADMI – *Achnathiidum minutissimum*, ADPY- *A. pyrenaicum*, AINA – *Amphora inariensis*, CLNT – *Cocconeis lineata*, COPL – *C. pseudolineata*, CPED – *C. pediculus*, GOLI – *Gomphonema olivaceum*, GTER – *G. tergestinum*, UULN – *Ulnaria ulna*.

Principal Component Analysis (PCA) has shown seasonal changes in diatom assemblages of two investigated rivers. The first two PCA axes explained 71.9% and 83.4% of the variance within the group of dominant diatom species in River Rača and River Studenica, respectively (Tab. 4 and 5). In the Rača River, the most dominant taxon in all seasons was *Cocconeis lineta*, but with higher abundance during the winter and summer periods (Fig. 4). *Amphora inariensis* and *Meridion circulare* were dominant only in spring 2011, while *C. pediculus*, *Diatoma ehrenbergii,* and *Gomphonema pumilum* in spring 2012 (Tab. 6). The higher abundance of *Gomphonema olivaceum* and *Ulnaria ulna*

Table 4. Summarized results of principal component analysis (PCA) of the diatom community structure (only dominant taxa included) in River Rača

Axes	**1**	**2**	**3**	**4**
Eigenvalues	0.458	0.261	0.098	0.084
Cumulative percentage variance of species data	45.8	71.9	81.7	90.1
Total variance				1.00
Sum of all eigenvalues				1.00

Table 5. Summarized results of principal component analysis (PCA) of the diatom community structure (only dominant taxa included) in River Studenica.

Axes	**1**	**2**	**3**	**4**
Eigenvalues	0.657	0.176	0.105	0.028
Cumulative percentage variance of species data	65.7	83.4	93.9	96.6
Total variance				1.00
Sum of all eigenvalues				1.00

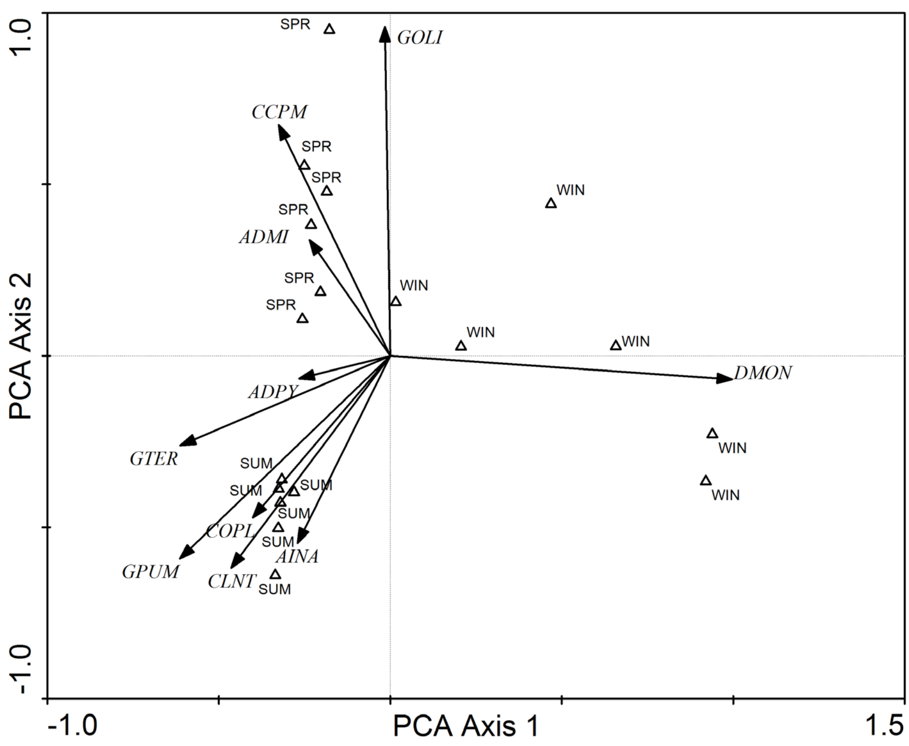

Figure 5. PCA biplot, the first two axes (River Studenica). triangles – samples, arrows – dominant diatom species. ADMI – *Achnnathiidum minutissimum*, ADPY- *A. pyrenaicum*, AINA – *Amphora inariensis*, CLNT – *Cocconeis lineata*; COPL – *C. pseudolineata*, CCPM – *Cymbella compacta,* DMON – *Diatoma moniliformis*, GOLI – *Gomphonema olivaceum*, GPUM – *G. pumilum*, GTER – *G. tergestinum*.

Table 6. Dominant taxa during different seasons at Rača and Studenica rivers

Rivers	Rača				Studenica			
Taxa	**Spring 2011**	**Summer 2011**	**Winter 2011**	**Spring 2012**	**Spring 2011**	**Summer 2011**	**Winter 2011**	**Spring 2012**
Achnanthidium minutissimum (Kützing) Czarnecki	18.8-21.7	11.4	15.2					12.8-16
Achnanthidium pyrenaicum (Hustedt) H.Kobayasi	11.6	25.3			13.4-31.5	11.4-40.7	29	10.3-13.1
Amphora inariensis Krammer	10.9-18.4							
Cocconeis lineata Ehrenberg	10.9-19.5	29-75.4	10.1-52.4	14.2-30.5		11.2-21.6		10.22-12.3
Cocconeis pediculus Ehrenberg				16-41.7				
Cocconeis pseudolineata (Geitler) Lange-Bertalot	15.5		12.8-28.9					
Cymbella compacta Østrup					13.7			
Cymbella parva (W.Smith) Kirchner								21
Diatoma ehrenbergii Kützing				53.7				
Diatoma moniliformis (Kützing) D.M.Williams							14.3-66.4	
Meridion circulare (Greville) C.Agardh	13.6-36.4							
Gomphonema olivaceum (Hornemann) Ehrenberg			19.1		15-44.5		16.1-30.9	13.2
Gomphonema pumilum (Grunow) E.Reichardt & Lange-Bertalot				22.9		17.9-23.1		14.7
Gomphonema tergestinum (Grunow) Fricke					16.4	21.6-23.2		30.2-34.8
Ulnaria ulna (Kützing) Compère			21.04					

was spotted during winter 2011. The higher dominance of species genus *Achnantidium* was observed in the spring 2011 (Table 6, Fig. 4). In the Studenica River, the most dominant taxon in all seasons was *Achnanthidium pyrenaicum*, with a higher abundance during the summer 2011. Also, the increased relative abundance of *Gomphonema pumilum* and *G. tergestinum* was observed during the summer 2011 (Fig. 5), and spring 2012 (Table 6). *G. olivaceum* reached the highest frequency in the spring 2011 (Fig. 5). However, *Diatoma moniliformis* was dominant only in winter 2011 (Fig. 5), while *Cymbella compacta* and *C. parva* was dominant in spring 2011 and 2012, respectively (Table 6, Fig. 5).

Discussion

Epilithic diatom communities recorded in the Rača and Studenica rivers are similar to community composition found in the other rivers in Serbia (Simić 1996, Nikitović & Laušević 1999, Krizmanić 2009, Andrejić et al. 2012), indicating that the community is composed of several dominant taxa and a large number of taxa occurring sporadically (Van Dam 1982, Kelly & Whitton 1995, Allott & Flower 1997). Genera *Gomphonema*, *Navicula,* and *Nitzschia* were the most numerous in both investigated rivers. Previous studies have shown that these three genera are dominant in the epilithic community in the other rivers in Serbia (Andrejic et al. 2012, Jakovljević et al. 2016a, b, Vasiljević et al. 2017, Ćirić et al. 2018), as well as in similar river conditions in other countries (Seve & Goldstein 1981, Ní Chatháin & Harrington 2008, Lobo et al. 2010, Sevindik & Kucuk 2016).

So far, about 800 taxa of pennate diatom have been recorded within the territory of Serbia (unpublished database, Krizmanić). The largest number of diatom taxa for Serbia are common for other countries in Europe, Asia, North and South America. (Stoermer et al. 1999, Lange-Bertalot 2001, Metzeltin et al. 2005, Solak et al. 2016). In our study, 11 taxa were identified for the first time in Serbia. Generally, these taxa are rarely found and are represented in the samples with low relative abundance. Some of them, such as *Gomphonema parvuliforme* and *G. ristovskae* have been described relatively recently (Levkov et al. 2016). Sometimes, it is difficult to distinguish similar taxa, e.g. *Reimeria ovata* and *R. sinuata*. For a long time *Cymbella sinuata* var. *ovata* Hustedt was considered to be a synonym of *C. sinuata* W.Gregory (homotypic synonym of *R. sinuata*) (Kociolek & Stoermer 1987), but more recently, Hartley (1996) made the transfer to *Reimeria*. More detailed morphological studies have shown that *R. ovata* and *R. sinuata* can be differentiated by valve shape, valve width and areolae shape (Levkov & Ector 2010).

The highest values of Shannon�How diversity index in the Rača and Studenica rivers were during the spring periods (2011, 2012), while the lowest were during the winter (Fig. 3). A similar investigation in Turkey have shown the lowest value of Shannon‘s diversity index during the spring (Akar & Şahin 2017; Gümüş & Gönülol 2018). Lowest diversity can occur as a result of changes due to some chemical or physical "stress", availability of substrate, leading to a poorer community dominated by several species that are euryvalent relative to a given stressor (Soininen & Heino 2007).

Comparing the dominant taxa in the Rača and Studenica rivers during the different seasons their proper shift is not observed. In general, a higher relative abundance species of the genus *Achnanthidium* and *Cocconeis* is observed in both rivers (Table 6). Species of the genus *Achnanthidium* was mostly spotted during the spring period in both rivers, while *Cocconeis* species in the Rača River in winter and in the Studenica River in summer period (Figs 4, 5). However, in the Studenica River species of the genus *Gomphonema* were present mainly in spring and summer with higher relative abundance. According to Ní Chatháin and Harrington (2008) *Cocconeis placentula* was recorded with higher relative abundance during the early summer, while species of genus *Gomphonema* were more represented in the colder period of the year. The study of Yang et al. (2015) has shown a higher relative abundance of *C. placentula* and lower relative abundance of *A. minutissimum* during the winter, while in the spring they found a higher relative abundance of *G. olivaceum* and *G. parvulum*.

Achnanthidium minutissimum, taxon characteristic for limestone substrate, was more dominant taxa in the Rača River than in the Studenica River where is more prevalent *A. pyrenicum*. *A*. *minutissimum* is a good indicator of high dissolved oxygen levels, sensitive to organic pollution and is considered an indicator of good water quality (Wojtal & Sobczyk 2006, Kwandrans et al. 1998). *A. pyrenaicum*, as well as *A. minutissimum* is a common taxon in limestone, in oligo- to mesotrophic flowing waters (Lange-Bertelot et al. 2017). In the Studenica River *A. pyrenaicum* with relative abundance up to 30% is recorded during the spring 2011 at increased concentrations of total phosphorus (59.17 μg/l) compared to other seasons (Table 2, 4). Although *A. pyrenaicum* and *A. minutissimum* are widespread taxa found in waters with different nutrient levels, *A. minutissimum* can be dominant taxa in eu- and hypertrophic waters (Lange-Bertalot et al. 2017), whereas this is not the case with *A. pyrenaicum*.

According to Van Dam et al. (1994) *Cocconeis lineata* is characteristic for eutrophic waters, which is inconsistent with our results. In the Rača River, the higher relative abundance of *C. lineata* (up to 75.4%) was during seasons with a lower concentration of total phosphorus (summer and winter 2011, spring 2012). This deviation can be explained by the fact that *C. lineata* and *C. pseudolineata* until recently belonged to the *C. placentula* group (Jahn et al. 2009), so their habitat, distribution and ecology are not yet sufficiently known (Lange-Bertalot et al. 2017).

Gomphonema pumilum is considered to be the most common and widespread species of the genus *Gomphonema.* In Germany, it is found in limestone, oligosaprobic to β-mesosaprobic waters, medium to high trophic levels (Lange-Bertalot et al. 2017). *G. olivaceum*, as a widespread species, has been recorded in different types of freshwaters, ranging from oligo- to β- and α-mesosaprobic, mainly eutrophic waters with medium electrolyte concentration (Van Dam et al. 1994, Levkov et al. 2016, Lange-Bertalot et al. 2017). *G. tergestinum* in comparison with *G. olivaceum* tolerates only low concentrations of organic nitrogen and according to Van Dam et al. (1994) is characteristic for oligosaprobic and oligo- to mesotrophic waters. In the Studenica River, the higher relative abundance of *G. tergestinum* corresponded with higher concentrations of nitrate during the spring 2012 (Table 2, 4). Also, in the Studenica River, the higher relative abundance of *Diatoma moniliformis* was observed during the winter period where the nitrate concentration was lower (1.28 mg/l). According to Stenger-Kovács et al. (2007) this is a sensitive species, characteristic for waters with low trophic status.

As we mentioned, in Serbia many habitats in terms of diatom research are unexplored such as Rača and Studenica rivers. The results of this research provide data on diatom diversity supplementing the floristic list and point that during the different seasons dominant diatom taxa do not show the proper shift. This type of research is necessary for better monitor diatom sensitivity to different changes and stressors in Serbian rivers.

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