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Ephemeroptera, Plecoptera, and Trichoptera assemblages of karst springs in relation to some environmental factors: a case study in central Bosnia and Herzegovina

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Abstract: In this study we analyzed changes of Ephemeroptera, Plecoptera, and Trichoptera (EPT) assemblages in 50 springs along the Cvrcka River mainstream in central Bosnia and Herzegovina. Springs were divided into 3 groups: rheocrene, rheopsammocrene, and piped springs. The objectives of our survey were to analyze changes in EPT communities in response to some environmental factors and anthropogenic activities. Twenty-eight morphologically distinct taxa were identified. EPT diversity was positively correlated to discharge and areal coverage of sand and stones but negatively correlated with areal coverage of algae. Canonical correspondence analysis revealed that the most important factors in structuring EPT spring assemblages were macrophytes, pH value, water temperature, and conductivity, while a significant influence of altitude for EPT species assemblages was not found. EPT assemblages in springs were dominated by collector-gatherers and shredders. Our results indicate that piped springs significantly differed in EPT taxa distribution and diversity and imply that anthropogenic activities (capturing) would change natural conditions with a decrease in EPT index. The studied springs presented high diversity and some of them constitute the habitat for rare or endemic species (e.g., *Drusus crenophylax*) of the Balkan Peninsula, giving a high conservation value to these habitats.

Key words: Springs, EPT assemblage, diversity, environmental factors, functional feeding groups

1. Introduction

Springs are characterized by emerging groundwater that creates aquatic–terrestrial and groundwater–surface water ecotones (Webb et al., 1998) and they thus make an important contribution to the regional biodiversity of freshwater ecosystems (Ward and Tockner, 2001; Boulton, 2005). Springs are a particularly interesting habitat for invertebrates as the lower and usually stable temperature creates optimal conditions for stenothermal cold-water organisms (Illies, 1952; Erman and Erman, 1995; Fischer et al., 1998; Buczyński et al., 2003). Persistence and stability of habitat conditions (van der Kamp, 1995) are considered to be the reason for variety and diversity of species in the springs (Erman and Erman, 1990). Due to this stability, the impact of disturbance caused by seasonal changes on the macroinvertebrate assemblage is lower, while types of disturbance caused by other factors, for example by anthropogenic activities (capturing), are more pronounced. Therefore, the studies on this topic become more important.

The macroinvertebrate composition of springs can be influenced by various environmental factors: physicochemical factors (Orendt, 2000), hydrological factors (Ilmonen and Paasivirta, 2005), substratum composition (Hahn, 2000), or altitude (Barquín and Death, 2006). On the other hand, spring biocenosis is affected by fauna migrating from neighboring biotopes and by anthropopressure (Buczyński et al., 2003).

This study is focused on Ephemeroptera, Plecoptera, and Trichoptera (EPT) assemblages of springs along the Cvrcka River mainstream (NW Republic of Srpska, Bosnia and Herzegovina). The EPT groups considered in this study show a high degree of endemism and abundance in freshwater ecosystems in the region of the central Balkan Peninsula (Paunović et al., 2006; Savić et al., 2011, 2013; Vitecek et al., 2015), but knowledge on their ecology in spring habitats is almost entirely lacking. Possibly, this is the first paper providing data on the ecology of the EPT insects inhabiting springs in the Balkans. In view of the high human pressure that threatens the ecological status of spring ecosystems, it is important to verify the EPT assemblage and environmental factors that influence the groups considered in this study. On other hand, it is worth mentioning that the study of springs lying along

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river mainstreams and impacts on these springs and their organisms is often neglected.

Having all this in mind, the aims of this study were to analyze diversity and distribution patterns of EPT assemblages and to evaluate effects of environmental factors and anthropogenic action (piped springs) on the spatial patterns of the EPT assemblages within the studied springs.

2. Materials and methods

2.1. Study site

All springs examined are situated in the canyon of the Cvrcka River in the northwestern part of the Republic of Srpska, in Bosnia and Herzegovina. The rocks are carbonate and limestone and derive from clastic sediments, mainly from the period of the upper Kreda. The vegetation was represented by deciduous forests dominated by common beech (*Fagus sylvatica* L.). An overview of characteristics of springs examined is given in Table 1.

2.2. Data collection

EPT taxa were collected in 50 springs along 12 km of the Cvrcka River basin (Figure 1). Macrozoobenthos was collected with a hand net (mesh size 350 µm) in the eucrenal zone of each spring as far as possible without damaging the habitat. Samples were collected for each spring once in September and October 2012 and 2013. Specimens were identified using a stereomicroscope (Leica EZ4 HD) to species level. Identification was done according to Belfiore (1983) and Bauernfeind and Soldán (2012) for Ephemeroptera, and Wallace et al. (2003) and Waringer and Graf (2013) for Trichoptera. Plecoptera were determined by Wolfram Graf personally.

Substrate composition (stones, gravel, sand, clay, anoxic mud, detritus), cover of stream organic matter (leaf litter), and vegetation cover (algae, moss, macrophytes) were determined by visual estimation (Armitage et al., 1995; Mori and Brancelj, 2006). Substrates were grouped into five classes of frequency (Table 2) according to Hahn (2000): 0 (absent), 1 (little), 2 (medium), 3 (much), and 4 (throughout).

In every spring the pH value was measured by pH-meter (HI 98127, accuracy of 0.1). Conductivity was measured by conductometer (Nahita, accuracy of 2 cF) and oxygen concentration by oximeter (HI 9142, accuracy of 0.1 mg/L). Water discharges were determined by eye and grouped into three classes according to von Fumetti et al. (2006): 1 (<1 L/min), 2 (>1 and <5 L/min), and 3 (>5 and <20 L/min).

2.3. Data analysis

Springs were divided into 3 groups: rheocrene (RH), rheopsammocrene (RP), and piped (spring water emerging from an artificial pipe, PI) (Table 1). One-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test, as a post hoc test, were applied using R software to determine the significance of differences in the elevation, water temperature, conductivity, pH, oxygen concentration, number of species, number of individuals, and % index of Ephemeroptera, Plecoptera, and Trichoptera index (Lewin et al., 2013) between the three spring types. For the other observed parameters (i.e. discharge, macrophytes, moss, leaf litter, detritus, anoxic mud, clay, sand, gravel, stones), the Kruskal–Wallis test was used to determine the significance of differences between the three spring types, since parameter distribution showed a lack of normality. In order to determine the dependence of the EPT index and number of EPT species on environmental factors, correlation analysis and Pearson's correlation coefficient were used. Prior to analysis, we checked the normality of data using the Kolmogorov–Smirnov test. For all statistical analysis, as the level of significance $P = 0.05$ was used.

Canonical correspondence analysis (CCA) (Ter Braak, 1986) was applied to test the influence of environmental variables on the EPT assemblages. Environmental variables may be highly correlated or "redundant" with one another. In such cases, CCA is sensitive to overfitting problems (McCune, 1997). This could lead to arbitrary inflation of the variance explained due to randomly covarying factors that are not ecologically related to the assemblage structure. To avoid the overfitting problem, we performed a forward selection of environmental variables (Ter Braak, 1986; Blanchet et al., 2008; Legendre et al., 2011). At each regression step, the variable that adds the most to the explained variance in the data is selected. The statistical significance of the null hypothesis that the selected variables are unrelated to EPT assemblage was tested by means of unrestricted Monte Carlo permutation tests (999 permutations) (Ter Braak and Wiertz, 1994). All classification and ordination methods were done using the FLORA software package (Karadžić et al., 1998; Karadžić and Marinković, 2009).

The Shannon diversity index (H') (Krebs, 2001) and index of %EPT were calculated (%EPT = sum of all individuals of Ephemeroptera, Plecoptera, and Trichoptera divided by the sum of all collected macroinvertebrates × 100) (Lewin et al., 2013).

3. Results

For the faunistic analyses we considered 50 springs (Table 1) for which EPT taxa were identified, with 28 taxa in total. Highest species richness was recorded among the Trichoptera (17 taxa), followed by the Ephemeroptera (6) and Plecoptera (5). All identified taxa are listed in Table 3.

The most frequent EPT taxa encountered were the Ephemeroptera *Baetis rhodani*, recorded in 11 springs,

Table 1. Geographical position and the values of environmental parameters of 50 studied springs: latitude (°N), longitude (°E), altitude (Al), discharge (D), temperature (WT), conductivity (Cond), oxygen content (O_2), and pH value (pH).

Spring number	Spring type	°N	°E	Al (m)	D	Temp (°C)	Cond (µS/cm)	O_2 (mg/L)	pH
1	RH	44°33.896′	17°25.509′	352	2	11.4	300	7.1	7.5
2	RH	44°33.944′	17°25.493′	351	1	13.9	400	8.1	8.1
3	RH	44°33.216′	17°24.100′	372	1	16.2	500	4.9	7.3
4	RH	44°33.214′	17°24.092′	371	1	11.8	400	7.5	7.7
5	RH	44°33.156′	17°23.872′	403	1	15.5	400	7.6	7.9
6	PI	44°33.162′	17°23.870′	405	1	16.9	500	6.6	7.7
7	PI	44°33.171′	17°23.853′	408	2	14.1	400	7.1	7.7
8	RH	44°32.878′	17°23.603′	470	4	9.6	300	9.0	8.0
9	RH	44°32.932′	17°23.562′	393	3	10.1	300	8.5	7.6
10	RH	44°32.797′	17°23.386′	421	1	16.0	300	6.6	8.0
11	RH	44°32.792′	17°23.378′	430	1	17.1	400	7.0	8.1
12	RH	44°32.676′	17°23.244′	441	2	12.5	300	7.6	7.7
13	RH	44°32.659′	17°23.240′	444	1	13.8	300	7.4	8.1
14	RP	44°32.547′	17°23.277′	487	2	10.7	300	7.6	7.7
15	RP	44°32.548′	17°23.292′	487	1	10.7	300	6.8	7.7
16	RP	44°32.654′	17°23.334′	486	1	10.9	400	7.5	8.1
17	RH	44°32.556′	17°23.298′	438	2	12.0	300	6.6	8.0
18	RH	44°32.453′	17°23.156′	453	3	12.0	300	6.2	7.9
19	RH	44°32.381′	17°23.157′	459	3	12.2	300	5.7	7.8
20	RH	44°32.254′	17°23.117′	455	3	12.3	400	7.1	8.0
21	RH	44°32.256′	17°23.121′	501	3	12.3	300	7.2	8.0
22	RH	44°32.274′	17°23.116′	472	2	11.1	400	7.5	7.9
23	RH	44°32.149′	17°23.150′	492	2	11.6	400	7.5	8.2
24	RH	44°32.018′	17°23.015′	588	4	13.0	300	5.2	7.9
25	RH	44°32.001′	17°22.944′	513	2	12.0	300	7.6	8.1
26	RH	44°32.017′	17°22.854′	489	4	13.3	300	5.9	7.9
27	PI	44°34.235′	17°25.738′	340	1	15.6	500	4.0	7.7
28	RH	44°33.219′	17°24.428′	437	1	14.4	400	6.3	7.7
29	RH	44°33.174′	17°24.476′	455	2	13.0	400	7.0	7.8
30	RH	44°33.088′	17°24.555′	491	3	12.9	400	7.1	7.6
31	RP	44°33.135′	17°24.160′	431	1	12.0	400	6.3	7.7
32	PI	44°33.131′	17°24.000′	382	1	13.9	400	6.8	7.8
33	RH	44°33.067′	17°23.780′	402	2	10.7	300	7.1	8.0
34	RP	44°33.490′	17°24.654′	394	2	14.0	500	6.5	8.2
35	RH	44°31.619′	17°21.510′	649	1	11.2	400	6.1	8.1
36	RH	44°31.422′	17°21.405′	666	2	10.5	400	5.1	7.5
37	RH	44°31.385′	17°21.294′	688	3	10.0	400	4.3	7.6
38	RH	44°31.567′	17°20.993′	720	1	13.8	400	7.1	7.7
39	PI	44°31.660′	17°20.635′	707	2	13.4	500	7.4	7.7
40	PI	44°32.870′	17°22.721′	745	3	11.7	300	7.6	7.7
41	PI	44°32.621′	17°22.765′	681	1	7.9	400	7.6	8.0
42	RH	44°32.834′	17°22.866′	666	1	12.2	400	7.0	7.8
43	RH	44°31.648′	17°21.955′	604	2	10.7	400	6.8	8.1
44	RH	44°31.498′	17°21.848′	627	3	9.5	300	4.5	7.8
45	RP	44°31.556′	17°21.910′	600	1	12.2	400	6.6	8.2
46	RH	44°30.633′	17°18.718′	802	1	11.6	400	6.0	7.8
47	PI	44°30.615′	17°18.715′	808	1	11.7	800	7.5	7.8
48	PI	44°30.625′	17°18.789′	780	2	9.8	400	5.7	7.7
49	RP	44°30.631′	17°18.928′	784	1	9.9	500	7.3	8.0
50	RH	44°30.737′	17°19.043′	734	3	9.5	500	7.4	7.7

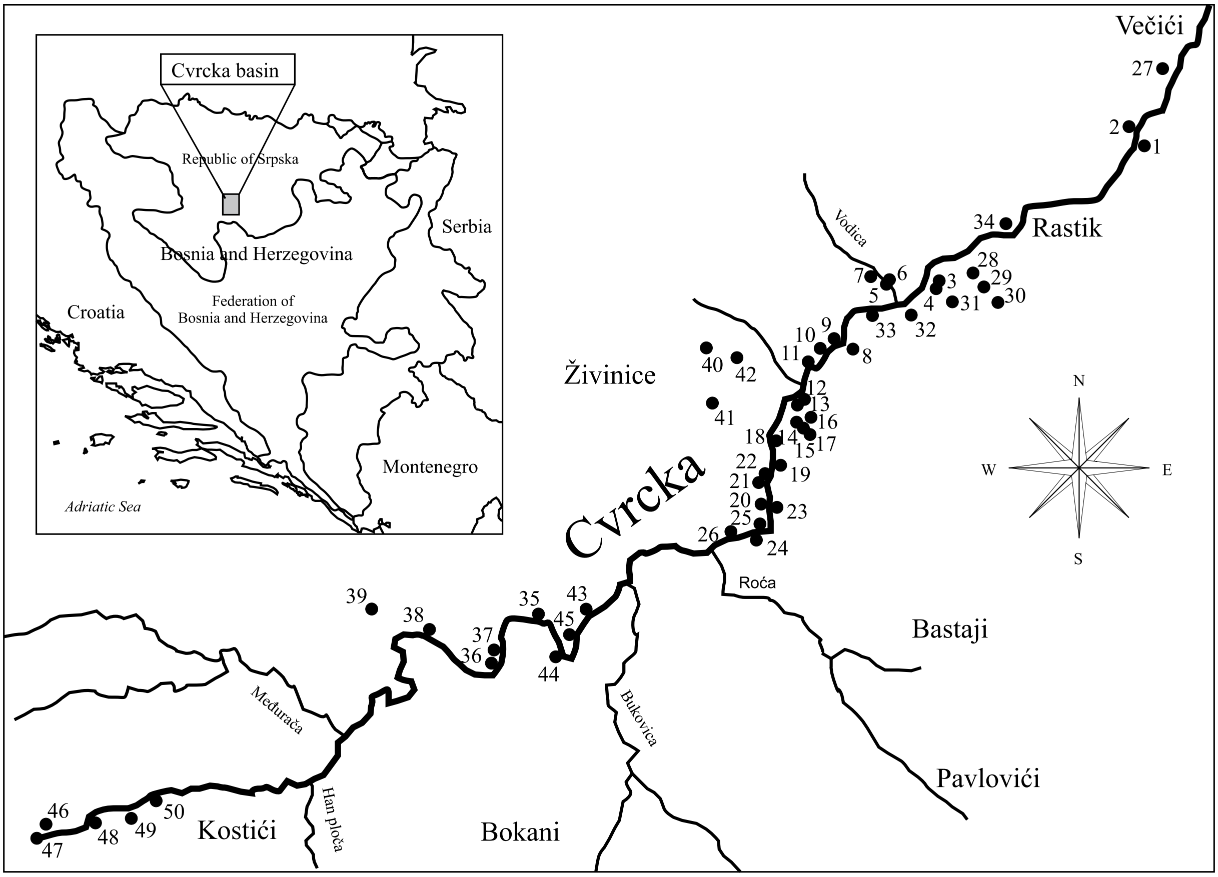

Figure 1. Map of the study area. The numbers correspond to spring numbers in Table 1.

and *Ecdyonurus* sp., recorded in 10 springs. The most frequent Trichoptera taxon was *Sericostoma* sp., detected in 8 springs, while *Leuctra hirsuta* was the most frequent Plecoptera, found in 3 springs.

The only species recorded in all three types of springs examined was *Baetis rhodani* (Table 3). On the other hand, 17 species were recorded from one type of spring: 13 species were restricted to rheocrenes, while 4 species were found exclusively in rheopsammocrene springs (Table 3). None of the species collected were restricted to piped springs.

The greatest species diversity was recorded in spring 24, with 8 species, while four springs had 5 recorded species each (springs 10, 18, 19, and 21).

The greatest value of EPT index was attained in spring 47 (Figure 2A), characterized by the greatest altitude among all studied springs.

Shannon's diversity index ranged from 0 to 1.78 (Figure 2B). Using the Kruskal–Wallis test it was confirmed that there was a significant difference in average values of Shannon's index among the three spring types (P = 0.040). The RH spring type had the highest average value, while the PI spring type had the lowest value of this index.

The Kolmogorov–Smirnov test showed that values of some of the studied parameters, i.e. elevation, water temperature, conductivity, pH, oxygen concentration, number of species, number of individuals, and EPT index, followed the normal distribution pattern in each of the three studied type of springs (rheocrenes, rhepsammocrenes, and piped springs). Using one-way ANOVA, it was confirmed that conductivity (F = 5.863; P = 0.005) and number of EPT species (F = 3.480; P = 0.039) differed significantly among springs types (Figure 3). Post hoc analysis, the LSD test, was performed in order to determine individual differences. The LSD analysis revealed that piped springs significantly differed from the two other spring types regarding conductivity (P = 0.001) and the number of EPT species (P = 0.011).

The Kolmogorov–Smirnov test showed that value distributions of other studied parameters, i.e. discharge and the areal coverage of the following substrate types: macrophytes, mosses, leaf litter, detritus, anoxic mud, clay, sand, gravels, and stones, were significantly different from normal distribution. The Kruskal–Wallis test did not show statistically significant differences in discharge and areal coverage of macrophytes. Regarding the remaining eight

Table 2. Substrate types (five classes of frequency) of 50 springs: 0 (absent), 1 (little), 2 (medium), 3 (much), and 4 (throughout).

Spring number	Stones	Gravel	Sand	Clay	Anoxic mud	Detritus	Leaf litter	Algae	Moss	Macrophytes
1	3	2	1	0	0	0	3	0	1	0
2	3	2	2	0	0	0	1	0	0	0
3	0	0	0	2	1	1	1	0	1	2
4	1	1	0	2	0	2	3	0	1	0
5	4	2	1	0	0	1	3	0	1	0
6	0	0	0	0	3	3	4	0	0	0
7	2	2	1	2	0	2	2	0	0	0
8	4	1	1	0	0	0	1	0	1	0
9	3	2	3	0	0	1	2	0	1	0
10	2	0	1	0	0	2	2	0	4	2
11	1	1	2	0	0	2	2	0	3	1
12	2	1	1	0	0	1	2	0	3	1
13	2	1	1	0	1	1	2	0	3	1
14	2	2	1	0	0	2	4	0	1	1
15	2	0	1	2	0	3	4	0	1	1
16	4	2	1	0	0	2	3	0	2	1
17	4	1	1	1	0	1	2	0	2	0
18	3	2	3	0	0	2	2	0	2	1
19	4	1	2	0	0	1	2	0	1	2
20	3	2	3	0	0	1	2	0	0	0
21	3	2	3	0	0	1	2	0	1	0
22	4	2	2	0	0	0	2	0	2	0
23	3	2	2	0	0	3	3	0	3	3
24	2	2	2	3	0	0	1	0	1	1
25	3	1	1	0	0	1	3	0	3	0
26	4	2	2	0	0	0	1	0	1	0
27	0	1	1	0	4	1	2	2	0	0
28	3	1	2	0	0	1	1	0	1	1
29	3	2	2	0	0	1	1	0	3	3
30	4	1	1	0	0	1	3	0	1	0
31	1	1	1	0	0	1	4	0	0	0
32	1	1	3	0	3	2	1	0	1	0
33	3	1	2	0	0	1	3	0	1	0
34	2	3	1	2	0	1	1	0	2	3
35	1	1	3	0	0	1	1	0	0	3
36	3	2	3	0	0	1	2	0	1	0
37	3	2	3	0	0	1	1	0	1	0
38	3	1	1	2	0	2	2	0	1	2
39	0	0	0	0	3	2	0	1	1	1
40	0	0	0	0	0	3	1	3	1	0
41	0	0	0	0	0	2	2	1	0	0
42	2	2	2	2	0	2	3	0	1	0
43	3	2	2	0	0	2	3	0	2	0
44	3	2	1	2	0	1	0	0	1	0
45	0	2	3	0	0	2	4	0	0	0
46	3	1	0	4	0	0	1	0	1	0
47	1	1	0	1	0	0	3	0	0	0
48	4	1	1	1	0	0	1	0	1	0
49	2	1	1	2	0	3	2	0	1	2
50	2	1	2	0	0	0	1	0	1	1

Table 3. The occurrence of EPT species in the studied springs. A - abbreviation of the species name; No_{PI} – number of PI springs inhabited by a particular species; No_{RH} – number of RH springs inhabited by a particular species; No_{RP} – number of RP springs inhabited by a particular species.

	A	No_{PI}	No_{RH}	No_{RP}	Spring number
Ephemeroptera					
Baetis rhodani (Pictet, 1843)	Br	1	9	1	8, 13, 17, 21, 24, 36, 37, 43, 44, 48, 49
Ecdyonurus sp.	Ec	1	9	0	5, 6, 8, 9, 19, 21, 24, 26, 36, 37
Electrogena sp.	El	1	7	0	2, 10, 18, 20, 21, 24, 36, 48
Habroleptoides confusa Sartori & Jacob, 1986	Hc	0	5	0	5, 10, 17, 20, 21
Ephemera danica Müller, 1764	Ed	0	4	0	10, 20, 21, 22
Caenis sp.	Ca	0	1	0	1
Plecoptera					
Perla marginata/pallida	Pm	0	1	0	30
Perla sp.	Pe	0	1	0	35
Nemurella pictetii Klapálek, 1900	Np	0	2	0	3, 4
Leuctra cf. *hirsuta*	Lh	1	2	0	7, 13, 46
Leuctra sp.	Le	0	0	1	49
Trichoptera					
Diplectrona sp.	Di	0	2	1	30, 43, 45
Diplectrona atra McLachlan, 1878	Da	0	1	1	14, 29
Polycentropus sp.	Po	0	0	1	15
Plectrocnemia sp.	Pl	0	4	1	4, 5, 36, 46, 49
Wormaldia sp.	Wo	0	0	1	45
Rhyacophila sp.	Rh	0	3	1	10, 19, 29, 45
Goera pilosa (Fabricius, 1775)	Gp	0	3	0	2, 9, 42
Silo sp.	Si	0	2	0	24, 35
Limnephilus sp.	Li	0	0	1	49
Drusus crenophylax Graf & Vitecek, 2015	Dr	0	7	0	2, 8, 9, 12, 18, 19, 24
Chaetopteryx sp.	Ch	1	2	0	17, 24, 41,
Chaetopteryx sp.	Cp	0	1	0	24
Halesus sp.	Ha	0	1	0	18
Potamophylax sp.	Pt	0	7	0	1, 4, 8, 10, 18, 19, 43
Crunoecia sp.	Cr	0	1	3	14, 23, 31, 34
Ernodes sp.	Er	1	1	0	25, 47
Sericostoma sp.	Sr	0	8	0	11, 13, 17, 19, 20, 24, 29, 35

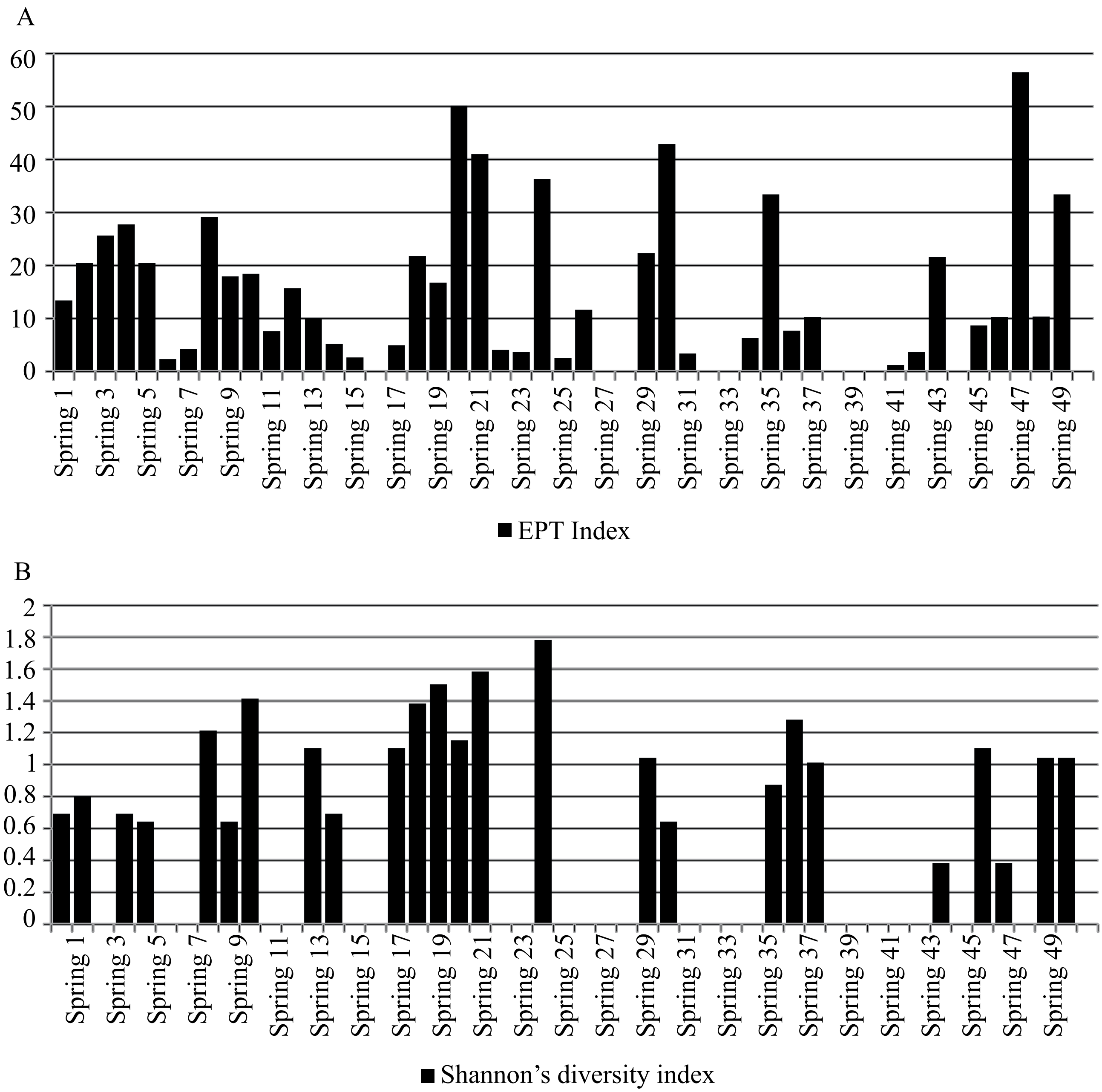

Figure 2. Values of: A) EPT index of studied springs, B) Shannon's diversity index.

parameters, i.e. areal coverage of the following substrate types: mosses, leaf litter, detritus, anoxic mud, clay, sand, gravels, and stones, there were statistically significant differences among the three types of springs, and the highest values of significance were recorded for areal coverage of algae (P = 0.000) and anoxic mud (P = 0.002).

Correlation analysis (Pearson's coefficient correlation) showed that the EPT index was in negative correlation with areal coverage of detritus (R = –0.281; P = 0.048).

Number of EPT species showed significant positive correlation with discharge (R = 0.315; P = 0.027) and areal coverage of sand (R = 0.345; P = 0.015) and stones (R = 0.310; P = 0.030), while it had a negative correlation with areal coverage of algae (R = –0.297; P = 0.038).

Canonical correspondence analysis (Figure 4) revealed that the most important environmental variables in determining the faunistic variability of the assemblage were the areal coverage of macrophytes, pH, and water temperature.

EPT assemblages were classified into 7 groups, following von Fumetti and Nagel (2011). Of the seven expected groups, four were recorded in our study, i.e.

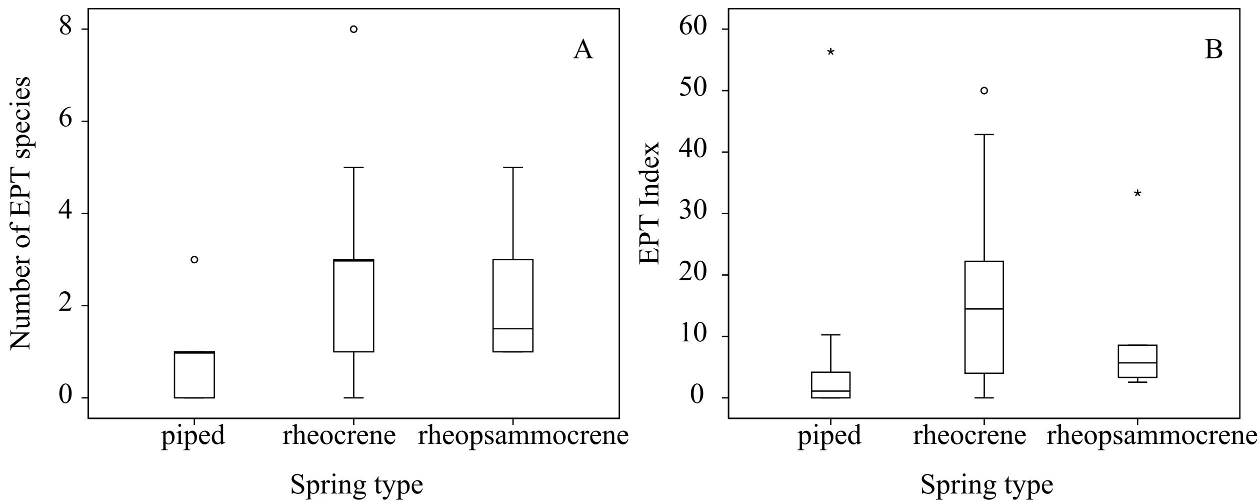

Figure 3. Box-and-whisker plot of the number of EPT species (A) and EPT index (B) among spring types.

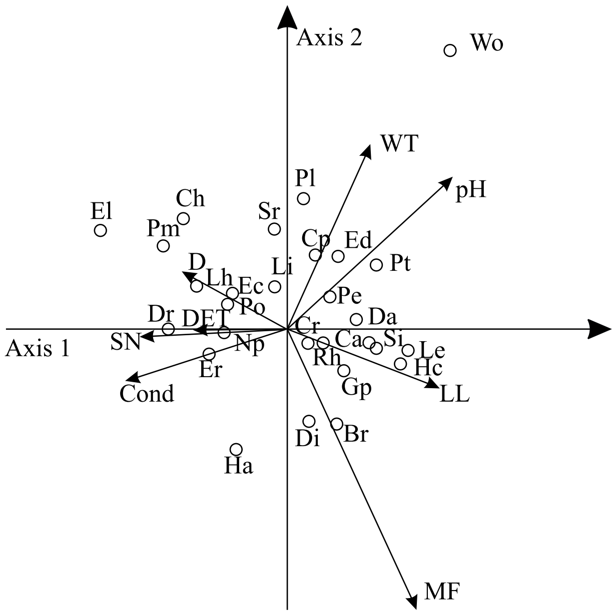

Figure 4. CCA analyses of the EPT taxa assemblages. WT – Water temperature; pH – pH value; D – discharge; LL – leaf litter; MF – macrophytes; Cond – conductivity; SN – sand; DET – detritus.

predators (Pr), scrapers (Sc), collector-gatherers (Co-Ga), and shredders (Sh). Of the 50 springs considered, 12 have three feeding types and 11 have two feeding types, while one type was found in the other springs (Figure 5). EPT assemblages of studied springs were dominated by shredders with 62.04%, followed by equal percentages of collector-gatherer and scraper organisms (17.58%), while predators were represented with the lowest percentage (2.78%).

4. Discussion

Regarding taxonomic richness, EPT assemblages of studied springs along the Cvrcka River mainstream were dominated by Trichoptera, which confirms previous studies of EPT taxa in springs (Erman and Erman, 1995; Maiolini et al., 2011). The most frequent species that we recorded belonged to the genera *Nemoura*, *Plectrocnemia*, and *Limnephylus*. In our study Ephemeroptera was more abundant than Plecoptera. Maiolini et al. (2011) showed that in Alpine springs Ephemeroptera was less abundant than Plecoptera. The latter authors reported the same number of Ephemeroptera (6 species) from 90 springs of Trentino (Italian Alps) as we found in our study. Ilmonen et al. (2009) recorded a slightly larger number of species of Ephemeroptera (8 species) in 153 springs in Finland during a 7-year study. Low abundance of mayflies in springs could be related to the thermal ability of mayflies that are generally adapted to warmer conditions (Pritchard et al., 1996). Our results show that this is not always true, and that abundance of mayflies in springs can be especially high.

Among the 6 taxa of Ephemeroptera, most species were rheophilous, i.e. *Baetis rhodani*, *Habroleptoides confusa*, *Ephemera danica*, *Ecdyonurus* sp., and *Electrogena* sp. All the springs of our study are connected with the river over the year, so it is possible that most of the detected mayflies species use springs as a refugium, especially in the period when the river level rises higher than the riverbank, and the floodwaters sometimes recharge spring brooks throughout the flooded areas.

The springs in our study were divided into three group based on environmental characteristics and also anthropogenic impact (piped springs). These three types of springs showed significant differences regarding the

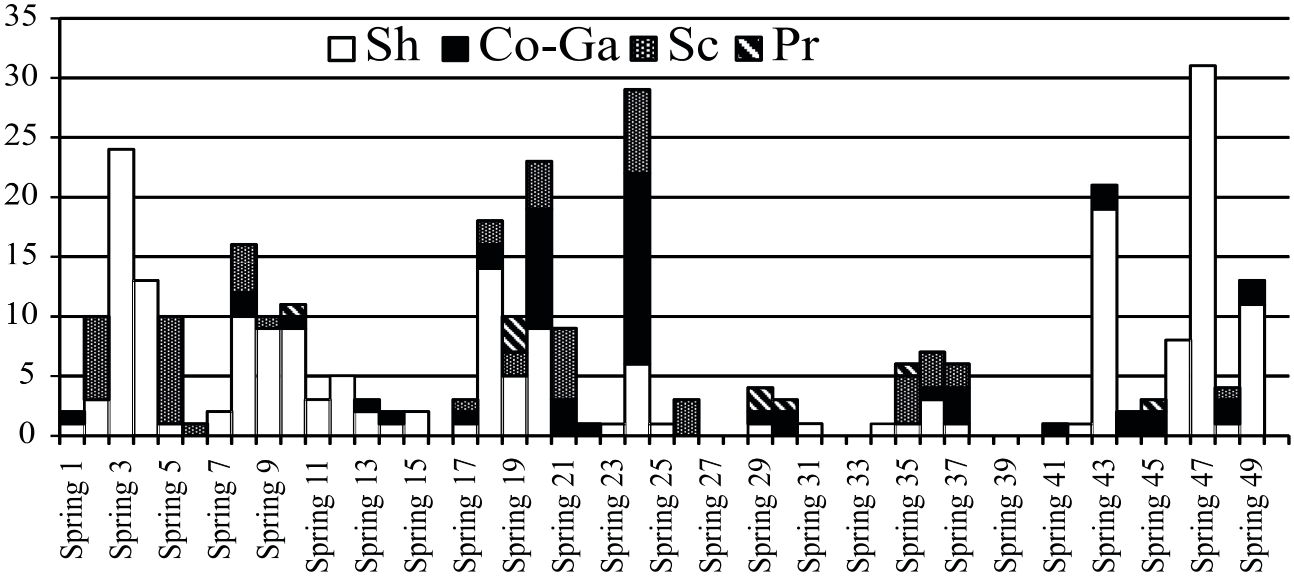

Figure 5. Functional feeding type composition of EPT assemblage.

number of EPT species, with the group of piped springs characterized by the lowest number of EPT species. The higher abundance of EPT taxa in rheocrenes compared with rheopsammocrenes highlights the importance of discharge for the spring fauna (von Fumetti and Nagel, 2012). In our study the number of EPT species was positively correlated with discharge ($R = 0.315$; $P = 0.027$), which is in confirmation of previous studies (Smith et al., 2003; Mori and Brancelj, 2006; von Fumetti et al., 2006; von Fumetti and Nagel, 2012). However, the results of CCA did not identify discharge as the most important factor for structuring EPT spring assemblages.

Our results match the conclusions by Harding et al. (1998), who showed a decline in the values of the EPT index at localities under some kind of anthropogenic impact. The studied types of springs differed significantly in the areal coverage of algae and anoxic mud in their substratum composition. The results of correlation analysis revealed that number of EPT species is negatively correlated with areal coverage of algae. Algae indicate a higher level of primary production, which leads to eutrophication (Dodds, 2006) and consequently to species decreasing. On other hand, some studies (e.g., Uwadiae, 2010) showed that mud in temperate streams can be considered as a poor substrate, probably because of its anoxic condition resulting from the decomposition of high organic matter load. Piped springs had low values of EPT index (Figure 2A). PI differed significantly from the two other types of springs in substratum composition (mainly anoxic mud and detritus; sand and leaf litter in rheopsammocrenes, and stones and gravel in rheocrenes).

The results of CCA analysis (Figure 4) in our study revealed macrophytes as a good predictor for EPT species richness. The macrophytes are highly significant in structuring the insect community in water ecosystems (Bowden et al., 2007). The localities with the greatest number of EPT taxa (springs 10, 18, 19, and 49 each hosted five taxa and spring 24 hosted 8 taxa) happen to be the same localities where macrophytes were recorded.

Elevation was not recognized as a significant factor in structuring EPT assemblage, in agreement with the findings of Verdonschot (2006). On the other hand, the results of our study are not consistent with the results by Zollhöfer (1999), Barquin and Deth (2009), and Maiolini et al. (2011), who considered altitude a good predictor of species richness and species composition. We think that this discrepancy was caused by the fact that springs studied in our study have a smaller range of altitudes (351–808 m a.s.l.) than the springs studied by, for example, Maiolini et al. (2011), where the range was 170–2792 m a.s.l. Figure 4 shows that, in addition to the presence of macrophytes, significant roles in structuring EPT assemblages belong to pH, temperature, and conductivity, as was already stated by Levin et al. (2013). Levin et al. (2013) showed that Ephemeroptera, Plecoptera, and Trichoptera belong to the taxa that are highly thermally sensitive. For example, the thermal limit for Heptagenidae (represented in our paper by the genus *Ecdyonurus*) ranged from 11.7 to 25.5 °C (Dallas and Ketley, 2011).

The Kruskal–Wallis test showed that there is no pattern in the distribution of feeding types in studied EPT spring assemblages. Springs in our study belong to the group characterized by gathering-collectors and shredders.

Those springs, usually with extensive carbonate deposits, lie between two extremes: springs dominated by scrapers and characteristic for lotic environments, and those that are mostly inhabited by filtering-collectors, associated with a lentic environment (von Fumetti and Nagel, 2011).

There are several anthropogenic activities (e.g., capturing, but also small hydropower plants fed by small mountain rivers such as the Cvrcka River that are currently under construction) that can potentially impact small spring habitats along river mainstreams. The studied springs presented high diversity and some of them constitute the habitats for rare or endemic species (e.g., stenoendemic *Drusus crenophylax*) in the Balkan Peninsula, giving a high conservation value to these habitats.

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