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Ecological patterns of Chironomidae assemblages in Dynaric karst springs

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Abstract – Springs are one of important freshwater habitats in the Dynaric Mountains. Nevertheless, there were no intensive studies on dipteran communities in the region. Here we present an ecological analysis of Chironomidae communities recorded from a set of 27 springs along the Cvrcka River mainstream (the Republic of Srpska, Bosnia and Herzegovina). Environmental classification of Cvrcka springs divide them into three groups reflecting the level of human impact. Chironomidae communities divide investigated springs into three groups more dependent on bottom substrate quality. CCA indicates that the hard bottom and altitude are primary (significant) factors determining midge assemblages. Secondary factors influencing communities are oxygen concentration and conductivity. There are clear differences in diversity and abundance in these three types of spring communities. Type II aggregates natural sites for Cvrcka valley. Samples characterized by high abundance of *Chironomus* seems to be an outliers in Cvrcka canyon. Eucronon and hypocronon communities are distinct, but no differences in the diversity level or the environmental assemblage relation were recorded for both mesohabitats. This study proves that solely environmental classification of spring habitats reflects well human impact, but invertebrate communities may not clearly follow general classification, reacting to a set of natural and altered conditions.

Key-words: Springs / Chironomidae / crenobiology / Dynaric Mountains

Résumé – Les caractéristiques écologiques des assemblages de Chironomidae dans les sources karstiques dinariques. Les sources sont l'un des habitats d'eau douce importants dans les montagnes dinariques. Néanmoins, il n'y avait pas d'études intensives sur les communautés de diptères dans la région. Ici, nous présentons une analyse écologique des communautés de Chironomidae étudiées à partir d'un ensemble de 27 sources le long du cours principal de la rivière Cvrcka (République de Srpska, Bosnie-Herzégovine). La classification environnementale des sources Cvrcka les divise en trois groupes reflétant le niveau de l'impact humain. Les communautés de Chironomidae divisent les sources étudiées en trois groupes dépendant surtout de la qualité du substrat. La CCA indique que la dureté du substrat et l'altitude sont des facteurs primaires (significatifs) déterminants des assemblages de chironomes. Les facteurs secondaires qui influent sur les communautés sont la concentration en oxygène et la conductivité. Il existe des différences nettes dans la diversité et l'abondance de ces trois types de communautés de source. Le type II regroupe des sites naturels de la vallée Cvrcka. Les échantillons sont caractérisés par une grande abondance de *Chironomus* et semble être un cas atypique dans le canyon Cvrcka. Les communautés de l'eucronon et de l'hypocronon sont distinctes, mais aucune différence dans le niveau de diversité ou de la relation à l'environnement de l'assemblage n'a été trouvée pour ces deux mésohabitats. Cette étude prouve que la classification de l'environnement des habitats de source reflète bien l'impact humain, mais les communautés d'invertébrés peuvent ne pas bien suivre ce classement général, en réaction à un ensemble de conditions naturelles et altérées.

Mots-clés : Source / Chironomidae / crénobiologie / montagne dinarique

1 Introduction

Springs are ecotones between groundwater and surface waters (Webb *et al.*, 1998) as well as between aquatic and

terrestrial biocenoses (Cantonati *et al.*, 2006). They reveal stable abiotic conditions in contrast to the rhithral and potamal zone (Van der Kamp, 1995). Chironomidae larvae are members of spring zoobenthos in both types of sites, eucronal (or spring source) and hypocronal (or springbrook) ones.

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Fig. 1. Map of the study area.

In comparison to other groups of insects, Chironomidae are poorly studied in springs due to the difficulty of their larvae determination. However, it is known that ecological patterns of Chironomidae assemblages in springs can be influenced by various environmental factors (Lencioni *et al.*, 2011; Mori and Brancelj, 2006) with an emphasis on anthropogenic activities (Ferrington, 1998), for example, by capturing springs (Lencioni *et al.*, 2012). Although Chironomidae larvae are important community members of spring zoobenthos in Balkan Peninsula (Mori and Brancelj, 2006) and other European regions (Wagner *et al.*, 1998), ecological patterns of their assemblages in springs of Dinaric karst are insufficiently known. This study focused on Chironomidae larvae assemblage of springs of Dinaric karst along the Cvrcka River mainstream (the NW Republic of Srpska, Bosnia and Herzegovina), where some of the investigated springs remain natural while the water quality and habitats of the others are substantially changed by human impact. Chironomidae larvae of Cvrcka River springs were not previously investigated, apart from Vilenjska Vrela spring (Filipović *et al.*, 2009). Nevertheless, they determined collected chironomid larvae only to the family level, providing information on their density at the bottom unit area. Those data were an integral part of the research of spring macrozoobenthos community. The same approach is applied while investigating the other springs of the catchment area of the Vrbanja River, which left tributary is the Cvrcka River, (e.g. Pavlović *et al.*, 2011) as well as the springs of the wider area of the Republic of Srpska (Pavlović *et al.*, 2009, 2012; Mršić *et al.*, 2009; Savić *et al.*, 2011).

This study is conducted in the framework of a broader project focused on benthic fauna in Cvrcka River springs. One

part of the springs is located in the canyon of the Cvrcka River. It is known that canyons as well as springs are refuges of many relicts and endemic species. That is why in the last years there have appeared several papers focused on the invertebrate fauna of Cvrcka River springs with the description of new probably steno-endemic species Hirudinea (Grosser *et al.*, 2014), Gastropoda (Glöer and Pešić, 2014) and Trichoptera (Vitecek *et al.*, 2015). All this further actualizes the importance of the research.

The aim of this study is: (1) to analyze the diversity and the distributional patterns of Chironomidae taxa in the springs along the Cvrcka River mainstream (the NW Republic of Srpska, Bosnia and Herzegovina), (2) to recognize the main environmental factors that influence Chironomidae assemblages in springs of Dinaric karst, (3) to verify how environmental classification of the springs is followed by chironomid communities themselves.

2 Materials and methods

2.1 Study area

The mainstream of the Cvrcka River (the NW Republic of Srpska, Bosnia and Herzegovina) is 14 745 km long with its source (785 m a.s.l.) in Kostići village and the water mouth (315 m a.s.l.) in the Vrbanja River, downstream of Večiči village (Figure 1). The climate of the Vrbanja River basin is temperate with high annual precipitation (the first maximum of precipitation is in spring and the second in autumn). Winter is the driest period of the year. The bedrock of the Cvrcka River

basin consists mainly of limestone and the river flows predominantly through a karst canyon. There are numerous karstic springs in the river valley and its near vicinity (Rajčević and Crnogorac, 2011).

The study included springs along 12 km of the Cvrcka River mainstream valley. This valley section is covered mostly by deciduous forest. Rheocrenes, rheopsammocrenes and captured springs were investigated (Appendix 1).

2.2 Field Sampling

Chironomidae larvae were collected from 27 springs of the Cvrcka River basin. Main spring sets (PSS) representing broad springs series typical for the Cvrcka River basin, aggregates 26 springs (Appendices 2 and 4). It was used to recognize general pattern of assemblages, and macrohabitats quality. Springs S 3, S 6 from PSS set and additionally site S 31 were sampled seasonally in spring, summer and autumn. They formed together EHSS spring set (Appendices 3 and 5) and were analyzed separately to find ecological distinctness between eucrenon and hypocrenon microhabitats. Chironomids from PSS were collected during September and October 2012 and 2013 (Appendix 2). From EHSS near Rastik village chironomids were taken seasonally during the year, specific for the source (eucrenal) and water flow directly downstream (hypocrenal) (Appendix 3). Samples were collected with a hand net (350 μ m mesh apertures) from all the microhabitats of the investigated springs. All the collected chironomids were preserved in 96% ethanol.

2.3 Environmental variables

Water temperature and pH values were measured with pH-meter HI 98127 accuracy 0.1, air temperature with thermometer accuracy 0.5 °C, conductivity with conductometer Nahita accuracy 2 cF and oxygen concentration with oximeter HI 9142 accuracy 0.1 mg·L⁻¹. Spring positions were recorded with GPS Oregon 550. Water discharge was determined by eye and grouped in classes: 1 (<1 L·min⁻¹), 2 (>1 and <5 L·min⁻¹), 3 (>5 and <20 L·min⁻¹) according to Fumetti *et al.* (2006). Substrate types were categorized in five classes of frequency: 0 (absent), 1 (little), 2 (medium), 3 (much), 4 (throughout) according to Hahn (2000).

2.4 Material identification

The material was determined mostly with Moller Pillot and Klink (2003) and Brooks *et al.* (2007). Ecological interpretation of taxa occurrence and their environmental preferences follow Moller Pillot (2009a, 2009b, 2013), Vallenduuk and Moller Pillot (2007), Wiederholm (1983), Moller Pillot and Klink (2003) and Brooks *et al.* (2007).

2.5 Statistical analyses

Multivariate statistics were performed for ecological interpretation of the data. Centered and standardized environmental data from the PSS were classified by Euclidean Distance

similarity index and illustrated by complete linkage dendrogram (Figure 2A). For classification of biota samples, Bray-Curtis similarity index on the square root transformed data was used, its results are illustrated by group average dendrogram (Figure 2B). PCA for PSS was undertaken on centered and standardized environmental data to recognize habitat diversification of the site groups used in the previous cluster analysis. Its results are presented on Figure 3, at first with marked by different colors groups of sites distinguished with environmental classification (Figure 3A, compare with Figure 2A) and secondly with sites marked after biota assemblages classification (Figure 3B, compare with Figure 2B). This is only picturing treatment, both plots (Figures 3A and 3B) illustrate the same mathematically PCA. SIMPER analysis was performed to test differences within faunal composition of the above mentioned groups A, B and C, and I, II and III (Table 3). Detrended Correspondence Analysis (DCA) was done to recognize variability gradient. As there were more than 7.1 SD units on the first two DC axes, Canonical Correspondence Analysis (CCA) was performed to find an environmental relation in taxa distribution among the samples (Figure 4, Appendix 6). Rare species were downweighted with method available in CANOCO 4.5 software. Biotic data for CCA were previously log transformed. Branches and algae were excluded from the analysis due to autocorrelation.

Because of low abundances (see Appendix 8) eukrenon-hypocrenon biotic data were not transformed. Because CCA didn't gave satisfactory results for EHSS data, Non-Metric MultiDimensional Scaling (NMDS) was performed on Bray-Curtis similarity index with 25 restarts, to find a general biota compositional pattern among the samples (Figure 5). Similarity Percentage (SIMPER) analysis was done to test differences between eucrenon and hypocrenon fauna (Table 4). Principal Component Analysis (PCA) was performed on centered and standardized environmental data to find diversification of the eucrenon and hypocrenon sampling sites (not illustrated).

Shannon diversity index was calculated on both EHSS and PSS data sets. Statistical differences for diversity index between the groups were analyzed by the t-test for the EHSS data set and the ANOVA for the PSS data set.

Canoco 4.5 statistical software was used for computing DCA and CCA, C2 software for centering and standardizing environmental data (except for CCA computed by Canoco) and PRIMER 6 for all the other multivariate analysis.

3 Results

3.1 Environmental features in the springs

Water temperature of the PSS (S 2–S 48) ranged between 7.9 and 17.1 °C (mode: 13.9 °C) with coefficient of variation 19.20% and air temperature near the spring ranged between 15.0 and 27.0 °C (mode: 17.0 °C) with coefficient of variation 18.36%. All the springs which belong to the PSS had alkaline pH value which varies around a mean of 7.83 ± 0.22 from 7.3 to 8.2 with coefficient of variation 2.82%. Conductivity of the PSS ranged between 3 and 5 cF (mean $\pm$ SD: 3.85 ± 0.67 cF) with coefficient of variation 17.55% and oxygen

Fig. 2. (A) Similarity distance between sites in groups A, B and C reflecting environmental characteristics of the PSS. (B) Bray-Curtis similarity of Chironomidae assemblages within the PSS.

concentration varied around a mean of $6.75 \pm 1.11 \text{ mg} \cdot \text{L}^{-1}$ from 4.0 to $8.5 \text{ mg} \cdot \text{L}^{-1}$ with coefficient of variation 16.41%.

Descriptive statistics of physical and chemical characteristics of the EHSS were analyzed by spring parts (Table 1).

Discharge at both site sets (PSS and EHSS) ranged from $<1 \text{ L} \cdot \text{min}^{-1}$ to >5 and $<20 \text{ L} \cdot \text{min}^{-1}$. Substrate composition of the PSS consisted of: anoxic mud, detritus, leaf litter, dead branches, moss, roots, macrophytes, clay, sand, gravel, stones, lime sinter, calcareous sinter, algae and waste materials (Appendix 4). Except algae and waste materials, all the substrate components were present in the EHSS, too (Appendix 5).

3.2 Assemblage composition

473 specimens from 23 taxa were collected from the PSS (S 2–S 48) in September and October 2012 and 2013 (Table 2). They represented four chironomid subfamilies (Tanypodinae, Prodiamesinae, Orthoclaudiinae and Chironominae). Subfamily Orthoclaudiinae accounted for 52% (12 taxa) of the total, followed by Chironominae (seven taxa or 30%), Tanypodinae (three taxa or 13%) and Prodiamesinae (one taxon or 4%).

All the collected chironomids were larvae. From one to seven taxa were found per spring. Only two springs (S 40

Fig. 3. (A) Results of PCA showing environmental characteristics of the PSS under classification into abiotic defined groups A, B, C. (B) Results of PCA showing environmental characteristics of the PSS under biotic classification into groups I, II, III, O (outliers).

and S 41) hosted more than 50 individuals. The most frequent taxon was *Micropsectra* type A (87 specimens present in 12 springs or 46% of the total 26 springs with chironomids) and the most abundant taxon was *Chironomus* (252 individuals).

140 specimens from 15 taxa, including larvae and singular pupal exuviae, were collected from springs S 3, S 6 and S 31 (EHSS) (Table 2). Chironomidae were collected from 16 or app. 67% of the total 24 samples (Appendix 3). From one to five taxa were found per sample. Only one sample (e3103) hosted more than 20 individuals. The most frequent and the most abundant taxon was *Prodiamesa olivacea* (present in

eight or 50% of the total 16 samples with chironomids) with 36 collected individuals.

3.3 Community patterns

Altogether 26 springs from the PSS may be divided into three groups (A, B and C) according to environmental conditions (Figure 2A).

To recognize environmental patterns in PSS, PCA was performed. The first, second and third PC axes explain respectively 21.8%, 17.3% and 10.6% variation of environmental variables. Results of PCA on the PSS (Figure 3A) show that

Fig. 4. Results of CCA for the PSS.

the sites in group A reveal a strong gradient in waste and algae amount. These sites are characterized by intermediate values and near 0 variation in temperature, discharge, macrophyte occurrence and calcium concentration, as well as by high content of anoxic mud and low concentration of lime stones. The sites from group B are distinct in high macrophyte abundance and calcium content. These sites reveal higher water temperature but lower discharge than groups A and C, intermediate to low algae and waste amount, the highest lime stone concentration and the lowest amount of anoxic mud. The sites from

group C manifest a relatively small variation of sediment composition (lime stones to anoxic mud), high discharge, macrophyte and calcium concentration and low temperature. Table 3 presents taxa mostly associated for each of site groups and dissimilarity in taxonomic composition between each of the groups. *Micropsectra* type A is characteristic for groups A and C. *Chironomus* is characteristic for the sites from group A, *Prodiamesa olivacea* show higher contribution to group C, whereas *Paraphaeonocladus* type A and *Brillia bifida* are characteristic for group B.

Table 1. Physical and chemical characteristics of the EHSS.

	Spring part	Water temperature (°C)	pH value	Conductivity (cF)	Oxygen concentration (mg·L ⁻¹)
Minimum	eucrenal	8.2	7.3	3	4.0
	hypocrenal	8.7	7.4	3	5.9
Maximum	eucrenal	15.2	8.0	6	7.5
	hypocrenal	15.2	8.0	5	7.8
Mean	eucrenal	11.98	7.65	4.08	6.77
	hypocrenal	12.01	7.72	3.83	7.11
St. Dev.	eucrenal	2.24	0.2	0.9	0.97
	hypocrenal	2.23	0.17	0.72	0.58
Coef. Var. (%)	eucrenal	18.66	2.64	22.05	14.28
	hypocrenal	18.55	2.15	18.72	8.17

Fig. 5. Results of the NMDS analysis showing a general gradient in chironomid assemblages in the eucrenon-hypocrenal spring zone.

The chironomid assemblages from the PSS may be divided into three groups (Figure 2B).

Most of the springs aggregates in group II, two smaller supplementary groups are I and III. Outliers encompasses 4 sites (S 44, S 40, S 41 and S 20) which do not belong to any cluster. PCA (Figure 3B) shows environmental conditions that favor such defined communities recorded at the study sites. Assemblages of the springs in group I form on the lime stone bottom, species belonging to communities I avoid anoxic mud. Algae and waste do not seem to correlate to occurrence of this group, while temperature, discharge, amount of calcium and macrophyte presence reveal a strong gradient within the sites of group I. The communities from group II are present on diverse bottom from anoxic mud to lime stones habitats. They reveal a strong variation along discharge, temperature, macrophyte amount and calcium content values. The species from this group clearly avoid waste and algae. The species from group III occur generally on the lime stone bottom, without anoxic mud content and do not seem to have a clear

preference in terms of discharge, temperature, waste, algae and macrophyte appearance. The outlier assemblages reveal a stronger correlation with algae and waste appearance. The species from this group prefer higher discharge, lower temperature and avoid habitats with macrophytes but reveal a strong variation according to anoxic mud and lime stone content.

Table 3 clearly indicates that the community groups specified in the biotic site classification (I, II, III) are much better defined, have higher internal similarity and are more dissimilar to each other than assemblages of A, B and C groups separated it on the habitat site classification. *Brillia bifida* and *Rheocricotopus effusus* are characteristic representatives of assemblages type I., while *Micropsectra* type A and *Prodiamesa olivacea* are distinct for assemblages type II. *Paraphaenocladus* type A and *Zavreliomyia* are typical of springs from group III and *Chironomus* separates outliers from the others.

ANOVA found significant difference ($P = 0.0001$) for the Shannon diversity index between PSS assemblages of site groups I, II and III. Highest diversity reveal assemblage type II (mean: 1.19, SD: ± 0.35), lowest assemblage type I (mean: 0.35, SD: ± 0.45), intermediate values keeps type III (mean: 0.53, SD: ± 0.30).

Results of CCA (Figure 4, Appendix 6) summarize main trends of chironomid-environmental relation. The first two axes explain 32.5% of species-environment relation. Two environmental factors significantly influence chironomid communities: stones (explaining 6.98% of variation with $P = 0.012$) and elevation (explaining 7.34% of variation with $P = 0.014$). The factor which has almost a similar significance in terms of influence on chironomid communities is oxygen concentration (explaining 5.19% of variation with $P = 0.11$). Ten factors are associated with Axis 1: elevation and detritus positively, while stones, moss, gravel, sand, air temperature, clay, leaf litter and water temperature negatively. Two factors are associated with Axis 2: oxygen concentration positively and conductivity negatively. Discharge is associated with Axis 1 and Axis 2 positively. Although most of the environmental factors, including significant ones, are associated with Axis 1, they differentiate only assemblages of springs from 40 and 41 from outliers and taxa *Chironomus* and *Limnophyes* from all the other assemblages and species. *Chironomus* and *Limnophyes* tend to occur on higher elevated sites more enriched by detritus. According

Table 2. List of Chironomidae taxa collected in all the investigated spring sites.

Subfamily	Taxa	Taxacode	PSS	EHSS
Tanypodinae	<i>Apsectrotanypus trifascipennis</i>	<i>Aps tri</i>	+	+
	<i>Macropelopia</i>	<i>Macpel</i>	+	+
	<i>Zavrelimyia</i>	<i>Zavrelim</i>	+	+
Prodiamesinae	<i>Prodiamesa olivacea</i>	<i>Proliv</i>	+	+
Orthocladiinae	<i>Brillia bifida</i>	<i>Brbif</i>	+	+
	<i>Corynoneura</i> sp. 1	<i>Corn 1</i>	+	
	<i>Corynoneura</i> cf. <i>antennalis</i>	<i>Coryn an</i>	+	
	<i>Heterotrissocladius marcidus</i> – type	<i>Het mar</i>	+	
	<i>Limnophyes</i>	<i>Limnop</i>	+	
	<i>Metriocnemus fuscipes</i> – type	<i>Metfus</i>	+	
	<i>Parametriocnemus stylatus</i> – type	<i>Prm sty</i>		+
	<i>Paraphaenocladius</i> sp. A	<i>ParaphA</i>	+	+
	<i>Paratrissocladius excerptus</i>	<i>Part ex</i>	+	
	<i>Paratrissocladius</i>	<i>Partriss</i>		+
	<i>Rheocricotopus effusus</i> – type	<i>Rheeff</i>	+	+
	<i>Rheocricotopus fuscipes</i> – type	<i>Rhefus</i>	+	
	<i>Synorthocladius semivirens</i>	<i>Synsem</i>	+	
	<i>Thienemannia</i>	<i>Thienm</i>	+	
Chironominae	<i>Chironomus</i>	<i>Chirono</i>	+	+
	<i>Polypedilum bicrenotum</i> – type	<i>Pol bic</i>	+	
	<i>Micropsectra</i> type A	<i>Mic A</i>	+	+
	<i>Micropsectra bidentata</i> – type	<i>Mic bid</i>	+	+
	<i>Micropsectra contracta</i> – type	<i>Mic con</i>	+	+
	<i>Micropsectra insignilobus</i> – type	<i>Mic ins</i>	+	+
	<i>Micropsectra junci</i> – type	<i>Micjun</i>	+	
	<i>Tanytarsus nemorosus</i> – type	<i>Tan nem</i>		+

to CCA, all the other taxa are more associated with lower elevated sites with the mineral bottom, including coarse fractions such as stones, leaf litter and moss. These taxa are found in higher temperatures. A much stronger variation is revealed by taxa and assemblages according to the gradient in oxygen concentration and conductivity. Axis 2 divides assemblages from group I, which is associated with higher oxygen concentration and lower conductivity, from assemblages II, which are more related to higher conductivity and appear in lower oxygen concentration. Groups III and outliers reveal intermediate oxygen and conductivity conditions.

3.4 Eucrenon-hypocrenon distinctness

The analyzed environmental EHSS data do not provide clear separation of the eucrenon and hypocrenon zone. PCA indicates that eucrenon sites (not illustrated) reveal slightly higher values of temperature and macrophyte amount, while hypocrenon stretches have higher oxygen concentration, branches and sand on the bottom but this pattern is very weak and insignificant. According to community composition, they have clearly distinct assemblages (Figure 5). Hypocrenon communities manifest a strong gradient in assemblage composition from near similar to eucrenon to very distinct from one, whereas eucrenon assemblages are more concentrated.

Prodiamesa olivacea and *Paraphaenocladius* type A separate eucrenon from hypocrenon zone communities (Table 4) but *Micropsectra* type A inhabits both types of mesohabitats. Both zones do not differ clearly in biodiversity and species richness. Eucrenon assemblages seem to reveal higher larvae abundance, whereas hypocrenon communities reveal slightly higher evenness.

A *t*-test found no significant difference in mean values for Shannon diversity index between eucrenon and hypocrenon ($P = 0.250$).

4 Discussion

Chironomidae larvae are an important component of mountain spring communities in the Balkan Region (Mori and Brancelj, 2006) and other European countries (Wagner *et al.*, 1998). As many as 20% of all chironomid species appear in spring habitats in the Holarctic region (Ferrington, 1998). In 27 springs of Cvrcka valley, there were 23 taxa recorded, whereas in the Italian Alps the number of species ranges from 81 taxa collected from 124 springs (Lencioni *et al.*, 2012) to as many as 104 species/groups in 81 springs (Lencioni *et al.*, 2011). Although it is difficult to distinguish truly crenobiotic species, many chironomid taxa are recognized to be crenophilous (Marziali *et al.*, 2010; Lencioni

Table 3. A) Results of SIMPER analysis for PSS assemblages of site groups A, B and C. B) Results of SIMPER analysis for PSS assemblages of site groups I, II, III and O (outliers).

Group A Average similarity: 20.00			Groups C&A Average dissimilarity = 85.42			Group I Average similarity: 34.55			Groups II & I Average dissimilarity = 98.23		
Group B Average similarity: 15.51			Groups C&B Average dissimilarity = 89.36			Group II Average similarity: 38.74			Groups III & I Average dissimilarity = 97.24		
Group C Average similarity: 14.23			Groups A&B Average dissimilarity = 87.92			Group III Average similarity: 23.49			Groups III & II Average dissimilarity = 88.09		
Species	Av.Abund	Av.Sim	Sim/SD	Contrib %	Cum. %	Outliers Average similarity: 12.52			Outliers & I; & II; & III Average dissimilarity = 99.41; = 96.25; = 97.12		
A						Species	Av.Abund	Av.Sim	Sim/SD	Contrib %	Cum. %
Mic A	1.42	7.63	0.60	38.17	38.17	I					
Chirono	3.48	6.96	0.41	34.81	72.99	Br bif	0.60	20.00	0.57	57.88	57.88
Proliv	0.71	2.08	0.39	10.38	83.37	Rheeff	0.68	14.55	0.61	42.12	100.00
Paraph A	0.29	1.25	0.22	6.27	89.64	II					
Mic con	0.49	0.77	0.22	3.86	93.50	Mic A	2.43	24.04	2.90	62.06	62.06
B						Proliv	1.14	7.84	0.76	20.23	82.29
Paraph A	0.71	5.73	0.49	36.98	36.98	Paraph A	0.43	1.54	0.30	3.98	86.27
Br bif	0.30	4.44	0.25	28.66	65.64	Part ex	0.40	1.35	0.30	3.47	89.74
Mic A	0.74	1.80	0.26	11.58	77.22	Mic ins	0.37	1.03	0.31	2.65	92.40
Zavrelim	0.44	1.58	0.26	10.16	87.38	III					
Rheeff	0.20	1.11	0.15	7.17	94.55	Paraph A	1.13	17.89	1.04	76.14	76.14
C						Zavrelim	0.84	5.60	0.32	23.86	100.00
Mic A	1.31	4.91	0.57	34.52	34.52	Outliers					
Proliv	0.86	4.10	0.42	28.83	63.35	Chirono	5.49	12.52	0.41	100.00	100.00
Part ex	0.46	2.08	0.26	14.60	77.96						
Zavrelim	0.70	2.05	0.29	14.41	92.37						

et al., 2011). Therefore, they have a higher potential for spring bioassessment and conservation than other insects. Nevertheless, Lencioni *et al.* (2011) and Lencioni *et al.* (2012) report only few taxa to achieve high abundance and frequency within their site sets. As in the Dinaric Mountains, in the Alps and other Holarctic localities, Orthoclaadiinae are the species richest group (Ferrington, 1998; Marziali *et al.*, 2010), whereas in Cvrcka River valley the presence of Diamesinae was not recorded. This may indicate some degradation of springs in this valley compared to the Alpine highlands (Lencioni *et al.*, 2011). The midge fauna of mountain springs is relatively diverse. Species richness tends to increase from the uplands to mountain elevations 1250–2250 m a.s.l., due to higher habitat heterogeneity (Lencioni *et al.*, 2011; Lencioni *et al.*, 2012).

The research in Cvrcka River valley prove that environmental and biotic classification of springs may not match. Environmental classification provides three clear groups of sites which indicate human impact on the habitat, landscape transformation and spring typology. Springs which belong to group A are under a strong human influence, particularly springs S 39, S 40 and S 41, which are captured springs in the central part of villages and their water is extensively used for drinking and watering livestock. Spring S 27 and S 6 are also captured, but they are located outside villages. Rheocrene springs S 3 and S 4 are located on the forest edge near Rastik village. All the other springs avoided a direct human impact. Most of the springs from group B are located in a valley with a deciduous forest. Some of the springs are captured, but they are not in use. Most of the springs from group C are upstream

Table 4. Results of SIMPER analysis for EHSS assemblages.

Group e Average similarity: 17.30				
Group h Average similarity: 8.76				
Groups e & h Average dissimilarity = 87.20				
Species	Av.Sim	Sim/SD	Contrib %	Cum. %
e				
Pro liv	8.70	0.65	50.31	50.31
Mic A	4.99	0.38	28.84	79.15
Mic con	1.59	0.39	9.18	88.33
Aps tri	1.27	0.24	7.37	95.70
h				
Paraph A	4.80	0.37	54.81	54.81
Mic A	1.73	0.22	19.77	74.58
Rhe eff	1.36	0.22	15.54	90.11

(S 46 and S 48) or downstream (S 2, S 7, S 9 and S 32) in relation to Cvrcka River canyon. They are more easily accessible to people compared to the springs from group B, but their water is not used intensively, due to the fact that most of them are located in the forest. Lencioni *et al.* (2011, 2012) and Ivković *et al.* (2015) prove human impact on terrestrial spring surroundings and canopy cover has a strong influence

on midge communities. Nevertheless, chironomid communities in Cvrcka valley divide sites into three groups and this biotic classification does not match the one based solely on environmental characteristics. Midge communities are also much more dissimilar than springs according to their environmental character. High faunistic dissimilarity between springs, even located nearby, is indicated by Lencioni *et al.* (2011). PCA indicates that midge communities in the Dinaric Mountains are strongly correlated to the bottom character, whereas such conditions as temperature and discharge are variable within assemblage types. Algae and waste are an important factor that influences species composition. Peryphyton is an important food supply for many midge species. Waste is often an artificial substrate for algae, especially if the natural bottom is composed of fine sediments. Algal vegetation is also enhanced by supply of nutrients, therefore eutrophicated, garbled springs should have specific chironomid fauna. It is symptomatic that communities type II, which reveal highest diversity, do not exist in garbled springs with ample algae vegetation. Assemblage II with *Micropsectra* and *Prodiamesa olivacea* seems to be natural, typical for the Cvrcka River basin, as it comprises the highest number of sites. *Prodiamesa olivacea* is also the most common species in springs in the Volga basin (Chuzhekova, 2014). It is likely that communities type I with *Brillia bifida* and *Rheocricotopus effusus* as well as III with *Paraphaenocladius* and *Zavrelimyia* inhabit two accessory natural spring types, and their occurrence depends on oxygen saturation and bottom type. Outliers reveal strong *Chironomus* domination. They are rheocrenes of cold, fast flowing water on higher elevation. They seem to be less typical for Cvrcka valley and associated with more garbled sites with ample algae vegetation. Springs S 40 and S 41 are situated in a village. These are huge captured springs with a high flow rate. Spring S 20 is located in the forest but in proximity of a settled area. Spring 44 is located in a small cave. This habitat diversity can be the reason why its assemblages do not aggregate with any other. CCA clearly separates sites of higher *Chironomus* and *Limnophyes* domination as being higher elevated, with more detritus on the bottom. A similar pattern was found in the Evortas River basin (Southern Greece). The mountain spring assemblages revealed higher *Chironomus* domination. *Limnophyes* and *Chironomus* tend to co-occur in the Evortas springs in more degraded sites (Karaouzas and Plóciennik, 2016). Ferrington (1998) indicates that springs influenced by human impact, frequented by cattle, are inhabited by taxa typical to lower-order enriched streams and have higher domination of Chironomini, namely *Chironomus* species. Lencioni *et al.* (2012) indicate *Limnophyes* to be also associated with captured springs. This species prefers the hygropetric zone in limnocrenes and appears on the rocky bottom with bryophytes (Lencioni *et al.*, 2011). Barquín and Death (2004) suggest that moss mats may accumulate detritus and provide good habitat for algae development. This may explain higher dominance of the above mentioned taxa in some of the springs, disturbed and natural as well. All the other species recorded in Cvrcka valley are associated with lower elevation and the stony bottom with a more mineral fraction. It is symptomatic that only stones and elevation shaped assemblages to a statistically significant degree. Factors such as presence of hard bottom, temperature, current and stream per-

manence associated with these two significant factors might have an indirect influence on the communities. Elevation and temperature are factors influencing chironomid communities on the broad, geographical scale (Ferrington, 1998). Mori and Brancelj (2006), Marziali *et al.* (2010) and Lencioni *et al.* (2011, 2012) indicate altitude, peryphyton, sediment quality, temperature and flow character to be the main drivers of biotic diversity in springs. In the Italian Alps, there is a strong positive correlation of habitat quality and chironomid diversity with altitude. Lencioni *et al.* (2012) find bed modification to cause diversity decrease whereas moderate eutrophication to favor species richness. In fact, all the other communities and species in Cvrcka River springs are more spread along the second CCA axis. Oxygen concentration and conductivity may play an important role while they remain insignificant according to the Monte Carlo Permutation test. We suppose they may be underestimated in this case. Communities II aggregating sites typical of Cvrcka valley occur in higher conductivity and lower oxygen concentration, whereas types I and III appear usually in better oxygen conditions and lower conductivity. Assemblages variability along the second CCA axis may in fact reflect water chemical composition. We have only information on pH, oxygen concentration and conductivity but other compounds such as nutrients and/or *e.g.*: sulfides, not measured, may have an influence on chironomids. In the Italian Alps (Marziali *et al.*, 2010, Lencioni *et al.*, 2011, 2012) pH, conductivity and water trophy are primary conditions differing highland pristine springs from upland disturbed ones. This pattern is not so relevant in boreal springs where mean annual temperature changing through climatic zones in a longitudinal gradient plays the main role. Ferrington (1998), Staudacher and Füreder (2007) draw attention to microhabitat complexity as the main driver of aquatic insect species composition, biodiversity and abundance. In the Eastern Alps, microhabitat heterogeneity and moisture of the spring zones – from fully aquatic, semiaquatic, to terrestrial – is linked to insect diversity. Chironomidae were spread through all those zones. In Eastern Alpine springs, chemical factors (such as conductivity) and altitude have only a weak influence on invertebrate communities (Staudacher and Füreder, 2007). Species composition and community structure manifest variability not only between spring types spread along Cvrcka valley but also in microhabitats within springs. In this study, no differences in diversity or species richness were observed between eu- and hypocrenon. McCabe (1998) reviews a number of examples from North America and Europe where a decrease or an increase of species richness with distance from the source was observed, so there is no general pattern, whereas individual gradients in species distribution were commonly observed. Rheocrene springs are proved to contain diverse niches and are species rich in chironomids (Lencioni *et al.*, 2012) and other insects (Cianficoni *et al.*, 1998). It is difficult to separate clearly defined types of springs, a gradual transition from one spring type to another is what can be observed instead (Lencioni *et al.*, 2011). NMDS and SIMPER analyses show that eucrenon and hypocrenon have different community composition in Cvrcka valley. Eucrenon assemblages are more specific due to higher uniformity of such habitats. Hypocrenon assemblages reveal a gradient ranging from more

similar to more distinct from eucronon. This is visible in the characteristic species groups for these two kinds of mesohabitats. In other studies *Prodiamesa olivacea* species was found in helocrenes and rheo-limnocrenes, *Micropsectra* species was recorded in the hygropetric zone with mineral sediment and bryophytes, *Paraphaenocladus* is usually found in brooks and telmatic margins of spring on the soft bottom, whereas *Apsectrotanypus trifascipennis* and *Rheocricotopus effusus* are typical for soft sediments in small flowing waters and helocrenes (Moller Pillot, 2013; Vallenduuk and Moller Pillot, 2007; Lencioni et al., 2011). Clear differences in assemblage composition indicated by NMDS and SIMPER are not confirmed by PCA which does not confirm environmental differences between these two kinds of habitats.

5 Conclusions

The investigated springs defy simple classification. Communities reflect well environmental parameters measured, but the environmental parameters alone do not give information how the community uses them. Whereas habitat structure, namely bottom composition, discharge and temperature, may explain to some extent community diversification, in this case only elevation and hard-bottom availability were significantly important factors. It seems that other, chemical factors, such as oxygen concentration, nutrients or diverse compounds responsible for conductivity that influence groundwater quality, may also play an important role for chironomids in springs in the Dinaric Mountains, but only investigation of a larger area and more detailed environmental data may prove that. Ecology of Balkan Peninsula springs remains relatively poorly recognized compared to temperate Europe, Apennine and Iberian Peninsulas. Unique climatic and geological conditions indicate a significant need for broader research of spring fauna of the region, especially due to the role which it plays in karstic landscape. Chironomidae are a key group of habitat quality indicators especially on the species level. Taxa, which here were recognized to be 'characteristic' for certain habitat quality, comprise high number of species (e.g. *Chironomus*, *Limnophyes*, diverse *Micropsectra* morphotypes) and are only ecological units. They leave only restricted space for exact interpretation or an ecological relation of environment and community. Further studies in the region should pay more attention to a wide database (including also a robust number of individuals) and high taxonomical resolution.

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References

Barquín J. and Death R.G., 2004. Patterns of invertebrate diversity in streams and freshwater springs in Northern Spain. *Arch. Hydrobiol.*, 161, 329–349.

Brooks S.J., Langdon P.G. and Heiri O., 2007. The Identification and use of Palaeoartctic Chironomidae Larvae in Palaeoecology,

QRA Technical Guide No. 10, Quaternary Research Association, London, 276 p.

Cantonati M., Gerecke R. and Bertuzzi E., 2006. Springs of the Alps, sensitive ecosystems to environmental change: from biodiversity assessments to long-term studies. In: Lami A. and Boggero A. (eds.), Ecology of high altitude aquatic systems in the Alps. Developments of Hydrobiology. *Hydrobiologia*, 562, 59–96.

Chuzhekova T.A., 2014. Spatio-temporal structure of macroinvertebrate community in springs and springbrooks of middle Volga basin. Kaamos Symposium 8-9.12.2014, Oulu. Abstracts of talks.

Cianficconi F., Carallini C. and Moretti G.P., 1998. Trichopteran fauna of Italian springs. In: Botosaneanu L. (ed.), Studies in Crenobiology, The biology of springs and springbrooks. Backhuys Publishers, Leiden, 125–140.

Ferrington L.C. Jr., 1998. Generic composition of Chironomid fauna in springs of North America. In: Botosaneanu L. (ed.), Studies in Crenobiology, The biology of springs and springbrooks. Backhuys Publishers, Leiden, 141–155.

Filipović S., Pavlović N., Pavlović B.P. and Savanović D., 2009. Stanje taksonocena zoobentosa krenona u slivu Vrbanje: 1. Vilenska vrela. In: Ilić P. (ed.), Zbornik radova. Naučno-stručni skup sa međunarodnim učešćem "Zaštita i zdravlje na radu i zaštita životne sredine", Institut zaštite, ekologije i informatike, Naučnoistraživački institut, Banja Luka, 323–329.

Fumetti S., Nagel P., Scheiffhacken, N. and Baltes B., 2006. Factors governing macrozoobenthic assemblage in perennial springs in north-western Switzerland. *Hydrobiologia*, 568, 467–475.

Glöer P. and Pešić V., 2014. *Belgrandiella bozidarcurcici* n. sp., a new species from Bosnia and Herzegovina (Gastropoda: Hydrobiidae). *Arch. Biol. Sci.*, 66, 461–464.

Grosser C., Pešić V. and Dmitrović D., 2014. *Dina sketi* n. sp., a new erpobdellid leech (Hirudinida: Erpobdellidae) from Bosnia and Herzegovina. *Zootaxa*, 3793, 393–397.

Hahn H.J., 2000. Studies on classifying of undisturbed spring in Southwestern Germany by macrobenthic communities. *Limnologia*, 30, 247–259.

Ivković M., Miliša M., Baranov V. and Mihaljević Z., 2015. Environmental drivers of biotic traits and phenology patterns of Diptera assemblages in karst springs: The role of canopy uncovered. *Limnologia* 54, 44–57.

Karouzias I. and Plóciennik M. 2016, Spatial scale effects on Chironomidae diversity and distribution in a Mediterranean River Basin. *Hydrobiologia*, 767, 81–93.

Lencioni V., Marziali L. and Rossaro B., 2011. Diversity and distribution of chironomids (Diptera, Chironomidae) in pristine Alpine and pre-Alpine springs (Northern Italy). *J. Limnol.*, 70, 106–121.

Lencioni V., Marziali L. and Rossaro B., 2012. Chironomids as bioindicators of environmental quality in mountain springs. *Freshw. Sci.*, 31, 525–541.

Marziali L., Lencioni V. and Rossaro B., 2010. The chironomids (Diptera: Chironomidae) from 108 Italian Alpine springs. *Verh. Int. Ver. Theor. Angew. Limnol.*, 30, 1467–1470.

Mc Cabe D.J., 1998. Biological communities in springbrooks. In: Botosaneanu L. (ed.), Studies in Crenobiology, The biology of springs and springbrooks, Backhuys Publishers, Leiden, 221–228

Moller Pillot H.K.M., 2009a. A key to the larvae of the aquatic Chironomidae of the North-West European Lowlands, private print, not published, 77 p.

Moller Pillot H.K.M., 2009b. Chironomidae Larvae, Biology and Ecology of the Chironomina, KNNV Publishing, Zeist, 270 p.

Moller Pillot H.K.M., 2013. Chironomidae Larvae, Volume 3: Biology and Ecology of the Aquatic Orthocladinae, KNNV Publishing, Zeist, 312 p.

- Moller Pillot H.K.M. and Klink A.G., 2003. Chironomidae larvae. Key to higher taxa and species of the lowlands of Northwestern Europe. – CD-ROM, ETI, Amsterdam.
- Mori N. and Brancelj A., 2006. Macroinvertebrate communities in karst springs of two river catchments in the Southern Limestone Alps (the Julian Alps, NW Slovenia). *Aquatic Ecol.*, 40, 69–83.
- Mršić M., Maksimović T., Pajčin R. and Filipović S., 2009. Stanje taksocena zoobentosa krenona u slivu Strižne i Vojskove. In: Ilić P. (ed.), Zbornik radova. Naučno-stručni skup sa međunarodnim učešćem “Zaštita i zdravlje na radu i zaštita životne sredine”, Institut zaštite, ekologije i informatike, Naučnoistraživački institut, Banja Luka, 331–338.
- Pavlović N., Pavlović P.B., Pajčin R., Filipović S., Dmitrović D. and Mršić M., 2009. Stanje taksocena zoobentosa krenona u slivu Sutjeske. In: Ilić P. (ed.), Naučno-stručni skup sa međunarodnim učešćem “Zaštita i zdravlje na radu i zaštita životne sredine”, Institut zaštite, ekologije i informatike, Naučnoistraživački institut, Banja Luka, 427–440.
- Pavlović N., Pavlović B.P., Dmitrović D., Pajčin R. and Filipović S., 2011. Zoobentos izvora gornjeg dijela sliva Vrbanje. *Skup*, 4, 13–23.
- Pavlović N., Balta M. and Dmitrović D., 2012. Longitudinalni raspored zoobentosa rječice Krupe pritoke Vrbasa. In: Redžić S. (ed.), Zbornik radova. Međunarodni naučni skup “Struktura i dinamika ekosistema Dinarida – stanje, mogućnosti i perspektive”, Akademija nauka i umjetnosti Bosne i Hercegovine, Sarajevo, 57–72.
- Rajčević V. and Crnogorac B.Č., 2011. Rijeka Vrbanja – Fiziogena svojstva sliva i riječnog sistema. “ARTPRINT”, Banja Luka, 276 p.
- Savić K., Pavlović N. and Dmitrović D., 2011. Stanje taksocena zoobentosa izvora slivnog područja Sane na Kozari. *Skup*, 3, 3–12.
- Staudacher K. and Füreder L., 2007. Habitat Complexity and Invertebrates in Selected Alpine Springs (Schütt, Carinthia, Austria). *Int. Rev. Hydrobiol.*, 92, 465–479.
- Vallenduuk H.J. and Moller Pillot H.K.M., 2007. Chironomidae Larvae of the Netherlands and Adjacent Lowlands. General Ecology and Tanypodinae, KNNV Publishing, Zeist, 143 p.
- Van der Kamp R.O., 1995. The hydrogeology of springs in relation to the biodiversity of spring fauna: a review. In: Ferrington L.C. Jr. (ed.), Biodiversity of aquatic insects and other invertebrates in springs. *J. Kans. Entomol. Soc.*, 68, 4–17.
- Vitecek S., Previšić A., Kučinić M., Bálint M., Keresztes L., Waringer J., Pauls S.U., Malicky H. and Graf W., 2015. Description of a new species of *Wormaldia* from Sardinia and a new *Drusus* species from the Western Balkans (Trichoptera, Philopotamidae, Limnephilidae). *Zookeys*, 496, 85–103.
- Wagner R., Fischer J. and Schnabel S., 1998. The Dipteran community of Central European springs: a summary. In: Botosaneanu L. (ed.), Studies in Crenobiology, The biology of springs and springbrooks, Backhuys Publishers, Leiden, 157–165.
- Webb D.W., Wetzel M.J., Reed P.C., Philippe L.R. and Young T.C., 1998. The macroninvertebrate biodiversity, water quality, and hydrogeology of ten karst springs in the Salem Plateau of Illinois. In: Botosaneanu L. (ed.), Studies in crenobiology: the biology of springs and springbrooks, Backhuys Publishers, Leiden, 39–48.
- Wiederholm T., 1983. Chironomidae of the Holarctic region. Keys and diagnoses. Part 1. Larvae. *Entomol. Scand. Suppl.*, 19, 457.

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Appendix 1: General characteristics of springs sampled along the Cvrcka River mainstream. The spring code follows general spring classification according to benthological studies in the Cvrcka River.

Spring code	Longitude	Latitude	Elevation (m)	Spring type	Land use
S 2	44°33.944'N	17°25.493'E	351	rheocrene	forest edge
S 3	44°33.216'N	17°24.100'E	372	rheocrene	forest edge
S 4	44°33.214'N	17°24.092'E	371	rheocrene	forest edge
S 5	44°33.156'N	17°23.872'E	403	rheocrene	forest
S 6	44°33.162'N	17°23.870'E	405	captured	forest edge
S 7	44°33.171'N	17°23.853'E	408	rheocrene	forest edge
S 9	44°32.932'N	17°23.562'E	393	rheocrene	forest
S 10	44°32.797'N	17°23.386'E	421	rheocrene	forest
S 11	44°32.792'N	17°23.378'E	430	rheocrene	forest
S 13	44°32.659'N	17°23.240'E	444	rheocrene	forest
S 15	44°32.548'N	17°23.292'E	487	rheopsamocrene	forest
S 17	44°32.556'N	17°23.298'E	438	rheocrene	forest
S 20	44°32.254'N	17°23.117'E	455	rheocrene	forest
S 23	44°32.149'N	17°23.150'E	492	rheocrene	forest
S 25	44°32.018'N	17°23.015'E	588	rheocrene	forest
S 27	44°34.235'N	17°25.738'E	340	captured	forest edge
S 31	44°33.135'N	17°24.160'E	431	rheopsamocrene	forest
S 32	44°33.131'N	17°24.000'E	382	captured	forest
S 36	44°31.422'N	17°21.405'E	666	rheocrene	forest
S 38	44°31.567'N	17°20.993'E	720	captured	forest
S 39	44°31.660'N	17°20.635'E	707	captured	village
S 40	44°32.870'N	17°22.721'E	745	captured	village
S 41	44°32.621'N	17°22.765'E	681	captured	village
S 43	44°31.648'N	17°21.955'E	604	rheocrene	forest
S 44	44°31.498'N	17°21.848'E	627	rheocrene	forest
S 46	44°30.633'N	17°18.718'E	802	rheocrene	forest
S 48	44°30.625'N	17°18.789'E	780	captured	forest

Appendix 2: Date of sampling at PSS (S 2–S 48) in September and October 2012 and 2013.

Spring code	Date of sampling	Spring code	Date of sampling
S 2	11.09.2012.	S 23	19.09.2012.
S 3	11.09.2012.	S 25	19.09.2012.
S 4	11.09.2012.	S 27	23.09.2012.
S 5	11.09.2012.	S 32	23.09.2012.
S 6	11.09.2012.	S 36	26.09.2012.
S 7	11.09.2012.	S 38	26.09.2012.
S 9	12.09.2012.	S 39	06.10.2012.
S 10	12.09.2012.	S 40	06.10.2012.
S 11	12.09.2012.	S 41	06.10.2012.
S 13	12.09.2012.	S 43	06.10.2012.
S 15	17.09.2012.	S 44	06.10.2012.
S 17	17.09.2012.	S 46	20.09.2013.
S 20	19.09.2012.	S 48	20.09.2013.

Appendix 3: Date of sampling at three springs (S 3, S 6 and S 31) (EHSS) by spring parts (eucrenal and hypocrenal) and four seasons during 2013.

Spring code	Date of sampling	Spring part	Spring part code
S 3	10.03.2013.	eucrenal	e3103
		hypocrenal	h3103
	10.06.2013.	eucrenal	e3106
		hypocrenal	h3106
	14.08.2013.	eucrenal	e3148
		hypocrenal	h3148
	24.09.2013.	eucrenal	e3249
		hypocrenal	h3249
S 6	10.03.2013.	eucrenal	e6103
		hypocrenal	h6103
	10.06.2013.	eucrenal	e6106
		hypocrenal	h6106
	14.08.2013.	eucrenal	e6148
		hypocrenal	h6148
	24.09.2013.	eucrenal	e6249
		hypocrenal	h6249
S 31	10.03.2013.	eucrenal	e31103
		hypocrenal	h31103
	10.06.2013.	eucrenal	e31106
		hypocrenal	h31106
	14.08.2013.	eucrenal	e31148
		hypocrenal	h31148
	24.09.2013.	eucrenal	e31249
		hypocrenal	h31249

Appendix 4: Physical and chemical characteristics from measurements in September and October 2012 and 2013 and substrate composition of 26 investigated springs (PSS) (S2–S48) of the Cvrcka river basin. Discharge (estimated by eye): 1 (<1 L·min⁻¹), 2 (>1 and <5 L·min⁻¹), 3 (>5 and <20 L·min⁻¹), 4 (>20 L·min⁻¹). Substrate type classes of frequency: 0 (absent), 1 (little), 2 (medium), 3 (much), 4 (throughout).

Spring code	Physical and chemical					Substrate composition															
	Water temperature (°C)	Air temperature (°C)	pH value	Conductivity (cF)	Oxygen conc. (mg/L)	Discharge	Anoxic mud	Detritus	Leaf litter	Dead branches	Moss	Roots	Macrophytes	Clay	Sand	Gravel	Stones	Lime sinter	Calcareous sinter	Algae	Waste materials
S2	13.9	17	8.1	4	8.1	1	0	0	1	1	0	0	0	0	2	2	3	0	0	0	0
S3	16.2	23	7.3	5	4.9	1	1	1	1	0	1	1	2	2	0	0	0	0	0	0	0
S4	11.8	23	7.7	4	7.5	1	0	2	3	3	1	0	0	0	1	2	1	2	1	0	0
S5	15.5	26	7.9	4	7.6	1	0	1	3	2	1	0	0	0	0	0	0	0	0	0	0
S6	16.9	26	7.7	5	6.6	1	3	3	4	2	0	0	0	0	0	0	2	0	0	0	0
S7	14.1	26	7.7	4	7.1	2	0	2	2	1	0	2	0	2	1	2	2	0	0	0	0
S9	10.1	23	7.6	3	8.5	3	0	1	2	1	1	0	0	0	3	2	3	1	0	0	0
S10	16.0	27	8.0	3	6.6	1	0	2	2	1	4	1	2	0	1	0	2	0	4	0	0
S11	17.1	27	8.1	4	7.0	1	0	2	2	1	3	0	1	0	2	1	1	0	4	0	0
S13	13.8	26.5	8.1	3	7.4	1	1	1	2	1	3	0	1	0	1	1	2	2	3	0	0
S15	10.7	17	7.7	3	6.8	1	0	3	4	2	1	1	1	2	1	0	2	2	1	0	0
S17	12.0	18	8.0	3	6.6	2	0	1	2	1	2	1	0	1	1	1	4	2	0	0	0
S20	12.3	15.5	8.0	4	7.1	3	0	1	2	1	0	0	0	0	3	2	3	1	0	0	0
S23	11.6	17	8.2	4	7.5	2	0	3	3	2	3	1	3	0	2	2	3	2	4	0	0
S25	12.0	20	8.1	3	7.6	2	0	1	3	1	3	0	0	0	1	1	3	2	3	0	0
S27	15.6	19	7.7	5	4.0	1	4	1	2	0	0	0	0	0	1	1	0	0	0	2	0
S32	13.9	21	7.8	4	6.8	1	3	2	1	1	1	0	0	0	3	1	1	1	0	0	0
S36	10.5	20.5	7.5	4	5.1	2	0	1	2	1	1	0	0	0	3	2	3	2	0	0	0
S38	13.8	25.5	7.7	4	7.1	1	0	2	2	2	1	1	2	2	1	1	3	2	0	0	0
S39	13.4	15	7.7	5	7.4	2	3	2	0	1	1	0	1	0	0	0	0	0	0	1	2
S40	11.7	18	7.7	3	7.6	3	0	3	1	0	1	0	0	0	0	0	0	0	0	3	0
S41	7.9	22	8.0	4	7.6	1	0	2	2	0	0	0	0	0	0	0	0	0	0	1	0
S43	10.7	23.5	8.1	4	6.8	2	0	2	3	2	2	1	0	0	2	2	3	2	1	0	0
S44	9.5	20	7.8	3	4.5	3	0	1	0	0	1	0	0	2	1	2	3	1	0	0	0
S46	11.6	17	7.8	4	6.0	1	0	0	1	1	1	0	0	4	0	1	3	1	0	0	0
S48	9.8	18	7.7	4	5.7	2	0	0	1	1	1	0	0	1	1	1	4	2	1	0	0

Appendix 5: Physical and chemical characteristics from measurements during 2013 and substrate composition of three investigated springs (S3, S31 and S6) (EHSS) of the Cvrčka river basin which are analyzed by seasons and spring parts. Discharge (estimated by eye): 1 (<1 L·min⁻¹), 2 (>1 and <5 L·min⁻¹), 3 (>5 and <20 L·min⁻¹). Substrate type classes of frequency: 0 (absent), 1 (little), 2 (medium), 3 (much), 4 (throughout).

Spring part code	Water temperature (°C)	Air temperature (°C)	pH value	Conductivity (cF)	Oxygen concentration (mg/L)	Discharge	Anoxic mud	Detritus	Leaf litter	Dead branches	Moss	Roots	Macrophytes	Clay	Sand	Gravel	Stones	Lime sinter	Calcareous sinter	Algae	Waste materials
e3103	10.4	14	7.7	3	7.0	2	0	2	2	2	1	1	1	2	3	1	1	0	0	0	0
h3103	9.6	14	7.9	3	7.4	2	0	2	1	1	1	1	3	1	1	1	1	0	0	0	0
e3106	10.5	25.5	7.7	4	6.1	2	3	2	1	1	1	1	2	2	1	1	1	0	0	0	0
h3106	10.6	25.5	7.7	3	6.6	2	0	2	1	1	1	3	3	2	1	1	1	0	0	0	0
e3148	12.7	27	7.4	4	6.4	1	0	2	1	1	0	2	4	0	1	1	1	0	0	0	0
h3148	14.2	27	7.6	4	6.9	1	0	2	1	1	1	1	4	0	1	1	1	0	0	0	0
e3249	15.2	22	7.8	6	4.0	1	2	1	1	1	1	1	3	2	1	1	1	0	0	0	0
h3249	14.8	22	7.8	4	5.9	1	2	1	1	1	1	1	3	2	1	1	1	0	0	0	0
e6103	8.2	14	7.4	4	6.9	1	0	2	4	2	0	1	0	0	1	1	1	0	0	0	0
h6103	8.7	14	7.4	4	6.7	1	0	2	4	2	0	1	0	0	1	1	1	0	0	0	0
e6106	10.9	22	7.6	4	7.4	2	0	2	3	1	0	0	0	0	1	1	1	0	0	0	0
h6106	10.9	22	7.7	4	7.4	2	0	2	4	2	0	0	0	0	1	1	1	0	0	0	0
e6148	13.3	22	7.3	5	7.0	1	0	2	4	1	1	1	0	0	1	1	1	0	0	0	0
h6148	13.6	22	7.5	5	6.7	1	0	2	3	2	0	0	0	0	1	2	1	0	0	0	0
e6249	13.0	13.8	7.8	5	7.2	1	0	2	3	2	0	1	1	0	0	1	1	1	0	0	0
h6249	12.6	13.8	7.8	5	7.2	1	0	2	3	2	0	1	0	0	1	2	1	1	0	0	0
e31103	9.5	16	7.6	3	7.5	3	3	3	2	1	1	1	0	1	2	1	1	1	0	0	0
h31103	9.6	16	7.8	3	7.8	3	0	1	1	2	1	1	0	0	2	3	2	1	0	0	0
e31106	11.0	23	7.7	3	7.2	3	2	2	2	2	1	1	1	2	2	2	2	1	0	0	0
h31106	10.9	23	7.7	3	7.8	3	0	0	1	1	1	1	0	0	1	2	3	1	0	0	0
e31148	15.2	26	7.8	4	7.0	1	2	1	1	1	1	0	0	1	1	2	2	1	1	0	0
h31148	15.2	26	7.8	4	7.1	1	1	2	1	1	1	1	0	1	1	2	3	1	1	0	0
e31249	13.9	19	8.0	4	7.5	1	2	2	2	2	2	1	1	1	1	2	2	1	0	0	0
h31249	13.4	19	8.0	4	7.8	1	0	2	2	2	1	0	0	1	2	2	3	1	0	0	0

Appendix 6:

Main parameters of CCA for the PSS.

Axes	1	2	3	4	Total inertia		
Eigenvalues			0.802	0.603	0.533	0.431	5.585
Species-environment correlations			0.987	0.899	0.984	0.888	
Cumulative percentage variance							
of species data			14.4	25.2	34.7	42.4	
of species-environment relation:			18.5	32.5	44.8	54.8	
Sum of all eigenvalues							5.585
Sum of all canonical eigenvalues							4.327

Appendix 7: List of Chironomidae species and number of individuals collected at 26 springs representing the PSS (S 2–S 48) in September and October 2012 and 2013.

Spring code	S 2	S 3	S 4	S 5	S 6	S 7	S 9	S 10	S 11	S 13	S 15	S 17	S 20	S 23	S 25	S 27	S 32	S 36	S 38	S 39	S 40	S 41	S 43	S 44	S 46	S 48
Taxa code	Number of individuals																									
<i>Aps tri</i>		3	1					4		1															2	
<i>Macpel</i>				6				1				1										1			8	
<i>Zavrelin</i>	3	1	3		5	4			1					1			3	5							3	
<i>Proliv</i>												1		1	1											
<i>Br bif</i>			1						1			1														
<i>Corn 1</i>							1																			
<i>Coryn an</i>																		3				1				
<i>Het mar</i>																										
<i>Linnop</i>																										
<i>Met fus</i>				1					2	5	1					1									1	
<i>ParaphA</i>													3				2					2			3	
<i>Part ex</i>			2			1		1																		
<i>Rheeff</i>												1							1							
<i>Rhefus</i>			1															1					1			
<i>Synsem</i>																										
<i>Thienm</i>																						1				
<i>Chirono</i>	1																									
<i>Pol bic</i>		3	2	1	3	18		2	14		5					3	2	14				81			2	
<i>Mic A</i>			5		18																				2	
<i>Mic bid</i>		1																							1	
<i>Mic con</i>		1				2												7		6					1	
<i>Mic ins</i>									1		1							2							1	
<i>Micjun</i>																										
No. taxa	1	6	7	3	3	5	2	4	5	2	4	3	1	1	1	2	3	6	1	3	2	2	2	1	7	
No. indiv.	3	10	15	8	26	28	3	8	19	6	8	3	3	1	1	4	7	32	1	9	169	82	3	1	18	

Appendix 8: List of Chironomidae species and number of individuals collected at three springs (S 3, S 6 and S 31) representing EHSS by spring parts (eucrenal and hypocrenal) at four seasons during 2013.

Spring code	S 3						S 6						S 31			
Spring part code	e3103	h3103	e3106	h3106	e3148	e3249	e6103	h6103	e6106	e6148	h6148	e6249	h6249	e31103	h31103	h31106
Taxa code	Number of individuals															
<i>Aps tri</i>			4			2					1	1				
<i>Macpel</i>				1	2											
<i>Zavrelin</i>									1							
<i>Proliv</i>			1		5	1	5			4	9	9	2			
<i>Br bif</i>																1
<i>Prm sty</i>	1															
<i>ParaphA</i>		1						1						3	6	
<i>Partriss</i>						2										
<i>Rheeff</i>				1		1		2								
<i>Mic A</i>	3						7			7	4	10	4			
<i>Mic bid</i>	1						1		1							
<i>Mic con</i>	5						1		3	1	2					
<i>Mic ins</i>				1		1										
<i>Chirono</i>	19		1													
<i>Tan nem</i>				1												
Number of taxa	5	1	3	4	2	5	4	2	3	3	4	3	2	1	1	1
Number of individuals	29	1	6	4	7	7	14	3	5	12	16	20	6	3	6	1