

## Environmental factors affecting mayfly assemblages in tufa-depositing habitats of the Dinaric Karst

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**Abstract** – Remarkably, unlike other parts of Europe, the ecology of mayflies in the southeastern regions is still poorly known. Here we present the first comprehensive study of Ephemeroptera in the tufa-depositing habitats of the Dinaric Karst. The study was conducted in Plitvice Lakes National Park monthly during a one-year period (2007–2008) in different types of habitats (springs, streams, mountainous rivers, tufa barriers). The aims of the study were to determine mayfly composition, abundance, spatial distribution and habitat preferences, and to examine the environmental factors important for the structuring of mayfly assemblages in Plitvice Lakes National Park. The mayfly fauna of tufa-depositing habitats was composed of 14 species (20 taxa). Water temperature, pH and ammonium concentration were the most important environmental variables explaining mayfly assemblages. Mayfly assemblages grouped according to habitat type. Generally, the most favourable habitat type was mountainous stream, tufa barriers were less favourable, and the least favourable were springs. Our results confirmed that mayflies are a powerful tool as descriptors of their environment, as the presence or absence of certain mayflies was strongly influenced by physico-chemical water properties.

**Keywords:** case study / ecology / abiotic factors / Ephemeroptera / Southeast Europe

**Résumé** – **Facteurs environnementaux influençant la composition des éphémères dans les tufières du Karst Dinarique.** De manière surprenante, et contrairement à d'autres parties de l'Europe, l'écologie des éphémères de la zone sud orientale est encore largement inconnue. Dans ce travail, nous présentons la première étude approfondie des éphémères habitant des tufières. L'étude a été menée au Parc National de Plitvice Lacs durant un an (2007–2008) dans différents types d'habitats (sources, ruisseaux, rivières montagneuses, barrières de tuf). Les objectifs de cette étude étaient de déterminer la composition, l'abondance, la distribution spatiale et les habitats préférentiels des éphémères, d'une part, et d'examiner quels facteurs environnementaux étaient responsables de leur structuration, d'autre part. La faune des éphémères des tufières est composée de 14 espèces (20 taxons). La température de l'eau, le pH et la concentration en ammonium sont les variables environnementales les plus importantes qui expliquent la composition des éphémères. Celle-ci est fonction des types d'habitats. De manière générale, le type d'habitat le plus favorable est celui représenté par les ruisseaux, suivi par les barrières de tuf, les sources étant les moins favorables. Les résultats confirment la puissante capacité des éphémères à décrire leur environnement, puisque la présence ou l'absence de certaines espèces est fortement influencée par les propriétés physico-chimiques de l'eau.

**Mots-clés** : étude de cas / écologie / facteurs abiotiques / Ephemeroptera / sud-est de l'Europe

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## 1 Introduction

Ephemeroptera (mayflies) is an order of aquatic insects that plays an important role in running and standing waters, representing a very large proportion of the aquatic ecosystem biomass (Brittain and Sartori, 2003; Bauernfeind and Soldán, 2012).

Water temperature, dissolved oxygen concentration, nutrients, water velocity and substrate type are the most important natural environmental factors driving freshwater communities, including mayflies (Giller and Malmqvist, 1998; Moog, 2002; Allan and Castillo, 2007; Choudhary *et al.*, 2014). Stream insects often show longitudinal zonation along habitats following the downstream gradient in physico-chemical water properties (Vannote *et al.*, 1980; Giller and Malmqvist, 1998; Bauernfeind and Humpesch, 2001). The majority of mayfly species prefer the meta- and hyporhithral sections (upper reaches) of the fast-flowing streams and rivers, and ecologically intact large potamal rivers (Bauernfeind and Moog, 2000; Bauernfeind and Soldán, 2012). On the other hand, crenal (spring) sections of streams, high mountains and metapotamal river sections (lower reaches) usually have low mayfly species diversity (Bauernfeind and Soldán, 2012).

The longitudinal succession of macroinvertebrate communities, including mayflies, reflects the temperature regime of a river, the differences in other environmental factors, but also the changes in food webs (Giller and Malmqvist, 1998; Moog, 2002; Schmidt-Kloiber and Hering, 2015). Consequently, changes in mayfly assemblages are often used as an important parameter to detect and assess temperature increases induced by climate change, or to assess river degradation (*e.g.* hydromorphological changes) and stress intensity (Schmidt-Kloiber and Hering, 2015).

Karst is a set of morphological, hydrological and hydro-geological terrain features built of water-soluble rock. Due to anthropogenic pressure and the specific hydrology and soil morphology (Sánchez-Fernández *et al.*, 2004), karst habitats are particularly endangered. These fragile ecosystems are inhabited by numerous rare and endangered species (Bonacci *et al.*, 2008; Bonacci, 2009). The karstification process has resulted in a mosaic of climatic and environmental conditions with diverse physiographic traits. Geographical isolation, local influences of climate and altitude and high habitat heterogeneity may have favoured the maintenance of a high level of speciation, and a high number of discontinuous populations of phylogenetically distinct origin (Bonacci *et al.*, 2008; Previšić *et al.*, 2009, 2014; Ivković and Plant, 2015). The Dinaric Mountains are the largest continuous karst landscape in Europe (Mihevc *et al.*, 2010), extending over approximately 60,000 km<sup>2</sup>, with an extremely complicated hydrological network (Bonacci and Jelin, 1988; Bonacci *et al.*, 2013). The mountain range lies along the western Balkan Peninsula, stretching in a north-south direction from northeastern Italy to Albania (Bonacci, 2009). Freshwater karst habitats are characterized by an exceptional phenomenon: secondary calcium-carbonate deposition – tufa. Tufa is a product of the physico-chemical characteristics of water, geological bed and the present biota. The biological component is a crucial factor in the genesis and maintenance of cascade lake systems with tufa barriers (barrage lakes), such as Plitvice Lakes National Park (Srdoč, 1985).

Faunistic surveys and ecological studies involving various aquatic insect groups were recently conducted in the Dinaric Karst area, *e.g.* stoneflies (Popijač and Sivec, 2009), caddisflies (Previšić *et al.*, 2007; Šemnički *et al.*, 2012), blackflies (Ivković *et al.*, 2013a), aquatic dance flies (Ivković *et al.*, 2013b) and riffle beetles (Mičetić Stanković *et al.*, 2015). However, the ecological preferences of mayfly assemblages in this region are still poorly known (*e.g.* Hrovat *et al.*, 2009; Vilenica *et al.*, 2016a, b). Matoničkin and Pavletić (1961, 1967); Habdija *et al.* (1994) and Habdija *et al.* (2004) investigated mayflies only sporadically as a part of invertebrate benthic communities. Moreover, most of the previous identifications are ambiguous, as the identification tools were not cited or current revision of sampled material is not possible.

Therefore, the main goals of this study were to

- determine the composition, abundance and spatial distribution of mayflies;
- determine the mayfly habitat preferences and
- examine which environmental factors are important in structuring mayfly assemblages in the selected tufa-depositing karst freshwater ecosystem of Plitvice Lakes National Park.

## 2 Material and methods

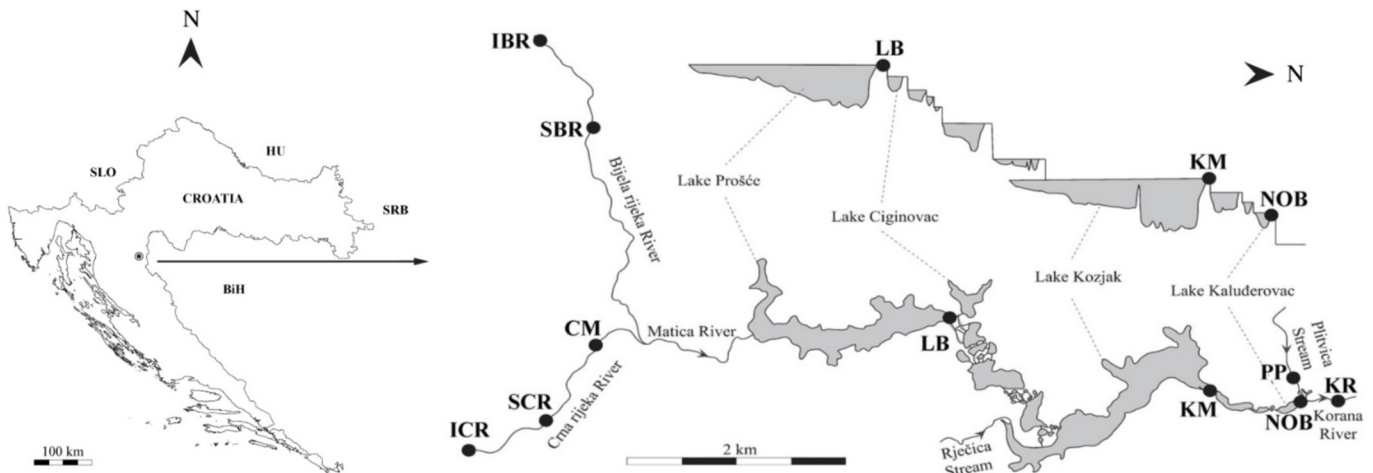
### 2.1 Study area

Plitvice Lakes National Park is located in the Dinaric Karst region in Croatia. The Plitvice Lakes' barrage lake system consists of 16 oligotrophic, dimictic and fluvial lakes divided and interconnected by tufa-depositing barriers (Fig. 1). After the confluence, the small mountainous rivers Bijela rijeka and Crna rijeka form the Matica river, the main surface-water supplier of the lakes (Stilinović and Božičević, 1998).

The climate is transitive, moderately mountainous (Šikić, 2007). The main rainfall occurs in autumn and winter, while the air temperature during the winter drops to –25 °C and during the summer rises above 30 °C (Makjanić, 1958). The area is rich in a range of karst habitat types (Stilinović and Božičević, 1998) and well known for its high endemism, especially in freshwater (Bănărescu, 2004), which is why Plitvice Lakes National Park (NP) was designated as a UNESCO world natural heritage site in 1979 (Stilinović and Božičević, 1998).

Sampling was conducted in various habitats at 10 study sites. According to their altitude and habitat type, lotic habitats were classified into four categories (Fig. 1, Tab. 1):

- Rheocrene springs of upper lotic habitats (small mountainous rivers): spring of the Bijela rijeka river (elevation 720 m) (IBR) and spring of the Crna rijeka river (675 m) (ICR).
- Downstream sections of upper lotic habitats (small mountainous rivers): upper reaches of the Bijela rijeka river (716 m) (SBR), middle reaches of the Crna rijeka river (670 m) (SCR) and lower reaches of the Crna rijeka river (665 m) (CM).
- Three tufa barriers, situated between upper and lower lotic habitats: Tufa barrier Labudovac (630 m) (LB), Tufa barrier Kozjak-Milanovac (545 m) (KM) and Tufa barrier Novakovića Brod (505 m) (NOB).
- Tufa-depositing lower lotic habitats at the end of the cascade system represented by downstream sections of the canyon type mountainous stream Plitvica stream (555 m) (PP) and mid-altitude large river Korana river (390 m) (KR).



**Fig. 1.** Location of Plitvice Lakes NP in Croatia and position of 10 study sites in Plitvice Lakes NP. Legend: IBR = Bijela rijeka river spring, SBR = Bijela rijeka river upper reaches, ICR = Crna rijeka river spring, SCR = Crna rijeka river middle reaches, CM = Crna rijeka river lower reaches, KR = Korana river, PP = Plitvica stream, KM = Tufa barrier Kozjak-Milanovac, LB = Tufa barrier Labudovac, NOB = Tufa barrier Novakovića Brod.

## 2.2 Mayfly sampling

Mayfly larvae were sampled monthly from February 2007 to February 2008 with other macroinvertebrates using a Surber sampler (0.1 m<sup>2</sup> surface area and 0.5 mm mesh size). In the CM, a D-frame hand net (with 0.5 mm mesh size) was used. Every month, at each site, three samples were taken, with the exception of the IBR, where four samples were taken. Samples were conserved in 80% ethanol.

## 2.3 Environmental factors

Physico-chemical water properties were measured monthly at each study site (Tab. 1): oxygen concentration, oxygen saturation, water temperature (using the oximeter WTW Oxi 330/SET), pH (using the pH-meter WTW ph 330), conductivity (with the conductivity meter WTW LF 330), alkalinity (by titration with 0.1 M HCl), water velocity (with P-670-M velocimeter), water depth (with handheld meter) and nutrients (ammonium by HRN ISO 70-3:1998 method, nitrates and nitrites by HRN ISO 7890-3:2001 method and orthophosphates by HRN ISO 6878:2001 method). Water velocity and water depth were measured at each sampled microhabitat. Altitude and distance from the springs were determined for each study site. Geographical coordinates were read using a GPS Garmin Oregon 550, and subsequently processed in ArcGIS software.

## 2.4 Data analysis

For all data, the Shapiro–Wilk normality test was performed. All mayfly data were log-transformed prior to analyses.

In order to determine differences in physico-chemical water properties between habitat types, one-way ANOVA for water velocity and Kruskal–Wallis *H* test with multiple comparisons test for other environmental factors were applied.

Non-metric multidimensional scaling analysis (NMDS) was applied to show similarities in the composition of mayfly assemblages among study sites using the Bray–Curtis similarity index. The results of the hierarchical cluster analysis are superimposed on NMDS ordination.

The relationship of mayflies to environmental factors were analysed from various aspects: (i) Kruskal–Wallis *H* test with multiple comparisons test and indicator value method (IndVal) were used to determine the preference of each recorded taxon for a certain habitat type, with Monte Carlo permutation test (4999 permutations) and threshold value of the proportion determining the characteristic indicator taxa of 55% (Dufrene and Legendre, 1997); (ii) Spearman's rank correlation coefficient (*R*) for analysis of the relationship of mayfly assemblages (number of individuals, number of taxa) and each of the recorded species with physico-chemical water properties; (iii) Canonical correspondence analysis (CCA) with Monte Carlo permutation test of significance (with 999 permutations) was used for identification and measurement of the mayfly associations and environmental conditions. The CCA analysis included 20 taxa and 9 physico-chemical water parameters. Prior to the CCA analysis, the full draftsman's plot excluded orthophosphates and nitrites.

Analyses were performed in Statistica, version 10.0 (Statsoft Inc., Tulsa, PC-ord ver. 5.0), Primer 6 software package (Clarke and Gorley, 2006) and CANOCO for Windows (ver. 4.02) (Ter Braak and Šmilauer, 1998).

## 3 Results

### 3.1 Environmental factors

Kruskal–Wallis and multiple comparisons of mean ranks for all groups separated the four habitat types (springs, upper lotic habitats, tufa barriers and lower lotic habitats), based on their physico-chemical water properties (Tabs. 1 and 2).

**Table 1.** Characteristics of the study sites in Plitvice Lakes National Park. Abbreviations of the study sites are presented in Figure 1.

Study site	Upper lotic habitats										Lower lotic habitats	
	IBR	ICR	SBR	SCR	CM	LB	KM	NOB	PP	KR		
Habitat type	Springs					Tufa barriers						
Latitude	N 44°50'05"	44° 50' 14"	N 44°50'04'	N 44°50'10'	N 44°50'22'	N 44°52'17'	N 44°53'39'	N 44°54'07'	N 44°54'07'	N 44°55'33'		
Longitude	E 15°33'43'	15° 36' 28"	E 15°33'33'	E 15°36'30'	E 15°35'59'	E 15°35'59'	E 15°36'32'	E 15°36'38'	E 15°36'27'	E 15°37'09'		
Altitude (m)	720	675	715	670	665	630	545	505	555	390		
Substrate	Pebbles and sand, Bryophytes	Pebbles and sand, Bryophytes	Pebbles and sand, Angiosperms, Bryophytes	Pebbles and sand, Angiosperms, Bryophytes	Pebbles and sand, Angiosperms	Pebbles, Bryophytes on tufa, Tufa with detritus	Pebbles, Bryophytes on tufa, Tufa with detritus, Sand	Pebbles, Bryophytes on tufa, Tufa with detritus, Sand	Pebbles, Bryophytes on tufa, Tufa with detritus, Sand	Pebbles, Bryophytes on tufa, Tufa with detritus, Sand		
Water temperature (°C)	Average 7.5	7.9	8.5	8.1	8.3	12.1	12.1	12.8	9.4	9.9		
	Min/max 7.3/7.8	7.7/8.2	7.2/9.9	7.1/9.7	6.9/9.6	2.5/20.5	3.1/22.9	3.3/22.9	3.2/15.4	1.7/19.8		
O <sub>2</sub> (mg L <sup>-1</sup> )	Average 10.3	10.7	10.5	10.8	11.1	10.3	10.1	10.2	10.5	11.1		
	Min/max 7.6/11.8	8.3/11.7	8.2/11.8	7.9/12.5	8.8/13.1	6.7/12.3	8.7/12.0	8.4/12.4	8.7/13.0	9.0/14.1		
O <sub>2</sub> (%)	Average 91.1	94.8	94.1	94.3	100.3	100.1	99.1	100.1	101.7	101.1		
	Min/max 65.2/101.8	87.0/105.7	71.2/106.6	68.8/115.9	96.7/111.1	59.7/139.2	72.0/113.6	77.3/117.1	75.7/122.5	79.0/121.0		
pH	Average 7.6	7.7	7.9	7.7	8.2	8.3	8.2	8.4	8.3	8.3		
	Min/max 6.9/7.8	7.4/8.2	7.5/8.4	7.7/8.6	7.9/8.4	6.8/8.7	6.9/8.4	8.2/8.7	6.8/8.9	6.8/8.7		
Conductivity (µS cm <sup>-1</sup> )	Average 483	417	481	418	426	383	382	362	424	361		
	Max 463/505	405/424	472/498	403/426	406/481	366/426	354/443	334/387	409/444	321/385		
Alkalinity (mg L <sup>-1</sup> CaCO <sub>3</sub> )	Average 262	237	267	237	240	216	214	211	248	205		
	Min/max 235/295	210/260	230/295	210/290	215/280	210/260	200/220	185/230	225/280	180/215		
Water velocity (cms <sup>-1</sup> )	Average 9.4	12.4	14.9	5.5	-	19.1	19.3	9.2	13.1	11.2		
	Min/max 12.35	10.00	10.52	13.13	35.24	10.98	15.46	15.78	15.57	29.87		
Water depth (cm)	Average 1.01	0.77	1.06	0.78	0.73	0.65	0.70	0.66	0.09	0.74		
	Min/max 0.50/1.22	0.64/1.10	0.66/1.42	0.58/0.91	0.64/0.82	0.36/0.87	0.36/1.13	0.37/0.77	0.62/1.16	0.48/1.05		
Nitrates (mg L <sup>-1</sup> )	Average 0.03	0.02	0.03	0.01	0.01	0.02	0.03	0.02	0.03	0.03		
	Min/max 0.01/0.07	0.01/0.08	0.01/0.07	0.01/0.02	0.01/0.03	0.01/0.03	0.01/0.07	0.01/0.03	0.01/0.07	0.01/0.06		

**Table 2.** Significant differences in physico-chemical water properties between habitat types in Plitvice Lakes NP based on Kruskal–Wallis  $H$  test with multiple comparisons test. Sample size ( $N$ )=31, \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$ .

Differences between habitat types	Physico-chemical water parameter	$H$	$p$
Tufa barriers and springs	Water temperature	27.25	***
	pH	20.87	***
	Conductivity	14.70	**
	Nitrates	16.90	**
Tufa barriers and upper lotic habitats	Oxygen concentration	22.32	***
	Alkalinity	12.58	**
	Water temperature	27.25	**
	Nitrates	16.90	**
	Conductivity	14.70	*
Tufa barriers and lower lotic habitats	Oxygen concentration	22.32	**
Springs and lower lotic habitats	pH	20.87	**
	Water temperature	27.25	*

### 3.2 Population aspects and relation of mayflies to environmental factors

A total of 14 mayfly species (20 taxa), belonging to 13 genera and 7 families, were recorded. Early instars or damaged specimens were identified to the genus or family level (six taxa) (Tab. 3). Springs and one tufa barrier had the lowest number (four) of mayfly species (Tab. 3). The highest diversities and abundances were recorded in the lower lotic habitats, especially at the site Plitvica stream (PP) (Tab. 3). *Baetis rhodani* (Pictet, 1843) and *B. cf. nubecularis* (Eaton, 1989) were the most widely distributed species, while *Serratella ignita* (Poda, 1761) was the most abundant. On the contrary, *Procladius pennulatum* (Eaton, 1870) was the rarest recorded species (Tab. 3).

NMDS analysis (Fig. 2a and b) showed clustering of the sites according to habitat type. Sites located in the upper lotic habitats (IBR, SBR and SCR) were clearly separated from the tufa barriers (LB, NOB and KM) and lower lotic habitats (PP and KR), which clustered together. A single study site located in the downstream sections of the upper lotic habitats (CM) clustered with the lower lotic habitats and tufa barriers.

Preferences for a certain habitat type was significant for four mayfly species: *Rhithrogena braaschi* Jacob, 1974 (IndVal=62.3,  $p < 0.05$ ) for upper lotic habitats; *Caenis horaria* (Linnaeus, 1758) (IndVal=55.6,  $p < 0.01$ ) for tufa barriers and *Centroptilum luteolum* (Müller, 1776) (IndVal=58.6,  $p < 0.05$ ) and *S. ignita* (IndVal=99.7,  $p < 0.001$ ) for lower lotic habitats.

Significant differences between abundances were recorded for two mayfly species. According to Kruskal–Wallis  $H$  test and multiple comparisons of mean ranks for all groups, abundances of *Rh. braaschi* were significantly different between the tufa barriers and springs ( $p < 0.05$ ), and between the tufa barriers and upper lotic habitats ( $p < 0.05$ ), as this species was absent from the tufa barriers and was most abundant in the upper lotic habitats (Tab. 3). Abundances of *S. ignita* strongly differed between the lower lotic habitats and

other habitat types: springs ( $p < 0.01$ ), tufa barriers ( $p < 0.01$ ) and upper lotic habitats ( $p < 0.05$ ). The species was absent from the springs, present in very low numbers in tufa barriers and upper lotic habitats and highly abundant in the lower lotic habitats (Tab. 3).

The eigenvalues for the first two CCA axes (Fig. 3) were 0.373 and 0.224 and accounted for 53.2% of the variability in species–environment relations, while the eigenvalues accounted for 80.1% of the overall variability. The Monte Carlo permutation test showed that the species–environment ordination was statistically significant (first axis:  $F$ -ratio = 4.876,  $p = 0.002$ ; overall: trace = 1.122,  $F = 3.048$  and  $p = 0.002$ ), indicating that mayfly assemblages were significantly related to the tested set of environmental variables. Axis 1 was related to water temperature ( $R = 0.646$ ) and pH ( $R = 0.632$ ) and Axis 2 to ammonium concentrations ( $R = 0.690$ ), indicating that these were the most important parameters in explaining patterns of the mayfly assemblages.

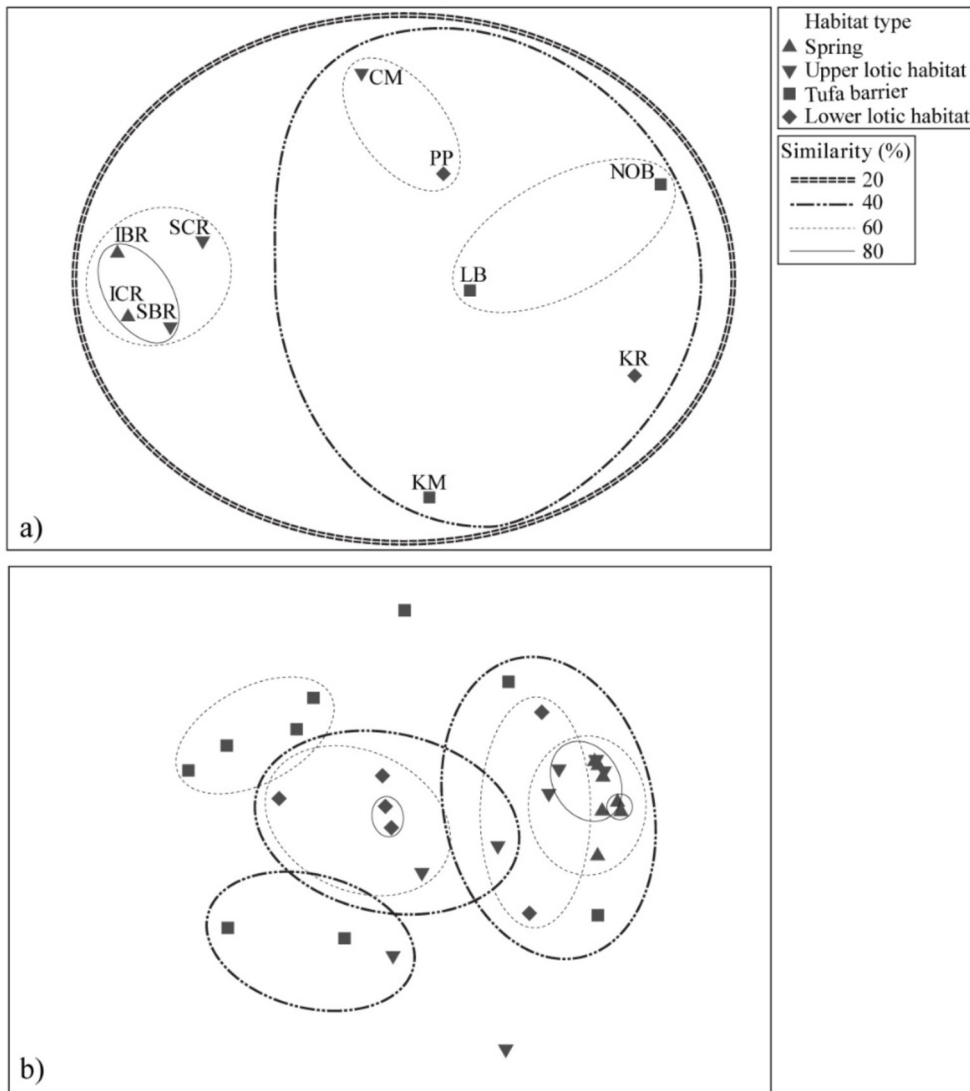
The number of mayfly taxa ( $S$ ) was positively correlated with conductivity ( $R = 0.21$ ,  $p < 0.001$ ), alkalinity ( $R = 0.19$ ,  $p < 0.001$ ) and the concentration of nitrate ions ( $R = 0.14$ ,  $p < 0.01$ ). The number of mayfly individuals ( $N$ ) was positively correlated with conductivity ( $R = 0.18$ ,  $p < 0.001$ ), alkalinity ( $R = 0.16$ ,  $p < 0.01$ ), concentrations of ammonium ( $R = 0.13$ ,  $p < 0.01$ ) and nitrate ions ( $R = 0.19$ ,  $p < 0.05$ ), water temperature ( $R = 0.11$ ,  $p < 0.05$ ) and water velocity ( $R = 0.12$ ,  $p < 0.05$ ). A negative correlation was determined between mayfly abundance and water depth ( $R = -0.17$ ,  $p < 0.001$ ). Significant correlations with physico-chemical water properties were recorded for 13 species (Tab. 4).

## 4 Discussion

Aquatic organisms in Plitvice Lakes NP are confronted with specific environmental conditions, such as low nutrient availability, low water temperature and high alkalinity, which

**Table 3.** Mayfly abundance (shown as a number of individuals per m<sup>2</sup>) and distribution in Plitvice Lakes NP: (a) springs and upper lotic habitats, (b) tufa barriers and lower lotic habitats. Abbreviations of the study sites are presented in [Figure 1](#).

Habitat type Mayfly taxa/Microhabitat	Springs							Upper lotic habitats								
	IBR1	IBR2	IBR3	IBR4	ICR1	ICR2	ICR3	SBR1	SBR2	SBR3	SCR1	SCR2	SCR3	CM1	CM2	CM3
<b>(a) Springs and upper lotic habitats.</b>																
Baetidae juv.	304	368	510	224	0	5049	0	5360	2880	7191	448	5967	0	0	80	0
<i>Alainites muticus</i> (Linnaeus, 1758)	0	48	0	96	0	0	0	0	0	0	0	0	0	0	32	0
<i>Baetis</i> sp. juv.	32	144	918	16	384	3519	96	352	1952	1683	128	4182	0	112	0	0
<i>Baetis cf. nubecularis</i> (Eaton, 1898)	16	64	1275	16	0	3876	240	400	176	2448	544	3468	0	0	0	0
<i>Baetis rhodani</i> (Pictet, 1843)	528	416	459	672	464	1632	416	2400	1584	3366	960	969	16	880	256	32
<i>Centroptilum luteolum</i> (Müller, 1776)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Procloeon pennulatum</i> (Eaton, 1870)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caenis</i> sp. juv.	0	0	0	0	64	4	1	0	0	0	0	0	0	0	16	0
<i>Caenis horaria</i> (Linnaeus, 1758)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ephemerellidae juv.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Serratella ignita</i> (Poda, 1761)	0	0	0	0	0	0	0	0	0	0	0	0	0	96	112	0
<i>Torleya major</i> (Klapalek, 1905)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ephemera danica</i> Müller, 1764	0	0	0	0	0	0	0	0	0	0	16	0	0	144	48	16
Heptageniidae juv.	0	0	0	0	0	0	0	0	0	0	2896	357	16	128	96	16
<i>Ecdyonurus submontanus</i> Landa, 1969	0	0	0	0	0	0	0	0	0	0	48	0	0	432	320	0
<i>Rhithrogena braaschi</i> Jacob, 1974	1424	112	561	224	96	306	3008	12272	592	612	9632	306	0	1344	32	0
Leptophlebiidae juv.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	64	80
<i>Habrophlebia lauta</i> Eaton, 1884	0	0	0	0	0	0	0	0	0	0	0	0	0	0	224	16
<i>Paraleptophlebia submarginata</i> (Stephens, 1835)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	416	16
<i>Siphonurus croaticus</i> Ulmer, 1920	0	0	0	0	0	0	0	0	0	0	0	0	48	0	96	0
<b>(b) Tufa barriers and lower lotic habitats.</b>																
Habitat type Mayfly taxa/Microhabitat	Tufa barriers						Lower lotic habitats									
	NOB1	NOB2	NOB3	LB1	LB2	LB3	KM1	KM2	KM3	KR1	KR2	KR3	PP1	PP2	PP3	
Baetidae juv.	0	459	16	612	0	80	0	153	0	160	918	80	1536	1020	128	
<i>Alainites muticus</i> (Linnaeus, 1758)	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Baetis</i> sp. juv.	80	663	0	918	0	0	0	459	0	64	0	16	7840	0	0	
<i>Baetis cf. nubecularis</i> (Eaton, 1898)	112	3417	16	4590	0	0	0	0	0	0	0	0	4414	0	0	
<i>Baetis rhodani</i> (Pictet, 1843)	0	0	0	1377	0	0	16	1989	0	576	1785	464	1728	408	0	
<i>Centroptilum luteolum</i> (Müller, 1776)	272	459	256	0	432	16	16	0	0	288	0	64	0	15198	1936	
<i>Procloeon pennulatum</i> (Eaton, 1870)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	
<i>Caenis</i> sp. juv.	16	0	0	0	0	16	0	0	0	0	0	0	0	0	0	
<i>Caenis horaria</i> (Linnaeus, 1758)	16	0	48	918	192	16	0	0	0	0	0	0	0	0	0	
Ephemerellidae juv.	0	0	0	0	0	0	0	0	0	48	357	0	0	0	0	
<i>Serratella ignita</i> (Poda, 1761)	16	0	0	0	0	0	0	0	0	240	1989	208	17360	2448	128	
<i>Torleya major</i> (Klapalek, 1905)	224	612	0	0	0	0	0	0	0	16	0	0	0	0	0	
<i>Ephemera danica</i> Müller, 1764	1200	0	1120	0	1936	1552	16	0	32	800	0	512	0	9486	976	
Heptageniidae juv.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Ecdyonurus submontanus</i> Landa, 1969	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Rhithrogena braaschi</i> Jacob, 1974	0	0	0	0	0	0	0	0	0	0	0	0	0	102	0	
Leptophlebiidae juv.	0	5967	0	612	32	48	0	0	32	0	0	0	48	1887	144	
<i>Habrophlebia lauta</i> Eaton, 1884	0	0	0	0	0	0	0	0	0	0	0	0	0	1224	224	
<i>Paraleptophlebia submarginata</i> (Stephens, 1835)	96	153	80	1836	0	0	0	0	48	0	0	0	0	208	320	
<i>Siphonurus croaticus</i> Ulmer, 1920	0	0	0	0	16	0	0	0	0	0	0	0	0	208	112	

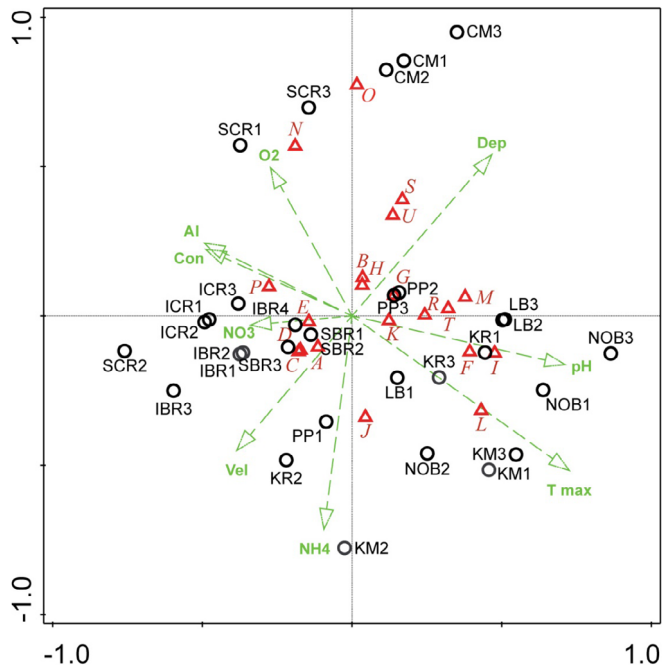


**Fig. 2.** NMDS analysis of the study sites in the different habitat types in Plitvice Lakes NP based on the composition of mayfly fauna shown (a) pooled per study site and (b) per microhabitat. Study sites: IBR = Bijela rijeka river spring, SBR = Bijela rijeka river upper reaches, ICR = Crna rijeka river middle reaches, SCR = Crna rijeka river lower reaches, CM = Crna rijeka river lower reaches, KR = Korana river, PP = Plitvica stream, KM = Tufa barrier Kozjak-Milanovac, LB = Tufa barrier Labudovac, NOB = Tufa barrier Novakovića Brod.

also most likely resulted in a relatively low diversity of mayfly assemblages (e.g. Hrovat *et al.*, 2009; Vilenica *et al.*, 2016a, b). The results obtained from the mayfly fauna are in accordance with data obtained from physico-chemical analyses of the water. Our results clearly indicate differences in physico-chemical water characteristics throughout Plitvice Lakes NP. As expected, the lowest variations were recorded in the springs and spring zones, where environmental conditions are stable regardless of stream type (Jones and Mulholland, 2000). On the other hand, sites situated downstream of the barrage-lake system, and sites at tufa barriers and in the lower lotic habitats, showed higher fluctuations in environmental factors. In accordance with the literature (e.g. Berner and Pescador, 1988; Bauernfeind and Soldán, 2012), the lowest mayfly species' richness was recorded in the springs and spring areas. Mayfly diversity is usually highest in meta- and hyporhithral stream sections (upper reaches) (Bauernfeind and Soldán, 2012). However, downstream sections of lower lotic habitats had the most diverse mayfly assemblages due to the rhithral character, more suitable environmental conditions

(e.g. higher water temperature), a variety of available microhabitats and food resources (see also in Vilenica *et al.*, 2014). Generally, tufa barriers were a less favourable habitat than the lower lotic habitats, which could be due to the more prominent fluctuations in oxygen concentrations, water temperature and available nutrients. On the other hand, tufa barriers were a more favourable habitat type than upper lotic habitats, due to the higher amount of organic matter. The barriers are situated between lakes, which is why they represent natural lake outlet habitats where organic matter accumulates, thus enhancing conditions for detritivorous insects such as mayflies (Obelić *et al.*, 2005; Šemnički *et al.*, 2012; Ivković *et al.*, 2013a).

Similar to the previously investigated freshwater systems of the Dinaric Karst (Vilenica *et al.*, 2016b), water temperature and pH were amongst the most important variables that determined mayfly assemblages and their distribution (e.g. Gerhardt, 1990; Moog, 2002; Petrin, 2011; Bauernfeind and Soldán, 2012). In addition, habitat type (springs, upper lotic habitats, tufa barriers and lower lotic habitats) and ammonium concentrations also played an important role.



**Fig. 3.** F1 × F2 plane of CCA analysis showing 20 mayfly taxa, 10 study sites encompassing 31 microhabitats in Plitvice Lakes NP and nine selected environmental variables. Legend: Study sites (black circle symbols): IBR = Bijela rijeka river spring, SBR = Bijela rijeka river upper reaches, ICR = Crna rijeka river spring, SCR = Crna rijeka river middle reaches, CM = Crna rijeka river lower reaches, KR = Korana river, PP = Plitvica stream, KM = Tufa barrier Kozjak-Milanovac, LB = Tufa barrier Labudovac, NOB = Tufa barrier Novakovića Brod. Environmental factors (green arrow symbols): T max = maximum water temperature (°C); O<sub>2</sub> = O<sub>2</sub> (mg L<sup>-1</sup>); pH = pH value; Con = Conductivity (μS cm<sup>-1</sup>); Al = Alkalinity (mg L<sup>-1</sup> CaCO<sub>3</sub>); Vel = Water velocity (cms<sup>-1</sup>); Dep = Water depth (cm); NO<sub>3</sub> = Nitrates (mg L<sup>-1</sup>); NH<sub>4</sub> = Ammonium (mg L<sup>-1</sup>). Taxa (red triangle symbols): A = juvenile Baetidae, B = *Alainites muticus*, C = juvenile *Baetis* sp., D = *Baetis* cf. *nubecularis*, E = *Baetis rhodani*, F = *Centroptilum luteolum*, G = *Procloeon pennulatum*, H = juvenile *Caenis* sp., I = *Caenis horaria*, J = juvenile Ephemerellidae, K = *Serratella ignita*, L = *Torleya major*, M = *Ephemera danica*, N = juvenile Heptageniidae, O = *Ecdyonurus submontanus*, P = *Rhithrogena braaschi*, R = juvenile Leptophlebiidae, S = *Habrophlebia lauta*, T = *Paraleptophlebia submarginata*, U = *Siphonurus croaticus*.

The NMDS analysis showed a grouping of mayfly assemblages according to habitat type (see also in Siegloch *et al.*, 2014; Lencioni and Spitale, 2015), with a clear separation of upper lotic habitats from lower lotic habitats and tufa barriers. In addition to differences in the physico-chemical water properties, upper lotic habitats differed from tufa barriers and lower lotic habitats in the microhabitat composition. In the upper lotic habitats, microhabitats mainly consisted of pebbles, sand, angiosperms and bryophytes, while in tufa barriers and lower lotic habitats, pebbles/tufa, bryophytes on tufa, tufa with detritus and silt dominated.

Several of the recorded species showed a significant preference for habitat type. To date, larvae of *Rh. braaschi* have been recorded in the rhithral sections (upper reaches) of

brooks and smaller rivers lying at elevations from 600 to 1000 m (Bauernfeind and Soldán, 2012). The distribution of this species in Plitvice Lakes NP was in accordance with its known habitat preferences (Jacob, 1974; Vidinova, 2003; Bauernfeind and Soldán, 2012), in the springs and upper lotic habitats, at sites with a low and constant water temperature. On the other hand, our results indicate that tufa barriers are less favourable for the species, which should be investigated in more detail. The limnophilic (lentic) species *C. horaria*, predominantly inhabiting the lakes (Bauernfeind and Soldán, 2012), was recorded at the tufa barriers but in low abundance. As much higher abundances of this species were recorded in the surrounding lakes (Vilenica *et al.*, 2014), low numbers of larval specimens at the tufa barriers are most likely the consequence of drift from upstream lakes (Sertić Perić *et al.*, 2011). Two eurytopic and eurythermic species, *C. luteolum* and *S. ignita*, found most commonly in habitats with moderate (<18 °C) and warmer water (≥18 °C) (Schmidt-Kloiber and Hering, 2015), preferred the lower lotic habitats in Plitvice Lakes NP, where such conditions were recorded.

Considering their water temperature requirements, the majority of the mayfly species recorded in the current study were eurytherms (Schmidt-Kloiber and Hering, 2015), with the exception of the warm stenotherms *P. pennulatum* and *C. horaria* (Bauernfeind and Soldán, 2012; Schmidt-Kloiber and Hering, 2015). Accordingly, these two species were recorded only at sites with the highest water temperatures, namely the tufa barriers (max. temp. NOB 22.9 °C; LB 20.5 °C; KM 22.9 °C) and lower lotic habitats (max. temp. KR 19.8 °C; PP 15.4 °C). Several additional species recorded in the lower lotic habitats and tufa barriers were also correlated with higher water temperatures, such as the eurythermic *S. ignita*, two species preferring moderate water temperatures (<18 °C), *Ephemera danica* Müller, 1764 and *Torleya major* (Klapálek, 1905) (Kamler, 1965; Céréghino and Lavandier, 1998; Bauernfeind and Soldán, 2012; Schmidt-Kloiber and Hering, 2015), and *B. cf. nubecularis*. Since the taxonomic status of the latter species is unresolved, its ecological preferences cannot be compared.

Another environmental factor strongly influencing mayfly assemblages was pH. Majority of mayfly species inhabit freshwaters with a neutral to alkaline pH, whereas they are highly sensitive to low pH values (e.g. Gerhardt, 1990; Petrin, 2011). pH values of the karst streams and rivers usually range from 6.5 to 8.5, due to the geological effects of karst substrate (Štambuk-Giljanović, 2005). In accordance with the recorded pH range (6.8–8.9), the majority of mayfly taxa recorded in our study preferred a neutral to alkaline environment (Jazdzewska and Górczynski, 1991; Schmidt-Kloiber and Hering, 2015), with *Rh. braaschi* and *B. rhodani* inhabiting sites with a neutral pH and *C. horaria*, *E. danica* and *T. major* preferring sites with a more alkaline pH.

Conductivity and alkalinity also had a strong influence on both diversity and abundance of mayflies in Plitvice Lakes NP, with e.g. *B. rhodani* (Dolisy and Dohet, 2003), *Alainites muticus* (Linnaeus, 1758) (López-Rodríguez *et al.*, 2010) and *Rh. braaschi* tolerating higher values and *C. horaria*, *E. danica* and *T. major* selecting sites with lower values.

The requirement for an adequate oxygen content in water is commonly high in mayflies (Bauernfeind and Soldán, 2012). Generally, oxygen concentrations in karst rivers fluctuate



**Table 4.** Spearman's rank significant correlation coefficient (*R*) for mayfly species and physico-chemical water properties in Plitvice Lakes NP. Sample size (*N*)=366, \*\*\* *p* < 0.001, \*\* *p* < 0.01, \* *p* < 0.05.

Species	Environmental factor	<i>R</i>	<i>t</i> ( <i>N</i> -2)	<i>p</i> -Value
<i>Alainites muticus</i>	Nitrate ions	-0.14	-2.74	**
	Conductivity	0.12	2.37	*
	Alkalinity	0.10	1.99	*
<i>Baetis cf. nubecularis</i>	Water velocity	0.31	6.31	***
	Water depth	-0.25	-4.91	***
	Ammonium ions	0.14	2.67	**
	Water temperature	0.11	2.09	*
<i>Baetis rhodani</i>	pH	-0.18	-3.46	***
	Conductivity	0.30	5.99	***
	Alkalinity	0.27	5.41	***
	Nitrate ions	0.15	2.94	**
	Water velocity	0.13	2.51	*
	Water depth	-0.12	-2.23	*
<i>Centroptilum luteolum</i>	Water velocity	-0.34	-6.99	***
	Water depth	0.19	3.61	***
	Oxygen concentration	-0.14	-2.64	**
<i>Caenis horaria</i>	Conductivity	-0.19	-3.69	***
	Alkalinity	-0.18	-3.44	***
	Water temperature	0.15	2.79	**
	pH	0.15	2.81	**
	Nitrate ions	-0.11	-2.02	*
	Oxygen concentration	-0.12	-2.34	*
<i>Ephemera danica</i>	Water temperature	0.18	3.51	***
	Water velocity	-0.35	-7.04	***
	Water depth	0.23	4.52	***
	pH	0.23	0.52	***
	Conductivity	-0.24	-4.73	***
	Alkalinity	-0.24	-4.77	***
	Nitrate ions	-0.14	-2.65	**
	Oxygen concentration	-0.12	-2.39	*
<i>Serratella ignita</i>	Water temperature	0.14	2.74	**
	Water velocity	-0.13	-2.58	*
<i>Torleya major</i>	Conductivity	-0.19	-3.83	***
	Alkalinity	-0.18	-3.46	***
	pH	0.17	3.21	**
	Water temperature	0.11	2.05	*
<i>Ecdyonurus submontanus</i>	Water depth	0.13	2.57	*
<i>Rhithrogena braaschi</i>	pH	-0.26	-5.06	***
	Conductivity	0.29	5.94	***
	Alkalinity	0.29	5.72	***
	Nitrate ions	0.21	4.00	***

**Table 4.** (continued).

Species	Environmental factor	<i>R</i>	<i>t</i> ( <i>N</i> -2)	<i>p</i> -Value
<i>Habrophlebia lauta</i>	Water velocity	-0.24	-4.66	***
	Water depth	0.16	3.03	**
	Conductivity	0.12	2.38	*
<i>Paraleptophlebia submarginata</i>	Water velocity	-0.17	-3.56	***
	Water depth	0.11	2.05	*
<i>Siphonurus croaticus</i>	Water velocity	-0.23	-4.40	***
	Water depth	0.13	2.51	*
	Alkalinity	0.12	2.26	*
	Ammonium ions	-0.12	-2.27	*

between 10 and 12 mg/L (Štambuk-Giljanović, 2005). In the current study, the water was well oxygenated (average in the range 10.1–11.1 mg/L) though several species, such as *C. luteolum*, *E. danica* and *C. horaria*, exhibited a correlation with lower concentrations of dissolved oxygen. These species usually inhabit slow-flowing or standing water bodies (Bauernfeind and Soldán, 2012; Schmidt-Kloiber and Hering, 2015), often associated with lower oxygen concentrations compared to fast flowing streams (Giller and Malmqvist, 1998; Wetzel, 2001). As such, they were generally more abundant in the downstream sections of lower lotic habitats, Plitvica stream and Korana river, on sandy substrates with lower water velocity.

Plitvice Lakes NP is an oligotrophic freshwater system (e.g. Habdija *et al.*, 2004; Špoljar *et al.*, 2007). However, several sites (springs and lower lotic habitats) exhibited slightly higher concentrations of nitrates and ammonium ions due to increased demands of tourism for waste disposal in recent decades (Petrik, 1961; Matoničkin and Pavletić, 1967). This may have had a positive effect on the algal growth (Fried *et al.*, 2003) and quantity of food, making these sites more suitable habitats for a higher abundance and higher number of mayfly taxa. Nevertheless, the values of nitrates and ammonium could increase more in the near future. This should be taken in account while protecting this valuable karst river system and its communities. Two species were correlated with ammonium concentrations, *B. cf. nubecularis* with higher and *Siphonurus croaticus* Ulmer, 1920 with lower values, indicating higher and lower tolerance, respectively. The potential sensitivity of *S. croaticus* to ammonium should be further studied.

Mayfly assemblages were also influenced by water velocity, and the majority of species preferred habitats with the higher water speed. This was not surprising since large number of mayfly species inhabit well oxygenated running waters with moderate to high current, having a certain preference for specific water velocity (Schmedtje and Colling, 1996; Bauernfeind *et al.*, 2002; Menetrey *et al.*, 2008; Bauernfeind and Soldán, 2012). Water velocity also influences the distribution of organic matter (Habdija *et al.*, 2004; Miliša *et al.*, 2006), which is another factor having an impact on mayfly distribution (e.g. Williams and Hynes, 1974; Fjellheim, 1996; Habdija *et al.*, 2004). In the studied hydrosystem, water velocity was inversely proportional to

water depth, therefore the rheo- to limnophilic *E. danica* and *Paraleptophlebia submarginata* (Stephens, 1835) and limno- to rheophilic *Habrophlebia lauta* Eaton, 1884 preferred sites with greater water depth and lower water velocity. On the other hand, *B. cf. nubecularis* and *B. rhodani* preferred sites with lower water depth and greater velocity. Thus, the highest diversity and/or abundance of mayflies in Plitvice Lakes NP were recorded at study sites with greater water velocity and lower water depth (e.g. Tufa barrier Novakovića-Brod) and with higher pH and water temperature (e.g. lower lotic habitats), which is in accordance with the habitat preferences of most mayfly species (e.g. Bauernfeind and Humpesch, 2001; Bauernfeind and Soldán, 2012).

In conclusion, the mayfly assemblages of Plitvice Lakes NP clearly reflected the high heterogeneity of the investigated karst hydrosystem, where their significant preferences for certain habitat types and physico-chemical water properties were recorded. Due to the specific abiotic factors, such as relatively low annual water temperature, high alkalinity and low productivity (e.g. Habdija *et al.*, 2004; Špoljar *et al.*, 2007), herbivorous and detritivorous mayflies encounter poorer food resources (Bauernfeind and Soldán, 2012). This leads to relatively low species richness in the karst system of Plitvice Lakes NP (Hrovat *et al.*, 2014; Vilenica *et al.*, 2016a). Nevertheless, some interesting faunistic aspects were recorded, such as taxonomically intriguing species of *Baetis* from the *alpinus* group, which presents intermediate morphological characteristics between *Baetis alpinus* Pictet, 1845 and *B. nubecularis* (see also in Vilenica *et al.*, 2014, 2015). Future investigations should focus on a detailed taxonomic revision of *B. alpinus* group. Furthermore, species with a wide (e.g. *B. rhodani*, *C. horaria*, *S. ignita*), patchy (e.g. *E. submontanus*, *S. croaticus*) and Balkan (e.g. *Rh. braaschi*) distribution (Bauernfeind and Soldán, 2012; Schmidt-Kloiber and Hering, 2015) were identified. Since the presence or absence of certain mayflies was strongly influenced by the physico-chemical water properties, this study confirmed that they are a powerful tool as descriptors of their environment. Future studies should include systematic ecological study throughout the entire Plitvice Lakes NP area, encompassing the lakes and a wider range of microhabitats. Due to the very sensitive bio-dynamics of tufa deposition in this hydrosystem, long-term monitoring of the inhabiting species should be also conducted. Despite the specifics are in the hydrology of

the Dinaric Karst system, the current study showed that the standard methodology for mayfly assemblage monitoring is applicable.

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