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Microhabitat selection and distribution of functional feeding groups of mayfly larvae (Ephemeroptera) in lotic karst habitats

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Abstract – Research examining mayfly ecology in karst streams and rivers has increased in recent years, though microhabitat preferences remain poorly characterized. We examined mayfly assemblage taxonomy, functional feeding groups and microhabitat preferences in two contrasting lotic Dinaric karst catchments, one pristine and one anthropogenically impacted. At monthly intervals over a one-year period, all major microhabitats (*i.e.* dominated by boulders, cobbles, sand, silt, mosses, or angiosperms) were sampled at sites spanning springs, upper, middle and lower river reaches, and tufa barriers. In both catchments, mayfly species richness was comparable among microhabitats, while mayfly abundance was highest on mosses and lowest on silt. NMDS ordination did not group assemblages according to microhabitat type, which may reflect the greater influence of physical and chemical water properties. In both catchments and all microhabitats, mayfly assemblages were dominated by grazers/scrapers at upstream sites and by detritivores at downstream sites. Active filter feeders were more abundant in microhabitats with silt substrates and lower current velocities. This study demonstrated that certain mayfly species strongly preferred a specific microhabitat type, reflecting their water current preferences and feeding strategies, while other species shifted between microhabitats, likely in search of food resources and shelter. The results emphasize the importance of habitat heterogeneity in supporting diverse communities in karst rivers.

Keywords: Substrate type / water velocity / water depth / mayfly assemblages / feeding groups

Résumé – Choix des microhabitats et répartition des groups trophiques fonctionnels de larves d'éphémères (Ephemeroptera) dans des milieux lotiques et karstiques. Ces dernières années ont vu un accroissement des recherches menées sur l'écologie des éphémères dans les cours d'eau en milieux karstiques, mais leurs préférences à l'échelle du microhabitat restent peu connues. Nous avons étudié la composition taxonomique des éphémères, leurs groupes trophiques fonctionnels et leurs préférences pour les microhabitats dans deux bassins versants du Karst dinarique très différents ; l'un intact, l'autre soumis à des influences anthropogènes. Sur une période d'un an, mensuellement, les principaux microhabitats (par ex. composés principalement de blocs, galets, sable, limon, mousses ou angiospermes) ont été échantillonnés dans des stations couvrant les sources, le cours supérieur, moyen et inférieur des cours d'eau, ainsi que les tufières. Dans les deux bassins, la richesse spécifique en éphémères est comparable entre microhabitats, tandis que l'abondance est maximale dans les mousses et la plus faible dans le limon. Les représentations en NMDS ne regroupent pas les assemblages suivant le type de microhabitats, suggérant ainsi que les paramètres physico-chimiques jouent un rôle plus important. Dans les deux bassins et dans tous les microhabitats la composition en éphémères est dominée par les brouteurs/racleurs dans les sites en amont, et par les détritivores dans les sites en aval. Les filtreurs actifs sont plus abondants dans les microhabitats composés de limon et à faible vitesse de courant. Cette étude confirme que certaines espèces d'éphémères montrent une préférence marquée pour certains types de microhabitats, illustrant ainsi leurs affinités pour certaines vitesses de courant ainsi que leurs préférences alimentaires, tandis que d'autres espèces passent d'un microhabitat à l'autre, probablement à la recherche de nourriture ou d'un abri. Nos résultats mettent

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l'accent sur l'importance de l'hétérogénéité des habitats afin de maintenir des communautés diverses dans les cours d'eau karstiques.

Mots clés : Substrat / vitesse du courant / profondeur de l'eau / composition en éphémères / groupes trophiques

1 Introduction

Microhabitat preferences of freshwater macroinvertebrates are essential for studying correlations between species and their environment, and in turn for creating an adequate foundation for the conservation of aquatic habitats and their biodiversity (*e.g.* Urbanič *et al.*, 2005; Sarr *et al.*, 2013; Álvarez-Troncoso *et al.*, 2017). Together with the physical and chemical characteristics of water, microhabitat heterogeneity is another key factor influencing the composition of benthic macroinvertebrate assemblages, with individual species often associated with particular microhabitat types (Corkum *et al.*, 1977; Giller and Malmqvist, 1998; Bauernfeind and Moog, 2000; Subramanian and Sivaramakrishnan, 2005; Arimoro and Muller, 2010; Leitner *et al.*, 2015; Milesi *et al.*, 2016; Sroka *et al.*, 2016). Microhabitat preferences are closely related to substrate type, water velocity and depth, organic matter deposits, food availability, turbulence and hydraulic parameters (Gordon *et al.*, 1992; Lampert and Sommer, 1997; Miliša *et al.*, 2006). The availability of appropriate microhabitats allows macroinvertebrates, including mayfly larvae, to seek refuge from predators and to acquire sufficient food resources (*e.g.* Corkum *et al.*, 1977; Hawkins, 1985; Dedieu *et al.*, 2015). Both organic substrates (especially macrophytes) and coarse inorganic sediments support high mayfly species richness (Bauernfeind and Moog, 2000; Baptista *et al.*, 2001; Dinakaran and Anbalagan, 2006; Bauernfeind and Soldán, 2012), as they trap more organic matter and provide habitat for periphyton (*e.g.* Rounick and Winterbourn, 1983; Webster *et al.*, 1987; Dedieu *et al.*, 2015), an important food resource for mayfly larvae.

Based on their feeding strategies and exposure to predation risk, most mayfly species depend on certain microhabitats during their larval stages. Grazers and scrapers prefer microhabitats dominated by macrophytes and/or inorganic sediments coated in diatom-rich biofilms, whereas shredders and gatherers/collectors occur in substrates containing decomposing coarse and fine particulate organic matter (FPOM; *e.g.* Lamp and Britt, 1981; Buffagni *et al.*, 1995; Bauernfeind and Soldán, 2012). Filter feeders also consume decomposing FPOM either deposited or suspended in the water column. Active filter feeders inhabit fine-grained inorganic substrates associated with lower water velocities, where their gill movements create a water current that actively traps the suspended FPOM. In contrast, passive filter feeders require current velocities to be sufficient to distribute FPOM food particles directly to the larvae (*e.g.* Bae and McCafferty, 1994, Schmedtje and Colling, 1996; Moog, 2002; Bauernfeind and Soldán, 2012).

The present study was conducted in the Dinaric karst, the largest continuous karst landscape in Europe (Mihevc *et al.*, 2010) extending over approximately 60,000 km^2. Dinaric karst habitats are recognized as a biodiversity hotspot (Bănărescu, 2004; Bonacci *et al.*, 2008; Previšić *et al.*, 2009; 2014; Ivković and Plant, 2015), though freshwater habitats in southern Europe are increasingly impacted by anthropogenic activities including river regulation, resulting in a rapid decline in freshwater biodiversity (Freyhof, 2012; Schwarz, 2012). Habitat alterations have a negative impact on mayfly assemblages leading to the rapid disappearance of many species (*e.g.* Bauernfeind and Moog, 2000; Brittain and Sartori, 2003; Monaghan *et al.*, 2005). River regulations change hydrological, physical and chemical habitat conditions, and microhabitat composition, which shifts from heterogeneous to homogeneous, becoming dominated by fine-grained substrates (Baxter, 1977; Ward and Stanford, 1979; Graf, 2005). Over the past decade, an increasing number of studies have examined the diverse aquatic insect assemblages of the Dinaric karst (*e.g.* Previšić *et al.*, 2007; Popijač and Sivec, 2009; Šemnički *et al.*, 2012; Ivković and Plant, 2015), however to date, mayflies have mainly been studied in relation to habitat preferences determined by physical and chemical water characteristics (Vilenica *et al.*, 2014; 2016a; 2016b; 2017), whereas information regarding their microhabitat preferences remains scarce (*e.g.* Savić *et al.*, 2010). Due to the specificity of karst habitats, several interesting mayfly taxa have been recorded (Vilenica *et al.*, 2016a; 2016b; 2017). Moreover, comprehensive data on microhabitat preferences are so far known for just 58.1% of European mayfly species (see in Buffagni *et al.*, 2017). Thus, collecting the missing data and comparing microhabitat selection of widely distributed species in karst habitats with literature data (Buffagni *et al.*, 2017) represent an interesting subject of research. Therefore, the main aim of this study was to examine the taxonomic and functional feeding group composition of mayfly assemblages in microhabitats located in different habitats types/river reaches of two contrasting lotic catchments in the Dinaric karst, one pristine and one impacted by anthropogenic activities.

2 Methods

2.1 Study area

The study was conducted in two catchments spanning two biogeographical regions in the Dinaric Western Balkan region of Croatia: Plitvice Lakes National Park in the Alpine region (44°51′ N, 15°34′ E), and the Cetina River and its tributary, the Ruda River, in the Mediterranean region (43°26′ N, 16°41′ E; Fig. 1) (EEA, 2008). The Plitvice Lakes catchment has a temperate humid climate with warm summer, while the Cetina River catchment has a temperate humid climate with hot summer (Köppen climate classification; Šegota and Filipčić (2003)).

The Plitvice barrage lake system is located in the area of Plitvice Lakes National Park, designated as a UNESCO natural World Heritage Site in 1979 (IUCN, 1979). The Plitvice Lakes catchment consists of 16 oligotrophic and oligo-mesotrophic, dimictic, fluvial lakes interconnected by lotic tufa barriers (second stream order; Stahler, 1952) (Fig. 1a). Two small
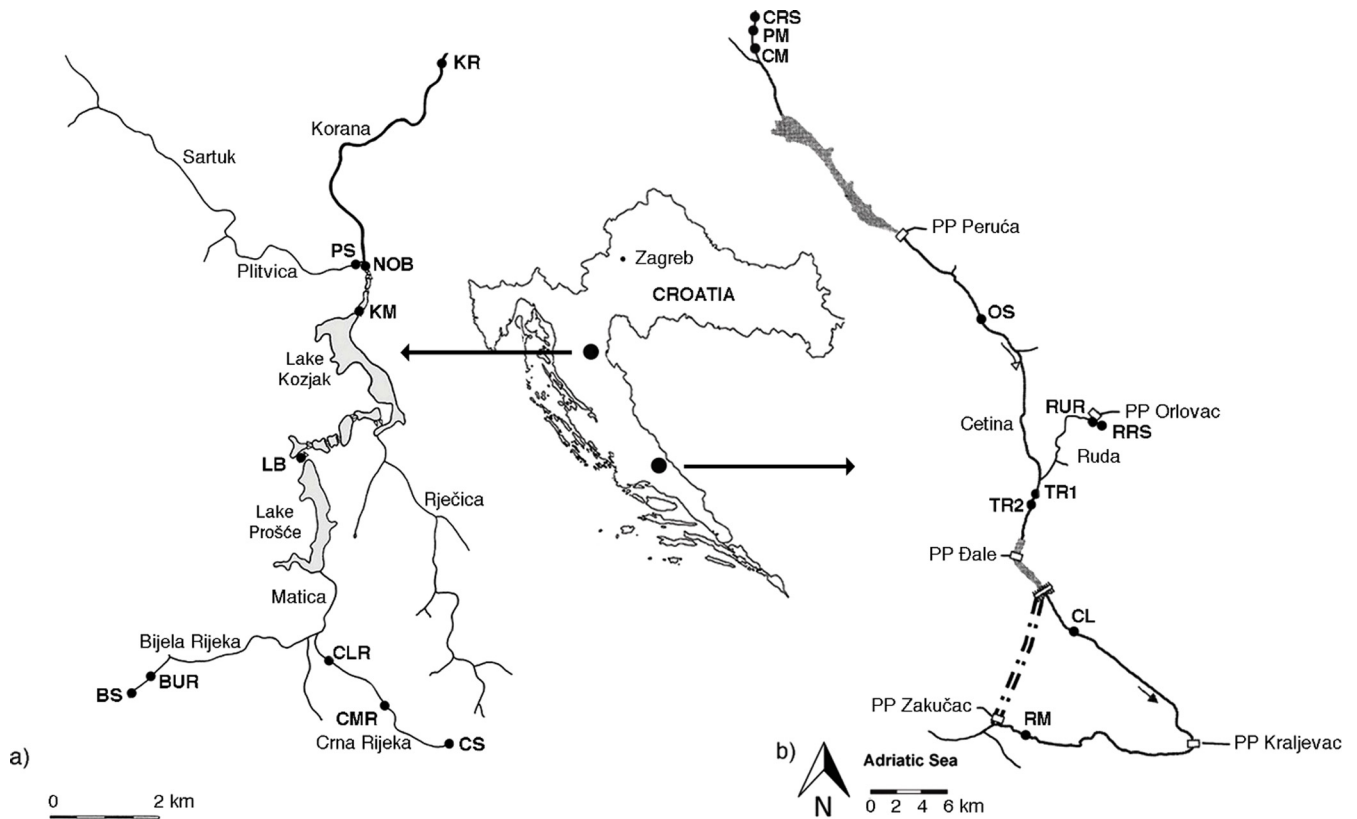
Fig. 1. Location of the study area in Croatia with study sites of microhabitat sampling. a) Plitvice Lakes National Park: Upper lotic habitats: BS − Bijela Rijeka River spring, BUR − Bijela Rijeka River upper reaches, CS − Crna Rijeka River spring, CMR − Crna Rijeka River middle reaches, CLR − Crna Rijeka River lower reaches; Tufa barriers: KM − Tufa barrier Kozjak-Milanovac, LB − Tufa barrier Labudovac, NOB − Tufa barrier Novakovića Brod; Lower lotic habitats: KR − Korana River, PS − Plitvica Stream. b) Cetina and Ruda Rivers: Springs: CRS − Cetina River spring (Glavaš), RRS − Ruda River spring; Upper reaches: PM − Preočki Most, CM − Crveni Most, RUR − Ruda River upper reaches; Middle reaches: OS − Obrovac Sinjski, TR1 − Trilj1, TR- Trilj2; Lower reaches: CL − Čikotina Lađa, RM − Radmanove Mlinice. PP − power plant; (dashed line shows subterranean water channel). Figures are modified from Vilenica *et al.* (2014) and Vilenica *et al.* (2016b).

mountainous rivers (first stream order) upstream of the barrage lake system, Bijela Rijeka River and Crna Rijeka River, join to form the Matica River (second stream order), the main surface-water supplier of the lakes (Stilinović and Božičević, 1998). Mean annual air temperature during the study period (year 2007) was 11.4 °C, with a mean minimum temperature of −0.5 °C in December, and a mean maximum of 21.1 °C in July. Annual rainfall during the study period was 1661 mm, with a minimum of 12 mm in April and maximum of 228 mm in October (Meteorological and Hydrological Institute of Croatia; www.meteo.hr).

The Cetina River (first and second stream order) stretches for 105 km from its spring source to its mouth at the Adriatic Sea (Fig. 1b). It is characterized by the inflow of numerous lateral springs (UNEP/MAP/PAP, 2000) along its course, including the Ruda River encompassed within this study. Mean annual air temperature during the study period (year 2004) was 12.9 °C, with a mean minimum temperature of 2.5 °C in December, and a mean maximum of 23.2 °C in July. Annual rainfall during the study period was 1347 mm, with a minimum of 8 mm in July and a maximum of 214 mm in April (Meteorological and Hydrological Institute of Croatia; www.meteo.hr). The Cetina River catchment is impacted by anthropogenic pressures including five hydropower plants,

the inflow of untreated domestic and agricultural effluents, and groundwater abstraction for public water supply (UNEP/MAP/PAP, 2000; see also Vilenica *et al.*, 2016b).

2.2 Sampling strategy

Microhabitats were sampled at ten study sites in each catchment. In the Plitvice Lakes catchment, sites encompassed the following habitat types: i) springs (BS, CS) and downstream sections (BUR, CUR, CLR) of small mountainous rivers (first stream order); Bijela Rijeka River and Crna Rijeka River located upstream of the barrage lake system (upper lotic habitats); ii) tufa barriers (LB, KM, NOB) (second stream order); and iii) the canyon-type mountainous Plitvica Stream (PS) (first stream order) and the mid-altitude large Korana River (KR) (second stream order), located downstream of the barrage lake system (lower lotic habitats) (Fig. 1a; for details, see Vilenica *et al.*, 2017). In the Cetina River catchment, the ten study sites represented the following habitat types: i) springs (CRS, RS); ii) upper reaches (PM, CM, RUR) (first stream order); iii) middle reaches (OS, TR1, TR2); and iv) lower reaches (CL, RM; Fig. 1b) (second stream order).

Sampling in the Plitvice Lakes catchment was conducted monthly from February 2007 to February 2008, and in the

Table 1. Study sites and dominant microhabitats sampled in the Plitvice Lakes and Cetina River catchments.

Plitvice Lakes catchment		Cetina River catchment	
Study site	Microhabitats	Study site	Microhabitats
Bijela Rijeka River spring (BS)	cobbles, angiosperms, mosses, sand	Cetina River spring (CRS)	boulders, angiosperms, sand, silt
Bijela Rijeka River upper reaches (BUR)	cobbles, angiosperms, mosses	Cetina River upper reaches – Preočki Most (PM)	boulders, angiosperms, sand
Crna Rijeka River spring (CS)	cobbles, mosses, sand	Cetina River upper reaches – Crveni Most (CM)	boulders, angiosperms, sand
Crna Rijeka River middle reaches (CMR)	cobbles, mosses, silt mixed with leaf litter	Cetina River middle reaches – Obrovac Sinjski (OS)	boulders, angiosperms, sand
Crna Rijeka River lower reaches (CLR)	cobbles, angiosperms, silt mixed with leaf litter	Cetina River middle reaches – Trilj 1 (TR1)	silt
Tufa barrier Kozjak-Milanovac (KM)	cobbles, mosses, silt mixed with leaf litter	Cetina River middle reaches – Trilj 2 (TR2)	silt
Tufa barrier Labudovac (LB)	mosses, sand, silt mixed with leaf litter	Cetina River lower reaches – Čikotina Lađa (CL)	boulders, mosses, sand
Tufa barrier Novakovića Brod (NOB)	cobbles, mosses, silt mixed with leaf litter	Cetina River lower reaches – Radmanove Mlinice (RM)	boulders, mosses, sand
Korana River (KR)	cobbles, mosses, sand	Ruda River spring (RRS)	boulders, mosses, sand
Plitvica Stream (PS)	cobbles, sand, silt mixed with leaf litter	Ruda River upper reaches (RUR)	boulders, mosses, sand

Cetina River catchment between August 2004 and August 2005 to capture taxa with diverse and contrasting life cycles (Vilenica *et al.*, 2016c).

At each site and in each month, triplicate samples were taken in all microhabitat types accounting for $\geq 5\%$ of the channel area. In the Plitvice Lakes catchment, microhabitat types comprised those dominated by submerged native angiosperms, mosses, cobbles, sand, and silt mixed with leaf litter; while in the Cetina River, catchment microhabitat types consisted of those dominated by submerged native angiosperms, mosses, boulders, sand, and silt (Tab. 1).

2.3 Mayfly sampling and identification

In association with collection of each individual sample, inorganic substrate categories were defined based on Wentworth (1922). Water velocity was measured using a P-670-M velocimeter (Dostmann electronic) and depth was measured using a depth-meter.

At most sampling points, macroinvertebrates including mayfly larvae were collected using Surber samplers (mesh size: 0.5 mm; surface area in the Cetina River catchment 33×33 cm; surface area in the Plitvice Lakes catchment 14×14 cm on mosses [due to very high macroinvertebrate abundance] and 25×25 cm in other microhabitats). At sites in the lower reaches of the Crna Rijeka River (CLR) in the Plitvice Lakes catchment and two sites in the Cetina River catchment (TR1, TR2), deep water prevented Surber sampling and macroinvertebrates were collected over a comparable surface area using a D-frame hand net (mesh size 0.5 mm). Samples were preserved in 80% ethanol.

Mayflies were identified to the lowest possible taxonomic level (species, genus, or occasionally family, depending on the instar) using resources listed in Vilenica *et al.* (2015). All voucher specimens were deposited at the Department of Biology, Faculty of Science, University of Zagreb, Croatia. Each taxon was enumerated and abundance expressed as individuals m^{-2} to allow comparison of different-sized samples.

2.4 Data analysis

One-way ANOVA tests with the unequal N HSD *post hoc* test were used to identify differences in water velocity and depth between microhabitat types.

The studied catchments differ in terms of key environmental influences on mayfly assemblages (*i.e.* habitat complexity, habitat diversity, and water physical and chemical properties) and therefore support contrasting assemblages (Bauernfeind and Soldán, 2012). All analyses were therefore conducted separately for samples collected from the Plitvice Lakes and Cetina River catchments, to allow comparison of patterns based on samples containing distinct but overlapping mayfly assemblages. Only species data were included in the analyses.

Hierarchical cluster analysis (HCA) and non-metric multidimensional scaling (NMDS) ordination based on a Bray-Curtis similarity matrix were used to examine variability in mayfly assemblage composition among microhabitats (Ramette, 2007). HCA results were superimposed on the
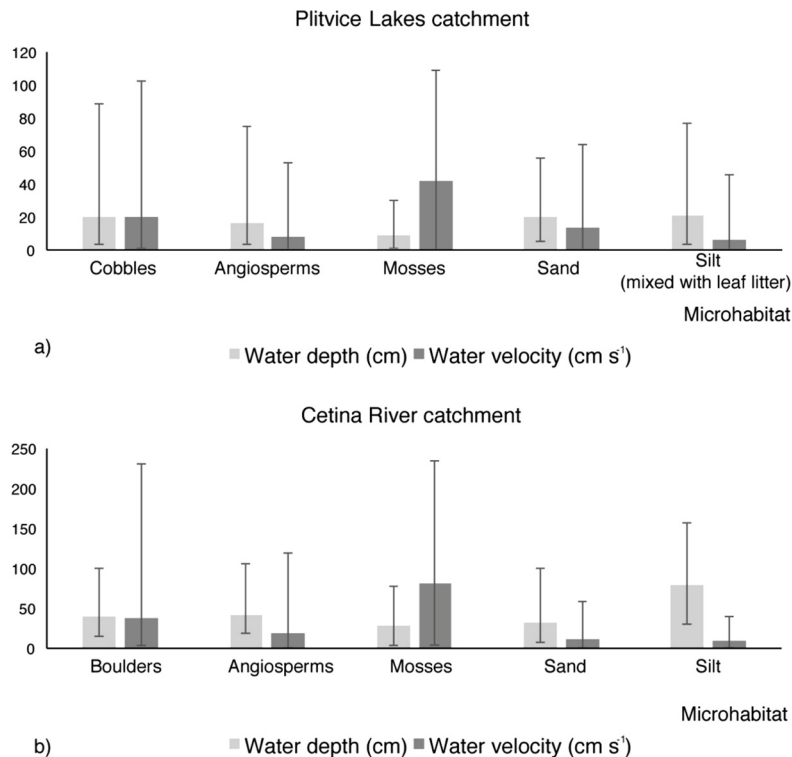
Fig. 2. Water depth and velocity (mean ± SD) in different microhabitat types measured over a one-year period in a) the Plitvice Lakes catchment and b) the Cetina River catchment.

NMDS ordination, to determine similarity percentage between microhabitats located in different study sites. Data were log-transformed prior to analysis.

The functional feeding group composition of mayfly assemblages in different microhabitats was classified using Buffagni *et al.* (2009), Buffagni *et al.* (2017) and Bauernfeind and Soldán (2012). The functional feeding group of each individual species is presented as a proportion of the assemblage. Since most taxa do not exclusively feed on a single food resource, the assignment of taxa to a particular category is based on the ten-point assignment scale (Schmidt-Kloiber and Hering, 2015). Using the given points and percentage of each species within the assemblage, the functional feeding group composition of mayfly assemblages at each microhabitat type was calculated.

To determine differences among microhabitats in species richness, total abundance, the abundance of individual species and feeding group proportions, non-parametric Kruskal-Wallis H tests were used, followed by multiple-comparison *post hoc* tests.

Indicator Value Analysis (IndVal; Dufrêne and Legendre, 1997) was used to identify species indicative of a microhabitat type. This analysis is based on the specificity and fidelity measured for each taxon in an assemblage, with indicator values ranging from 0 to 100% and reaching a maximum when all individuals of a taxon are recorded in only a single microhabitat type (high specificity) and when the taxon is present in all samples of that microhabitat type (high fidelity). Thus, species indicative of a particular microhabitat, have high and significant percentage IndVals (>55%) and were considered symmetrical indicators. The indicator values were tested

for statistical significance using Monte Carlo tests with 4,999 permutations.

Spearman's rank correlation coefficients were used to assess the correlation of species richness, total abundance, and the abundance of individual species with water velocity and water depth.

NMDS analysis was conducted in Primer 6 (Clarke and Gorley, 2006). One-way ANOVA, Kruskal-Wallis H test and Spearman's rank correlation coefficient were calculated in Statistica 13.0 (Dell Inc., 2016), and IndVal was performed using PC-ORD version 5.0 (McCune and Mefford, 2006).

3 Results

3.1 Water depth and water velocity analysis

Water depth (one-way ANOVA, $F = 5.72$, $df = 4.27$, $p < 0.01$) and velocity ($F = 15.36$, $df = 4.27$, $p < 0.001$) differed among microhabitats in the Plitvice Lakes catchment. Depth was lower in microhabitats with mosses compared to those with cobbles, sand, and silt mixed with leaf litter (unequal N HDS *post hoc* tests, $p < 0.001$; Fig. 2a). Velocity was higher in mosses compared with all other microhabitats ($p < 0.01$). Additionally, velocity was also higher in the cobbles microhabitat compared to silt mixed with leaf litter ($p < 0.05$; Fig. 2a).

In the Cetina River catchment, depth ($F = 11.72$, $df = 4.21$, $p < 0.001$) and velocity ($F = 8.40$, $df = 4.21$, $p < 0.001$) also differed among microhabitats. Depth was higher in the silt microhabitat compared to sand, mosses ($p < 0.001$), boulders ($p < 0.01$) and angiosperms ($p < 0.05$; Fig. 2b). Water velocity

was higher in the mosses microhabitat compared to sand, silt ($p < 0.01$) and angiosperms ($p < 0.05$), and was higher in the boulders microhabitat compared to silt ($p < 0.05$; Fig. 2b).

3.2 Mayfly assemblages

A total of 14 mayfly species (Tab. 2) were recorded in the Plitvice Lakes catchment. NMDS ordination (Fig. 3a) showed that mayfly assemblages generally did not group based on microhabitat type. Species richness was comparable among microhabitats (Kruskal-Wallis H test, multiple comparisons *post hoc* test, H [4, $N = 31$] = 6.25, $p > 0.05$), ranging between a total of 11 species each in the microhabitats with angiosperms, sand, and silt mixed with leaf litter, to eight species in moss microhabitats. Abundance was highest in mosses and lowest in silt mixed with leaf litter (H [4, $N = 31$] = 10.87, $p < 0.05$; Tab. 2). Mayfly abundance was negatively correlated with water depth (Spearman's rank correlation, $R = -0.17$, $p < 0.001$) and positively with velocity ($R = 0.12$, $p < 0.05$).

In the Cetina River catchment, a total of 21 mayfly species (Tab. 2) were recorded. Assemblages generally did not group based on microhabitat type in the NMDS ordination space (Fig. 3b). Species richness (H [4, $N = 25$] = 0.17, $p > 0.05$) and abundance (H [4, $N = 25$] = 8.15, $p > 0.05$) were comparable among microhabitats, with a total of 19 species recorded on sand, 17 on boulders, 16 on mosses, 11 on angiosperms and eight on silt. Silt microhabitats had the lowest abundance, while mosses had the highest, though these differences were not statistically significant. Mayfly species richness (Spearman's rank correlation, $R = -0.11$, $p < 0.05$) and abundance ($R = -0.23$, $p < 0.001$) were negatively correlated with water depth and positively with water velocity (S, $R = 0.32$, $p < 0.001$; N, $R = 0.41$, $p < 0.001$).

3.3 Microhabitat preferences of mayfly species

In both catchments, a high number of species were collected from all microhabitats within the catchment (Tab. 2). Yet, some species showed a significant preference for a particular microhabitat type.

In the Plitvice Lakes catchment, *Baetis* cf. *nubecularis* was indicative of moss microhabitats (IndVal = 72.50, $p < 0.01$), with no significant indicators identified for any other microhabitat. Additionally, *B.* cf. *nubecularis* was more abundant in mosses compared to silt mixed with leaf litter (Kruskal-Wallis and multiple comparisons *post hoc* tests; H [4, $N = 22$] = 16.28, $p < 0.01$) and sand ($p < 0.05$; Tab. 2). *Baetis rhodani* was more abundant in mosses compared to silt mixed with leaf litter (H [4, $N = 28$] = 14.53, $p < 0.01$), *Ephemera danica* was most abundant in sand and absent from mosses (H [4, $N = 21$] = 13.22, $p < 0.05$; Tab. 2), and *Rhithrogena braaschi* was most abundant in cobbles and absent from silt mixed with leaf litter (H [4, $N = 19$] = 10.19, $p < 0.05$; Tab. 2).

Serratella ignita (Spearman's rank correlation, $R = -0.18$, $p < 0.01$), *B.* cf. *nubecularis* ($R = -0.18$, $p < 0.01$) and *B. rhodani* ($R = -0.12$, $p < 0.05$) were negatively correlated with water depth, whereas *C. luteolum* ($R = -0.22$, $p < 0.01$) and *E. danica* ($R = -0.20$, $p < 0.01$) were positively correlated. *Baetis* cf. *nubecularis* ($R = 0.36$, $p < 0.001$), *B. rhodani* ($R = 0.14$, $p < 0.05$) and *Rh. braaschi* ($R = 0.16$, $p < 0.05$) were positively correlated with water velocity, while *C. luteolum* ($R = -0.49$,

$p < 0.001$), *E. danica* ($R = -0.39$, $p < 0.001$), *Habrophlebia lauta* ($R = -0.40$, $p < 0.001$) and *Paraleptophlebia submarginata* ($R = -0.19$, $p < 0.01$) were negatively correlated.

In the Cetina River catchment, *Baetis lutheri* was indicative of moss microhabitats (IndVal = 87.00, $p < 0.001$; Kruskal-Wallis and multiple comparisons *post hoc* tests; H [2, $N = 12$] = 9.99, $p < 0.01$; Tab. 2) and *Rh. braaschi* of boulder microhabitats (IndVal = 60.3, $p < 0.01$). Other mayfly species had low IndVal values.

Baetis rhodani (Spearman's rank correlation, $R = -0.12$, $p < 0.05$) was negatively correlated with water depth, while *Ecdyonurus macani* ($R = 0.18$, $p < 0.05$) and *S. ignita* ($R = 0.14$, $p < 0.05$) were positively correlated. *Baetis lutheri* ($R = 0.44$, $p < 0.001$), *Ecdyonurus venosus* ($R = 0.26$, $p = 0.001$), *Epeorus assimilis* ($R = 0.32$, $p < 0.001$), *Rh. braaschi* ($R = 0.32$, $p < 0.001$), *Baetis melanonyx* ($R = 0.28$, $p < 0.05$) and *Ephemerella mucronata* ($R = 0.41$, $p < 0.05$) were positively correlated with water velocity while the abundances of *E. danica* ($R = -0.38$, $p < 0.001$) and *Ephemera lineata* ($R = -0.18$, $p < 0.01$) were negatively correlated.

3.4 Functional feeding groups

In the Plitvice Lakes catchment, microhabitats with cobbles had a higher proportion of grazers compared to microhabitats with silt mixed with leaf litter (Kruskal-Wallis H test, multiple comparisons *post hoc* test; H [4, $N = 31$] = 11.34, $p < 0.05$) and a lower proportion of gatherers/collectors compared to microhabitats with mosses (H [4, $N = 31$] = 14.49, $p < 0.01$). Additionally, microhabitats with silt mixed with leaf litter had a higher proportion of active filter feeders compared to microhabitats with mosses (H [4, $N = 31$] = 12.32, $p < 0.05$). Furthermore, all microhabitats in the upper lotic habitats were dominated by grazers/scrapers followed by gatherers/collectors. In the lower reaches of the Crna Rijeka River, active filter feeders also occurred in microhabitats on silt mixed with leaf litter. Microhabitats located at tufa barriers and lower lotic habitats had a higher proportion of gatherers/collectors while active filter feeders were most numerous in microhabitats at tufa barriers. In the latter two habitat types, mosses had the highest proportion of gatherer/collectors, sand and silt mixed with leaf litter were dominated by gatherers/collectors and active filter feeders, while on the cobbles, all three feeding groups occurred (Fig. 4).

Feeding group proportions were comparable among microhabitats in the Cetina River catchment (H [4, $N = 25$] = 4.61, $p > 0.05$). However, mayfly assemblages in microhabitats located in springs and upper reaches were dominated by grazers/scrapers followed by gatherers/collectors. All microhabitats in the middle and lower reaches had the highest proportion of gatherers/collectors. Active filter feeders appeared in the middle reaches and were distributed in all microhabitats situated downstream, with the highest proportion on sand (Fig. 5).

4 Discussion

Although the two studied catchments differed in the extent of anthropogenic pressure, mayfly assemblages were relatively diverse at both (Vilenica *et al.*, 2016a; 2016b; 2017), while some similarities were seen in microhabitat selection. In both

Table 2. Abundance (N, individuals m⁻²) and abundance % of mayfly taxa in various microhabitats of the two studied catchments: PL – Plitvice Lakes catchment, CR – Cetina River catchment; indet. – undetermined individuals due to very juvenile stage.

| Taxa	PL Cobbles		CR Boulders		PL Angiosperms		CR Angiosperms		PL Mosses		CR Mosses		PL Sand		CR Sand		PL Silt + leaf litter		CR Silt	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Baetidae indet.	784	12.5	278	1.8	1479	38.8	667	8.5	2448	13	518	3	571	13.2	440	7.7	37.3	2.9	0	0
Baetis sp. indet.	144	2.3	1686	11	699	18.3	1583	20.1	3834	20.4	4168	23.9	45.6	1.1	770	13.5	0	0	10	0.7
Alainites muticus (Linnaeus, 1758)	0	0	0	0	26.7	0.7	0	0	0	0	0	0	19.2	0.4	0	0	2.7	0.2	0	0
Baetis liebenauae Keffermüller, 1974	0	0	1.25	0.01	0	0	257	3.3	0	0	10	0.1	0	0	17.5	0.3	0	0	50	3.6
Baetis lutheri Müller-Liebenau, 1967	0	0	280	1.8	0	0	0	0	0	0	1920	11	0	0	7.5	0.1	0	0	0	0
Baetis melanonyx (Pictet, 1843)	0	0	5744	37.6	0	0	0	0	0	0	2195	12.6	0	0	338	5.9	0	0	0	0
Baetis cf. nubecularis Eaton, 1898	134	2.1	0	0	80	2.1	0	0	3650	19.4	0	0	51.2	1.2	0	0	2.7	0.2	0	0
Baetis rhodani (Pictet, 1843)	768	12.3	2836	18.5	752	19.7	1593	20.3	1512	8	2255	12.9	392	9	1620	28.5	8	0.6	10	0.7
Nigrobaetis niger (Linnaeus, 1761)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.8	0.1	0	0	0	0
Centroptilum luteolum (Müller, 1776)	72	1.2	0	0	0	0	0	0	51	0.3	65	0.4	1053	24.3	18.8	0.3	371	29.1	5	0.4
Procloeon bifidum (Bengtsson, 1912)	0	0	0	0	0	0	0	0	0	0	2.5	0.01	0	0	5	0.1	0	0	0	0
Procloeon pennulatum Eaton, 1870	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caenis sp. indet.	2	0.03	3.8	0.02	5.3	0.1	6.7	0.1	0	0	1115	6.4	0	0	1.3	0.02	2.7	0.2	45	3.3
Caenis beskidensis Sowa, 1973	0	0	46.9	0.3	0	0	0	0	0	0	158	0.9	0	0	22.5	0.4	0	0	0	0
Caenis horaria Linnaeus, 1758	2	0.03	0	0	0	0	0	0	102	0.5	0	0	38.4	0.9	0	0	10.7	0.8	0	0
Caenis macrura Stephens, 1835	0	0	262.5	1.7	32	0.8	6.7	0.1	0	0	1606	9.2	0	0	545	9.6	0	0	190	13.8
Heptageniidae indet.	378	6	380	2.5	0	0	0	0	39.7	0.2	2.5	0.01	0	0	13.8	0.2	5.3	0.4	0	0
Ecdyonurus sp. indet.	0	0	144	0.9	0	0	0	0	0	0	25	0.1	0	0	96.3	1.7	0	0	0	0
Ecdyonurus insignis (Eaton, 1870)	0	0	6.3	0.04	0	0	23.3	0.3	0	0	0	0	0	0	0	0	0	0	0	0
Ecdyonurus macani Thomas & Sowa, 1970	0	0	120	0.8	107	2.8	0	0	0	0	30	0.2	0	0	18.8	0.3	0	0	0	0
Ecdyonurus submontanus Landa, 1969	60	1	0	0	0	0	3.3	0.04	0	0	0	0	0	0	0	0	0	0	0	0
Ecdyonurus venosus (Fabricius, 1775)	0	0	58.8	0.4	0	0	0	0	0	0	8	0.05	0	0	10	0.2	0	0	0	0
Epeorus assimilis Eaton, 1885	0	0	66.3	0.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhithrogena braaschi Jacob, 1974	3558	56.8	1935	12.7	278	7.3	283	3.6	141	0.8	485	2.8	653	15.1	556	9.8	0	0	30	2.2
Ephemera sp. indet.	0	0	37.5	0.3	0	0	13.3	0.2	0	0	333	1.9	0	0	62.5	1.1	0	0	0	0
Ephemera danica Müller, 1764	270	4.3	5	0.03	16	0.4	0	0	0	0	105	0.6	1087	25.1	253	4.4	616	48.3	0	0
Ephemera lineata Eaton, 1870	0	0	91.3	0.6	0	0	56.7	0.7	0	0	348	2	0	0	145	2.6	0	0	130	9.4
Ephemerellidae indet.	6	0.1	226	1.5	0	0	1130	14.4	39.7	0.2	558	3.2	0	0	203	3.6	0	0	0	0
Ephemerella mucronata (Bengtsson, 1909)	0	0	151	1	0	0	250	3.2	0	0	0	0	0	0	2.5	0.04	0	0	0	0
Serratella ignita (Poda, 1761)	44	0.7	705	4.6	37.3	1	1770	22.5	6369	33.8	1064	6.1	195	4.5	320	5.6	21.3	1.7	910	65.9
Torleya major (Klapalek, 1905)	34.4	0.6	61.3	0.4	0	0	0	0	68	0.4	398	2.3	0	0	50	0.9	0	0	0	0
Leptophlebiidae indet.	0	0	0	0	21.3	0.6	0	0	293	1.6	0	0	125	2.9	0	0	50.7	4	0	0
Habrophlebia lauta McLachlan, 1884	0	0	0	0	74.7	2	0	0	0	0	0	0	76.8	1.8	0	0	40	3.1	0	0
Paraleptophlebia submarginata (Stephens, 1835)	12	0.2	173	1.1	176	4.6	213	2.7	284	1.5	100	0.6	12.8	0.3	173	3	77.3	6.1	0	0
Siphlonurus croaticus Ulmer, 1920	0	0	0	0	32	0.8	3.3	0.04	0	0	0	0	16	0.4	1.3	0.02	26.7	2.1	0	0
Total (number of individuals m⁻²)	6269		15299		3816		7860		18832		17466		4335		5693		1275		1380	
Number of mayfly species	10		17		11		11		8		16		11		19		11		8	
Number of mayfly taxa	15		24		15		16		13		23		14		26		15		9	

a)

b)
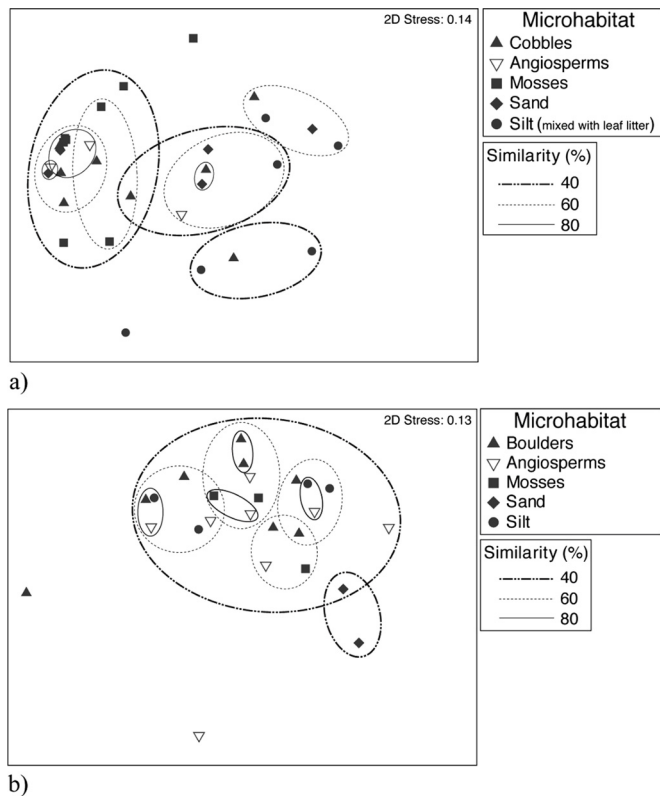
Fig. 3. Non-metric multidimensional scaling (NMDS) ordination of mayfly assemblages based on Bray-Curtis similarity coefficient (group average linking) and their log transformed abundances based on microhabitat type in: a) Plitvice Lakes catchment and b) Cetina River catchment. Results are shown pooled per sampling point (microhabitat).

catchments, species richness was comparable among microhabitats, which could be related to flow conditions, oxygen and nutrient availability (*e.g.* Ciborowski and Clifford, 1983; Collier, 1994). These results are also a consequence of the varying compositions of mayfly assemblages, with different proportions of species exhibiting a range of substrate preferences, *i.e.* from species found on a wide range of substrates (*e.g. S. ignita, B. rhodani*) to microhabitat specialists (*e.g. E. assimilis, Rh. braaschi, E. danica*) (Buffagni *et al.*, 2009; 2017). Additionally, previous studies showed that many mayfly species shift between microhabitats during their life cycles in search of adequate food resources and shelter (Ciborowski and Clifford, 1983; Holomuzki and Messier, 1993; Collier, 1994; Bauernfeind and Soldán, 2012), and this merits further detailed inspection in this study area.

Water velocity, depending on water discharge, is of exceptional importance in microhabitat selection, as besides directly influencing benthic invertebrates, it also controls the distribution of substrate and food, and influences oxygen concentration (Ciborowski, 1983). Thus, the highest mayfly abundances in both studied catchments were recorded at microhabitats such as mosses or coarse sized sediment (*i.e.* cobbles, boulders) associated with higher water velocity, smaller depth and higher amounts of available food resources (Plenković-Moraj *et al.*, 2002; Miliša *et al.*, 2006; Špoljar *et al.*, 2007). The fine substrate is generally known to support

relatively poor macroinvertebrate species richness (Minshall, 1984), which was confirmed for mayflies in the regulated catchment of the Cetina River. However, this is most likely a consequence of the generally poor environmental conditions related to the accumulation of muddy sediment in the Cetina River (see Vilenica *et al.*, 2016b). On the contrary, though in markedly lower numbers, a large part of the recorded species in the pristine catchment of the Plitvice Lakes inhabited silt mixed with leaf litter, which could have provided more substantial food resources in the otherwise oligotrophic water quality (Špoljar *et al.*, 2007; Gligora Udovič *et al.*, 2017).

The NMDS analyses indicated the lack of a specific preference for microhabitat type among the mayfly assemblages. Additionally, the majority of species showed no exclusive preference for a specific substrate type. Vilenica *et al.* (2016b; 2017) showed that mayfly assemblages in the Plitvice Lakes catchment primarily grouped according to the habitat type, and according to the particular river reaches in the Cetina River catchment. This could indicate higher mayfly preference for the specific physical and chemical water properties important in defining river sections and/or habitat types, rather than for a particular substrate.

Nevertheless, some mayfly species showed a distinct preference for a specific microhabitat. For instance, in the Cetina River catchment, *B. lutheri* preferred microhabitats with mosses, which is not in agreement with its typical preferences, as the species is listed as a mesolithal habitat specialist, occasionally recorded from akal and macrophytes (Buffagni and Desio, 1994; Buffagni *et al.*, 2009; 2017). In the Cetina River catchment, however, microhabitats with mosses had the highest water velocities, which is possibly the reason for the preference shown by this rheophilous species (see also in Vilenica *et al.*, 2016a). Though the eurytopic and rheophilous *B. rhodani* inhabited all available microhabitats in both catchments, it showed a preference for microhabitats with higher water velocity (Buffagni *et al.*, 2009; 2017). In addition, it showed a significant preference for substrate type in the Plitvice Lakes catchment, where it favoured microhabitats on mosses, and avoided microhabitats on silt mixed with leaf litter. Moreover, as the highest abundance of young larval instars of Baetidae was also recorded on mosses, this was likely the most suitable substrate providing food and shelter for younger instars. In accordance with literature data (Buffagni *et al.*, 2009; 2017), statistically supported specific microhabitat dominance was also seen in *E. danica* in both catchments and *B.* cf. *nubecularis* in the Plitvice Lakes catchment. The rheo- to limnophilous and predominantly psammal species *E. danica* preferred microhabitats on sandy substrates associated with slower water current (Buffagni *et al.*, 2009; Bauernfeind and Soldán, 2012; Buffagni *et al.*, 2017), while *B.* cf. *nubecularis* favoured microhabitats on mosses associated with the fastest current. Though the taxonomical status of the latter species is not yet resolved, the larvae of the *Baetis alpinus* group typically prefer lotic habitats exposed to fast currents, and tend to inhabit stony substrates from crenal to metarhithral sections of fast flowing streams (Bauernfeind and Soldán, 2012). This could indicate a species preference for higher water velocity rather than for the mossy substrate, since these were the sites of significantly higher water velocity. In both catchments, the rheophilous *Rh. braaschi* preferred microhabitats on coarse sediment (cobbles,
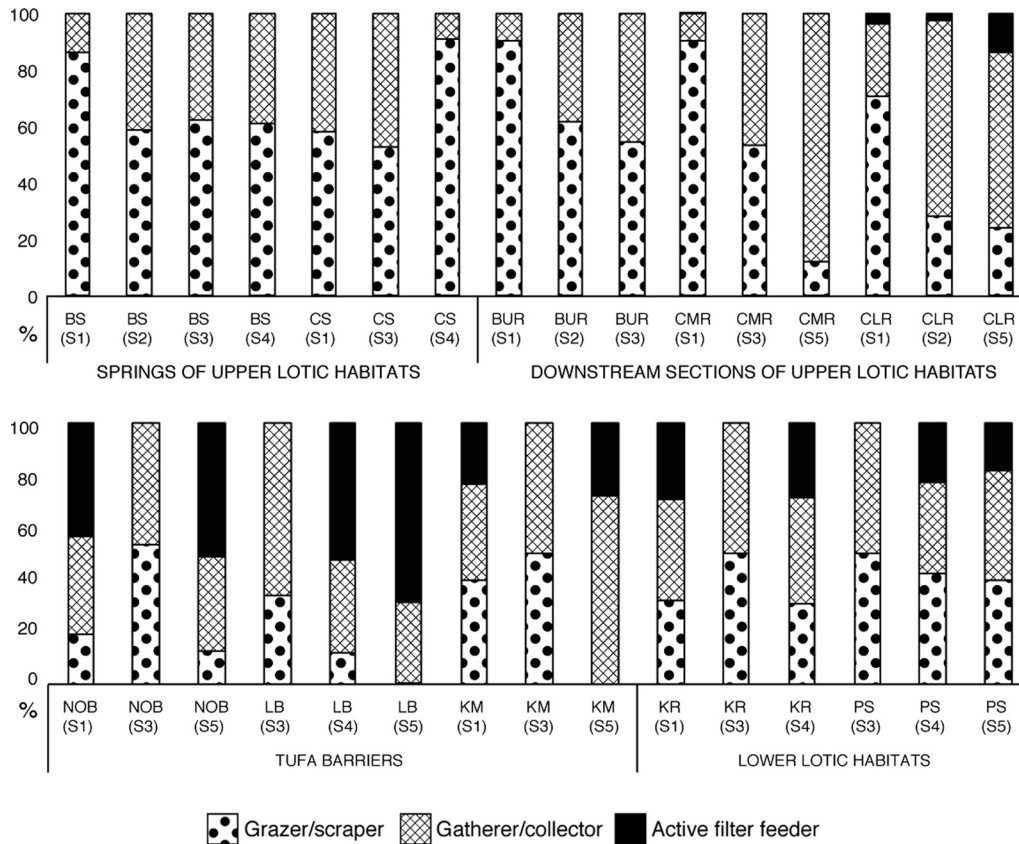
Fig. 4. Percentage abundance of diverse functional feeding groups of mayfly assemblages and their distribution in various habitats and microhabitats in the Plitvice Lakes catchment. Legend: S1 − cobbles; S2 − angiosperms; S3 − mosses; S4 − sand; S5 − silt mixed with leaf litter. For abbreviations of study site names see Fig. 1.

boulders) and faster water current, which is in accordance with its typical microhabitat selection (Buffagni *et al.*, 2009; 2017).

Finally, though several species did not show an exclusive preference for a specific substrate type, they were found to favour certain microhabitats. Thus, significant preferences for microhabitats with higher current and lower water depth were shown for the rheophilous *B. melanonyx*, rheo- to limnophilous *E. venosus* and *E. mucronata*, and the rheobiont *E. assimilis* in the Cetina River catchment (Buffagni *et al.*, 2009; Bauernfeind and Soldán, 2012; Buffagni *et al.*, 2017). On the other hand, several species favoured microhabitats with lower water velocity and greater water depth, such as the limnophilous *C. luteolum*, limno- to rheophilous *H. lauta*, rheo- to limnophilous *P. submarginata* in the Plitvice Lakes catchment, as well as the limno- to rheophilous *E. lineata* in the Cetina River catchment (Buffagni *et al.*, 2009; Bauernfeind and Soldán, 2012; Buffagni *et al.*, 2017). Interestingly, in the pristine Plitvice Lakes catchment, *S. ignita* preferred microhabitats with smaller water depth, while in the regulated Cetina River catchment, this species favoured microhabitats with greater water depth, which could be related to its broad ecological tolerance (Buffagni *et al.*, 2009; 2017).

In both catchments, mayfly assemblages shifted from being grazer/scraper-dominated in all microhabitats in the springs and upstream sites to detritivore-dominated downstream. Due to the morphology of the studied karst hydro-systems, which are mainly characterized by high water velocity and presence of coarse sized sediment and aquatic vegetation with trapped organic matter and overgrown with periphyton (*e.g.* Bonacci, 1987; Plenković-Moraj *et al.*, 2002), the highest proportion of grazers (*e.g. Rh. braaschi*, *E. assimilis*) and gatherers (*e.g. C. macrura*, *P. submarginata*) (Buffagni *et al.*, 2009; 2017) is not surprising. In the Cetina River catchment and in the Bijela Rijeka River in the Plitvice Lakes catchment, riparian vegetation forms an open canopy. It enables algal growth that benefits grazers, and macrophyte growth that benefits gatherers due to the accumulation of organic particles on the vegetation (Miliša *et al.*, 2006). Active filter feeders (*e.g. E. danica*, *E. lineata*) appeared in microhabitats located the middle reaches of the Cetina River (see in Vilenica *et al.*, 2016b) and lower reaches of the Crna Rijeka River, especially on silty substrates, where slower water velocity caused an accumulation of fine sediment and organic matter (Vilenica *et al.*, 2016b; 2017). In the Plitvice Lakes catchment, tufa barriers − natural lake outlets, had the highest proportion of active filter feeders in the majority of microhabitats due to the accumulation of organic particles on barrier substrates (Obelić *et al.*, 2005).

In conclusion, as previously recorded for other aquatic insects (*e.g.* Šemnički *et al.*, 2012), the mayfly assemblage composition and structure in both studied river systems are influenced by microhabitat characteristics (*i.e.* water velocity, water depth and substrate type) only to a certain degree, and are more reliant on the availability of different food resources (*e.g.* Plenković-Moraj *et al.*, 2002; Miliša *et al.*, 2006) and the
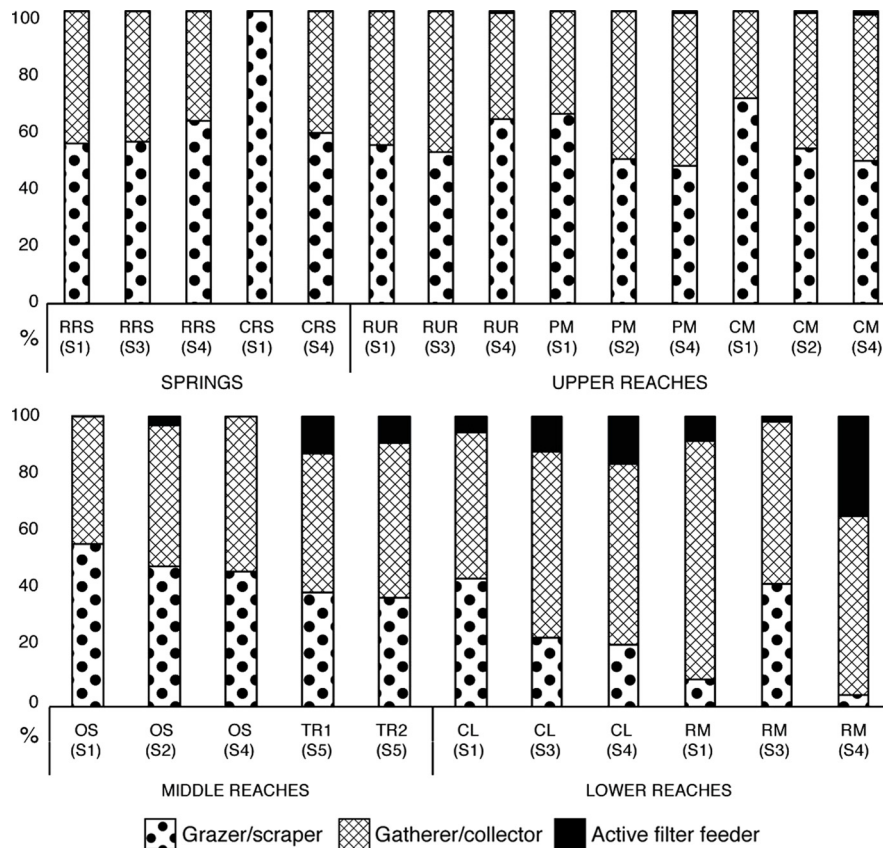
Fig. 5. Percentage abundance of diverse functional feeding groups of mayfly assemblages and their distribution in various habitats and microhabitats in the Cetina River catchment. Legend: S1 − boulders; S2 − angiosperms; S3 − mosses; S4 − sand; S5 − silt. For abbreviations of study site names see Fig. 1.

physical and chemical water properties (Vilenica *et al.*, 2014; 2016a, b; 2017). These are related to the position within the barrage-lake system in the Plitvice Lakes catchment and to the longitudinal distribution in the Cetina River catchment. Even though still greatly under-studied, the Dinaric Karst area represents one of the global biodiversity hotspots (Bãnãrescu, 2004; Griffiths *et al.*, 2004; Ivković and Plant, 2015), which is also highly threatened due to numerous anthropogenic pressures (Štambuk-Giljanović, 2001; Obelić *et al.*, 2005; Chatzinikolaou *et al.*, 2006; Tierno de Figueroa *et al.*, 2013). Since mayflies are widely used as bioindicators of freshwater ecosystems (Landa and Soldán, 1991), data on mayfly ecology, *i.e.* microhabitat preferences presented here, represent the necessary background for further research and conservation practices in karst streams and rivers.

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