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# Community structure and distribution of Ephemeroptera and Plecoptera larvae in lowland karst rivers in Slovenia

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Mayfly and stonefly larval community compositions were investigated in lowland karst rivers. From January to December 2005, 49 benthos samples were taken using a multi-habitat approach. Environmental variables were measured in about monthly intervals. Altogether, 26 mayfly taxa (20 species) and 17 stonefly taxa (13 species) were recorded. Eight species were common, whereas 16 taxa were rare. Only *Baetis rhodani* and *Ephemerella ignita* occurred frequently at all sites. Significant differences were observed among sampling sites in diversity and richness metrics but neither in abundance nor in values of index of biocenotic regions. Spatial and seasonal differences amongst assemblages were determined by NMS. The relationship between mayfly and stonefly assemblage structure and seasonally varied environmental factors were investigated using canonical correspondence analysis (CCA). Mayfly and stonefly larval taxa distribution was best correlated to an eutrophication gradient represented by conductivity, nitrate concentration and dissolved oxygen concentration.

**Keywords:** mayflies; stoneflies; diversity; multivariate analysis; lowland karst river; karst spring; Slovenia

## Introduction

Mayfly and stonefly larvae have an important role in running water ecosystems, especially in non-polluted lotic systems, where they are usually dominant taxa. They can be of importance for water management as they are widely accepted as bioindicators (Landa and Soldan 1995; Bauernfeind and Moog 2000; Beketov 2004).

Karst systems are distinct freshwater ecosystems, which provide an interface between subterranean and surface waters (Smith, Wood and Gunn 2003). However, the influence of the subterranean water on downstream changes largely depends on discharge and distance between upper and lower watercourse sections. Many karst streams have a spatial and temporal difference, and sometimes an extreme discharge regime, due to various and mostly complicated hydrological conditions (Meyer and Meyer 2000). Moreover, due to high permeability of substrate, karst rivers also

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exhibit hard water and low water temperature variation, but the latter may vary according to the level of the groundwater influence (Gams 2003). The Dinaric Karst, the largest connected karst area in Europe, covers the region between the Friuli plain (Italy) in the northwest and the Albanian mountains in the southeast. Ecologically the area is part of the ecoregion Dinaric western Balkan (Illies 1978; Urbanič 2008a) and the bioregion Sub-Dinaric hills and plains (Urbanič 2008b).

Studies addressing a relationship between macroinvertebrate communities and environmental variables in karst streams can be found. However, most studies were either related to karst springs (e.g. Smith et al. 2003; Mori and Brancelj 2006) or temporary karst streams (Meyer and Meyer 2000). On the other hand, existing publications including perennial karst rivers are mainly related to the individual macroinvertebrate group or specific environmental variables (e.g. Habdija, Radanović, Primc-Habdija and Špoljar 2002; Urbanič and Toman 2006; Tanaka, Ribas and de Souza 2008).

In this study, the community composition and distribution of mayfly and stonefly larvae in three karst rivers are characterised. The main objectives of the study were (1) to compare site characteristics and community composition among sites; (2) to relate the community structure to the selected ecological factors that varied among sampling dates, and (3) to rank selected ecological factors according to their importance in determining the distribution of mayfly and stonefly larvae.

## Methods

### *Study area*

Stonefly and mayfly larval community composition was studied in karst spring rivers located in the Bela Krajina, southeast part of Slovenia (Figure 1). Kolpa River is a main watercourse of the region. The main tributary to Kolpa River is Lahinja River. The Krupa River joins the Lahinja River after only 2.5 km of waterflow. At these three rivers five sampling sites were selected (Table 1). One sampling site (KrKr) was situated in the headwater section of Krupa River, approximately 100 m from the spring. Three sampling sites (LaML, LaBu and LaPr) were located along the course of the Lahinja River, in the upper, middle and lower sections. The last sampling site (KoKr) was selected in the middle section of the Kolpa River.

### *Mayfly and stonefly larvae*

Samples were collected from January 2005 to December 2005 in approximate monthly intervals except in August in Kolpa River and in April and July in all three rivers due to high water levels. Altogether, 49 quantitative benthic samples were collected at the selected sites. Samples were taken using multihabitat sampling methods. Mayfly and stonefly larvae were identified using taxonomic keys in Aubert (1959), Kis (1974), Raušer (1980), and Bauernfeind and Humpesch (2001). For the identification of stoneflies, we also consulted a reference collection of the Slovenian Museum of Natural History, Ljubljana.

### *Environmental variables*

For each sampling site two groups of environmental variables were recorded. First group variables e.g. altitude, distance to source, stream order,

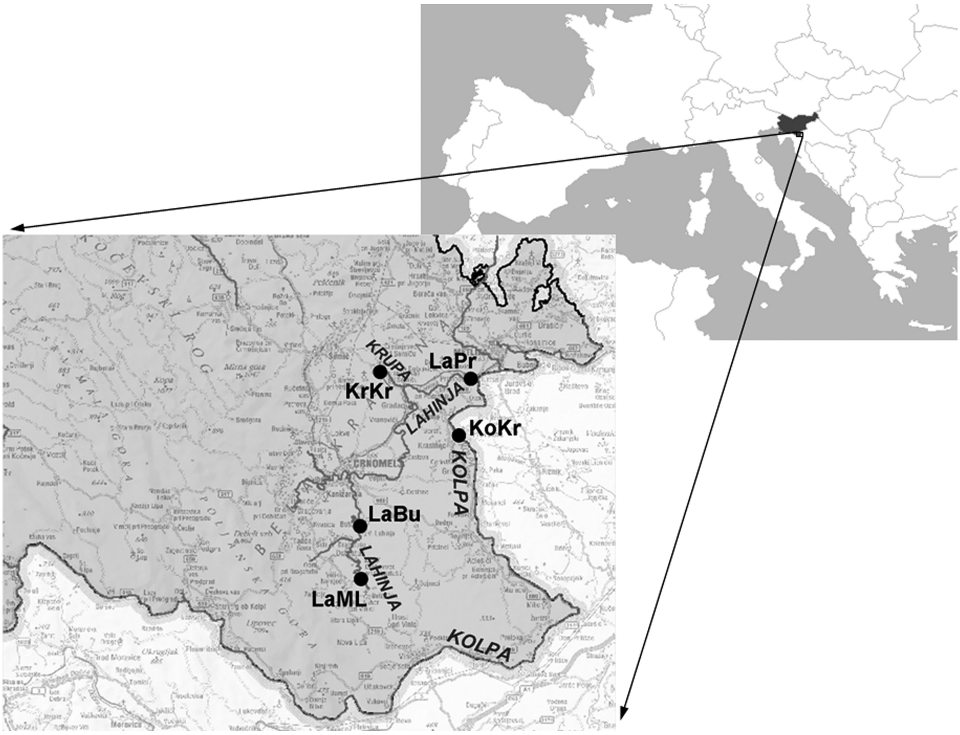

Figure 1. Map of research area with five selected sampling sites in Bela krajina, SE Slovenia.

Table 1. Main characteristics of the sampling sites.

| River<br>Location<br>Sampling site code | Krupa<br>Krupa<br>KrKr | Kolpa<br>Krasinec<br>KoKr | Lahinja<br>Mala Lahinja<br>LaML | Lahinja<br>Butoraj<br>LaBu | Lahinja<br>Primostek<br>LaPr |
|---|---|---|---|---|---|
| Altitude (m) | 139 | 138 | 148 | 143 | 131 |
| Gauss-Krüger Y | 5517304 | 5522502 | 5516212 | 5516392 | 5523705 |
| Gauss-Krüger X | 5054461 | 5050181 | 5040354 | 5044158 | 5053864 |
| Stream order (Strahler) | 1 | 5 | 1 | 3 | 4 |
| Slope (‰) | 1 | 1.10 | 1.29 | 0.58 | 1.23 |
| Distance to karst source (km) | 1.05 | 101.80 | 0.97 | 8.69 | 34.57 |
| Stream width (m) | 20.2 | 90.0 | 4.5 | 14.0 | 40.6 |
| Predominant substratum | Mesolithal | Microlithal | Argyllal | Mesolithal | Mesolithal |

Gauss–Krüger coordinates and slope were determined using Slovenian Environment Agency's Interactive Nature Protection Atlas. Besides these, predominant substratum was recorded only once. On the other hand, 10 variables (Table 2) were recorded each time on the same dates as mayfly and stonefly larvae were sampled.

Table 2. Mean and range (minimum–maximum) of environmental variables used in CCA.

| River | | Krupa | Kolpa | Lahinja | Lahinja | Lahinja |
|---|---|---|---|---|---|---|
| Location | | Krupa | Krasinec | Mala Lahinja | Butoraj | Primostek |
| Environmental variable | Code | KrKr | KoKr | LaML | LaBu | LaPr |
| Water depth (m) | Depth | 1.20 (1.10–1.30) | 0.36 (0.09–1.04) | 0.61 (0.50–0.74) | 0.29 (0.20–0.48) | 0.44 (0.28–0.72) |
| Water temperature (°C) | Temperature | 10.8 (10.3–11.1) | 9.4 (0.1–22.5) | 10.4 (8.8–11.8) | 9.5 (2.1–15.9) | 10.2 (3.9–14.9) |
| Dissolved oxygen (mg/L) | Dissolved $O_2$ | 10.1 (9.4–11.1) | 11.7 (7.5–14.9) | 10.6 (9.5–11.8) | 10.4 (7.5–13.2) | 11.1 (9.2–13.2) |
| Oxygen saturation (%) | $O_2$ saturation | 92 (85–101) | 101 (88–110) | 96 (88–106) | 91 (76–109) | 99 (82–117) |
| Conductivity ($\mu$S/cm) | Conductivity | 423 (390–446) | 308 (214–347) | 387 (338–410) | 391 (352–426) | 414 (380–442) |
| pH | pH | 7.7 (7.3–8.1) | 8.2 (7.2–8.8) | 7.9 (7.5–8.4) | 8.1 (7.6–8.5) | 8.1 (7.5–8.4) |
| Nitrate ($NO^{3-}$) (mg/L) | $NO_3$ | 4.4 (1.4–5.5) | 2.3 (0.3–2.9) | 4.3 (3.0–6.5) | 4.3 (2.6–6.0) | 4.0 (2.2–5.2) |
| Current velocity (m/s) | Velocity | 0.39 (0.14–0.73) | 0.58 (0.22–1.20) | 0.52 (0.14–0.98) | 0.24 (0.01–0.58) | 0.59 (0.21–1.06) |
| Total suspended solids (mg/L) | TSS | 36.0 (0.1–86.0) | 31.0 (0.7–61.0) | 49.4 (0.3–107.0) | 51.4 (1.3–97.0) | 56.0 (0.2–106.0) |
| Total solids (mg/L) | TSS + TDS | 276 (101–498) | 207 (51–419) | 259 (91–537) | 266 (99–498) | 278 (104–510) |

### *Data analysis*

Taxa richness and abundance were calculated to assess differences between mayfly and stonefly larvae communities of sampling sites. In addition, Shannon–Wiener, Simpson, Margalef and Evenness indices were used to compare species diversity and richness of sampling sites.

To compare weather differences in distance to source, the Index of biocenotic region (IBR) was calculated using Asterix 3.01. Differences in calculated metrics among sampling sites were compared using one-way ANOVA (analysis of variance) with a post-hoc test to compare all sampling site pair combinations using SPSS 13.0.

Besides the comparison of sampling sites, differences in the community composition among samples were analysed by non-metric multidimensional scaling (NMS) using a Bray–Curtis similarity measure and log transformation of abundance data option was selected in the program WinKyst 1.0 (Šmilauer 2003). In addition, the relationship between selected environmental variables and mayfly and stonefly taxa distribution was quantified by Canonical Correspondence Analysis (CCA) using the CANOCO 4.0 software (Ter Braak and Šmilauer 1998). Ten variables only recorded at all benthos-sampling occasions were used (Table 2). For the analyses, the matrices of the biotic and environmental variables were log transformed. To assess the statistical significance of the relation between species and measured variables, the Monte Carlo permutation test (999 permutations) was done.

## Results

### *Mayfly and stonefly larval community composition*

In total, 26 taxa of mayfly (20 species) and 17 taxa (13 species) of stonefly larvae were identified. The frequency occurrence and the cumulative number of taxa are given in Table 3. The highest taxa richness (27) was observed at sampling site LaBu, where also the highest number of mayfly taxa (19) was recorded. On the other hand, stoneflies were represented in highest number in the Kolpa River (10 taxa), which was the second most taxa rich (24 taxa) site. At the source sampling sites KrKr and LaML the least number of species was recorded; 17 and 16 taxa were found, respectively.

Eight species were recorded at all sites, but only *Baetis rhodani* and *Ephemerella ignita* were common as a frequency higher than 0.5 was recorded at all sites. Six species were less common with at least at one sampling site frequency lower than 0.5. *Centroptilum luteolum* and *Ephemera danica* were uncommon at site KoKr, *Paraleptophlebia submarginata* at site LaPr, *Habrophlebia fusca* at both latter sites, whereas *Nemoura cinerea* and *Brachyptera tristis* were uncommon at more than one sampling site. In addition, 16 rare species were recorded only at one site. However, at sites where they occurred they could be abundant. *Baetis fuscatus, Heptagenia sulphurea*, and *Rhithrogena* sp. were common at site KoKr, *Leuctra fusca* at site LaPr, *Isoperla inermis* at site KrKr and *Isoperla lugens* at site LaBu. Six taxa were found only in one sample. These taxa were *Ephemerella notata*, *Leuctra nigra*, *Brachyptera risi*, *Perlodes* sp., *Siphonoperla* sp., and *Baetis* sp.-juv. All other 19 taxa were recorded at two to four sampling sites and only *Electrogena* sp. and *Nemurella pictetii* were common at all sites where they occurred.

There was no statistical significant difference in abundance among sampling sites (one-way ANOVA, $F = 1.52$, $P > 0.05$, Table 4). Mean values below

Table 3. The list of 43 taxa of mayfly and stonefly larvae, the number of taxa and the occurrence frequency (n/N) of taxa at five sampling sites ($n$ = number of dates on which the taxa occurred, $N$ = number of dates samples). See Table 1 for sampling site codes.

| Taxon name | Taxon code | Sampling site<br>KrKr ($N = 10$) | <br>KoKr ($N = 9$) | <br>LaML ($N = 10$) | <br>LaBu ($N = 10$) | <br>LaPr ($N = 10$) |
|---|---|---|---|---|---|---|
| **Ephemeroptera** | | | | **n/N** | | |
| *Baetis fuscatus* | Bae_fus | | 0.8 | | | |
| *Baetis scambus/fuscatus* | Bae_f_s | | | | 0.3 | 0.4 |
| *Baetis liebenauae* | Bae_lib | | | | 0.4 | |
| *Baetis lutheri* | Bae_lut | | 1 | 0.1 | 0.3 | 0.3 |
| *Baetis niger* | Bae_nig | | | 1 | 0.7 | |
| *Baetis rhodani* | Bae_rho | 0.8 | 1 | 1 | 0.9 | 1 |
| *Baetis* sp.-juv. | Bae_spp | 0.1 | | | | |
| *Baetis vernus* | Bae_ver | | | 0.6 | 0.9 | 0.1 |
| *Centroptilum luteolum* | Cen_lut | 0.9 | 0.3 | 0.8 | 1 | 0.9 |
| *Procloeon pennulatum* | Cen_spp | | | | 0.4 | 0.1 |
| *Caenis luctuosa* | Cae_luc | | | 0.1 | 0.5 | 0.7 |
| *Caenis rivulorum* | Cae_riv | | | | 0.5 | 0.3 |
| *Ephemerella ignita* | Epm_ign | 0.8 | 0.8 | 0.6 | 0.8 | 1 |
| *Ephemerella notata* | Eph_not | | 0.1 | | | |
| *Ephemerella major* | Epm_maj | 0.2 | 0.2 | | | |
| *Ephemera danica* | Eph_dan | 0.7 | 0.4 | 1 | 0.6 | 0.8 |
| *Ephemera vulgata* | Eph_vul | | | | 0.4 | 0.1 |
| *Ecdyonurus* sp. *helveticus* group | Ecd_hel | | | | 0.2 | 0.4 |
| *Ecdyonurus* sp. *venosus* group | Ecd_ven | 1 | 1 | | | |
| *Electrogena* sp. | Ele_spp | 0.9 | | 1 | 1 | 0.6 |
| *Heptagenia sulphurea* | Hep_sul | | 0.7 | | | |
| *Rhithrogena* sp. | Rhi_spp | | 0.9 | | | |
| *Habrophlebia fusca* | Hab_fus | 0.7 | 0.1 | 0.9 | 0.5 | 0.2 |
| *Habrophlebia lauta* | Hab_lau | | 0.1 | | 0.1 | |
| *Paraleptophlebia submarginata* | Pal_sub | 1 | 1 | 1 | 0.8 | 0.3 |
| *Siphlonurus aestivalis* | Sip_aes | | | | 0.3 | |
| **Plecoptera** | | | | **n/N** | | |
| *Siphonoperla* sp. | Siph_spp | | 0.1 | | | |
| *Capnia bifrons* | Cap_bif | | 0.1 | | 0.2 | |
| *Leuctra albida/fusca* | Leu_a_f | 1 | 0.7 | 0.1 | 0.4 | |
| *Leuctra fusca* | Leu_fus | | | | | 0.6 |
| *Leuctra nigra* | Leu_nig | | 0.1 | | | |
| *Leuctra prima* | Leu_pri | | | | 0.2 | |
| *Nemoura avicularis* | Nem_avi | 0.2 | | | | |
| *Nemoura cinerea* | Nem_cin | 0.4 | 0.1 | 0.8 | 0.5 | 0.7 |
| *Nemurella pictetii* | Nemu_pic | 0.7 | | 0.8 | | |
| *Isoperla inermis* | Iso_ine | 1 | | | | |
| *Isoperla lugens* | Iso_lug | | | | 0.5 | |
| *Perlodes* sp. | Per_spp | | 0.1 | | | |
| *Brachyptera tristis* | Bra_tri | 0.4 | 0.6 | 0.1 | 0.2 | 0.1 |
| *Brachyptera risi* | Bra_ris | 0.1 | | | | |
| *Taeniopteryx nebulosa* | Tae_neb | | 0.2 | | 0.4 | 0.1 |
| *Taeniopteryx schoenemundi* | Tae_sch | | 0.6 | | 0.1 | 0.1 |
| *Taeniopteryx* sp.-juv. | Tae_spp | | 0.1 | 0.2 | | |
| **Number of taxa** | | **17** | **24** | **16** | **27** | **20** |
| **Number of mayfly taxa** | | **10** | **14** | **11** | **19** | **15** |
| **Number of stonefly taxa** | | **7** | **10** | **5** | **8** | **5** |

500 specimens/m$^2$ were recorded at all sampling sites, but at site LaML, a higher mean abundance was observed. High observed abundance values at the latter site were mainly due to high abundance of *Baetis rhodani*. On the other hand, at all sites some extreme values occurred (Figure 2), this indicates during the year abundance might vary greatly according to sampling date.

Differences among sampling sites were statistically significant for all diversity and richness metrics (one-way ANOVA, $F = 2.72–7.56$, $P < 0.05$, Table 4) but evenness with $P > 0.05$. However, for most metrics there was quite high annual variation in calculated values (Figures 2 and 3). The post-hoc LSD tests revealed that in many cases only one or two sampling sites were statistically different ($P < 0.05$) from other sites. The highest number of taxa was recorded at site KoKr in the Kolpa River, where also the highest number of rare species were found.

There was no significant difference among sites (one-way ANOVA, $F = 2.17$, $P > 0.05$, Table 4) in value of the Index of biocenotic region. However, the $P$-value is very close to the statistically significant boundary used ($P < 0.05$) (Table 4) and in the upper section of Lahinja River, IBR values were statistically ($P < 0.05$) lower compared to the downstream sections.

Table 4. One-way ANOVA ($N$ = number of data, $F = F$ value and $P$ = statistical significance) results for abundance, diversity metrics of mayfly and stonefly larvae.

| Metric | $N$ | $F$ | $P$ |
|---|---|---|---|
| Abundance (ind m$^{-2}$) | 48 | 1.52 | 0.212 |
| Number of Ephemeroptera and Plecoptera taxa | 48 | 5.68 | 0.001 |
| Number of Ephemeroptera taxa | 48 | 5.06 | 0.002 |
| Number of Plecoptera taxa | 48 | 5.40 | 0.001 |
| Shannon-Wiener Diversity | 48 | 3.64 | 0.012 |
| Simpson Diversity | 48 | 2.72 | 0.041 |
| Margalef Diversity | 48 | 7.56 | 0.000 |
| Evenness | 48 | 1.71 | 0.164 |
| IBR – Index of Biocoenotic region | 48 | 2.17 | 0.088 |

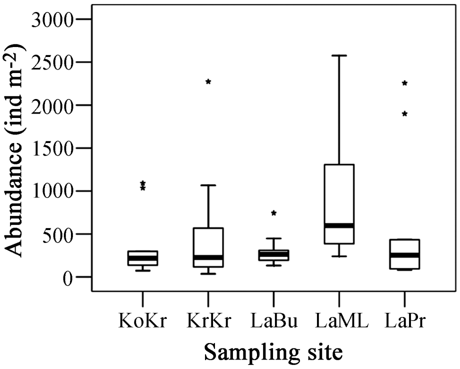

Figure 2. Box-plot of abundance of mayfly and stonefly larvae for five sampling sites. See Table 1 for sampling site codes.

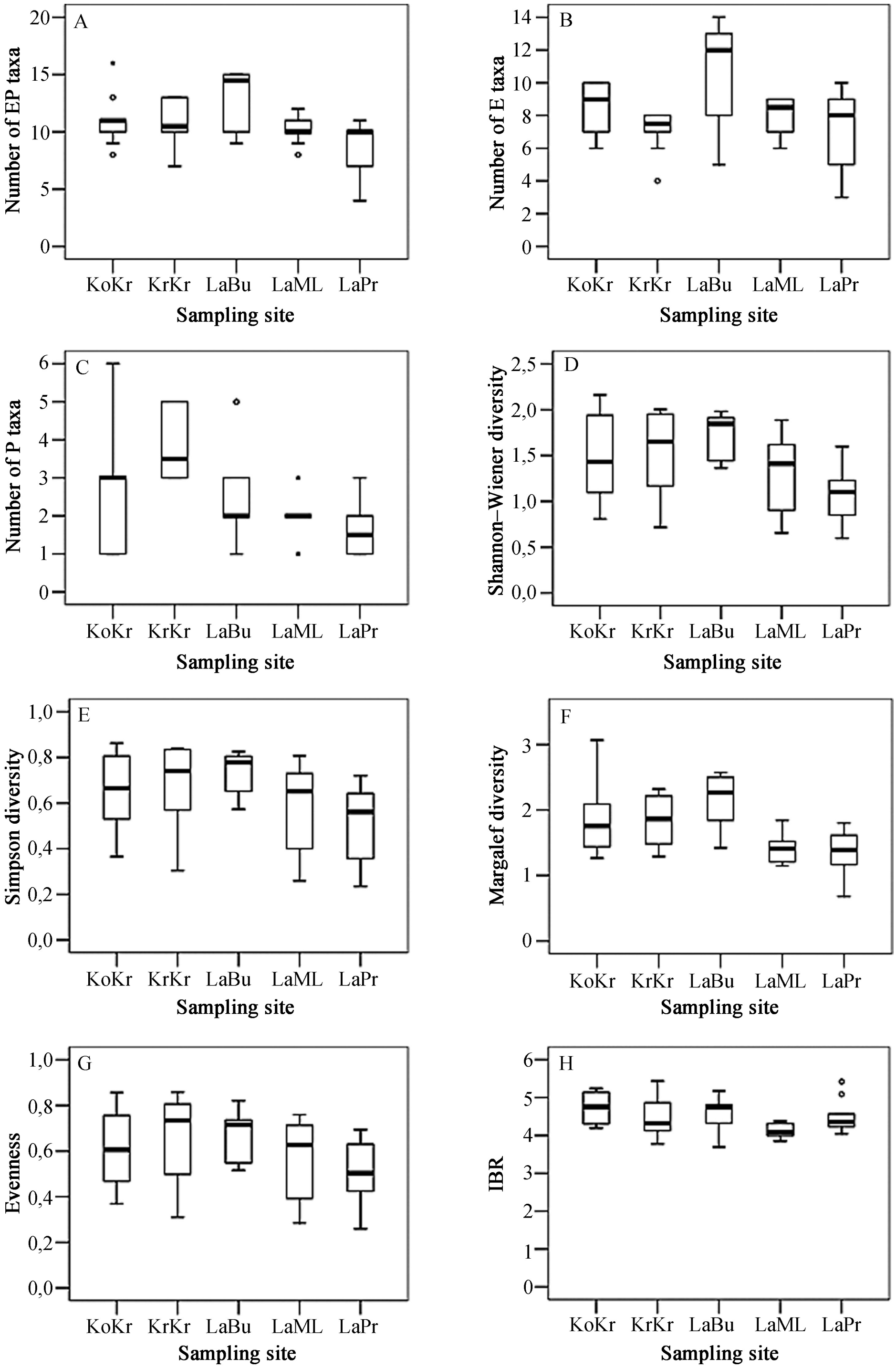

Figure 3. Box-plots of (A) number of mayfly and stonefly taxa, (B) number of mayfly taxa, (C) number of stonefly taxa, (D) Shannon–Wiener diversity index, (E) Simpson diversity index, (F) Margalef diversity index, (G) Evenness and (H) Index of Biocenotic Region for five sampling sites. For site codes see Table 1.

### *Mayfly and stonefly community composition in relation to environmental variables*

NMS showed spatial and seasonal differences amongst assemblages of sampling sites (Figure 4). In general, spatial differences in the community composition were greater than the seasonal ones, although in some cases seasonal fluctuations exceed spatial ones. The most similar assemblages were between sites LaML in upper and LaBu in the middle section of the Lahinja River, whereas assemblage site KoKr was the most distinct. NMS also showed a significant difference between species assemblages within the community at upstream sites KrKr and LaML. Site LaML was less distinct from sampling sites LaBu and LaPr at lower sections of Lahinja River than from site KrKr.

The relationships between mayfly and stonefly assemblage structure and selected environmental variables were investigated using canonical correspondence analysis (CCA). At first, marginal and conditional effects were calculated (Table 5). The highest share of marginal and conditional effects was explained by conductivity. This variable was shown to be the best predictor of the distribution of mayfly and stonefly larvae, followed by water depth and dissolved oxygen.

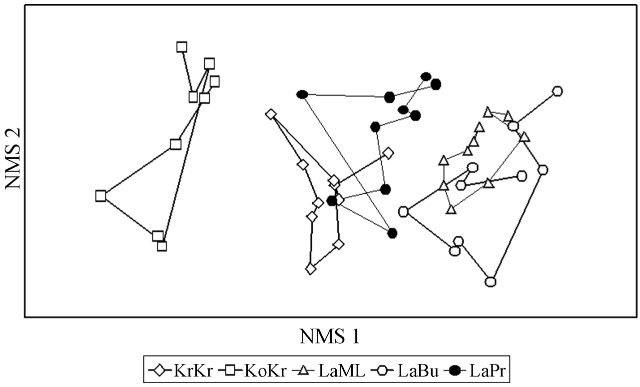

Figure 4. NMS ordination diagram of 49 samples with mayfly and stonefly taxa data. Stress = 0.13.

Table 5. Marginal and conditional effects of the environmental variables, their Lambda score and *P*-value. See Table 2 for variable codes.

| Marginal effect | Lambda 1 | Conditional effect | Lambda A | *P* |
|---|---|---|---|---|
| Conductivity ($\mu$S/cm) | 0.19 | Conductivity ($\mu$S/cm) | 0.19 | 0.001 |
| Water depth (m) | 0.16 | Water depth (m) | 0.13 | 0.001 |
| Dissolved $O_2$ (mg/L) | 0.14 | Dissolved $O_2$ (mg/L) | 0.11 | 0.001 |
| $NO^{-3}$ (mg/L) | 0.12 | $O_2$ saturation (%) | 0.08 | 0.006 |
| Temperature (°C) | 0.12 | Current velocity (m/s) | 0.05 | 0.055 |
| $O_2$ saturation (%) | 0.08 | Temperature (°C) | 0.05 | 0.097 |
| pH | 0.07 | $NO^{-3}$ (mg/L) | 0.05 | 0.186 |
| Current velocity (m/s) | 0.07 | pH | 0.03 | 0.353 |
| Total solids (mg/L) | 0.04 | TSS (mg/L) | 0.02 | 0.632 |
| TSS (mg/L) | 0.02 | Total solids (mg/L) | 0.02 | 0.771 |

CCA showed that the investigated environmental variables could explain up to 73.5% of the total variability of the taxa composition (Table 6). Most of the relationship between taxa and environmental variables was explained by the first ordination axis. The first and second axis together explained 52.5% of the variance in the taxa composition. Therefore, the first two axes were used for illustration of the results. A Monte Carlo permutation test confirmed the statistical significance ($P = 0.001$) of all canonical axes.

The results of the CCA are presented in Figure 5. In the CCA biplot, axes represent the most important environmental gradients along which the fauna was distributed. The first ordination axis reflected an eutrophication gradient mostly related to the conductivity, nitrates and dissolved oxygen concentration. The conductivity and nitrates increase from the positive to the negative end of the axis, whereas dissolved oxygen decreases. The second ordination axis reflected water depth gradient which indicated that the water depth had the next largest effect on the distribution of mayfly and stonefly larvae. Water depth increases from the negative to the positive end of axis 2.

Table 6. Summary of the CCA for 49 samples.

| | Ordination axes | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Total variance |
| Eigenvalues | 0.234 | 0.152 | 0.123 | 0.090 | 1.923 |
| Species–environment correlations | 0.862 | 0.761 | 0.764 | 0.761 | |
| Cumulative percentage variance | | | | | |
| of species data | 12.2 | 20.1 | 26.4 | 31.1 | |
| of species–environment relation | 31.9 | 52.5 | 69.2 | 81.5 | |
| Sum of all eigenvalues | | | | | 1.923 |
| Sum of all canonical eigenvalues | | | | | 0.735 |

Notes: Monte Carlo permutation test. Test of significance of first canonical axis: eigenvalue = 0.234 (*F*-ratio = 5.267, *P*-value = 0.0010). Test of significance of all canonical axes: trace = 0.735 (*F*-ratio = 2.351, *P*-value = 0.0010).

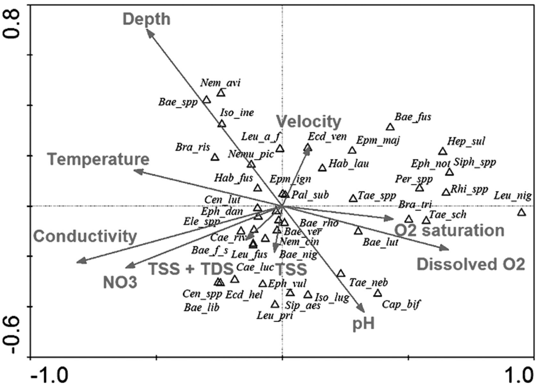

Figure 5. CCA ordination diagram with taxon scores (triangles) and selected environmental variables (arrows). See Table 3 for taxa codes and Table 2 for environmental variable codes.

On the ordination diagram (Figure 5) four ecological groups of mayfly and stonefly taxa were recognised. Species in group 1 (*Ecdyonurus* sp. *helveticus* group), *Baetis liebenauae*, *Procloeon pennulatum*) were associated with high conductivity, temperature and $NO^{3-}$ concentration and low dissolved oxygen concentration. On the other hand, species in group 2 (*Heptagenia sulphurea*, *Siphonoperla* sp., *Ephemerella notata*, *Rhithrogena* sp., *Perlodes* sp.) were in negative correlation with those environmental variables. Group 3 was characterised by species that had affinity to deep water (*Nemoura avicularis*, *Isoperla inermis*, *Baetis* sp.-juv.). Species included in group 4 (*Capnia bifrons*, *Taeniopteryx nebulosa*, *Isoperla lugens*, *Siphlonurus aestivalis*, *Leuctra prima*, *Ephemera vulgata*) were found in shallow and well oxygenated water with high pH.

## Discussion

### *Diversity and distribution*

The mayfly and stonefly fauna of the karst rivers Krupa, Kolpa and Lahinja are relatively diverse with few common and many rare species, which is in accordance with a species-abundance distribution theory (for overview see McGill et al. 2007). Common species found at all sites in high frequency were *Baetis rhodani* and *Ephemerella ignita*. Both species are known as ubiquitous, very tolerant and widely distributed in Central Europe (Derka 2003; Soldan, Zahrádková, Helešic, Dušek and Landa 1998). Other common species occurred infrequently at least at one site. The frequency of *Centroptilum luteolum* and *Ephemera danica* was low in Kolpa River. Both species prefer rhithral conditions but can also be found in the potamal zone (Moog 1995). Sampled sections of the Kolpa River have a potamal character and therefore both species can be uncommon. On the other hand, both species were also found in the crenal zone in high frequency. *Habrophlebia fusca* was rarely found at downstream sites, which is in accordance with known ecological distribution for a species which prefers crenal and rhithral zones (Moog 1995). Both common stonefly species, *Nemoura cinerea* and *Brachyptera tristis*, are rare at many sites, but are common in karst rivers (Sivec 1996). Some species were rare and restricted to a sampling site because of specific habitat requirements. *Isoperla inermis* is a characteristic species of karst springs. In Slovenia, it is reported only in Krupa River (Sivec 2001). *Ecdyonurus venosus* group and *Rhithrogena* sp. are said to be typical rhithral taxa, while *Heptagenia sulphurea* and *Baetis fuscatus* are reportedly typical potamal species (Moog 1995; Derka 2003). However, in our study all those species showed similar habitat preferences in Kolpa River, and a tendency of *Baetis fuscatus* and *Heptagenia sulphurea* to inhabit large lowland rivers was evident. Despite observed differences in the distribution of some taxa, there was, among sampling sites, no statistically significant difference ($P > 0.05$) in the mean value of the Index of Biocoenotic region, although sites represent a gradient from spring to over 100 km in the distance to source (Table 1). The Index of biocenotic region was not statistically significantly different among sampling sites, which suggests that the spatial succession of the community was not expressed.

The mayfly community reached the highest species diversity in the middle section of Lahinja River, whereas the stonefly community did so at the Kolpa River. Also, at both sites, the highest total diversity was recorded, but in the Kolpa River a greater number of rare taxa was found. At the latter site, higher variation in water temperature and water depth was observed, which might influence the presence of rare taxa as less stable conditions which were present throughout the year.

The lowest number of species was recorded at spring sites, which is in accordance with a River continuum concept (Vannote et al. 1980). According to the continuum model, biodiversity in headwaters is limited mainly by low thermal heterogeneity, low light and low nutrients. In our spring sites low variation of listed ecological factors was observed mainly in thermal heterogeneity. Moreover, all diversity and richness indices were significantly different among sampling sites (Table 4), although the pattern is not in accordance with the ideal biodiversity pattern exhibited by mayflies and stoneflies (Ward 1998). At spring sites higher values were observed compared to downstream sites (Figure 3). Nevertheless, in our study other types of streams with almost no altitudinal gradient are considered than in the Ward (1986) study and this might influence the results.

### *Relation between mayfly and stonefly community composition and environmental variables*

In general, spatial between-site differences in community composition were greater than the seasonal ones, but also temporal variability was substantial. This was evident especially at most downstream sites where disturbance was greater and in accordance with nonequilibrium theories of community structure (Conell 1978). Along the NMS axes, no upstream–downstream gradient was observed, although the Kolpa River was clearly divided from other sampling sites. However, the dissimilarities between most upstream sampling sites KrKr and LaML were greater than between sampling sites in the upper section (KrKr) and in the lower section (LaPr) or between the sampling sites in the same river (LaML, LaBu, LaPr). That no upstream–downstream gradient is present is also evident from comparison of the IBR values where no significant difference was observed among sampling sites (Table 4). As most environmental variables at spring sites KrKr and LaML were comparable, the main factor affecting mayfly and stonefly larval communities was probably mineral substrate composition as this was the only observed difference. At sampling site LaPr the predominant substratum was argyllal while at sampling site KrKr it was mesolithal. That substratum is an important ecological factor affecting benthos communities has been stressed in several papers (e.g. Minshall 1984). Moreover, Urbanič and Toman (2007) found that argyllal is a key factor affecting the distribution of caddisfly larvae in a Pannonian lowland ecoregion, but is less important in Dinaric western Balkan. However, from the latter ecoregion no sites with a high percentage of argyllal substratum were included in the analyses.

CCA of the mayfly and stonefly community composition indicated that, among all seasonally fluctuating factors, conductivity was the dominant factor influencing the mayfly and stonefly communities within the examined karst systems. The conductivity was shown to be the important factor influencing the distribution of mayfly and stonefly species within many riverine systems (Soldán et al. 1998; Krno 2003). Geological bedrock, flow size and eutrophication are mentioned to have a determining influence on its values (Krno 2003). Soldán et al. (1998) and Krno (2003) found the conductivity to be an important indicator of eutrophication. In our study, the conductivity, together with nitrate and dissolved oxygen concentration, reflected an eutrophication gradient, which represents a key factor in the distribution of mayfly and stonefly taxa.

Water depth was found to also be an important variable in determining the distribution of mayfly and stonefly larvae in our study. Water depth is an important environmental variable already known to influence community composition and distribution especially in relation to intermittency and also in karst spring systems (Meyer and Meyer 2000). In addition, some authors (Urbanič and Toman 2007) mention that an absence of riffles can heavily influence benthic community composition. Moreover, water depth was also reported to be among the most important environmental variables affecting mayfly distribution (Burian 1997). In our study water depths vary among sampling occasions at most sites (Table 1).

The spatial distribution of mayfly and stonefly species show differences in their habitat preferences, which is confirmed by the four ecological groups established in the CCA analysis (Figure 5). *Baetis liebenauae*, *Procloeon pennulatum* and *Ecdyonurus* sp. *helveticus* group included in the first group preferred habitats where high conductivity, temperature, nitrate concentration and low oxygen concentration occurred. Those species were recorded only in the middle and lower section of the Lahinja River (Table 3). However, in Europe the species of the *Ecdyonurus helveticus* group are typical spring or upper rhithral species (Derka 2003), whereas *Baetis liebenauae* prefers small lowland streams with slow flowing waters (Buffagni and Gomba 1996).

In the second group are taxa (*Heptagenia sulphurea*, *Siphonoperla* sp., *Ephemerella notata*, *Rhithrogena* sp., *Perlodes* sp.) that occurred in well-oxygenated and cold waters with low conductivity and nitrate concentration. Those species were found only in Kolpa River but mainly in the cold season (Table 3). *Ephemerella notata* is a hyporhithral or epipotamal species (Soldán et al. 1998). Also *Heptagenia sulphurea* is known as a typical species of large lowland rivers (Bauernfeind and Moog 2000; Derka 2003). Other taxa, e.g. *Rhithrogena* spp. are rhithral and inhabit the riffle section of streams and rivers, but may also emerge further downstream (Bauernfeind and Moog 2000). However, the diversity of species in *Rhithrogena* may be significantly reduced by eutrohication, increasing temperature and by a smaller variety of current/substratum or riffle/pools types (Bauernfeind and Moog 2000).

The third group of taxa (*Nemoura avicularis*, *Isoperla inermis*, *Baetis* sp.-juv.) showed an affinity for deep water. All those species were found in the spring section of Krupa River (Table 3) in a relatively stable environment with small seasonal variation of environmental factors (Table 2).

The last group of mayfly and stonefly taxa (*Capnia bifrons*, *Taeniopteryx nebulosa*, *Isoperla lugens*, *Siphlonurus aestivalis*, *Leuctra prima*, *Ephemera vulgata*) preferred shallow water and were positively correlated to dissolved oxygen concentration and pH. All these taxa showed an affinity to a crenal habitat (Table 3). Among these species *Ephemera vulgata* is a burrowing species found in still waters, but can also be found in the potamon (Moog 1995). Leaf barriers in riverine flood-ponds or dense vegetation in more stagnant waters are typical habitats of *Siphlonurus aestivalis* (Bauernfeind and Moog 2000), whereas *Capnia bifrons* and *Leuctra prima* are creophilous species with a high affinity to oxygenated cold waters and they are among the earliest stonefly species, hatching in winter (Sivec 1996; Krno 2003).

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