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MAYFLIES OF THE BEDNJA RIVER, CROATIA

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Here we present the results of a mayfly study conducted on a hydromorphologically altered lowland river in Croatia, the Bednja River. Twenty-two mayfly species were recorded, *Baetis fuscatus* (Linnaeus, 1761) and *Serratella ignita* (Poda, 1761) being the most widespread and *Paraleptophlebia submarginata* (Stephens, 1836) and *Cloeon dipterum* (Linnaeus, 1761) the rarest species. A significant increase in mayfly species richness was observed along the river course. Nevertheless, cluster analysis showed that their assemblages were not grouped according to the study position along the Bednja River. The lowest species richness and diversity were recorded for the spring area. Surprisingly, the highest species richness was found at a site with moderate hydromorphological alterations, and the highest diversity at a site with severe hydromorphological alterations. This could be because the river is connected to numerous lateral streams and a variety of microhabitats are available. New distribution records in Croatia are presented for some rare species in Croatian freshwater habitats, such as *Heptagenia longicauda* (Stephens, 1836). *Ephemera lineata* Eaton, 1870 was recorded for the first time in the Pannonian lowland ecoregion (ER 11) in Croatia. *Caenis* cf. *pseudorivulorum* Keffermüller, 1960 has not yet been recorded in Croatian freshwater habitats, but since our identifications are based on juvenile specimens, its occurrence is still to be confirmed.

Key words: Ephemeroptera, lowland river, human impact, species richness, rare species

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U radu predstavljamo rezultate istraživanja vodencvjetova na hidromorfološki izmijenjenoj nizinskoj rijeci Bednji u Hrvatskoj. Zabilježene su 22 vrste, pri čemu su *Baetis fuscatus* (Linnaeus, 1761) i *Serratella ignita* (Poda, 1761) bile najraširenije vrste, a *Paraleptophlebia submarginata* (Stephens, 1836) i *Cloeon dipterum* (Linnaeus, 1761) najrjeđe vrste. Duž riječnog toka zabilježen je značajan porast u broju vrsta vodencvjetova. No, klaster analiza je pokazala da njihove zajednice nisu grupirane s obzirom na položaj istraživanih lokacija duž rijeke Bednje. Najniži broj vrsta i raznolikost zabilježeni su za izvorišno područje. Iznenađujuće, najviši broj vrsta zabilježen je na lokaciji s umjerenim hidromorfološkim promjenama, a najveća raznolikost na lokaciji s velikim hidromorfološkim promjenama. Razlog bi mogao biti to što je Bednja povezana s brojnim bočnim pritokama te su u rijeci prisutna različita mikrostaništa. Prikazani su novi podaci o rasprostranjenosti nekih rijetkih vrsta u hrvatskim slatkovodnim staništima, kao što je *Heptagenia longicauda* (Stephens, 1836). *Ephemera lineata* Eaton, 1870 je zabilježena po prvi puta za Panonsku nizinsku ekoregiju (ER 11) u Hrvatskoj. *Caenis* cf. *pseudorivulorum* Keffermüller, 1960 još nije bila zabilježena za hrvatska slatkovodna staništa, no kako se naša determinacija temeljila na juvenilnim primjercima, njen nalaz još treba biti potvrđen.

Ključne riječi: Ephemeroptera, nizinska rijeka, utjecaj čovjeka, broj vrsta, rijetke vrste

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INTRODUCTION

Ephemeroptera is a hemimetabolous amphineutic insect order, whose nymphs inhabit a wide range of freshwater habitats, while adults complete their life cycle in a terrestrial environment (Bauernfeind & Soldán, 2012). Their diversity is highest in the upper reaches of fast flowing rivers and streams, and in the lower reaches of ecologically undisturbed lowland rivers, where they may also occur in high abundances (Bauernfeind & Moog, 2000; Williams, 1980). They are highly dependent on microhabitat composition and physico-chemical water properties such as water temperature, oxygen content, pH, nutrient concentration, and water velocity (e.g., Vilenica *et al*., 2017; 2018). Because mayfly species are sensitive to anthropogenic disturbance of their habitats, they are considered valuable indicators of freshwater health, and are widely used for water quality assessment and long-term monitoring (Buffagni *et al*., 2001; Moog & Hartmann, 2017).

Many European lowland rivers are exposed to various anthropogenic pressures. They are hydromorphologically altered, including the channelization of the river course and habitat degradation due to the construction of hydroelectric power plants (Lucić *et al*., 2015; Schwarz, 2012). Rivers are also exposed to various types of pollution of urban, pharmaceutical and agricultural origin (Previšić *et al*., 2020; Vilenica *et al*., 2020). As riverine habitats are heavily modified, their connectivity is disturbed, altering flow regimes, substrate composition and water quality as well as allowing the introduction of invasive species, which negatively impacts native river biota and modifies their communities (e.g., Vilenica *et al*., 2019; 2020).

Research on mayflies in Croatia started about a decade ago and resulted in the identification of a relatively high number of 85 mayfly species in the Croatian fauna (see in Vilenica *et al*., 2021). However, there are still gaps in our knowledge of the distribution and habitats of many species, especially in the Pannonian lowland ecoregion (ER 11; Illies, 1978) (Vilenica *et al*., 2021). With the aim of increasing our knowledge of the mayfly fauna of lowland rivers in Croatia, we studied the mayfly fauna of the Bednja River with the main objective of determining the mayfly species richness, diversity and abundance along the river course.

MATERIALS AND METHODS

Study area

The Bednja River is a mid-sized Pannonian lowland river (ER 11, Illies, 1978) with a length of 106 km (Čanjevac *et al.*, 2022) and a catchment area of about 600 km^2. It is the largest tributary of the Drava River in Croatia, situated in the northern part of the country (Fig. 1). The climate in its catchment area is temperate humid (type Cfb, Köppen climate classification). The average air temperature in the warmest month does not exceed 22°C and the average temperature in the coldest month does not fall below -3°C (Šegota & Filipčić, 2003). During the study period, in 2015, the average monthly air temperature in the study area was 11.6°C (± 7.6°C), while the average total annual precipitation was 897 mm (± 114.6 mm) (the data were obtained by personal calculation of raw data from the meteorological station Varaždin received upon request to the Croatian Meteorological and Hydrological Service). The discharge regime of the river is classified as peri-Pannonian pluvial-nival (Čanjevac, 2013).

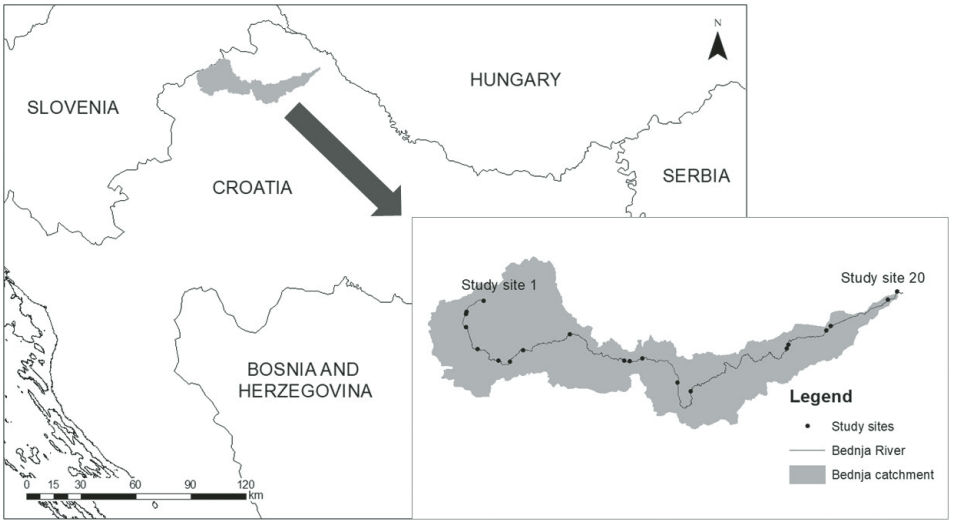

Fig. 1. Map of the study area with the Bednja River catchment and the location of the 20 study sites along its course.

Land use in the river catchment is near natural, urban or agricultural (extensive agriculture in the form of pasture and intensive agriculture in the form of complex cropping). At the time of this study, urban settlements along the river did not have wastewater treatment plants. The 20 study sites differ in terms of physico-chemical water parameters and morphological alterations (see Vidaković Maoduš *et al*., 2022; Vilenica *et al*., 2022). Study sites 1, 3, 4, 14, 16, 18 and 20 are in good morphological condition, while others are moderately (study sites 2, 8, 9, 10, 12, 13 and 15) or severely altered (5, 6, 7, 11, 17 and 19) (see also in Vilenica *et al*., 2022).

Sampling protocol and species identification

Aquatic macroinvertebrates, including mayflies, were sampled in summer 2015 (from June 30 to July 7) using a hand net (0.5 mm mesh size), according to the "multi-habitat method" (AQEM consortium, 2002). A total of 20 subsamples were collected at each site, covering approximately 1.25m^2 of stream bottom area. The subsamples corresponded to the dominant substrate types at each site (covering >5% of the sampling area). Substrates consisted mainly of fine sediment (sand, silt, mud), lithal (stones, gravel), xylal (dead plant material and woody debris) and aquatic vegetation (submerged and emergent).

All collected specimens were preserved in 96% ethanol and sorted in the laboratory. Mayflies were identified to the lowest possible taxonomic level (very juvenile and/or damaged individuals were identified only to genus or family level) using the relevant identification keys (Bauernfeind & Humpesch, 2010; Malzacher, 1984; Müller-Liebenau, 1969). The voucher specimens are deposited in the collection of the first author, at the Department of Biology, Faculty of Science, University of Zagreb, Croatia.

Data analyses

Shannon and Simpson diversity indices at study sites with respect to mayfly composition and abundance were determined using the PRIMER 6 software package (Clarke & Gorley, 2006).

The Kruskal–Wallis H test was used to determine differences in mayfly assemblages among the study sites, i.e., differences in species richness, diversity and abundance. Spearman's rank correlation coefficient (R) was used to determine differences in mayfly assemblages along the longitudinal course of the river, i.e., to analyse the correlation between mayfly species richness, diversity, and abundance and distance from the source of the river. These analyses were performed in Statistica, version 10.0 (Statsoft Inc., 2010).

To assess the similarity of mayfly assemblages among the study sites, a cluster analysis was performed based on Bray-Curtis similarity index. For cluster analysis, similarity profile analysis (SIMPROF) was performed to identify statistically significant evidence of genuine clusters of sites. These analyses were conducted using the PRIMER 6 software package (Clarke & Gorley, 2006).

RESULTS

In all, 22 mayfly species were recorded (Tab. 1). *Baetis fuscatus* (Linnaeus, 1761) and *Serratella ignita* (Poda, 1761) were the most common species, present at 18 study sites, and *Baetis fuscatus* was also the most abundant species (Table 1). *Paraleptophlebia submarginata* (Stephens, 1836) and *Cloeon dipterum* (Linnaeus, 1761) were the rarest species, present only at study site 17 (Tab. 1).

Significant differences were recorded in species richness (Kruskal-Wallis H test, H (19, N =7 1) = 36.59, p = 0.008) and abundance (H (19, N = 71) =3 6.23, p = 0.009) among study sites. The lowest number of mayfly species, four, was recorded at study site 1, while the highest species richness was recorded at study site 12 (13 species) (Tab. 1, Fig. 2). Study site 17 had the highest Shannon (1.86) and Simpson diversity (0.79) indices, while the lowest values of both indices were recorded for study site 1 (0.61 and 0.31, respectively) (Tab. 1). The highest mayfly abundance was recorded at study site 18 (4571 individuals/m^2), and the lowest at study site 4 (24 individuals/m^2) (Tab. 1, Fig. 2). In addition, mayfly species richness increased significantly with distance from the river source (Spearman's rank correlation coefficient, R = 0.566, p = 0.009).

In the cluster analysis, study sites mostly did not group according to their position along the river course (Fig. 3).

DISCUSSION

Our study revealed a rather high Bednja River mayfly diversity although a high number of study sites are under anthropogenic stress, i.e. influenced by hydromorphological alterations. As explained by Vilenica *et al*. (2022), more than a quarter of Croatian mayflies inhabit this river system, most likely due to its connectivity with numerous tributaries along the river course the considerable variety of available microhabitats. However, as the sampling protocol was not designed to collect mayflies specifically, the sampling period was probably too late for the species that emerge during the spring (Bauernfeind & Soldán, 2012). In order to obtain a more detailed

Tab. 1. Mayfly species (presented as number of individuals per m^2) recorded at 20 study sites along the Bednja River in Croatia.

Mayfly species/Study site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Baetis fuscatus (Linnaeus, 1761)		1		7	10	546	419	174	214	3	748	233	152	175	115	465	9	3324	352	1273
Baetis buceratus Eaton, 1870		153	43	6		12	30	151	132		679	1	3			65	31	918	119	255
Baetis rhodani (Pictet, 1843)	198	112	22		5	182	367	347	23		64	1	7	1		21		78		
Baetis vernus Curtis, 1834		157	234		60	29	67	116	10		25		7	6	22	70				70
Baetis liebenauae Keffermüller, 1974											36					6		27		
Baetis lutheri Müller-Liebenau, 1967											69	8								
Centroptilum luteolum Müller, 1776			10	7								25							1	
Cloeon dipterum (Linnaeus, 1761)																	6			
Procloeon bifidum (Bengtsson, 1912)					5					8		1		2	2	1	26	6		
Procloeon pennulatum Bengtsson, 1915			1											5					3	
Caenis luctuosa (Burmeister, 1839)					2	3	2	1		226	1	6			4	2	1	26	26	49
Caenis cf. *pseudorivulorum* Keffermüller, 1960												1		5	17		4		6	15
*Serratella ignit*a (Poda, 1761)		10	22		4	38	89	54	5	36	174	118	26	149	184	385	61	183	50	106
Ecdyonurus macani Thomas & Sowa, 1970			4			2					2									
Electrogena ujhelyii (Sowa, 1981)	66	16	94	1		2	3				2	2				2			1	
Heptagenia flava Rostock, 1878					10	10					2		10	14	7	10	7	3		9
*Heptagenia longicaud*a (Stephens, 1836)												1	8		5	6		3	2	
Habrophlebia lauta McLachlan, 1884	1	46	258	3		5	3	2		1					6				1	1
Paraleptophlebia submarginata (Stephens, 1836)																	10			
Ephemera danica Müller, 1764	1		2			1				1				8						
Ephemera lineata Eaton, 1870												2	2	2	2		1			2
Potamanthus luteus (Linnaeus, 1767)										1	1	6			1	1	8	3		2
Species richness	4	7	10	5	7	11	8	7	5	7	12	13	8	10	11	12	11	10	10	10
Abundance (ind/m^2)	266	495	688	24	96	830	980	845	384	278	1803	405	215	367	365	1034	164	4571	561	1782
Shannon index	0.61	1.49	1.52	1.46	1.29	1.07	1.29	1.47	1.01	0.64	1.37	1.18	1.09	1.18	1.35	1.31	1.86	0.84	1.11	1.00
Simpson index	0.31	0.74	0.72	0.78	0.59	0.52	0.66	0.74	0.57	0.38	0.67	0.58	0.48	0.61	0.64	0.65	0.79	0.43	0.55	0.46

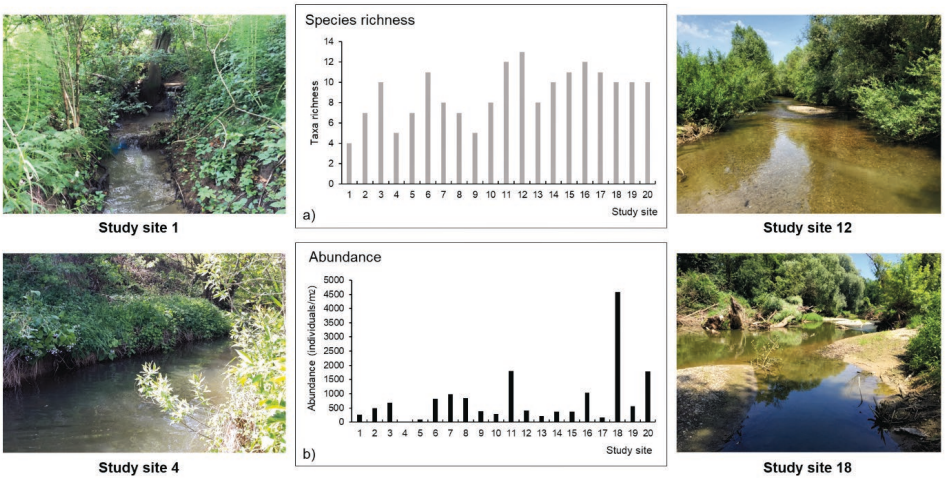

Fig. 2. Mayfly a) species richness and b) abundance at 20 study sites along the Bednja River, Croatia. Study site 1 has the lowest species richness and study site 12 the highest. The lowest abundance is found at study site 4 and the highest at study site 18.

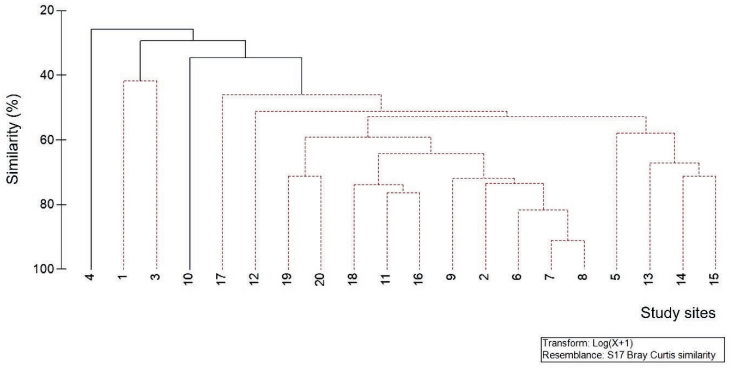

Fig. 3. Cluster analysis (using SIMPROF analysis) of mayfly assemblages at 20 study sites along the Bednja River, Croatia, based on Bray-Curtis Similarity.

mayfly species list, at least two more sampling events should be conducted, in spring and autumn. Although mayfly species richness increased along the river course, as expected (Bauernfeind & Soldán, 2012), it is possible that the study sites did not show any particular pattern in similarity of their mayfly assemblages as a function of site location along the river due to the interconnectedness with side streams and the different substrate compositions at the study sites. The lowest mayfly species richness and diversity was recorded near the river source which is not surprising since mayfly diversity tends to be low in such extreme habitats (Bauernfeind & Moog, 2000; Bauernfeind & Soldán, 2012; Vilenica *et al.*, 2017). Surprisingly, the highest species richness was recorded at one of the moderately altered study sites, study site 12. Despite the hydromorphological stress, this site still has several microhabitats (akal, psammal, microlithal and xylal) that provide sufficient habitat conditions for 13 mayfly species.

The most numerous among them were widespread species such as *Baetis fuscatus,* and *Serratella ignita* (e.g., Vilenica *et al*. 2015, 2021). Most of these 13 species were previously reported from various substrate types, such as *Baetis fuscatus, Baetis rhodani, Electrogena ujhelyii*, but some have specific preferences for e.g. fine substrates, such as *Heptagenia longicauda, Ephemera lineata, Potamanthus luteus* (AQEM consortium, 2002; Moog *et al*., 1999; Schmedtje & Colling, 1996; Schmidt-Kloiber & Hering, 2015). Since some of the species were present in extremely low abundances (i.e., one individual per m^2), such as *Procloeon bifidum, Heptagenia longicauda, Caenis* cf. *pseudorivulorum*, it is possible that their occurrence at this study site was accidental and that they drifted from one of the tributaries in the vicinity or from more natural upstream sites (Sertić Perić *et al*., 2018, see also Vilenica *et al*., 2022). Another reason could be the assessment method on the basis of which hydromorphological alteration was evaluated in this study. The study site was previously channelized, and the altered morphology was the main contributor to the site's classification in the moderately altered group. Although the site has since been left to succession (which is particularly evident from the age of the riparian willows), it is still considered morphologically modified according to the European standard EN 15843:2010. Another unexpected result was the highest mayfly diversity occurring at the highly hydromorphologically altered site 17, which is most likely related to the more balanced abundance of the mayfly species collected, as the total number of mayfly individuals was quite low and most of the species collected at this site were of low abundance. Their occurrence at this site should be re-evaluated in future studies.

The highest abundance of mayflies was recorded at study site 18, which is in good hydromorphological condition and characterized by a high diversity of microhabitats and flows. This suggests that although hydromorphologically altered sites may support relatively high numbers of mayfly species, their populations are much better established at sites that are in a natural or near natural condition. However, these results may also imply that mayfly bioindicative traits (e.g., Sartori & Brittain, 2015) should be further investigated with a focus on hydromorphological degradation. Surprisingly, only five species, mainly widespread (Vilenica *et al*., 2015; 2021), were detected in the lowest abundances at one of the most morphologically natural sites, study site 4. However, this study site is located immediately downstream of the outlet of Trakošćan Lake and a hotel without wastewater treatment, which could affect the site. In addition, a considerable amount of silt was observed in the sediment during sampling, which may result in a loss of macroinvertebrate diversity by reducing the available interstitial habitat and available periphyton food supply (Jowett, 2003). This situation should be investigated further in future studies.

Species common in Croatian lotic habitats (e.g., Vilenica *et al*., 2015; 2021), rheo to limnophil *Baetis fuscatus* and *Serratella ignita* (Poda, 1761) (Buffagni *et al*., 2022; Schmidt-Kloiber & Hering, 2015), were the most frequently recorded species along the Bednja River, recorded at almost all study sites. On the other hand, rheo to limnophil *Paraleptophlebia submarginata* (Stephens, 1836) and limnophil *Cloeon dipterum* (Linnaeus, 1761) (Buffagni *et al*., 2022; Schmidt-Kloiber & Hering, 2015) were the rarest species, recorded only at the heavily altered study site 17. The lotic *Paraleptophlebia submarginata* (Buffagni *et al*., 2022; Schmidt-Kloiber & Hering, 2015) has already been recorded from anthropogenically impacted habitats (e.g., Elbrecht *et al*., 2016; Vilenica *et al*. 2019), while the eurytopic and euryvalent *Cloeon dipterum* is known to be tole-

rant to various extreme habitat conditions (e.g., Bauernfeind & Soldán, 2012; Buffagni *et al*., 2022; Schmidt-Kloiber & Hering, 2015; Vilenica *et al*., 2016). Some species recorded in the Bednja River are considered rare in the Pannonian lowland ecoregion (ER 11) in Europe (based on spatial and temporal scales: species distribution range, historical records and current number of records in this ecoregion) (Buffagni *et al*., 2022; Schmidt-Kloiber & Hering, 2015): *Baetis liebenauae, Ephemera lineata, Caenis luctuosa, Caenis* cf. *pseudorivulorum* and *Heptagenia longicauda*. Of these species, several can be considered rare in Croatian freshwater habitats. *Heptagenia longicauda* has been recorded in only two rivers, the Bukovska Dobra River (ER 5) (Vilenica *et al*., 2015) and the Mura River (ER 11) (Dekić *et al*., 2016). Although it is not considered rare in Croatia, *Ephemera lineata* has so far been recorded only in streams, rivers and lakes in the Dinaric Western Balkan Ecoregion (ER 5) in Croatia (Vilenica *et al*., 2015; 2019). To date, *Caenis pseudorivulorum* has not been recorded in Croatian freshwater habitats (Vilenica *et al*., 2021). However, since our identifications are based on juvenile individuals, it is necessary to collect mature nymphs and confirm whether this species is new to the Croatian fauna. Alternatively, the identification of such young instars could be confirmed with DNA barcoding.

In conclusion, to protect European riverine habitats adequately, it is essential to have good knowledge of the species that inhabit them. Our study revealed new data on mayfly assemblages and species habitat preferences in anthropogenically impacted lowland rivers. Some of the distributional evidence for rare species in Croatian freshwater habitats can be considered new. We recommend those results be used in future assessment of mayfly species conservation status and protection of lowland rivers in Croatia. In order to obtain the true assemblage composition of the studied river system, we suggest extending the study period to all four seasons (i.e. spring, summer, autumn, winter). Moreover, the Bednja River tributaries should be also included in future studies.

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