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DINARIC KARST INTERMITTENT RIVERS HARBOUR SOME RARE MAYFLIES (INSECTA, EPHEMEROPTERA)

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While investigating the aquatic macroinvertebrate fauna of four intermittent Dinaric karst rivers in Croatia, we confirmed or recorded new distribution data and ecological features for several mayfly species rare in Croatian freshwater habitats: *Nigrobaetis niger* (Linnaeus, 1761), *Procloeon pennulatum* (Eaton, 1870) and *Paraleptophlebia werneri* Ulmer, 1920. To our knowledge, this is the first record of *N. niger* in intermittent lotic habitats. We discuss their substrate preferences in the studied habitats as well as their relationships with measured physico-chemical water parameters. The newly obtained results confirm that our knowledge about Croatian mayfly fauna and species ecological requirements in intermittent Mediterranean rivers is still incomplete and is increasing with systematic studies.

Key words: Ephemeroptera, IRES, temporary habitats, lotic habitats, environmental parameters, *Nigrobaetis niger*, *Procloeon pennulatum*, *Paraleptophlebia werneri*

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Tijekom istraživanja slatkovodnih beskralješnjaka povremenih tekućica dinarskog krša u Republici Hrvatskoj, potvrdili smo ili zabilježili nove podatke o rasprostranjenosti i ekološkim značajkama nekoliko vrsta vodencvjetova koje se smatraju rijetkima u hrvatskim slatkovodnim staništima: *Nigrobaetis niger* (Linnaeus, 1761), *Procloeon pennulatum* (Eaton, 1870) i *Paraleptophlebia werneri* Ulmer, 1920. Prema našim spoznajama, *N. niger* je po prvi puta zabilježen u povremenim tekućicama. Osim rasprostranjenosti, u ovom radu raspravljamo i o njihovom izboru mikrostaništa te odnosu s fizikalno-kemijskim parametrima vode u istraživanim povremenim tekućicama. Ovi rezultati potvrđuju da naše znanje o fauni vodencvjetova Republike Hrvatske te o njihovim ekološkim zahtjevima u povremenim tekućicama još uvijek nije potpuno te se povećava sa svakim sustavno provedenim istraživanjem.

Ključne riječi: Ephemeroptera, IRES, povremena lotička staništa, okolišni čimbenici, *Nigrobaetis niger*, *Procloeon pennulatum*, *Paraleptophlebia werneri*

INTRODUCTION

Intermittent rivers and streams (IRES) are hydrologically highly dynamic and complex freshwater ecosystems that periodically cease to flow and run dry. They

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occur worldwide, covering more than half of the global river network. They are also common in Europe, especially in the Mediterranean region, where they are even dominant in some river networks (Larned *et al.*, 2010; Skoulikidis *et al.*, 2017; Tockner *et al.*, 2009). Due to climate change and increasing demands for water, intermittent lotic habitats are expanding worldwide, and there is evidence that some perennial rivers changed their hydrology becoming intermittent during the past few decades (Gleick, 2003). Although intermittent have lower taxonomical diversity than perennial rivers and streams, many studies showed that they can also support high biodiversity (see in Stubbington *et al.*, 2018). Aquatic taxa of IRES exhibit traits associated with resistance (e.g. protection from desiccation, diapauses) or resilience (e.g. efficient dispersal, various locomotion types) to flow intermittence (Leigh *et al.*, 2015).

Mayflies are an amphibious insect order, spending their nymphal life in aquatic habitats and adult life in terrestrial habitats. Their nymphs can be found in both standing and running waters, where they are dependent on a wide range of physical and chemical water parameters, such as oxygen concentration, pH, water temperature, water velocity, substrate composition, and nutrient availability (e.g. Bauernfeind & Soldán, 2012; Vilenica *et al.*, 2018a, 2017a, 2016). Very often, mayflies are among the most abundant groups of aquatic macroinvertebrates in freshwater habitats, having an important role in secondary production as a significant food source for a wide range of aquatic and terrestrial predators (Elliott *et al.*, 1988; Sartori & Brittain, 2015). Due to their high sensitivity to habitat alterations (Firmiano *et al.*, 2017; Vilenica *et al.*, 2019, 2020), they are widely used in bio-monitoring programmes worldwide (Sartori & Brittain, 2015).

Mayfly-focused research in Croatia started less than a decade ago and revealed a relatively high number of mayfly species in Croatian freshwater habitats (Vilenica *et al.*, 2021). Nevertheless, there are still gaps in our knowledge about the distribution and ecological requirements of many species (Vilenica *et al.*, 2021), and based on the current knowledge, some of them can even be considered rare. As there is still much to be learned about the ecology and biodiversity of IRES, we have conducted research in four intermittent Mediterranean karst rivers in Croatia. The aim of this study is: 1) to present the distribution of several rare mayfly species recorded in IRES, based on literature and new data; and 2) to provide further insight into their habitat choice and relationship with environmental factors.

MATERIAL AND METHODS

Study area

Croatian territory extends over two limno-ecological regions: the Dinaric Western Balkan Ecoregion (ER5) and the Hungarian Lowlands Ecoregion (ER11) (NN 66/16, 2016). Our study area is in the Croatian part of ER5. The climate of the area is temperate humid with hot summer (Cfa, Köppen classification) with the average temperature of the warmest month above 22°C (Šegota & Filipčić, 2003). The average annual air temperature is around 14°C and the average annual rainfall is around 1000 mm (Zaninović *et al.*, 2008). This study encompassed four geographically close intermittent rivers – the Krčić, Čikola, Guduča and Miljašić Jaruga Rivers (Fig. 1, Tab. 1). Along each river, three study sites were chosen, with increasing distance from the river source (1 = closest to the source, 3 = furthest from the source).

Tab. 1. Characteristics of the study sites along four intermittent Dinaric karst rivers in Croatia.

River	Krčić			Guduča		
Locality	1	2	3	1	2	3
Coordinates, N	44.02734	44.02761	44.04190	43.92848	43.92358	43.89061
Coordinates, E	16.31899	16.30671	16.25358	15.83178	15.80045	15.79960
Substrate composition (%)	60% phytal, 20% mesolithal, 20% microlithal	60% phytal, 20% mesolithal, 20% microlithal	40% phytal, 40% lithal, 10% xylal, 10% psammal	60% phytal, 20% megalithal, 20% mesolithal	20% mesolithal, 20% xylal, 60% phytal	10% microlithal, 10% mesolithal, 80% phytal
River	Čikola			Miljašić Jaruga		
Locality	1	2	3	1	2	3
Coordinates, N	43.84345	43.84572	43.83771	44.19420	44.20716	44.21980
Coordinates, E	16.25727	16.17832	16.04927	15.27819	15.25737	15.23919
Substrate composition (%)	80% phytal, 20% megalithal	60% phytal, 30% argylal, 10% akal	10% phytal, 20% microlithal, 20% xylal, 50% megalithal	70% phytal, 30% mesolithal	50% phytal, 30% microlithal, 20% argylal	80% phytal, 20% argylal

Sampling protocol and species identification

Aquatic macroinvertebrates, including mayfly nymphs, were collected on April 15th and 16th 2021, using a standard benthic macroinvertebrate sampling method, a Surber sampler (25 cm x 25 cm, mesh size 500 µm). At each study site, four replicate samples were collected, proportional to the microhabitats present. Substrates consisted mainly of fine sediment (sand, silt, mud), lithal (stones, gravel), and aquatic vegetation (submerged and emergent); detailed substrate composition of the study sites is presented in Tab. 1.

All samples were immediately preserved in 96% ethanol, subsequently sorted and identified in the laboratory. Mayfly nymphs were identified using Bauernfeind & Humpesch (2001). Taxonomy follows Waltz *et al*. (1994) and Bauernfeind & Soldán (2012). The voucher specimens are deposited in the first author's collection, at the Department of Biology, Faculty of Science, University of Zagreb, Croatia.

At each study site, the following physico-chemical water parameters were measured: water temperature, dissolved oxygen concentration and saturation (using the oximeter WTW Oxi 330/SET), conductivity (with the conductivity meter WTW LF 330), pH (using the pH-meter WTW ph 330), water body width and depth (using a hand meter/measuring tape) and water velocity (using the SonTek Flow Tracker). Chemical analyses of water were conducted in the laboratory using Standard Analytical Procedure (APHA, 1992) for measuring alkalinity, water hardness, chemical oxygen demand, concentrations of nitrites, nitrates, and orthophosphates (Tab. 2).

Data analysis

Distribution maps were created in QGIS desktop 3.4.6. Ink (http://www.qgis.org) using literature data and new findings.

Tab. 2. Physico-chemical water parameters along four intermittent Dinaric karst rivers in Croatia.

Physico-chemical water parameters/Rivers	Krčić		Guduča		Čikola		Miljašić Jaruga	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Water temperature (°C)	8.4	0.3	14.5	1.1	12.1	0.4	15.0	0.5
Oxygen saturation (%)	98.8	2.6	99.8	6.7	96.8	0.9	118.0	4.2
Dissolved oxygen (mg/L)	11.29	0.32	10.15	0.85	10.22	0.16	11.99	0.47
pH	8.03	0.32	7.79	0.30	8.18	0.12	7.87	0.13
Conductivity (µS/cm)	333	13	487	8	489	26	615	16
Water velocity (m/s)	0.60	0.09	0.60	0.31	0.38	0.22	0.17	0.06
Water depth (cm)	27	10	35	19	35	18	26	16
COD (mg O_2/L)	3.03	0.71	4.62	1.19	3.77	0.67	3.45	1.69
Nitrates (mg N/L)	0.319	0.166	0.230	0.175	0.187	0.097	0.199	0.108
Nitrites (mg N/L)	0.000	0.000	0.039	0.051	0.009	0.005	0.013	0.003
Phosphates (mg P/L)	0.017	0.002	0.024	0.012	0.009	0.007	0.022	0.007
Alkalinity (mg $CaCO_3$/L)	120	12	143	15	149	19	156	8
Water hardness (mg $CaCO_3$/L)	189.9	35.9	229.9	35.9	302.6	64.9	281.8	40.8

Abundances (number of collected individuals) of each of the recorded mayfly species were correlated with physico-chemical water properties data using Spearman's rank correlation coefficient. Analyses were performed using Statistica 13.0 (TIBCO Software Inc., 2017).

RESULTS AND DISCUSSION

While investigating the aquatic macroinvertebrate fauna of the four intermittent Dinaric karst rivers in Croatia, we have confirmed or recorded new distribution data and ecological features for several mayfly species rare in Croatian freshwater habitats: *Nigrobaetis niger* (Linnaeus, 1761), *Procloeon pennulatum* (Eaton, 1870) and *Paraleptophlebia werneri* Ulmer, 1920 (e.g. Vilenica *et al*., 2014, 2015, 2016, 2017b, 2018b, 2020). All three species are considered rare on the European scale, due to their scarcity in several ecoregions. *Nigrobaetis niger*, a species with Palearctic distribution (Bauernfeind & Soldán, 2012), is considered rare in ER3 (i.e. present only in Italy), ER7 (Eastern Balkans), ER12 (Pontic Province), ER13 (Western Plains) and ER14 (Central Plains). Furthermore, *P. pennulatum,* a species with Holarctic distribution (Bauernfeind & Soldán, 2012), is rare in ER4 (Alps) and ER8 (Western Highlands) while *P. werneri,* a species with western Palaearctic distribution, is rare in ER10 (the Carpathians) and ER11 (Hungarian Lowlands) (Buffagni *et al*., 2009, 2021).

Nigrobaetis niger (Linnaeus, 1761)

In this study, rheo- to limnophile *N. niger* was recorded in the Krčić (study site 3), Čikola (study sites 1 and 2) and Guduča Rivers (study sites 2 and 3) (Figs. 1a, b, c). In previous studies, the species was recorded at four lotic habitats in the Hungarian Lowlands ecoregion (ER11) (the Kraljevec, Čemernica (Vilenica *et al*., 2015), Dankovića klada Streams (Vilenica *et al*., 2016), and the Glina River (Vilenica *et al*., 2015))

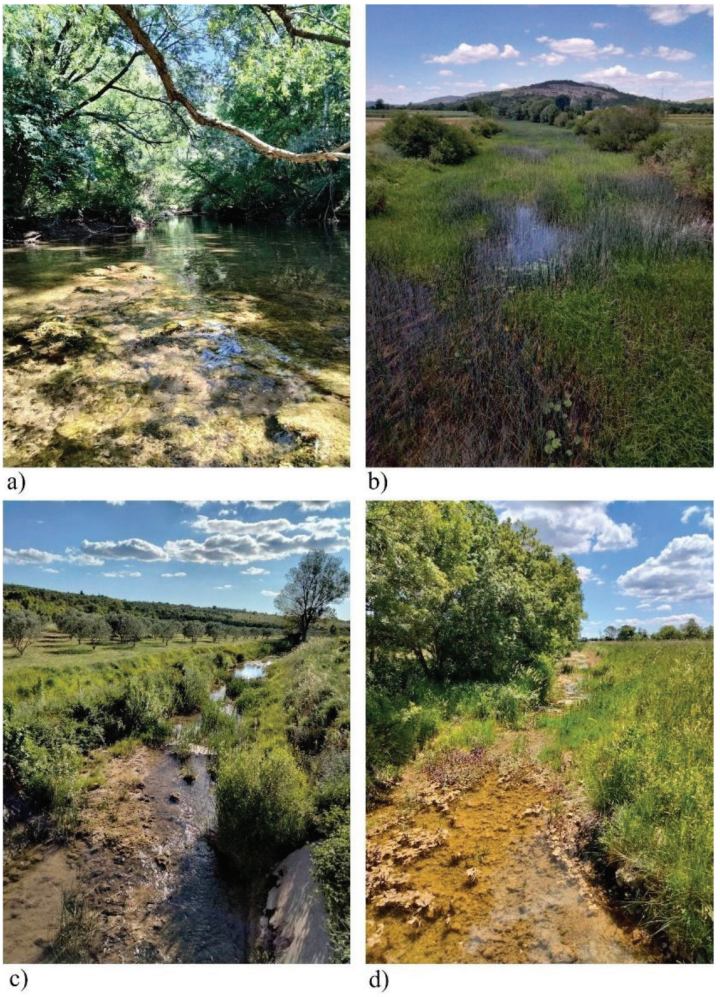

Fig. 1. Examples of the study sites aloong four intermittent rivers in Croatia inhabited by rare mayflies: a) Krčić river (site 3, habitat of *Nigrobaetis niger*), b) Čikola river (site 1, habitat of *Nigrobaetis niger* and *Procloeon pennulatum*), c) Guduča river (site 2, habitat of *Nigrobaetis niger*) and d) Miljašić Jaruga river (site 2, habitat of *Paraleptophlebia werneri*).

and four in the Dinaric Western Balkan ecoregion (ER 5) (the Suha Ričina and Brodic Streams, and the Otuča and Vrba Rivers (Fig. 2a; Vilenica *et al*., 2015)). This species was reported from hypocrenal to epipotamal river sections (Buffagni *et al*., 2009, 2021), most commonly from the rhithralic parts (Bauernfeind & Soldán, 2012), which was also the case in the Croatian watercourses (Vilenica *et al*., 2015, 2016). To our knowledge, the species is now recorded in IRES for the first time.

In the studied intermittent rivers, the highest abundances of *N. niger* were collected from macrophytes (Fig. 3a), but the species was also present on lithal and xylal (stony substrates and dead vegetation parts). The species is known as a microhabitat specialist for macrophytes, although it was also recorded from other substrates, such as micro/mesolithal (pebbles, boulders) and particulate organic matter (Buffagni *et*

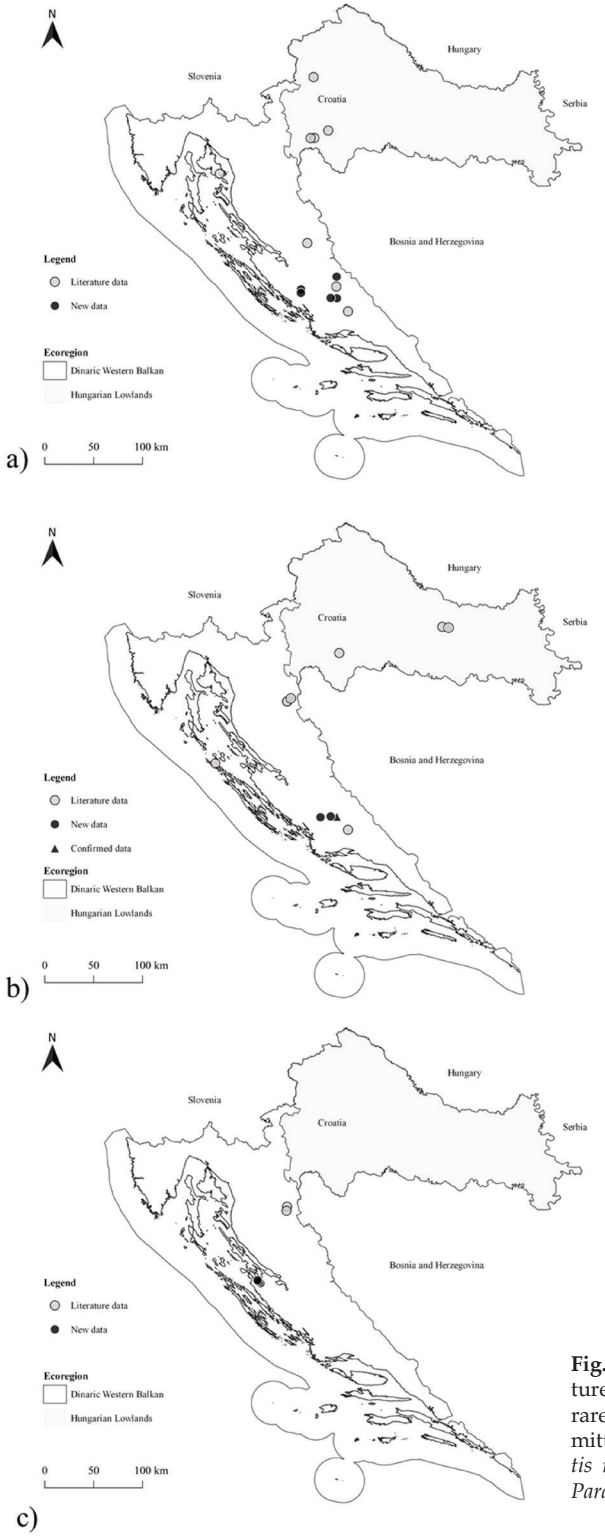

Fig. 2. Distribution maps (with literature records and new data) for three rare species recorded at four intermittent rivers in Croatia: a) *Nigrobaetis niger*, b) *Procloeon pennulatum*, c) *Paraleptophlebia werneri*.

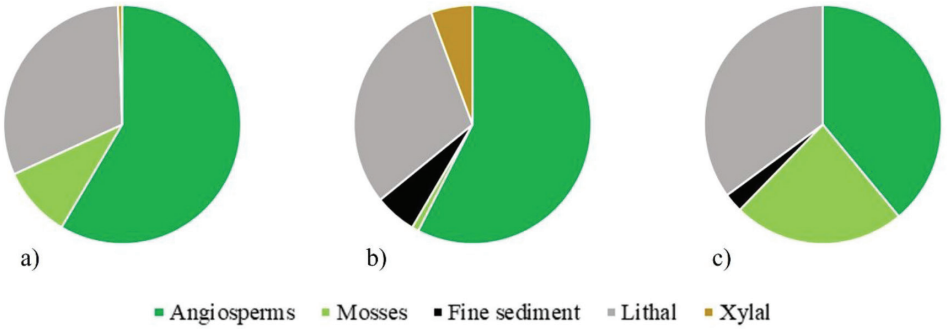

Fig. 3. Species occurrence on the dominant substrate types in the four investigated intermittent rivers in Croatia: a) *Nigrobaetis niger*, b) *Procloeon pennulatum*, c) *Paraleptophlebia werneri*.

al., 2009, 2021). Abundance of *N. niger* was positively correlated with chemical oxygen demand (R = 0.364, p = 0.011), nitrite concentration (R = 0.385, p = 0.007) and alkalinity (R = 0.299, p = 0.039); in the Čikola and Guduča Rivers it was most abundant at sites characterized by high anthropogenic pressure and the vicinity of farmland. These results could also indicate that *N. niger* is able to tolerate water pollution.

Procloeon pennulatum (Eaton, 1870)

During our study, we have collected rheo- to limnophile *P. pennulatum* only in the Čikola River, at all three sites (Fig. 2b). In Croatia, the species was recorded predominantly in ER5, where it was found at a total of five sites: the Plitvica Stream and the tufa barrier Novakovića Brod in the Plitvice Lakes National Park (Vilenica *et al*., 2017b), the Lepenica Reservoir (Vilenica *et al*., 2020), and the Čikola and Vrba Rivers (Vilenica *et al*., 2015). Three sites are located in ER11: the Petrinjčica River (Vilenica *et al*., 2015) and the Ćeralinica and Djedovica Streams in Papuk Nature Park (Fig 2b; Vilenica *et al*., 2018b). Our current records confirm data presented in Vilenica *et al.* (2015), who already recorded the species at site 1 in the Čikola River. Nevertheless, since these authors did not investigate other sites on this river, our records from the study sites 2 and 3 can be considered new (Fig. 1b). In Croatian freshwater habitats, the species was previously recorded from both lotic and lentic sites (rivers, streams, tufa barriers, reservoirs) (Vilenica *et al*., 2015, 2017b, 2018b). The species can be found at hypocrenal to metapotamal river sections (Buffagni *et al*., 2009, 2021), although Bauernfeind & Soldán (2012) emphasize its preference for rhithralic sections of smaller brooks, where the nymphs can be found mostly in sections with very slow currents. The species was previously reported from intermittent streams (e.g. Armitage & Bass, 2013), and is listed as one of the taxa with traits that enable the species to resist drought, i.e. it can resist dry periods in the egg stage (Macan, 1978; Buffagni *et al*., 2009, 2021).

The highest abundances of *P. pennulatum* were collected from macrophyte substrates (Fig. 3b), which corroborates previous findings regarding its microhabitat preferences (see also Buffagni *et al*., 2009, 2021). We have also recorded it from fine sediment (sand, mud, silt), xylal (dead parts of aquatic vegetation) and lithal (stony substrates) (Fig. 3b), similar to the studies that showed its occasional occurence its occasional occurrence on particulate organic matter, woody debris, micro/mesolithal

(pebbles, gravel) and psammal (sand) (Buffagni *et al*., 2009, 2021). The abundance of this species was positively correlated with water pH (R = 0.446, p = 0.001); it is known to prefer neutral to alkaline pH values (Buffagni *et al*., 2009, 2021). The negative correlations with oxygen saturation (R = –0.351, p = 0.014) and dissolved oxygen concentration (R = –0.377, p = 0.008) could be related to its preference for habitats with warm water (>= 18°C) (Buffagni *et al*., 2009, 2021), but also to its absence from well-oxygenated fast flowing intermittent rivers, such as the Krčić and Guduča Rivers. The species also showed a negative correlation with the concentration of phosphates in water (R = –0.623, p = < 0.001), which could reflect its potential sensitivity to agricultural pollution.

Paraleptophlebia werneri Ulmer, 1920

Rheophile *P. werneri* was recorded in only one intermittent river included in this study, the Miljašić Jaruga River (Figs. 1d, 2c), at all three study sites. The habitat choice of this species in Croatian waterbodies (Vilenica *et al*., 2014, and new data) is in accordance with the literature data, but this is the first time it was recorded from temporary habitats in Croatia. Prior to this research, it was recorded only at two sites in ER5: Plitvica Stream and Prošće Lake in the Plitvice Lakes National Park (Fig. 2c; Vilenica *et al*., 2014). The current results have increased the known distribution area of the species towards the south in ER5, while it remains unknown for ER11. The species was reported from epirhithral to epipotamal river sections, with a preference towards zones with moderate to high current. It was also often found in lentic habitats, such as fishponds (Buffagni *et al*., 2009, 2021; Bauernfeind & Soldán, 2012). *Paraleptophlebia werneri* was frequently reported from waterbodies that dry out during the summer (Bauernfeind & Soldán, 2012; White *et al*., 2018), which is why it is among the species that are indicative of hydrological conditions, i.e. indicator species for temporary waterbodies (Bratton, 1990; Buffagni *et al*., 2009, 2021). The species is adapted to intermittent flows *via* the production of drought-resistant eggs (Wright *et al*., 1984). Adaptations in oviposition, length of embryogenesis, egg quiescence, larval growth rate, current speed adaptation and habitat range are among the traits of this species listed as potentially advantageous in intermittent habitats (Řezníčková *et al*., 2010).

Our data show that *P. werneri* is mostly abundant on macrophytes (Fig. 3c), which is in line with the data reported in literature (Buffagni *et al*., 2009, 2021; Bauernfeind & Soldán, 2012). We also collected it from fine sediment and lithal (stony substrates) (Fig. 2), similar to previous studies that found the species on fine sediment and particulate organic matter (Buffagni *et al*., 2009, 2021). The habitat choice of this species (lotic as well as lentic habitats) combined with its microhabitat preferences for macrophyte vegetation, could explain the negative correlation with water velocity in the studied intermittent rivers (R = –0.515, p = < 0.001). There are no data on temperature preferences of the species (Buffagni *et al*., 2009, 2021), but in our study its abundance was positively correlated with water temperature (R = 0.381, p = 0.007). Positive correlations were recorded with oxygen saturation (R = 0.574, p = < 0.001) and dissolved oxygen concentration (R = 0.593, p = < 0.001) which could be related with species' preference for habitats with submerged aquatic macrophytes (Bauernfeind & Soldán, 2012), that are often rich in oxygen (e.g. Frodge *et al*., 1990). Positive correlations were also recorded between species abundance and phosphate concentration (R = 0.327, p

= 0.023) and conductivity (R = 0.546, p = < 0.001), which could indicate a tolerance to agricultural pollution, similar to *P. pennulatum*.

In conclusion, as most of the available literature data regarding the presented mayfly species occurrence in IRES was obtained from Central European habitats, our records in the Mediterranean Dinaric karst intermittent rivers can be considered new. To properly protect (intermittent) habitats and their biota, it is essential to have good knowledge of the species that inhabit them. Our results indicate the importance of IRES for maintaining local mayfly diversity, as they harbour environmental conditions adequate to provide habitats for several rare species. The results presented here could contribute to filling the gaps in our knowledge of the distribution and occurrence frequency of rare Croatian mayflies, as well as their ecological requirements, which could help in future assessments of the species' conservation status, both in the country and in their whole distribution area.

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