

# Littoral mayfly assemblages in South-East European man-made lakes

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## ABSTRACT

Man-made freshwater habitats have been widely used to successfully modify the environment for human benefit, including purposes of irrigation, flood control or electricity generation. Over the past decade, our knowledge about the mayfly fauna and its ecology in South-East Europe has been growing, yet the data related to lentic assemblages remain very scarce. In this study, littoral mayfly assemblages were investigated at artificial and heavily modified lentic habitats in the Dinaric Western Balkan (n=21) and Pannonian Lowland (n=15) ecoregions. Mayfly nymphs were sampled during the summer months of 2016 or 2017. At each sampling site, ten samples were collected using a benthos hand net. A total of 21 mayfly species were recorded, though species richness per site was rather low (i.e., between zero and seven species). *Cloeon dipterum* (Linnaeus, 1761) was the most frequent species recorded, while *Caenis horaria* Linnaeus, 1758 was the most abundant. This study showed that the Croatian mayfly fauna is still growing, with the first record of *Siphonurus aestivalis* Eaton, 1903 for the country. The assemblage structure was mainly dominated by lower reaches and lentic (potamal and littoral) elements and detritivores (gatherers/collectors and active filter feeders). Orthophosphates and chemical oxygen demand had the highest influence on mayfly assemblages, reflecting a higher level of pollution at sites in the Pannonian Lowland ecoregion, and consequently markedly lower mayfly abundances. As mayflies are widely used as bioindicators of freshwater ecosystems, detailed information about their assemblages in heavily modified and artificial habitats could contribute to future conservation activities of freshwater habitats and their communities. Additionally, these results could be applied in creating a monitoring system for artificial lakes according to the requirements of the European Water Framework Directive.

## INTRODUCTION

Historically, man-made freshwater habitats have been widely used to successfully modify the environment for human benefits, such as recreational purposes, fish farming, flood control, irrigation or as a source of drinking water for livestock (Seaman and Sprague, 1991; Petriere, 1996). In recent years, reservoirs are the most commonly created artificial water-bodies (Schwarz, 2012),

predominantly formed by using a dam or a lock to store water, for the primary purpose of electrical energy generation (Petrere, 1996). Reservoirs are complex systems that represent a transition between lakes and rivers (Wetzel, 2001; Irz *et al.*, 2002) and their shores are most commonly altered. In most cases, water over the dam loses its lotic properties such as flow and mechanical aeration and becomes slow-flowing (Polak, 2004). Damming causes greater hydrological changes, such as river basin erosion downstream, rising water temperatures and increased sedimentation and water fluctuations (Petts, 1984; Wang and Hu, 2009; Carmignani and Roy, 2017). The impoundment has a higher nutrient intake, which accelerates eutrophication, frequently resulting in oxygen deficiency and reduced water transparency (Wiatkowski, 2011). These changes in turn modify the composition and structure of biological communities and their lower diversity (Baxter, 1977; Vollenweider and Kerekes, 1982; Brittain and Saltveit, 1989; Wetzel, 2001, Céréghino *et al.*, 2002; Poff and Zimmerman, 2010) and disable certain important ecological processes, such as fish migration. Sensitive taxa are lost, while tolerant taxa prevail, producing assemblage turnover (Davies and Jackson, 2006; King and Baker, 2014). The inhabiting communities often show signs of “rithralization” (e.g. caused by channel straightening) or “potamalization” (e.g. caused by impounding). For instance, when the distribution of longitudinal zonal associations and/or trophic structure of a certain river zone differs from its reference state, the river system has been altered indicating a certain level of ecological disturbance (Moog,

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Contributions: ZM, designed and supervised the research; MV, identified the specimens, analysed the data and wrote the paper; ZM, NV, collected samples; NV, sorted samples. All authors edited the drafts and approved the final version of the manuscript.

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2002). Nevertheless, certain researchers (Baxter, 1977; Irz *et al.*, 2006, Vilenica *et al.*, 2016a) argue that heavily modified and artificial lentic habitats could also be important from the biodiversity conservation aspect, as they provide habitats for many animal species (birds, fish, insects etc.) and affect the enhanced development of surrounding vegetation.

Inhabiting a wide range of freshwater habitats, mayflies are often one of the most abundant taxonomic groups, contributing approximately 25% of the total benthic macroinvertebrate production (Elliott *et al.*, 1988). Their species richness is generally much higher in lotic habitats, especially in the upper reaches (rithral sections) of fast-flowing streams and rivers, and lower reaches (potamal sections) of slow-flowing natural lowland rivers. On the other hand, springs (crenal sections) and lentic habitats harbour much lower mayfly species richness (Bauernfeind and Moog, 2000; Bauernfeind and Söldan, 2012). In accordance with the available microhabitats, which offer different food resources, the composition of mayfly assemblages will be composed of varying ratios of grazers/scrapers, gatherers/collectors and filter feeders (Bauernfeind and Söldan, 2012; Vilenica *et al.*, 2018). Grazers/scrapers consume epilithic algae and fine particulate organic matter (FPOM), while detritivores (gatherers/collectors, active and passive filter feeders) feed on decomposing coarse particulate organic matter (CPOM) and FPOM (Lamp and Britt, 1981; Buffagni *et al.*, 1995; Bauernfeind and Söldan, 2012).

In order to develop effective conservation and management of freshwater habitats, biological assessments are highly recommended (Hughes *et al.*, 1986; Stoddard *et al.*, 2008). Numerous studies have shown that mayflies are highly sensitive to anthropogenic alterations of their habitats, and are among the first benthic macroinvertebrates to disappear (Di Giovanni *et al.*, 2003; Brittain and Sartori, 2003). Consequently, they have been widely used as a particularly valuable taxonomic group for biomonitoring programmes (Lenat, 1988; Lenat and Penrose, 1996; Bauernfeind and Moog, 2000; Ferro and Sites, 2007). This study was conducted in the framework of the project “Development of a classification system to assess the ecological potential of artificial and heavily modified surface water bodies”, intended to provide much-needed information on the ecology of benthic biota, including mayflies, in man-made lentic habitats. Moreover, Croatia has few natural, large, lentic habitats (*i.e.* lakes), and some artificial habitats are already proving to be important habitats for certain rare aquatic insects, such as dragonflies (Vilenica *et al.*, 2016a).

Therefore, the aims of this study were to: i) identify mayfly species richness and distribution, ii) analyse the composition and structure of mayfly assemblages (in terms of longitudinal zonal associations and feeding

groups), and iii) determine the main environmental factors that influence mayflies in the studied man-made lakes.

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## METHODS

### Study area

The study encompassed a total of 36 man-made lakes, of which 21 were located in the Dinaric Western Balkan ecoregion (ER 5) and 15 in the Pannonian Lowland ecoregion (ER 11) (Illies, 1978) (Figs. 1 and 2). The majority of these sites are reservoirs built on smaller or larger rivers, while several are anthropogenically-impacted natural lakes (*e.g.*, Prološko Blato, Sakadaš, Njivice) or artificial lakes and gravel pits (*e.g.*, Ponikve, Šoderica Koprivnica, Rakitje, Novo Čiče).

### Sampling protocol

Mayfly nymphs were sampled together with other benthic macroinvertebrates, during the low water period from July to September in 2016 or 2017. At all 36 study sites, the littoral zone was sampled at two or more locations (depending on reservoir size). Sampling was conducted by applying a modified proportional stratified sampling approach (Urbanič *et al.*, 2012).

Each sampling location covered an area of 25 m lakeshore up to 1 m depth. At each location, ten replicates were collected using a benthic hand net (25 x 25 cm surface area, 500 µm mesh-size). Samples were taken in microhabitats covering at least 10% of the area, proportionate with their coverage at the sampling sites. We defined the substrate categories according to the AQEM consortium (2002). To generate the “lithal” variable, we summed up the coverage percentages of the megalithal, macrolithal, mesolithal and microlithal. To generate the “fine sediment” variable, we summed the coverage percentages of the akal, psammopelal, pelal and psammal substrate (Tab. 1).

Mayflies were identified to the lowest possible taxonomical level (very juvenile and/or very damaged individuals were identified only to the genus level) using *e.g.* Malzacher (1984) and Bauernfeind and Humpesch (2001). All voucher specimens are deposited at the Department of Biology, Faculty of Science, University of Zagreb, Croatia.

### Environmental parameters

At the littoral zone of each study site, the following environmental parameters were measured at the time of benthic macroinvertebrate sampling: water temperature, dissolved oxygen concentration (using the oximeter WTW Oxi 330/SET), conductivity (with the conductivity meter WTW LF 330) and pH (using the pH-meter WTW

**Tab. 1.** Environmental parameters at 36 man-made lakes in Croatia. Study site abbreviations are presented in Fig. 1.

Ecoregion (ER)	Study site / Environmental parameter	Alt	A	Max depth	Mean depth	Tw	Oxy	Con	pH	BOD <sub>5</sub>	COD <sub>Mn</sub>	NO <sub>3</sub> <sup>-</sup>	TN	TOC	PO <sub>4</sub> <sup>3-</sup>	WLeVF	I-AG (%)	NAT (%)	Substrate
Dinaric Western Balkan Ecoregion (ER 5)	B	717	0.50	7.2	2.7	23.8	9.3	195	8.25	1.21	1.40	0.48	0.64	1.55	0.003	3	0.0	98.6	Li, Fs, Py
	BU	41	2.90	19.5	9.1	28.0	8-9	308	8.13	0.76	2.15	0.12	0.37	2.84	0.004	3	1.4	69.0	Li, Fs, Py, XI, CPOM
	BR	187	0.03	18.0	10.0	21.0	10.8	620	8.38	0.84	1.30	0.26	0.52	0.77	0.003	2	9.8	74.0	Li, Fs, Py
	GO	307	0.17	6.0	3.0	18.1	10.8	986	8.10	0.66	1.40	0.16	0.31	0.78	0.003	3	0.2	89.6	Li, Fs, Py, CPOM
	GP	430	0.43	6.5	5.4	12.5	11.4	428	8.05	2.83	1.74	0.47	0.72	1.77	0.012	5	19.6	63.3	Li, Fs
	KR	554	8.64	75.5	37.8	18.3	8.7	287	8.16	2.50	1.05	0.30	0.78	1.83	0.004	5	25.5	52.3	Li, Fs
	LP	730	0.80	18.0	13.5	27.1	8.7	139	8.09	1.18	1.59	0.32	0.51	1.83	0.002	5	0.0	100.0	Li, Fs
	LE	182	1.70	42.5	21.0	21.3	11.7	299	8.19	1.56	1.82	0.84	1.22	2.78	0.016	4	0.0	81.3	Li, Fs, Py
	LO	767	2.30	44.7	33.8	21.9	8.8	136	7.55	1.08	1.16	0.47	0.62	1.66	0.003	5	0.0	96.0	Li, Fs
	NJ	2	0.75	10.0	4.0	29.9	7.3	620	7.92	1.36	2.67	0.20	0.30	3.46	0.002	2	13.2	50.9	Li, Fs
	OP	575	0.89	6.7	2.9	26.5	9.5	305	8.00	2.42	1.45	0.33	0.43	1.16	0.008	3	0.0	60.6	Li, Fs, Py
	PE	330	20.09	31.0	20.8	23.4	9.8	297	8.24	1.30	1.60	0.15	0.45	1.53	0.002	5	5.1	86.5	Li, Fs, Py
	PO	19	1.20	5.0	3.0	30.0	12.0	490	8.20	1.46	1.63	0.10	0.29	2.16	0.002	4	2.3	87.5	Fs, Py
	PR	284	1.00	20.0	6.3	17.3	10.8	437	8.13	2.45	2.82	0.54	0.58	1.18	0.003	2	16.9	67.2	Li, Fs, Py
	PB	269	2.10	5.3	4.0	29.7	9.4	250	8.12	1.59	2.10	0.08	0.61	2.71	0.002	5	0.0	83.1	Li, Fs, Py
	RA	9	0.65	7.5	5.8	23.4	10.6	266	8.29	1.00	1.61	0.12	0.47	1.37	0.003	4	2.3	80.9	Li
RI	387	1.90	39.6	16.0	26.8	8.3	255	8.12	0.71	1.38	0.20	0.49	2.35	0.001	5	12.8	68.3	Li, Fs	
SA	320	1.70	6.2	3.0	16.4	3.4	384	7.46	1.88	1.72	0.74	1.12	2.32	0.012	4	1.9	82.0	Fs, Py	
ST	553	1.70	6.5	4.3	24.8	8.7	284	8.20	2.92	1.16	0.20	0.32	1.42	0.007	4	0.0	47.3	Li	
TR	60	0.40	4.0	2.7	22.4	8.7	241	7.53	1.63	1.80	0.26	0.42	2.53	0.002	2	0.0	88.5	Li, Fs	
VL	122	0.30	10.0	4.0	28.7	10.3	339	8.15	1.46	4.50	0.11	0.65	3.96	0.003	4	38.2	0.0	Li, Fs, Py	
Pannonian Lowland Ecoregion (ER 11)	BJ	80	1.25	5.0	3.0	21.7	3.2	666	7.95	6.31	6.82	0.07	1.01	8.78	0.026	1	64.8	23.1	Fs, Py, XI, CPOM
	SO	128	1.50	20.0	8.0	21.5	10.1	469	8.19	2.34	2.28	0.34	0.71	3.67	0.005	1	72.6	7.7	Li, Fs, Py
	RK	120	2.00	5.0	2.5	25.3	8.3	432	8.25	1.71	3.33	0.52	1.05	3.02	0.007	1	7.0	47.3	Li, Fs
	BO	137	1.72	15.1	7.0	25.8	9.8	247	8.46	3.85	6.03	0.17	1.04	9.23	0.009	4	3.3	94.4	Li
	LA	123	0.50	11.0	5.5	24.3	12.2	179	9.29	5.09	6.63	0.18	0.96	9.46	0.010	4	4.7	84.2	Fs
	JO	93	0.79	1.4	1.0	26.7	12.0	412	8.51	6.84	7.98	0.48	1.80	9.87	0.032	2	86.5	3.8	Fs
	NC	103	0.90	40.0	15.0	23.7	9.1	448	8.24	1.42	1.95	3.86	3.98	1.01	0.010	1	65.8	11.8	Li, Fs
	PA	104	2.72	6.3	2.7	25.3	4.6	189	8.70	4.07	6.46	0.34	1.02	6.20	0.028	4	23.3	58.9	Li, Fs
	SK	79	0.12	7.0	4.0	22.3	4.2	552	8.02	5.37	6.84	0.37	1.40	8.03	0.037	1	0.0	82.2	Fs
	PP	96	0.75	6.5	2.0	23.3	10.1	150	7.80	12.16	17.38	0.38	2.45	8.26	0.027	4	21.0	70.6	Li, Fs
Pannonian Lowland Ecoregion (ER 11)	JA	115	6.40	7.0	4.0	19.8	11.6	537	8.28	1.44	1.87	1.27	1.45	1.78	0.010	2	9.3	42.4	Li, Fs, Py
	GR	98	1.26	5.0	3.0	21.3	8.0	558	8.03	5.10	6.81	0.30	0.99	7.29	0.027	3	77.9	16.6	Py, CPOM
	CK	168	10.50	13.2	7.0	25.6	10.4	235	8.85	1.61	1.40	0.76	1.37	1.96	0.008	3	57.5	28.4	Li, Fs, CPOM
	DU	150	16.60	13.4	8.0	27.6	10.2	222	8.40	1.49	1.32	0.76	1.54	1.77	0.006	4	58.3	28.6	Li, Fs, Py
	VA	191	2.85	8.7	5.0	24.5	9.9	249	8.40	2.96	3.99	0.86	1.10	4.41	0.015	3	12.5	72.6	Li, Fs

Alt, altitude (m asl); A, surface area (km<sup>2</sup>); max depth, maximum depth (m); Tw, water temperature (°C); oxy, dissolved oxygen content (mg L<sup>-1</sup>); Con, conductivity (μS cm<sup>-1</sup>); pH, pH; BOD<sub>5</sub>, biological oxygen demand (mg O<sub>2</sub> L<sup>-1</sup>); COD<sub>Mn</sub>, chemical oxygen demand (mg O<sub>2</sub> L<sup>-1</sup>); NO<sub>3</sub><sup>-</sup>, nitrates (mg N L<sup>-1</sup>); TN, Total nitrogen (mg N L<sup>-1</sup>); TOC, total organic carbon (mg L<sup>-1</sup>); PO<sub>4</sub><sup>3-</sup>, orthophosphates (mg P L<sup>-1</sup>); WLeVF, water level fluctuations size classes: (1) 0.00-0.25 m (2) >0.25-0.75 m (3) >0.75-1.00 m (4) 1.00 m-5.00 m (5) >5.00 m; I-AG, percentage of intensive agricultural land use in the catchment; NAT, percentage of natural and near natural areas in the catchment; Substrate, substrate composition (Li-Lithal, Fs-Fine sediment, Py-Phytal, XI-Xylal).

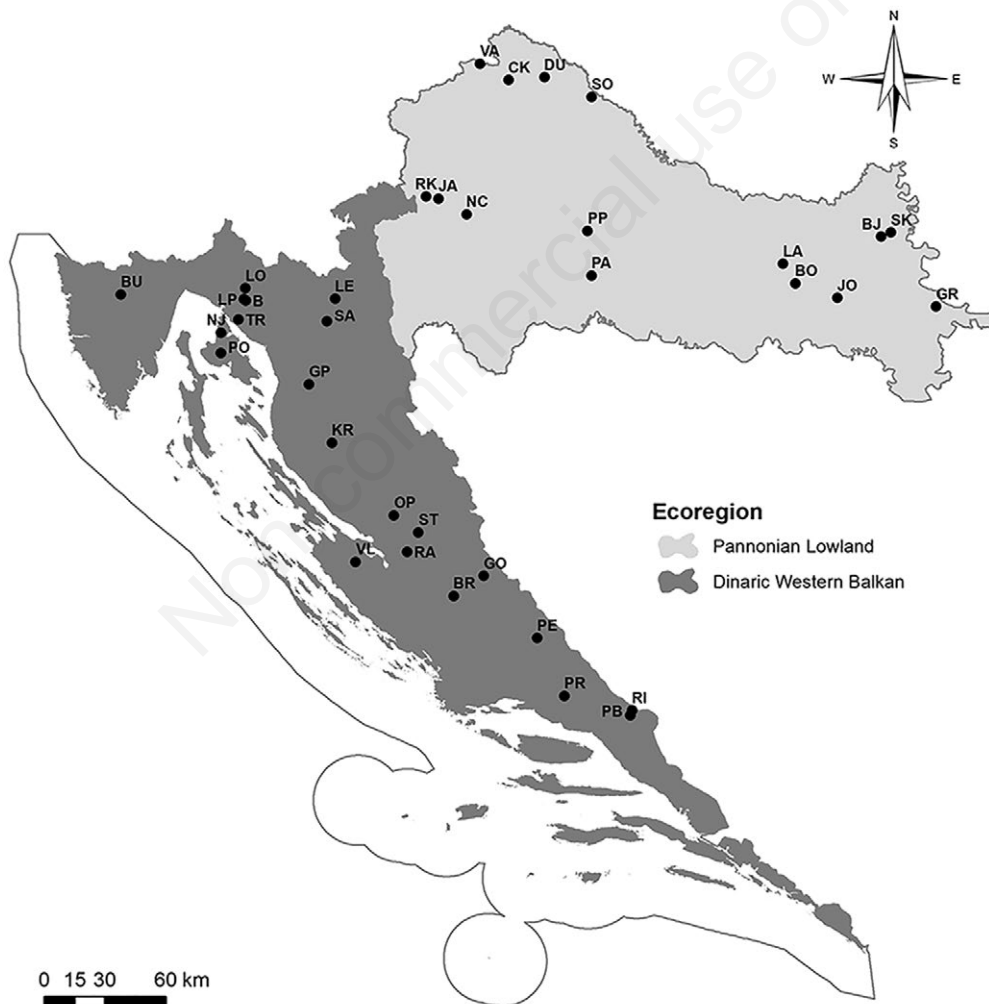
ph 330). The remaining environmental parameters are presented as the mean value of six composite samples from the euphotic zone collected over a six-month period (April-September 2016 or 2017) (Tab. 1). Water chemistry analyses were carried out according to the APHA standard methods (1992). Variables describing land use in catchments were calculated with GIS tools, using the Corine Land Cover classification (CLC Hrvatska, 2013).

Two categories of land use were distinguished: natural and semi-natural areas (CLC classes 3, 4, and 5), and areas characterized by intensive agricultural practices (CLC categories 2.1, 2.2, 2.4.1 and 2.4.2). Seasonal water level fluctuation values were grouped into five categories: (1) 0.00-0.25 m, (2) 0.25-0.75 m, (3) 0.75-1.00 m, (4) 1.00-5.00 m, (5) >5.00 m (Tab. 1) (Peterlin and Urbanič, 2013).

### Data analyses

The similarity of mayfly assemblages between study sites was examined using Hierarchical cluster analysis (HCA) and non-metric multidimensional scaling (NMDS) ordination based on the Bray-Curtis similarity matrix. Species data were log transformed prior to analyses. Only sites with mayfly records were included in the analysis.

In order to determine river influence on lentic waterbodies and their assemblages, the composition of mayfly assemblages was examined in terms of trophic structure and longitudinal zonal associations of species at each study site (using the classification of Buffagni *et al.*, 2009, 2018). The longitudinal zonal association and functional feeding group of each individual species are presented as a proportion within the assemblage. Most



**Fig. 1.** Geographical position and locations of the 36 study sites. Study sites: **ER 5:** B, Bajer; BU, Butoniga; BR, Brljan; GO, Golubić; GP- Gusić polje; LP, Lepenica; LE, Lešće; LO, Lokve; NJ, Njivice; OP, Opsenica; PE, Peruća; PO, Ponikve; PR, Prančević; PB, Prološko blato; RA, Razovac; RI, Ričice; SA, Sabljaci; ST, Štikada; TR, Tribalj; VL, Vlačine. **ER 11:** BJ, Biljsko jezero; SO, Šoderica Koprivnica; RK, Rakitje; BO, Borovik; LA, Lapovac; JO, Jošava; NC, Novo Čiče; PA, Pakra; SK, Sakadaš; PP, Popovac; JA, Jarun; GR, Grabova; CK, Čakovec; DU, Dubrava; VA, Varaždin.

taxa do not occur exclusively in one biocoenotic region and do not feed exclusively on a single food resource. Therefore, the assignment of taxa to a particular category is based on the ten-point assignment scale (see Schmidt-Kloiber and Hering, 2015). Using the given points and percentage of each species within the assemblage, the longitudinal zonation preferences and functional feeding group composition of mayfly assemblages at each study site were calculated.

Canonical correspondence analysis (CCA) was used to ordinate mayfly occurrence with respect to environmental variables. The analysis was performed using data for 21 taxa (rare species were downweighted) and 16 environmental variables. The Monte Carlo permutation test with 499 permutations was used to test the statistical significance of the relationship between all taxa and all variables. The Pearson's correlation coefficient was applied to examine the strength and significance of the reservoir land cover catchment

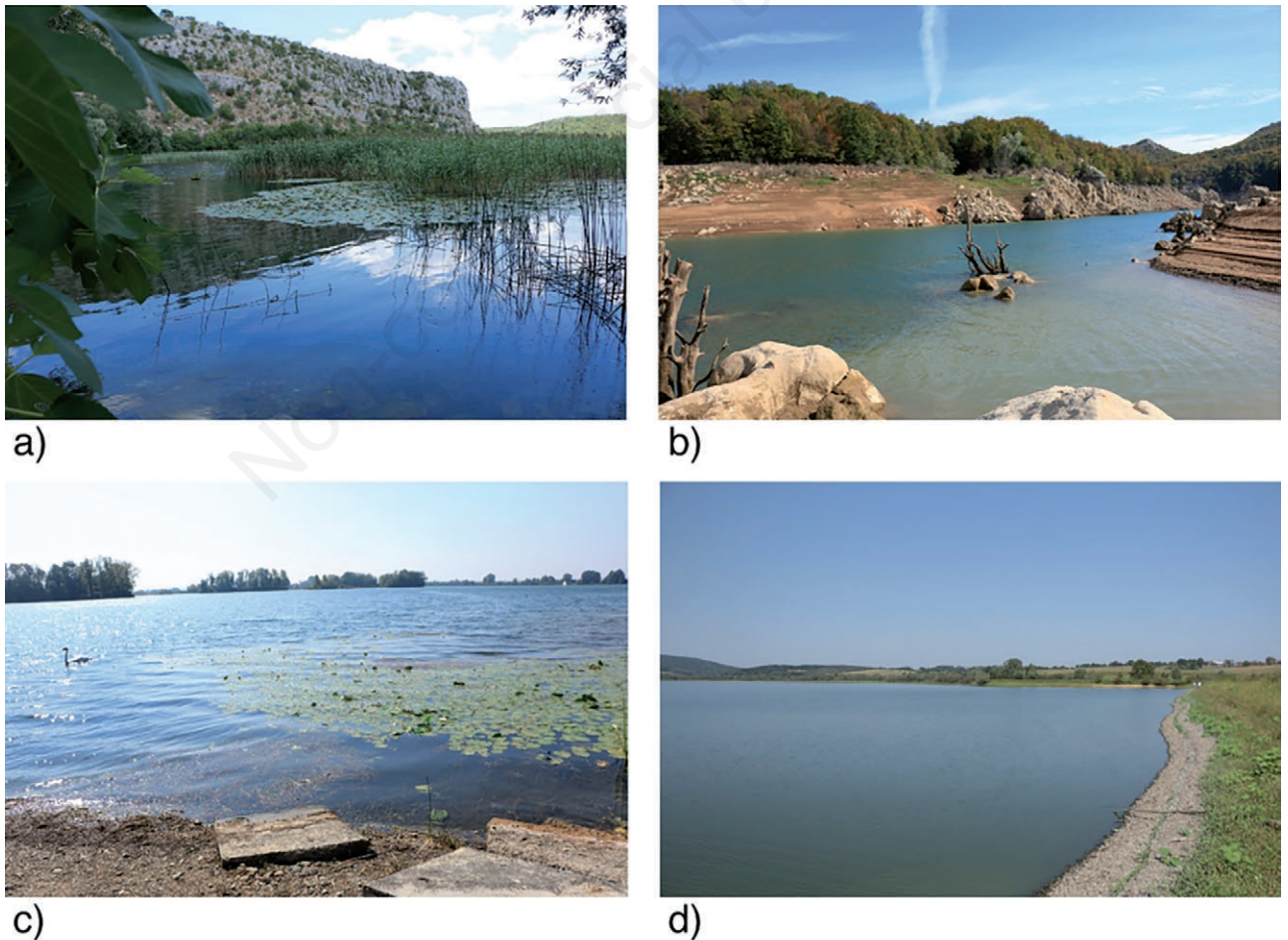
characteristics and orthophosphates as the major eutrophication driver.

The Bray-Curtis similarity index, HCA and NMDS analyses were conducted in Primer 6 (Clarke and Gorley, 2006). The CCA analysis was performed using CANOCO 5.00 (ter Braak and Šmilauer, 2012). Pearson's coefficient of correlation was calculated using Statistica 10.0 (Statsoft, 2010). All figures were processed with Adobe Illustrator CS6.

## RESULTS

### Mayfly assemblages

A total of 27,769 individuals were collected and identified, belonging to 21 species (Tab. 2). The number of individuals is presented per meter square (Tab. 2). The most widespread was *Cloeon dipterum* (Linnaeus, 1761), recorded at 27 study sites, while *Caenis horaria* Linnaeus,



**Fig. 2.** Examples of the study sites in the Dinaric Western Balkan ecoregion a) Brljan, b) Krušćica and in the Pannonian Lowland ecoregion c) Šoderica Koprivnica and d) Popovac.

**Tab. 2.** Mayfly taxa recorded at 36 man-made lentic habitats in Croatia. Taxa codes are those used in the CCA analysis.

Ecoregion (ER) Mayfly taxa/Study site	Dinaric Western Balkan Ecoregion (ER 5)																						
	Taxa codes	B	BU	BR	GO	GP	KR	LP	LE	LO	NJ	OP	PE	PO	PR	PB	RA	RI	SA	ST	TR	VL	
<i>Caenis</i> sp.*	a	0	0	0	0	0	0	0	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caenis horaria</i> Linnaeus, 1758	b	746	0	0	0	715	0	1640	0	4003	0	2010	0	0	0	122	125	64	2635	131	45	328	
<i>Caenis lactea</i> (Burmeister, 1839)	c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caenis luctuosa</i> (Burmeister, 1839)	d	0	114	1	0	0	2	0	12	0	0	0	0	0	0	0	91	78	0	0	118	134	
<i>Caenis</i> cf. <i>rivulorum</i> Eaton, 1884	e	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caenis robusta</i> Eaton, 1884	f	0	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Alainites muticus</i> (Linnaeus, 1758)	g	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baetis</i> sp.*	h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Baetis liebenauae</i> Keffermüller, 1974	i	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Baetis rhodani</i> (Pictet, 1843)	j	0	0	21	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cloeon</i> sp.*	k	0	0	0	0	0	1	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0
<i>Cloeon dipterum</i> (Linnaeus, 1761)	l	81	32	2	731	233	1	2	67	2	6	814	0	27	9	1	0	0	1907	0	5	29	
<i>Cloeon simile</i> Eaton, 1870	m	0	0	0	0	0	2	0	0	0	11	0	474	28	0	0	34	0	0	0	13	0	0
<i>Procloeon bifidum</i> (Bengtsson, 1912)	n	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Procloeon pennulatum</i> (Eaton, 1870)	o	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Centropitulum lateolum</i> (Müller, 1776)	p	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Paraleptophlebia</i> cf. <i>submarginata</i> (Stephens, 1835)	r	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ephemera glaucops</i> Pictet, 1843	s	0	230	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
<i>Ephemera lineata</i> Eaton, 1870	t	0	0	32	0	0	0	0	0	0	0	0	2	0	0	0	4	0	0	0	0	0	0
<i>Ephemera danica</i> Müller, 1764	u	0	0	0	0	0	0	0	0	0	0	37	0	0	0	0	0	0	0	0	0	0	0
<i>Ephemera vulgata</i> Linnaeus, 1758	v	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Serratella ignita</i> (Poda, 1761)	w	0	0	8	60	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Siphonurus aestivalis</i> Eaton, 1903	x	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Potamanthus luteus</i> (Linnaeus, 1767)	y	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Species richness		3	4	7	4	3	3	4	4	2	2	3	2	2	4	3	4	2	2	2	3	3	
Abundance (individuals/m <sup>2</sup> )		828	400	66	820	950	5	1646	117	4006	18	2861	476	70	14	123	254	142	4542	144	169	491	

To be continued on next page

Tab. 2. Continued from previous page.

Taxa codes	Pannonian Lowland Ecoregion (ER II)														
	BJ	SO	RK	BO	LA	JO	NC	PA	SK	PP	JA	GR	CK	DU	VA
<i>Caenis</i> sp.*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caenis horaria</i> Linnaeus, 1758	0	104	61	39	0	2	50	4	0	0	17	119	14	174	2
<i>Caenis lactea</i> (Burmeister, 1839)	0	0	0	0	3	0	0	0	0	0	0	0	2	0	0
<i>Caenis luctuosa</i> (Burmeister, 1839)	0	0	206	60	57	19	110	10	0	0	57	1	817	1482	141
<i>Caenis</i> cf. <i>rivulorum</i> Eaton, 1884	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caenis robusta</i> Eaton, 1884	4	0	0	0	0	0	0	3	34	0	0	20	0	0	0
<i>Alainites muticus</i> (Linnaeus, 1758)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baetis</i> sp.*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baetis liebenauae</i> Keffermüller, 1974	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baetis rhodani</i> (Pictet, 1843)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cloeon</i> sp.*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cloeon dipterum</i> (Linnaeus, 1761)	2	6	5	2	1	0	0	0	4	0	0	4	1	16	85
<i>Cloeon simile</i> Eaton, 1870	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Procloeon bifidum</i> (Bengtsson, 1912)	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Procloeon pennulatum</i> (Eaton, 1870)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Centroptilium luteolum</i> (Müller, 1776)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paraleptophlebia</i> cf. <i>submarginata</i> (Stephens, 1835)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ephemera glaucops</i> Pictet, 1843	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ephemera lineata</i> Eaton, 1870	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ephemera danica</i> Müller, 1764	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
<i>Ephemera vulgata</i> Linnaeus, 1758	0	0	0	2	0	0	0	0	0	0	2	0	0	0	0
<i>Serratella ignita</i> (Poda, 1761)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Siphonurus aestivalis</i> Eaton, 1903	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Potamanthus luteus</i> (Linnaeus, 1767)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Species richness	2	3	3	4	3	2	2	3	2	0	3	4	5	4	4
Abundance (individuals/m <sup>2</sup> )	6	111	271	102	61	21	159	17	38	0	76	144	834	1676	230

\*Juvenile or damaged individuals, not identifiable to the species level. Study site abbreviations are presented in Fig. 1.

1758 was the most abundant. Several species were recorded at a single site, with *Alainites muticus* (Linnaeus, 1758), *Procladius bifidus* (Bengtsson, 1912) and *P. pennulatum* (Eaton, 1870) as the rarest species (Tab. 2). The record of *Siphonurus aestivalis* Eaton, 1903 is a first for Croatia.

Species richness ranged between zero species recorded at Popovac and seven at Brljan. Abundance was highest at Sabljaci (4542 individuals/m<sup>2</sup>), and lowest at Krušćica (5 individuals/m<sup>2</sup>), both located in the Dinaric Western Balkan ecoregion. Nevertheless, sites in this ecoregion had markedly higher abundance than sites in the Pannonian Lowland ecoregion (Tab. 2).

A 20% similarity of mayfly assemblages was recorded among the study sites. In NMDS analysis (Fig. 3, 2D stress=0.1), sites were partially grouped in several clusters with 40% similarity based on their position in one of the ecoregions.

At the majority of sites, assemblage structure was dominated by lower reaches and lentic elements (potamal and littoral elements), and by detritivores (gatherers/collectors and active filter feeders) (Fig. 4). Golubić, Lešće and Prančević had a higher percentage of spring and upper reaches (crenal and rhithral) elements compared to other sites, while Golubić, Peruća, Njivice and Ponikve had similar shares of grazer/scrappers and detritivores.

### Mayflies and environmental variables

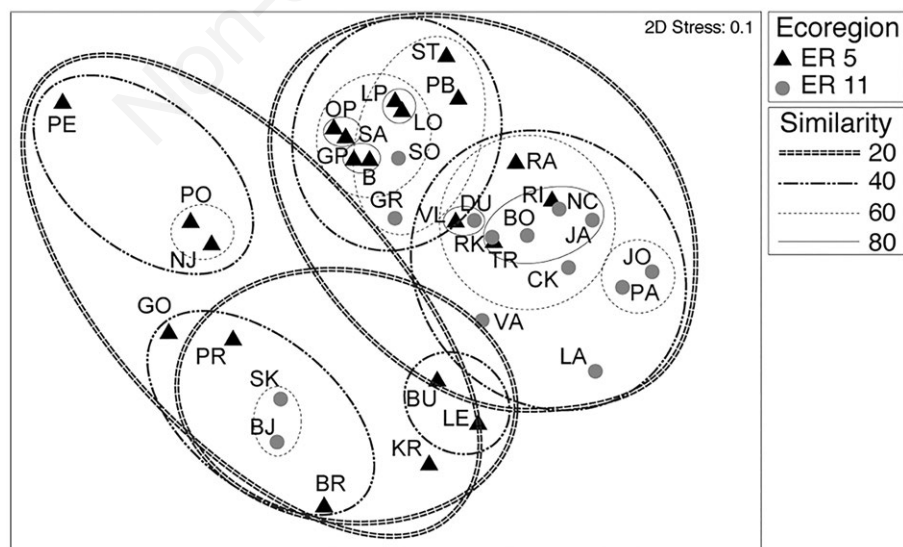
The results of the ordination of species and environmental data of the CCA are presented on the F1 ×

F2 ordination plot (Fig. 5). The eigenvalues for the first two CCA axes were 0.45 and 0.31 and explained 55.5% of the species-environment relations. The Monte Carlo permutation test showed that the species-environment ordination was significant (first axis: F-ratio=8.37, P=0.002; overall: trace=1.37, F=2.37, P=0.002) indicating that mayfly assemblages were significantly related to the tested set of environmental variables. Axis 1 was related to orthophosphates (R=0.66) and chemical oxygen demand (R=0.56), indicating that these were the most important parameters in explaining patterns of mayfly assemblages.

Additionally, a positive correlation was found between intensive agriculture and orthophosphates (Pearson's correlation coefficient; r=0.43; P<0.01), while natural and semi-natural areas correlated negatively with orthophosphates but without statistical significance.

### DISCUSSION

The studied man-made lentic habitats showed relatively high total mayfly species richness, with 25% of Croatian mayfly fauna (Vilenica *et al.*, 2015, 2016c; Dekić *et al.*, 2016). Nevertheless, the richness per site was relatively low in comparison to the literature (Bauernfeind and Soldán, 2012; Vilenica *et al.*, 2014; Vilenica *et al.*, 2016b, 2016c). In addition to generally low mayfly diversity in lentic habitats, the low species richness recorded at most sites is likely the result of the strong



**Fig. 3.** Non-metric multidimensional scaling (NMDS) ordination of mayfly assemblages based on the Bray-Curtis similarity coefficient (group average linking) and their log-transformed abundances at 36 man-made lakes in Croatia. Study site abbreviations are presented in Fig. 1.



anthropogenic pressures on these habitats, resulting in high organic and inorganic pollutant loads, high daily and seasonal water level fluctuations, low habitat heterogeneity and consequently poorer food resource diversity. Anthropogenic pressures on freshwater habitats cause loss of environmental quality by altering soil use, riparian habitats, nutrient balance, sedimentation, substrate composition (Nessimian *et al.*, 2008), resulting in changes to species composition, abundance and assemblage structure (Allan, 2004; Yoshimura, 2012; Brasil *et al.* 2014).

These results indicated that the eurytopic and euryvalent *Cloeon dipterum* (Bauernfeind and Soldán, 2012; Buffagni *et al.*, 2009; 2018) was the most widespread species. The predominantly lentic *Caenis*

*horaria* (Bauernfeind and Soldán, 2012; Buffagni *et al.*, 2009; 2018) was most abundant, which was also seen in the natural lakes in the same region (Vilenica *et al.*, 2014). As expected, the assemblage composition mainly consisted of taxa of potamal (lower reaches) and lentic (*e.g. Caenis horaria*, *C. lactea*, *C. robusta*) or wide range habitat type preferences (*e.g. Cloeon dipterum*, *Centroptilum luteolum*, *Ephemera danica*, *Serratella ignita*) (Bauernfeind and Soldán, 2012; Buffagni *et al.*, 2009; 2018). Sites under the strong influence of medium and large karst rivers (*i.e.*, sites Brljan, Golubić, Lešće and Prančević) were an exception due to a higher percentage of spring and upper reaches (crenal and rhithral) elements. High abundance of the eurytopic *Cloeon dipterum*, and presence of lotic species such as

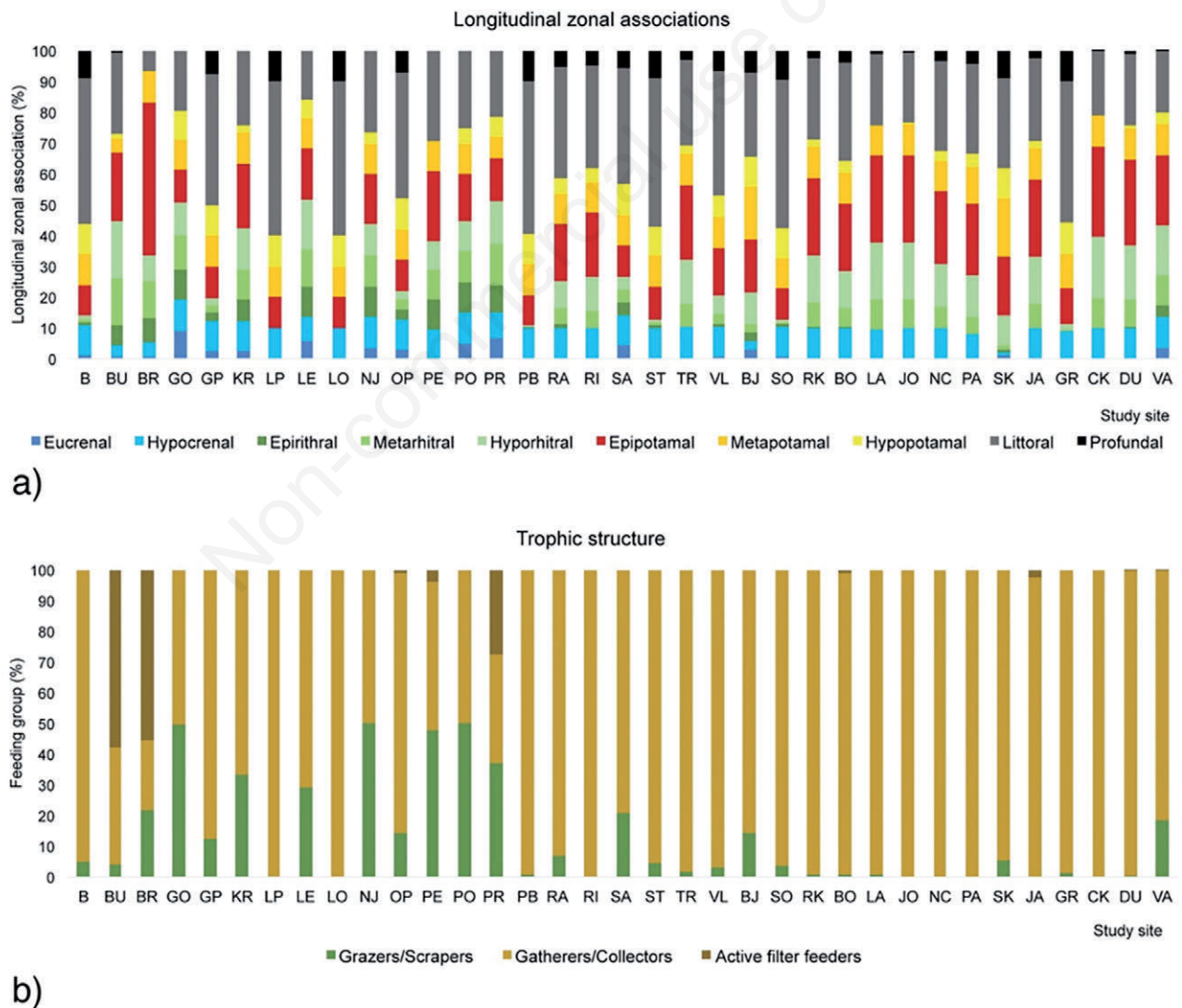


Fig. 4. a) Longitudinal zonal associations and b) trophic structure of mayfly assemblages at 36 man-made lakes in Croatia. Study site abbreviations are presented in Fig. 1.

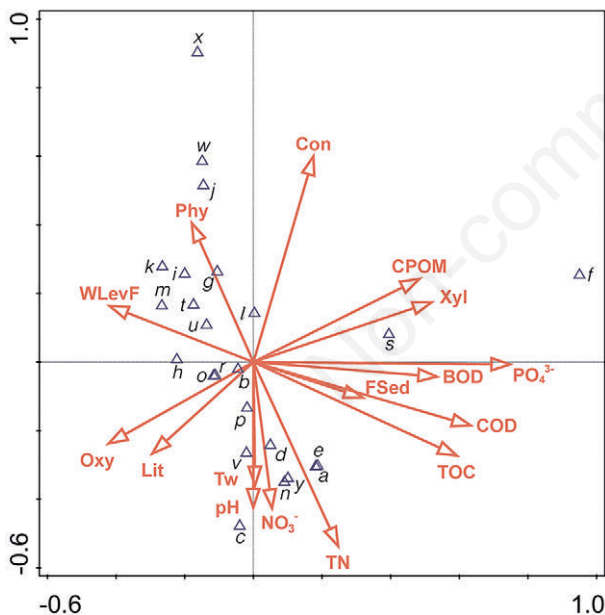
*Alainites muticus*, *Baetis liebenauae* and *Baetis rhodani* were recorded (Bauernfeind and Soldán, 2012; Buffagni *et al.*, 2009; 2018). Due to the low microhabitat diversity with a high level of sedimentation, the domination of detritivores is not surprising. Sites with higher shares of lithal and phytal (Golubić, Peruća, Njivice and Ponikve) elements had similar shares of detritivores and grazers/scrapers, due to the presence of species such as *Baetis rhodani*, *Cloeon dipterum*, *C. simile*, *Serratella ignita* (Bauernfeind and Soldán, 2012; Buffagni *et al.*, 2009, 2018).

Due to the low number of species recorded at the majority of sites, and the rather similar species composition, the NMDS analysis showed poor separation of study sites based on mayfly assemblages. Only partial separation was observed based on the position of a site within the ecoregion, as species such as *Ephemera glaucops*, *Baetis liebenauae* and *Alainites muticus* were observed only in ER 5, and species such as *Potamanthus luteus*, *Caenis lactea*, *Ephemera vulgata* and *Proclotron bifidum* inhabited only man-made lakes in ER 11. This is partially in agreement with the studies of Vilenica *et al.* (2015) and Dekić *et al.*

(2016), where *A. muticus* and *C. lactea* were also recorded only in ER 5 and ER 11, respectively. Other mentioned species were previously recorded from both ecoregions (Vilenica *et al.*, 2015). Moreover, the record of *E. glaucops* is the first confirmation of the species presence in Croatia, as it was reported only by Bauernfeind and Soldán (2012), without reference to exact localities (see also in Vilenica *et al.*, 2015).

Since mayflies have clear preferences for physical and chemical characteristics of their environment, such as specific water velocity, water temperature, nutrient concentrations, hydraulic conditions (Brittain and Saltveit, 1989) and river section (Buffagni *et al.*, 2007; 2009), they are highly sensitive to habitat alteration and are among the first macroinvertebrate taxa to disappear, which was confirmed with the results of the CCA analysis. According to the analysis, mayfly assemblages were most influenced by orthophosphate concentration and chemical oxygen demand, reflecting the level of anthropogenic pollution and eutrophication, especially in ER 11. Effects of intensive agriculture on littoral benthic assemblages are indirect, such as habitat destruction and nutrient loading, which leads to eutrophication processes (Fraterrigo and Downing, 2008). Numerous studies on aquatic macroinvertebrates, including mayflies, showed that eutrophication is the main water quality problem in reservoirs leading to shifts in their biological assemblages and loss of species diversity (Vollenweider and Kerekes, 1982; Brittain and Saltveit, 1989; Wetzel, 2001; Lock and Goethals, 2013; Phillips *et al.*, 2016; Chirwa and Chilima, 2017; Mazur *et al.*, 2017; Buczyńska *et al.*, 2018). Therefore, sites located in the Pannonian Lowland ecoregion are under greater influence of various kinds of pollution from land use, industry, agriculture (for 7 of 15 man-made lakes, the share of intensive agriculture in the catchment is greater than 57%), and their mayfly assemblages are characterized by markedly lower abundances, sometimes even with lower species richness. Beketov *et al.* (2013) showed that in areas with intensive agricultural activities where the water was contaminated with pesticides, invertebrate species richness dropped by 42%.

Annual drawdowns can decrease the taxonomic richness of benthic invertebrates and their densities are often lowest in lakes with large (*e.g.* >3 m) drawdown amplitudes (Carmignani and Roy, 2017; Grimås, 1965). Mayflies were not recorded in the Popovac Reservoir, while in the Kruščica Reservoir they were present in very low abundances (only 5 individuals per square meter). Both reservoirs are characterized by large water level fluctuations. Nevertheless, when all the investigated reservoirs were considered, no significant correlations were determined between mayfly population densities and annual changes in water level fluctuations. The samples were collected during the low water summer period, and



**Fig. 5.** F1 x F2 plane of the Canonical Correspondence Analysis (CCA) based on 21 mayfly taxa and 16 environmental variables. For the abbreviations of the taxa codes (blue triangle symbols) see Tab. 2. Environmental variables (red arrow symbols): Phy, phytal; WLevF, water level fluctuation; Oxy, dissolved oxygen concentration; Lit, lithal; Tw, water temperature;  $\text{NO}_3^-$ , nitrates; TN, total nitrogen; TOC, total organic carbon; COD, chemical oxygen demand; BOD, biological oxygen demand; FSed, fine sediment;  $\text{PO}_4^{3-}$ , orthophosphates; Xyl, xylal; CPOM, coarse particulate organic material; Con, conductivity.

we assume that mayflies, as r-selected life history strategists, can rapidly recover in this season (Brittain and Saltveit, 1989; Carmignani and Roy, 2017).

Systematic research on Croatian mayflies began relatively recently (see in Vilenica *et al.*, 2015), which is why our knowledge still not complete, and is growing with each new study. The results presented here are an important contribution to the knowledge of Croatian mayflies, presenting new distributional data, new habitat type preferences and tolerance to anthropogenic disturbances for the recorded species. Moreover, several species considered to be rare in Croatian freshwaters were recorded, such as *Caenis lactea*, *Cloeon simile* and *Ephemera glaucops*, while the find of *Siphonurus aestivalis* is a new species record for the Croatian mayfly fauna (Vilenica *et al.*, 2015, 2016b; Dekić *et al.*, 2016), raising the number of species to a total of 83. The species has a wide European distribution, inhabiting macrophytes or leaf litter in lentic sections of rivers, ponds and littoral zone of the lakes, usually at elevations lower than 600 m (Bauernfeind and Soldán, 2012). In the present study, the species was recorded in Golubić Reservoir in ER 5, as a lake strongly influenced by the Butižnica River and the presence of required microhabitats.

## CONCLUSIONS

With a new species record for the country, this study showed that the knowledge of the Croatian mayfly fauna is still growing. Moreover, our results enrich the previously scarce knowledge about the relationship between littoral mayfly assemblages and environmental parameters in man-made lakes. Despite the relatively high total species richness, the number of mayfly species per site is rather low due to severe changes in habitat morphology, hydrology, and physicochemical water parameters. The difference between assemblages in the Dinaric Western Balkan ecoregion and Pannonian Lowland ecoregion was rather high, as the more polluted habitats in the Pannonian Lowland ecoregion supported much poorer mayfly assemblages, especially in terms of their abundance. Damming caused a high level of assemblage “potamalization”, as they were dominated by lentic species together with species with wide ecological tolerance. Only sites under strong influence of karst rivers had slightly different assemblage composition, including lotic species.

Identifying and understanding the relationships between mayflies and environmental conditions in anthropogenically pressured habitats can contribute to species conservation, habitat management and restoration activities. Additionally, these results could be applied in creating a monitoring system for man-made lakes according to the requirements of the European Water Framework Directive.

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