



Benthic Macroinvertebrate Assemblages in Karst Springs Ecosystems in Bulgaria

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Abstract: Ecosystems of karst springs are insufficiently studied in Bulgaria. This study was focused on bottom macroinvertebrate assemblages from this river type. Their taxonomic diversity, community structure and habitat preferences in relation with the environmental factors were examined. During the summer of 2020, seven rivers originating from karst spring areas were sampled at sites being located at different distances from the sources. Diverse communities regarding their taxonomic richness and abundance were found, with 148 taxa enlisted, identified to the lowest possible level (species, genus or family). The strong variability of the number of taxa (S, from 6 to 51), absolute abundance (N, from 351 to 7956 specimens) and the values of the structural parameters (diversity indices, dominance and evenness) proved the highly variable characters of these benthic communities, which predetermined the low level of similarity at the taxon level (less than 21%). The correlation among biota and environmental variables such as altitude, distance to source, substratum type and physical and chemical parameters reflected both abiotic specificities and their impact on the benthic communities at a local and catchment scale.

Key words: macrozoobenthos, community structure, environmental heterogeneity, Bulgaria

Introduction

Karst springs are the natural outflows of karst groundwater onto the surface. They are transitional ecosystems between groundwater and surface aquatic habitats (BOTOSANEANU 1998, BARQUÍN & SCARSBOOK 2007). As such, they have heterogeneous and variable characteristics, which harbour specific biotic communities (KOPERSKI et al. 2011). Although differing in the way they are formed, karst springs have a number of common features, such as unstable discharge regime and highly variable flow,

depending on the elevation of the water table. Karst waters are considered as one of the global and sensitive water sources under a high risk of pollution (STEVANOVIĆ 2018).

Recently, the knowledge on the springs' biota has increased significantly due to the active studies on this aquatic environment. The influence of the low fluctuations of water temperature (VON FUMETTI et al. 2017), relatively constant water chemistry (GLAZIER 1991, ORENDT 2000) and discharge regime (flow variability versus flow permanence) (WOOD et al. 2005) have been considered to be im-

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portant factors determining spring macroinvertebrate communities (SMITH & WOOD 2002).

Karst terrains occupy c. 23% of the Bulgarian territory and include about 90 karst springs (POPOV 1970). Most typical are the large springs giving rise to rivers. According to the national typology in Bulgaria, such aquatic ecosystems belong to the R15 River Type *Karst springs and other source communities*. They are quite variable in terms of hydro-morphological characteristics and their macroinvertebrate fauna is still poorly studied in Bulgaria. The available data on the zoobenthos diversity of spring areas are related mainly to faunistic studies on systematics of some insect groups. BRAASCH & JOOST (1971) reported two stonefly species (Plecoptera) from the spring area of Botunya River (NE Bulgaria). From the nearby karst area, VIDINOVA & RUSSEV (1997) mentioned the finding of a mayfly species (Ephemeroptera) in the karst spring Ruska Bela (Mezdra Municipality, NE Bulgaria). Scarce data on caddisflies (Trichoptera) from springs are presented by KUMANSKI (1969, 1975a, b, 1979, 1985, 1988, 2004) based mostly on records from the 1920s till the 1970s.

The focus of the current study was the bottom macroinvertebrate assemblages with their taxonomic diversity and abundance, community structure, degree of similarity and relations to the environment.

Materials and Methods

Study area

For the purposes of the study, seven rivers of karst origin in various regions in Bulgaria were sampled in August and September 2000 (Table 1). They belonged to Ecoregion 12 (Pontic Province) and Ecoregion 7 (Eastern Balkans) and to the following river catchments: Iskar (ZlaPa), Osam (Maara), Provadiyska (Devne), Veleka (Aider and Dokuz), Mesta (Iz-

tok) and Struma (Petro) (Fig. 1, Table 1). The sampling sites were located at different distances to the sources and only two of them could be considered as samples of the source areas (ZlaPa and Maara).

Environmental variables

The following physical and chemical water parameters were measured *in situ* using portable multi-parameter instruments: temperature (WT), hydrogen ion concentration (pH), conductivity (EC, $\mu\text{S}\cdot\text{cm}^{-1}$), dissolved oxygen concentration (DO, $\text{mg}\cdot\text{dm}^{-3}$) and saturation (O_{SAT} %). The content of some biogenic elements was analysed in a certified laboratory: total nitrogen (TN, $\text{mg}\cdot\text{dm}^{-3}$), ammonium nitrogen ($\text{NH}_4\text{-N}$, $\text{mg}\cdot\text{dm}^{-3}$), nitrite nitrogen ($\text{NO}_2\text{-N}$, $\text{mg}\cdot\text{dm}^{-3}$), nitrate nitrogen ($\text{NO}_3\text{-N}$, $\text{mg}\cdot\text{dm}^{-3}$) and total phosphorus (TP, $\text{mg}\cdot\text{dm}^{-3}$) as well as biological oxygen demand (BOD, $\text{mg}\cdot\text{dm}^{-3}$) (Table 2).

A description of the relative proportion of bottom substrata (in %) was also performed before sampling, distinguishing the following types: bedrock (Bdr), big stones >40 cm (St>40), stones 20-40 cm (St_20-40), middle-sized stones 6-20 cm (St_6-20), small stones 2-6 cm (St_2-6), gravel (Gr), sand (Sa), silt (Si), higher plants (HP) and coarse woody debris (CDW) (Table 2).

Benthic macroinvertebrate sampling

The collection of macroinvertebrates followed the pro-rata multi-habitat sampling approach, using a hand net (mesh size 500 μm) and a set of hydrobiological sieves for sandy and silt substrata (CHESHMEDJIEV et al. 2011) according to the standards BDS EN ISO 10870:2012 and EN 16150:2012. The laboratory processing included separation of the macroinvertebrates by systematic groups, followed by taxonomic determination to the lowest possible level and enumeration of specimens. The abundance of each taxon was presented as a percentage of the total numbers per sample. All collected and deter-

Table 1. List of the sampling sites with geographical attributes.

River	Site	Site code	Coordinates		Altitude (m)	Distance to the source (km)
			N	E		
Zlatna Panega	at spring area	ZlaPa	43.5304	24.9381	181	0.2
Maarata	upstream Krushuna Village	Maara	43.14765	25.020051	144	0.25
Devnenska	before the river mouth	Devne	43.179109	27.626486	4	25
Aidere	at Stoilovo Village	Aider	42.03337	27.51272	213	13
Dokuzak	downstream Malko Tarnovo	Dokuz	42.028969	27.503175	241	10
Iztok	before the mouth in Mesta River	Iztok	41.89277	23.54382	759	16
Petrovska	before the mouth in Pirinska Bistritsa River	Petro	41.40653	23.43201	174	27

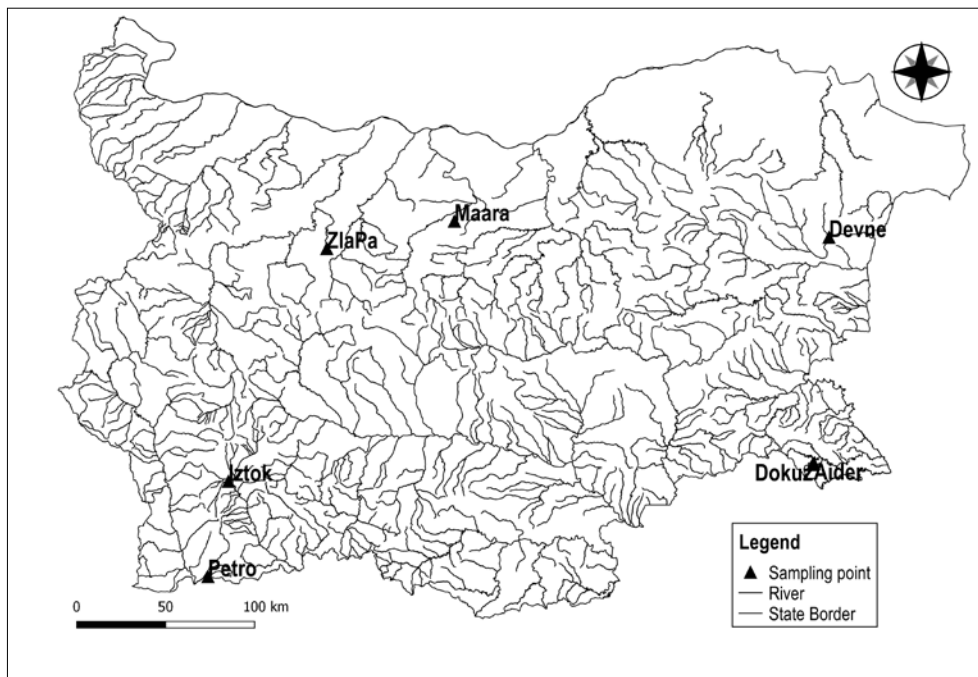


Fig. 1. Map with the location of the studied sites (for site abbreviations, see Table 1).

mined specimens were preserved and stored in 80 % ethanol. The nomenclature of the macroinvertebrates in the enclosed taxonomic list followed in general Fauna Europaea (DE JONG et al. 2014). Determination keys and morphological descriptions for different macroinvertebrate groups or single taxa were used (HRBÁČEK 1980, NAGEL 1989, SCHMIDT 1993, WARINGER & GRAF 2011, BAUERNFIEND & SOLDÁN 2012, ANDERSEN et al. 2013, BITUŠÍK & Hamerlík 2014, DE WALT et al. 2021).

Data analyses

In order to characterise the bottom macroinvertebrate communities, the following metrics and indices were calculated: number of taxa (S), total abundance (N), relative abundance per taxon (%), frequency of occurrence of the species (pF, %, DE VRIES 1937), species diversity – H (SHANNON & WEAVER 1949), species richness – d (MARGALEF 1958), evenness (e) (PIELOU 1966) and index of dominance (c) (SIMPSON 1949).

Pearson correlation was performed using the Data Analyses tool of Microsoft Excel (2010). The presence of statistically significant correlations between the physical and chemical water parameters, measured *in situ* and the parameter “number of taxa per site” on one hand and ten types of substrata, distance to the source and the altitude on the other was tested. Principal Component Analysis (PCA) in the CANOCO 5 program for Windows was applied to explore the inherent patterns within distance to the

source, altitude, substratum types and the measured environmental factors – WT, DO, TN, TP, BOD, pH and EC.

Primer v6 statistical package was used to determine the similarity between the macrozoobenthos of the surveyed sites. The macroinvertebrate taxon richness for each site was obtained and Bray-Curtis index of similarity between the sites was calculated. A cluster analysis (Ward’s method, complete linkage) was performed based on both presence/absence and abundance of taxa. Non-parametric distance-based linear model regression was also used to analyse the relationship between the biological (abundance) and environmental (physical and chemical variables) data matrices. Prior to analyses, biological data were square-root transformed and environmental data were log transformed and normalised.

Results

Environmental variables

The study sites were located in two altitude ranges: up to 200 m and up to 800 m a.s.l., between 4 m (Devne) and 759 m a.s.l. (Iztok). The water temperature and pH varied in narrow ranges: from 14.1°C to 18.6°C and from 7.55 to 8.4, respectively (Table 2). A more significant difference between the minimum and maximum values were recorded for EC (with a difference of 371 $\mu\text{S}\cdot\text{cm}^{-1}$), oxygen indicators (DO with a difference of 5.47 $\text{mg}\cdot\text{dm}^{-3}$ and O_{sat} – 55.3%), BOD (11.59 $\text{mg}\cdot\text{dm}^{-3}$), TN (8.55 $\text{mg}\cdot\text{dm}^{-3}$) and ni-

Table 2. Values of physical and chemical parameters and distribution of the substratum types per site (%). For site codes, see Table 1.

Sites	ZlaPa	Maara	Devne	Aider	Dokuz	Iztok	Petro
Parameters							
T °C	15	16.7	17.9	15.4	14.8	14.1	18.6
EC	346	495	667	473	543	296	410
pH	7.55	8.01	7.9	8.3	8.4	7.61	7.61
DO	6.35	9.62	4.15	9.23	9.08	9.2	7.7
O _{SAT}	61.7	98.9	43.6	94.7	91.2	94	89
BOD	2.13	1.52	12.5	1.16	0.91	1.79	1.17
TN	1.04	8.97	0.96	0.42	2.39	1.17	0.56
NH ₄ N	0.01	0.01	0.03	0.01	0.01	0.11	0.01
NO ₂ N	0.01	0.01	0.067	0.01	0.01	0.07	0.01
NO ₃ N	0.94	8.52	0.665	0.409	0.688	0.59	0.57
TP	0.031	0.023	0.163	0.01	0.01	0.13	0.042
Substratum type							
Bedrock	0	0	0	40	50	20	0
Stones >40	0	0	0	0	0	50	20
Stones 20-40	0	5	10	0	0	10	50
Stones 6-20	0	25	0	20	20	0	0
Stones 2-6	10	5	10	10	20	0	10
Gravel	5	5	0	10	0	0	0
Sand	45	30	0	20	10	20	0
Silt	30	0	20	0	0	0	0
Higher plants	10	20	50	0	0	0	10
Coarse woody debris	0	10	10	0	0	0	10

trates (8.11 mg.dm⁻³) (Table 2). No significant correlations were found except a negative one bordering on significance between EC and altitude ($r = -0.713$; $p = 0.072$).

Concerning the microhabitat diversity, overall ten types of substrata in different proportions were identified at the observed localities. The small stones (St₂₋₆) were present at all sites except Iztok but with low percentages (up to 20 %). The most pronounced variety of substrata was registered at Maara (7 types), followed by ZlaPa, Devne, Aider, Petro (5 types) and Dokuz and Iztok (4 types). The dominance of one type (45-50 %) or related ones (e.g., different sizes of stones) was observed at all the studied sites, except for Maara as mentioned above (Table 2).

Taxon diversity

During the study, we found in total 148 taxa, of which 67 were determined to the species level, 57 to the generic level and 21 to the family level. They all belonged to 20 benthic systematic groups, 71 families and 98 genera. The nematodes, water mites

and gammarids were not identified in detail (Table 3). Regarding the proportion of the numbers of families, genera and species established within each systematic group, the most diverse (with more than ten taxa) were Oligochaeta, Gastropoda, Ephemeroptera, Coleoptera, Trichoptera and Diptera (Fig. 2, Table 3). The groups Hydrozoa, Gordioidea, Decapoda and Megaloptera were the poorest ones, found with one taxon each (Table 3).

Despite the rich taxon diversity recorded, the frequency of occurrence of individual species as a whole remained quite low (Table 3). The most common were only two species: *Asellus aquaticus* (Isopoda) and *Baetis rhodani* (Ephemeroptera) ($pF = 57.14$), followed by *Pisidium (Casertiana) casertanum* (Bivalvia) and *Ibisia marginata* (Diptera) ($pF = 42.86$). The rest of the species were found twice (18 species, $pF = 28.57$) or only once (42 species, $pF = 14.29$). None of the caddis species was recorded at more than two sites. The genus *Hydropsyche* was the most frequent one, recorded at four sites (Table 3). Of the family Chironomidae (Diptera), representatives of 13 genera were registered, of which

Table 3. List of the macroinvertebrate taxa established during the study with the relative abundance (%) and frequency of occurrence (pF).

Taxa	ZlaPa	Maara	Devne	Aider	Dokuz	Iztok	Petro	pF
Hydrozoa								
<i>Hydra</i> sp.			0.10			0.62		
Turbellaria								
<i>Dugesia lugubris</i> – polychroa		0.09	0.10					28.57
<i>Dugesia</i> sp.				1.04				
<i>Girardia tigrina</i> (Girard, 1850)			0.25					14.29
<i>Polycelis felina</i> (Dalyell, 1814)		3.32				41.34		28.57
Nematoda, indet.				0.06		0.22		
Gordioidea								
<i>Gordius</i> sp.		0.04						
Oligochaeta								
<i>Stylaria lacustris</i> (Linnaeus, 1767)			0.90					14.29
<i>Aulodrilus plurisetia</i> (Piguet, 1906)			0.10					14.29
<i>Psammoryctides albicola</i> (Michaelsen, 1901)				0.12				14.29
<i>P. barbatus</i> (Grube, 1861)		0.17						14.29
<i>Limnodrilus claparedianus</i> Ratzel, 1869			0.05			3.64		28.57
<i>Limnodrilus</i> sp.	2.28			0.31		0.56	2.03	
<i>Potamothrix hammoniensis</i> (Michaelsen, 1901)			0.05					14.29
<i>Rhyacodrilus coccineus</i> (Vejdovsky, 1876)						0.11	0.51	28.57
Tubificinae, gen. sp.	1.42		0.10	0.31		0.11		
<i>Lumbriculus variegatus</i> (Müller, 1774)			0.05	0.67				28.57
Lumbriculidae, gen. sp.	1.14							
Lumbricidae, gen. sp.		0.04					0.76	
Hirudinea								
<i>Erpobdella octoculata</i> (Linnaeus, 1758)		0.04				0.22		28.57
Erpobdellidae, gen. sp., juv.			0.25			1.23		
<i>Glossiphonia complanata</i> (Linnaeus, 1758)			0.20			0.11		28.57
Gastropoda								
<i>Bithynia tentaculata</i> (Linnaeus, 1758)			1.61					14.29
<i>Potamopyrgus antipodarum</i> (Gray, 1843)			1.16					14.29
<i>Bythinella</i> sp.		15.59						
Hydrobiidae, gen. sp.			14.78					
<i>Valvata piscinalis</i> (O. F. Müller, 1774)	13.68		28.91					14.29
<i>Acroloxus lacustris</i> (Linnaeus, 1758)				0.06				14.29
<i>Radix auricularia</i> (Linnaeus, 1758)	0.28							14.29
<i>Peregriana labiata</i> (Rossmässler, 1835)	0.28							14.29
<i>Physella acuta</i> (Draparnaud, 1805)			0.70					14.29
<i>Physa fontinalis</i> (Linnaeus, 1758)			0.45					14.29
<i>Ancylus fluviatilis</i> O. F. Müller, 1774	0.28							14.29
<i>Gyraulus (Armiger) crista</i> (Linnaeus, 1758)			0.05					14.29
<i>G. (Torquis) laevis</i> (Alder, 1838)			0.10					14.29
<i>Planorbis planorbis</i> (Linnaeus, 1758)			0.10					14.29
<i>Theodoxus fluviatilis</i> (Linnaeus, 1758)			5.88					14.29
Bivalvia								

Table 3. Continuation.

Taxa	ZlaPa	Maara	Devne	Aider	Dokuz	Iztok	Petro	pF
<i>Pisidium (Pseudeupera) subtruncatum</i> Malm, 1855			6.28					14.29
<i>P. (Casertiana) casertanum</i> (Poli, 1791)		0.04	11.56				1.78	42.86
<i>Pisidium</i> sp.							0.51	
Amphipoda								
<i>Gammarus</i> sp.	35.33	64.60	1.81	91.58	96.06		0.51	
Isopoda								
<i>Asellus aquaticus</i> (Linnaeus, 1758)	3.42		5.93			4.15	0.25	57.14
<i>Jaera</i> sp.			0.05					
Decapoda								
<i>Potamon ibericum</i> (Bieberstein, 1809)							0.76	14.29
Hydracarina, indet.	11.40		11.76				0.25	
Ephemeroptera								
<i>Baetis buceratus</i> Eaton, 1870			0.05			0.90		28.57
<i>B. fuscatus</i> (Linnaeus, 1761)						1.23		14.29
<i>B. melanonyx</i> (Pictet, 1843)						0.11		
<i>B. muticus</i> (Linnaeus, 1758)		0.04				6.16		28.57
<i>B. pavidus</i> Grandi, 1949							4.06	14.29
<i>B. rhodani</i> (Pictet, 1843)		0.47		0.24		4.15	17.77	57.14
<i>B. vernus</i> Curtis, 1834						4.26		14.29
<i>Baetis</i> sp.			0.15	0.18		6.72		
<i>Centroptilum luteolum</i> Müller, 1776	9.97	0.04						28.57
<i>Ecdyonurus (Helvetoraeticus)</i> sp.						0.22		
<i>E. (Ecdyonurus)</i> sp.							5.08	
<i>Ecdyonurus</i> sp.				0.06			6.60	
<i>Ephemerella danica</i> Müller, 1764		2.71			0.38			28.57
<i>Habroleptoides</i> sp.				0.06				
<i>Ephemerella ignita</i> Poda, 1761						4.93		14.29
<i>Caenis macrura</i> Stephens, 1835							7.61	14.29
<i>C. pseudorivulorum</i> Keffermüller, 1960						0.28		14.29
<i>C. robusta</i> Eaton, 1884			1.16					14.29
Odonata								
<i>Ischnura elegans</i> (Linden, 1820)			0.35					14.29
Coenagrionidae, gen. sp.			0.90					
<i>Calopteryx splendens</i> (Harris, 1780)			0.10				2.03	28.57
<i>C. virgo</i> (Linnaeus, 1758)	3.42							14.29
<i>Platycnemis pennipes</i> (Pallas, 1771)			0.75					14.29
<i>Libellula</i> sp.			0.05					
<i>Gomphus vulgatissimus</i> (Linnaeus, 1758)							6.09	14.29
<i>Onychogomphus forcipatus</i> Linnaeus, 1758						0.11	2.54	28.57
<i>Brachytron pratense</i> (Müller, 1764)							1.78	14.29
Aeshnidae, gen. sp.		0.04						
Plecoptera								
<i>Leuctra albida</i> Kempny, 1899						0.67		14.29
<i>L. fusca fusca</i> (Linnaeus, 1758)							15.23	14.29

Table 3. Continuation.

Taxa	ZlaPa	Maara	Devne	Aider	Dokuz	Iztok	Petro	pF
Coleoptera								
<i>Platambus maculatus</i> (Linnaeus, 1758) Ad	1.14					0.11		28.57
Dytiscidae, gen. sp. La						0.11		
Dytiscidae, gen. sp. Ad			0.05					
<i>Gyrinus</i> sp. Ad							1.27	
<i>Haliplus ruficollis</i> (De Geer, 1774)			0.20					14.29
<i>Helophorus</i> sp. La						0.11		
Hydrophilidae, gen. sp. La			0.05					
<i>Limnebius</i> sp. La		0.04						
<i>Ochthebius</i> sp. La						0.11		
<i>Hydraena</i> sp. Ad				0.67				
Dryopidae, gen. sp. Ad				0.06				
<i>Elmis</i> sp. La		0.04						
<i>Esolus</i> sp. La		7.88						
<i>Limnius</i> sp. La							1.02	
<i>Oulimnius</i> sp. La				0.85				
<i>O.</i> sp. Ad				1.22				
Elmidae, gen. sp. La				0.37	1.34			
Elmidae, gen. sp. Ad	3.42			0.06	1.44			
<i>Helodes</i> sp.			0.05	0.24				
Coleoptera, indet. Ad		0.17						
Heteroptera								
<i>Gerris</i> sp.		0.09						
Gerridae, gen. sp.							0.51	
<i>Velia</i> sp.		0.17						
Veliidae, gen. sp.			0.05					
<i>Mesovelgia</i> sp.		0.04	0.05					
<i>Plea minutissima minutissima</i> Leach, 1817			0.25					14.29
Megaloptera								
<i>Sialis</i> sp.		0.04						
Trichoptera								
<i>Hydropsyche angustipennis</i> (Curtis, 1834)	0.28							14.29
<i>H. bulbifera</i> McLachlan, 1878						0.56		14.29
<i>H. incognita</i> Pitsch, 1993						3.03	2.28	28.57
<i>Hydropsyche</i> sp.		0.04				8.74	0.51	
<i>Plectrocnemia</i> sp.	0.28							
<i>Polycentropus</i> sp.						0.22		
<i>Lype reducta</i> (Hagen, 1868)		0.09						14.29
<i>Tinodes</i> sp.		0.17			0.19			
<i>Brachycentrus maculatus</i> (Fourcroy, 1785)						0.06	8.63	28.57
<i>Halesus digitatus</i> (Schrank, 1781)						0.17		28.57
<i>Halesus</i> spp.							0.25	
Limnephilidae, gen. sp.		0.04						
<i>Helicopsyche</i> cf. <i>bacescui</i> Orghidan & Botosaneanu, 1953				0.06				14.29

Table 3. Continuation.

Taxa	ZlaPa	Maara	Devne	Aider	Dokuz	Iztok	Petro	pF
Sericostoma flavicorne/personatum		0.78		0.06				28.57
Hydroptila sp.	0.57		0.20					
Orthotrichia sp.			0.30					
Oxyethira sp.			0.15					
Hydroptilidae, gen. sp.			0.25					
Gen. sp., pupae		0.04	0.35					
Rhyacophila sp.		0.04					0.25	
Diptera								
Chironomidae								
Polypedilum sp.	3.42		0.25	0.18		1.57	3.81	
Paratanytarsus sp.				1.16		0.45		
Micropsectra sp.	5.70							
Tanytarsini, gen. sp.		0.43						
Brillia sp.						0.34		
Corynoneura sp.	1.14							
Cricotopus sp.			0.35				3.30	
Epoicocladus sp.						0.11		
Eukiefferiella sp.						0.56		
Tvetenia sp.						0.11		
Ablabesmyia sp.	1.14				0.10			
Conchapelopia sp.		1.98	0.20			0.22		
Procladius sp.							1.27	
Simuliidae, gen. sp.		0.09				1.12		
Tipulidae								
Tipula sp.							0.25	
Limoniidae								
Hexatoma sp.				0.06			0.25	
Athericidae								
Ibisia marginata (Fabricius, 1781)		0.30		0.12			0.25	42.86
Athericidae, gen. sp.					0.48			
Tabanidae								
Tabanus sp.		0.04						
Muscidae								
Limnophora riparia (Fallen, 1824)						0.22		14.29
Ceratopogonidae								
Bezzia sp.		0.09						
Dixidae								
Dixella sp.		0.09						
Psychodidae								
Psychoda sp.		0.04						
Tonnoiriella sp.				0.06				
Stratiomyidae								
Oxycera sp.				0.12				
Sciomyzidae, gen. sp.			0.40					

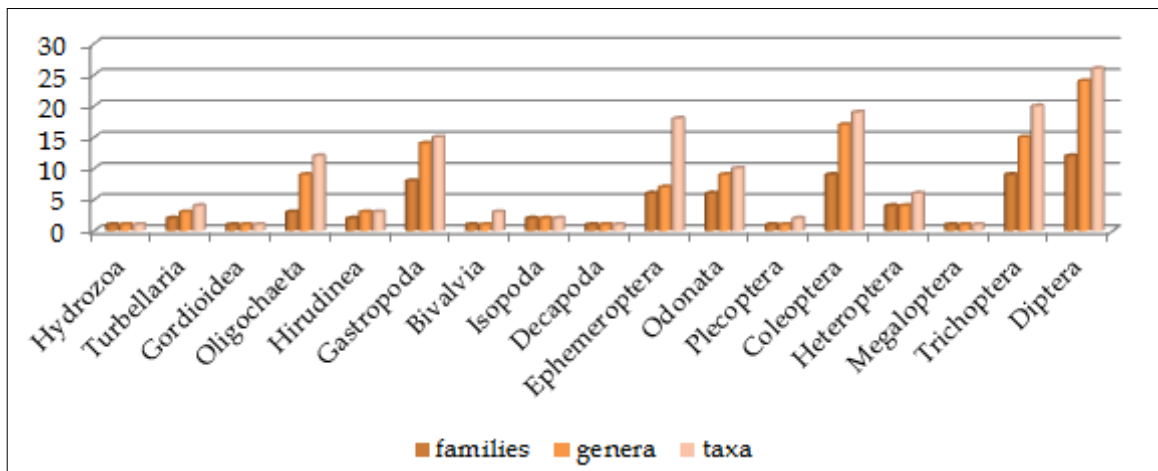


Fig. 2. Diversity of taxa at different taxonomic levels per benthic groups.

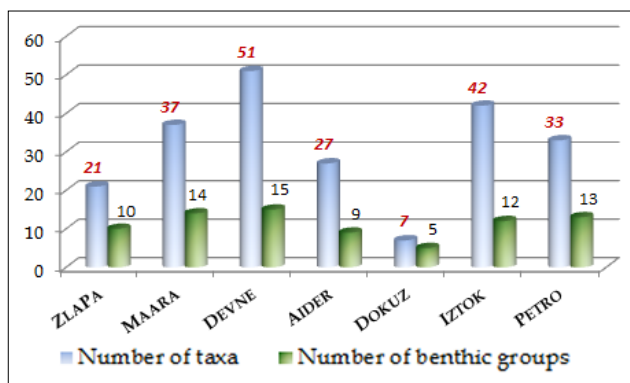


Fig. 3. Taxa richness at the studied sites. For site abbreviations, see Table 1.

only the genera *Polypedilum*, *Cricotopus*, *Paratanytarsus* and *Conchapelopia* were recorded two times or more within the studied sites (Table 3).

The taxon richness of the macrozoobenthos measured by the number of taxa (S) varied widely. The most diverse communities were established in Devne (51 taxa), followed by Iztok (42), Maara (37) and Petro (33). The poorest was the community in the Dokuzak River, where the number of recorded taxa was only seven. In parallel with this, the number of benthic groups per site generally followed the same pattern as the number of taxa, varying in wide ranges: from 15 (Devnenska River at its mouth) to five (Dokuzak River) (Fig. 3).

Abundance

The absolute abundance of the macroinvertebrates at each site also varied considerably: from 351 specimens (ZlaPa) to 7,956 specimens (Devne). The main systematic groups with the highest abundance were Gastropoda and Amphipoda (more than 4,000 ind. within all samples), followed by Bivalvia (more than 1000 ind.), Turbellaria, Hydracarina

and Ephemeroptera (between 800 and 1000 ind.) and Isopoda, Coleoptera, Trichoptera and Diptera (less than 600). The detailed analysis showed strong dominance of individual taxa and groups (with total abundance more than 1000 individuals each), which was due to the higher abundance of the communities in five of the sampling sites (Fig. 4a). The most abundant taxa were the family Hydrobiidae (Gastropoda) (at Maara and Devne, Fig. 4c, d), *Valvata piscinalis* (Gastropoda) (Devne, Fig. 4d), *Gammarus* sp. (Amphipoda) (Fig. 4c, d, e, f), Hydracarina (Devne, Fig. 4d), *Polycelis felina* (Turbellaria) (Iztok, Fig. 4g) and *Pisidium casertanum* (Bivalvia) (Devne, Fig. 4d), with a number of individuals varying between 738 and 2300 ind. per sample (Fig. 4).

Structure of communities

The values of the coenotic indices of diversity “H”, species richness “d”, dominance “c” and evenness “e” varied considerably among the separate sites (Fig. 5 A, B). Diversity indices were higher for Devne, Iztok and Petro ($H > 3.4$ and $d > 12$) while the lowest values were established for the Dokuz: $H = 0.32$, $d = 1.99$ and $e = 0.11$, accompanied with the very high dominance ($c = 0.92$). Although with lower values compared to the above-mentioned sites, the coenotic indices for ZlaPa showed a balanced macroinvertebrate community. The situation was the opposite for Maara and Aider sites, with quite low Shannon diversity and reverse trend of the ratio between evenness and dominance, particularly pronounced for Aider ($c = 0.84$) (Fig. 5B).

Taxonomic similarity

The cluster dendrogram of similarity among the sampling sites proved the notably different character of the studied benthic communities, which was predetermined by the low level of compliance.

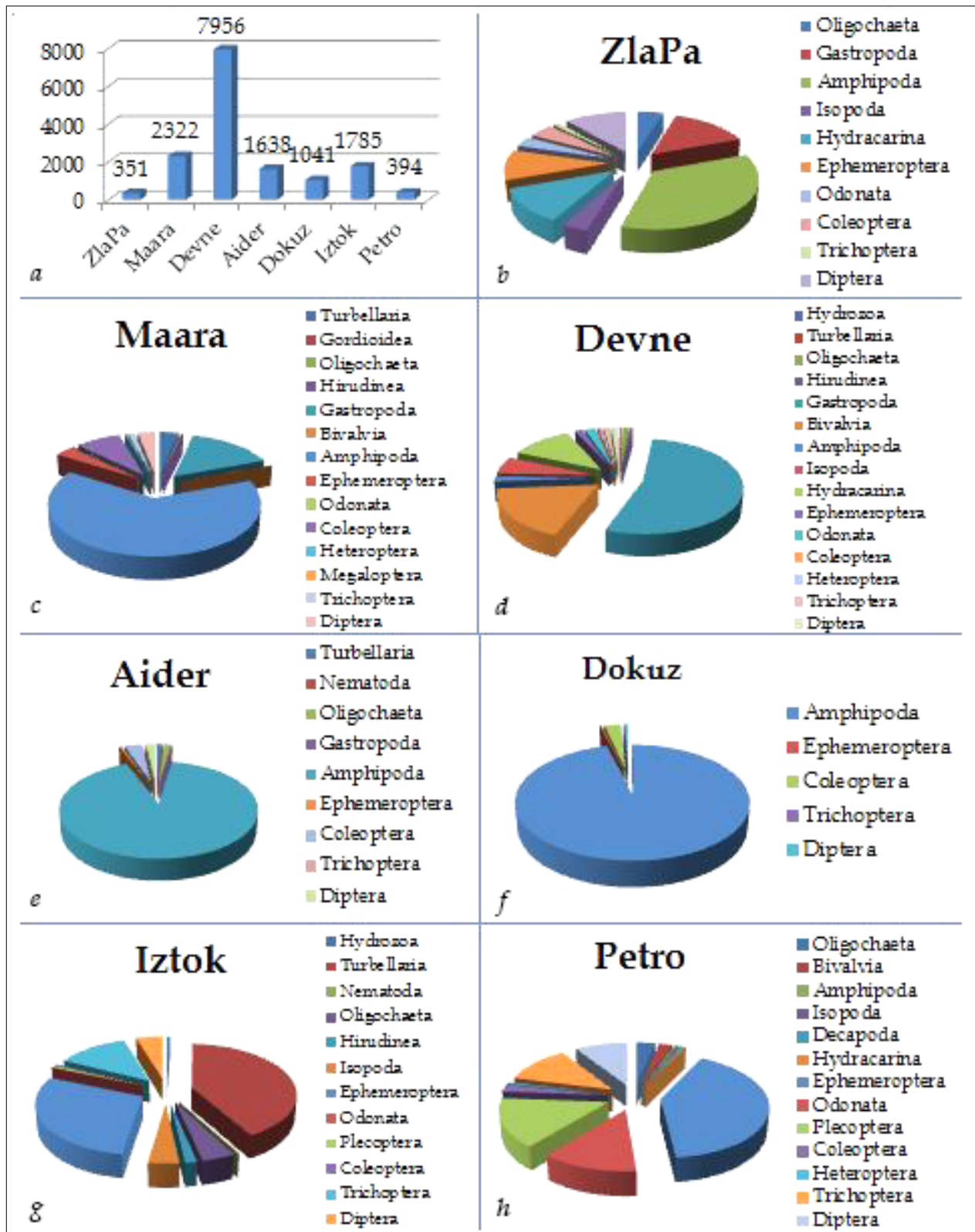


Fig. 4. Total numbers (a) and abundance of the benthic groups per sampling site (b, c, d, e, f, g, h). For site abbreviations, see Table 1.

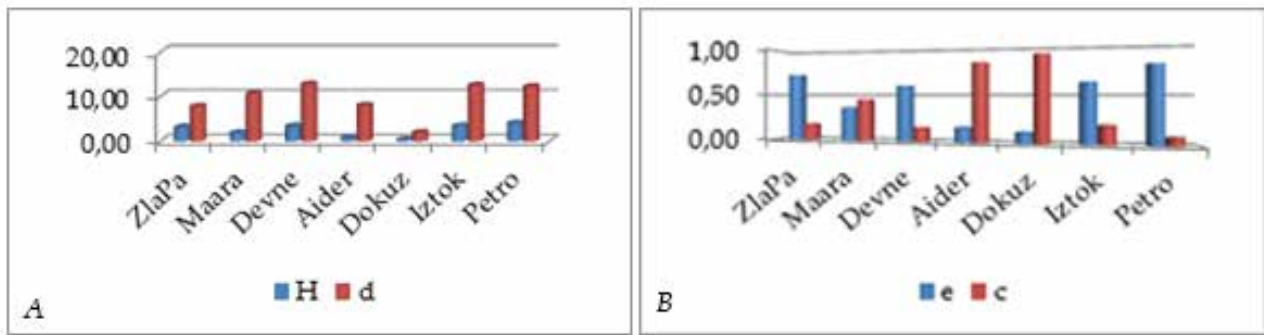


Fig. 5. Values of the coenotic indices: **A** – indices of diversity “H”, “d”; **B** – dominance “c” and evenness “e”. For site abbreviations, see Table 1.

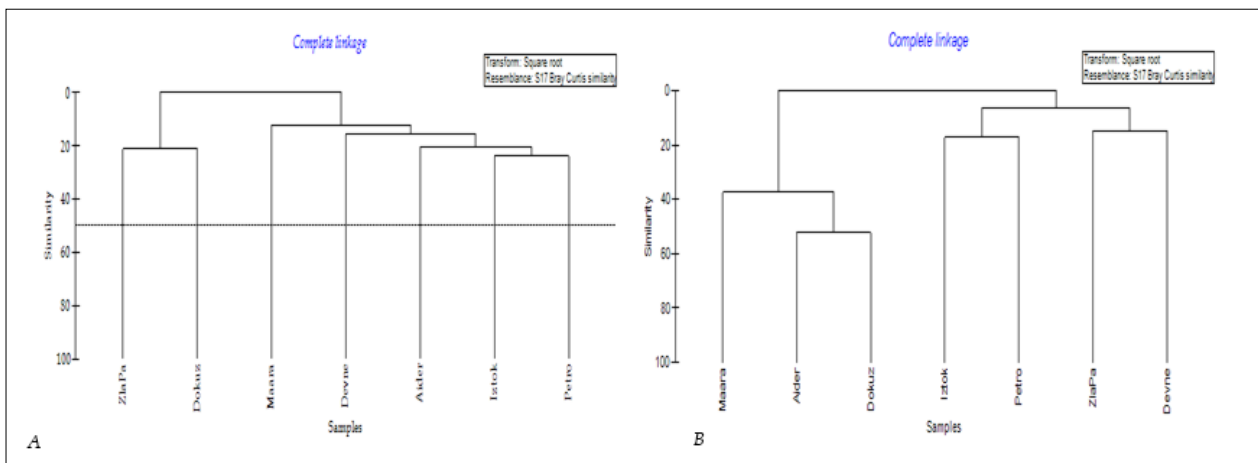


Fig. 6. Cluster dendrograms of similarity between the sampling sites based on: **A** – presence/ absence of taxa, **B** – abundance. For site abbreviations, see Table 1.

ZlaPa and Dokuz only were in a separate cluster, where the least similar benthic taxa and systematic groups have been established. Overall, the resemblance among the sites was relatively low and did not exceed 21% (Fig. 6A). The second dendrogram of similarity based on the abundance of taxa per sites, grouped into a separate cluster Maara, Aider and Dokuz, where strong dominance of the family Gammaridae was registered. The rest of the sites were separated in a group with relatively low level of similarity (Fig. 6B).

Relation with the aquatic environment

A PCA, presented on a correlation biplot diagram, summarised the variation of the environmental factors within the studied sites/ rivers (Fig. 7). PCA statistics showed that the first two principal components (eigenvalues $\lambda_1 = 0.347$; $\lambda_2 = 0.240$) cumulatively explained 58.77% of the total variance of the data.

Axis 1 reflected two gradients: the first positively correlated with WT (0.730), BOD (0.762), HP (0.905), CWD (0.600) and Si substrata (0.536); the

second correlated negatively with the DO (− 0.853) and separated the Devne with both high WT, high percentage of HP substratum (50%) and lowest values of the DO. The first axis was also positively associates to distance to the source (0.511), TP (0.596) and middle-sized stone substratum (0.518), and separated the Petro sampling site, which was the most remote from its spring and the middle-sized stones were presented to the greatest extent.

Axis 2 was also followed two gradients: the first one positively correlated with altitude (0.865) and St>40 substratum (0.947) and separated the Iztok as this site was located at the highest altitude and predominant substratum consisted of bigger stones. The second gradient was negatively related with pH (− 0.707), total nitrogen (− 0.297), gravel (− 0.265) and small-sized stone substrata. It separated the Maara with the highest value of TN, Dokuz – with the highest values of pH and Maara, Dokuz and Aider with the largest proportion of gravel and small-sized stone substrata. The ZlaPa site did not group with any of the other sites (Fig. 7), both due to its proximity to the spring

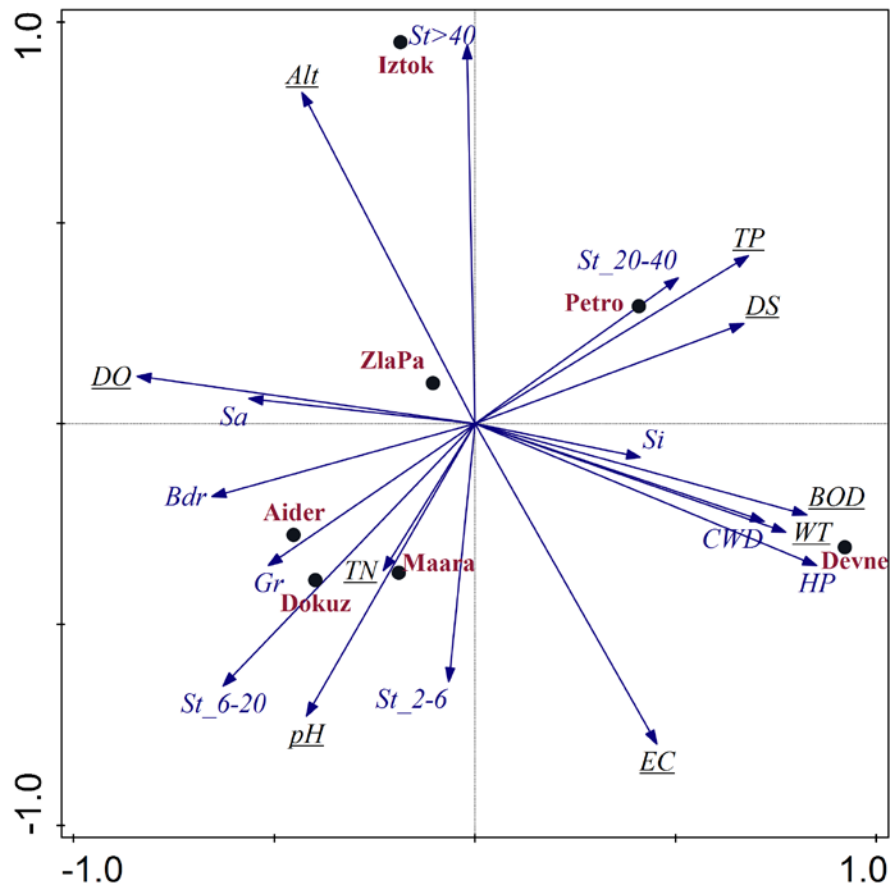


Fig. 7. PCA correlation biplot based on the altitude, distance to the sources, substratum types and physical and chemical parameters of seven sites of the studied karst rivers. For abbreviations, see Tables 1 and 2.

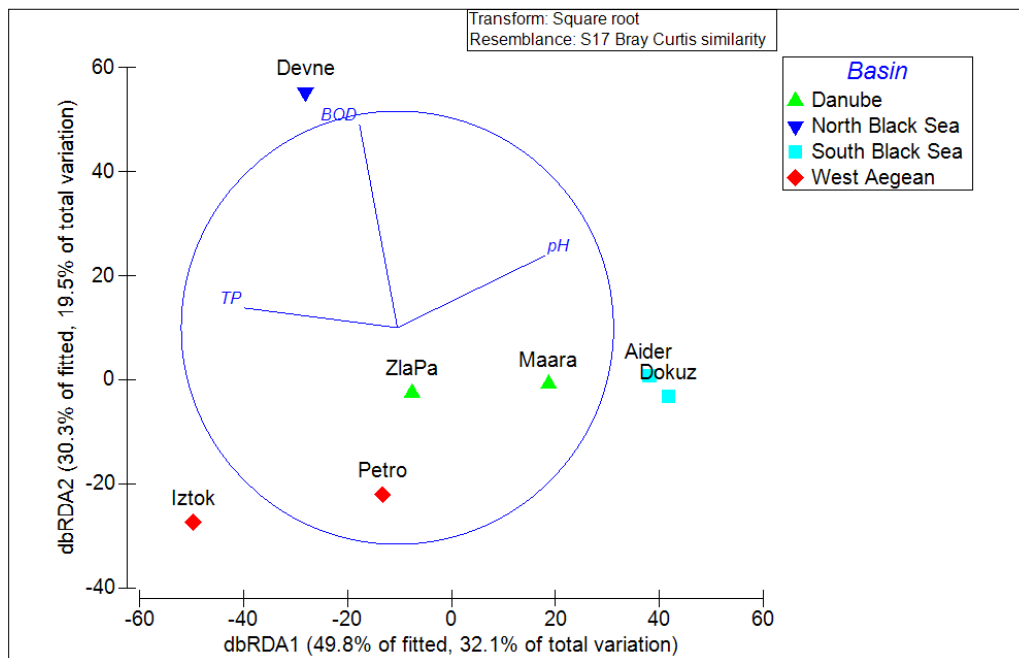


Fig. 8. Distance-based Redundancy Analysis (dbRDA) plot diagram of the biological and environmental variables matrices (the first axis explained 32.1% and the second axis – 19.5% of the total variation of macroinvertebrate abundance by taxa, in correspondence to the environmental variables (TP, BOD and pH) at the studied sampling sites. For site abbreviations, see Table 1.

source and the predominant sandy substratum at the sampling site (45%).

According to the non-parametric distance-based linear model regression, only biological oxygen demand (BOD), pH and total phosphorus (TP) as variables had statistical significance (0.049, 0.047 and 0.015, respectively). The sampling sites were grouped on the ordination plot by basin where the rivers were located (Danube, North Black Sea, South Black Sea and West Aegean) (Fig. 8). Only Devne (North Black Sea Basin) had considerably higher value of BOD together with high concentration of TP (Table 2). This corresponded with the values of oxygen parameters, which were the lowest at this sampling site. Iztok and Petro (West Aegean Basin) had the lowest pH and higher values of TP, while in turn Aider and Dokuz (South Black Sea Basin) were with the highest pH values and the lowest TP concentration (Table 2). The sampling sites within the Danube River Basin (ZlaPa and Maara) were located at an intermediate position between the West Aegean and South Black Sea Basins, mainly due to their values of pH and TP: ZlaPa was closer to the group of West Aegean Basin and Maara was closer to the South Black Sea Basin group (Fig. 8).

Discussion

Karst springs and other spring ecosystems is a specific river type in Bulgaria and refers to river sections near the springs. By definition, this type includes river sections located no further than 5 km away from the springs (PASSPORTS OF NATIONAL RIVER TYPES IN BULGARIA). It is a highly heterogeneous “point” type, common throughout the territory of Bulgaria in both eco-regions – Eastern Balkans (7th) and Pontic Province (12th). The most typical are the large karst springs, giving rise to rivers and having a strong influence of groundwater.

Because of the high habitat complexity and the large number of different types, springs are considered as unique aquatic habitats that contribute significantly to local and regional biodiversity (CANTONATI et al. 2012). According to HYNES (1970), the studied karst ecosystems belong to distinct categories in terms of their spring nature, i.e., limnocrone (ZlaPa) and rheocrone (all others).

Compared to the data from other European regions, the benthic macroinvertebrate fauna of the karst ecosystems in Bulgaria (148 taxa, 20 benthic systematic groups, 74 families, 98 genera enlisted, see Table 3) can be defined as quite rich and diverse at regional scale. MORI (2003) reported 76 taxa and 33 families from 16 karst springs from the Slove-

nian part of the Julian Alps. Forty-nine families or subfamilies and four higher taxa (Hydrozoa, Turbellaria, Nematoda and Hydracarina) were found in the springs from Kraków-Częstochowa Upland, Poland (DUMNICKA et al. 2007).

We established taxa, which are both commonly found in diverse aquatic environments and others that are very rare. E.g., within the caddisflies, all the three taxa of the genus *Hydropsyche* identified to the species level are defined as common or very common for Bulgaria, while *B. maculatus* is very rarely found in the country but, when recorded, it is very abundant (KUMANSKI 1988). This is the case also in this study. Most of the genera of the family Chironomidae recorded are common in Bulgaria, with the exception of the genus *Epoicocladius*, which is relatively rarely found in other Bulgarian rivers, lakes and dams. The mayfly *B. rhodani*, which exhibits the highest pF in our study, could be found in a wide range of microhabitats, yet due to its preference for moderate water velocity (VILENICA et al. 2018); this species reaches the highest number of individuals preferably at stony substrata (Iztok and Petro sites).

Concerning the taxon richness, our data confirmed that midge taxa were much more dissimilar than the environmental characteristics of the springs they inhabited (PŁÓCIENNIK et al. 2016). This was valid even for springs that were located nearby (LENCIONI et al. 2011). Chironomids did not achieve high abundance and frequency (LENCIONI et al. 2011, 2012). Nevertheless, the subfamily Orthoclaudiinae stood out as the richest group (also confirmed by PŁÓCIENNIK et al. 2016), with seven taxa out of 13 recorded for the whole family.

The springs are characterised by the stability of many environmental parameters such as water temperature, certain water chemistry parameters and bottom substratum type (DUMNICKA et al. 2007). However, relatively permanent environmental parameters could be disturbed due to the longitudinal changes downstream the outflowing streams.

Due to the different distance of the sampling sites from the sources and the river catchments affiliation, in some cases we have recorded significant variations in abiotic characteristics between the sites. The most pronounced difference in values was observed for Devnenska River, the upper stretch of which was dried up and the sampling site was located in a section with very slow water flow. At this site, we recorded the lowest concentration and saturation of oxygen, most likely also due to the presence of abundant submerged higher vegetation (Fig. 9). These levels of oxygen were directly related to the extreme high value of BOD (12.5 mg.dm⁻³).



Fig. 9. General view of the Devnenska River before the river mouth (Photo by B. Gyosheva).

Obviously, the overgrowth of aquatic plants and algae consumes dissolved oxygen, even when they decompose. In addition, the concentrations of TP and $\text{NH}_4\text{-N}$ had higher values than at the other sites, which indicated a significant organic load on the river in this section and corresponded to moderate ecological status (RBMP-BSRB, 2016-2021). WILLIAMS et al. (1997) also reported significant differences among the spring communities associated with different levels of urbanisation and water chemical conditions.

It is considered that the karst systems have characteristically high levels of water hardness, alkalinity and pH (JENNINGS 1985, cited after RIGHI-CAVALLARO et al. 2010). The values of pH registered during the study were in the alkaline spectrum (7.55 and above). GOVONI et al. (2018) found that the chironomid abundances were negatively correlated with pH values, i.e., non-chironomid taxa dominated the spring communities at higher pH. According to our data, the abundance of chironomid larvae (as a percentage of the total numbers) was higher at the stations with lower pH (ZlaPa, Iztok and Petro). CARROLL & THORP (2014) found that chironomid midges were not the most numerous group within the insects in spring brooks and they represented 1–3 % of the overall abundance in each spring during the summer. Our results confirmed these data, since the abundance of the chironomid larvae varied between 1 and 3% of the total abundance, with

the exception of ZlaPa and Petro samples where the percentage was 11% and 8%, respectively. In those samples, the lower abundance of the representatives of the other benthic taxa could be a possible reason for the higher percentage of chironomid larvae.

GLAZIER (1991) offered a hypothetical framework to explain the dominance of non-insect taxa in hard-water limestone springs, emphasising on the importance of a non-emergent lifestyle and high population densities in springs, as peracaridans, molluscs and triclads dominated the hard-water limestone springs. This fact is confirmed by our results, with the abundant presence of *P. felina* (Turbellaria) at Iztok and Maara, species of the family Hydrobiidae as well as *Valvata piscinalis* and *Theodoxus fluviatilis* (Gastropoda) at Devne and *Bythinella* sp. (Gastropoda) at Maara River site.

Many authors stressed on particular environmental factors influencing the diversity and abundance of benthic macroinvertebrates. According to DUMNICKA et al. (2007), substratum type is found to be the main discriminatory factor with regard to the fauna density, while faunal composition is related to the geographical position of springs. Altitude and dissolved oxygen content were found to be the most important environmental variables determining the EPT community structure in springs (PASTUCHOVA 2006). The author distinguished two types: (1) EPT communities of natural streams and (2) EPT communities of disturbed streams, where two types of

stressors were identified: organic pollution and low discharge. The orders Trichoptera, Odonata and Plecoptera are dependent on water temperature and on nitrates, phosphates and pH, which in turn depend on underground flow and influence upstream sites during different seasons. Moreover, the insect community is formed under specific physical and chemical conditions along the river course (RAĐA & ŠANTIĆ 2014). In karst rivers, HABDIJA et al. (2002) found significant differences in caddis community structure depended on vegetation cover associated with substratum type and river area.

Conclusions

Based on the obtained results, we can conclude that the benthic communities in the studied karst rivers are characterised by very diverse taxonomic composition. We recorded almost 150 taxa from seven karst springs as the most taxon-rich groups were the classes Oligochaeta and Gastropoda and the orders Ephemeroptera, Coleoptera, Trichoptera and Diptera. Overall, the communities of the studied karst springs were very different compared to each other as evidenced by their low level of similarity. This was likely owing to their variable flow and the different environmental conditions. Our analyses suggest that the habitat heterogeneity due to the substratum types as well as the natural change in the environmental factors downstream of each of the studied rivers are among the most important factors for the diversity and abundance of benthic communities. Future research will help to elucidate the mechanism of benthic community formation in these specific ecosystems.

Acknowledgements: We are grateful to MSc Borislava Gyocheva for photographs from the rivers within the Black Sea watershed. The field studies were funded by the World Bank, project # 71 957 35/17.4.2020, DICON-UBA; the analysis of the results has been carried out in the framework of the National Science Program “Environmental Protection and Reduction of Risks of Adverse Events and Natural Disasters”, approved by the Resolution of the Council of Ministers № 577/17.08.2018 and supported by the Ministry of Education and Science (MES) of Bulgaria (Agreement № D01-230/06.12.2018).

References

- ANDERSEN T., CRANSTON P. S. & EPLER J. H. (Eds.) 2013. The larvae of Chironomidae (Diptera) of the Holarctic Region – Keys and diagnoses. *Insect Systematics & Evolution*, Suppl. 66: 1–571.
- BARQUÍN J. & SCARSBROOK M. R. 2007. Management and conservation strategies for cold water springs. *Aquatic Conservation* 18(5): 580–591.
- BAUERNFEIND E. & SOLDAN T. 2012. The mayflies in Europe (Ephemeroptera). Ollerup, Denmark: Apollo Books. 781 p.
- BDS EN ISO 10870:2012 Water quality – Guidelines for the selection of sampling methods and devices for benthic macroinvertebrates in fresh waters.
- BITUŠÍK P. & HAMERLIK L. 2014. Identification key for Chironomidae larvae of Slovakia. Part 2. Tanypodinae. Belianum, Vydavateľstvo Univerzity Mateja Bela v Banskej Bystrici, Slovakia, 96 p. (In Slovak).
- BOTOSANEANU L. (Ed.) 1998. Studies in crenobiology. The biology of springs and springbrooks. Backhuys Publishers, Leiden, 261 p.
- BRAASCH D. & JOOST W. 1971. Neue Plecopterenfunde aus Bulgarien. *Entomologische Nachrichten* 15 (6):58–66.
- CANTONATI M., GERECKE R. & BERTUZZI E. 2012. Springs of the Alps – sensitive ecosystems to environmental change: from biodiversity assessments to long-term studies. In: Lami A. & Boggero A. (Eds.): *Ecology of High Altitude Aquatic Systems in the Alps*. *Hydrobiologia* 562: 59–96, DOI 10.1007/s10750-005-1806-9
- CARROLL T. M. & THORP J. H. 2014. Ecological shifts in diversity and functional traits in zoobenthic communities of karst springs. *Hydrobiologia* 738: 1–20. DOI 10.1007/s10750-014-1907-4
- CHESHMEDJIEV S., SOUFI R., VIDINOVA Y., TYUFEKCHIEVA V., YANEVA I., UZUNOV Y. & VARADINOVA E. 2011. Multi-habitat sampling method for benthic macroinvertebrate communities in different river types in Bulgaria. *Water Research and Management* 1(3): 55–58.
- DE JONG Y., VERBEEK M., MICHELSEN V., BJØRN P., LOS W., STEEMAN F., BAILLY N., BASIRE C., CHYLARECKI P., STLOUKAL E., HAGEDORN G., WETZEL F.T., GLÖCKLER F., KROUPA A., KORB G., HOFFMANN A., HÄUSER C., KOHLBECKER A., MÜLLER A., ANTON A., STOEVE P. & PENEV L. 2014. Fauna Europaea – all European animal species on the web. *Biodiversity Data Journal* 2: e4034. doi: 10.3897/BDJ.2.e4034.
- DE VRIES D. 1937. Methods used in scientific plant sociology and in agricultural botanical grassland research. *Herbage Review* 5 (4): 36–61.
- DEWALT R. E., NEU-BECKER U. & STUEBER G. 2021. Plecoptera species file online. Version 5.0/5.0. [retrieval date]. Available at: [Plecoptera.SpeciesFile.org].
- DUMNICKA E., GALAS J. & KOPERSKI P. 2007. Benthic invertebrates in karst springs: Does substratum or location define communities? *International Review of Hydrobiology* 92:452–464.
- FUMETTI VON S., WIGGER F. & NAGEL P. 2017. Temperature variability and its influence on macroinvertebrate assemblages of alpine springs. *Ecohydrology* 10 (7): 1878 p. 10.1002/eco.v10.710.1002/eco.1878
- GLAZIER D. S. 1991. The fauna of North American temperate cold springs: patterns and hypotheses. *Freshwater Biology* 26 (3): 527–542. 10.1111/j.1365-2427.1991.tb01417.x
- GOVONI D. P., KRISTJÁNSSON B. K., ÓLAFSSON J. S. 2018. Spring type influences invertebrate communities at cold spring sources. *Hydrobiologia* 808: 315–325. <https://doi.org/10.1007/s10750-017-3434-6>
- HABDIJA I., RADANOVIĆ I., PRIMC-HABDIJA B. & ŠPOLJAR M. 2002. Vegetation cover and substrate type as factors influencing the spatial distribution of trichopterans along a karstic river. *International Review of Hydrobiology* 87 (4): 423–437.
- HRBÁČEK J. 1980. Klíč vodních larev hmyzu. *Československá*

- Akademie Věd, Praha, 524 pp.
- HYNES, H.B.N. 1970. *The Ecology of Running Waters*. Univ. Toronto Press, Liverpool.
- KOPERSKI P., DUMNICKA E. & GALAS J. 2011. Abiotic parameters determining fauna composition in karstic springs. *Polish Journal of Ecology* 59 (1): 153–163.
- MARGALEF R. 1958. *Information Theory in Ecology*. *General Systems* 3: 36–71.
- KUMANSKI K. 1969. Contribution à l'étude des Trichoptères en Bulgarie. II (In Bulgarian, summaries in Russian and French). *Bulletin de l'Institut de zoologie et musée* 29: 175–181.
- KUMANSKI K. 1975a. La famille Glossosomatidae (Trichoptera) en Bulgarie. *Acta Zoologica Bulgarica* 3: 48–58.
- KUMANSKI K. 1975b. New trichopterological data from Bulgaria (Insecta, Trichoptera). *Acta Zoologica Bulgarica* 2: 58–69. (In Bulgarian, with Russian and English summaries).
- KUMANSKI K. 1979. The Family Hydroptilidae (Trichoptera) in Bulgaria. *Acta Zoologica Bulgarica* 13: 3–20.
- KUMANSKI K. 1985. Fauna Bulgarica. Vol. 15. Trichoptera, Annulipalpia (in Bulgarian). In: JOSIFOV M. (Ed). Sofia: Bulgarian Academy of Sciences 244 p.
- KUMANSKI K. 1988. Fauna Bulgarica. Vol. 19: Trichoptera, Integripalpia. JOSIFOV M. (Ed.). Sofia: Bulgarian Academy of Sciences, 356 p.
- KUMANSKI K. 2004. Trichoptera (Insecta) from the Eastern Rhodopes (Bulgaria and Greece). In: BERON P. & POPOV V. (Eds): *Biodiversity of Eastern Rhodopes (Bulgaria and Greece)*. [Biodiversity of Bulgaria 2.] [Pensoft Series Faunistica No 41.], 515–524.
- LENCIONI V., MARZIALI L. & ROSSARO B. 2011. Diversity and distribution of chironomids (Diptera, Chironomidae) in pristine Alpine and pre-Alpine springs (Northern Italy). *Journal of Limnology* 70: 106–121.
- LENCIONI V., MARZIALI L. & ROSSARO B. 2012. Chironomids as bioindicators of environmental quality in mountain springs. *Freshwater Science* 31: 525–541.
- MARGALEF, R. 1958. *Information theory in ecology*. *General Systematics* 3: 36–51.
- MORI N. 2003. Macroinvertebrate communities of karst springs in the Julian Alps in relations to environmental factors. *Natura Sloveniae* 5(2): 5–17.
- NAGEL P., 1989. *Bildbestimmungsschlüssel der Saprobien: Makrozoobenthon*. Gustav Fischer Verlag Stuttgart/New York: 183 p.
- ORENDT C. 2000. The chironomid communities of woodland springs and springbrooks, severely endangered and impacted ecosystems in a lowland region of eastern Germany (Diptera: Chironomidae). *Journal of Insect Conservation* 4: 79–91
- PASSPORTS FOR CERTAIN TYPES OF RIVERS AND LAKES. [HTTP://WWW.BD-DUNAV.ORG/UPLOADS/CONTENT/FILES/UPRAVLENIE-NAVODITE/AKTUALIZACIA_PURB/SWMI/PRILOJENIE%202_2_1_1.PDF](http://www.bd-dunav.org/uploads/content/files/upravlenie-navodite/aktualizacia_purb/swmi/prilojenie%202_2_1_1.pdf)
- PASTUCHOVA Z. 2006. Macroinvertebrate assemblages in conditions of low-discharge streams of the Cerovar vrchovina highland in Slovakia. *Limnologia* 36: 241–250.
- PIELOU E. 1966. Species diversity and pattern diversity in the study of ecological successions. *Journal of Theoretical Biology* 10: 370–383.
- PLÓCIENNIK M., DMITROVIĆ D., PEŠIĆ V. & GADAWSKI P. 2016. Ecological patterns of Chironomidae assemblages in Dinaric karst springs. *Knowledge and Management of Aquatic Ecosystems* 417 (11), <https://doi.org/10.1051/kmae/2015044>
- POPOV V. 1970. THE SPREAD OF KARST IN BULGARIA AND SOME OF ITS FEATURES. *Bulletin of the Institute of Geography of the Bulgarian Academy of Sciences* 13: 5–17 (In Bulgarian).
- RADA B. & ŠANTIĆ M. 2014. Community structure of aquatic insects in the karstic Jadro River in Croatia. *Journal of Insect Sciences* 14 (54): 1–10.
- RIGHI-CAVALLARO K., ROCHE K. F., FROEHLICH O. & CAVALLARO M. R. 2010. Structure of macroinvertebrate communities in riffles of a Neotropical karst stream in the wet and dry seasons. *Acta Limnologica Brasiliensia* 22 (3): 306–316. DOI: 10.4322/actalb.02203007
- RIVER BASIN MANAGEMENT PLAN OF THE BLACK SEA REGION, 2016-2021. (cited as RBMP 2016-2021) (In Bulgarian).
- SCHMIDT P. 1993. A key to the larval Chironomidae and their instars from Austrian Danube region streams and rivers. Part I: Diamesinae, Prodiamesinae and Orthoclaudiinae. Federal Institute for Water Quality, Wien-Kaisermuehlen. Wasser und Abwasser, Supplement 3.
- SHANNON C. & WEAVER W. 1949. *The mathematical theory of communication*. University of Illinois Press, Urbana, Chicago.
- SIMPSON E. 1949. Measurement of Diversity. *Nature* 163: 688 p.
- SMITH H. & WOOD P. J. 2002. Flow permanence and macroinvertebrate community variability in limestone spring systems. *Hydrobiologia* 487: 45–58.
- STEVANOVIĆ Z. 2018. Global distribution and use of water from karst aquifers. *Geological Society London Special Publications* 466(1): SP466.17 DOI: 10.1144/SP466.17
- VIDINOVA Y. & RUSSEV B. 1997. Distribution and ecology of the representatives of some Ephemeropteran families in Bulgaria. In: LANDOLT P. & SARTORI M. (Eds): *Ephemeroptera & Plecoptera: Biology – Ecology – Systematics*, (MTL) Fribourg, pp. 139–146.
- VILENICA M., BILIĆ M., MIČETIĆ STANKOVIĆ V. & KUČINIĆ M. 2018. Mayfly ecological traits in a European karst spring: species, microhabitats and life histories. *Community Ecology* 19(3): 248–258, DOI: 10.1556/168.2018.19.3.6
- WARINGER J. & GRAF W. 2011. *Atlas of Central European Trichoptera Larvae*. Dinkelscherben: Erik Mauch Verlag, 468 p. <https://www.nhbs.com/atlas-of-central-european-trichoptera-larvae-atlas-der-mittleuropaischen-kocherfliegenlarven-book>
- WILLIAMS D., WILLIAMS N. & CAO Y. 1997. Spatial differences in macroinvertebrate community structure in springs in southeastern Ontario in relation to their chemical and physical environments. *Canadian Journal of Zoology* 75 (9): 1404–1414, <https://doi.org/10.1139/z97-767>
- WOOD P.J., GUNN J., SMITH H. & ABAS-KUTTY A. 2005. Flow permanence and macroinvertebrate community diversity within groundwater dominated headwater streams and springs. *Hydrobiologia* 545: 55–64, DOI 10.1007/s10750-005-2213-y.