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Macroinvertebrate Communities of sub-Mediterranean Intermittent Rivers in Bulgaria: Association with Environmental Parameters and Ecological Status

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Abstract. Intermittent rivers and ephemeral streams drain more than 50% of the land surface on Earth. Yet, their ecology remains insufficiently understood. In Bulgaria, temporary rivers are typically small- or medium-sized rivers (national type R14), flowing in areas with sub-Mediterranean climate. We present the first data focused explicitly on macrozoobenthos from intermittent rivers in four Bulgarian river basins within the drainage of the Aegean Sea. We identified 114 taxa from nine rivers (5÷33 taxa/site), with abundance varying between 61 and 994 ind.m^{-2}. The most common were taxa of Ephemeroptera and Chironomidae, followed by the crab *Potamon ibericum.* There were considerable differences among macroinvertebrates at different sites at taxon level, with similarities among samples increasing when using lower taxonomic resolution. The distinctness of communities was likely a reflection of the high variability in environmental conditions and local human impacts. Redundancy analysis identified key groups for the sites with fast flow (e.g. Ephemeroptera, Trichoptera, Plecoptera, Coleoptera and Diptera Varia, taxa associated with altitude and the higher share of stone substrata). Most of the river sites were classified as having high ecological status according to the Bulgarian legislation. Only the sites in the Vrabcha and Dereorman Rivers were with moderate status; these were the sites with the lowest taxon richness. We could speculate that the structuring of the benthic community was affected by other factors that have not been accounted for in the present study, i.e. great annual fluctuations in river flow, characteristic for R14, or by loading with nutrients or other pollutants.

Key words: benthic invertebrates, temporary rivers, environmental factors, ecological status, Southern Bulgaria.

Introduction

Intermittent or temporary rivers and streams have highly variable flow that periodically ceases along parts of their courses (Matthews, 1988; Uys & O'Keeffe, 1997). Worldwide, together with ephemeral streams, intermittent rivers drain more than half of the land surface on our planet (Datry et al., 2014). Nonetheless, their ecology is not sufficiently studied.

In Bulgaria, intermittent rivers are common in areas with sub-Mediterranean

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climate (Isheva & Ivanov, 2016; Wolfram et al., 2016). These are small- or medium-sized rivers, classified as R14 under the national river typology (Cheshmedjiev et al., 2013). Bulgarian intermittent rivers belong to the four Bulgarian river basins (of Struma, Arda, Maritsa and Tundzha Rivers) within the drainage of the Aegean Sea.

Wolfram et al. (2016) identified two subtypes within R14 national river type: R14a with low slope and altitude, with low to medium flow velocity and R14b with high slope and maximum altitude in the catchment area, with medium high flow velocity. The authors verified and refined the Bulgarian river typology, as well as the class boundaries for the ecological status of R14 based on an adapted version of the Biotic index (as described in Cheshmedjiev & Varadinova, 2013), other biological quality elements and environmental factors. Wolfram et al. (2016) did not provide any details on the benthic invertebrate communities inhabiting rivers of R14 national river type.

This is the first faunistic study to focus explicitly on macrozoobenthos of intermittent rivers in Bulgaria. Our aims were to: (i) analyse the basic environmental parameters (i.e. altitude, water temperature, conductivity, pH, oxygen content and saturation, substrata), (ii) study the benthic invertebrate communities (their abundance, diversity and dominant taxa at both lowest possible taxon level (TL) and at major-groups level (GL), (iii) test for associations between environmental factors and macroinvertebrate communities (at GL) and (iv) to assess their ecological status based on benthic macroinvertebrates (at TL).

Material and Methods

Study area

Nine sites representative of intermittent rivers in Bulgaria were selected and sampled once in June 2020 (Fig. 1, Table 1). The selection criteria were defined for the purposes of a bigger project (see Acknowledgements), aiming at developing a new classification system for assessment of the ecological status, based on the biological quality element macrozoobenthos. Thus, the selected river sections represented various ecological situations: from relatively unaffected to anthropogenically impacted (as identified by East Aegean RBMP, 2016-2021; West Aegean RBMP, 2016-2021). All sampled rivers were situated in Southern Bulgaria and belonged to the basins of the Struma (Ludata and Vrabcha Rivers), Tundzha (Fishera and Dereorman Rivers), Arda (Kodzha Dere, Chataldzhevitsa, Kulidzhinska and Aterinska Rivers) and Maritsa (Luda River) Rivers (Fig. 1).

Sample collection and processing

Bottom habitat structure was described on site: the presence of stone, gravel, sand and silt was recorded. Physical and chemical water parameters, i.e. water temperature (WT), oxygen concentration (DO [$mg.dm^{-3}$]) and saturation (OSat [%]), conductivity (Cond [$\mu S.cm^{-1}$]) and pH, were measured in situ using portable Windaus Labortechnik Package and HANNA multi-parameter instruments. Water samples were processed following ISO 5667-3: 2018.

Macroinvertebrates were collected by wading in the river for ca. 100 m, following an adapted version of the pro-rata multi-habitat procedure described in Cheshmedjiev et al. (2011) and in accordance with BDS EN ISO 10870:2012 and EN 16150:2012 standards. The method was regulated by the national water legislation trough Regulation No H-4 (2012). Invertebrate samples were cleaned from coarse substrata, washed through a sieve (500 μm) on site, transferred into containers with 70% alcohol and transported to the laboratory. Benthic specimens were sorted by systematic groups, enumerated and then identified to the lowest possible level.

Analyses

Pearson correlations and their level of significance among water parameters were identified using R 4.0.3. (R Core Team, 2020) and the PerformanceAnalytics package (Peterson et al., 2014). Principal component analysis (PCA), based on the correlation matrix by centring and standardisation, was used to summarise the major patterns of variation of environmental variables (i.e. altitude, physical and chemical water parameters and substrata).

Log-transformed absolute abundances were used in analyses on macroinvertebrate communities. Cluster analyses were performed using Bray-Curtis similarities for taxa (TL) and major groups (GL) with PRIMER-E Vers. 6. Redundancy analysis (RDA) explored associations among the correlation matrices of 16 macroinvertebrate groups and seven environmental parameters. Parameters identified by RDA to have the lowest contribution on explaining associations among environmental factors and communities were excluded from further statistical analyses. Ordinations (PCA and RDA) were performed using CANOCO Vers. 5 statistical package for Windows.

The ecological status of the studied river sections was assessed using the Biotic index (BI) and the Total number of taxa (TNT) according to a river-type specific scale developed in the national legislation (Regulation No H-4, 2012).

Results

Environmental factors

The study sites were situated between 50 and 582 m a.s.l. (Table 1). The lowest temperature was recorded at the site in the Ludata River (13.7°C at 582 m a.s.l.). The highest value of conductivity was measured at the site in the Dereorman River (1332 $\mu S.cm^{-1}$), followed by the Fishera River (669 $\mu S.cm^{-1}$). Pearson correlations identified a significant association ($p=0.032$) between pH and conductivity and an association bordering on significance between pH and altitude ($p=0.077$; Fig. 2).

According to the results of the PCA, the first three principal components (PCs) explained 81.91% of the total variance in environmental data (eigenvalues $\lambda 1 = 0.362$; $\lambda 2 = 0.321$; $\lambda 3 = 0.137$). Axis 1 represented two environmental gradients: the first was related to conductivity and silt; this gradient separated the Dereorman River with the highest values of these parameters. The second gradient of PC1 was associated with water temperature and the presence of sand and separated the Vrabcha and Luda Rivers from the rest of the river sites (Fig. 3). Axis 2 was also related to two gradients: the first was associated with altitude and stone substratum that separated the Ludata, Kodzha Dere and Aterinska Rivers. The second gradient of PC2 was related to oxygen concentration (used also as a proxy for oxygen saturation as identified by the PCA). The highest values of dissolved oxygen were measured in Fishera (its position on the biplot was associated also with the higher conductivity) and Luda Rivers. Chataldzhevitsa River was separated as the site with the highest pH and an altitude of 406 m a.s.l.

Biota: diversity and dominant taxa

We identified 114 taxa of 53 families and 16 systematic groups of bottom invertebrates in the studied R14 rivers (Table 2).

The number of taxa varied between five (Dereorman River) and 33 (Kulidzhinska River) taxa per site (Table 2), while the absolute abundance varied between 61 (Vrabcha River) and 994 (Kulidzhinska River) $ind.m^{-2}$ (Fig. 4). The most common species were the mayflies *B. rhodani* (recorded at six of the nine sites), *C. macrura*, *E. ignita*, *E.* (*E.*) *dispar* and the chironomid *Cr. sylvestris*-gr. (all recorded at five sites), followed by the crab *P. ibericum* and the chironomid *Conchapelopia* sp. (recorded at four of the sites). The most abundant was *Gammarus* sp. with absolute abundance of 550 $ind.m^{-2}$ (or relative abundance of 96.32%), recorded from the Dereorman River. The family Simuliidae (Insecta: Diptera) was recorded at six of the sites.

Table 1. Rivers with sampling sites, codes and environmental characteristics.

N	River	Site	ID	Altitude Alt [m a.s.l.]	Temperature WT [°C]	Conductivity Cond [μS.cm^{-1}]	pH	Dissolved oxygen DO [mg.dm^{-3}]	Oxygen saturation OSat [%]	Substrata presence Stone	Gravel	Sand	Silt
1	Ludata	Yanovski bridge after Senokos site	Luda_YS	582	13.7	443	8.15	7.3	81	x	x	x	
2	Vrabcha	Upstream of delta, E79 road before Strumyani Town	Vrab_D	129	18.9	442	8.06	7.3	85		x	x	
3	Fishera	500 m upstream the border with Turkey	Fish_B	104	15.6	669	7.66	8.6	89	x	x	x	x
4	Dere-orman	Delta, bridge on Yambol - Elhovo road	Der_D	109	15.4	1332	7.71	7.4	74	x	x		x
5	Luda	Before confluence in Byala Rver	Luda_C	50	18.3	170	8.01	8.3	74		x	x	
6	Kodzha Dere	Malko Kamenyane Village	Kod_MK	284	17.2	358	8.07	7.2	87	x		x	
7	Chatal-dzhevitsa	Delta under dam of Borovitsa Reservoir	Chat_D	406	16.9	94	8.67	7.3	81	x	x	x	
8	Kuli-dzhinska	Bryagovets Village, 800 m from Ivailovgrad Reservoir	Kuli_D	139	18.1	476	8.02	7.8	86	x	x	x	
9	Aterinska	Bridge between Ivailovgrad Town and Svirachi Village	Ater_IS	180	14.2	406	7.96	7.3	71	x	x	x	x

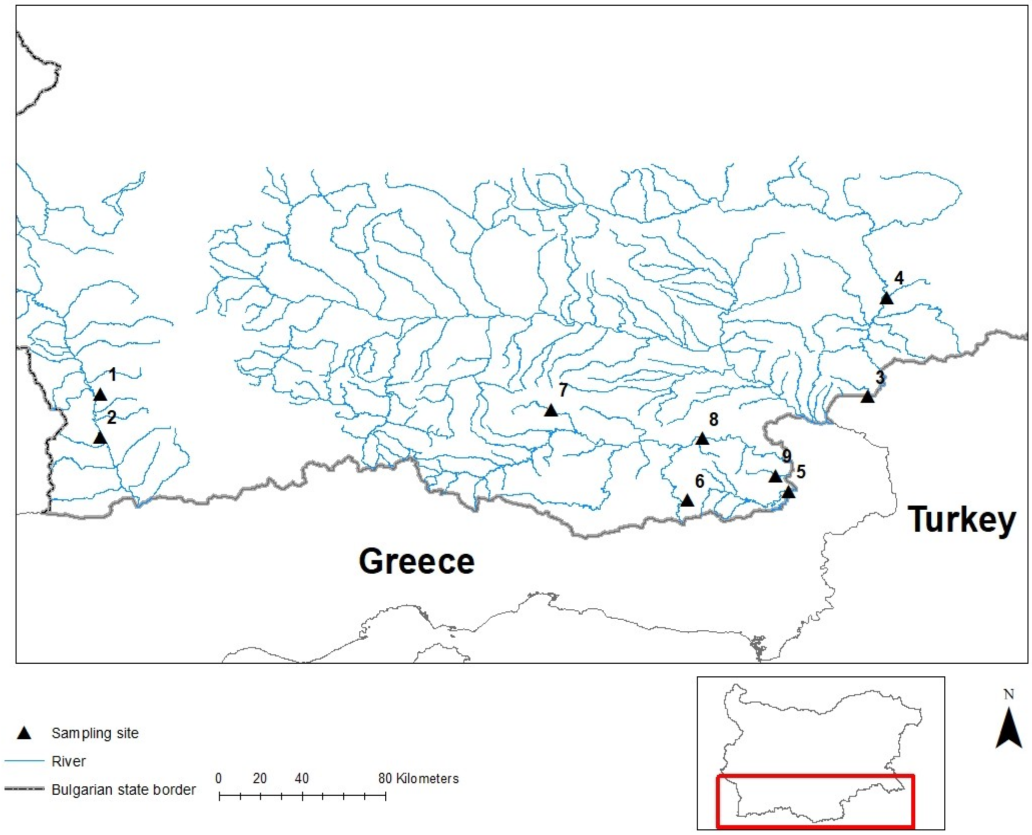

Fig. 1. Map of Bulgaria with sampling area and sites. See Table 1 for site numbers.

Table 2. List of taxa recorded at each of the sites. Site numbers are as in Table 1.

Group	Taxa	Sites								
		1	2	3	4	5	6	7	8	9
Oligochaeta	Oligochaeta indet.									x
	Lumbricidae Gen. sp.		x			x			x	
Hirudinea	*Erpobdella octoculata* (Linnaeus, 1758)	x	x							
Hydracarina	*Lebertia inaequalis* (Koch, 1837)						x	x		x
Gastropoda	*Belgrandiella* sp.								x	
	Radix labiata (Rossmässler, 1835)			x		x				x
	Radix sp.	x								
	Physella acuta (Draparnaud, 1805)		x							
	Planorbis planorbis (Linnaeus, 1758)			x						
	Ancylus fluviatilis O. F. Müller, 1774		x						x	x
Amphipoda	*Gammarus* sp.				x		x		x	x
Isopoda	*Asellus aquaticus* (Linnaeus, 1758)			x						
Decapoda	*Potamon ibericum* (Bieberstein, 1809)			x			x		x	x
Ephemeroptera	*Acentrella sinaica* Bogoescu, 1931					x				
	Alainites muticus (Linnaeus, 1758)								x	
	Baetis rhodani (Pictet, 1843)	x	x				x	x	x	x
	B. fuscatus (Linnaeus, 1761)					x			x	
	B. vernus Curtis, 1834	x								
	B. melanonyx (Pictet, 1843)	x								
	B. scambus Eaton, 1870							x		
	B. subalpinus Bengtsson, 1917		x							
	B. vardarensis Ikonomov, 1962								x	
	Procloeon bifidum (Bengtsson, 1912)					x				
	Cloeon simile Eaton, 1870			x						
	Caenis macrura Stephens, 1835	x	x	x			x	x		
	Oligoneuriella rhenana (Imhoff, 1852)					x	x		x	
	Ephemera danica Müller, 1764	x								
	Ephemerella ignita (Poda, 1761)					x	x	x	x	x
	Ecdyonurus (*Ecdyonurus*) *dispar* (Curtis, 1834)					x	x	x	x	x
	Ecdyonurus (*E.*) *insignis* (Eaton, 1870)							x		
	Ecdyonurus (*Helvetoraeticus*) sp.	x								x
	Electrogena affinis (Eaton, 1887)							x		x
	E. quadrilineata (Landa, 1969)						x			
	Rhithrogena bulgarica Braasch, Soldan & Sowa, 1985	x						x	x	
	Siphlonurus aestivalis (Eaton, 1903)									x
	Habrophlebia eldae Jacob & Sartori, 1984								x	

	Habrophlebia sp.						x			
	Habroleptoides sp.							x		
Plecoptera	*Leuctra* sp.	x							x	
	Nemoura sp.	x								
	Isoperla grammatica (Poda, 1761)	x							x	x
	Perla sp.							x		
Odonata	*Anax imperator* Leach, 1815	x						x		
	Calopteryx splendens (Harris, 1782)			x						
	Calopteryx sp.			x						
	Cordulegaster sp.			x						
	Gomphus sp.	x								
	Ophiogomphus sp.		x							
	Onychogomphus sp.							x		
Trichoptera	*Agapetus orchipes* Curtis, 1834									x
	Agapetus sp.								x	
	Cyrnus trimaculatus (Curtis, 1834)								x	
	Plectrocnemia conspersa conspersa (Curtis, 1834)							x		
	Polycentropus ierapetra Malicky, 1972						x			
	Hydropsyche angustipennis (Curtis, 1834)									x
	H. bulbifera McLachlan, 1878	x							x	
	H. incognita Pitsch, 1993								x	
	H. gr. *instabilis*							x		
	H. gr. *incognita*							x		
	Hydropsyche sp.	x							x	
	Hydropsychidae Gen. sp.									x
	Sericostoma sp.	x								x
	Rhyacophila sp.	x				x			x	
	Potamophylax sp.	x								
	Halesus digitatus (Schrank, 1781)	x						x		
	H. tesselatus (Rambur, 1842)						x			
	Limnephilidae Gen. sp.	x								
	Wormaldia sp.							x		
Megaloptera	*Sialis lutaria* (Linnaeus, 1758)			x						
Coleoptera	Dytiscidae Gen. sp.	x		x		x				
	Platambus maculatus (Linnaeus, 1758)								x	
	Elmidae Gen. sp.	x					x			
	Limnius volckmari (Panzer, 1793)					x			x	
	Hydraena sp.								x	
	Limnichidae Gen. sp.					x			x	
Hemiptera	Gerridae Gen. sp.			x	x					x

	Microvelia sp.	x								
	Veliidae Gen. sp.				x					x
	Mesovelia sp.									x
	Nepa cinerea Linnaeus, 1758					x				
	Ablabesmyia sp.			x			x			
Diptera (Chironomidae)	*Aspectrotanypus* sp.		x							
	Cladotanytarsus sp.			x						
	Chironomus sp.				x					
	Conchapelopia sp.		x	x					x	x
	Cricotopus sylvestris-Gr.			x		x	x		x	x
	Cricotopus (*Isocladius*) sp.								x	
	Cricotopus sp.						x			
	Dicrotendipes sp.			x						
	Kiefferulus tendipediformis (Goetghebuer, 1921)			x						
	Macropelopia sp.	x								
	Microtendipes sp.			x		x	x			
	Micropsectra sp.	x								
	Orthocladius sp.			x						
	Paramerina sp.			x						
	Paratanytarsus sp.			x						
	Paratrissocladius sp.							x		
	Procladius sp.			x						
	Polypedilum convictum (Walker, 1856)		x						x	x
	Polypedilum sp.		x	x			x			
	Prodiamesa olivacea (Meigen, 1818)		x		x					
	Psectrocladius sp.			x						
	Tanytarsus sp.			x						
	Brillia sp.	x	x							x
	Simuliidae Gen. sp.	x				x	x	x	x	x
Diptera varia	*Atherix* sp.						x		x	x
	Tipula sp.	x	x							
	Dicranota sp.	x								x
	Limoniidae Gen. sp.							x	x	x
	Hexatoma sp.					x				
	Tabanus sp.	x						x		
	Empididae Gen. sp.			x			x			x
	Psychodidae Gen. sp.									x
	Ceratopogonidae Gen. sp.		x				x			

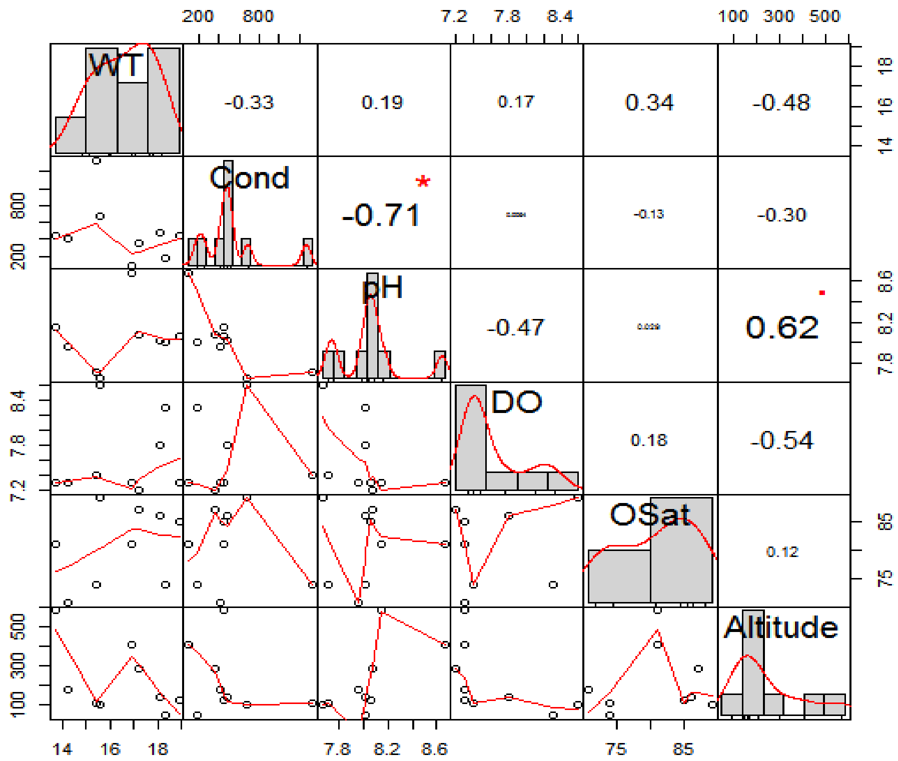

Fig. 2. Pearson correlations among environmental parameters. Asterisk (*) denotes statistically significant correlations. Parameter abbreviations are according to Table 1.

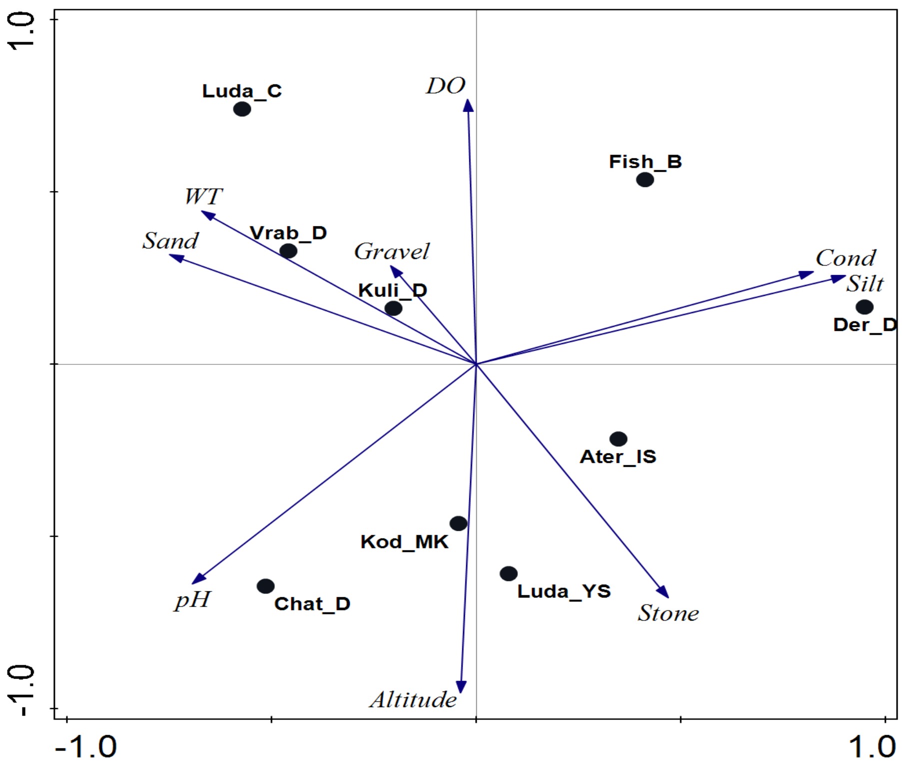

Fig. 3. Ordination biplot diagram based on principal component analysis of altitude, water parameters and four substratum types in the nine river sites (for codes see Table 1).

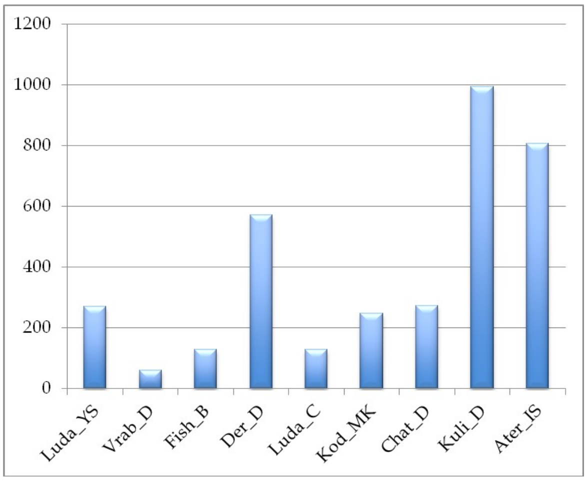

Fig. 4. Absolute abundance of macro-invertebrates at each site (for codes see Table 1).

The major groups included Oligochaeta, Hirudinea, Hydracarina, Gastropoda, Amphipoda, Isopoda, Decapoda, Ephemeroptera, Plecoptera, Odonata, Trichoptera, Megaloptera, Coleoptera, Hemiptera, Chironomidae, Diptera Varia (excluding Chironomidae). The most diverse were the groups of Ephemeroptera (25 taxa), Chironomidae (24 taxa) and Trichoptera (19 taxa). The most frequent were taxa of Chironomidae (recorded at all sites), followed by Ephemeroptera and Diptera varia (at eight sites) and by Trichoptera and Gastropoda (at six sites; Table 2).

Mayflies were dominant or subdominant (based on their abundance) at all but two sites (Fig. 5). Most numerous were *B. rhodani, E. ignita* and *E. (E.) dispar*, but due to a variety of microhabitats we also found some rare species, e.g. *A. sinaica*, *Pr. bifidum*, *Electrogena* spp., *H. eldae*, usually occurring in small populations. We recorded six families of caddisflies, of which the most common were Hydropsychidae (*Hydropsyche* spp.) at five sites, Polycentropodidae and Limnephilidae at three. Representatives of four subfamilies of Chironomidae were recorded: Tanypodinae, Orthocladiinae, Prodiamesinae and Chironominae (tribes Tanytarsini and Chironomini). From the sample in the Fishera River, we recorded the highest number of taxa of the family (14), while at the other sites the number of taxa varied between one and six. Snails of four families were recorded at six of the sites (Table 2). The most common were the taxa belonging to Planorbidae (recorded at four sites).

Taxon-level cluster analyses indicated that benthic assemblages were rather variable at the different sites and the similarities among sites were low (max= 44.60%; mean=17.42%; Fig. 6A). The most distinct from the others were the sites in the Dereorman, Fishera and Vrabcha Rivers, while the least different were the Kodzha Dere, Kulidzhinska, Aterinska and Chataldzhevitsa Rivers. Group-level cluster analyses suggested higher similarities among sites (max=75.48%; mean=46.43%; Fig. 6B) and this motivated us to explore the associations between biota and their environment at GL.

Biota: association with environment and ecological status

Three of the parameters (WT, DO and OSat) had similar values among sites and little contribution to explaining the associations among environmental factors and communities (as demonstrated by RDA), therefore they were excluded from further statistical analyses.

According to the RDA ordination of biota and the seven environmental variables retained in the analyses (Fig. 7), 77.3% of the total variance in benthic invertebrate communities was explained by the first three axes (eigenvalues $\lambda 1$ = 0.399; $\lambda 2$ = 0.273; $\lambda 3$ = 0.101). Axis 1 was positively related to conductivity (9.6% explained variation) and silt substratum (29.2% explained variation) and separated the Dereorman River, where most abundant were gammarids of order Amphipoda (96% of the total abundance, Fig. 4), followed by order Hemiptera. Subdominant at the site in the Fishera River was order Hemiptera; that was the only site where the orders Megaloptera and Isopoda were recorded (Figs. 5 and 7). Altitude (9.8% explained variation) and stone substratum (11.3% explained variation) were associated with taxa of orders Ephemeroptera, Trichoptera, Plecoptera, Coleoptera and Diptera Varia (i.e. elmids, simuliids), taxa that were mostly recorded in the Ludata, Kulidzhinska, Aterinska and Kodzha Dere Rivers. The highest abundance of class Gastropoda was found in the Vrabcha River. Taxa of order Odonata were more frequent on gravel (4.9% explained variation) and sand substrata (14.3% explained variation). Shorter gradients related to water mites (Hydracarina) and order Decapoda were evident along PC2 and correlated with the site in the Kodzha Dere River. Altitude and pH explained 11.6% of the total variation in communities and determined the position in the ordination of Chataldzhevitsa River; the lowest absolute and relative abundance of Chironomidae were recorded at the same site.

Our results regarding the ecological assessment based on BI and TNT indicated that all river sites but two were classified as having high ecological status. Both metrics and TNT suggested moderate ecological status at the sites in the Dereorman and Vrabcha Rivers, correspondingly (Fig. 8).

Discussion

Intermittent rivers provide diverse and dynamic conditions that are associated with their intermittence, which in turn shape distinctive invertebrate communities (Datry et al., 2014). Additionally, man-driven climate change or anthropogenic factors such as pollution or modification of riverbed morphology and river flow trigger alterations in the structure and diversity of macrozoobenthos. Our results suggest diverse communities inhabit R14 in Southern Bulgaria, with mayflies predominating in most of them. Cluster analyses indicated considerable differences among macroinvertebrates at different sites at taxon level, with similarities among samples increasing when using group-level resolution. To some extent, important for the grouping of rivers was their affiliation to river basins. In addition, seven of the measured environmental parameters explained more than 3/4 (grouped in gradients along the first three PCs) of the total variation in macrozoobenthic communities.

The most taxon-rich was the site in the Kulidzhinska River, characterised as undisturbed according to the East Aegean RBMP (2016-2021). This was confirmed also by the recorded moderate to low conductivity, high oxygen saturation and its high ecological status (based on both BI and TNT). Relatively taxon-rich were also the Ludata and Aterinska Rivers. Aterinska River was assessed as having high ecological status according to the East Aegean RBMP (2016-2021) and in the present study. In 2009–2011, Stoyanova et al. (2014) studied taxa of Ephemeroptera, Plecoptera and Trichoptera (EPT taxa) at six sites along the Ludata River. The authors also recorded high diversity of these sensitive taxa at all sites but one that was impacted by the toxic compounds in the seepage waters from a mine upstream the site.

The macroinvertebrate communities in the Dereorman, Fishera and Vrabcha Rivers, were the least similar to the rest of the studied rivers. The lowest taxon richness was recorded for the Dereorman River, due to organic pollution from agriculture as recorded also by Borisova et al. (2013). The lowest macroinvertebrate abundance and the second lowest richness at the site in the Vrabcha River we attribute to the higher share of sand substratum, which is associated with lower organic matter and food availability and the resulting low biotic diversity (Leitao et al., 2014; Tolonen et al., 2001).

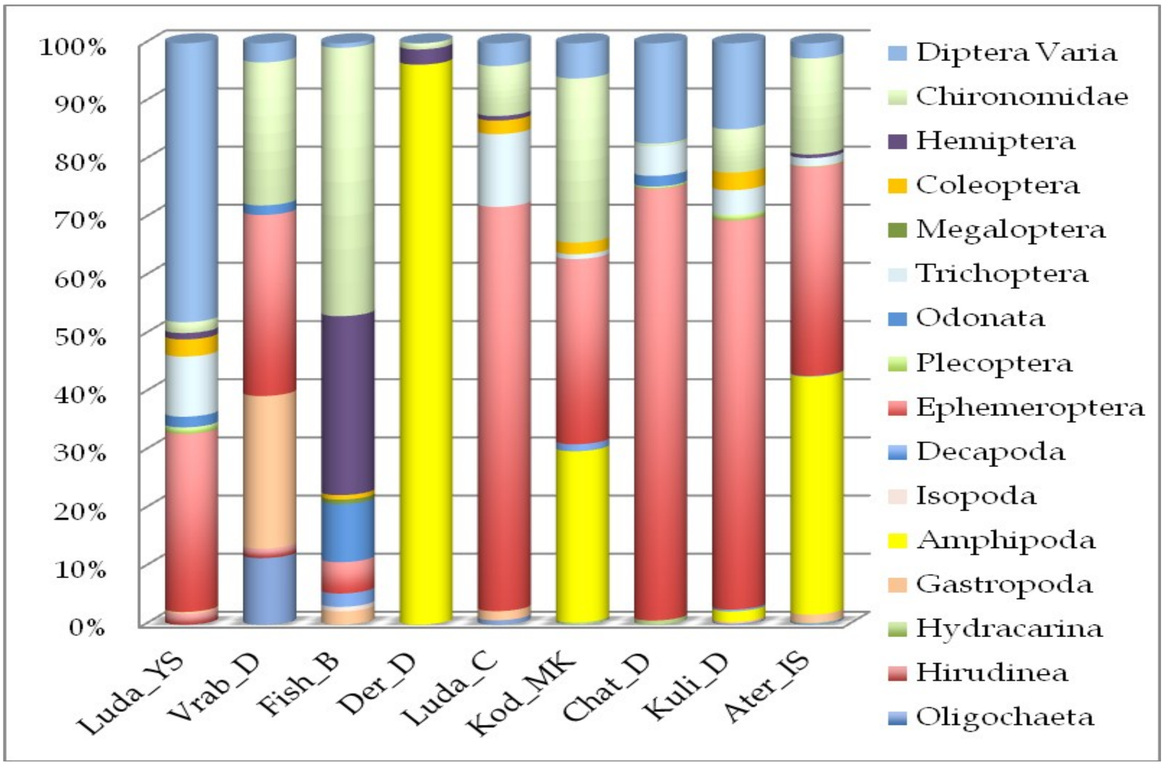

Fig. 5. Relative abundance (%) of the benthic groups at each of the nine sites (for codes see Table 1).

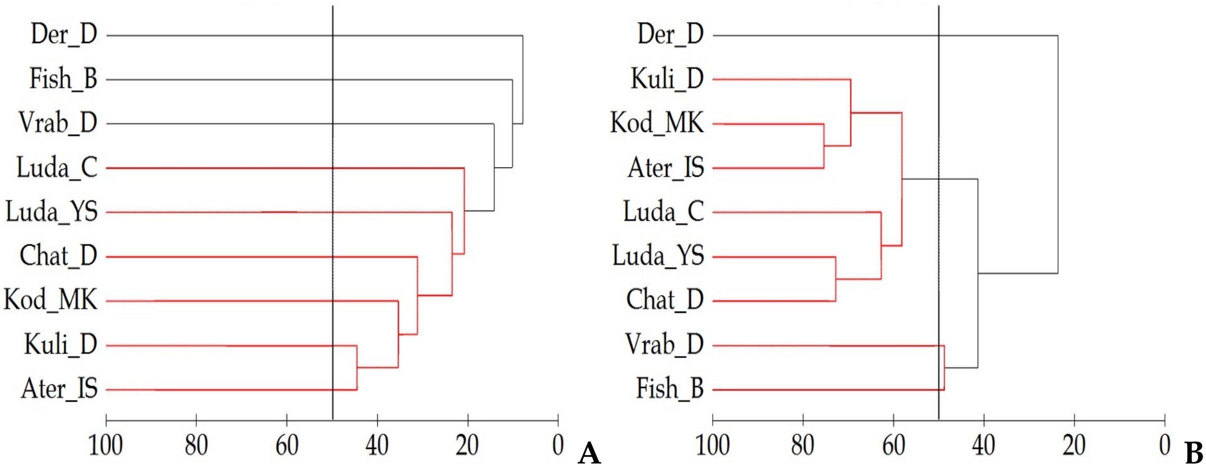

Fig. 6. Bray-Curtis similarity among samples based on macroinvertebrate communities at taxon level (A) and at group level (B). Vertical lines denote 50% of similarity between couples of samples (for codes see Table 1).

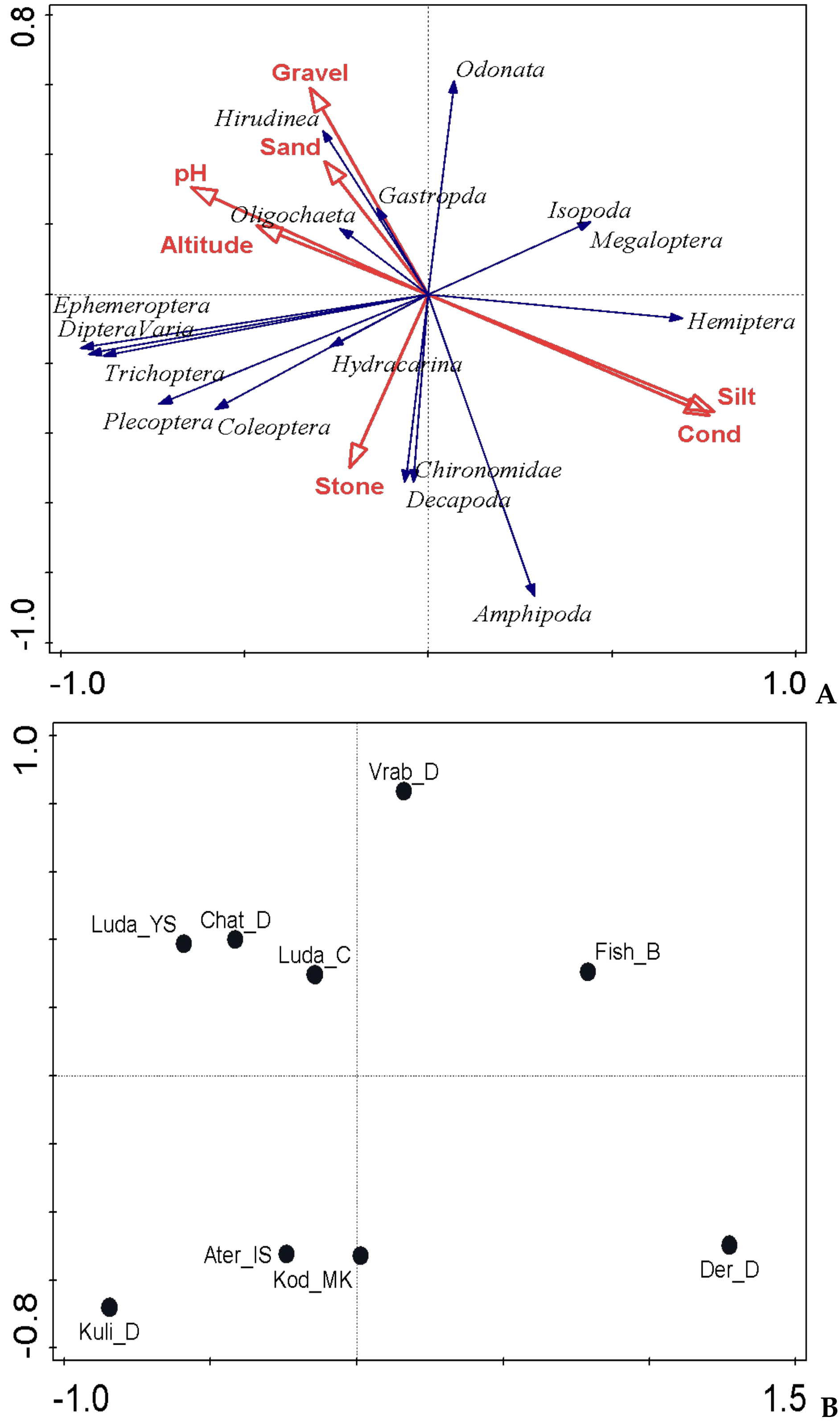

Fig. 7. Redundancy analysis ordinations of environmental variables and macroinvertebrate groups at the studied river sites. (A) associations among the seven environmental variables and the distribution patterns of benthic groups and (B) ordination of river sites (for codes see Table 1).

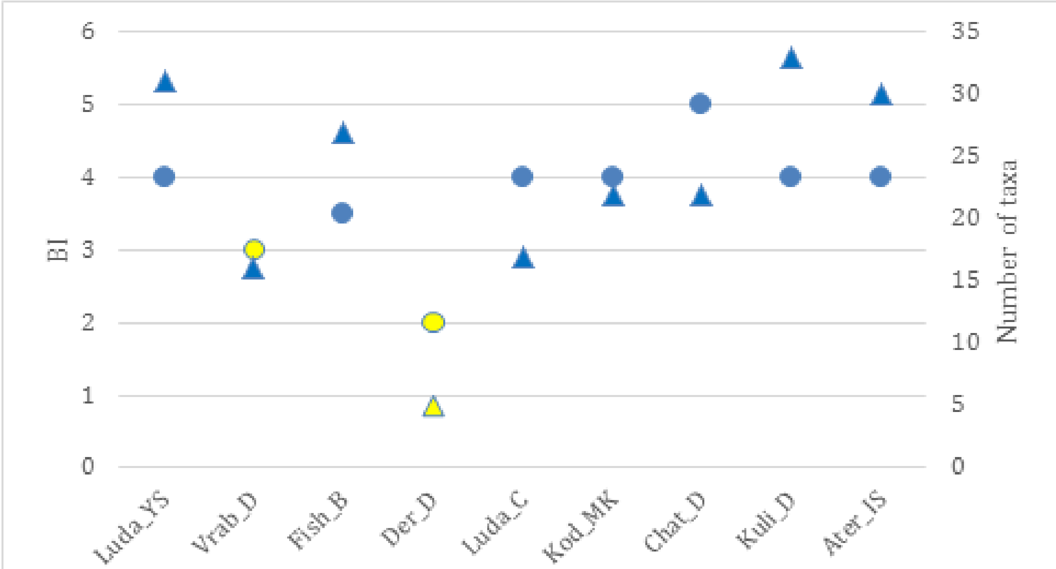

Fig. 8. Ecological status at each of the studied sites. Circles denote values of the Biotic index, triangles - of the Total number of taxa per site, blue - high ecological status and yellow - moderate ecological status (for codes see Table 1).

Another possible explanation could be the local anthropogenic impact these sites are subjected to, as suggested by the recorded high conductivity at the former two sites and their moderate ecological status (East Aegean RBMP, 2016-2021; West Aegean RBMP, 2016-2021). In addition, the site in the Vrabcha River is impacted by the Struma Motorway, the nearby Strumyani Town and a factory for the production of marble, limestone and granite. We recorded 27 taxa at the site in the Fishera River, where various substrata were recorded, thus suggesting a relatively high diversity. However, 14 of the recorded taxa were of the family Chironomidae, unlike the other taxon-rich sites where between one and four taxa of Chironomidae were recorded. A combination among various local impacts at these sites and the availability of suitable microhabitats likely resulted in the lower number of the more sensitive taxa.

Wolfram et al. (2016) identified two subtypes within the R14 national river type: R14a and R14b, as discussed in the Introduction. The presence of families of mayflies, caddisflies and stoneflies along with elmids and simuliids suggest that the undisturbed river sites within the Struma and Arda River Basins (Ludata, Chataldzhevitsa, Kulidzhinska, Aterinska and Kodzha Dere) are fast-flowing and resemble R14b. High similarities among the four rivers from the Arda River Basin were recorded also by Wolfram et al. (2016), where based on macrozoobenthos and other biological quality elements the rivers from the basin of the Arda River were classified as R14b river subtype within the Mediterranean middle-sized R-M2 river type. Moreover, typical of this European river type are Chironomidae, Baetidae, Simuliidae, Heptageniidae, Leuctridae, Elmidae, Ephemerellidae, Hydropsychidae, Gammaridae, Oligochaeta, Hydracarina, Nemouridae, Leptophlebiidae, Limoniidae, Limnephilidae (Feio, 2011). Most of those taxa were recorded also during the current study. On the other hand, Fishera and Dereorman Rivers of the basin of the Tundzha River match the description of R14a (sensu Wolfram et al., 2016).

R-selected chironomids was the only group that was present at all nine sites, owing to their high motility and tolerance to dynamic hydrological conditions (Evtimova

& Donohue, 2016; Langton & Casas, 1999). However, according to Puntí et al. (2009), they are potentially good indicators of ecological status in Mediterranean rivers on the Iberian Peninsula and individual taxa have differing requirements to environmental conditions. For instance, *Micropsectra* gr. are found at river sites with good quality (Marziali et al., 2010), as confirmed also by the records of this genera in the Ludata River at Senokos Village. Thus, it is important to explore the taxonomic structure of family Chironomidae. The other abundant group was Ephemeroptera. Mayflies are sensitive to human disturbances but the common and widespread species of the order are also good colonisers (Brittain, 1982). Overall, insect taxa dominated in terms of diversity and abundance. Their life-cycle strategies ensure thriving in undisturbed intermittent rivers and streams with lower intermittence: change between larval aquatic and aerial adult stages promote survival in relatively natural wet/dry periods, respectively (Datry et al., 2014; Stubbington et al., 2011).

We found a high association between the environmental factors and the benthic invertebrate communities (GL) of Bulgarian intermittent rivers. Our RDA identified key taxa, characteristic of this river type and its two subtypes. The distinctness of communities (at TL) was likely a reflection of the high variability in environmental conditions and local human impacts. Over the last century, among the main disturbances in Medditerranean rivers and streams are alteration of land use, river morphology, water management (Aguiar & Ferreira, 2005; Hooke, 2006). Local impacts could trigger the reduction or even disappearance of sensitive taxa and their replacement by more tolerant ones (e.g. amphipods, isopods, hemipterans and tolerant chironomids). According to our analyses, the taxonomic structure of the studied communities could be explained only partially by the measured environmental parameters. While aquatic invertebrate communities of R14 are type-specific under undisturbed conditions, taxonomic structure is altered when the rivers are affected by various anthropogenic pressures. We could speculate that the taxonomic composition of the studied sites was affected by factors that have not been accounted for in the present study, i.e. the great annual fluctuations in river flow, characteristic for R14, or by loading with nutrients or other pollutants.

Conclusions

Under unaffected conditions of the aquatic environment in the studied intermittent rivers, benthic communities are characterised by high taxonomic richness with a dominant representation of Ephemeroptera, Chironomidae and Trichoptera. The influence of different types of local impacts (agricultural activity, local industry and proximity to a highway) had adverse effects on the integrity of the ecosystem, which impacts the taxonomic richness and ecological status assessed through macrozoobenthos.

Further studies incorporating different seasons, hydrological metrics and organic loading would enable us to better explain the structure of the macrozoobenthos inhabiting intermittent rivers and will bring additional clarity regarding this river type and its representative benthic communities.

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