



Community Structure of Mayflies (Insecta: Ephemeroptera) in a Biodiversity Hotspot as Revealed by Multivariate Analyses

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Abstract: The aim of this study was to examine the community structure of the order Ephemeroptera in the Eastern Part of the Black Sea Region of Turkey by using multivariate analyses such as Canonical Correspondence Analysis (CCA) and Two-Way Indicator Species Analysis (TWINSPAN). For this purpose, 12,117 individuals of Ephemeroptera belonging to seven families, 11 genera and 28 species were sampled at 24 collecting sites and identified. Environmental variables measured at each collecting site were dissolved oxygen (DO), water temperature, electrical conductivity (EC), pH, PO₄-P, NO₃-N, NO₂-N, NH₄-N and SO₄. The study area covered the eastern part of the Black Sea Region of Turkey, as it is a sub-ecoregion of the Caucasus Biodiversity Hotspots. With CCA analysis, the relationship between the mayfly species and the environmental variables was analysed for the first time for the region. In addition, TWINSPAN analysis determined the indicator species at the collecting sites. According to the results of CCA, *Baetis milani*, *B. gemellus*, *Alainites muticus* and *Electrogena quadrilineata* were positively related to PO₄-P but negatively related to temperature. *Baetis rhodani*, *B. lutheri*, *B. fuscatus*, *Electrogena affinis*, *E. starmachi*, *Caenis macrura*, *C. luctuosa* and *Epeorus zaitzevi* were positively related to SO₄, NO₂-N, EC and pH but negatively related to dissolved oxygen. *Rhithrogena iridina*, *R. zelinkai*, *Ecdyonurus picteti*, *E. helveticus*, *Epeorus sylvicola*, *E. znojko*, *E. caucasicus* and *Habroleptoides confusa* were positively correlated with DO but negatively correlated with SO₄, EC, NO₂-N and pH. *Rhithrogena semicolorata*, *R. puytoraci*, *R. beskidensis*, *Seratella ignita* and *Potamanthus luteus* were positively correlated with temperature but negatively correlated with PO₄-P. In addition, according to the results of TWINSPAN, the collecting sites divided into two main group (Group A and B). *Epeorus sylvicola*, *E. znojko* and *E. caucasicus* were assigned as the indicator species for the sites in Group A. However, there were no indicator species assigned for Group B.

Key words: Biodiversity, Canonical Correspondence Analysis, indicator species, reference site, TWINSPAN, water quality.

Introduction

Many environmental variables determine the composition and the structure of aquatic communities. These variables interact with communities and also interact with each other. Benthic macroinvertebrates in running waters depend on many physical and chemical variables, such as water temperature, dis-

solved oxygen, pH, electrical conductivity, velocity, bed structure, riparian vegetation cover, concentration of N or P compounds, etc. (HAWKES 1979, DE PAUW & HAWKES 1993, DE PAUW et al. 2006). These variables affect the benthic macroinvertebrate community structure, both directly and indirectly. The relationships of these environmental variables with the benthic macroinvertebrate community structure

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can be determined using multivariate analysis.

The insect order Ephemeroptera (mayflies) is an important component of freshwater environments. Mayflies are a member of benthic invertebrate communities as one of the most deterministic biological quality elements in running waters. Many quality indices (BMWP and its versions, ASPT, BBI, etc.) used in the EU Water Framework Directive (WFD) to determine habitat quality of aquatic environments are based directly or indirectly on species of the Ephemeroptera (COUNCIL OF EUROPEAN COMMUNITIES 2000, 2003). Besides, in the concept of “reference condition”, which is of great importance in WFD, the Ephemeroptera is one of the major groups (along with Plecoptera and Trichoptera) containing multiple important indicator species in reference habitats (COUNCIL OF EUROPEAN COMMUNITIES 2000).

In the present study, we apply Canonical Correspondence Analysis (CCA) and Two-Way Indicator Species Analysis (TWINSPAN) to analyse the community structure of the species of the order Ephemeroptera. Multivariate analysis methods have been previously applied to study benthic macroinvertebrate communities in Turkey (KAZANCI et al. 2008, 2009, 2017, KAZANCI & DÜGEL 2000, 2008, 2010, GIRGIN et al. 2003, 2010, DÜGEL & KAZANCI 2004, KALYONCU & GÜLBOY 2009, DURAN & AKYILDIZ 2011, DURAN 2006).

The aims of the present study were to: (1) analyse the mayfly community structure by using multivariate analysis methods, such as CCA and TWINSPAN, (2) explore the relationship of species of the Ephemeroptera with some environmental variables, (3) give information about habitat preferences of the species and compare them with the literature and (4) complete unknown habitat preferences information of some species.

Materials and Methods

Study area

The Eastern part of the Black Sea Region was chosen (Fig. 1) because it is an important biodiversity area in Turkey. This area is a sub-ecoregion of the Caucasus Biodiversity Hotspot (KAZANCI 2013), which is one of the 25 biodiversity hotspots of the World (MYERS et al. 2000). The Caucasus is one of the WWF’s Global 200 Ecoregions, identified as globally exceptional for biodiversity. This hotspot area covers 580,000 km² (CEPF 2003) and consists of the territories of the North Caucasus situated in Russia, Georgia, Azerbaijan, Turkey, Iran and Armenia (Fig. 2). This area has many unpolluted or

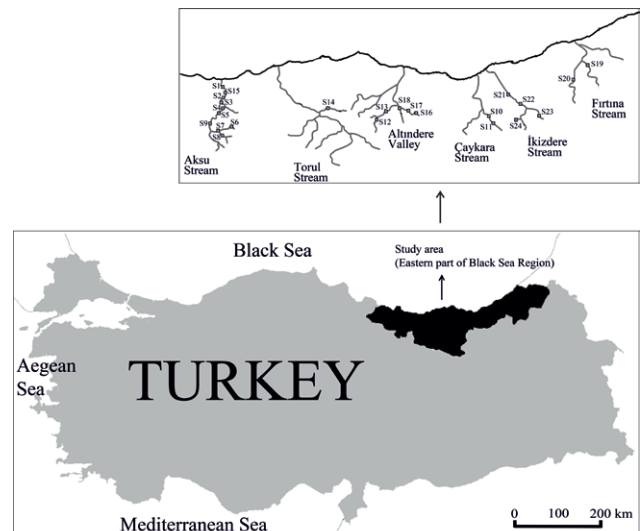


Fig. 1. Study area: the Eastern part of the Black Sea Region of Turkey.



Fig. 2. Caucasus Biodiversity Hotspot area and its sub-ecoregion, Eastern Part of the Black Sea Region of Turkey.

slightly polluted mountain streams with environmental and hydromorphological characteristics favourable for many species of Ephemeroptera.

Sampling sites

Streams in the Eastern part of the Black Sea Region of Turkey were sampled at 24 sites (Fig. 1). When determining the sampling sites, some circumstances were considered, such as (1) suitable stream zones preferred by Ephemeroptera, (2) reference and non-reference habitats (to compare the species composition) and (3) potamal and rhithral stream zones (to compare the differences in the mayfly communities). When determining whether the collecting sites have reference habitat conditions or not, some environmental features were noted during the field studies: absence of settlements around the sites and struc-

Table 1. Water quality classes and ranges of the variables.

Variables	Water Quality Classes				Reference
	I	II	III	IV	
Temperature (°C)	≤ 25	≤ 25	25 - 30	> 30	MINISTRY OF FORESTRY AND WATER AFFAIRS 2015
Dissolved oxygen (mg/l)	> 8	8.0 - 6.0	6.0 - 3.0	< 3	MINISTRY OF FORESTRY AND WATER AFFAIRS 2016
pH	6.5 - 8.5	6.5 - 8.5	6.0 - 6,5 and 8.5-9.0	< 6.0 or > 9.0	MINISTRY OF FORESTRY AND WATER AFFAIRS 2015
NO ₃ -N (mg/l)	0.00 - 3.00	3.00 - 10.00	10.00 - 20.00	> 20.00	MINISTRY OF FORESTRY AND WATER AFFAIRS 2016
NO ₂ -N (mg/l)	0.00 - 0.01	0.01 - 0.06	0.06 - 0.12	> 0.12	MINISTRY OF FORESTRY AND WATER AFFAIRS 2015
NH ₄ -N (mg/l)	0.00 - 0.20	0.20 - 1.00	1.00 - 2.00	> 2.00	MINISTRY OF FORESTRY AND WATER AFFAIRS 2015
SO ₄ (mg/l)	0 - 200	0 - 200	200 - 400	> 400	MINISTRY OF FORESTRY AND WATER AFFAIRS 2004
PO ₄ -P (mg/l)	0.00 - 0.06	0.06 - 0.19	0.19 - 1.00	> 1.00	KLEE 1991

tures such as bridge, dams, reservoirs, water channels as well as no drainage into the water, according to the criteria suggested by BUFFAGNI et al. (2001). In the next step, the sites with high water quality (according to the physical and chemical variables) and with high species richness were used as supporting criteria to determine the reference sites for streams in the studied area.

Benthic macroinvertebrate samples

The samples were collected using a standard D-shaped hand-net from 24 sites in 2008 (July), 2009 (June) and 2011 (July). One-time sampling was performed in each site. Benthic materials were kept in a 4% formaldehyde solution and transported to the laboratory. The macroinvertebrates were sorted, washed with water and then transferred in 80% ethanol. Individuals of the order Ephemeroptera were then separated and identified to the species level using appropriate identification guides (e.g. BAUERNFEIND 1994, 1995, BAUERNFEIND & HUMPEsch 2001, BAUERNFEIND & SOLDAN 2012, BELFIORE 1983, BELFIORE & BUFFAGNI 1994, EISELER 2005, HAYBACH 1999, KLUGE 1988, 1994, 1997, LANDA 1969, MALZACHER 1984, MULLER-LIEBENAU 1969, SINITSHENKOVA 1976, 1981, STUDEMANN et al. 1992, SOLDAN & LANDA 1999, TOMKA & RASCH 1993, UJHELYI 1959, WEBB & MCCAFFERTY 2008, ZURWERRA et al. 1986). In addition, the original species descriptions were used when the keys did not allow unambiguous identification. For species taxonomy, the *World Checklist of Freshwater Ephemeroptera Species* by BARBER-JAMES et al. (2013) was followed.

The abundance (%) of the species in the collecting sites was based only on individuals of the

Ephemeroptera. The habitat status evaluation (according to the results of BMWP) of the collecting sites was according to KAZANCI et al. (2010) and KAZANCI & TÜRKMEN (2016). In the literature comparisons of habitat preferences of the species, oligosaprobic environments (high quality) were matched with the Class I habitat quality (high quality), β -mesosaprobic environments (good quality) were matched with the Class II habitat quality (good quality) and α -mesosaprobic environments (moderate quality) were matched with the Class III habitat quality (moderate quality).

Physical and chemical variables

Dissolved oxygen (DO), water temperature, electrical conductivity and pH were measured by using YSI 550 oxygen meter and YSI multiprobe system in the field, while benthic macroinvertebrate samples were collected. Furthermore, water samples were taken from sampling sites and transported back to the laboratory for the analyses of PO₄-P, NO₃-N, NO₂-N, NH₄-N and SO₄ concentrations by using DR/890 data logging colorimeter according to HACH (2005). Water quality classes based on these variables were determined according to the MINISTRY OF FORESTRY AND WATER AFFAIRS OF TURKEY (2004, 2015, 2016) and KLEE (1991) (Table 1).

Statistical analysis

Multivariate analysis methods, Canonical Correspondence Analysis (CCA) and Two-Way Indicator Species Analysis (TWINSPAN) were applied to analyse the community structure and habitat preferences of the species. CANOCO software (version 4.5) was used for CCA. CAP software (version 4.1.3)

was used for TWINSpan. CCA is an environmental-variable analysis. It links “regression methods” to demonstrate the dependence on the environment of a single species and “ordination methods”, which reveal the distribution of species in a community (TER BRAAK 1986, TER BRAAK & SMILAUER 2002, LEPS & SMILAUER 2003). TWINSpan is a divisive clustering method for animal communities. It generates indicator species by using the species composition of sampling sites. The clusters of sampling sites are ordered such that similar clusters are near each other according to their community structure (HILL 1973, 1979).

Results

We collected 12,117 individuals of the order Ephemeroptera from all 24 sampling sites. They belonged to seven families, 11 genera and 28 species (Table 2). When individual numbers from all sites were considered, the individual numbers of three baetid species (*B. rhodani*, *B. gemellus* and *B. lutheri*) constituted

approximately half of all individuals. The proportion of the individuals of the baetid and the heptageniid species represented 62% and 30%, respectively. The baetid species (except *B. fuscatus*) showed high dominance at all non-reference sites (Table 3) with low habitat quality. However, the high dominance of baetid species was also observed at some reference sites (S4, S5, S6, S7, S8, S9, S11, S12, S13, S14) with high habitat quality. At these sites, heptageniid species were also dominant. The high dominance of heptageniids were observed at all reference sites (Table 4), with the most abundant being *E. caucasicus*, *E. znojkoii*, *E. sylvicola*, *R. semicolorata* and *R. beskidensis*. At sites S10, S19, S22 and S23, the three most dominant species were only heptageniids (Table 4). The ephemereid *Serratella ignita* dominated at the non-reference sites S1, S2 and S15 but high dominance of this species was also observed at reference sites S20 and S21 (Table 4). *Potamanthus luteus* exhibited high dominance only at S3 (Table 4), which was a non-reference site (Table 3).

Table 2. List of Ephemeroptera species found at the sampling sites*.

	Families		Genera		Species	Authors		
1	Baetidae	1	<i>Alainites</i>	1	<i>A. muticus</i>	(Linnaeus, 1758)		
			2	<i>Baetis</i>	2	<i>B. fuscatus</i>	(Linnaeus, 1761)	
		3			<i>B. gemellus</i>	Eaton, 1885		
		4			<i>B. lutheri</i>	Müller-Liebenau, 1967		
		5			<i>B. milani</i>	Godunko, Prokopov & Soldan, 2004		
		6	<i>B. rhodani</i>	(Pictet, 1843)				
2	Heptageniidae	3	<i>Ecdyonurus</i>	7	<i>E. helveticus</i>	Eaton, 1883		
				8	<i>E. macani</i>	Thomas & Sowa 1970		
				9	<i>E. picteti</i>	(Meyer-Dür, 1864)		
				10	<i>E. starmachi</i>	Sowa, 1971		
				4	<i>Electrogena</i>	11	<i>E. affinis</i>	(Eaton, 1883)
						12	<i>E. quadrilineata</i>	(Landa, 1969)
		5	<i>Epeorus</i>			13	<i>E. caucasicus</i>	(Tshernova, 1938)
				14	<i>E. sylvicola</i>	(Pictet, 1865)		
				15	<i>E. zaitzevi</i>	Tshernova 1981		
				16	<i>E. znojkoii</i>	(Tshernova, 1938)		
				6	<i>Rhithrogena</i>	17	<i>R. beskidensis</i>	Alba-Tercedor & Sowa, 1987
		18	<i>R. iridina</i>			Kolenati, 1859		
		19	<i>R. puytoraci</i>			Sowa & Degrange, 1987		
		20	<i>R. semicolorata</i>			(Curtis, 1934)		
		21	<i>R. zelinkai</i>			Sowa & Soldan, 1984		
3	Leptophlebiidae	7	<i>Habroleptoides</i>	22	<i>H. confusa</i>	Sartori & Jacob, 1986		
4	Ephemeridae	8	<i>Ephemera</i>	23	<i>E. danica</i>	Müller, 1764		
5	Potamanthidae	9	<i>Potamanthus</i>	24	<i>P. luteus</i>	(Linnaeus, 1767)		
6	Ephemereididae	10	<i>Serratella</i>	25	<i>S. ignita</i>	(Poda, 1761)		
7	Caenidae	11	<i>Caenis</i>	26	<i>C. luctuosa</i>	(Burmeister, 1839)		
				27	<i>C. macrura</i>	Stephens, 1836		
				28	<i>C. martae</i>	Belfiore, 1984		

*Order of the families follows HUBBARD (1990). Genera and species have been arranged in alphabetical order.

Table 3. Reference and non-reference sites and their habitat status with water quality classes.

Sites	N of species	N of ind.	RHC	Evaluation of Habitat Status	WQP and corresponding WQC							
					T (°C)	DO (mg/l)	pH	PO ₄ -P (mg/l)	NO ₃ -N (mg/l)	NO ₂ -N (mg/l)	NH ₄ -N (mg/l)	SO ₄ (mg/l)
S1	14	675	-	Class III	I	I	III	II	I	II	I	I
S2	11	482	-	Class II	I	II	I	II	I	II	I	I
S3	8	78	-	Class II	I	I	I	II	I	I	I	I
S4	8	112	+	Class II	I	I	I	II	I	I	I	I
S5	6	53	+	Class II	I	I	I	I	I	II	I	I
S6*	14	708	+	Class I	I	I	IV	IV	I	I	I	I
S7*	15	1158	+	Class I	I	I	I	IV	I	I	I	I
S8*	12	346	+	Class II	I	I	IV	IV	I	II	I	I
S9*	4	96	+	Class II	I	I	IV	IV	I	II	I	I
S10*	13	207	+	Class I	I	I	IV	IV	I	I	I	I
S11*	10	428	+	Class I	I	I	III	IV	I	I	I	I
S12*	10	138	+	Class I	I	I	IV	IV	I	I	I	I
S13*	10	76	+	Class I	I	I	IV	IV	I	I	I	I
S14*	8	415	+	Class II	I	I	III	IV	I	I	I	I
S15*	10	410	-	Class II	I	I	I	IV	I	II	I	I
S16*	11	1466	-	Class II	I	I	I	IV	I	II	I	I
S17*	11	1302	-	Class II	I	I	I	III	I	II	I	I
S18*	11	1623	-	Class I	I	I	I	IV	I	I	I	I
S19*	18	341	+	Class I	I	I	I	III	I	I	I	I
S20*	15	583	+	Class I	I	I	I	III	I	I	I	I
S21	13	535	+	Class II	I	I	I	II	I	II	I	I
S22	12	499	+	Class II	I	I	I	II	I	II	I	I
S23	13	286	+	Class II	I	I	I	III	I	I	I	I
S24	7	100	-	Class II	I	I	I	II	I	II	I	I

*The sites with episodic acidification; RHC, Reference Habitat Condition; WQP, Water Quality Parameters; WQC, Water Quality Classes; T, water Temperature; DO, Dissolved Oxygen; PO₄-P, Orthophosphate-phosphorus; NO₃-N, Nitrate-nitrogen; NO₂-N, Nitrite-nitrogen; NH₄-N, ammonium-nitrogen; SO₄, Sulphate.

According to the water temperature, DO, NO₃-N, NH₄-N and SO₄, the water quality corresponded to the Class I in all sites (DO water quality corresponded to the Class II in Site 2). For NO₂-N, the water quality matched to either Class I or II in all sites (Table 3, Fig. 3). In regards of the state of the habitats, 16 sites were classified as reference habitats and eight sites as non-reference habitats (Table 3). According to BMWP, nine sites were determined as corresponding to the Class I and 14 sites belonged to the Class II habitat quality. Only one site corresponded to the Class III habitat quality (Table 3).

In order of their importance as revealed by the CCA, variables affecting taxon distribution were: pH, temperature, DO, EC, PO₄-P, NO₂-N and SO₄ (Fig. 4). In the CCA, 24 sites and 25 species represented in at least two sites were analysed. Therefore, *E. macani*, *C. martae* and *E. danica* were not included in the CCA, since these species were observed only in one site. The P value of the first canonical axis was 0.005 and P value for all canonical axes

was 0.002 (Table 5). That means the relationship between environmental variables and the species were statistically significant.

The TWINSpan dendrogram was divided into two main groups: Group A and Group B (Fig. 5). The main differentiations between these two groups were their pollution status and stream zones. The sites in Group A were situated in the rhithral zones of the streams and were unpolluted, or slightly polluted. They exhibited reference habitat conditions, except for S16, S17, S18 and S24. The sites in Group B were situated in the potamal zones of the streams (except S14) and were moderately polluted sites. With the exception of S14, these sites did not present reference habitat conditions.

Discussion

Up to now, 55 species and three subspecies have been recorded from the studied area (AYDINLI 2017). After the description of *Epeorus bicolliculatus* by

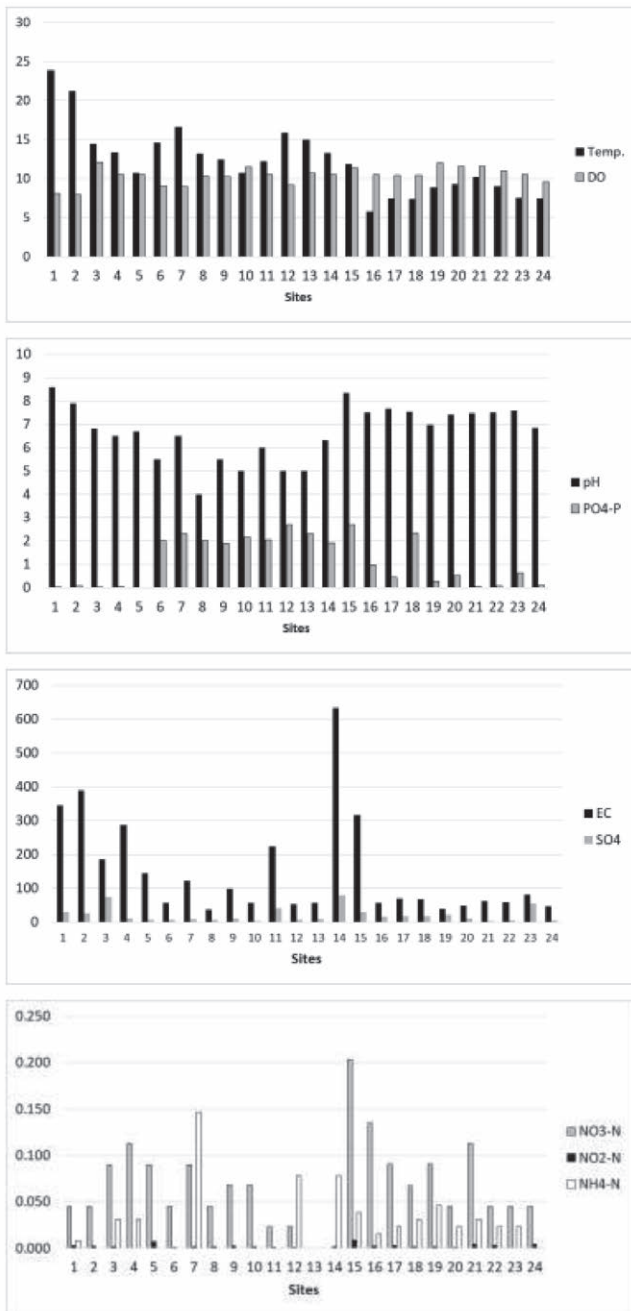


Fig. 3. Temperature (°C), dissolved oxygen (DO, mg/l), pH, electrical conductivity (EC, µs/cm), NO₃-N (mg/l), NO₂-N (mg/l), NH₄-N (mg/l), PO₄-P (mg/l) and SO₄ (mg/l) at the sampling sites.

HRIVNIAK et al. (2017), this number has increased to 56. However, the ecological characteristics of species of the Ephemeroptera in the Eastern part of the Black Sea Region of Turkey are poorly known. Of them, 25 and 28 are included in the CCA and TWINSPAN analyses, respectively.

In the first quadrant of the CCA diagram, the determinant variable is PO₄-P and the sites are S4, S11, S16, S17, S18, S23 and S24. One of the reasons for the high PO₄-P concentration in running waters is

the presence of organic debris due to dense riparian vegetation. This causes dissolved phosphate compounds to enter into the aquatic ecosystems (MEYER 1979). Sites S4, S11, S16, S23 and S24 have 100% riparian vegetation cover along the streamside. This may cause high PO₄-P concentrations at these sites due to organic debris and allows these sites to be situated close to PO₄-P in CCA. Another reason for the high PO₄-P concentration in streams at high altitudes is snowmelt. According to MEYBECK (1982), the average PO₄-P content of rain and snow is 5 µg/l and even this proportion causes high PO₄-P concentrations in unpolluted streams. The first melting water from the snowpack is an especially important water quality threat (KAZANCI 2009). Sites S16, S17, S18, S23 and S24 are located at the highest altitudes in this study (1919 m–2679 m a.s.l.). Water samples have been collected from these sites during the melting period, which can explain the high PO₄-P concentrations at these sites. According to the classification of KLEE (1991), the water quality of these sites ranges from Class III-IV, regarding PO₄-P. As in CCA, PO₄-P is the determinant variable for these sites.

The species in the first quadrant are *B. milani*, *B. gemellus*, *A. muticus* and *E. quadrilineata*. These species are positively related to PO₄-P but negatively related to temperature. Phosphorus is one of the most limiting nutrients in freshwater ecosystems and PO₄-P is the form of P available for uptake (WELCH & JACOBY 2004). The species in this quadrant have a high tolerance to high PO₄-P concentrations (up to 2.69 mg/l for this study). *Alainites muticus* and *E. quadrilineata* mainly prefer oligosaprobic and β-mesosaprobic habitats (BAUERNFEIND et al. 2002, SPORKA 2003). The sites where *A. muticus* and *E. quadrilineata* have been found belong to the Classes I and II habitat quality. Additionally, ERBA et al. (2003) have stated that the absence of *A. muticus* individuals in the Pioverna Stream (Italy) in the summer might be due to high temperatures. Here, we also have found that *A. muticus* is negatively related to temperature (Fig. 4). There is not much information about the habitat preferences of *B. gemellus* and *B. milani*. GODUNKO et al. (2004) have reported *B. milani* in a stream with a fast current and rocky-bed structure downstream in the Crimea. TÜRKMEN & ÖZKAN (2011) suggest that the habitat preferences of *B. milani* is β-mesosaprobic. According to our results, *B. gemellus* and *B. milani* are situated in the first quadrant with *A. muticus* and *E. quadrilineata*. Therefore, can be assumed that the habitat preferences of *B. gemellus* and *B. milani* are similar to those of *A. muticus* and *E. quadrilineata*. In addi-

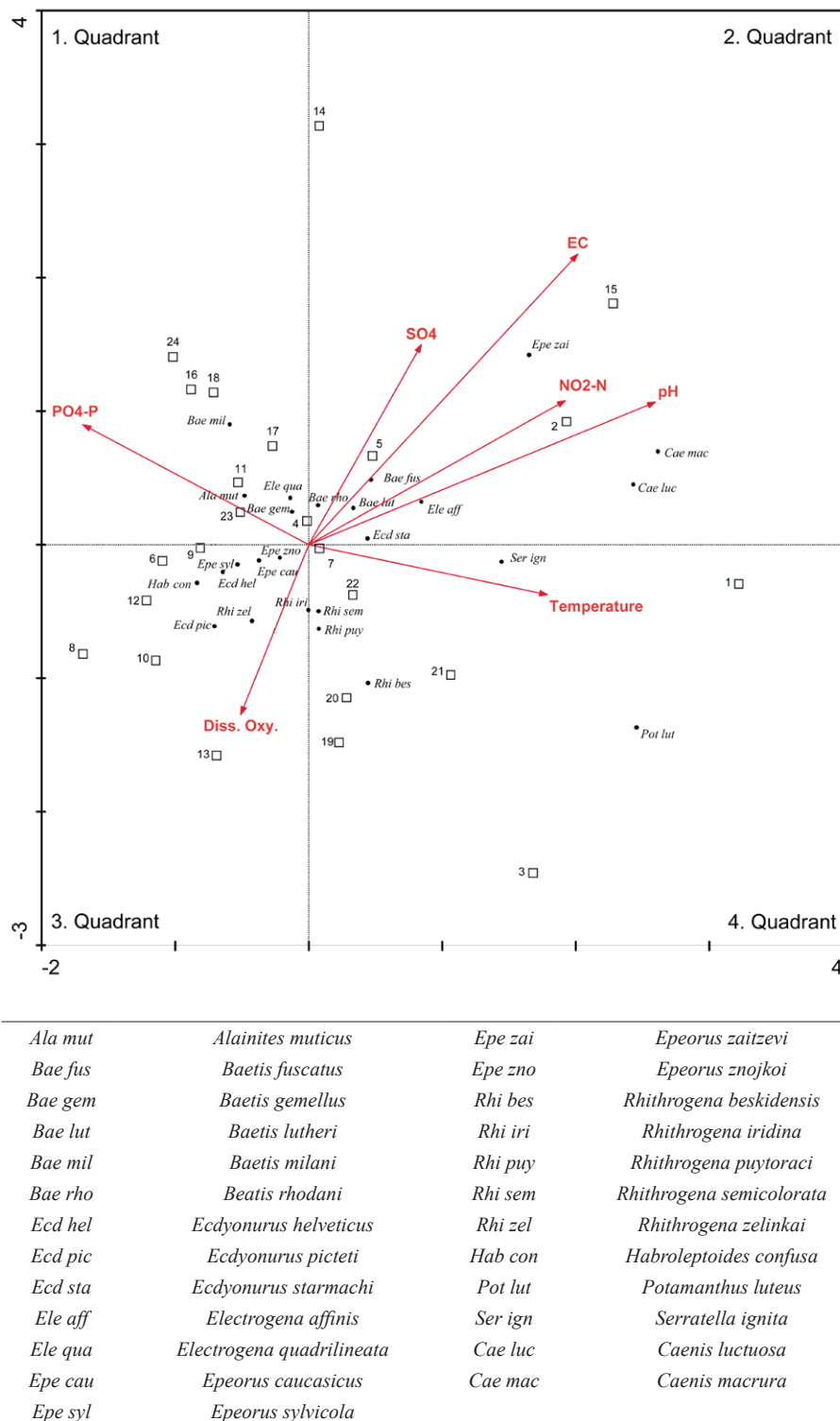


Fig. 4. CCA diagram of the analysis of the mayfly communities (•: Species, □: Sites). Monte Carlo permutation test ($p \leq 0.005$).

tion, the high tolerance to $PO_4\text{-P}$ indicates that these species can present in β -mesosaprobic habitats.

The environmental variables indicating habitat degradation are in the second quadrant of the CCA diagram (determinant variables are SO_4 , $NO_2\text{-N}$, EC and pH). The sites in this quadrant are S2, S5, S14

and S15. While S5 and S14 exhibit reference habitat conditions, S2 and S15 do not. One of the reference sites, S14, is situated in an irrelevant place in CCA. Therefore, there are no determinant environmental variables for S14. Although S5 is characterised by reference habitat conditions, it is situated in the sec-

Table 4. Abundance (%) of species of Ephemeroptera within the study area.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24
<i>A. muticus</i>	-	-	-	0.89	-	0.56	0.52	-	-	-	-	-	9.21	-	-	7.03	6.84	1.54	1.47	-	-	3.81	5.94	35.00
<i>B. fuscatus</i>	33.04	0.62	-	-	-	-	-	-	-	1.93	6.78	2.17	-	6.99	12.68	10.03	8.53	4.81	3.52	2.57	5.42	-	-	-
<i>B. gemellus</i>	1.04	0.62	6.41	17.86	45.28	21.75	1.81	10.69	48.96	4.35	21.73	7.25	13.16	26.27	8.29	17.53	22.04	25.82	6.74	4.80	8.97	4.01	7.34	20.00
<i>B. lutheri</i>	33.48	23.86	-	26.79	11.32	0.99	16.15	6.36	-	5.80	2.57	-	7.89	9.64	30.98	15.76	9.83	9.49	4.11	10.12	12.34	5.01	-	-
<i>B. milani</i>	-	-	-	-	-	-	-	-	-	0.97	8.18	26.09	-	11.57	-	1.98	0.92	1.29	0.29	-	-	-	-	-
<i>B. rhodani</i>	6.37	5.19	58.97	2.68	9.43	38.84	17.53	3.47	27.08	11.11	32.71	-	7.89	44.82	24.63	44.88	45.93	45.84	2.35	2.74	4.67	3.61	2.45	16.00
<i>E. helveticus</i>	-	-	-	-	-	-	0.60	1.16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.05	-
<i>E. macani</i>	0.30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>E. picteti</i>	-	-	-	-	-	-	-	10.40	-	-	-	-	-	-	-	-	-	-	0.59	1.03	-	-	8.39	-
<i>E. starmachi</i>	0.15	-	-	-	-	-	0.17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.40	-
<i>E. affinis</i>	1.19	1.24	1.28	-	-	0.14	-	-	-	-	-	-	-	-	0.24	0.14	0.23	0.68	0.29	-	0.19	-	-	-
<i>E. quadrilineata</i>	0.15	0.21	1.28	-	-	1.41	0.35	-	-	-	-	-	-	-	0.24	0.27	0.23	0.43	-	1.03	-	-	4.55	9.00
<i>E. caucasicus</i>	-	-	-	2.68	9.43	23.73	22.37	25.43	-	16.91	10.98	5.07	7.89	0.24	-	1.91	3.07	5.18	16.72	7.55	20.37	22.24	13.29	5.00
<i>E. sylvicola</i>	-	-	-	47.32	-	0.71	0.69	28.61	15.63	8.70	4.67	19.57	1.32	0.24	-	0.20	0.69	1.42	3.52	0.86	0.56	2.40	-	-
<i>E. zaitzevi</i>	0.15	-	-	-	-	-	-	-	-	-	-	-	-	0.24	-	-	-	-	-	-	-	-	-	-
<i>E. znojkoii</i>	-	1.87	1.28	0.89	22.64	1.69	6.39	3.76	-	14.49	9.58	9.42	3.95	-	-	0.27	1.69	3.51	11.14	1.89	14.02	16.23	15.38	13.00
<i>R. beskidensis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21.11	16.30	5.05	5.61	-	-
<i>R. iridina</i>	-	-	-	-	1.89	0.56	5.79	-	-	2.42	-	0.72	-	-	-	-	-	-	2.64	2.06	2.80	8.02	6.64	-
<i>R. puytoraci</i>	-	-	2.56	-	-	0.56	5.01	6.65	-	10.14	-	-	-	-	1.46	-	-	-	4.40	10.12	3.93	6.41	-	-
<i>R. semicolorata</i>	1.48	3.53	-	-	-	1.27	18.39	0.58	-	19.32	-	21.74	35.53	-	-	-	-	-	9.97	14.75	9.16	16.03	13.29	2.00
<i>R. zelinkai</i>	-	-	-	-	-	0.56	4.15	0.87	-	1.93	0.47	1.45	6.58	-	-	-	-	-	3.52	3.09	-	-	7.34	-
<i>H. corifusa</i>	-	-	-	0.89	-	7.20	0.09	2.02	8.33	1.93	2.34	6.52	6.58	-	-	-	-	-	0.29	-	-	-	12.94	-
<i>E. danica</i>	-	-	1.28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>P. luteus</i>	3.26	-	26.92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. ignita</i>	18.81	62.24	-	-	-	-	-	-	-	-	-	-	-	20.73	-	-	-	-	7.33	21.10	12.52	6.61	-	-
<i>C. luctuosa</i>	0.15	0.41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>C. macrura</i>	0.44	0.21	-	-	-	-	-	-	-	-	-	-	-	-	0.49	-	-	-	-	-	-	-	-	-
<i>C. martae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.24	-	-	-	-	-	-	-	-	-

Table 5. Summary of CCA.

Axes			1	2	3	4	Total inertia
Eigenvalues			0.235	0.147	0.118	0.071	1.235
Species-environment correlations			0.923	0.849	0.875	0.869	
Cumulative percentage variance							
	of species data		19.1	31.0	40.5	46.3	
	of species-environment relation		36.0	58.4	76.4	87.3	
Sum of all eigenvalues							1.235
Sum of all canonical eigenvalues							0.654
Summary of Monte Carlo							
Test of significance of first canonical axis:	eigenvalue	=	0.235				
	F-ratio	=	3.770				
	P-value	=	0.0050				
Test of significance of all canonical axes:	Trace	=	0.654				
	F-ratio	=	2.578				
	P-value	=	0.0020				

ond quadrant where indicator variables of organic pollution are present. The reason is that the physical structure of S5 is not suitable for efficient sampling. The number of individuals in S5 is the lowest during the study. Since CCA has evaluated the species composition and physical and chemical variables together, S5 is considered as having degraded habitat conditions; however, this is not accurate. Sites S2 and S15 lack reference habitat conditions and the stream types of these sites differ from the other sites in this quadrant. These habitats are not reference sites because there are settlements and agricultural fields nearby.

The species in the second quadrant are *B. rhodani*, *B. lutheri*, *B. fuscatus*, *E. affinis*, *E. starmachi*, *C. macrura*, *C. luctuosa* and *E. zaitzevi*. They are positively related to SO_4 , $\text{NO}_2\text{-N}$, EC and pH but negatively related to dissolved oxygen. The source of SO_4 in natural waters is from rocks, fertilisers and atmospheric precipitation (WETZEL 2001). Especially in non-industrial areas, the primary source of SO_4 is rain and snow. In freshwater, the source of N is from precipitation, N-fixation and surface inputs and groundwater drainage (WETZEL 2001). High $\text{NO}_2\text{-}$ concentration may be a result of bacterial denitrification of organic compounds and this situation indicates organic pollution in freshwater ecosystems (KELSO et al. 1997, 1999, WETZEL 2001). In natural waters, pH values can be high if riparian vegetation is dense (WETZEL 2001). All sites in this quadrant have dense riparian vegetation along riversides. Additionally, DOW & ZAMPELLA (2000) have stated that there is a positive correlation between EC and

pH. All these variables are associated with organic pollution. The species in this quadrant demonstrate tolerance to high concentrations of SO_4 and $\text{NO}_2\text{-N}$, high values of EC and pH and low concentrations of dissolved oxygen. The most significant relationships between environmental variables and species in this quadrant are EC with *E. zaitzevi* and pH with *C. macrura* and *C. luctuosa*. These *Caenis* spp. are tolerant to high pH values in running waters (PERU & THOMAS 2004, BUFFAGNI et al. 2009). *Baetis lutheri*, *E. affinis* and *E. starmachi* prefer oligosaprobic and β -mesosaprobic habitats (BAUERNFEIND et al. 2002, SPORKA 2003). *Baetis rhodani* is a widespread species that can be found in a variety of running-water ecosystems (TIERNEY et al. 1998, BAUERNFEIND et al. 2002). Therefore, it has a high tolerance to organic pollution and low oxygen concentrations. Thus, *B. rhodani* and *B. lutheri* have been found in oligosaprobic, β -mesosaprobic and α -mesosaprobic habitats, *E. affinis* and *E. starmachi* have been registered in β - and α -mesosaprobic habitats. *Caenis macrura* and *C. luctuosa* prefer mostly beta-mesosaprobic habitats but they can also be found in α -mesosaprobic habitats (BAUERNFEIND et al. 2002, SPORKA 2003). The sites where *C. macrura* and *C. luctuosa* occur belong to the Classes II and III. There is no information regarding the habitat preferences of *E. zaitzevi*. One common characteristic of the species in this quadrant is that all of them can be found in β -mesosaprobic habitats. Therefore, *E. zaitzevi* can prefer β -mesosaprobic habitats. Due to the presence of *C. macrura* and *C. luctuosa* together in the same quadrant, we can assume that *E. zaitzevi*

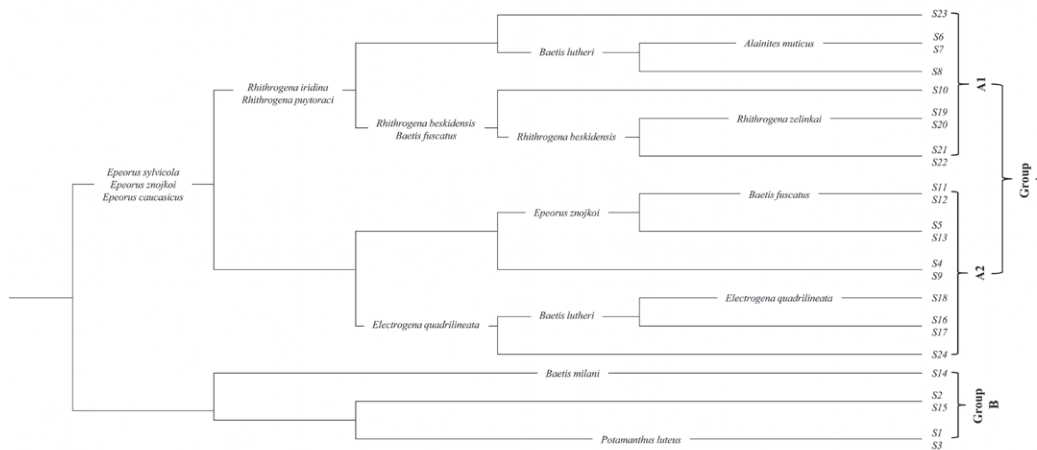


Fig. 5 TWINSpan grouping of sampling sites and indicator species.

may also prefer α -mesosaprobic habitats. Moreover, the habitat quality of the sites where *E. zaitzevi* occurs belong to the Classes II and III.

In the third quadrant of the CCA diagram, the determinant variable is dissolved oxygen (DO). The sites in this quadrant are S6, S8, S9, S10, S12 and S13. All these sites have reference habitat conditions. The species in this quadrant are *R. iridina*, *R. zelinkai*, *E. picteti*, *E. helveticus*, *E. sylvicola*, *E. znojtkoi*, *E. caucasicus* and *H. confusa*. These species are positively correlated with DO but negatively correlated with SO_4 , EC, NO_2 -N and pH. In other words, these species are sensitive to low DO concentrations and high SO_4 , NO_2 -N concentrations and high EC and pH values. The species in this quadrant are highly sensitive to organic pollution and habitat degradation. Species of *Rhithrogena* and *Epeorus*, two of the most typical representatives of rhithral zones (BISS et al. 2002, TACHET et al. 2010), generally prefer moderate or fast currents (SCHMEDTJE & COLLING 1996), low temperatures (TACHET et al. 2010) and oxygen-rich waters. In a study conducted to determine the indicators of running waters in the western part of Turkey, KAZANCI et al. (2008) found that *Rhithrogena* spp. are positively related to dissolved oxygen but negatively related to NO_2 -N, NH_4 -N and PO_4 -P and that *Epeorus* spp. are slightly positively correlated with dissolved oxygen but negatively correlated with temperature. *Rhithrogena iridina*, *R. zelinkai*, *E. picteti*, *E. helveticus* and *H. confusa* mostly prefer xenosaprobic habitats. They can also be found in oligosaprobic habitats, albeit rarely (BAUERNFEIND et al. 2002, SPORKA 2003). There is no information regarding the habitat preferences of *E. sylvicola*, *E. znojtkoi* and *E. caucasicus*. One common characteristic of the species in this quadrant is that all of them could be found in

xenosaprobic habitats. Therefore, *E. sylvicola*, *E. znojtkoi* and *E. caucasicus* may prefer xenosaprobic habitats. They may also prefer oligosaprobic and β -mesosaprobic habitats to a lesser extent, according to the results of the CCA. Moreover, these species are found at sites belonging to the Classes I and II by the present study.

In the fourth quadrant of the CCA diagram, the determinant variable is temperature (T); the sites are S1, S3, S7, S19, S20, S21 and S22. Of these, S7, S19, S20, S21 and S22 have reference habitat conditions. The stream type of S1 and S3 differ from the other sites, as also demonstrated by the CCA: S1 and S3 are situated far from the other sites in this quadrant. The species are *R. semicolorata*, *R. puytoraci*, *R. beskidensis*, *S. ignita* and *P. luteus*. They are positively correlated with T but negatively correlated with PO_4 -P. In other words, these species have high tolerance to high temperatures and low tolerance to high PO_4 -P concentrations. *Serratella ignita* and *P. luteus* have high tolerance to environmental variables. They mostly prefer β - and α -mesosaprobic habitats. Rarely, they can be found in oligosaprobic habitats, as well (BAUERNFEIND et al. 2002, SPORKA 2003). Although *S. ignita* is in the fourth quadrant, it is positively related to the variables in the second quadrant (SO_4 , NO_2 -N, EC and pH) because this species is situated very closely to the second quadrant. The sites where *S. ignita* and *P. luteus* are found belong to the habitat quality Classes I, II and III. *Rhithrogena semicolorata*, *R. puytoraci* and *R. beskidensis* are sensitive to organic pollution. *Rhithrogena puytoraci* mostly prefers xenosaprobic habitats. Rarely, it can also be found in β -mesosaprobic habitats (BAUERNFEIND et al. 2002, SPORKA 2003). *Rhithrogena beskidensis* mostly prefers β -mesosaprobic habitats but is also rarely found

in xenosaprobic habitats (BAUERNFEIND et al. 2002, SPORKA 2003). *Rhithrogena semicolorata* mostly prefers β -mesosaprobic habitats and can rarely be found in xenosaprobic and oligosaprobic habitats (BAUERNFEIND et al. 2002, SPORKA 2003). In this study, the sites where *R. semicolorata*, *R. puytoraci* and *R. beskidensis* are recorded as belonging to the habitat quality Classes I and II. Although *R. semicolorata*, *R. puytoraci* and *R. beskidensis* are in the fourth quadrant, they are much more related to DO rather than temperature, as these species are situated very near the third quadrant.

The TWINSPAN has identified two main groups, Group A and Group B. There are 19 sites clustered together in Group A and the indicator species of this group are *Epeorus sylvicola*, *E. znojko* and *E. caucasicus*. These species are found in the same CCA quadrant (Fig. 4) and they are elucidated as sensitive to low concentrations of dissolved oxygen, high concentrations of SO_4 , NO_2 -N and high values of EC and pH. Group A consists of two subgroups, A1 and A2. There are nine sites in A1 (S6, S7, S8, S10, S19, S20, S21, S22 and S23). All these sites have reference habitat conditions and *Rhithrogena iridina* and *R. puytoraci* are assigned as indicator species for A1. *Alainites muticus* is the indicator species of S6 and S7 and *Baetis lutheri* – for S6, S7 and S8. These sites are under effects of episodic acidification. Such “episodic acidification” is a common phenomenon for the region and has been observed at sites between S6-S20. KAZANCI (2009) has reported this situation in the Eastern part of the Black Sea Region and the Yeşilirmak River Basin for the first time. KAZANCI (2009) has observed high PO_4 -P values with low pH and has defined it as episodic acidification. Spring runoff from melting snow causes episodic acidification in some streams. KAZANCI (2009) has stated that atmospheric discharge and natural process can affect nature of episodic acidification (see also JACKS et al. 1986, DAVIES et al. 1993). For this reason, the water quality classes according to pH and PO_4 -P of the sites with episodic acidification are the Classes III and IV. However, this situation is temporary and the species that inhabit the streams of the region are adapted to this phenomenon. A high PO_4 -P concentration (2.01 mg/l, 2.3 mg/l and 2.01 mg/l, respectively) and low pH values (pH 5.5, pH 6.5 and pH 4, respectively), which are the indicator of episodic acidification, have been observed at these sites. While other physical and chemical variables correspond to the Class I water quality, PO_4 -P concentration and pH values of these sites correspond to the Class IV water quality. According to BRAUKMANN & BISS (2004),

A. muticus and *B. lutheri* can be found in predominantly neutral to episodically weakly acidic waters. Therefore, pH preferences of *A. muticus* and *B. lutheri* are from neutral to alkaline (BUFFAGNI et al. 2009). *Rhithrogena zelinkai* is the indicator of S19 and S20. It mostly prefers clean, unpolluted habitats (xenosaprobic) but it can be also found in oligosaprobic habitats (SPORKA 2003). These sites belong to the Class I according to their habitat quality and had reference habitat conditions. *Rhithrogena beskidensis* is the indicator species of S19, S20, S21 and S22 and has been found in only at these sites. The habitat qualities of these sites correspond to the Class I (S19 and S20) and the Class II (S21 and S22). The species prefers mostly β -mesosaprobic habitats but it can also be found in oligosaprobic habitats. *Rhithrogena beskidensis* and *B. fuscatus* are the indicator species of S10, S19, S20, S21 and S22. There is not any indicator species assigned for S10, as this site has been included in this group subsequently. Therefore, it can be assumed that *B. fuscatus* is somehow related to S10. The site S10 is under the effect of episodic acidification, too. High PO_4 -P concentration (2.17 mg/l) and low pH value (pH 5) have been observed in S10. While other physical and chemical variables indicate the Class I water quality, PO_4 -P concentrations and pH values of these sites correspond to the Class IV water quality. BRAUKMANN & BISS (2004) stated that *B. fuscatus* can be found in predominantly neutral to episodically weakly acidic waters. Therefore, the pH preference of *B. fuscatus* is neutral to alkaline (BUFFAGNI et al. 2009).

There are ten sites in A2 (S4, S5, S9, S11, S12, S13, S16, S17, S18 and S24). No indicator species are assigned for A2 but these sites are related to *E. sylvicola*, *E. znojko* and *E. caucasicus*. A2 has two branches. One of them includes S4, S5, S9, S11, S12 and S13, and they with reference habitat conditions; the other one includes S16, S17, S18 and S24, none having reference habitat conditions. Reference sites and non-reference sites are clustered together in A2. *Baetis fuscatus* is the indicator species of S11 and S12 where episodic acidification has been observed. High PO_4 -P concentrations (2.05 mg/l and 2.69 mg/l, respectively) and low pH values (pH 6 and pH 5) have been observed here. While other physical and chemical variables correspond to the Class I water quality, PO_4 -P concentrations and pH values of these sites are relevant to the Classes III and IV. *Epeorus znojko* is the indicator of S5, S11, S12 and S13. Among these sites, episodic acidification is not observed. Only at S5, all physical and chemical variables correspond to the Class I. However, although PO_4 -P concentrations and pH values correspond to

the Classes III and IV in S11, S12 and S13, all the other physical and chemical variables are relevant to the Class I (Table 3). It can be assumed that *B. fus-catus* is an indicator species for reference sites with episodic acidification and *E. znojko* is an indicator species for reference sites without episodic acidification. The other branch of A2 consists of S16, S17, S18 and S24. These are all non-reference sites and the indicator species of them is *E. quadriline-ata*. Despite the fact that all these are non-reference sites, their habitat quality corresponds to the Class I (S18) and the Class II (S16, S17 and S24). *Electrotrigena quadrilineata* mostly prefers oligosaprobic and β -mesosaprobic conditions (SPORKA 2003). The common feature of these sites is their lowest water temperature during the study (5.74°C for S16, 7.37°C for S17, 7.3°C for S18 and 7.37°C for S24). According to the CCA, these sites are situated at the opposite side of the temperature gradient. Moreover, *E. quadrilineata*, which is assigned as the indicator species of S16, S17 and S24 in TWINSpan, is situated at the opposite side of temperature in the CCA. This suggests that *E. quadrilineata* is sensitive to high water temperature.

In the second main group (Group B), five sites (S1, S2, S3, S14 and S15) are clustered together and there are no indicator species assigned to this group by the analysis. All these sites lack reference habitat conditions and are the most polluted sites (except S14). *Potamanthus luteus* is the indicator species of S1 and S3. The physical and chemical as well as hydromorphological characteristics of S1 and S3 are similar. Site S1 has the highest water temperature (23.86°C), the highest pH value (8.58), the third highest EC value (346 $\mu\text{s}/\text{cm}$) and the second lowest dissolved oxygen (8.04 mg/l). Site S3 has the second highest SO_4 concentration (74 mg/l). According to BUFFAGNI et al. (2009), *Potamanthus luteus* has a wide temperature range and prefers temperatures mostly $>18^\circ\text{C}$. This species largely prefers β -mesosaprobic habitats but can also be found in α -mesosaprobic habitats. Sites at which *P. luteus* has been found belong to the Classes II and III. *Baetis milani* is an indicator species of S14. This site is clustered together with the other sites in Group B, despite its reference habitat conditions. There are no habitat degradation or anthropogenic impacts and the physical and chemical variables (except $\text{PO}_4\text{-P}$ concentration and pH values due to episodic acidification) correspond to the Class I. For these reasons, S14 is determined to be a reference site. However, the species composition at this site indicates the opposite and it is clustered with the non-reference sites by TWINSpan.

Conclusion

The community structure of the Ephemeroptera was analysed using multivariate analysis, CCA and TWINSpan. These analyses allowed exploring the relationship between the observed species diversity and several environmental variables. We used the CCA to analyse the relationship of the species with seven physical and chemical variables (temperature, dissolved oxygen, pH, electrical conductivity, $\text{NO}_2\text{-N}$, SO_4 and $\text{PO}_4\text{-P}$). The TWINSpan analysis allowed identifying the indicator species at the collecting sites; thus, we identified the indicator species of reference or non-reference sites. The habitat characteristics of some species with unknown habitat preferences were also determined. According to the results, *B. gemellus* and *B. milani* preferred oligosaprobic and β -mesosaprobic habitats. *Epeorus zaitzevi* preferred mostly β -mesosaprobic and rarely α -mesosaprobic habitats. *Epeorus sylvicola*, *E. znojko* and *E. caucasicus* preferred mostly xenosaprobic and rarely oligosaprobic or β -mesosaprobic habitats. Furthermore, the fauna of the Ephemeroptera of the studied area has many shared species with Europe and the Caucasus. Only three out of 28 species, *E. caucasicus*, *E. zaitzevi* and *E. znojko* are restricted in the Caucasus Region. Therefore, this study brings new information to the distributional and ecological knowledge of European and Caucasian species of Ephemeroptera.

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