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## Assessment on the Ephemeroptera distribution (Insecta) in relation with aquatic parameters in different rivers from Aninei Mountains (SW Romania)

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**Abstract**. The wide distribution and high tolerance to physical and chemical factors makes it possible for Ephemeroptera to be used as a bioindicators. The objective of this paper was to bring some new information regarding the distribution of the ephemera species in western Romania, together with the analysis of species distribution in relation to certain physical-chemical parameters. The samples were taken from the three river basins of the Aninei Mountains (Bârzava, Caraș and Nera), totalizing a number of 27 sites located on the tributary and on the main course of the rivers. We identified a number of 14 species, the most common being *Ecdyonurus venosus*, *Ecdyonurus torrentis*, *Bäetis rhodani* and *Serratella ignita*. The statistical interpretation, related to physicochemical factors showed a decisive effect of the water temperature, of dissolved oxygen, magnesium, nitrate and cyanide, concerning the distribution of some common species of mayflies.

Key Words: Ephemeroptera, diversity, Aninei Mountains, Bârzava, Nera, Caraș.

**Sažetak**: Velika rasprostranjenost i izdržljivost fizičko-hemijskih faktora omogućava korišćenje efemeroptera kao bioindikatori. Cilj ovog dela je sakupljanje što više informacija o rasprostranjenosti vrsta efemeroptera u zapadnom predelu Rumunije, kao i analiza rasprostranjenosti odredjenih vrsta u odnosu sa fizičko-hemijskim parametrima. Dokazi su preduzeti iz tri hidrografska sliva iz Planine Aninei (Birzava, Karaš I Nera) iz kojih su proizišle 27 stanica sa pritoka i na glavnoj putanji reke. 14 vrsta efemeroptera bile su identifikovane, a najčešće prisutne bile su *Ecdyonurus venosus, Ecdyonurus torrentis, Bäetis rhodani i Serratella ignita.* Statistike, zajedno sa fizičko hemijskim faktorima, pokazuju odlučujuci efekat temperature vode, rastvorenog kiseonika, magnezijuma, nitrata i cianura nad rasprostranjenosti zajedničkih vrsta. **Ključne riječi:** Efemeroptera, raznovrsnost, Planine Aninei, Birzava, Nera, Karaš.

**Rezumat**. Larga distribuție și toleranța mare la factorii fizico-chimici face posibilă utilizarea efemeropterelor ca bioindicatori. Obiectivul acestei lucrari este de a aduce noi informații cu privire la distribuția speciilor de efemeroptere în vestul României, alături de analiza distribuției anumitor specii în relație cu parametrii fizico-chimici. Probele au fost prelevate din cele trei bazine hidrografice din Munții Aninei (Bârzava, Caraș și Nera) totalizând 27 de stații situate pe tributari și pe cursul principal al râului. 14 Specii de efemeroptere au fost identificate, cele mai frecvent întâlnite fiind *Ecdyonurus venossus, Ecdyonurus torrentis, Bäetis rhodani* și *Serratella ignita.* Interpretarea statistică în relația cu factorii fizico-chimici arată un efect decisiv al temperaturii apei, oxigenul dizolvat, magneziu, nitrați și cianuri, asupra distribuției speciilor comune. **Cuvinte cheie**: Ephemeroptera, diversitate, Munții Aninei, Bârzava, Nera, Caraș.

**Introduction**. The importance of Ephemenoptera insects lies in the fact that it represents one of the dominant groups of benthic fauna, with an important role in the matter and energy transfer of the lotic ecosystems. By their microphage nutrition mode, they help to reduce the amount of detritus, while the larval stages (and adults in a small way) represent a significant percentage in fish feed. The distribution of these organisms is closely related with the environmental factors, reason for the entire taxonomic group is being used as a bioindicator in the studies targeting water quality (De Haas et al 2002; Fialkowski et al 2003; Brittain & Sartori 2009). Studies regarding the correlation between the environmental factors and the ephemeroptera larvae diversity have been made in Romania by Petrovici (2009) in the hydrographic basin of the Crisul Repede River.

The Aninei Mountains are located in the south-western Romania and include two national parks: Semenic-Cheile Carașului National Park and Cheile Nerei-Beușnița National Park, being predominant of calcareous nature (Bleahu & Rusu 1965). This geographical unit is drained by the waterways of three main rivers: Bârzava, Caraș and Nera. The Bârzava River collects its waters from the northern and northeastern part of the Aninei Mountains and from the western part of the Semenic Mountains, crossing a substratum of crystalline schist. The upstream stretch of the Caraș River drains the central-western Aninei Mountains, of calcareous composition. The Nera River crosses the southern sector of these mountains, draining the waters from the southern and central-eastern sector (Pârvulescu 2009). This area was strongly impacted by industrialization, in the form of dams, water catchments, mining (Pârvulescu & Hamchevici 2010). This paper approaches the following issues: i) a first list of the ephemeroptera (larvae) fauna from these three river basins, for which have not been done any studies on the fauna at the level of this group and ii) determination of the environmental factors that the spread of these species is depending on, in the aquatic ecosystems.

#### Material and Method

Sampling. The samples were collected in August 2009, from the catchment areas of Bârzava (8 sites), Caraş (11 sites) and Nera (8 sites), from Aninei Mountains (see Figure 1). Qualitative samples were collected using a hand net with meshes of 250  $\mu$ m. A total of 5 kicks were collected proportionately from all major habitat types over the length of the reach. The collected material was preserved in 70% alcohol (in the field) and then identified in the laboratory to species using Landa (1969), Belfiore (1983), Studemann et al (1986), Bauernfeind & Humpesch (2001).

Several general physical-chemical indicators were measured in each of the sampling stations: water temperature, pH, dissolved oxygen (DO), total hardness (Hrd), anionic surfactants (AS), tannin+lignin (Tan+Lig), cyanide (Cyan), chemical oxygen demand (COD), dissolved inorganic nitrogen forms (N-nitrate, N-nitrite, N-ammonia) and soluble reactive phosphorous (SRP). These indicators were recorded by *in-situ* measurements with HACH Lange field equipment.

To perform the statistical analysis, we used the STATISTICA software version 7.0 for Windows (StatSoft Inc.) and PAST. Principal Component Analysis (PCA) was undertaken for the habitat parameters for a better illustration of their variation over the sampling sites. The eigenvalues and the percentage explained by the first both axis were estimated. One-way ANOVA test were applied for every parameter in order to reveal differences between presence and absence of the species.



Figure 1. Sampling sites in Aninei Mountains

*Study sites*. <u>The Bârzava catchment area</u>: B1: Crivaia stream, 45°12'10"N 22°00'38"S, 700 m; B2: Grindești stream, 45°13'14"N 22°00'33"S, 630 m; B3: Breazova stream, 45°15'10"N 22°02'58"S, 480 m; B4: Lișcov stream, 45°17'32"N 22°02'33"S, 380 m; B5: Stârnic stream, 45°18'13"N 22°02'43"S, 330 m; B6: Râul Alb stream, 45°17'32"N 21°59'41"S, 430 m; B7: Bârzava river, upstream to Reșița city, 45°17'39"N 21°56'15"S, 275 m; B8: Cuptoare stream, 45°16'36"N 21°57'35"S, 360 m;

The Caraş catchment area: C1: Gelugu stream, 45°16'01"N 21°48'31"S, 230 m altitude; C2: Nermed stream, 45°13'59"N 21°52'26"S, 275 m; C3: Oraviţa stream, upstream to Oraviţa city, 45°03'07"N 21°45'02"S, 390 m; C4: Jitin stream, 45°07'13"N 21°48'02"S, 395 m; C5: Natra stream, 45°06'21"N 21°46'09"S, 295 m; C6: Lisava stream, 45°06'22"N 21°46'39"S, 300 m; C7: Călugăra stream, 45°01'45"N 21°45'15"S, 300 m; C8: Cândeni stream, 44°56'49"N 21°44'08"S, 295 m; C9: Topliţa stream, 45°10'56"N 21°56'56"S, 465 m; C10: Celnicu Mare stream, 45°06'48"N 21°51'46"S, 435 m; C11: Gârlişte river, 45°06'23"N 21°50'53"S, 420 m;

<u>The Nera catchment area</u>: N1: Staier stream: <u>45°02'38"N 21°51'34"S</u>, 540 m; N2: Minis spring, <u>45°01'29"N 21°49'24"S</u>, 540 m; N3: Beiu river, <u>44°55'22"N 21°46'28"S</u>, 240 m; N4: Nera river, upstream to the Cheile Nerei-Beușnița National Park, <u>44°50'19"N 21°51'18"s</u>, 200 m; N5: Bresnic stream, <u>44°50'27"N 21°51'21"S</u>, 200 m; N6: Ducin stream, <u>44°52'31"N 21°53'47"S</u>, 280 m; N7: Moceriș stream, <u>44°53'36"N 21°53'53"S</u>, 298 m; N8: Lăpușnic stream, <u>44°55'03"N 21°55'37"S</u>, 298 m.

Habitat was different for each site: deciduous forests (sites B3-B5, B7, B8, C2-C9, C11, N1, N3, N4, N5, N7 and N8), mixed forests (site B1, B2, B6 and N2), crops and grasslands (sites C1, C10 and N6). Vegetation coverage of the riverbed has different values: 0 - 50% (sites B1-B8, C3, C5-C9, N1-N4, N8) and between 51-98% for the remaining sites. The average values of the width (m) and depth (m) of the catchment area where the stations were placed were: Bârzava river: 2.3 m [95% CI: 1.52-3.08], 0.26 m [95% CI: 0.17-0.35]; Caraş river: 1.93 [95% CI: 1.38-2.48], 0.22 [95% CI: 0.15-0.29]; Nera river: 2.15 [95% CI: 1.15-3.15] and 0.34 [95% CI: 0.09-0.59] respectively. Average values of the air and water temperature (<sup>0</sup>C) for each of the 3 hydrographic basins were calculated: Bârzava river: 23.59 [95% CI: 22.27-24.91] and 16.8 [95% CI: 15.55-18.05]; Caraş river: 19.68 [95% CI: 17.77-21.59] and 15.04 [95% CI: 14.01-16.07]; Nera river: 21.62 [95% CI: 19.7-23.54] and 15.35 [95% CI: 13.98-16.72].

#### **Results and Discussions**

#### Mayflies diversity

In the three basins present in the Aninei Mountains have been identified 14 ephemeroptera species (Table 1), belonging to six superfamilies and eight families. The best represented is the Heptageniidae Family, followed by Baëtidae Family, preferring well oxygenized waters, rapids, from the mountain area.

The genus *Ecdyonurus* (Eaton 1868) was represented with the largest number of recorded species – 4 species. Three species within the genera *Baetis* (Leach, 1815), two species within the genera *Ephemera* (Linnaeus, 1758) and one species within the genera *Caenis* (Stephens, 1835), *Serratella* (Edmunds, 1959), *Paraleptophlebia* (Lestage, 1917), *Oligoneuriella* (Ulmer, 1924) and *Habrophlebia* (Eaton, 1881).

Table 1

Catchment Area	Sampling stations	Baëtis rhodani	Baëtis vernus	Baëtis muticus	Caenis luctuosa	Serratella ignita	Ephemera vulgata	<i>Ephemera</i> danica	Ecdyonurus dispar	Ecdyonurus venosus	cdyonurus austriacus	Ecdyonurus torrentis	<i>Nigoneuriella rhenana</i>	Habrophlebia lauta	Paraleptophlebia submarginata
	<b>D</b> 1									-	Ē		0		
Bârzava	B1 B2	x				x				X		x			
	D2 D2					X				x		×		v	
		~				x				X		x		X	
	D4 85	x				×		×		×		×			
	B6	Y				~		~		×		^		×	
	B7	X				×				~				~	
	B8	x				~				×		×			
	C1	X			×					X		A			
raș	C2				~					x					
	C3	x		x						×		x		×	
	C4	x		x		х				x					
	C5									x		x			
	C6						х			x					
S	C7				x					x					
	C8	x								x		x			
	C9									x					
	C10														х
	C11	х													
Nera	N1	x													
	N2	x								х		х			
	N3					x									
	N4					x	х					x	х		
	N5	x	х												
	N6								х	х	х				
	N7	х							х	x		x			
	N8									х		x			
Frequency		44.4	3.7	7.4	7.4	25.9	7.4	3.7	7.4	63	3.7	40.7	3.7	11.1	3.7

Occurrence of mayfly (Ephemeroptera) species at studied localities along the Bârzava, Caraș and Nera Rivers from Aninei Mountains, 2009.

Baëtidae Family Leach, 1815 - Small Minnow Mayflies

This family is considered by Clifford (1980) as being the most abundant in the Holarctic region. They are species common to rivers and slow flowing waters. The entire family is listed by Hilsenhoff (1988) and Bode et al (2002) as having a tolerance value for organic population of 5. Of this family, one genus with 3 species has been identified in the Aninei Mountains.

**Baëtis rhodani** Pictet, 1873. This species was by far the most common and abundant of the Ephemeroptera in Aninei Mountains, preferring well-oxygenated waters, rapid mountain sector. It withstands at high temperature variations, being encountered between 5 - 19  $^{\circ}$ C, with a optimum between 11 - 14  $^{\circ}$ C. It was found by Clifford (1980) after the

data synthesis from over 100 studies, as the most abundant species in the Palaearctic region. Schoenemund (1930) reports it as being present in all rivers, with no special environmental requirements relating to water quality and Hellawell (1986) ranks it among the moderately tolerant species to pollution. It was found in the Aninei Mountains with high frequency in all the three rivers investigated. Referring to this species, Găldean (1992) predicts a progressive development of this species in the future, in the detriment of others that are not opportunistic.

**Baëtis vernus** Curtis, 1834. It prefers submontane waters, with temperatures between  $13-17^{\circ}$ C, with an optimum at  $14^{\circ}$ C. It is one of the most tolerant species when it comes to water quality degradation, being found on almost all types of substratum. However, it prefers the habitats with submerged vegetation in the river sections dominated by transport and sedimentation phenomena. It is famous for its resistance to ammonia concentrations of up to 5 ppm and CBO (biochemical oxygen consume) values of up to 10 ppm. In Aninei Mountains, it was only identified in Breșnic stream (tributary of the Nera River), with its origin coming from a coal mining.

**Baëtis muticus** (Linneus, 1758) sin. *Baëtis pumilus* (Burmeister 1839). It is a species of hilly or submontane, euriterma, with the optimum at 13-20<sup>o</sup>C (Bogoescu 1958). It lives preferentially on a sand and oozy substrate, with finely chopped detritus sediments and can be also found on submerged vegetation (Petrovici 2009).

In the Aninei Mountains it was identified only in Caraș river hydrographic basin, in two tributaries (Oravița and Jitin streams), upstream of the localities with the same name.

#### Caenidae Family Newman, 1853 - Small Squaregilled Mayflies

The entire family is listed by Hilsenhoff (1988) and Bode et al (2002) as having a tolerance value for organic population of 6, measured on a scale ranging from 0-intolerant to 10-tolerant.

**Caenis luctuosa** (Burmeister 1839) sin. *Caenis moesta* (Bengtsson 1917). Undemanding species can be encountered both in large waters, torrents or lakes (Perán et al 1999). It is present in the areas with sandy, clay or clay-loam substrate, but can also be found adhering to stones. It prefers less rushed and more warm waters with oozy and sandy substrate, rich in fine detritus, characteristic to river sectors where the transportation and deposition processes are dominant (Petrovici 2009). It is a tolerant species, at high mineralization, and also regarding organic water pollution (Belfiore 1983). In the Aninei Mountains it was identified only in a tributary of the river Caraș (Gelugu Stream), after crossing the Lupac village that causes an increased eutrophication of the water.

#### **Ephemerellidae Family** Klapálek, 1909 - Spiny Crawler Mayflies

Hilsenhoff (1988) and Bode et al (2002) give the first grade to this family, considering it to be the most sensitive species at the water quality modifications.

**Serratella ignita** (Poda 1761). It is a crawling, common species in the mountain streams as in lowland waters. This species appears to have preference for places where there was a good, fairly fast flow of clean water with a depth of more than 10 cm, and some emergent vegetation (Langford & Bray 1969). Omnivourous species, undemanding regarding feeding, is fed on detritus as well as on algaes and the corpses of congeners or of others aquatic species. During the ovarian maturation, in the case of the larvae from which will derive the females, have been reported cases of cannibalism, they were found looting larvae of the same species. Having adaptations to several types of habitat, it is found both in fast streams, and in the gently flowing waters, being described big differences in size and coloration depending on the habitat. Thus, in fast mountain streams, the larvae are smaller and darker, and in plain waters are bigger and of lighter colors (Schoenemund 1930). Găldean (1992) predicted for this opportunistic species a progressive development in the rivers of Romania, while reducing to extinction of others. We have met it in all the three

rivers of the Aninei Mountains, being one of the most common species in this catchment basin.

#### **Ephemeridae Family** Latreille, 1810 - Common Burrower Mayflies

Hilsenhoff (1988) and Bode et al (2002) give to this family the degree number 3, because it's representatives were found in all three hydrographic basins.

**Ephemera vulgata** Linne, 1746. Edmunds & McCafferty (1996) noted that this species prefers the substrates composed of muddy and fine sand deposits, where it digs galleries with the extensions of the mandibles. In the Aninei Mountains it was identified in two of the three hydrographic basins: Caraş (in Lisava tributary) and Nera (at the entrance of this river- Cheile Beuşnița - Nera National Park).

**Ephemera danica** Müller, 1764. This species was described by Kimmins (1954) as common and abundant on sandy substrates in lakes, rivers and streams, with a preference for alkaline waters. It lives in high speed waters, on a sandy or loamy substrate and doesn't have as a habit the digging of galleries. In the early stages of life, it prefers the microhabitats with sand, and as a nymph in the last stage, it prefers those composed of sand and gravel. In the Aninei Mountains, it was identified only in a tributary of the Bârzava River, named Stârnic.

#### Heptageniidae Family Needham, 1901 - Stream Mayflies

All the species belonging to this family are rheophile species, having the body strongly flattened, and living in well oxygenized and fast waters, from the creeks and river areas characterized by processes of erosion (Petrovici 2009). Hilsenhoff (1988) and Bode et al (2002) give the degree number 3 to this family, because of the tolerance to water degradation.

**Ecdyonurus dispar** (Curtis, 1834). It lives in fast mountain waters, but downs even at the smoothes ones from the plains. This species has been identified only in two tributaries of the Nera river: Ducin and Moceriș streams.

*Ecdyonurus venosus* (Fabricius, 1775). It was the most common species found in this study (in 63% of the sites investigated).

*Ecdyonurus austriacus* (Kimmins, 1958). It was identified in only one tributary of the Nera river, Ducin stream.

*Ecdyonurus torrentis* (Kimmins, 1942). It was met with a high frequency (40.7%), being present in all three rivers investigated.

#### Oligoneuriidae Family Ulmer, 1914

Hilsenhoff (1988) and Bode et al (2002) give this family note 2, because of the tolerance to water degradation. It is a family that depends on a high oxygen content of water.

#### Oligoneuriella rhenana Imhoff, 1852

This species is very sensitive to diminution of oxygen contents in water. It is characteristic of that part of river, where the transportation phenomena has prevalence (corresponds to the metharithron, where the substratum is formed from middle sized stones, among which organic debris, gravel sands and are accumulated. The nymphs of *O. rhenana*, typically litophilous, are usually present in rivers with fast flowing waters and cobble or rocky substrata; they possess a characteristic sub-triangular head and the inner margin of the femur and tibia of the forelegs possesses a double row of long setae (Fenoglio et al 2005). Hence, on the basis of their trophic role, they ecologically belong to the functional feeding group of filterers (Engblom 1996). We have found this larva only in Nera River, at its entry in Cheile Nerei-Beuşniţa National Park, where they meet the conditions of habitat as described above.

#### **Leptophlebiidae Family** Banks, 1900 - Pronggilled Mayflies

Hilsenhoff (1988) and Bode et al (2002) grants this family the note 3, because of the tolerance to water degradation.

**Habrophlebia lauta** Eaton, 1884. It lives in small mountain creeks, with a slender body that allows it to find shelter in the dimples and cracks of rocks, as well as in the submerged vegetation. It was identified in Bârzava river Dam, upstream to the Breazova and in a tributary (White River), and in Caraș River (Oravița tributary, upstream to the city with the same name).

**Paraleptophlebia submarginata** Stephens, 1836. Rare and scarce. 1 Nymph was collected at Celnicu Mare stream (tributary of Caraș River) from sandy substrata where dead leaves and twigs had accumulated in ripples. Kimmins (1954) described the habitat of this species as slow streams where the substratum was sand with packets of leaves. Also, the author it quotes as rheophila, hemistenoterma.

As far as the similarity in the preferences for a specific habitat (Figure 2) is concerned, significant links were noticed among few mayflies' species in the Aninei Mountains. However, affinities are noticed between the *E. vulgata* and *O. rhenana* and between *E. dispar* and *E. austriacus*. *Caenis luctuosa* is associated negatively with all other species. This species, although it supports large variations of the physical and chemical factors, was identified only there where, due to a massive eutrophization of the water, no other species of mayflies have been identified.



Figure 2. The association of mayfly's species in the rivers of Aninei Mountains. (single linkage, coph. corr. 0.6196).



Figure 3. The PCA ordination of the physical-chemical parameters in Aninei Mountains Rivers.

The first and second axis of the PCA ordination of physical-chemical parameters (Figure 3) explained 40.6% of their variance. The eigenvalues of the first axis are 3.5 and respectively 3.2. It can be seen as N-species, phosphorous, detergents, oxygen deficit, all good indicators of organic pollution, are in negative correlation with altitude and dissolved oxygen. In order to find a link between the water quality (as given by the selected physical-chemical indicators) and the absence/presence of commonest mayflies species, the obtained data sets were subject to ANOVA analysis, between the set of data from the sampling stations where species was found and the set of data from the sampling station where the species was not found (Table 2). The diagrams which show the frequency (Figs 4-9) emphasize the interval of values for the presence or the absence of this species.

Table 2

ANOVA LESIS IOF THE SELECTED PHYSICAL-CHEMICAL INDICATORS,	
measured in 2009 in the Aninei Mountains Rivers under investigation for the commone	est
mayflies (significant ANOVA p values in bold)	

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	, (5	•	,	
Variable	Ecdyonurus venosus	Baëtis rhodani	Ecdyonurus torrentis	Serratella ignita
Altitude	0.62911	0 57674	0 31669	0 13387
Shadow	0 12981	0.83537	0.59801	0 12012
Airtomporaturo	0.85882	0.46545	0.17612	0.53405
Mater temperature	0.03002	0.40343	0.17012	0.05495
water temperature	0.60381	0.06184	0.24187	0.06831
рН	0.36510	0.93503	0.58219	0.07571
Dissolved oxygen	0.03752	0.33383	0.74982	0.01901
Conductivity	0.77124	0.81036	0.91001	0.58444
Total hardness	0.98794	0.59518	0.86635	0.37972
Calcium	0.72995	0.39547	0.81895	0.17661
Magnesium	0.93667	0.84937	0.65201	0.03248
Anionic surfactants	0.12309	0.16127	0.40876	0.49708
Tannin+lignin	0.79203	0.90386	0.74673	0.79203
COD	0.22795	0.21614	0.39015	0.49615
Cyanide	0.00506	0.55014	0.86818	0.35723
N-nitrate	0.15354	0.03395	0.24800	0.49111
N-nitrite	0.40993	0.65829	0.41951	0.53457
N-ammonia	0.27893	0.33791	0.22348	0.74534
SRP	0.09081	0.91390	0.51166	0.49213



Figure 4. Diagram of ANOVA test (left) and histogram of the sampling frequency (right) with and without *E. venosus* for dissolved oxygen.



Figure 5. Diagram of ANOVA test (left) and histogram of the sampling frequency (right) with and without *E. venosus* for cyanides.



Figure 7. Diagram of ANOVA test (left) and histogram of the sampling frequency (right) with and without *B. rhodani* for N-nitrate.



Figure 8. Diagram of ANOVA test (left) and histogram of the sampling frequency (right) with and without *S. ignita* for dissolved oxygen.



Figure 9. Diagram of ANOVA test (left) and histogram of the sampling frequency (right) with and without *S. ignita* for magnesium.

According to the ANOVA test some parameters have a decisive effect on the distribution of the commonest mayflies' species in Aninei Mountains: dissolved oxygen, magnesium, Nnitrate and cyanide. Ecdyonurus venosus prefers a high quantity of dissolved oxygen, with an optimum around 8.7 mg L<sup>-1</sup>, while the statistical results show a preference of *S. ignita* species for lower values of the oxygen, of 7.9 mg L<sup>-1</sup>. A higher concentration of water dissolved magnesium ion is prefered by S. ignita species, with an optimum around 9.7 mg L<sup>-</sup> <sup>1</sup>. Also, Bäetis rhodani is tolerant to higher concentrations of N-nitrate, with optimum around 0.5 mg L<sup>-1</sup>. The *E. venosus* species shows cyanide intolerance (measured in a low span in this region 0.001-0.019 mg  $L^{-1}$ ). There was no evidence for a preferential distribution of mayflies as far as the following parameters are concerned: altitude (measured in the interval 190 - 900 m), shadow (measured in the interval 0 - 100%), water temperature (measured in the interval 13.3 - 23.7), chemical oxygen demand (measured in the interval  $2.5 - 5.8 \text{ mg L}^{-1}$ ), total hardness (measured in the interval 0.69 -28.7 mg  $L^{-1}$ ), calcium (measured in the interval 13.8 – 76.2), tannin+lignin (measured in the interval  $0.1 - 2.3 \text{ mg L}^{-1}$ ), N-nitrite (measured in the interval  $0.001 - 0.124 \text{ mg L}^{-1}$ ), Nammonia (measured in the interval  $0.007 - 2.78 \text{ mg L}^{-1}$ ) and soluble reactive phosphorus (measured in the interval  $0.02 - 1.27 \text{ mg L}^{-1}$ ).

**Conclusions**. In the Aninei Mountains, the most commonly encountered species were: *Ecdyonurus venossus, Ecdyonurus torrentis, Bäetis rhodani* and *Serratella ignita*. The Bârzava hydrographic basin has the lowest species diversity.

A good indicator species of dissolved oxygen is *Ecdyonurus venosus* also sensitive to chemical pollutants. *Baëtis rhodani* is a good indicator species for low organic pollution waters. To obtain statistically significant data on the sensitivity of mayfly species studies needed to cover a wider area, so that some species can be caught more frequently like in the Aninei Mountains.

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