Studii şi Cercetări	Mai 2018	Biologie	27/2	34-41	Universitatea”Vasile Alecsandri” din Bacău

SEASONAL DYNAMICS OF BIOTA FROM SEVERAL SPRINGS LOCATED IN THE APUSENI MOUNTAINS, ROMANIA

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Key words: *invertebrate communities, seasonal dynamics, diatoms, environmental factors*

INTRODUCTION

Springs, defined as the place of appearance on the surface of groundwaters as a result of sediment permeability, represent unique ecotonal ecosystems, where groundwater, surface waters and terrestrial ecosystems interact [1]. Currently classified as groundwater-dependent ecosystems, they are important hotspots of biodiversity due to their heterogeneity in typology, their habitat complexity and their particular environmental conditions [5]. Springs are considered to be ”islands” in the riverine systems, harboring a well-defined, constant, often endemic biota [4]. Springs can also represent sources of drinking water [1] and in many cases they have cultural importance for humans (e.g. the ”living spring water” in the Romanian folklore). Even if springs are stable habitats, they are particularly sensitive to human impacts, like pollution, changes in land use and habitat degradation [5].

Variations in water velocity discriminate three classic types of springs [4; 5]: 1. limnocrenes or pool springs (lentic habitats with no water currents); 2. helocrenes or seepage springs (swampy area with slow-flowing waters) and 3. rheocrenes or flowing springs (lotic habitats). Intermediate types can be distinguished, based mainly on the characteristics of their biotic communities: algae as photoautotrophs and invertebrates, both meiofauna and macroinvertebrates, as consumers [5].

Spring habitats were recognized as important biotopes by Illieş and Botoşăneanu [14], who described them as the ”crenon / crenal biocoenosis”. Since then, numerous studies described spring typologies, characteristics, flora and fauna in Europe [e.g. 5; 8; 10; 20; 22; 24 etc.].

However, spring habitats received little interest in Romanian literature, especially in case of the Apuseni Mountains. Previous studies considered different geographic areas [13; 21], or focused on one group (e.g. amphipods [7], water mites [6] etc.).

The aim of the present paper is to investigate the ecological characteristics of eucrenal biota from several sites located in the Apuseni Mountains, focusing on the seasonal dynamics of invertebrate assemblages. The novelty of this purpose resides from the fact that no information on this subject was available in the Romanian literature. No seasonal dynamics was hypothesized to occur for the invertebrate communities, since little variation in physico-chemical factors was observed.

MATERIAL AND METHOD

The invertebrates were sampled in 2017 in three seasons, as follows: the 18th of February 2017, the 14th of May 2017 and the 23rd of July 2017 (Table 1). Only summer samples were considered in case of algae.

The five sampling springs were located in the Apuseni Mountains, in a region drained by two rivers: the Someşul Cald and the Someşu Rece [12] (Figure 1). The main characteristics of the sampling sites are depicted in Table 1.

Physico-chemical parameters were measured in the field, using a Hanna HI98194 multiparameter. Qualitative samples were taken from bryophytes, sand and stones in case of algae (by cutting the vegetation, suctioning with a pipette or scrapping the surface of larger stones); and using a 250 µm mesh size net for invertebrates. Only eucrenon habitats were sampled.

All samples were preserved with formaldehyde in the field and later analyzed in the laboratory, following standard methods. Identification for invertebrates was carried out to different taxonomic levels [23; 28; 29]. In case of algae, identifications were made to the species level only in case of diatoms, since they usually represent the dominant periphytic group [9; 16; 17; 18].

Diatom frustules were cleaned using a standardized method with H_2SO_4 and $KMnO_4$ to remove the organic matter [30]. Two permanent mounts were prepared for each site and a total of 300-400 diatom valves were counted to assess the relative abundance.

Table 1. Description of the five sampling sites considered for the present study

Sampling site codes		Sampling date	Sampling site area	GPS coordinates	Altitude (m)	Spring type and human impact	Spring characteristics
S1	WI	18.02.2017	Muntele Rece	N: 46°39'11" E: 23°17'42"	851	limnocrene, not-modified	- forest area - substrate: mosses and other macrophytes; silt; sand - probably temporary
	SP	14.05.2017					
	SU	23.07.2017					
S2	WI	18.02.2017	Muntele Rece	N: 46°38'25" E: 23°19'46"	1036	rheocrene, not-modified	- forest area - substrate: silt, sand - covered by humans; used for human water source
	SP	14.05.2017					
	SU	23.07.2017					
S3	WI	18.02.2017	Măguri Răcătău	N: 46°39'14" E: 23°12'14	626	limnocrene, modified	- forest area - substrate: stones, sand - used for human water source
	SP	14.05.2017					
	SU	23.07.2017					
S4	WI	18.02.2017	Ruseşti-Prigoana	N: 46°41'55" E: 23°9'58"	565	limnocrene, modified	- agriculture and pasture area - substrate: silt, stones - used for animal water source
	SP	14.05.2017					
	SU	23.07.2017					
S5	WI	18.02.2017	Gilău	N: 46°46'0" E: 23°24'16"	387	limnocrene, not-modified	- agriculture area - substrate: sand, silt - shaded by trees
	SP	14.05.2017					
	SU	23.07.2017					

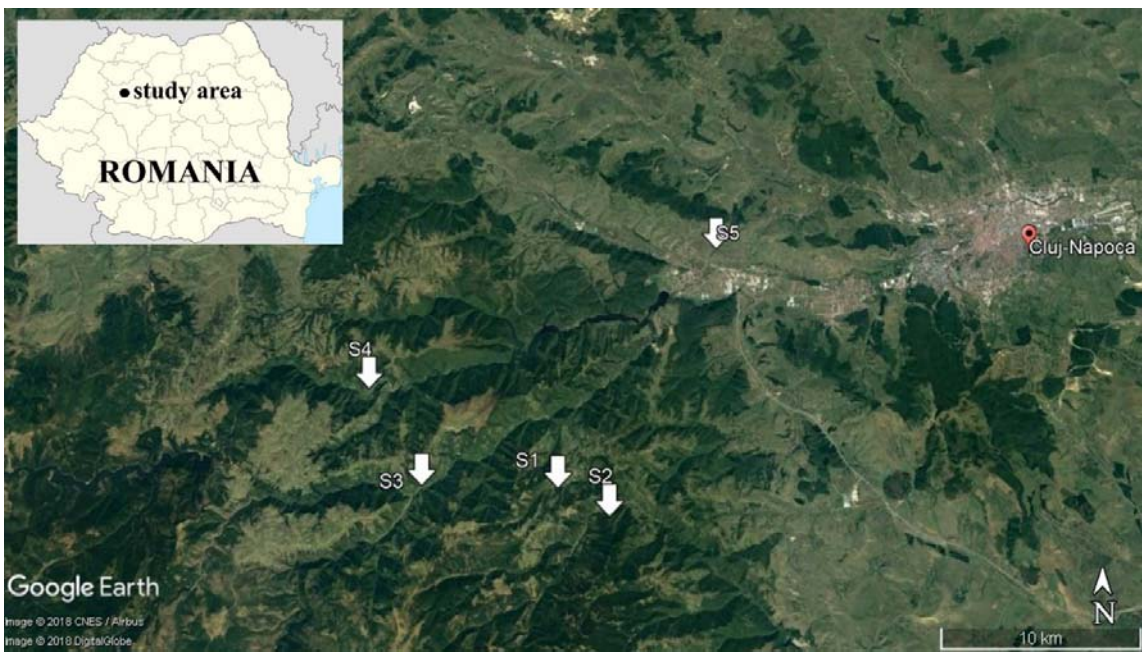

Figure 1. Location of the five sampling sites considered for the present study (abbreviations as in Table 1)

Quantitative analysis included: relative abundance, frequency, Shannon-Wiener diversity, Equitability and similarity (Jaccard and Bray-Curtis indices). Data were tested with the Shapiro-Wilk normality test. The nonparametric Kruskal-Wallis test was used to compare invertebrate communities sampled in different seasons from all five sampling springs.

A Canonical Correspondence Analysis (CCA) was constructed in order to visualize the invertebrate taxa, the five sampling sites and the main physico-chemical parameters on the same plot. Addinsoft XLSTAT Version 2018.5.51886 was used to process the data.

RESULTS AND DISCUSSIONS

Invertebrate communities and the environmental factors

A total number of 17 taxonomic groups was identified in the five sampling springs from the Apuseni Mountains. The number of taxa ranged from 7 in S4 and S5 to 12 in S3, with variations among seasons (Figure 2, Table 2). Four taxa were present in all sampling springs: oligochetes, amphipods, copepods and caddisflies. However, amphipods recorded the highest abundances, exceeding 90% in some cases (as for S3SP). High abundances were also calculated for chironomids (e.g. >60% for S3WI and S4SU). On the other hand, several taxa were limited to one or two sampling sites, like stoneflies, that recorded higher abundances only in S1 and S2 (>15%), or clams, found only in S3 (Table 2).

The Shannon-Wiener diversity and the Equitability followed a similar pattern as the taxon richness, with higher values in S1 and S2 (exceeding 1.3 and 0.6, respectively) and lower in the other three springs. The lowest values were recorded in S3SP, where amphipods represented almost 95% from the invertebrate community.

The higher number of taxa recorded in winter could be explained by different life cycles with emergent insects in certain seasons, or by summer droughts that could lead to water level decreases. The differences in taxon richness between S1, S2 and the rest of the sampling sites could be related to increases of human pressures in springs located at lower altitudes.

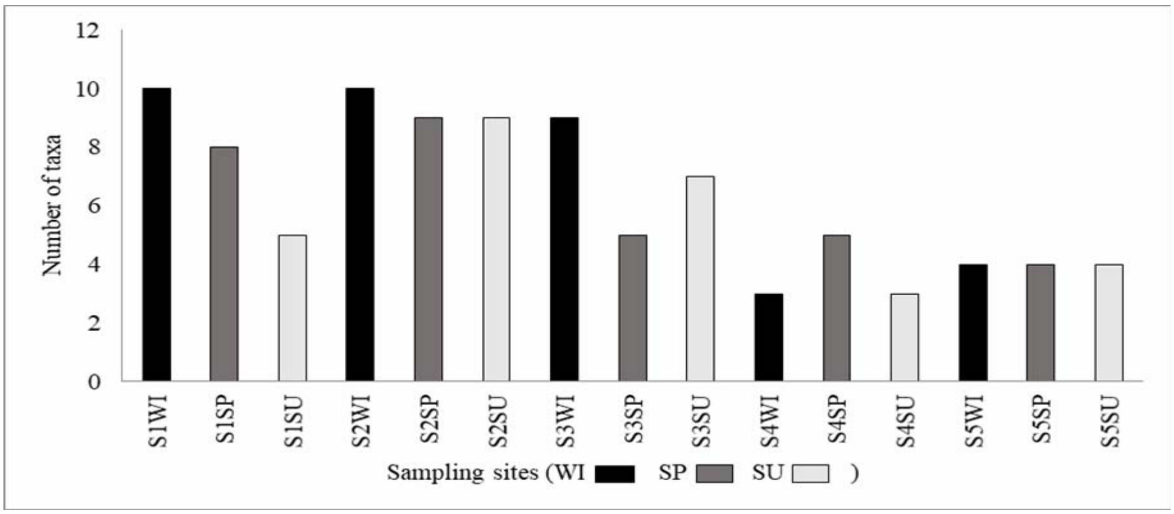

Figure 2. Total number of invertebrate taxa from the five sampling sites in winter, spring and summer 2017 (abbreviations as in Table 1)

Table 2. Relative abundance of benthic invertebrates identified at the five sampling sites in 2017 (Plecoptera identified to the genus level; Trichoptera and Diptera identified to the family level; abbreviations as in Table 1)

TAXA	**SAMPLING SPRINGS**														
	S1			**S2**			**S3**			**S4**			**S5**		
	WI	SP	SU	WI	SP	SU	WI	SP	SU	WI	SP	SU	WI	SP	SU
OLIGOCHAETA	0.6	1.0		34.1	20.8	26.3	6.3	2.0	21.5	54.5	9.1	39.5	44.4	3.5	22.6
NEMATODA				0.1			0.6								
PLATYHELMITHES				0.2		29.0		1.3							
HIRUDINEA	1.2		22.7				1.0	1.0							
BIVALVIA									1.3						
GASTROPODA		3.8											5.6		0.9
AMPHIPODA	8.0	30.5	4.5	0.2	12.5	4.8	28.9	94.7	64.6		9.1		47.2	84.5	74.8
COPEPODA	36.2		4.5	9.1	2.1	0.5			1.3	3.0				11.5	
OSTRACODA				0.4	8.3	10.2			1.3	42.4	18.2	0.5			1.7
PLECOPTERA (ALL)	29.5	49.6	22.7	17.0	16.7	23.7	0.3				9.1				
Leuctra	*2.5*	*4.8*			*2.1*	*1.1*									
Nemoura	*27.0*	*44.8*	*22.7*	*17.0*	*14.6*	*22.6*	*0.3*				*9.1*				
TRICHOPTERA (ALL)	4.9	2.9		1.0	6.3	2.2	0.6		3.8					0.5	
Hydropsidiidae							*0.3*								
Limmepliididae				*0.6*	*4.2*	*2.2*	*0.3*		*3.8*						
Polycentropodidae	*4.9*	*2.9*		*0.4*	*2.1*									*0.5*	
ODONATA		1.9													
DIPTERA (ALL)	19.6	10.5	45.5	37.8	33.3	3.2	62.2	1.0	6.3		54.5	60.0	2.8		
Chironomidae	*17.8*	*10.5*	*45.5*	*37.8*	*33.3*	*3.2*	*61.9*	*1.0*	*6.3*		*54.5*	*60.0*			
Empididae	*0.6*														
Tipulidae	*1.2*														
Ceratopogonidae													*2.8*		
Psychodidae							*0.3*								

The invertebrate abundances calculated for the five sampling sites in winter, spring and summer 2017 were related to the environmental variables (Figure 3). Conductivity values in S5 were high in all seasons, probably due to the agricultural land use surrounding the sampling site. Similar findings were reported for springs located in agricultural areas [15]. High percentages of amphipods were characteristic to sites S3 and S5, regardless of the season. Invertebrate communities were similar in S1 and S2, in all seasons, due to the high abundances of stoneflies, caused in turn by the fact that these two sampling springs were located at altitudes exceeding 800 m a.s.l. S4 was shown as a different group in figure 3, due to the high percentage of ostracods identified at this site. Ostracods are known to avoid fast-flowing springs [27], reaching higher abundances in limnocrene habitats, as S4. Thus, invertebrate communities from different seasons aggregated according to sampling sites, and not according to sampling seasons.

Diatom communities

A number of 76 diatom taxa was identified in the five springs sampled in summer 2017, as follows: S1 and S2 with the highest number of taxa, 38 and 40, respectively; S4 with 30 taxa; S3 with only 10 taxa, followed by S5 with 7 taxa. *Planothidium lanceolatum*, *Achnanthidium minutissimum* and *Gomphonema parvulum* had a frequency of 100%, while other three taxa: *Odontidium mesodon*, *Hantzschia amphioxys* and *Nitzschia palea* recorded a frequency of 80%. However, half of diatom taxa were present in only one out of five sampling springs. The Jaccard similarity recorded low values, not exceeding 0.35 (for S1, S2 and S4), depicting very different diatom communities.

The relative abundance of diatoms was calculated for four springs (S1, S2, S3, S4), because in S5 the algal abundance was too low to be taken into account. *Achnanthidium minutissimum* dominated the diatom community in S1, S2 and S3, with a relative abundance of 68%, 38% and 64.5%, respectively (Figure 4). *Planothidium lanceolatum* was also present, but in lower percentages (e.g. 10% in S2). *Tabularia fasciculata* (28%) dominated the diatom communities from S4, followed by *Fragilaria capucina*.

The highest Shannon-Wiener diversity (1.88) and Equitability (0.85) were estimated in S4. The Bray-Curtis similarity recorded the highest values between the algal communities from S1 and S3 (0.62).

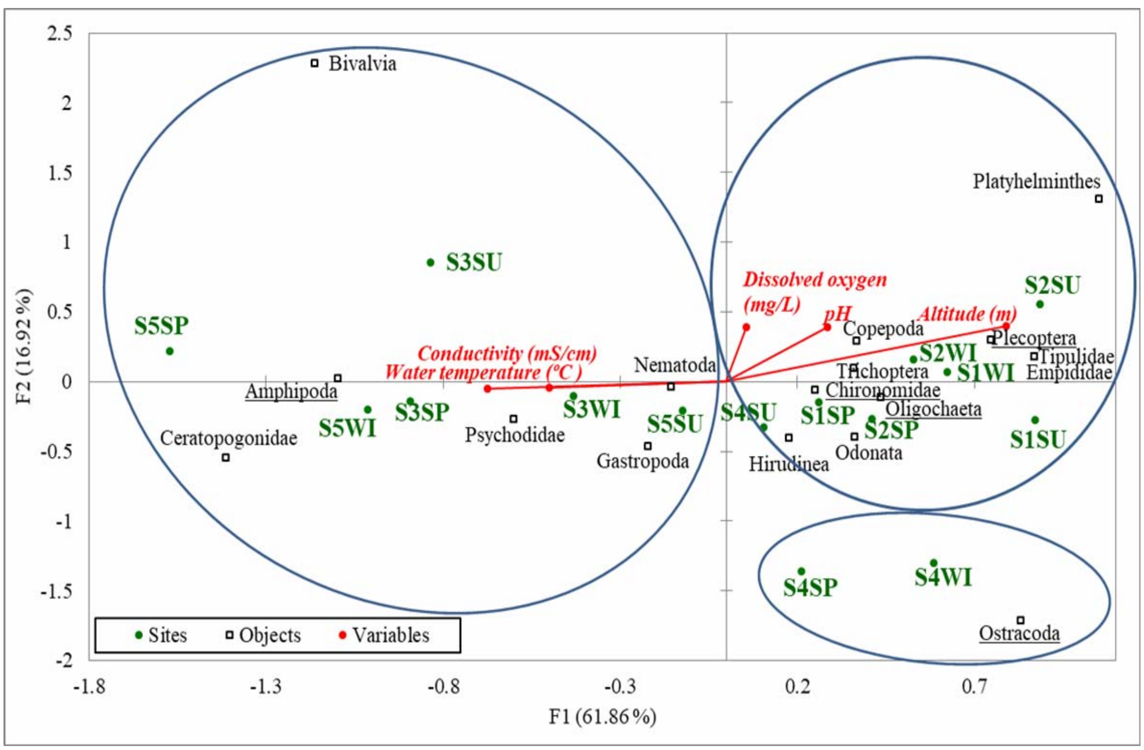

Figure 3. Canonical Correspondence Analysis CCA (axes F1 and F2: 78.78 %) based on invertebrate groups (squares) and physical parameters (vectors) measured in the five sampling springs (circles) (abbreviations as in Table 1)

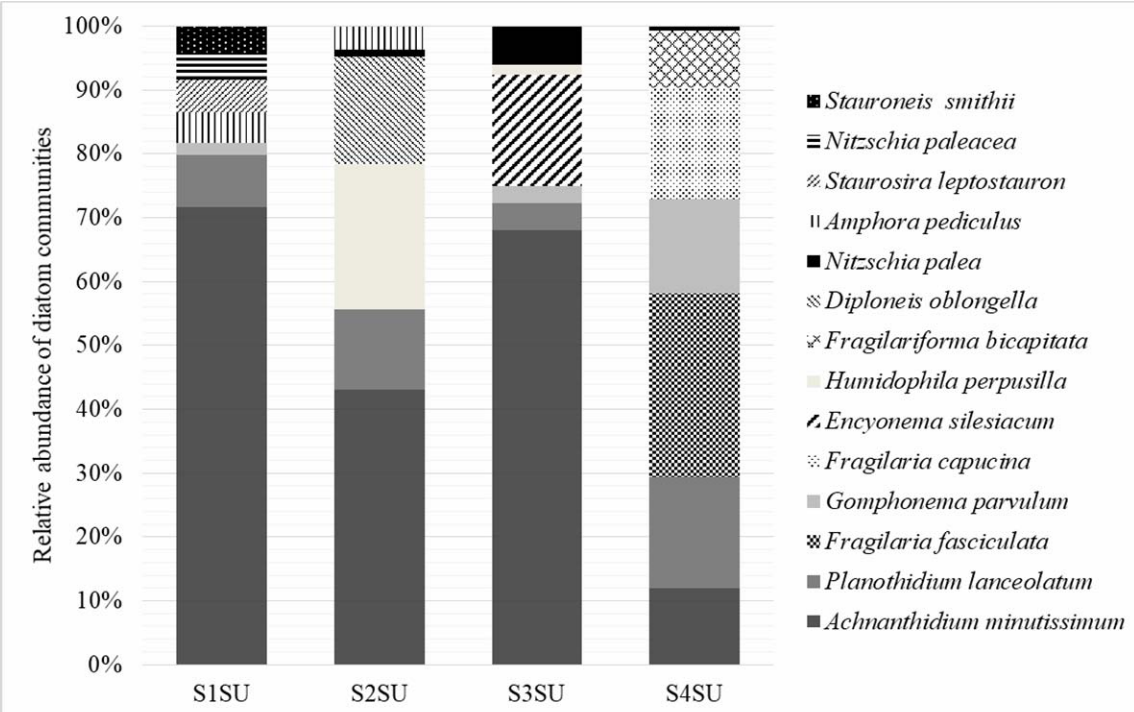

Figure 4. Relative abundance of diatoms in four of the studied springs (abbreviations as in Table 1)

Seasonal dynamics in springs

Seasonal changes were considered only in case of invertebrate communities, since diatoms were only sampled once, in summer. Strong patterns of seasonal variations were described in the literature for stream invertebrates [26]. However, seasonal dynamics in spring habitats was not so clearly defined, since space and time were found to be equally important for eucrenal macroinvertebrates [25]. Moreover, other studies reported differences in temporal variability depending on the sampling methods, with observed variations only in benthic traps and moss samples [2]. Microhabitat characteristics and favourable conditions could be more important drivers in spring diversity than seasonal changes [19].

The abundances of invertebrate taxa found in the five sampling sites in winter, spring and summer were tested using the non-parametric Kruskal-Wallis test. No statistically significant differences were obtained between seasonal data at each sampling site ($p>0.05$ in all cases). Tests carried out on Ephemeroptera and Trichoptera data led to similar results.

Figure 5 depicts the invertebrate taxa with abundances exceeding 5% identified in winter, spring and summer at every sampling site. The proportion of each taxon was calcultated from the total number of individuals counted at one site in all three seasons. In most cases, similar taxa appeared in all seasons at one site. The differences in their percentages could be explained by differences in the sampling effort or by differences in life cycles. Variations in community diversity and equitability were also depicted in Figure 5, since invertebrate communities from S1 and S2 were clearly more diverse and more equally distributed than the other three communities from S3, S4 and S5.

The lack of seasonal dynamics was also illustrated by the aggregation of invertebrate communities in relation with physico-chemical parameters according to sampling sites, and not according to sampling seasons (Figure 3).

The initial hypothesis was true: no seasonal dynamics was observed for invertebrates in the five sampling springs, even if they differed in altitude, habitat conditions and human impact. The cause for this lack of temporal variability might be the relative similarity in physico-chemical parameters in each sampling spring. However, subsequent research should consider wider spatial and temporal scales, in order to better extrapolate the results. As for diatoms, no significant differences were reported between seasonal samples in the literature [3; 11; 31]. However, further research is needed in order to investigate the diatom communities sampled in different seasons.

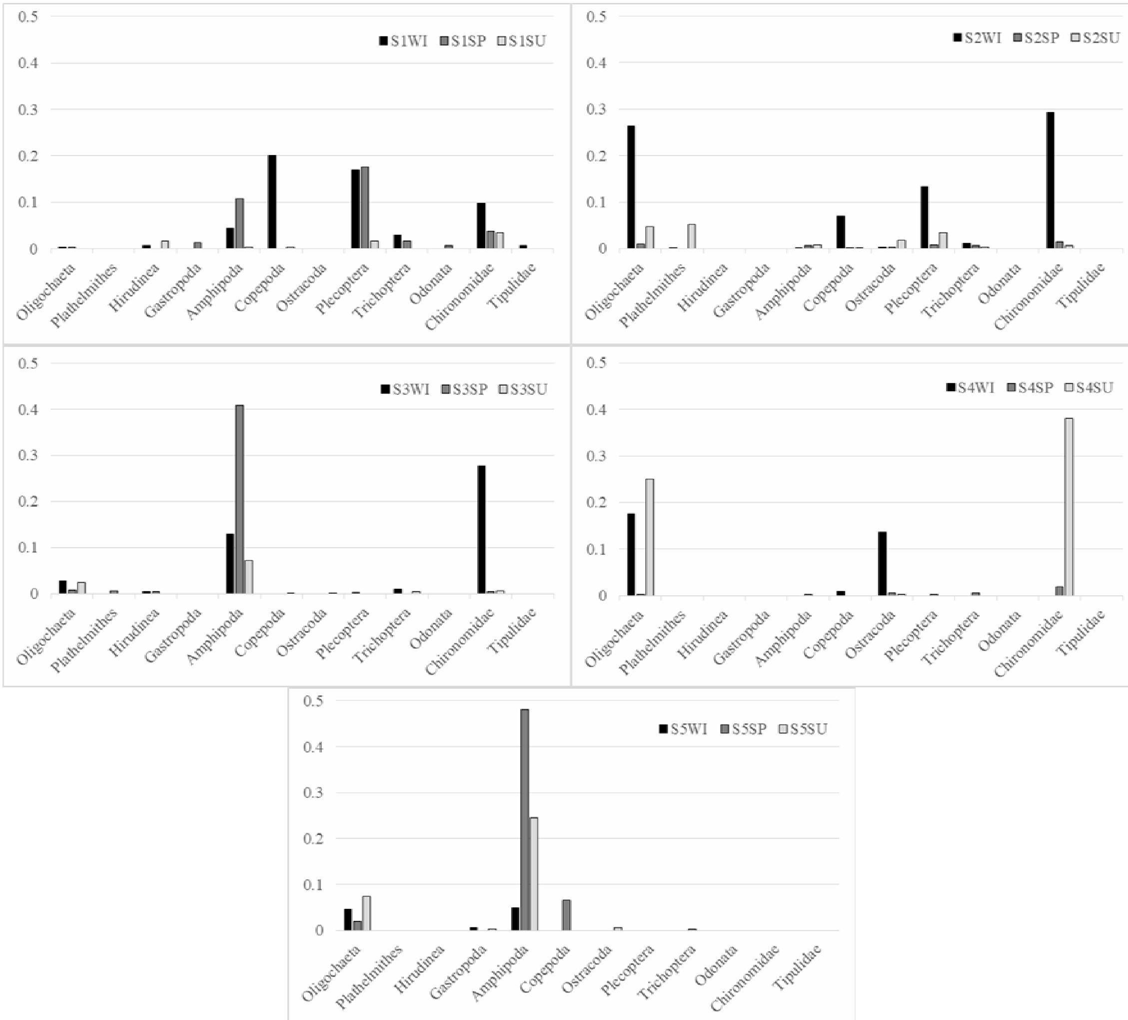

Figure 5. Invertebrate taxa identified in winter, spring and summer 2017, calculated as proportions of the total number of individuals at each sampling site in all three seasons (vertical axis: proportions; horizontal axis: taxonomic groups; abbreviations according to Table 1)

CONCLUSIONS

The qualitative structure of invertebrate communities from five springs located in the Apuseni Mountains showed oligochetes and amphipods as most frequent and most abundant taxa. Chironomids, stoneflies, caddisflies, copepods, ostracods, flat worms and leeches also reached high abundances, but they were not frequent. Diatom communities sampled in summer 2017 differed among the five springs, however the dominant taxa were similar in most of the sampling sites.

No seasonal dynamics was observed for invertebrate communities from the five springs. Due to the relative constant abiotic conditions, similar taxa were identified in the three seasons, differing only in their relative abundances.

ABSTRACT

Invertebrate and diatom communities from five springs located in the Apuseni Mountains (Transylvania, Romania) were considered for the present study. Invertebrates were sampled in winter, spring and summer 2017 using zoobenthic nets. Amphipodes and oligochetes were the most abundant and frequent taxa in all sampled springs. Stoneflies, ostracods and chironomids also recorded high abundances, but lower frequencies. No seasonal dynamics was observed, because no significant differences were identified between the sampling seasons. Similar taxa were present at every site, differing only in their relative abundances. This could be caused by the relative constancy of physico-chemical parameters measured in the sampling sites.

Diatoms were only considered in summer 2017 at the same sampling sites, and the dominant taxa were identified.

REFERENCES

1. BARQUÍN, J., SCARSBROOK, M., 2008 - Management and conservation strategies for coldwater springs. Aquatic Conservation: Marine and Freshwater Ecosystems, 18(5), 580-591;
2. BOTTAZZI, E., BRUNO, M. C., PIERI, V., DI SABATINO, A., SILVERI, L., CAROLLI, M., ROSSETTI, G., 2011 - Spatial and seasonal distribution of invertebrates in Northern Apennine rheocrene springs. Journal of Limnology, 70(1s), 77-92;
3. CANTONATI, M., 1998 - Diatom communities of springs in the Southern Alps. Diatom research, 13(2), 201-220;
4. CANTONATI, M., GERECKE, R., BERTUZZI, E., 2006 - Springs of the Alps - sensitive ecosystems to environmental change: from biodiversity assessments to long-term studies. Hydrobiologia, 562, 59-96;
5. CANTONATI, M., FÜREDER, L., GERECKE, R., JÜTTNER, I., COX, E. J., 2012 - Crenic habitats, hotspots for freshwater biodiversity conservation: toward an understanding of their ecology. Freshwater Science, 31(2), 463-480;
6. CÎMPEAN, M., GERECKE, R., 2006 - Water mites (Acari. Hydrachnidia) from the Retezat National Park (Romania). In The Retezat National Park, Transylv. Rev. Syst. Ecol. Res., Sibiu, 3: 63-74;
7. COPILAŞ-CIOCIANU, D., GRABOWSKI, M., PÂRVULESCU, L., PETRUSEK, A., 2014 - Zoogeography of epigean freshwater Amphipoda (Crustacea) in Romania: fragmented distributions and wide altitudinal variability. Zootaxa, 3893(2), 243-260;
8. DUMNICKA, E., GALAS, J., JATULEWICZ, I., KARLIKOWSKA, J., RZONCA, B., 2013 - From spring sources to springbrook: Changes in environmental characteristics and benthic fauna. Biologia, 68(1), 142-149;
9. ETTL, H., 1983 - Chlorophyta 1. Phytomonadina, In: Süßwasserflora von Mitteleuropa, Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D. (eds.), 9, Gustav Fischer Verlag, Stuttgart, pp. 807;
10. GERECKE, R., CANTONATI, M., SPITALE, D., ELISABETH, S. T. U. R., WIEDENBRUG, S., 2011 - The challenges of long-term ecological research in springs in the northern and southern Alps: indicator groups, habitat diversity, and medium-term change. Journal of Limnology, 70(1s), 168-187;
11. GESIERICH, D., KOFLER, W., 2010 - Epilithic diatoms from rheocrene springs in the Eastern Alps (Vorarlberg, Austria). Diatom Research, 25(1), 43-66;
12. GÎŞTESCU, P., 1990 - Fluviile Terrei. Editura Sport–Turism, Bucureşti, pp. 264;
13. GODEANU, S., 1974 - Contribuții la cunoaşterea sinecologică a zoocenozelor tinovului Gemenele din Parcul Național Retezat, Sargetia, Acta Mus. Dev., Ser. Sci. Nat., X, 155-175;
14. ILLIEŞ, J., BOTOŞĂNEANU, L., 1963, Problemes et methodes de la classification ecologique des eaux courantes, considerees surtout du point du vue faunistique. Mitt. Intern. Ver. T.A. Limnologie 12:1–57 ;
15. KNYSH, K. M., GIBERSON, D. J., VAN DEN HEUVEL, M. R., 2016 - The influence of agricultural land use on plant and macroinvertebrate communities in springs. Limnology and Oceanography, 61(2), 518-530;
16. KRAMMER, K., LANGE-BERTALOT, H., 1986 - Bacillariophyceae. 1. Teil: Naviculaceae. Süsswasserflora von Mitteleuropa, 2/1, Spektrum Akademischer Verlag, Heidelberg, pp. 876;
17. KRAMMER, K., LANGE-BERTALOT, H., 1988 - Bacillariophyceae. 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. Süsswasserflora von Mitteleuropa, 2/2, Spektrum Akademischer Verlag, Heidelberg, pp. 611;
18. KRAMMER, K., LANGE-BERTALOT, H., 1991 - Bacillariophyceae. 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. Süsswasserflora von Mitteleuropa, 2/3, Spektrum Akademischer Verlag, Heidelberg, pp. 599;
19. KŘOUPALOVÁ, V., BOJKOVÁ, J., SCHENKOVÁ, J., PAŘIL, P., HORSÁK, M., 2011 – Small Scale Distribution of Aquatic Macroinvertebrates in Two Spring Fens with Different Groundwater Chemistry. International Review of Hydrobiology, 96(3), 235-256;
20. MAIOLINI, B., CAROLLI, M., SILVERI, L., 2011 - Ephemeroptera, Plecoptera and Trichoptera in springs in Trentino (south-eastern Alps). Journal of Limnology, 70(1s), 122-133;
21. MOTAŞ, C., BOTOŞĂNEANU, L., NEGREA, Ş., 1962 - Cercetări aspra biologiei izvoarelor şi apelor freatice din partea centrală a Cîmpiei Romîne, Ed. Acad. R.P.R., pp. 366;
22. PEŠIĆ, V., GLIGOROVIĆ, B., SAVIĆ, A., BUCZYŃSKI, P., 2017 - Ecological patterns of Odonata assemblages in karst springs in central Montenegro. Knowledge & Management of Aquatic Ecosystems, (418), 3;
23. SANSONI, G., 2001 - Microinvertebrati Dei Corsi D’Acqua Italiani, Ed. Agenzia provinciale per la protezione dell’ambiente, pp. 191;

24. SAVIĆ, A., DMITROVIC, D., PEŠIĆ, V., 2017 - Ephemeroptera, Plecoptera, and Trichoptera assemblages of karst springs in relation to some environmental factors: a case study in central Bosnia and Herzegovina. Turkish Journal of Zoology, 41(1), 119-129;
25. SPITALE, D., LENCIONI, V., CANTONATI, M., 2012 - Relative importance of space and time in determining the biotic structure in the upper part of spring-fed streams. Freshwater Science, 31(2), 586-598;
26. ŠPORKA, F., VLEK, H. E., BULÁNKOVÁ, E., KRNO, I. J., 2006 - Influence of seasonal variation on bioassessment of streams using macroinvertebrates. In The Ecological Status of European Rivers: Evaluation and Intercalibration of Assessment Methods. Springer, Dordrecht. pp. 543-555;
27. STOCH, F., GERECKE, R., PIERI, V., ROSSETTI, G., SAMBUGAR, B., 2011 - Exploring species distribution of spring meiofauna (Annelida, Acari, Crustacea) in the south-eastern Alps. Journal of limnology, 70(1s), 65-76;
28. SUNDERMANN, A., LOHSE, S., BECK, L.A., HAASE P., 2007 - Key to the larval stages of aquatic true flies (Diptera), based on the operational taxa list for running waters in Germany, Ann. Limnol- Int. J. Lim., 43 (1), p. 61-74;
29. TACHET, P., RICHOUX, M., BOURNAUD, P., USSEGLIO-POLATERA, 2000 - Invertebres d'eau douce: systematique, biologie, ecologie, Ed. CNRS Editions, Paris, pp. 588;
30. TAYLOR, J.C., HARDING, W.R., ARCHIBALD, C.G.M., 2007 - A methods manual for the collection, preparation and analysis of diatom samples. WRC Report No TT 281/07. Water Research Commission, Pretoria, pp.11-23;
31. WEHR, J.D., 1981 - Analysis of seasonal succession of attached algae in a mountain stream, the North Alouette River, British Columbia. Canadian Journal of Botany, 59(8), 1465-1474.

ACKNOWLEDGEMENTS

The research was partly financed by the excellence scholarship awarded by the Babeş-Bolyai University, Cluj-Napoca, Romania (contract no. 37441/23.11.2016).

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