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DISCHARGE, SUBSTRATE TYPE AND TEMPERATURE AS FACTORS AFFECTING GASTROPOD ASSEMBLAGES IN SPRINGS IN NORTHWESTERN BOSNIA AND HERZEGOVINA

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Abstract: In this study, we analyzed changes to gastropod assemblages with regard to the seasonal variations of discharge, substrate composition and temperature in 19 springs in northwestern Bosnia and Herzegovina. Six aquatic gastropod species were identified, including *Bosnidilhia vreloana* Boeters, Glöer & Pešić, 2013 and *Islamia dmitroviciana* Boeters, Glöer & Pešić, 2013, two species endemic for the studied area. Surprisingly, we did not observe a significant influence of discharge on the composition of gastropod assemblages in the studied springs. This could mean that gastropod assemblages in the springs examined are well adapted to intermittent conditions (i.e. fluctuations in discharge). The results of canonical correspondence analysis (CCA) showed that specific substrate types can be good predictors for the occurrence of particular species. Interestingly, we found that spring specialists such as *Bythinella schmidti* and *Islamia dmitroviciana* also inhabit springs with intermittent dry periods. The studied springs should be considered at risk and some of their gastropod dwellers, such as the endemics *Bosnidilhia vreloana* and *Islamia dmitroviciana*, are possibly endangered, requiring conservation planning to protect these spring habitats.

Key words: springs; intermittent springs, gastropods; discharge; substrate types

INTRODUCTION

Springs, with exception of karst and intermittent springs, are often treated as stable ecosystems due to the low variability of their abiotic conditions (especially low temperature amplitudes), small impact of nutrients and relatively stable discharge regime [1,2]. Consequently, they are inhabited by organisms adapted to the stable abiotic conditions typical for this environment [3,4].

However, hydrological disturbances also occur in springs and may become important factors in shaping spring assemblages [5], especially in karst and intermittent springs, where heavy rainfall and drought periods influence the aquatic macroinvertebrates [6]. Many studies have shown that other abiotic factors, including hydrochemistry and substrate composition, also influence spring fauna [7-12]. The western Balkans harbor a high biodiversity that includes a high number of endemic species living in springs [13,14]. This has been attributed to the highly diverse geology and historic climate conditions of the region [15], as well to the fact that the Balkan Peninsula was a one of refuges of genetic diversity during the Pleistocene [16].

The importance of spring habitats for gastropod diversity is highlighted by the fact that the Balkans exhibit a large number of narrow-range endemics, most of them restricted to their type locality [13,17]. Spring snails tend to have poor dispersal characteristics and are probably more susceptible to factors promoting speciation, such as climatic and geological processes

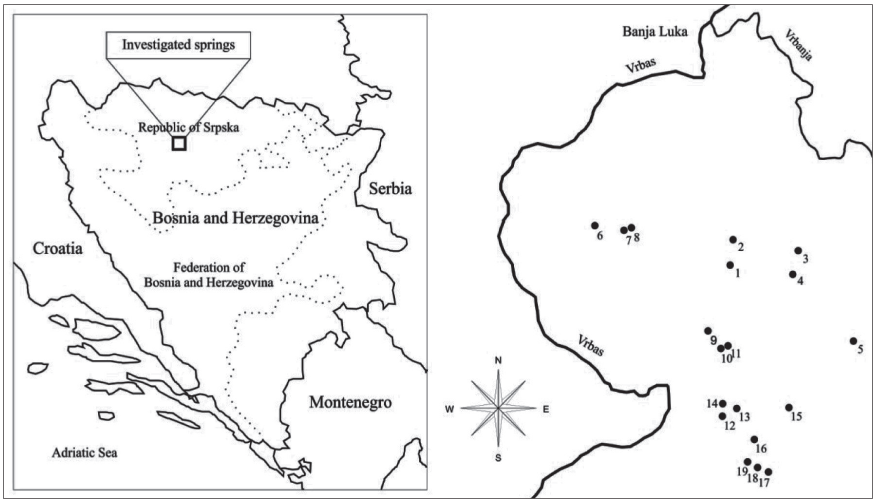

Fig. 1. Map of the study area. The numbers correspond to spring numbers in Table.1.

[18,19]. Recent studies on the diversity of spring snails in the mountainous region of Bosnia and Herzegovina found intriguing biogeographic patterns and several new species [13,20,21], including *Bosnidilhia vreloana* Boeters, Glöer & Pešić, 2013, a member of the monotypic moitessieriid genus *Bosnidilhia*, and *Islamia dmitroviciana* Boeters, Glöer & Pešić, 2013, both endemic to the studied area of northwestern Bosnia and Herzegovina [20].

However, the pattern of distribution of aquatic snails in springs has been poorly studied, despite the fact that these snails constitute a major faunistic component of spring environments [22]. Given the low levels of migration and gene flow between populations of spring snails [18] and the fact that most spring habitats are already threatened by human impacts [23] or even destroyed by draining or cattle trampling [24], further conservation planning and management is required to protect these habitats.

This study examines the influence of disturbances in discharge, substrate type and temperature on the taxonomic richness and seasonal patterns of aquatic gastropod assemblages in the studied springs of northwestern Bosnia and Herzegovina. All springs examined are situated around the city of Banja Luka in the Republic of Srpska in Bosnia and Herzegovina (Fig. 1). The springs are situated between 247 and 603 m a.s.l., with an average altitude of 411.79 m. The climate is temperate: winters are moderately cold and summers warm and wet. The landscape ranges from urban to agricultural areas and forest. After preliminary investigations, 19 relatively undisturbed springs (I1-I19) were selected.

MATERIALS AND METHODS

Experimental specimens

Aquatic gastropods were quantitatively sampled with a Surber sampler (34x33.5 cm=0.1139 m^2, 350-µm mesh width)in the eucrenal zone of each spring, taking care not to damage the habitat. Two seasonal surveys were carried out: in winter, 20-21 March, 2010 and summer, 14-15 August 2010. All samples were immediately preserved in 70% ethanol, and subsequently sorted and determined in the laboratory.

Study area

Water temperature was measured *in situ* on each sampling occasion. Discharge was assessed by eye and classified according to Hoffsten and Malmqvist [25]. The springs were divided into 4 groups according to discharge (0: without flow; 1: <1 L min^{-1}; 2: >1 and <5 L min^{-1}; 3: >5 and <20 L min^{-1}). The substrate types

Table 1. Selected characteristics of the investigated springs.

Springs	Latitude	Longitude	Altitude above sea level (m)	Mode of outflow	Type of spring	Land use
I1	44°43.724′	17°13.490′	526	Artificial pipe	Perennial	Urban area
I2	44°43.757′	17°13.550′	504	Artificial pipe	Perennial	Urban area
I3	44°43.628′	17°14.730′	462	Artificial pipe	Perennial	Meadow
I4	44°43.317′	17°14.613′	554	Artificial pipe	Perennial	Edge of forest
I5	44°42.470′	17°15.674′	603	Seeping	Perennial	Edge of forest
I6	44°43.974′	17°11.025′	461	Artificial pipe	Intermittent	Forest
I7	44°43.905′	17°11.564′	517	Artificial pipe	Perennial	Forest
I8	44°43.941′	17°11.701′	522	Artificial pipe	Intermittent	Forest
I9	44°42.620′	17°13.085′	425	Seeping	Perennial	Edge of forest
I10	44°42.398′	17°13.318′	482	Artificial pipe	Perennial	Edge of forest
I11	44°42.419′	17°13.419′	438	Artificial pipe	Intermittent	Forest
I12	44°41.550′	17°13.319′	289	Artificial pipe	Perennial	Meadow
I13	44°41.710′	17°13.335′	291	Flowing	Intermittent	Forest
I14	44°41.642′	17°13.576′	302	Artificial pipe	Perennial	Village
I15	44°41.654′	17°14.509′	377	Artificial pipe	Perennial	Village
I16	44°41.265′	17°13.897′	290	Artificial pipe	Perennial	Meadow
I17	44°40.850′	17°14.133′	275	Flowing/seeping	Perennial	Forest
I18	44°40.911′	17°13.934′	247	Flowing/seeping	Intermittent	Meadow
I19	44°40.992′	17°13.818′	259	Artificial pipe	Perennial	Forest

were categorized into five classes of frequency based on percentage of areal coverage [9]: 0: 0%; 1: 1-25%; 2: 26-50%; 3: 51-75%; 4: 76-100%. An overview of the springs' characteristics is given in Table 1.

Statistical analyses

All statistical analyses were performed using PRIMER version 7.0, SPSS version 17 and MVSP version 3.21. Normality tests (Kolmogorov-Smirnov) were performed on faunistic and environmental data, and accordingly appropriate nonparametric tests/analyses were used. The Kruskal-Wallis test was used to determine the significance (at $\alpha=0.05$) of differences in substrate composition between springs [26]. The Wilcoxon test [27] was used to determine differences between environmental factors of the three spring types examined in winter and summer. Categorical principal components analysis (CATPCA) for data was performed on raw, untransformed substrate data. Ordination of the springs was conducted using nonmetric multidimensional scaling (NMDS). Bray-Curtis similarity was used as the similarity index for the faunistic data. Faunistic similarities between samples were analyzed using the analyses of similarities (ANOSIM) procedure. ANOSIM was used to determine significant differences in all substrate types (in a multivariate sense) between the sampling seasons. CCA [28] was applied to test the influence of environmental variables on the gastropod assemblages.

RESULTS

A total of 6 species of the three families of freshwater gastropods (Moitessieriidae, Hydrobiidae and Lymnaeidae) were collected from 19 springs. Lymnaeidae was the most diverse family with three species (see Table 2). We found on average 1.05±0.93 species per spring, and diversity ranged from 0 (I13, I15) to 3 (I3, I5, I12, I16) species found. The recorded gastropod assemblages comprised of one stygobiotic species, *Bosnidilhia vreloana* (indicated by the completely reduced eye spots [20]), and 5 non-stygobiotic species. The assemblage density of springs varied from 0 individuals per square meter (ind/m²) and reached 685 ind/m² in I12. The hydrobiid *Bythinella schmidti* (Kuster, 1852)

Table 2. List of taxa recorded in the investigated springs; indicated as ind/m².

Spring	*Bosnidilhia vreloana*		*Bythinella schmidti*		*Galba truncatula*		*Islamia dmitroviciana*		*Radix balthica*		*Radix labiata*	
	w	s	w	s	w	s	w	s	w	s	w	s
I1	-	-	-	-	9	-	-	-	-	-	-	-
I2	-	-	-	-	9	-	-	-	18	-	-	-
I3	-	-	18	9	53	53	9	-	-	-	-	-
I4	-	-	9	-	-	-	53	-	-	-	-	-
I5	79	9	9	-	-	-	9	-	-	-	-	-
I6	-	-	53	-	-	-	26	-	-	-	-	-
I7	-	-	193	395	-	-	-	9	-	-	-	-
I8	-	-	70	-	-	-	-	-	-	-	-	-
I9	-	-	-	-	9	35	-	-	-	-	-	-
I10	-	-	114	-	35	-	-	-	-	-	-	-
I11	26	-	140	-	-	-	-	-	-	-	-	-
I12	-	-	-	9	-	219	149	457	-	-	-	-
I13	-	-	-	-	-	-	-	-	-	-	-	-
I14	-	-	132	-	26	-	-	-	-	-	-	-
I15	-	-	-	-	-	-	-	-	-	-	-	-
I16	-	-	-	-	26	35	44	-	-	-	88	-
I17	-	-	18	53	-	-	-	-	-	-	-	-
I18	-	-	-	53	-	325	-	-	-	-	-	-
I19	-	-	18	-	-	114	-	-	-	-	-	-

Abbreviations: w – winter; s – summer.

was recorded in 13 springs and it was the most abundant and frequent species, especially in the winter. The lymnaeid *Galba truncatula* (O. F. Müller, 1774) represented the most abundant and frequent taxon in the summer and was found in 10 springs.

In the studied springs, 13 different types of substrates were identified (Table 3-4). Discharge ranged from low in most springs to high in four springs in winter (I1, I2, I4, I15). The water temperature ranged from 8.5 to 17.5°C (mean 11.06±2.39). Water temperature was significantly different in some springs. The highest water temperature (17.5°C) as well as the larger seasonal amplitude (8.5°C) were recorded at I19. The Kruskal-Wallis test did not reveal statistically significant differences (α=0.05) in substrate composition between springs of different discharge capacity. A comparison of seasonal environmental values using the Wilcoxon test showed the presence of statistically significant seasonal differences in temperature (p=0.002), discharge (p=0.011), leaf litter (p=0.000) and clay (p=0.028). The CATPCA (Fig. 2), performed with the 13 substrate types recorded in 19 winter samples, showed that factors one (pca1 axis) and two (pca 2 axis) explained 28.7% and 21.2% of the total variance, respectively. Component one is dominated by anoxic mud and algae on the positive axis and by leaf litter on the negative axis. Component two is determined positively by roots

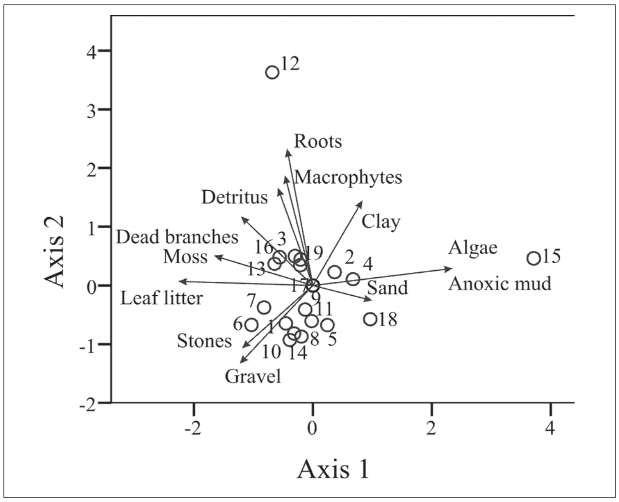

Fig. 2. Principal components analysis of the 19 investigated springs based on the substrate types recorded in winter. Circles – spring localities.

Table 3. Temperature and substrate composition of the 19 investigated springs (I1-I19) in winter 2010.

Spring	T	D	Substrate												
			Am	De	Ll	Db	Ms	R	M	C	S	G	St	A	Wm
I1	10	3	0	0	2	0	1	0	0	0	0	2	0	0	1
I2	9.5	3	0	3	3	0	0	0	0	0	1	0	0	0	0
I3	11	2	0	0	2	0	1	0	2	3	1	1	1	0	0
I4	11	3	0	0	4	0	0	0	0	4	0	0	0	0	0
I5	8.5	2	0	0	4	0	0	0	0	0	1	1	0	0	0
I6	8.5	2	0	0	3	1	1	0	0	0	0	2	2	0	0
I7	9	1	0	1	3	1	1	0	0	0	0	0	1	0	0
I8	9	1	0	0	4	0	0	0	0	0	0	1	1	0	0
I9	9	1	0	0	0	0	0	0	0	0	1	1	1	0	0
I10	10	2	0	0	2	0	1	0	0	0	1	1	1	0	0
I11	10	2	0	0	4	0	0	0	0	0	0	0	4	0	0
I12	9	1	0	2	3	1	1	2	1	0	0	0	0	0	0
I13	9	1	0	2	2	1	1	0	0	1	1	1	2	0	0
I14	12	2	0	0	1	0	1	0	0	0	1	1	2	0	0
I15	12	3	3	0	0	0	0	0	0	1	0	0	0	2	0
I16	9	1	0	2	3	2	0	0	0	0	0	1	0	0	0
I17	9.5	1	0	2	2	1	0	0	0	0	0	0	1	0	0
I18	8.5	1	0	1	0	0	0	0	0	4	1	1	1	0	0
I19	9	1	0	2	4	0	1	0	0	0	0	0	0	0	0

Discharge: 0: without flow; 1: <1 L min−1; 2: >1 and <5 L min−1; 3: >5 and <20 L min−1. Substrate type classes (percentage of area coverage): 0: 0%; 1: 1-25%; 2: 26-50%; 3: 51-75%; 4: 76-100%. Abbreviations: T – temperature, D – discharge, Am – anoxic mud, De – detritus, Ll – leaf litters, Db – dead branches, Ms – moss, R – roots, M – macrophytes, C – clay, S – sand, G – gravel, St – stones, A – algae, Wm – waste material.

Table 4. Temperature and substrate composition of the 19 investigated springs (I1-I19) in summer 2010.

Spring	T	D	Substrate												
			Am	De	Ll	Db	Ms	Rs	M	C	S	G	St	A	Dr
I1	12.9	2	0	1	0	0	0	0	0	0	0	3	0	0	-
I2	10.7	2	0	4	0	0	0	0	0	0	1	0	0	0	-
I3	11.5	2	0	3	0	0	1	0	2	2	1	1	1	0	-
I4	11	1	0	2	1	0	0	0	0	4	0	0	0	0	-
I5	13	1	0	2	0	0	0	0	0	3	1	1	0	0	-
I6	-	0	0	0	0	0	0	0	0	0	0	0	0	0	+
I7	9.1	1	0	1	2	1	1	0	0	0	0	2	2	0	-
I8	-	0	0	0	0	0	0	0	0	0	0	0	0	0	+
I9	14	1	0	0	0	0	1	0	0	0	1	1	1	0	-
I10	12	1	0	0	1	0	1	0	0	0	1	1	1	0	-
I11	-	0	0	0	0	0	0	0	0	0	0	0	0	0	+
I12	13.5	1	0	1	1	1	1	1	1	3	0	0	0	0	-
I13	13	1	0	0	0	1	1	0	0	3	0	0	3	0	-
I14	13	1	0	0	0	0	0	0	0	1	1	1	2	0	-
I15	12.9	2	3	0	0	0	0	0	0	1	0	0	0	2	-
I16	15	1	0	2	0	0	0	0	0	0	1	1	2	0	-
I17	15	1	0	1	0	1	0	0	0	2	1	1	2	0	-
I18	16	1	0	1	0	0	0	0	0	4	1	1	1	0	-
I19	17.5	1	0	1	1	0	1	0	0	3	1	1	0	0	-

Discharge: 0: without flow; 1: <1 L min−1; 2: >1 and <5 L min−1; 3: >5 and <20 L min−1. Substrate type classes (percentage of areal coverage): 0: 0%; 1: 1-25%; 2: 26-50%; 3: 51-75%; 4: 76-100%. Abbreviations: T – temperature, D – discharge, Am – anoxic mud, De – detritus, Ll – leaf litters, Db – dead branches, Ms – moss, R – roots, M – macrophytes, C – clay, S – sand, G – gravel, St – stones, A – algae, Wm – waste material, Dr – dried springs.

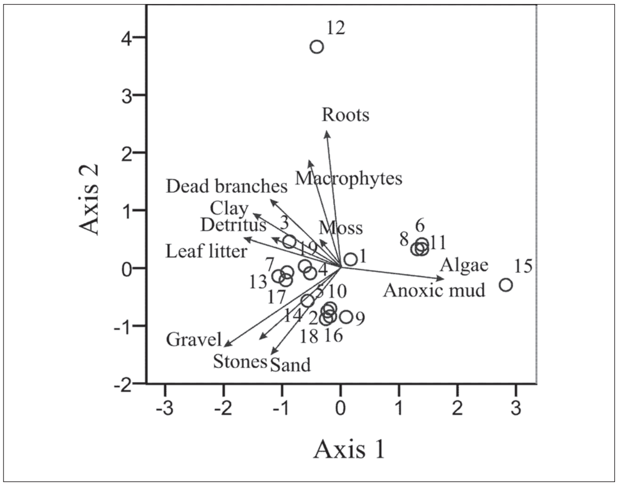

Fig. 3. Principal components analysis of the 19 investigated springs based on the substrate types recorded in summer. Circles – spring localities.

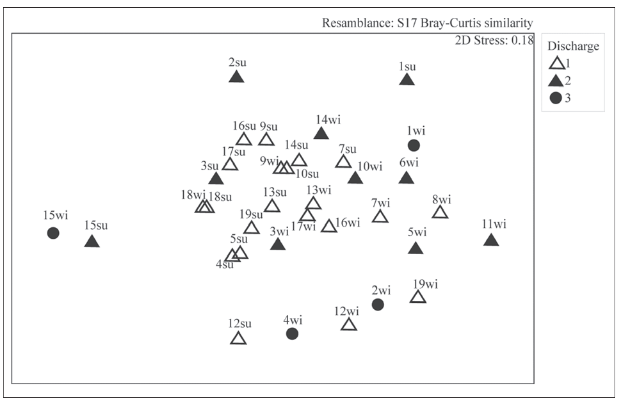

Fig. 4. Nonmetric multidimensional scaling (NMDS) of the investigated springs based on the substrate data. Similarity index: Bray-Curtis; factor: discharge (0: without flow;1: <1 L min^{-1}; 2: >1 and <5 L min^{-1}; 3: >5 and <20 L min^{-1}). Abbreviations: wi – winter, su – summer; spring localities as in tables 1.

and macrophytes and negatively by gravel. Results of CATPCA performed with the 12 substrate types observed in 16 summer samples, revealed that factors one and two explained 26.8% and 22.2% of the variance, respectively. Component one is dominated by anoxic mud and algae on the positive axis, and negatively by detritus and gravel. Component two is determined positive by macrophytes and plant structures (roots) and negative by sand (Fig. 3).

ANOSIM revealed significant differences in substrate composition in terms of the season of sampling (R=0.16 p=0.001). The substrate composition is significantly influenced by discharge (R=0.305 p=0.002). ANOSIM showed that springs with low discharge (>1 and <5 L min^{-1}) differ significantly by their substrate composition from springs with medium discharge (>1 and <5 L min^{-1}; R=0.232 p=0.01) and high discharge (>5 and <20 L min^{-1}; R=0.549 p=0.0031).

When faunistic data were used, ANOSIM showed that there is no significant influence of discharge (R=-0.079 p=0.9) on the gastropod assemblage. On the other hand, ANOSIM revealed significant differences in gastropod assemblage relative to the season of sampling (R=0.076 p=0.026). The NMDS applied to substrate composition did not show any grouping of springs, except for locality 15, which is well separated on the NMDS plot (Fig 4).

The CCA (Fig. 5) based on species data and environmental variables (Tables 2, 3 and 4) showed that the first axis explained 25.03% and the second 17.05% of the variance. CCA indicated gastropod species related to temperature, discharge and substrate types. Thus, *B. schmidti* and *B. vreloana* were found to prefer springs with a stone-rich substrate and higher discharge, while *G. truncatula* prefers springs with higher temperature and clay-rich substrate. *Islamia dmitroviciana* prefers springs with roots and detritus-rich substrate, *Radix balthica* prefers springs with moss and dead branches-rich substrate and *Radix labiata* prefers springs with dead branches rich-substrate.

DISCUSSION

Freshwater gastropod assemblages are influenced by many factors at regional and local scales, each of which varies considerably. Lodge et al. [29] showed that disturbance regimes, competition and predation were the most important variables affecting the small-scale distribution of freshwater snails. On the other hand, the role of hydrochemistry was not established on a local spatial scale for assemblages of freshwater snails [29], but some studies showed that factors such as dissolved free carbon dioxide [30] and temperature [31] play a significant role in the distribution or density and growth rate of some species.

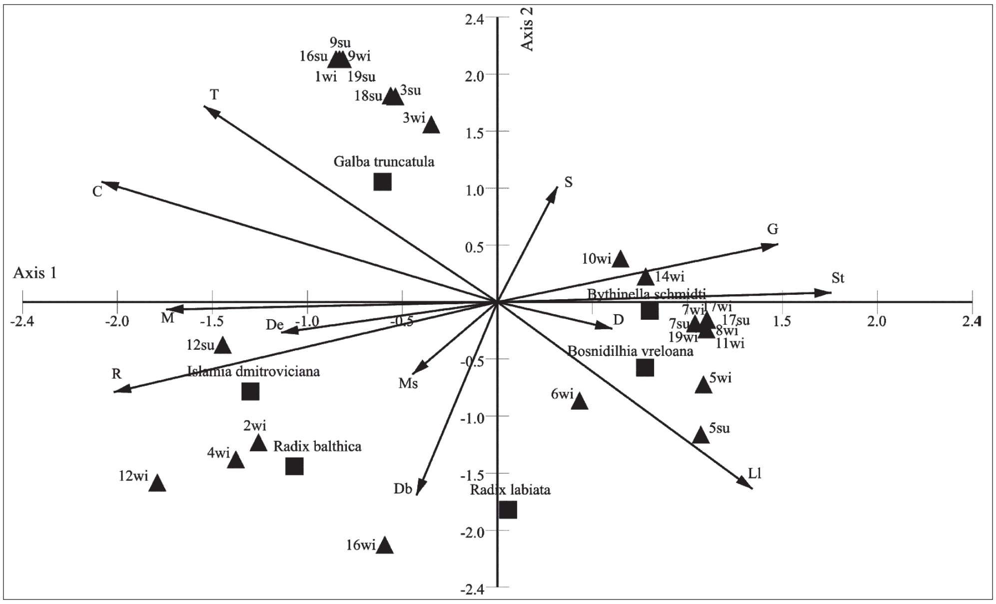

Fig. 5. CCA of the freshwater snail assemblages and environmental factors in the investigated springs. Abbreviations for vectors as in Tables 2-3; wi – winter, su – summer; squares – gastropod species; triangles – spring localities.

The results of our study confirmed that substrate composition is important to gastropod assemblages, which is consistent with several studies [32-34] that showed that substrate type influences the diversity of freshwater gastropod communities or particular species. These studies revealed that a sandy bottom covered with a thin layer of organic silt is the most suitable substrate for snails in rivers [34]. On the other hand, spring snails seem to prefer firm substrates, such as cobble, rocks, woody debris and aquatic vegetation, and are rarely found on or in soft sediment [35]. In our study, the highest abundances of gastropod assemblages in winter were recorded in the springs dominated by leaf litter. As demonstrated [36], leaf litter is particularly important as a source of energy in springs with little allochthonous material. In summer the highest densities of gastropod assemblages were recorded in the springs dominated by a clay-rich substrate, mostly due to the higher density of *Galba truncatula*. CCA showed that the latter species prefer springs with a higher water temperature and a clay-rich substratum.

Discharge stability or variability is the most important abiotic factor influencing spring fauna directly as well as indirectly through the shaping of substrate structure and composition [5,37,38]. In our study, springs with low discharge were found to be significantly different from springs with higher discharge with respect to substrate composition. However, surprisingly we did not found a significant influence of discharge on the composition of gastropod assemblages in the studied springs. This indicates that the gastropod assemblage in the springs examined in current study is well adapted to intermittent conditions (i.e. fluctuations in discharge). This might be explanation for the lower species richness in the studied springs [37,39].

On the other hand, ANOSIM analysis showed that there are significant differences in gastropod assemblage in terms of season of sampling, suggesting that a seasonal disturbance of abiotic interactions, such as discharge and substrate composition, is an important factor in the shaping of gastropod communities in springs. However, it is worth mentioning that the seasonal difference in gastropod assemblages might be the result of differences in biotic interactions such as predation and competition [33]. Increased predation by invertebrate predators [40] and changes in competitive ability (increased for pulmonate taxa and decreased for prosobranch, [41]) at the localities where food is a limiting factor may alter the habitat preferences of some species.

The results of our study are consistent with von Fumetti and Nagel [5] and confirms the absence of a correlation as described in the intermediate disturbance hypothesis, which predicts a peak in biodiversity at an intermediate level of disturbance [42]. Further, our results are not in accordance with studies showing that, because of the periodic disturbance, intermittent springs are usually not inhabited by spring specialists [1]. In our study, spring specialists such as *Bythinella schmidti* and *Islamia dmitroviciana* also inhabit springs with intermittent dry periods.

The results of CCA in our study showed that discharge, substrate type and temperature could be good predictors for the occurrence of particular species. *Bythinella schmidti* prefers springs with a higher discharge and a stony substrate. According to Zollhöfer [42] and Zollhöfer et al. [43], species of *Bythinella* are typical for springs exhibiting karst characteristics with highly fluctuating discharge conditions.

In groundwater-fed springs the gastropod assemblages often include species from the aquifer that feeds the spring (hypogean species) by a constant washout. Therefore, it is not surprising that we collected stygobiotic *Bosnidilhia vreloana* mainly in winter, when the discharge capacity of springs is generally higher.

Among the studied springs only a few natural springs are found; the majority were piped (spring water emerging from an artificial pipe). Such a situation indicates that the spring snail fauna of the studied area should be considered at risk. Given the susceptibility of springs to modification from land-use changes and water extraction, as well climate change (since 2010 two of the studied springs have dried up), further studies will require long-term monitoring to assess the impact on freshwater snail assemblages.

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