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Assemblages of Freshwater Snails (Mollusca: Gastropoda) from the Nišava River, Serbia: Ecological Factors Defining their Structure and Spatial Distribution

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Abstract: Monthly samples of freshwater snails (Mollusca: Gastropoda) were taken from ten localities along a 151 km long section of the Nišava River (Eastern Serbia) during a one-year study. Eleven taxa of four families were identified. The gastropod assemblages were primarily defined by concentration of total nitrogen and oxygen, while other significant factors were pH value and the percentage of stable substrate. Neither the number of species nor the abundance of gastropods showed any statistically significant correlation with the distance from the river mouth, the water residence time or the stream order. This indicates that the gastropod assemblages are more influenced by factors indicating the degree of anthropogenic impact than by hydrological characteristics connected to a particular sector of the river. Some of the investigated localities constitute habitats of *Theodoxus transversalis*, which is classified as endangered species by the IUCN Red List. Therefore, the study area deserves additional attention in terms of its conservation.

Key words: gastropod assemblages; environmental factors; diversity; river

Introduction

The Balkan region is one of the global hotspots of biodiversity (Griffith *et al.* 2004, Strong *et al.* 2008). Particularly the Balkan freshwater fauna has long been recognised as highly diverse with remarkable degree of endemism in all major taxa (Banarescu 2004, Gloër *et al.* 2007).

Gastropods occupy an important position in the food web and are ubiquitous and abundant in aquatic ecosystems (Covich *et al.* 1999). In freshwater ecosystems, they constitute an important link in circulation of organic matter and nutrients. Freshwater gastropods, which are hololimnic organisms, have limited mobility and therefore could be used as bioindicators of changes in their habitats (Amin *et al.* 2009, Hookham *et al.* 2014). They reflect the abiotic or biotic state of water habitats, which represents the impact of environmental changes on the habitat, the community and the ecosystem (Lewin 2014). Freshwater mollusks are known to play significant role in public and veterinary health and need to be studied in greater details (Husein *et al.* 2011; Saddozai *et al.* 2013). Some freshwater gastropods are ideal bioindicators of trophic stages of lakes, as well as for lotic environments (Choubisa 2010; Choubisa, Sheikh 2013, Blagojević *et al.* 2014). These are only some of the reasons for increased need in knowledge of the gastropod assemblages, which used to be poorly studied in this region.

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There are certain records on gastropod taxa from the territory of Serbia, but they are presented mostly in papers focusing on taxonomy (Glöer 2008, Novaković *et al.* 2013; Marković *et al.* 2014), therefore there are almost no data on the impact of environmental factors on the structure and composition of gastropod assemblages from Serbia.

To date, no long-term surveys on the environmental factors that determine the occurrence of mollusks in rivers (including the physical and chemical parameters of the water, bottom sediments and macrophytes) have been carried out, either in general (Lewin 2014) or in this particular area. There are also no data on the bioindicative role of snails on the territory of Serbia.

The importance of different ecological factors varies significantly from one ecological zone to another and even between water bodies, suggesting that it is necessary to perform local studies in order to identify important factors in each zone or water body (Imevbore *et al.* 1988; Ofoezie 1999; Hussein 2011). Lodge *et al.* (1987) reviewed ecological studies on freshwater gastropods and concluded that their biogeographical distribution is primarily controlled by physicochemical variables.

The objectives of the present survey were (1) to assess the spatial composition of the freshwater gastropod assemblages along the Nišava River continuum; (2) to identify key environmental factors influencing their variation; (3) to determine whether the freshwater gastropod assemblages are primarily defined by anthropogenic impact or by the hydrological characteristics related to the particular sector of the river; and (4) to determine if freshwater gastropods are suitable as biological indicators of water quality in this region.

Materials and Methods

The Nišava River was chosen for this research because it was possible to study almost the entire river length in Serbia, including parts with and without human impact. The Nišava River belongs to the Danube/Black Sea drainage basin. It originates in the Stara Planina Mt., Western Bulgaria, and it flows in SE-NW direction. It is the longest tributary of the Južna Morava River and also the largest one in terms of discharge (36 m^3/sec; Gavrilović, Dukić, 2002). It is 218 km long, of which 67 km flows through Bulgaria, and 151 km through Serbia.

Ten localities were chosen along the entire course of the Nišava River in Serbia (Fig. 1). The odd numbered localities are positioned upstream, and even numbered downstream of the settlements. Sampling was performed each month, from May 2006 to April 2007. All localities were sampled on a single day during each field trip.

Biochemical oxygen demand (BOD$_5$) was estimated using the standard methodology recommended by APHA (1999). Dissolved oxygen, pH and conductivity were measured using a WTW® Multi 340i probe. The concentration of total nitrogen (TN) and phosphorus (TP) were determined in the field, using a Photometer-SystemPC MultiDirect Lovibond® meter. Water turbidity was measured with a Lovibond® Checkit device.

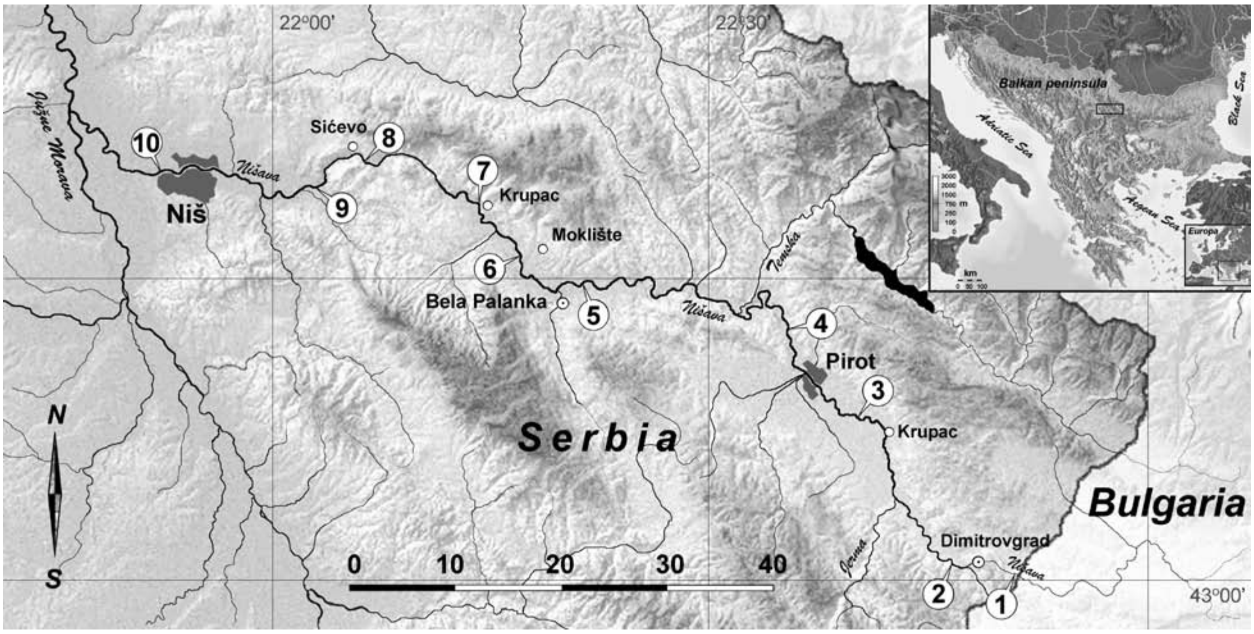

Fig. 1. Map of the studied area with localities

The percentage of substrate was observed visually; the classification of mineral substrates by particle size followed Wentworth (1922) and Verdonschot (1999). Since larger substrates require greater stream power for movement, they are physically more stable (Gurtz *et al.*, 1984); thus, pebbles, cobbles and boulders consolidated a stable substrate.

The watershed area was calculated using ArcGIS software (ESRI, 2009). Scanned topographic maps at 1:25000 scale were added in ArcMAP and then georeferenced; the polygons that corresponded to sub-areas were constructed using the contour lines with ArcMAP Calculate Geometry Tool. Water residence time was calculated with the formula

$$R = 0.8\, A_d^{06} / Q^{0.1} \qquad (1),$$

where R is water residence time at the sampling site (d), A_d is watershed area upstream of the sampling site (km^2), and Q is river discharge (m^3/s; Soballe, Kimmel, 1987).

In order to include various water depths periphyton was sampled in triplicates by gentle scraping with a brush (up to 10 cm^2 of surface) at the left bank, in the middle of the river, and at the right bank along a transect. In general, the stone was removed from the stream before scraping so that the scraped algae were not washed away (Lowe, Laliberte, 2007). Upon collection the periphyton was combined into a single sample, and its wet weight was measured. The percentage of canopy shadow was estimated by visual observation.

At each locality a 10 m long reach, characteristic of that site and on the part of the river where macroinvertebrates were sampled, was selected for visual estimation of macrophyte cover. Estimate of the macrophyte cover were determined once a month from April to September from 1x1 m quadrate across the entire width of the river over a 10 m river stretch. For each quadrate, the percentage cover of all macrophytes was estimated in % increments. Macrophyte cover per locality was calculated as the average of macrophyte cover from all quadrates.

Macrozoobenthos was sampled at each locality over a 50 m river stretch with a square frame kick net (35 × 35 cm, mesh size 300 μm). Three three-minute samples were taken during each visit at each location to include different substrates (boulder, cobble, pebble, sand, silt, and detritus) and flow regime zones. The net was held perpendicular to the flow and the substrate was vigorously disturbed in front of the net. As the substrate was disturbed, sampling moved progressively upstream. The three samples were then pooled, representing a single monthly sample for each site. This sampling procedure was previously evaluated by preliminary test sampling, and three replicates proved to be sufficient to capture the maximum number of taxa. All samples were elutriated in the field and the organisms were fixed in 4% formaldehyde solution and returned to the laboratory for sorting. The material was identified using identification keys for freshwater snails (Glöer 2002, 2015).

The species diversity was estimated using the Shannon's diversity index (H'), (Karadžić, Marinković, 2009). A repeated measures analysis of variance (ANOVA) and multiple range tests (Fisher's least significant difference (LSD) procedure) were applied using R software to test the significance of differences in the total phosphorus, total nitrogen, oxygen, and the number of species between localities.

Multivariate analyses permit a considerable level of understanding of community structure and its relationships with corresponding environmental properties (Ormerod, Edwards, 1987). We applied a cluster analysis in order to detect patterns of variation in the freshwater snail assemblage structure. For agglomerative classification, we used the UPGMA method (Sokal, Rohlf, 1995) in combination with the chi-square distance. The canonical correspondence analysis (CCA; ter Braak, 1986) was applied to test the influence of environmental variables on the freshwater snail assemblages. To explore the studied data set and to examine the similarities of the samples, PCA was used. As this data set contains measurements performed within different magnitude ranges, the PCA model was constructed for the standardised data. For determination of the number of significant components (PCs) of this data set, Guttman-Kaiser criterion was used.

Results

The analyses of environmental parameters showed that locality 4 had the highest cumulative average annual TP + TN concentration, highest total N and very high values of BOD$_5$. Locality 10 has the highest average periphyton mass, lowest turbidity and lowest oxygen concentration (Table 1). One of the shared features of localities 4 and 10 was the somewhat-higher percentage of agricultural areas (which is also the case with localities 1 and 2; Table 2).

ANOVA showed that there was a significant difference between localities in regard to the total phosphorus (F = 7.6, p = 0.0001, df = 9), total nitrogen (F = 5.6, p = 0.001, df = 9), and oxygen concentration (F = 7.00, p = 0.0001, df = 9). The post-hoc test showed that locality 10 (downstream Nis) differed significantly from other localities regarding both the total phosphorus (except from locality 4)

Table 1. The average annual values of environmental parameters at each locality (loc) studied along the Nišava River. TP-total phosphorus in mg/l; TN-total nitrogen in mg/l; N+P-sum of TN and TP in mg/l; O-oxigen in mg/l; BOD_5-biochemical oxygen demand in mg/l; TU-turbidity in NTU; CON-conductivity in S/cm; SS-stable substrate in %; CC-canopy cover in %; MC-macrophyte cover in %; MP-mass of peryphiton

	loc 1	**loc 2**	**loc3**	**Loc 4**	**loc 5**	**loc 6**	**loc 7**	**loc 8**	**loc 9**	**loc 10**
TP	0.02	0.08	0.04	0.10	0.07	0.07	0.07	0.06	0.07	0.11
TN	0.27	0.28	0.08	0.34	0.10	0.12	0.12	0.08	0.09	0.21
N+P	0.29	0.36	0.12	0.44	0.17	0.19	0.19	0.14	0.16	0.32
O	6.72	7.28	8.04	7.09	7.85	8.34	8.37	7.98	7.56	6.50
BOD_5	1.68	3.04	2.74	4.16	3.08	3.37	3.61	3.08	3.17	2.99
pH	7.56	6.54	6.17	6.59	6.98	6.56	6.30	7.34	6.15	6.54
TU	26.60	35.92	12.33	18.20	21.98	20.72	5.28	12.90	6.94	3.75
CON	496.00	536.42	460.08	459.42	395.75	403.83	414.17	411.08	413.58	575.67
SS	67.50	79.67	78.33	46.50	76.67	52.58	83.75	89.17	66.67	70.42
CC	28.33	10.42	8.75	26.67	10.00	30.42	27.92	21.25	51.67	7.92
MC	12	17	16	0	22	5	18	13	18	32
MP	7.57	10.74	7.45	8.13	7.72	5.86	7.23	7.01	7.89	13.31

Table 2. Geographical position and characteristics of each studied locality along the Nišava River. AL-Altitude (m); LA-Latitude (°N); LO-Longitude (°E); WRT-water residence time in d; DI-Distance from the river mouth (km); SO-Stream order; Land use (Corine 2010): AR- Artificial surfaces (%); AG-Agricultural areas (%); FS-Forest and semi-natural areas (%)

	Geographical position			**Hydrological characteristic**			**Land use**		
	AL	LA	LO	WRT	DI	SO	AR	AG	FS
1	466	43°00’956”	22°47’907”	2.86	140.15	4	5.22	33.32	61.46
2	445	43°00’996’	22°45’220”	3.68	135.06	5	4.56	35.22	60.22
3	381	43°07’825’	22°37’844”	5.57	111.38	6	2.48	31.86	65.66
4	359	43°11’428’	22°33’995”	6.07	101.14	6	2.65	34.03	63.32
5	281	43°13’909’	22°19’466”	7.57	66.27	7	1.84	30.21	67.95
6	276	43°14’633’	22°16’322”	7.81	60.27	7	1.78	30.26	67.96
7	266	43°17’635’	22°13’580”	7.98	52.16	7	1.71	30.28	68.01
8	233	43°19’893’	22°04’429”	8.11	35.1	7	1.72	30.43	67.85
9	224	43°18’424’	22°02’273”	8.19	30.32	7	1.69	30.42	67.89
10	205	43°19’330’	21°52’375”	8.69	14.04	7	2.21	32.19	65.6

and the oxygen concentration, and that locality 4 was significantly different from other localities regarding the nitrogen concentration.

Gastropods collected at the studied sites belong to 11 species within four families (Table 3). Three families were represented by three species each (Neritopsina, Lymnaeidae, Planorbidae). One family was represented with a single species (Melanoposidae). Considering the number of collected specimens, 90.15% were Lymnaeidae, 7.28% Neritidae, 1.65% Planorbidae and 0.92% Melanopsidae.

The greatest species richness occurred at localities 5, 6 and 7 (8 species). The species P. corneus was found at only one locality, while the species R. balthica was present at all studied localities (full names are given in Table 3).

ANOVA has shown significant difference in species richness between localities (F = 32.8, p = 0.000, df = 9), while the post-hoc test showed that locality 8 was significantly different from all other localities. At the same time this was the locality with the greatest value of Shannon’s diversity index (Fig. 2).

The greatest number of individuals was recorded at localities with the highest mean annual level of macrophyte cover: localities 5 and 7. Locality 10 was also among the localities with the highest annual mean of macrophyte cover (Table 1), but the number of individuals at this site was far lower due to high levels of pollution (it had already been placed in a separate group together with locality 4).

Since the results suggested that localities 4 (downstream Pirot) and 10 were polluted localities, we labelled them as the group A and all other localities as the group B, and reran the ANOVA based on these two classes of data. Oxygen concentration, species richness and collected specimens, were found to be higher in Group B ($F = 7.68$, $p = 0.01$, $df = 1$; $F = 12.71$, $p = 0.001$, $df = 1$ and $F = 8.056$, $p = 0.008$, $df = 1$, respectively), and both total phosphorus and total nitrogen were found to be significantly higher in Group A ($F = 22.95$, $p = 0.0001$, $df = 1$, and $F = 7.06$, $p = 0.013$, $df = 1$, respectively; Fig. 3).

In order to explore the studied data set and to examine the similarities of the samples, PCA was used. As this data set contained measurements performed within different magnitude ranges, the PCA model was constructed for the standardised data. For determination of the number of significant components (PCs) of this data set, Guttman-Kaiser criterion was used. The PCA (Fig. 4) model with three significant principal components described 83% of the data variance.

If we compare similarities obtained from environmental variables (Fig. 4) and similarities obtained from gastropod assemblages (Fig. 5) we can notice that localities 4 and 10 clearly differed from the others, while 7 and 8 are close to each other on both figures.

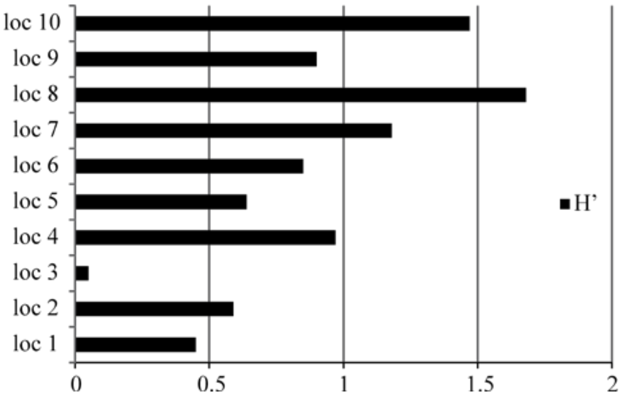

Fig. 2. Average annual values of Shannon's diversity index (H')

Table 3. The occurrence of gastropod species at each studied locality (loc) along the Nišava River. Abb – Abbreviation

Family/species	**Abb**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Melanopsidae											
Fagotia daudebartii (Prevost, 1821)	Fad					+			+	+	+
Neritidae											
Theodoxus danubialis C. Pfeiffer, 1828	Thd		+			+	+	+	+	+	
Theodoxus fluviatilis Linnaeus, *1758*	Thf					+	+	+	+	+	
Theodoxus transversalis C. Pfeiffer, 1828	Tht					+	+	+	+	+	
Limnaeidae											
Radix auricularia Linnaeus, 1758	Rau		+					+			+
Radix balthica (Linnaeus, 1758)	Rab	+	+	+	+	+	+	+	+	+	+
Stagnicola palustris (O.F. Muller, 1774)	Stp					+	+	+	+	+	+
Planorbidae											
Ancylus fluviatilis O.F. Muller, 1774	Anf	+		+	+	+	+	+	+	+	
Anisus vortex Linnaeus, 1758	Anv		+				+				
Planorbarius corneus (Linnaeus, 1758)	Plc		+								
Planorbis planorbis Linnaeus, 1758	Plp		+			+	+	+			+

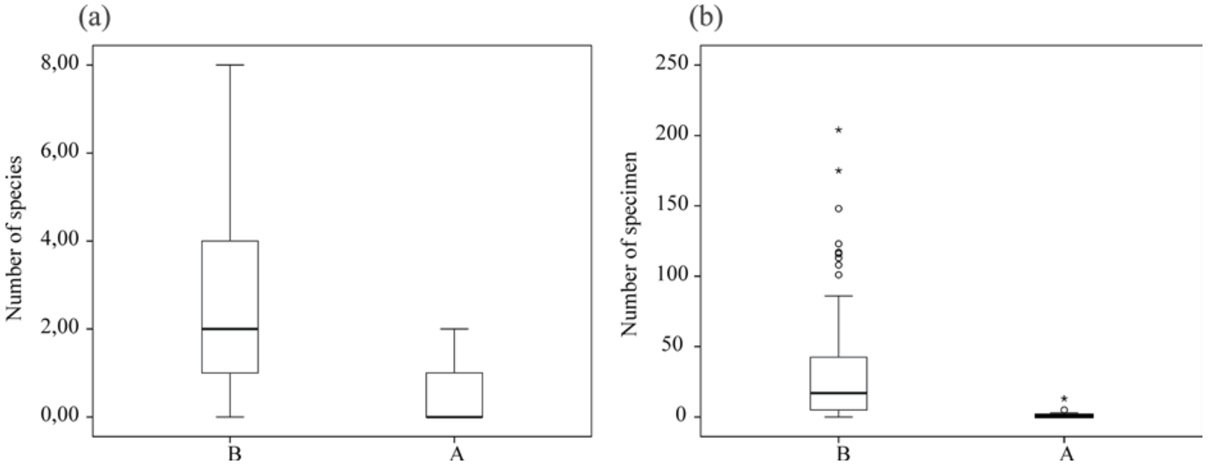

Fig. 3. Box-and-Whisker plot: (a) number of species; (b) number of specimens. Group A: localities 4 and 10; Group B: all other localities

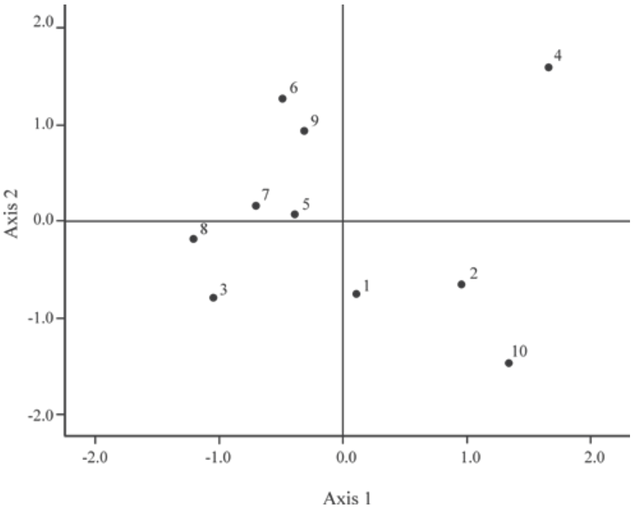

Fig. 4. Ordination of 10 localities generated by applying Principal Component Analysis to environmental variables

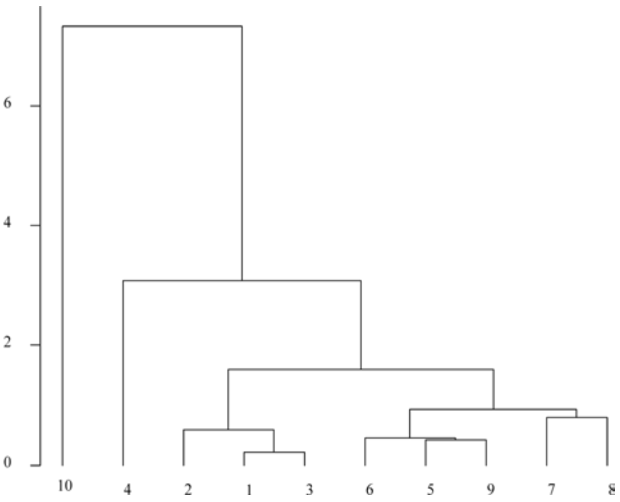

Fig. 5. Cluster analysis of the freshwater snail assemblages in the Nišava River. The number corresponds to number localities in Fig 1 and Table 2

Discussion

Radix balthica was recorded from all localities. This is euryoecious species but rare for the Balkans. Usually, this species prefers polluted water habitats (Gloer and Diercking 2010). However, throughout the study period the number of individuals recorded at locality 4 (two individuals) and locality 10 (six individuals) was significantly lower than in the localities of the less polluted group. One example is locality 7, which had the highest number of individuals of this species during the study period (218 individuals). This locality was also characterised by the highest annual mean of concentration of oxygen (Table 1).

Similar situation was recorded for species *Ancylus fluviatilis*, which was recorded in most localities, both polluted and unpolluted, but the number of individuals was also highest at locality 7. *Ancylus fluviatilis* is generally considered an indicator of good water quality, but it was also recorded from lightly to highly polluted localities as reported by Rueda *et al.* (2002). Therefore, it may be reasonable to conclude that the density of this species is indicative of habitat quality (Van Horne, 1983). Regularity is also noticeable in terms of the total number of gastropods (number of individuals of the whole assemblage), which was notably lower at localities 4 and 10.

Theodoxus fluviatilis, *T. transversalis* and *Stagnicola palustris* inhabit localities from the 7th stream order (Table 2 and Table 3), indicating a strong preference by these species for the lower section of the river. Theodoxus transversalis was recorded in five out of ten localities (Table 3). Once it used to have a wide distribution range in the Danube catchment area, but due to decrease in water quality it is now reduced to less than 20 known subpopulations (Solymos, Feher 2011, Pavlova *et al.* 2013). Due to this rapid decline and present rarity, *T. transversalis* is listed in Annexes II and IV of the European Habitat Directive and categorised as Endangered by IUCN (Solymos, Feher 2011). As stated by Solymos, Feher (2011) at most of the known sites *T. transversalis* are threatened by pollution and the invasive *T. fluviatilis* and it is likely that in the future some subpopulations along the whole Danube section may become extinct. Due to all of the above, the study area of this research deserves additional work in the aspect of conservation biology.

On the other hand, there were no species separated by having preference for the higher section of the river.

The Shannon's diversity index at locality 8 has the highest value in this study. This locality is characterised by: the highest level of stable substrate when compared with other localities, high concentration of oxygen and low concentrations of T+P. In comparison to locality 8, localities 6 and 7 have higher concentration of oxygen and T+P. Therefore the Shannon's diversity index might be limited primarily by the concentration of T+P and not by the oxygen concentration . When locality 3 was added to the analysis, it showed a higher concentration of oxygen but lower concentration of T+P than locality 8. However, locality 8 has significantly more stable substrate, indicating that the role of stable substrate is particularly high in structuring the gastropod assemblages. This is also visible in Fig. 6 where the intensity of vectors shows that SS, P+N and oxygen concentration have high significance in structuring the gastropod assemblages.

According to the Correlation analysis (Pearson correlation coefficient), gastropod density showed positive correlation with pH (R=0.189; sig=0.39), as concluded also by Lewin, Smolinski (2006) for the mollusk assemblages from Central Europe. This result confirmed the earlier investigations by Økland (1983) and Herrmann *et al.* (1993). Mollusk density also showed positive correlation with concentration of oxygen (R=0.318; sig=0.000). The number of species was positively correlated with oxygen level (R=398; sig=0.000) and substrate stability (R=372; sig=0.000). Concentration of total nitrogen was negatively correlated with both mollusk density (R=-0.289; sig= 0.001) and number of species (R=-0.379; sig=0.000). Russel-Hunter (1978) stated that the number of gastropod species and specimens was greater in eutrophic reservoirs compared to oligotrophic reservoirs. This was confirmed by our survey, which demonstrated a correlation between the gastropod density and the concentration of total nitrogen. Lewin, Smolinski (2006) found negative correlation between the number of species and concentration of phosphates, but this correlation was not significant in our sample. Jakubik, Lewandowski (2011), Ihtimanska *et al.* (2014) have also recorded lack of significant correlation between the concentration of phosphates and the number of recorded gastropod species.

The mass of periphyton did not show statistically significant correlation with either gastropod density or number of species.

According to the Correlation analysis (Pearson correlation coefficient), the correlations between the gastropod density and the number of species, and the distance from the river mouth, the water residence time or the stream order were no statistically significant. This indicates that the gastropod assemblages are more influenced by factors related to the degree of anthropogenic impact (higher values of total phosphorus concentration) than by hydrological characteristics that are connected to a particular sector of the river.

The analyses of gastropod assemblages showed evident grouping of localities into two groups: Group A – polluted localities (including localities 4 and 10) and Group B – unpolluted localities (the rest of the localities). Species richness and number of collected specimens were found to be higher in Group B (*F* = 12.71, *p* = 0.001, *df* = 1 and *F* = 8.056, *p* = 0.008, *df* =1, respectively). This result supports the use of gastropod assemblages as indicator of water quality. Some scientists assume that using gastropod assemblages for bioindicator purposes in this region would not provide the high resolution shown by groups much more commonly used for bioindicator purposes (Ephemeroptera, Plecoptera, Trichoptera etc.). However, the use of this group should not be avoided, as most representatives are large and more easily determined to species level than the representatives of the other three groups. EPT taxa scores provide higher resolution but only at species level, while their use on genus level is not reliable for bioindicator purposes in this region (Savic *et al.* 2011, Savic *et al.* 2013).

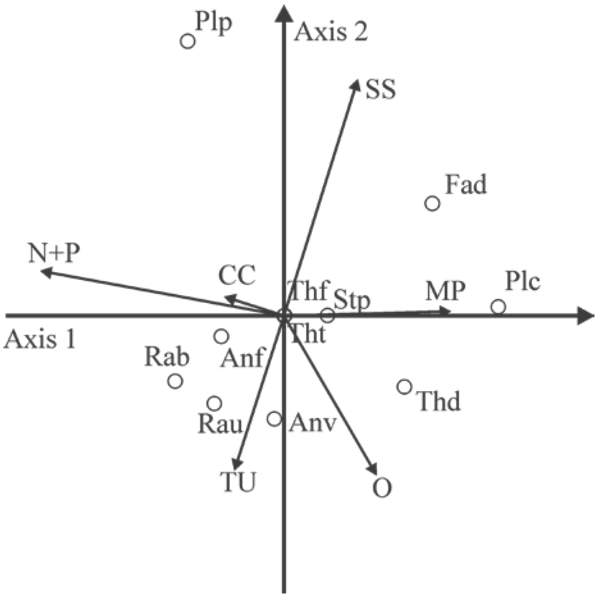

Fig. 6. CCA of the freshwater snail assemblage in the Nišava River

Conclusion

We found 11 species in the study area, including *T. transversalis* with the IUCN status of endangered species. Therefore the study area deserves additional attention from the standpoint of its conservation.

The gastropod assemblages were primarily defined by concentration of total nitrogen and oxygen, while other significant factors were pH value and the percentage of stable substrate. Neither the number of species, nor abundance of gastropods has shown any statistically significant correlation with the distance from the river mouth, the water residence time or the stream order. This indicates that the gastropod assemblages are influenced more by factors related to the degree of anthropogenic impact than by hydrological characteristics connected to particular sector of river.

Our results demonstrate that the gastropod assemblages are unreasonably neglected when choosing bioindicator groups in this region. Their inclusion may yield precious data on the present conditions in the ecosystem.

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