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HYDROBIOLOGICAL AND FAUNISTIC INVESTIGATION OF THE NEMATODE FAUNA (NEMATODA, NEMATHELMINTHES) OF THE URDINI EZERA GLACIAL LAKES, NORTHWESTERN RILA, WESTERN BULGARIA

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Abstract. Te present study gives for te first time detailed information about the free-living Nematode fauna of the Urdini ezera. A total of 47 species were found,belonging to 18 genera, 13 families and 6 orders. Seven of the species (marked by +), belonging to 2 genera (marked by o), are new for the Bulgarian hydrofauna.
Key words: Nematoda (Nemathelminthes) Urdini Ezera Lakes, Bulgaria.

Urdini Ezera lakes are situated in the Western part of the large Urdin circus in Northwestern Rila. The circus is open towards North-east and it is confined by Ushite peak (2547 m), Maliovitza peak (2730 m), Mermera peak and Dodov Vrah peak (2661 m) from where starts the southern part of the circus, Damga peak (2670 m) and Zeleni Rid hills. It is a group of six lakes which originate from Urdina River, a tributary of Cherni Iskar River.

Urdini Ezera lakes are produced through glacial and subsequent water activity and through meteorological forces and thus conserved typical remnants from glacial forms, as moraines at the outflow of the third and fifth lakes. However, some lakes represent tectonic cavities with no glacial forms, as the second and the fourth lakes.

Urdini Ezera lakes are relatively small and their surface varies between 7500 m^2 and 25 300 m^2.

In the Urdin circus, besides the Urdini Ezera lakes, there are seven high-mountain pools, with a depth varying from 0.3 to 0.9 m.

The first Urdino Ezero lake is the highest in the lakes group. It is located at 2375 m above the sea level, 1560 m southeast from Damga peak. Its southern coast is abrupt and rocky and gradually changing eastwards into a more even, the northern and northeastern coasts being flat and low and flat. All the shores of the lake, except the southern , are surfaced with vegetation. At the southern and south-western parts, where the highest depths are observed, the bottom is rocky, and at the western and northwestern parts it is sandy. The biggest part of the bottom which remains is covered with slime, with occasional spots due to ferro-bacteria. The main surface inflow comes from the western part of the lake, and its under-ground inflow comes from the southeastern part. The outflow from the lake is located at its northeastern part and it feeds the second lake which lays below it.

The second Urdino Ezero lake has the largest surface and volume within the lakes group. It is located to the north of the first Urdino Ezero lake at 2278 m above the sea level, 1300 m from Damga peak. Its southern and southeastern coasts are very abrupt and rocky and the rest is relatively more even and covered with vegetation. The lake is feeded from two inflows. The major one flows in the southern part of the lake, coming from the first Urdino Ezero lake and forming a big waterfall on its way. The minor inflow runs into the western part of the lake. The outflow from the second Urdino Ezero lake is located at the northeastern part of the lake, and it is the beginning of the Urdina river.

The third Urdino Ezero lake is located at 2339 m above the sea level, 800 m south of Damga peak. Most of its coasts are abrupt and covered with vegetation. The western and southwestern coasts are covered with a snow-drift during almost the whole year. The bottom is mainly covered with slime, the parts near the coasts being rocky, and sndy in its western part. There is an inflow at the lake’s western part. The outflow is located in the eastern part and it is a rather big stream. It is the longest within the group.

The fourth Urdino Ezero lake is located 1660 m southeast of Damga peak in a deep hollow, at 2336 m above the sea level. Its southern coast is a talus, with flat stones, and the other coasts are covered with grass. The bottom is rocky. The lake is feeded from an indistinct inflow in its eastern part. The inflow in the northern part of the lake forms a little waterfall and runs into Urdina river.

The Ribno Urdino Ezero lake is located 500 m east of Damga peak at 2338 m above the sea level. Its coasts are even and covered with vegetation. The southern coast is partly covered by a talus. The bottom is covered with slime, and sandy in its western part. The lake is feeded from several little streams in its western part, and from an underground inflow at its southern coast. The outflow is located at the lakes's eastern part and runs into Urdina river. It has the greatest catchment area within the lakes group.

The sixth Urdino Ezero lake is located on a little terrace, 1200 m east of Damga peak at 2295 m above the sea level. It has the smallest surface, vloume and depth within the group. The coasts are even, dry and covered with grass, except the eastern part, which is swampy. In the middle of the northern coast, there is a peatery in the form of a small peninsula, which was part of the lake in the past. The bottom is almost everywhere covered with slime. The lake is feeded at its northern part, from several little streams coming across the peatery. The outflow is located at the lakes's southeastern part and runs into Urdina river. Details on the six Urdini ezera lakes are given further in Table 1.

Table 1. Description of Urdini Ezera lakes (The First Urdino Ezero lake as Station 1, i.e. the highest station, through the Sixth Urdino Ezero lake as Station 6, i.e. the lowest lake)

Parameters	Stations					
	1	2	3	4	5	6
Length, m	115	220	246	146	217	128
Maximum width, m	99	163	157	124	105	91
Mean width, m	75	115	96	n.a.	n.a.	59
Water surface, m^2	8600	25 300	23 400	12 600	16 500	7500
Water volume, m^3	16 000	89 500	59 500	54 000	1 9000	6000
Coast line, m	347	600	750	420	595	450
Maximum depth, m	4.7	6.6	4.5	7.6	2.4	2.0
Minimum depth, m	1.9	3.6	2.5	4.3	1.2	0.8

MATERIAL AND METHOD

The material was collected by the author in 1999–2007. A total of 162 samples were collected. The determination and the presentation of the nematode species w as made according to Gagarin (1981). The determination of the nematode species' qualitative composition was made according to the formula of De Man (1886) and Teskey (1981). Before fixing and processing, a careful heating up to 60 °C was effected, as described in Filipjev (1918), by which nematodes become slacken and erected and thus more convenient for measuring. Freshwater nematodes are a rathe r delicate material and their conservation for a long period is only possible in a 4% formalin solution, since alcohol and other fixatives dehydrate their bodies.

The analysis of dominant quantitative presence was made according to the method of De Vries (1937)

LITERATURE REVIEW

The first publication of data on the benthic fauna of remote mountain lakes in Bulgaria was given by Valkanov (1932). Later, Valkanov (1934, 1938) reported 35 benthic species. Data on 61 free-living nematode were published by Stoichev (1996). Data about composition, distribution and ecology of free-living nematodes from Bulgarian inland waters were reported by Stoichev (1999 a). Recently Stoichev (1999 b) reported 21 free-living nematode species. Stoichev (2000 a, b) studied the benthic fauna of several glacial lakes in Rila mountain and reported 26 species in the lakes (21 chironomid and 5 nematode species) and 27 benthic species in their outflows. The present study gives for the first time detailed information about te free-living nematode fauna of the Urdini ezera glacial lakes.

RESULTS

A total of 47 species were found, belonging to 18 genera, 13 families, and 6 orders. Seven of te species (marked by +) and 2 of genera (marked by o), are new for te Bulgarian hydrofauna (Table 2). Table 2 gives information ot the frequency of occurrence (*pF*%), frequency of dominance (*F*%) and the range of dominance (*DT*) of the species found. According to the obtained frequency of presens date the following classification could be applied. This classification wus proposed for the first time by Stoichev (1996), Where the species fall to someone of the groups:

1. Very frequently species (*pF* 50 %): Dorzlaimus stagnalis, Monhzstera filiformis, Monhzstera stagnalis. Total: 5 species.
2. Frequently found species (*pF* – 10–50 %): Rabditis filiformis: Total 1 species.
3. Rarely found species (*pF* – 1–10 %): Eudorylaimus similes, Aporcelaimellus obtusicaudatus, Aporcelaimellus superbus: Total: 19 species.
4. Very rare species (*pF* 1 %): Nigolaimus clavicaudatus, Nigolaimus intermedius, Nigolaimus aquaticus: Total: 22 species.

Table 2. Species composition, distribution and dominance analysis of the nematodes found in Urdini Ezera lakes

Taxa	Stations						Dominant analisis		
	1	2	3	4	5	6	*pF*%	*DF*%	*DT*%
Nematoda									
Dorylaimida Pearse, 1942									
Dorylaimidae de Man, 1876									
Dorylaimus stagnalis Dujardin, 1848	x	x	x	x	x	x	92.59	77.16	83.33
Prodorylaimus acris (Thorne, 1933)	x						0.61		

Table 2 (cont.)

Eudorylaimus similis (de Man, 1876)		x	x				3.08		
Dorylaimus sp.	x						0.61		
Aporcelaimidae Heyns, 1965									
Aporcelaimellus obtusicaudatus (Bastian, 1865)				x			1.23		
Aporcelaimus superbus (de Man,1880)			x				1.84		
Aporcelaimus sp.				x			0.61		
Nigolaimoidea Thorne, 1935									
Nigolaimidae Thorne, 1935									
Nigolaimus clavicaudatus Altherr, 1953			x				0.61		
Nigolaimus intermedius (de Man,1880) +		x					0.61		
Nigolaimus aquaticus Thorne, 1930 +	x						0.61		
Monhysterida de Coninck et Sch. Stekhoven, 1933									
Monhysteridae de Man, 1876									
Monhystera filiformis Bastian, 1865	x	x	x	x	x	x	88.27	70.99	80.42
Monhystera stagnalis Bastian, 1865	x	x	x	x	x	x	83.33	54.93	65.91
Monhystera vulgaris de Man, 1880					x	x	2.46	0.61	24.79
Monhystera dispar Bastian, 1865			x	x			1.85		
Monhwstera uncispiculahim Gagarin, 1979		x					1.23		
Monhystera sp.	x						0.61		
Enoplida *Chitwood, 1933*									
Enoplidae *Dujardin, 1845*									
Enoploides fluviatilis Micoletzky, 1923			x	x			1.85		
Enoploides sp.		x					0.61		
Tripylidae de Man, 1876									
Tripyla selifera Bьetschli, 1873		x		x		x	4.32	1.23	28.47
Tripyla glomerans Bastian, 1865		x	x	x			5.55	0.61	
Tripyla sp.				x			0.61		
Tobrilus gracilis (Bastian,1865) Andrassy, 1959	x	x	x	x	x	x	74.69	42.59	57.02
Tobrilus longus (Leidy,1852) Andrassy, 1959			x		x		3.08		
Tobrilus stefanskii (Mikoletzky,1925) Andrassy, 1959		x					1.23		
Tobrilus sp.	x						0.61		
Araeolaimida de Coninck et Sch. Stekhoven, 1933									
Cylindrolaimidae Mikoletzky, 1922									
Cylindrolaimus communis de Man, 1880		x		x			1.85		

Table 2 (cont.)

Cylindrolaimus melancholicus de Man, 1880	x						1.23		
Cylindrolaimus sp.		x					0.61		
Rhabdolaimidae Chitwood, 1951									
Rhabdolaimus terrestris de Man, 1880 +o	x						0.61		
Chronogasteridae Chitwood, 1951									
Chronogaster typicus (de Man, 1921) de Coninck, 1935		x	x		x		3.70	1.85	50.00
Chronogaster boetgeri Kischke, 1956		x		x			1.23		
Chronogaster sp.			x				0.61		
Plectidae Oerley, 1880									
Plectus parietinus Bastian, 1865 +		x					0.61		
Plectus cirratus Bütschli, 1873	x	x	x	x	x	x	57.40	5.55	9.66
Plectus palustris de Man, 1880 +			x				0.61		
Plectus rhizophilus de Man, 1880 +				x			0.61		
Plectus tenuis Bastian, 1865	x		x	x			2.46		
Plectus sp.		x					0.61		
Tylocephalus Crossman, 1933									
Tylocephalus auriculatus (Butschli, 1873) Anderson, 1966+o			x				0.61		
Tylocephalus sp.			x				0.61		
Rhabditida Chitwood, 1933									
Rhabditidae Oerley, 1880									
Rabditis filiformis Bütschli, 1873	x	x	x	x	x	x	24.07	9.87	41.00
Rhabditis sp.				x			0.61		
Diplogasteridae Micoletzkky, 1922									
Diplogaster sp.			x				0.61		
Diplogaster rivalis (Leydi, 1854) Butschli, 1873			x		x	x	3.70	0.61	16.48
Mononchoides striatus (Butschli, 1876) Goodey, 1963			x				1.23		
Mononchoides sp.		x					0.61		
Panagrolaimidae Thorne, 1937									
Panagrolaimus hygrophiluss Bassen, 1940				x	x		1.23		

Beside with the species of high values of *pF* and *DT3* (*Dorylaimus stagnalis, Monhystera filiformis, Monhystera stagnalis*), species of high values of the range of dominans low presens and dominans, frequency, can be found (*Monhystera vulgaris, Tripyla selifera, Chronogaster typicus*).

The present data establish stenobiontic character of some species as well. The abundant development of these species is possible only in narrow limits of the enviromental conditions. Out of this limits they can not be found or they are quantitatively scanty.

The table shows that the following species have the highest ferquency of occurrence: *Dorylaimus stagnalis* – 92.59 % and *Monhystera filiformis* – 88.27%. (Total 5 species). Most of the species have a low value of the *pF*% and do not dominate the zoobenthos of the glacial mountain lake ecosystems. In a qualitative aspect the lakes are poor in benthic species. According to us, a possible reason could be the isolation of the lakes, their relatively small size and the interspecial struggle within the short vegetatative period. These factors are an obstacle to the colonization of the lakes and lead to an intensification of intraspecial struggle, which is probably the reason for the relatively small number of species. In these cases a particularly important role is attached to the first colonizer of the water body. The difference in the coasts and the bottoms of the lakes, as well as the relative lack of sediments and of higher aquatic vegetational are also factors contributing to the poor species composition.

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CONTRIBUTION TO THE STUDY OF FREE-LIVING FRESHWATER NEMATODE FAUNA (NEMATODA: NEMATHELMINTHES) FROM SINAPOVSKA RIVER, SAKAR MOUNTAIN, SOUTHEAST BULGARIA

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Abstract. The present research gives the first information about free-living freshwater nematode fauna from Sinapovska River, Sakar Mountain, Southeast Bulgaria. A total of 25 species were found, belonging to 11 genera, 8 families, and 5 orders. Two of the species and 2 of the genera are new for the Bulgarian hydrofauna.

Key words: Nematoda, Sinapovska river, Bulgaria, faunistics, hydrobiology, Southeast Bulgaria

First information about the free-living Nematode fauna in Bulgaria was given by Valkanov (1934, 1935) who reported 13 species of 6 genera. Russev (1979) added 2 species from the Bulgarian stretch of the Danube River. Recent more detailed information on the free-living freshwater nematodes in Bulgaria was given by Stoichev (1996a, 1996b), Kovachev and Stoichev (1996), and Stoichev (1998, 1999, 2007a, 2007b) and Stoichev and Chernev (2007). They reported 86 species of 43 genera, 22 families, and 6 orders. The present study gives information, for the first time, about the free-living nematode fauna from Sinapovska River.

INVESTIGATION AREA AND SAMPLE SITES

Sinapovska River springs from the Northern slopes of Sakar Mountain, Southeast Bulgaria. It comes from a number of flows and small rivers and flows in a narrow and shallow valley with gentle slopes (8° to 10°). The area around the springs is afforested with broad-leaved forests, but as a whole the afforestation is a small percentage of the catchment area. The valley slopes ae covered with bush, arable land, meadows and vineyards. The river flows to the North until the village Dobroselets, and then eastwards.

Above the village Dobroselets the river valley broadens significantly and separate small hills replace the slopes. The river meanders, more frequently downstream, and the turns become more curved. Near the village Sinapovo the river valley becomes very wide. The river flows near the foot of Sakar Mountain and, after receiving its biggest inflow, Azmak river, it flows into the Tundzha River.

MATERIAL AND METHODS

The material was collected in February, July, September and November 2005–2007. The following sites of Sinapovska river were sampled:

1. Upstream the village Hlyabovo;
2. Downstream the village Hlyabovo;
3. Upstream the village Dobroselets;
4. Downstream the village Dobroselets;
5. Upstream the village Chukurovo;
6. Downstream the village Chukurovo;
7. Upstream the village Sinapovo;
8. Downstream the village Sinapovo;
9. Upstream the estuary of Kalnitsa river;
10. Downstream the estuary of Kalnitsa river;
11. Before flowing into Tundzha river.

A total of 132 samples were collected. The nematode samples were rinsed with two screens with mesh diameter sizes of 500 μm and 150 μm respectively. An important preliminary procedure is that, before processing and fixing, the nematodes should be carefully heated up to 60 °C in a water bath. As a result they become slacken and erected, and more convenient for measuring. The freshwater nematodes are extremely sensitive and their proper conservation for a long period is only possible in 4% formaline solution (alcohol and other fixatives dehydrate their bodies). The formula of de Man (1886) was used to determine the species qualitative composition.

Table 1. Species composition, distribution and dominant analysis of the nematodes found in Sinapovska River.

Taxa	Stations											Dominance analysis		
	1	2	3	4	5	6	7	8	9	10	11	*pF*%	*DF*%	*DT*%
Dorylaimida Pearse, 1942														
Mononchidae Filipjev, 1934														
Mononchus aquaticus Goetzee, 1968				X	X	X						3.78	0.75	19.84
Mononchus truncatus Bastian, 1865			X	X	X	X	X	X	X	X		29.54	6.06	20.5
Mononchus sp.			X									0.75	-	-
Clarkus papillatus (Bastian, 1865) *o			X									1.51	-	-
Clarkus sp.				X								0.75	-	-
Miconchus stideri Steiner, 1914 *o		X										1.51	-	-
Miconchus sp.		X										0.75	-	-
Dorylaimidae de Man, 1876														
Dorylaimus stagnalis Dujardin, 1848	X	X	X	X	X	X	X	X	X	X	X	58.93	37.87	54.93
Dorylaimus sp.		X										0.75	-	-
Monhysterida de Coninck et Sch. Stekhoven, 1933														
Monhysteridae de Man, 1876														
Monhystera stagnalis Bastian, 1865	X	X	X		X	X	X	X	X	X	X	52.27	29.54	56.51
Monhystera paludicola de Man, 1880				X		X						6.06	-	-
Monhystera filiformis Bastian, 1865		X	X	X	X	X	X	X		X	X	53.78	23.48	43.65
Monhystera sp.					X							0.75	-	-
Chromadorida Chitwood, 1933														
Microlaimidae Micoletzky, 1913														
Prodesmodora circulata (Micoletzky, 1913)						X		X	X			2.27	0.75	33.03
Prodesmodora sp.						X						0.75	-	-
Araeolaimida de Coninck et Sch. Stekhoven, 1933														

Table 1. (cont.)

Halaphanolaimidae de Coninck et Sch. Stekhoven, 1933														
Paraphanolaimus behningi Micoletzky, 1923							X		X	X		1.51	-	-
Aphanolaimus aquaticus Dadax, 1897							X	X		X	X	3.78	-	-
Aphanolaimus sp.								X				0.75	-	-
Cylindrolaimidae Micoletzky, 1922														
Cylindrolaimus communis de Man, 1880	X	X		X	X	X						3.03	-	-
Cylindrolaimus melancholicus de Man, 1880			X									0.75	-	-
Cylindrolaimus sp.		X										0.75	-	-
Plectidae Oerley, 1880														
Plectus cirratus Bastian, 1865	X	X	X	X		X						14.39	0.75	5.21
Plectus inquirendus Andrassy, 1958		X	X									6.81	-	-
Rhabditida Chitwood, 1933														
Rhabditidae Oerley, 1880														
Protorhabditis *filiformis* Sudhaus,1976	X	X	X	X		X	X					11.36	1.51	13.29
Rhabditis sp.		X										0.75	-	-

The analysis of dominant quantitative presence (frequency of occurrence, *pF*; frequency of dominance, *DF*; range of dominance *DT*, in %) has been made according to the method of de Vries (1937) by Kozhova, 1970. The determination and the presentation of the species was made according to Gagarin (1981).

RESULTS AND DISCUSSION

A total of 25 species, belonging to 11 genera, 8 families, and 5 orders have been found. Two of the species (marked by*) and 2 of the genera (marked by o), are new for the Bulgarian nematods hydrofauna.

The results of the dominance analysis of the species are shown in Table 1. As shown in the table, Dorylaimus stagnalis has been found at all 11 sites. As a result of the obtained data on species' frequency of presence, the following clasification, proposed for the first time in Stoichev (1996a), can be applied:

1. Very frequently found (*pF*%>50%): *Dorylaimus stagnalis* Dujardin, 1848, Monhystera stagnalis Bastian, 1865, *Monhystera filiformis* Bastian, 1865 (a total of 3 species).

2. Frequently found (*pF*% 10–50%): e.g. *Mononchus truncates* Bastian, 1865, *Plectus cirratus* Bastian, 1865 (total 3 species).

3. Rarely found (*pF*% 1–10%): *Mononchus aquaticus* Goetzee, 1968 , Clarkus *papillatus* (Bastian, 1865), *Miconchus stideri* Steiner, 1914, etc. (total 9 species).

4. Very rarely found (*pF*% < 1%): *Mononchus* sp., *Cylindrolaimus melancholicus* de Man, 1876, etc. (total 10 species).

A comparison of the index *pF* and the range of dominance *DT* shows that the very frequent species dominate also qualitatively in the zobenthic complex.

Together with species having high *pF* and *DT* values (*Dorylaimus stagnalis*, Monhystera *stagnalis*, *Monhystera filiformis*), also species with high *DT* but low *pF* have been found (*Mononchus aquaticus*, *Prodesmodora circulate* (Micoletzky, 1913).

The present data establish the stenobiontic character of some species whose abundant development is only possible in narrow limits of the environmental conditions. Beyond these limits they can not be found, or they are quantitatively scanty.

At the places with great self-purificational capacity a well composed and usually constant qualitative composition can be found. The distribution of the species by habitats (Table 2) shows that the slime contains the greatest number of species (17), followed by slime and sand (14), gravel and sand (11), sand (5), gravel (4), and clay and sand (3). No species were found in clay. Table 2 shows also that many species are able to inhabit various habitats which could probably be explained by the tendency of nematode species to broaden their range, and by their eurybiontic character, as an expression of the biological progress of the group.

Table 2. Distribution of the nematodes found in the Sinapovska River by bottom substrata

Taxa	Gravel	Gravel & sand	Slime	Slime & sand	Sand	Clay & sand	Clay
Mononchus aquaticus Goetzee, 1968		X	X				
Mononchus truncatus Bastian, 1865			X	X			
Mononchus sp.		X					
Clarkus papillatus Bastian, 1865 *o			X				
Clarkus sp.			X	X			
Miconchus stideri Steiner, 1914 *o				X			
Miconchus sp.			X				
Dorylaimus stagnalis Dujardin, 1848	X	X	X	X	X	X	
Dorylaimus sp.			X				
Monhystera stagnalis Bastian, 1865	X	X	X	X	X	X	
Monhystera paludicola de Man, 1880			X				
Monhystera filiformis Bastian, 1865	X	X	X	X	X	X	
Monhystera sp.		X		X			
Prodesmodora circulata (Micoletzky, 1913)			X				
Prodesmodora sp.				X			
Paraphanolaimus behningi Micoletzky, 1923			X				
Aphanolaimus aquaticus Dadax, 1897			X	X			
Aphanolaimus sp.		X		X			
Cylindrolaimus communis de Man, 1880	X		X	X			
Cylindrolaimus melancholicus de Man, 1880			X	X	X		
Cylindrolaimus sp.		X					
Plectus cirratus Bastian, 1865		X	X	X			
Plectus inquirendus Andrassy, 1958		X					
Protorhabditis filiformis Sudhaus, 1976		X	X	X			
Rhabditis sp.					X		
Total	4	11	17	14	5	3	-

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ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“
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HYDROBIOLOGICAL AND FAUNISTIC INVESTIGATION OF THE NEMATODA FAUNA (NEMATODA, NEMATHELMINTHES) FROM BULGARIAN STRETCH OF THE VELEKA RIVER (SOUTHEAST BULGARIA)

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Abstract. The present study gives for the first time detailed information about the free-living Nematode fauna of the Veleka River. A total of 56 species were found, belonging to 25 genera, 16 families, and 6 orders. Tree of the species, 1 genera and 1 families are new for the Bulgarian hydrofauna.

Key words: Nematoda, Veleka River, Bulgaria, faunistics, hydrobiology

At the present moment the representatives of the nematode fauna have taken possession of the whole biosphere and inhabitt all biotops know to science.The enormous quantitly, of the nematods, which, according to some authors(Rees, 1940), amounts to severall millions of specimens per metre squared, determines their significance for the balance of the organic substances in the water basins. Their biomass is offen greater than that of the other multicellular organism.Ther nematods, along with the copepods appear to be a basic component of the meiobenthos of the continental water basins.

First information about the free-living Nematode fauna in Bulgaria was given by Valkanov (1934, 1935) who reported 13 species belonging to 6 genera. Information about fresh water nematodes was given by Russev (1979) who added 2 species from the Bulgarian-Romanian stretch of the Danube River. Recent more detailed information on the free-living fresh-water nematodes in Bulgaria was given by Stoichev (1996a, 1996b), Kovachev and Stoichev (1996), and Stoichev (1998) who reported 61 species of 35 genera, 22 families, and 6 orders.

The marine free-living nematods have been investigated more in detail (Stoykov, 1980).

The present study gives for the first time detailed information about the free-living Nematode fauna from the Bulgarian stretch of the Veleka River. A dominant analysis has been made too. (Fig. 1)

Fig. 1 Veleka River, Southeast Bulgaria.

The Veleka River flows from many karst spring in Turkey, near the village Kovchaz. The flow on Turkish territory is through a highly afforested area Fig. 1. The river crosses the Turkish-Bulgarian border at about 500 m near the protected area Moryane. In its upper reaches near the village Zvezdec the river flows in a deep narrow valley with a slope reaching $33^{o}/_{oo}$. At the village, the slope decreases to 4 $^{o}/_{oo}$. Below the village the river meanders. The river bed is 20 m wide,with a depth of 0.80–1.00 m. The river bottom is covered with sand,with isolated larger stones. Near the estuary of the river Karumluka the hills become lower and the slope decreasses to 25–30^{o}. The low-stemmed afforestation decreases to 50%. (Ivanov et al., 1964). The remaining part consist of cultivated lands. The longitudinal slope of the river becomes 2 $^o/_{oo}$. The river is 80 m wide. The bottom is covered with large gravel and sand. Until the inflow of the Broyanovski Dol river the valley is bed-like, and at the village Kosti it becomes trapezoid-like. The river bed is 10–12 m wide,with an average depth of 2 m. The river bottom is covered with sand. Niar Brodilovo village the valley widens to 1200 m. (Ivanov et al., 1964). The slopes are low and covered with low-stemmed vegetation. The depth reaches 2.5 m. The influence of the pressure coming from the sea can be observed. The river flows very slowly. The stretches of the river with switt flow disappear. The longitudinal slope of the river is 1.5–1.8 $^o/_{oo}$. After the Selmata tributary the heigth and the inclination of the valley slopes decrease. Its width reaches 1000 m the river, s with is almost constantly at 8–10 m, and its depth 2–4 m. The bottom is covered with clay and sand. At the estuary the rivers, s width becomes 50 m, and the depth 7–8 m. Below the bridge of the highway connecting Sinemorets and Ahtopol the river over flows its banks, makes a big elbow and flows into the sea.

MATERIAL AND METHODS

The material was collected by the author during the 4 seasons, for 8 consecutive years (2001–2008). A total of 288 samples were investigated. An "Eckmann-Birge" grab was used for the collection of the material from Veleka River.

The samples were collected from 9 sites in the river, as follows:

1. Upstream the village Zvezdets;
2. Downstream the village Zvezdets;
3. Upstream the village Gramatikovo;
4. Downstream the village Gramatikovo;
5. Upstream the village Kosti;
6. Downstream the village Kosti;
7. Upstream the village Brodilovo;
8. Downstream the village Brodilovo;
9. At the estuary.

An important preliminary procedure is that, before processing and fixing, the nematodes should be carefully heated up to 60 °C in a water bath. As a result they become slacken and erected. Being thus heated, they are more convenient for measuring. The fresh water nematodes are extremely sensitive and their proper conservation is only possible in 4% formaline solution (alcohol and other fixatives dehidratate their bodies). Thus fixed, they are preserved for a long period.

The preparation and determination are according to Gagarin (1981), on the basis of the formula of de Man (1886) determining the species qualitative composition. The analysis of dominant quantitative presence (frequency of occurrence, *pF*%; frequency of dominance, *DF*%; range of dominance *DT* %) was made according to the method of de Vries (1937).

RESULTS AND DISCUSSION

A total of 56 species were found, belonging to 25 genera, 16 families, and 6 orders. Three of the species (marked by +) and 1 of the genera (marked by o), and 1 of families (marked by !) are new for the Bulgarian hydrofauna. (Table 1) show that Dorylaimus stagnalis can be found in all Veleka river sites. Monhystera stagnalis, Monhystera filiformis and Rhabditis filiformis can be found nearly everywhere.

The results of the species' dominance analysis are expressed in Table 1.

A comparison of the frequency of occurrence, *pF*% and range of dominance *DT*% shows that the very frequent species dominate also qualitatively in the zoobenthic complex.

According to the classification of frequency of occurrence, the free-living fresh-water nematodes from the Bulgarian strech of the Veleka River can be grouped into four groups, as follows:

1. Very frequently found (*pF*% > 50%): *Dorylaimus stagnalis* Dujardin, 1868, *Tobrilus gracilis* (Bastian, 1865) Andrassy, 1959 etc. (total 4 species).
2. Frequently found (*pF*% 10–50%): *Monhystera dispar* Bastian, 1865, *Enoploides fluviatilis* Micoletzky, 1922 etc. (total 2 species).
3. Rarely found (*pF*% 1–10%): *Monhystera paludicola* de Man, 1880, *Penrancia agilis* (de Man, 1880) Filipjev, 1918, *Cylindrolaimus communis* de Man 1880, etc. (total 16 species).
4. Very rarely found (*pF*% < 1%): *Monhystera macraphis* Filipjev, 1930, *Mesodorylaimus potus* Heynis, 1963 etc. (total 34 species).

Besides the species with high *pF*% and *DT*% values (*Dorylaimus stagnalis, Monhystera stagnalis* Bastian, 1865), also species with high *DT*% and low *pF*% *(Enoploides fluviatilis* Micoletzky, 1923, Monhystera dispar, can be found.

The present data establish the stenohalinic character of some species as well. The abundant development of these species is only possible within narrow limits of the environmental conditions. Outside of these limits they cannot be found or they are quantitatively scanty.

The nematode complex of the species found in the Bulgarian Stretch of the Veleka River is represented by both eurihalinic and stenohalinic species. In the upper and the middle streams only fresh water nematode species were found. Downstream the village Brodilovo there is a mixture of fresh water and sea free living nematodes. In the estuary zone the eurihalinic free- living nematode species dominate, and also typical sea species (*Enoplus littoralis* Filipjev, 1918, *Enoplus moeoticus* Filipjev, 1916, *Enoploides amphiohi*, Filipjev, 1918 and *Viscosia glabra* Bastian, 1865).

Table 1. Species composition, distribution and dominant analysis of the nematodes found in Veleka River.

Taxa	Dominant analysis											
	1	2	3	4	5	6	7	8	9	*pF*%	*DF*%	*DT* %
MONHYSTERIDA de CONINCH et SCH. STEKHOVEN, 1933												
Monchisteridae de Man, 1876												
Monhystera stagnalis Bastian, 1865		x	x	x		x	x	x		67.70	41.66	61.53
Monhystera dispar Bastian, 1865				x	x		x			10.41	6.25	60.03
Monhystera paludicola de Man, 1880	x	x		x						8.68		
Monhystera filiformis Bastian, 1865	x		x	x	x	x	x	x		76.38	62.50	81.82
Monhystera macraphis Filipjev, 1930		x								0.34		
Monhystera sp.		x								0.34		
Penrancia agilis (de Man 1880), Filipjev,1918			x	x						1.04		
Penrancia sp.		x								0.34		
ARAEOLAIMIDA de CONINCH et SCH. STEKHOVEN, 1933												
Cylindrolaimidae Micoletzky, 1922												
Cylindrolaimus communis de Man, 1880		x		x		x				2.43		
Cylindrolaimus melancholicus de Man, 1880			x		x					1.73		
Cylindrolaimus sp.				x						0.34		
Chronogasteridae, Gagarin, 1975												
Chronogaster boettgeri Kischke, 1959					x					0.34		
Chronogaster sp.				x						0.34		
Plectidae Oerley, 1880												
Plectus cirratus Bastian, 1865	x		x			x	x			5.20		
Plectus sp.				x						0.34		
ENOPLIDA CHITWOOD, 1933												
Enoplidae Dujardin, 1845												
Enoploides fluviatilis Micoletzky, 1923	x	x		x	x		x			13.19	0.69	5.23

Table 1. (cont.) *Enoploides amphioxi* Filipjev, 1918 *									x	0.34		
Enoploides sp.									x	0.34		
Enoplus maeoticus Filipjev, 1916 *									x	1.38		
Enoplus littoralis Filipjev, 1918 *									x	1.04		
Enoplus sp.*									x	0.34		
Oncholaimidae												
Viscosia glabra Bastian, 1865 *									x	0.34		
Tripylidae de Man, 1876												
Tobrilus gracilis (Bastian, 1865), Andrassy 1959	x	x	x		x	x	x	x		67.70	52.08	76.92
Tripiloididae												
Bathilaimus cobbi Filipjev, 1922 *									x	0.34		
Bathilaimus sp. *									x	0.34		
RABDITIDA CHITWOOD, 1933												
Rhabditidae Oerley, 1880												
Rhabditis filiformis Buetschli, 1873			x		x	x				5.20		
Rhabditis sp.			x							0.34		
Aporcelaimidae Heyns, 1965												
Aporcelaimellus obtusicaudatus (Bastian 1865) Alther, 1968			x		x		x			3.12	1.04	33.33
Aporcelaimellus sp.			x							0.34		
Mononchidae Pearse, 1942												
Mononchus truncatus Bastian, 1865	x		x	x	x	x	x	x		9.72	5.20	53.49
Mononchus sp.	x									0.34		
Clarkus papillatus (Bastian, 1865) Jairajpuri, 1970			x							0.34		
Clarcus sp.		x								0.34		
Miconchus studeri (Steiner, 1914) Andrassy, 1958			x							0.34		
Miconchus sp.			x							0.34		
Mylonchulidae Jairajpuri, 1969												

Table 1. (cont.)

Mylonchulus brachyurus (Buetschli, 1873) Alther, 1953		x	x							0,34		
Mylonchulus sp.		x								0,34		
Dorylaimidae de Man, 1876												
Dorylaimus stagnalis Dujardin, 1848	x	x	x	x	x	x	x	x	x	88.54	71.87	81.17
Dorylaimus paradoxus Eliava, 1967		x		x						2.08		
Dorylaimus sp.		x								0.34		
Eudorylaimus carteri (Bastian, 1865) Andrassy, 1959		x	x	x	x	x				4.16		
Eudorylaimus sp.			x							0.34		
Laimidorus pseudostagnalis (Micoletzky, 1927) Siddiqi, 1969		x		x						1.73		
Laimidorus agilis (de Man, 1880) Siddiqi, 1969			x							0.69		
Laimidorus sp.			x							0.34		
Mesodorylaimus potus Heyns, 1963 +		x								0.69		
Mesodorylaimus meyli (Andrassy,1958) +	x									1.38		
Paradorylaimus filiformis (Bastian, 1865) Andrassy, 1969		x	x							0.69		
Paradorylaimus sp.	x									0.34		
CHROMADORIDA CHITWOOD, 1933												
Microlaimidae Micoletzky, 1922												
Prodesmodora circulata (Micoletzky, 1913) Micoletzky, 1925		x				x	x			4,86		
Prodesmodora sp.				x						0,34		
Microlaimus globiceps de Man, 1880			x							0,34		
Ethmolaimidae Filipjev et Sch. Stekhoven, 1941 !												
Ethmolaimus pratensis de Man, 1880 + o			x							0,34		
Ethmolaimus sp.		x								0,34		
Chromadoridae Filipjev, 1917												
Chromadorina viridis (Linstow, 1876) Wieser, 1954	x		x		x	x	x			6,59		
Chromadorina sp.		x								0,34		

The fact of finding typical sea fresh water nematode species downstream the village Brodilovo could be probably explained with an influx of sea water into the river, caused by heavy sea, and also with the constant circulation of fisherman boats and tourist yachts in the estuary zone of the river. Last but not least this can be caused by water birds which contribute to the dissemination of the sea species upstream the river. The low depth also favours the complete mixing of the water in the estuary zone, which also contributes to the dissemination and the complete mixing of eurihalinic fresh water nematodes with free- living sea nematodes.

The distribution of species is show in Table 2.It illustrates that the slime of species – 24, followed by sand (19), slime-sand (17), gravel-sand (16), gravel (14), fine sand – (9), clay and sand (2), and clay (0). The distribution of the nematodes bybottom substrata is inequal and mosaic. Thable 2, shows that the nematodes found in the Veleka river prefer soft substrata. A large part of the nemathodes inhabit both soft and solid substrata, which suggest that many of them have an euritopic nature. One of nemathodes (*Enoploides fluviatilis*) can be, probably because the stenotopic species.

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Table 2. Distribution of the nematodes found in the Veleka River by bottom substrata.

Taxa	Gravel	Grave -sand	Slime	Slime -sand	Sand	Fine sand	Clay-sand	Clay
Monhystera stagnalis Bastian, 1865	x	x	x	x	x	x	x	
Monhystera dispar Bastian, 1865	x		x	x	x			
Monhystera paludicola de Man, 1880	x		x					
Monhystera filiformis Bastian, 1865	x		x	x	x	x		
Monhystera macraphis Filipjev, 1930		x						
Monhystera sp.	x							
Penrancia agilis(de Man 1880), Filipjev,1918		x						
Penrancia sp.	x							
Cylindrolaimus communis de Man, 1880			x	x	x			
Cylindrolaimus melancholicus de Man, 1880		x						
Cylindrolaimus sp.			x					
Chronogaster boettgeri Kischke, 1959				x				
Chronogaster sp.			x					
Plectus cirratus Bastian, 1865	x	x	x	x	x	x		
Plectus sp.			x					
Enoploides fluviatilis Micoletzky, 1923	x		x	x	x	x		
Enoploides amphioxi Filipjev, 1918 *					x			
Enoploides sp.						x		
Enoplus maeoticus Filipjev, 1916 *					x	x		
Enoplus littoralis Filipjev, 1918 *						x		
Enoplus sp.*					x			

Table 2. (cont.) *Viscosia glabra* Bastian, 1865 *					x			
Tobrilus gracilis (Bastian, 1865) Andrassy, 1959	x	x	x	x	x	x		
Bathilaimus cobbi Filipjev, 1922 *					x			
Bathilaimus sp. *					x			
Rabditis filiformis Buetschli, 1873	x	x						
Rabditis sp.		x						
Aporcelaimellus obtusicaudatus (Bastian 1865) Alther, 1968			x	x				
Aporcelaimellus sp.			x					
Mononchus truncatus Bastian, 1865	x	x	x	x	x			
Mononchus sp.		x						
Clarkus papillatus (Bastian, 1865) Jairajpuri, 1970	x							
Clarcus sp.		x						
Miconchus studeri (Steiner, 1914) Andrassy, 1958			x					
Miconchus sp.		x						
Mylonchulus brachyurus (Buetschli, 1873) Alther, 1953			x					
Mylonchulus sp.			x					
Dorylaimus stagnalis Dujardin, 1848	x	x	x	x	x	x	x	
Dorylaimus paradoxus Eliava, 1967			x					
Dorylaimus sp.				x				
Eudorylaimus carteri (Bastian, 1865) Andrassy, 1959	x		x	x				
Eudorylaimus sp.		x						
Laimidorus pseudostagnalis (Micoletzky, 1927) Siddiqi, 1969			x					
Laimidorus agilis (de Man, 1880) Siddiqi, 1969		x						

Table 2. (cont.)

Laimidorus sp.		x						
Mesodorylaimus potus Heyns, 1963 +			x					
Mesodorylaimus meyli (Andrassy,1958) +				x				
Paradorylaimus filiformis (Bastian, 1865) Andrassy, 1969			x	x				
Paradorylaimus sp.			x					
Prodesmodora circulata (Micoletzky, 1913) Micoletzky, 1925				x				
Prodesmodora sp.					x			
Microlaimus globiceps de Man, 1880				x				
Ethmolaimus pratensis de Man, 1880 + o					x			
Ethmolaimus sp.			x					
Chromadorina viridis (Linstow, 1876) Wieser, 1954					x			
Chromadorina sp.					x			

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LONG-TERM CHANGES OF THE BOTTOM INVERTEBRATE FAUNA OF THE MESTA RIVER IN SOUTHWESTERN BULGARIA

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Abstract. A full list of the invertebrate species found in the macrozoobentos of the Mesta River contained in total 439 taxa as recorded since 1978. The best represented were the groups of Diptera (totally 153 taxa recorded, of them 79 taxa of Chironomidae), Oligochaeta (67), Ephemeroptera (65) Trichoptera (60), etc. The species content changed significantly after year 1990 when severe organic pollution was eliminated. The faunistic similarity was estimated on 56% for the periods before and after 1990. Some 164 taxa were still common for both periods of comparison. In total 123 taxa were not more regis-

tered, but another 153 new taxa appeared in the bottom invertebrate fauna after elimination of the pollution.
Key words: Invertebrates, Macrozoobenthos, Long-term Changes, Water Quality Improvement, Mesta River, Bulgaria

In the Bulgarian territory, the Mesta River (named Nestos in Greece) is a mountain stream with 1318 m average altitude, 126 km length and annual water discharge of 39 m^3/s at the state border, having 2770 km^2 of catchment area. The main river has 25 tributaries, the largest of them – Dospat, joins Mesta in the territory of Greece.

Since 1963, the organic pollution of the Mesta has increased drastically. Because of operation of two large plants in the town of Razlog (yeast, cellulose and cardboard manufactures), the industrial wastewater exceeded 90 000 m^3 per day. Besides of local wastewater treatment, the average daily levels of BOD_5 varied between 300–450 mg O_2/dm^3 as estimated for the accepting tributary Iztok. Such an unfavorable ecological situation remained until 1990, when the respective authorities have banned the operation of the main sources of pollution. Immediately, some improvements of the water quality within the river system of Razlozhka-Iztok-Mesta have been registered in 1990–1991 (Kovachev, 1992). Next studies showed relatively stable β-mesosaprobity at the bordering point of Hadzhidimovo in the period of 1992–1994 (Mihaylov et al., 1994).

By this way, the developments of the ecological state of the Mesta River could be defined in two large periods: until 1990, the period of heavy loading with organic matter of industrial origin, and after 1990, the period of recovery and stabilization of the ecological situation. The last estimation of the saprobic conditions showed remarkable improvement, especially for the sites having been impacted by severe organic pollution (Varadinova & Uzunov, 2002).

The bottom invertebrate communities have been never subject of special faunistic investigations. Most of the known studies concerned the macrozoobenthos mainly as a source of information about the water quality assessment by means both of saprobic and cenotic indices. Firstly, Kovachev (1977) mentioned 75 taxa for the course of the Mesta, but he did not provide any inventory of the established species. Later Kovachev & Uzunov (1986) assumed a list of 143 taxa with detailed information about locations and frequencies of occurrence and dominance of the species listed. Prof. Dr. B. Russev and Dr. I. Yaneva studied 3 selected locations along the Mesta River and recorded totally 147 invertebrate taxa established (Mihaylov et al., 1994). Several other investigations on the macrozoobenthos undertaken by Dr. S. Kovachev (1990–1991) and Dr. Y. Uzunov (1986, 1987, 1997) remained still not officially published. Uzunov & Kapustina (1993) published part of the available information concerning especially Oligochaeta. No data published yet about bottom macroinvertebrates of the Mesta River as obtained during the last study in 1999–2000.

The aims of this study were to represent recent data on bottom macroinvertebrates as obtained in 1999–2000 and to trace some long-term changes of the zoobenthos species diversity for the last 25 years.

MATERIAL AND METHODS

Recent studies on the macrozoobenthos of the Mesta River were carried out in summer months of years 1999 (August, September, October) and 2000 (June, July, August, September). The data obtained (49 collections from 8 permanent sites) were associated with the available information about invertebrate species of the Mesta River. Kovachev & Uzunov (1986) published aggregated data of their samplings in March, May and September 1978, June, September 1979, May, July 1980 and June, August, November 1981. Later on Uzunov & Molle (1992) published a part of the data on macroinvertebrates collected monthly from March until November 1986, April, June, July, September and October 1987. Kovachev (1992) undertook next studies on the macrozoobenthos in March, November 1990 and March, June, August, November 1991. The materials published by Michailov (1994) contained data on zoobenthos found during the studies of the Mesta River in October, November, December 1992 and March, April, June, September 1993. Some unpublished yet data of Dr. Uzunov (collected in August 1997) were associated also to the all those above.

The information system BIOMONITOR (developed and maintained by the Group on Bio-Indication & Environmental Assessment at the (Central Laboratory of General Ecology, at present IBER – Institute of Biodiversity and Ecosystem Research) contained more than 380 records about samplings within the basin of the Mesta River. A review of the data available showed that the bottom invertebrate fauna have been studied at more than 42 different locations, including some tributaries and glacial lakes. Along the Mesta River course, only 8 sites were recognized as common for all periods of study. The respective data (256 samplings) for these representative sites were further used in the present paper (Fig. 1).

Most of the materials have been gathered by a quantitative net (Surber's type) covering bottom surface of 0.1 m^2. Materials have been collected qualitatively in the studies before 1980 and in 1992–1994.

Taxonomic identification of some of the materials from 1999–2000 has been done by Assoc. Prof. Dr. I. Yaneva (Hirudinea, Ephemeroptera: Baetidae), Assoc. Prof. Dr. K. Kumanski (Trichoptera), Sen. Res. Dr. S. Stoichev (Diptera: Chironomidae), Assoc. Prof. Dr. Y. Uzunov and Dr E. Varadinova (Oligochaeta), MSc Y. Vidinova (Ephemeroptera). Dr. I. Dedov (CLGE) confirmed some determinations of Gastropoda and Bivalvia, and MSc. V. Tyufekchieva (Institute of Zoology) checked some of the Plecoptera.

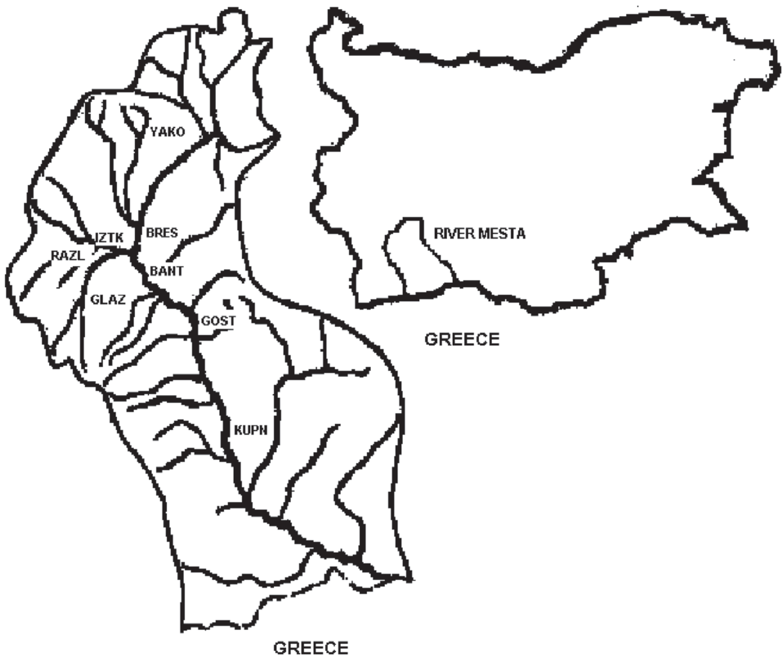

Fig. 1. Layout of the basin of Mesta River with locations of the sampling points.

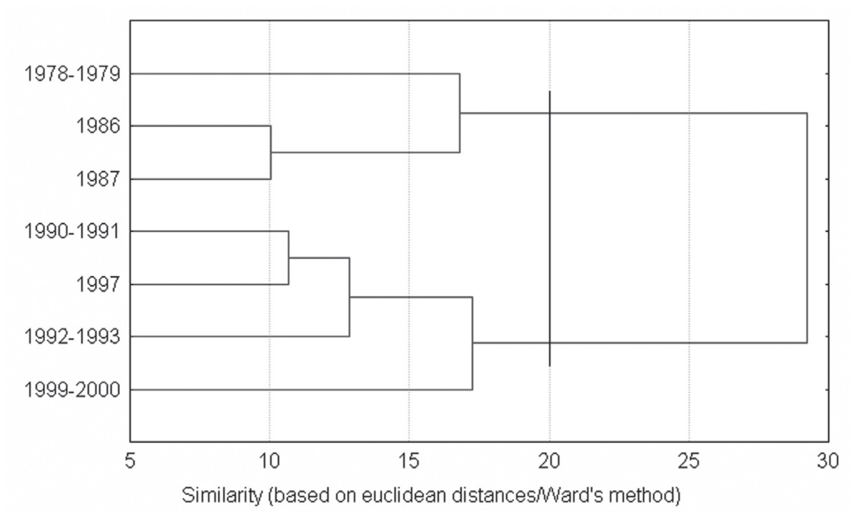

Fig. 2. Cluster analysis (Ward’s method) of taxonomic data for different periods of investigation.

Besides some above mentioned, the following colleagues have taken part in species determination in the past (1978–1997): Prof. Dr. B. Russev (Ephemeroptera, Plecoptera), Prof. Dr. P. Mihailova (Diptera: Chironomidae), Prof. Dr. V. Beshovsky (Odonata), Prof. Dr. M. Yosifov (Heteroptera), Assoc. Prof. Dr. S. Kovachev (Diptera: Simuliidae), Assoc. Prof. Dr. V. Gueorguiev (Coleoptera), Sen. Res. S. Andreev (Isopoda, Amphipoda), most of them from the Institute of Zoology and National Natural History Museum in Sofia.

Some of the older determinations were revised in order to meet current species checklists and catalogues on Bulgarian and European fauna (Kummanski, 1985, 1988 and later contributions; Nartshuk et al., 1990, Uzunov & Kapustina, 1993; Angelov, 2002; Vidinova, 2003, etc.). Where necessary, synonyms were replaced by their currently valid names, but in some cases, some doubtful identification (species *nomen dubia* or species *nomen nudum*) had to be kept due to the further statistical data processing.

The similarity index of Sörensen was used when comparing the inventory lists for different periods or sites. STATISTICA for Windows 5.5 was used for cluster analyses. Ward's method was run on the taxonomic data in order to find periods with similar community composition for different periods of study (Fig. 2).

RESULTS AND DISCUSSION

The list of all species recorded in macrozoobenthos communities along the Mesta River system (including tributary Iztok) is presented on Table 1. The order of genera and species follows the alphabet for reader's convenience.

Table 1. Bottom invertebrate fauna of the Mesta River System as recorded for different periods of study within years 1978–2000

Taxon	Periods of study						
	1978–1981	1986	1987	1991	1992–1994	1997	1999–2000
1	2	3	4	5	6	7	8
HYDROZOA							
Hydra sp.					*		*
TURBELLARIA							
Crenobia alpina Dana		*	*			*	*
**Dendrocoelum lacteum* (O. F. Müller)							*
Dugesia gonocephala (Duges)		*	*		*	*	*
Dugesia lugubris (Schmidt)					*		*
**Polycelis nigra* (O. F. Müller)							*
NEMATODA g.sp.		*	*			*	*
OLIGOCHAETA g. sp.			*				

Table 1. (cont.)

1	2	3	4	5	6	7	8
Aelosoma hemprichi Ehnrenberg	*						
Aulophorus furcatus O. F. Müller	*	*	*				*
Bothrioneurum vejdovskyanum Stolc				*	*		*
**Branchiura sowerbyi* Beddard							*
Chaetogaster crystallinus Vejdovsky	*						
Chaetogaster diaphanus (Gruithuisen)	*	*	*		*		*
Chaetogaster diastrophus (Gruithuisen)	*		*				
Chaetogaster sp.					*		
**Dendrobaena octaedra* Savigny							*
Dero digitata O. F. Müller	*					*	*
Dero obtusa D' Udekem	*	*	*				*
Dero nivea Aiyer			*				
Dero sp.						*	
Eiseniella tetraedra (Savigny)	*	*	*	*	*	*	*
Enchytraeus albidus Henle	*	*	*	*		*	*
Enchytraeidae g. sp.							*
Fridericia sp.		*	*	*		*	*
Haplotaxis gordioides Hartmann	*	*	*			*	
*Henlea sp.							*
Homochaeta naidina Bretscher	*						
Limnodrilus claparedeanus Ratzel	*	*	*				*
Limnodrilus hoffmeisteri Claparede	*	*	*	*	*	*	*
Limnodrilus profundicola (Verrill)		*	*	*	*		*
Limnodrilus udekemianus Claparede	*	*	*	*		*	*
Limnodrilus sp.				*	*		
Lumbriculus variegatus Grube	*	*	*	*		*	*
Lumbricidae g. sp. juv.							*
**Marionina* sp.							*
Mesenchytraeus armatus Levinson		*	*	*			*
Nais barbata O. F. Müller	*	*	*		*	*	*
Nais bretscheri Michaelsen	*	*	*	*	*		*
Nais communis Piguet	*	*	*				*
Nais elinguis O. F. Müller	*	*	*		*	*	*
Nais pardalis Piguet	*	*	*				*
Nais pseudobtusa Piguet	*	*	*	*			*
Nais simplex Piguet	*		*				
Nais variabilis Piguet	*	*	*				*
Nais sp.					*	*	

Table 1. (cont.) 1	2	3	4	5	6	7	8
Ophidonais serpentina O. F. Müller	*	*	*	*	*	*	*
**Paranais frici* Hrabe							*
Pristina aequiseta Bourne	*	*	*				*
**Pristina amphibiotica* Lastockin							*
Pristina bilobata Bretscher	*		*				
Pristina longiseta Ehrenberg	*		*				*
Pristina menoni Aiyer	*		*				*
Pristina rosea Piguet	*	*	*				*
Psammoryctides albicola (Michaelsen)	*	*	*	*	*	*	*
Psammoryctides barbatus (Grube)	*	*	*	*	*	*	*
Psammoryctides sp.					*		
Rhyacodrilus coccineus (Viejdovsky)	*	*	*	*	*		*
Rhyacodrilus sp.				*	*		
Rhynchelmis tetratheca Mich.	*		*				*
**Rhynchelmis vagensis* Hrabe							*
Rhynchelmis sp.		*	*	*	*	*	*
Slavina appendiculata D' Udekem	*					*	*
**Specaria josinae* Vejdovsky							*
Spirosperma ferox Eisen		*	*				
Stylaria lacustris L.	*	*	*	*	*		*
Stylodrilus heringianus Claparede	*	*	*				*
Stylodrilus parvus Hrabe & Cernosvitov		*	*				
Stylodrilus sp.				*	*	*	*
Tubifex tubifex O. F. Müller	*	*	*	*	*	*	*
Tubifex sp.					*		*
**Trichodrilus moravicus* Hrabe							*
Uncinais uncinata Oerst.	*						
Vejdovskyella comata (Vejd.)	*						
HIRUDINEA							
Dina lineata O. F. Müller			*	*	*		*
Erpobdella cf. monostriata Dagr.	*						
Erpobdella octoculata (L.)	*	*	*	*	*	*	*
Erpobdella sp.				*			*
**Glossiphonia complanata* L.							*
**Glossiphonia concolor* (Apathy)							*
Glossiphonia sp.				*			*

Table 1. (cont.) 1	2	3	4	5	6	7	8
Haemopis sanguisuga L.	*					*	*
Helobdella stagnalis (L.)	*	*			*	*	*
**Hemiclepsis marginata* O. F. Müller							*
Hirudo medicinalis L.	*	*				*	
GASTROPODA							
Ancylus fluviatilis O. F. Müller	*	*	*	*	*	*	*
**Anisus contortus* (L.)							*
Aplexa hypnorum (L.)			*				
**Bythinella* sp.							*
Limnaea (Galba) sp.		*	*				
**Limnaea (Radix) auricularia* (L.)							*
Limnaea (Radix) peregra O. F. Müller.				*	*		
Physa acuta Drap.	*		*				*
Planorbis planorbis L.	*	*	*	*			*
Planorbis sp.					*		
BIVALVIA							
Pisidium sp.				*	*	*	*
Sphaerium sp.					*		
MALACOSTRACA							
Asellus aquaticus L.	*	*	*	*	*	*	*
Astacus astacus L.	*						*
Gammarus balcanicus Schaeff.	*						
Gammarus sp.						*	*
Potamon potamius Chiav.			*				
HYDRACARINA g. sp.		*			*		*
EPHEMEROPTERA							
Baetis alpinus (Pictet)	*	*	*		*	*	*
Baetis buceratus Eaton	*	*	*		*		*
Baetis fuscatus(Linnaeus).	*	*	*	*	*		*
Baetis lutheri Müller-Liebenau	*		*		*		*
Baetis melanonyx (Pictet)	*	*	*				*
Baetis muticus (Linnaeus.)	*	*	*	*	*		*
Baetis pavidus Grandi	*						*
Baetis rhodani (Pictet)	*	*	*	*	*	*	*
Baetis scambus Eaton	*	*	*				*
Baetis vernus Curtis	*	*	*		*	*	*
Baetis sp. (*cf. lapponicus* Bengt.)	*		*				

Table 1. (cont.) 1	2	3	4	5	6	7	8
Baetis sp. *juv.*		*	*	*			*
Caenis horaria Limnecus.	*		*				
Caenis gr. macrura Stephens	*	*	*	*			*
Caenis luctuosa (Burmeister)	*		*			*	
Caenis macrura Stephens					*		*
Caenis pseudorivulorum Keffermüller							
Caenis robusta Eaton	*		*				
Caenis sp. *juv.*							*
*Centroptilum luteolum (*Müller)	*	*					*
*Cloeon dipterum (*Linnaeus.)	*				*		
Cloeon simile Eaton	*		*				
Ecdyonurus aurantiacus (Burmeister)					*		
**Ecdyonurus s vitoshensis* Jacob& Braasch							*
Ecdyonurus dispar (Curtis).	*	*	*				
**Ecdyonurus epeorides* Demoulin							*
**Ecdyonurus* sp. gr. *helveticus*							*
Ecdyonurus insignis (Eaton)	*		*		*		*
Ecdyonurus torrentis Kimmins	*		*				
Ecdyonurus venosus (Fabricius)	*	*	*	*	*		*
Ecdyonurus sp. gr. *venosus*			*			*	*
Electrogena macedonica Ikonomov		*					
**Electrogena quardilineata* (Landa)							*
**Epeorus alpicola* (Eaton)							*
Epeorus sylvicola (Pictet)	*	*	*	*		*	*
Epeorus jougoslavicus Samal	*	*	*	*	*		*
Ephemera danica O. F. Müller	*	*			*		*
Ephemera vulgata Linnaeus.			*				
**Ephemerella maculocaudata* (Ikonomov)							*
Ephemerella mesoleuca (Brauer)	*	*	*	*		*	*
Ephemerella mucronata (Bengtsson)	*	*	*	*	*	*	
Ephemerella notata Eaton	*		*				
Ephemerella sp. juv.							*
Habroleptoides contusa Saztorig Jacob	*	*	*		*		*
Habrophlebia lauta Eaton	*	*			*		*
Heptagenia coerulans Rostock	*		*				
Heptagenia flava Rostock	*						
**Heptagenia longicauda* (Stephens)							*
Heptagenia sulphurea (Müller)	*		*				

Table 1. (cont.)

1	2	3	4	5	6	7	8
*Kageronia fuscogrisea (*Retzius)	*						
*Oligoneuriella rhenana (*Imhoff)	*						
Paraleptophlebia cincta (Retzius)					*		
Paraleptophlebia submarginata (Stephens)	*	*	*		*		*
**Potamanthus luteus* (Limnaeus)							*
*Procloeon pennulatum (*Eaton)					*		
**Rhithrogena braaschi* Jacob							*
**Rhithrogena bulgarica* Braasch, Soldan & Sowa							*
Rhithrogena semicolorata (Curtis)					*		*
Rhithrogena sp. *gr. semicolorata*	*	*	*				
**Rhithrogena podhalensis Sowa & Soldan*							*
**Rhithrogena* sp. *gr. hybrida*							*
Rhithrogena loyolaea Navas	*						
Rhithrogena sp. juv.				*		*	*
Serratella ignita (Poda)	*	*	*	*	*	*	*
Siphlonurus aestivalis (Eaton)	*						
Siphlonurus lacustris (Eaton)					*		
Torleya major(Klapalek)	*			*			
PLECOPTERA							
Amphinemoura sp.	*		*				
Amphinemoura triangularis Ris.		*					
Brachyptera seticornis (Klapalek)		*	*		*		
**Brachyptera* sp. juv.							*
Bulgaroperla mirabilis Rauscher			*				
Capnia bifrons Newman			*				
**Capnia* sp.							*
**Chloroperla* sp. juv.							*
Dinocras megacephala Klapalek					*		
Dinocras sp.					*		
Perla sp. *(*gr. *burmeisteriana*)				*			
Perla marginata (Rauser)	*			*	*		*
Perla sp. *(*gr. *marginata)*		*	*			*	*
Perla sp.					*		*
Perlodes dispar Rambur			*				
Perlodes intricata (Pictet)					*		*
Perlodes microcephala Pictet		*	*				

Table 1. (cont.)

1	2	3	4	5	6	7	8
Perlodes sp.						*	*
Protonemoura sp.	*	*	*	*		*	*
**Taeniopterix* sp.							*
ODONATA							
Calopteryx splendens (Harris)	*		*		*		*
Calopteryx virgo L.	*		*				*
Gomphus vulgatissimus (L.)	*		*		*		
Onychogomphus forcipatus L.			*				*
Ophiogomphus cecilia (Fourcroix)		*	*		*	*	
Platicnemis pennipes (Pallas)					*		
TRICHOPTERA							
Agapetus sp.		*	*		*		
Allogamus uncatus Brauer					*		
Brachycentrus montanus Klap.		*	*				*
**Ceraclea* sp.							*
Chaetopteryx sp.		*			*		
Cheumatopsyche lepida (Pictet)	*		*				*
**Drusus discolor* (Rambur)							*
Drusus sp.	*	*	*	*	*	*	*
**Ecclisopteryx dalecarlica* Kolenati							*
Goera pillosa (Fabricius)		*					
**Glossosoma discophorum* Klap.							*
Glossosoma sp.					*		
Halesus cf. *digitatus* Schrk.		*			*		*
Hydropsyche angustipennis Curtis		*	*		*		
Hydropsyche bulbifera McL.		*	*		*		*
**Hydropsyche contubernalis* McL.							*
**Hydropsyche emarginata* Navas							*
Hydropsyche cf. *fulvipes* (Curtis)					*		*
Hydropsyche cf. *instabilis* Curtis	*	*	*		*		*
Hydropsyche modesta Navas					*		
Hydropsyche incognita Pitsch (syn. *pellucidula* Curtis)	*	*	*		*	*	*
Hydropsyche cf. *saxonica*McL					*		
Hydropsyche tabacarnui Botss.		*	*		*		*
Hydropsyche sp. gr. *gutata*	*						*
**Hydropsyche valkanovi* Kum.							*
Hydropsyche sp.		*	*	*	*	*	*

Table 1. (cont.) 1	2	3	4	5	6	7	8
**Hydroptila kalonichtis* Malicky							*
**Hydroptila* sp.							*
Lype reducta Hagen					*		
**Limnephilus sparsus* Curtis							*
Limnephilus sp.	*	*	*				*
Limnephilidae g.sp.					*		*
Micrasema minimum McL.	*	*	*				
Micropterna sp.		*					
Oecismus monedula Hagen	*	*	*	*	*	*	*
Oecismus sp.					*		
Odontocercum hellenicum Mal.					*		*
Oligoplectrum maculatum (Fourcroy)		*	*				
Philopotamus montanus Don.		*	*		*		*
**Philopotamus variegatus* (Scopoli)							*
**Plectrocnemia brevis* McL.							*
Plectrocnemia conspersa(Curtis)			*				*
Polycentropus flavomaculatus Pictet			*				
Polycentropus sp.		*				*	*
Potamophylax cingulatus Steph.		*			*		
Potamophylax sp.	*				*	*	*
Psychomyia pusilla Fabr.			*				*
**Rhyacophila armeniaca* Guer.							*
Rhyacophila loxias Schm.		*	*				*
**Rhyacophila moscaryi* Klapalek							*
Rhyacophila nubila Zett.	*	*	*		*		*
Rhyacophila obliterata McL		*					*
Rhyacophila gr. *vulgaris*	*	*	*		*		*
Rhyacophila tristis Pictet		*	*				*
Rhyacophila sp.			*	*		*	*
**Silo* sp.							*
Sericostoma flavicorne Schneider			*		*		*
Sericostoma sp	*		*		*		
Sericostomatidae g. sp.					*		
Stenophylax sp.	*	*	*		*	*	*
HETEROPTERA g. sp. juv.A237					*		
Gerris sp.			*				
Nepa rubra L.	*	*	*				*
Notonecta glauca L.			*				

Table 1. (cont.)

1	2	3	4	5	6	7	8
Sigara falleni (Fieb.)	*						
Ranatra linearis (L.)	*						
COLEOPTERA g.sp.							
Agabus sp.			*				
Donacia sp.				*		*	
Dryopsidae g. sp.					*		
Elmis aenea Mull.						*	
Elmis sp.		*	*		*		*
**Graphoderus bilineatus* (Deg.)							*
**Gyrinus distinctus* Aube							*
Gyrinus sp.	*	*	*			*	
Haliplus ruficolis Deg.					*		
**Helodes* sp.							*
Helophorus sp.			*				
Hydraena sp.					*		
Hydroporus marginatus (Duft.)					*		
Hydrophorus sp.			*				
Laccobius sp.					*		
Laccophylus hyalinus Degr.			*				
Riolus sp.					*		
Stenelmis sp.	*	*	*				*
DIPTERA: ATHERICIDAE g. sp.						*	*
Atherix marginata Fabricius		*	*			*	*
**Atherix ibis* E.							*
Atherix sp.	*		*		*		*
DIPTERA: BLEPHARICERIDAE g. sp.						*	*
Blepharicera fasciata West.	*		*				
**Blepharicera* sp.							*
Liponeura brevirostris Loew.	*			*			
Liponeura cinerascens Loew.	*						
Liponeura sp.					*		*
DIPTERA: CERATOPOGONIDAE g. sp.							*
Bezzia sp.	*	*	*	*	*		*
Culicoides sp.						*	*
DIPTERA: CHIRONOMIDAE g. sp.				*		*	*
Larsia curticalcar (Kieffer)					*		
Brillia longifurca Kieffer	*	*	*			*	

Table 1. (cont.) 1	2	3	4	5	6	7	8
Brillia modesta (Meigen)	*						
Brillia sp. juv.						*	
**Chironomus gr. plumosus* Kieffer							*
Chironomus riparius Meigen	*	*	*	*	*	*	*
Chironomus sp.				*			
Conchapelopia pallidula (Meigen)	*						
Cladotanytarsus mancus (Walker)					*		
**Cricotopus (C.) algarum* Kieffer							*
Cricotopus (C.) bicinctus Meigen	*	*	*				
Cricotopus (C.) annulator Goetghebuer	*						
Cricotopus (C.) curtus Hirvenoja	*						
**Cricotopus (C.) fuscus* (Kieffer)							*
Cricotopus (C.) tremulus (Linnaeus)	*						
Cricotopus (C.) trifascia Edwards	*						
Cricotopus (C.) tristis Hirv.	*						
Cricotopus (C.) vierriensis Goetghebuer	*	*	*				
Cricotopus (Cricotopus) sp.			*			*	
Cricotopus (I.) intersectus (Staeger)	*						
Cricotopus (I.) tricinctus (Meigen)	*						
Cricotopus (I.) sylvestris (Fabricius)	*				*		*
Cricotopus (Isocladius) sp.			*			*	
Cryptochironomus gr. *defectus* (Kieffer)	*				*		*
Cryptochironomus supplicans (Meigen)	*						
**Cryptochironomus* sp.							*
Demicriptochironomus vulneratus (Zetterstedt)	*						
Diamesa insignipes Kieffer	*						
**Diamesa* sp.							*
**Endochironomus* sp.							*
Eukiefferiella clypeata (Kieffer)	*						
Eukiefferiella claripennis (Lundberk)	*						
Eukiefferiella lobifera Goetghebuer	*						
Eukiefferiella gracei (Edwards)		*	*				
**Eukiefferiella similis* Goetghebuer							*
Eukiefferiella sp.		*	*			*	*
Glyptotendipes gripenkoveni (Kieffer)							*
Larsia atrocincta (Goetghebuer)	*						
**Dicrotendipes nervosus* (Staeger)							*

Table 1. (cont.)

1	2	3	4	5	6	7	8
**Dicrotendipes* sp.							*
Paralimnophyes hydrophilus (Goetghebuer)	*						
Limnophyes minimus (Meigen)	*						
**Limnophyes prolongatus* (Kieffer)							*
Macropelopia sp.		*	*			*	
Micropsetra viridiscutellata Goetghebuer (n. d.)	*						
Micropsectra sp.		*	*				
**Microtendipes chloris* (Meigen)							*
Odontomesa fulva (Kieffer)	*						
Orthocladius saxicola Kieffer	*				*		
Orthocladius sp.			*			*	
Orthocladius (Euorthocladius) rivulorum Kieffer	*						
Orthocladius (Euorthocladius) thienemanni Kieffer	*						
Synorthocladius semivirens (Kieffer)					*		
**Parakiefferiella bathophila* (Kieffer)							*
Parametriocnemus stylatus (Kieffer)	*						
Paratanytarsus confusus Palmen	*						
**Paratendipes nudisquam* (Edwards)							*
**Paratendipes* sp.							*
Polypedilum gr. *convictum* (Walker)	*	*	*				
Polypedilum pedestre Meigen		*	*		*		*
Polypedilum (Pentapedilum) exsectum (Kieffer)	*		*				
Polypedilum (Tripodura) scalaenum (Schrank)	*						
Polypedilum sp.			*		*		
Potthastia longimana (Kieffer) (Edwards)	*						
Procladius (Holotanypus) ferrugineus (Kieffer)	*						
Procladius sp.					*		*
Prodiamesa olivacea Meigen (syn. *dilatatus* (Van der Wulp))	*	*	*			*	*
Psectrocladius (Allopsectrocladius) obvius (Walker)	*						

Table 1. (cont.) 1	2	3	4	5	6	7	8
Psectrocladius sp.			*				
Pseudosmittia gracilis (Geotghebuer)	*						
Rheocricotopus effusus (Walker)	*		*				
Stictochironomus sticticus (Fabricius) (syn. _histrio_ (Fabr.)	*						
Synorthocladius semivirens (Kieffer)	*						*
Tanytarsus mendax Kieffer	*						
Tanytarsus gregarius Kieffer					*		*
Tanyrtarsus verralli (Goetghebuer)	*						
Tanytarsus sp.			*				
Telmatopelopia nemorum (Goetghebuer)	*						
Tvetenia calvascens (Edwards)	*				*		
DIPTERA: CULICIDAE g.sp.					*		*
Aedes sp.		*					
*Culex sp.							*
DIPTERA: CYLINDROTOMIDAE							
Phalacrocera sp.		*					
*DIPTERA: DIXIDAE g. sp.							*
Dixa sp.	*						*
DIPTERA: EMPIDIDAE g. sp.					*		
Wiedemannia sp.		*	*	*			*
DIPTERA: LIMONIIDAE g. sp.		*	*	*			*
Antocha sp.			*				*
*Dactylolabis sp.							*
Dicranota sp.	*	*	*		*	*	*
Hexatoma sp.					*	*	*
Ormosia sp.		*			*		
*DIPTERA: MUSCIDAE g. sp.							*
Limnophora sp.					*	*	
DIPTERA: PSYCHODIDAE g. sp.	*				*		*
Psychoda alternata Say	*	*	*	*		*	*
*Psychoda sp.							*
Tonnoiriella sp.		*	*				*
DIPTERA: SIMULIIDAE g. sp.						*	*
Cnetha brevidens Rubzov		*					
Cnetha codreanui Serban		*	*				
Cnetha cryophila Rubzov		*	*				

Table 1. (cont.) 1	2	3	4	5	6	7	8
Cnetha verna (Mucq.)			*				
Eusimulium serbicum (Baranov)	*	*	*		*		
Gnus ibariense Zivk. & Gren.	*		*				
Odagmia ornata (Meigen)	*	*	*	*	*		
Odagmia spinosa Doby et Debl.	*	*	*				
Odagmia sp.						*	
Prosimulium hirtipes (Fries)					*		
Prosimulium latimucro Enderlein			*		*		
Prosimulium rufipes Meigen	*	*					
Prosimulium tomosvaryi Enderlein		*	*				
Prosimulium sp.						*	*
Simulium argyreatum Meigen			*				
Simulium galeratum Edwards	*	*	*		*		
Simulium noeleri Enderlein			*				
Simulium monticola Friederichs		*	*				
Simulium morsitans Edwards	*	*	*				
Simulium rheophilum Knoz	*	*					
Simulium reptans (L.)	*	*	*		*		
Simulium variegatum (Meigen)	*	*	*	*	*		
Tetisimulium condici Baranov	*	*	*				
Wilhelmia balcanica (Enderlein)	*				*		
Wilhelmia pseudequina (Puri)	*	*	*	*	*		
Wilhelmia sp.						*	
DIPTERA: STRATIOMYIDAE g. sp.			*				*
Berris sp.			*				
Oxycera sp.		*					
*DIPTERA: SYRPHIDAE g. sp.							*
Erystalomyia tenax (L.)	*	*					
DIPTERA: TABANIDAE g. sp.	*						*
Tabanus sp.		*	*		*		*
DIPTERA: THAUMALEIDAE							
**Thaumalea* sp.							*
DIPTERA: TIPULIDAE g. sp.	*						*
Tipula sp.		*	*	*	*		*
LEPIDOPTERA							
Acentropus niveus Oliv.		*					

Total number of the taxa established for the period 1978–2000 was found on 439. The groups of Chironomidae (Diptera) with 79 taxa recorded, Ephemeroptera (65 taxa), Oligochaeta (67 taxa), Trichoptera (60 taxa), etc. were the best represented in the macrozoobenthos. Such species content characterized the Mesta River as a mountain stream with a dominating lithorheophilic community in the macrozoobenthos.

In general, 239 taxa were established for the same period – the highest figure ever recorded before for this river. Perhaps, the number of species could be much more if the sampling periods included all biological seasons. Totally, 67 taxa were firstly recorded in the bottom fauna of the Mesta River (noted on the table by asterisk*) during the last investigation (years 1999–2000). It should be noted here the findings of *T. moravicus, C. pseudorivulorum, E. epeoroides, I. alpicola, P. luteus, G. discophorum, H. contubernalis, H. emarginata, E. dalecarlica*, etc. in the alpine part, as well as another group of species like *B. sowerbyi, S. josinae, H. longicauda, P. brevis*, etc. in lower parts of the river course.

However, the similarity of the species content was estimated on 56% while comparing number of species established in two periods – before and after 1990. This was because only 164 taxa were still common for the two compared periods. The total number of taxa established before 1990 was 288 against 320 ones found after 1990. The figures above showed that some 124 taxa have not been registered anymore after elimination of the organic pollution in 1990. On contrary, another 155 taxa have appeared in the bottom invertebrate fauna of the Mesta River after 1990.

Some of the "lost" species like *P. friči, H. naidina, S. ferox, O. rhenana, E. torrentis, E. macedonica, R. loyolaea, C. robusta, E. dispar, N. picteti, G. pillosa, O. maculatum, B. modesta,* several species of *Cricotopus, Eukiefferiella, Orthocladius*, etc. were initially classified as "rare" by Kovachev & Uzunov (1986) due to their low frequency of occurrence in the samples studied (less than 5%). "Disappearance" of some more frequent species like polysaprobic *E. tenax*, larvae of Stratyomyidae, etc., was due to the radical improvements of the water quality in some sites after 1990.

The cluster analysis (Fig. 2) confirmed the splitting of studied period in two main sub-periods: before and after 1990, when the industrial organic impacts were eliminated. As it could be expected, the largest distance occurred between species content in the earliest (1978–1979) and latest period of studies (1999–2000). In practice, there were no significant differences of the invertebrate fauna established in 1986 and in 1987. The relatively close similarity of the benthos in 1990–1991 and 1997 was probably due to the single samplings in August 1997. However, it should be noted that invertebrates species content of for all 6 consequent samplings carried out within 1990–1991 (62 taxa registered) was almost the same for a single sampling in 1997 (78 taxa). Perhaps, some period of adaptation of the zoobenthos fauna took place just after the elimination of heavy organic pollution in 1990, and processes of reconstruction and enrichment of the species content followed in next years.

The trends towards an enrichment of the macrozoobenthos were evident when compare the mean number of taxa per sample for both of the period of comparison. Note, that sites were ordered following the saprobic gradient rather than real consequence along the river course (Varadinova & Uzunov, 2002). As seen on the Fig. 2, the mean species number at unaffected sites (JAKO, BRES) was found almost the same before and after 1990. Slight decrease of the mean species number for another clean site of GLAZ could be addressed possibly to the fast development of the winter tourism (ski-sport) in the upstream watershed of this site. The most evident changes were registered for the sites under organic pollution (RAZL, IZTK) and sites of active self-purification (BANT, GOST, KUPN).

These facts reflected the positive development trends of the invertebrate fauna of the Mesta River after elimination of the heavy organic pollution as predicted earlier (Kovachev & Uzunov, 1986). The data discussed above clearly show that the species diversity and richness of the invertebrate fauna is a quite dynamic characteristic. The species represented in the macrozoobenthos in different study periods reflected the dominant ecological situation, in our case as dependent on the saprobic situation and the rate of self-purification processes. Following the changes of dominant ecological situation, the zoobenthos species structure also gets changed. During this process, some of the species fall away, and new species appear in the species content of the benthic communities. In both cases, they were more sensitive, while a number of common species for the whole period of study were likely ecologically more tolerant.

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ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“
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THE MACROZOOBENTHOS OF THE RILSKA RIVER, SOUTHWEST BULGARIA

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Abstract: Rilska River is one of the left tributaries of the river Struma (Strimon) and a source of drinking water for the capital city of Sofia. In spite of that the hydrobiological data are quite poor especially these concerning bottom invertebrate fauna. This study represents the results from studies on the macrozoobenthos species composition carried out in 2007–2008 at 6 permanent sites along the river. In total 190 taxa from 192 found in the river macrozoobenthos during the studied period are reported here for the first time. The presence of sensitive to pollution taxons during the whole period of investigation, and at the all sites along the river was indicative for stable low-saprobic conditions in the river and its high self-purification capacity. Along the river continuum it was determined significant difference in the faunistic similarity between the upper and lower stretches of the river and a tendency of increasing species richness downstream the river was observed.

Key words: macroinvertebrate benthic fauna, species composition, dominant species, Rilska River.

Rilska River is one of the left tributaries of Struma River and it is a source of drinking water for the capital city of Sofia. The river springs from Ribni Ezera (Fish Lakes) at 2691 m a.s.l. and flows in west direction. The catchment area is 392 km^2 large and its natural annual outflow is 141.9 million km^3. The average slope of the river is quite high – 30 ‰. Its biggest tributary is Iliina River with total length of 16 км flowing out from the Sinyoto Ezero (Blue Lake) in Middle Rila Mts. In the upper course, the river forms deep and steep valley in the mountain, passing through Kocherinovo plain and mouthing the Struma River. The deep snow in winters, wet climate in summers and lots of circus lakes are the main water sources feeding the river. The main source of pollution of the Rilska River is the urban wastewater from few settlements along side the river course. The natural hydrological regime of the river is strongly influenced by several derivations, small hydropower plants and small accumulations for leveling. According to the West-Aegean Basin Directorate the rivers Rilska and Iliina are "probably at risk" not to achieve the environmental objectives of the Water Framework Directive 2000/60/EC.

This study presents the results of studies on the macrozoobenthos species composition as a basic biological quality element for ecological status classification of the riverine water bodies within the Rilska River watershed.

The former hydrobiological studies of Rilska River are quite poor. Kovachev & Uzunov (1977) investigated the macrozoobenthos at a site near Kocherinovo city studying the pollution of the Struma River. Later, in 1987, within the National Youth Action "Clean Rivers" financed by Ministry of Public Education (1989) two sites along the Rilska River (Pastra and Barakovo) have been studied too (not published results). During that study the benthic fauna was established rather poor and on the last site in the autumn benthos was missing at all. Known data contain some information about bottom invertebrates as for 11 simuliid species (Diptera: Simuliidae) reported by Kovachev (1976) and for 1 mayfly species (Ephemeroptera) as reported by Vidinova (2001). The only detailed information consist the study of Stoichev (2007) who reported totally 35 species free-living nematodes in Rilska River (4 of them new for the Bulgarian hydrofauna).

MATERIAL AND METHODS

The study of the macrozoobenthos in Rilska River was carried out monthly during the vegetation periods from June 2007 till November 2008 at six sites along the river (Table 1), selected as representative to reflect the ecological situations of the respective river stretch. Sixty eight quantitative hydrobiological (macrozoobenthic) samples have been gathered following the ISO 7828: 1985 (F) standard. After laboratory processing and species determination, dominant analysis was performed according to the method of De Vries (1937) (after Kovachev et al., 1979). The species similarity amongst the different sites along the river continuum was determined by Jaccard similarity index (1901) and the coefficient of Bray-Curtis (1957), presented by Cluster Agglomerative Analyse (PRIMER-E, Clarke & Warwick, 2001). The faunistic similarity with some Bulgarian mountain rivers was compared using the index of Sörensen (1948).

Table 1. Description of the studied sites along the Rilska River.

Name of the site	Altitude m a.s.l	Bottom substrate	Vegetation	Average t°	Average velocity, m/s
Manastirska River, upstream Kirirlova polyana site	1500	Stone, gravel	Coniferous vegetation	9.3	0,351
Iliina River, upstream Manastirska River	1450	Stone, gravel	Deciduous vegetation	10.75	0,485
Rislka R., downstream Iliina River	1395	Stone, gravel	Deciduous vegetation	11.9	0,443
Rilska River downstream the village of Pastra	706	Stone, gravel	Deciduous vegetation	14.2	0,38
Rilska River, at the village of Stob	465	Gravel, sand	Riparian woods and shrubs	15.27	0,503
Rilska River, at the mouth into the Struma River	370	Gravel, sand	Riparian woods and shrubs	17.3	0,627

RESULTS AND DISCUSSION

During the whole period of the study, totally 192 benthic taxa from 56 invertebrate families have been found (Table 2). From them 108 were determined to species level. For Iliina River 93 taxa from 42 families were found; of them *D. alpina* (Lumbricidae), *I. yougoslavicus* (Heptageniidae), dipterian larvae of *Liponeura* sp. (Blephariceridae) and *Hexatoma* sp. (Limoniidae) were not found in the Rilska River. The value of taxa richness was the highest at site 4 where in total 102 taxa have been registered for the studied period. Three groups were found as most rich of representatives: Oligochaeta (21 species), Ephemeroptera (20 species) and Diptera (20 species). Close to this was the species content of bottom invertebrates at site 6 with 93 registered taxa, where Oligochaeta and Ephemeroptera were presented with 23 taxa each. After that comes site 2 where representatives of Trichoptera (22 taxa) and dipterans larvae (21 taxa) prevail.

The macrozoobenthos of the other sites studied have had similar number of species established between 90 for site 3, 88 for the site 5, and 82 (with highest number of 20 dipteran species) for the site 1. These results respond to the principles of River Continuum Concept for increasing the number of species from head water to the middle waters.

Table 2. Species content, frequences and distribution of the macrozoobenthos along Rilska River.

Taxon	pF%	DF%	DT%	Site					
				1	2	3	4	5	6
1	2	3	4	5					
Dendrcoelidae									
Dendrocoelum lacteum (O. F. Müller, 1774)	5.88			*	*	*			
Dugesiidae									
Dugesia gonosephala (Duges, 1830)	13.23			*	*	*			*
Dugesia tigrina (Girard, 1850)	1.47								*
Dugesia sp.	2.94			*		*			
Planariidae									
Crenobia alpina (Dana, 1766)	19.11			*	*	*			
ANNELIDA									
APHANONEURA									
Aeolosomatidae									
Aeolosoma quaternarium Ehrenberg, 1831	1.47								*
OLIGOCHAETA									
Enchytraeidae g. sp.	50			*	*	*	*	*	*
Cognetia sp.	1.47						*		
Enchytraeus albidus Henle, 1937	4.41							*	*
Fridericia sp.	14.7			*	*		*	*	*
Henlea sp.	7.35			*	*	*			*
Lumbricillus lineatus (Műller, 1774)	2.94							*	
Mesenchytraeus armatus (Levinsen, 1884)	5.88			*		*		*	
Marionina cf riparia Bretscher, 1899	1.47							*	

Table 2. (cont.) 1	2	3	4	5					
Marionina sp.	8.82			*	*		*	*	*
Haplotaxidae									
Haplotaxis gordioides (Hartmann, 1821)	20.58			*	*	*		*	
Lumbricidae g. sp.	14.7			*		*	*	*	*
Dendrobaena alpina (Rosa, 1884)	1.47				*				
Dendrobaena octaedra (Savigny 1826)	1.47							*	
Eiseniela tetaedra (Savigny 1826)	19.11			*	*		*	*	*
Fitzingeria platyura (Fitzinger, 1883)	2.94							*	
Lumbricus rubellus Hoffmeister, 1843	1.47								*
Lumbriculidae g. sp.	1.47						*		
Lumbriculus variegatus (Műller, 1774)	13.23			*	*	*		*	*
Rhynchelmis sp. *cf. tetratheca* Michaelsen, 1920	2.94							*	*
Stylodrilus heringianus Claparede, 1862	41.17			*	*	*	*	*	*
Naididae									
Dero digitata (Müller, 1773)	1.47						*		
Nais alpina Sperber, 1948	4.41					*	*		
Nais barbata Műller, 1773	4.41							*	*
Nais bretscheri Michaelsen, 1899	10.29				*	*		*	*
Nais communis Piguet, 1906	11.76			*	*		*	*	
Nais elinguis Műller, 1773	14.7						*	*	*
Nais pardalis Piguet, 1906	16.17			*	*		*	*	*
Nais pseudoobtusa Piguet, 1906	35.29	1.47	4.17	*	*	*	*	*	*
Nais variabilis Piguet, 1906	8.82			*			*		*
Nais sp.	4.41					*	*		*
Pristina aequiseta Bourne, 1891	8.82						*	*	*
Pristina amphibiotica Lastockin, 1927	1.47						*		
Pristina (Pristinella) bilobata (Bretcher, 1903)	5.88						*		*
Pristina foreli Piguet, 1906	5.88							*	*

Table 2. (cont.)

1	2	3	4	5				
Pristina (Pristinella) rosea (Piguet, 1906)	8.82					*	*	*
Pristina sp.	1.47					*		
Tubificidae								
Limnodrilus hoffmeisteri Claparede, 1862	2.94						*	
Rhyacodrilus coccineus (Vejdovsky, 1875)	1.47							*
Tubifex tubifex (Műller, 1774)	1.47						*	
HIRUDINEA								
Erpobdellidae								
Dina lineata (O. F. Müller, 1774)	8.82			*			*	
Erpobdella monostriata (Lindenfeld & Pietruszynski, 1890)	1.47						*	
Erpobdella octoculata (Linnaeus, 1758)	4.41						*	*
Erpobdella sp.	1.47						*	
MOLLUSCA								
GASTROPODA								
Planorbidae								
Ancylus fluviatilis (O. F. Müller, 1774)	14.7				*	*	*	*
BIVALVIA								
Sphaeriidae								
Pisidium sp.	1.47							*
CRUSTACEA								
MALACOSTRACA								
AMPHIPODA								
Gammaridae								
Gammarus balcanicus Schäferna 1922	39.7	23.53	59.26	*		*	*	*
ARACHNIDA								
HYDRACARINA g.sp.	1.47				*			
INSECTA								
EPHEMEROPTERA								
Baetidae								

Table 2. (cont.) 1	2	3	4	5					
Baetis alpinus (Pictet, 1843)	75	11.76	15.68	*	*	*	*	*	*
Baetis fuscatus (Linnaeus, 1761)	30.88			*		*	*	*	*
Baetis lutheri Muller-Liebenau 1967	4.41								*
Baetis melanonyx (Pictet, 1843)	11.76						*	*	*
Baetis muticus (Linnaeus, 1758)	44.11			*	*	*	*	*	*
Baetis rhodani (Pictet, 1843)	73.53	8.82	12	*	*	*	*	*	*
Baetis scambus Eaton, 1870	30.88			*		*	*	*	*
Baetis vernus Curtis, 1834	4.41							*	
Baetis sp.	2.94				*				*
Pseudocentroptilum pennulatum Eaton, 1870	1.47						*		
Caenidae									
Caenis macrura Stephens, 1835	1.47								*
Caenis sp.	5.88						*		*
Ephemeridae									
Ephemera danica O. F. Müller, 1746	13.23						*	*	*
Ephemera sp.	1.47						*		
Ephemerellidae									
Ephemerella mucronata (Bengtsson, 1909)	2.94					*	*		
Ephemerella sp.	1.47								*
Serratella ignita (Poda, 1761)	22.05				*	*	*	*	*
Heptageniidae									
Ecdyonurus epeorides Demoulin, 1955	8.82			*		*			*
Ecdyonurus sp. gr. *helveticus*	41.17			*	*	*	*	*	*
Ecdyonurus sp. gr. *venosus*	20.58					*	*	*	*
Ecdyonurus sp.	58.82			*	*	*	*	*	*
Electrogena lateralis (Curtis, 1834)	1.47							*	
Epeorus sylvicola (Pictet, 1865)	38.23	1.47	3.84	*	*	*	*	*	*
Heptagenia sp.	4.41						*	*	*
Iron yougoslavicus (Samal, 1935)	1.47				*				
Rhithrogena braaschi Jacob, 1974	4.41				*	*		*	

Table 2. (cont.)

1	2	3	4	5					
Rhithrogena podhalensis Sova & Soldan, 1986	11.76				*	*			
Rhithrogena semicolorata (Curtis, 1834)	8.82	1.47	16.66	*				*	*
Rhithrogena sp. gr. *hybrida*	23.52			*	*	*	*	*	*
Rhithrogena sp. gr. *diaphana*	1.47								*
Rhitrogena sp.	47.05	7.35	15.62	*	*	*	*	*	*
Leptophlebiidae									
Habroleptoides confusa Sartori & Jacob 1986	30.88			*	*	*	*	*	*
PLECOPTERA									
Capniidae g. sp.	4.41			*					*
Capnia bifrons (Newman, 1839)	2.94					*			
Chloroperlidae									
Siphonoperla sp.	2.94				*				*
Leuctridae									
Leuctra fusca (Linnaeus, 1758)	11.76	1.47	12.5	*		*	*		*
Leuctra hippopus Kempny, 1899	10.29				*	*	*		*
Leuctra inermis Kempny, 1899	7.35				*	*	*		*
Leuctra nigra (Olivier, 1811)	5.88				*	*	*		
Leuctra pseudosignifera Aubert, 1954	30.88	1.47	4.76	*	*	*	*	*	*
Leuctra prima Kempny, 1899	1.47						*		
Leuctra sp.	17.64			*	*		*	*	*
Nemouridae									
Amphinemoura sp.	1.47							*	
Nemoura fulviceps Klapälek, 1902	2.94					*	*		
Nemoura sp.	8.82				*	*	*	*	
Protonemoura brevistyla (Ris, 1902)	1.47						*		
Protonemura montana Kimmins, 1941	17.64			*	*	*			*
Protonemoura sp.	4.41			*	*	*			
Perlidae									
Dinocras megacephala (Klapalek, 1907)	5.88					*	*		*

Table 2. (cont.) 1	2	3	4	5					
Dinocras sp.	1.47								*
Perla marginata (Panser, 1799)	39.7			*	*	*	*	*	
Perla sp.	27.94			*	*	*	*		*
Perlodidae									
Isoperla grammatica (Poda, 1761)	5.88					*	*		
Isoperla sp.	10.29			*	*		*	*	
Perlodes intricata (Pictet, 1841)	22.05			*	*	*	*		*
Perlodes microcephala (Pictet, 1833)	1.47					*			
Perlodes sp.	7.35			*	*	*			
Taenioptergidae									
Brachyptera seticornis (Klapalek, 1902)	1.47					*			
MEGALOPTERA									
Sialidae									
Sialis sp.	1.47			*					
TRICHOPTERA									
Brachycentridae									
Brachycentrus montanus Klapalek, 1892	2.94			*		*			
Micrasema minimum McLachlan, 1876	8.82			*	*	*		*	
Glossosomatidae									
Agapetus sp.	8.82			*	*		*		
Synagapetus sp.	4.41			*					
Goeridae g. sp.	2.94			*	*				
Goera cf. *pilosa* (Fabricius, 1775)	1.47			*					
Silo cf. *pallipes* (Fabricius, 1781)	4.41			*	*				
Helicopsychidae									
Helicopsyche sp.	1.47								*
Hydropsychidae									
Hydropsyche incognita Pitsch, 1993	14.7	4.41	30				*	*	*
Hydropsyche instabilis (Curtis, 1834)	16.17						*	*	
Hydropsyche tabacarui Botosaneanu, 1960	14.7				*	*	*		

Table 2. (cont.)

1	2	3	4	5					
Hydropsyche sp.	13.23			*	*	*	*	*	*
Limnephilidae									
Allogamus auricolliis (Pictet, 1834)	1.47			*					
Chaeteropterigini gen. sp.	19.11			*	*	*	*		
Consorophylax consors (McLachlan, 1880)	2.94								*
Drusus sp.	17.64			*	*	*	*	*	*
Halesus digitatus Schrank 1781	8.82				*	*	*	*	
Limnephilus vittatus (Fabricius, 1798)	5.88				*	*	*		
Potamophylax sp.	7.35				*		*		
Odontoceridae									
Odontocerum hellenicum Malicky, 1972	13.23	1.47	11.11	*	*	*	*	*	*
Philopotamidae									
Philopotamus cf. montanus (Donovan, 1813)	1.47							*	
Philopotamus sp.	5.88			*		*	*		*
Polycentropodidae									
Neureclipsis bimaculata (Linnaeus, 1758)	4.41			*	*		*		
Plectrocnemia sp.	1.47						*		
Polycentropus flavomaculatus (Pictet, 1834)	4.41				*		*		
Psychomiidae									
Psychomyia pusilla (Fabricius, 1781)	1.47								*
Sericostomatidae									
Notidobia ciliaris (Linnaeus, 1761)	11.76				*	*		*	
Oecismus monedula (Hagen, 1859)	14.7			*	*	*	*		*
Rhyacophilidae									
Rhyacophila cf. *armeniaca* Guerin-Meneville, 1843	7.35			*	*	*	*		
Rhyacophila nubila Zetterstedt, 1840	22.05			*	*		*		*
Rhyacophila cf. *Philopotamoides* McLachlan, 1879	1.47					*			
Rhyacophila torrentium Pictet 1834	13.23				*	*	*	*	

Table 2. (cont.) 1	2	3	4	5					
Rhyacophila tristis Pictet, 1834	13.23				*	*		*	
Rhyacophila gr. *vulgaris*	8.82				*		*		*
Uenoidae									
Thremma anomalum McLachlan, 1876	7.35				*	*	*		
COLEOPTERA									
Dytiscidae g. sp.	8.82			*				*	
Agabus sp.	1.47					*			
Hygrotus sp.	5.88				*	*			
Elmidae									
Elmis sp.	17.64			*	*	*	*		*
Stenelmis sp.	10.29				*		*		*
Helodidae									
Helodes sp.	2.94						*		*
DIPTERA									
Athericidae									
Atherix ibis (Fabricus 1798)	5.88			*	*		*		
Atherix sp.1	26.47			*	*	*	*		
Atherix sp. 2	1.47					*			
Blephariceridae g. sp.	7.35					*		*	*
Liponeura sp	1.47				*				
Ceratopogonidae g. sp.	35.29			*	*	*	*	*	*
Bezzia sp.	1.47						*		
Chironomidae									
Ablabesmyia gr. *curticalcar* (Kieffer, 1918)	4.41			*				*	
Chironomus gr. *riparius* Meigen, 1804	50	14.7	29.41	*	*	*	*	*	*
Chironomus sp.	5.88			*	*	*	*		
Cricotopus gr. *algarum* (Kieffer, 1911)	2.94					*		*	
Cricotopus gr. *silvestris* (Fabricius, 1794)	10.29			*	*	*		*	*
Cricotopus sp.	8.82				*	*		*	*
Cryptochironomus gr. *defectus* (Kieffer, 1913)	33.82	4.41	13.04	*	*	*	*	*	*

Table 2. (cont.) 1	2	3	4	5					
Cryptochironomus sp.	4.41					*	*		
Eukiefferiella gracei (Edwards, 1929)	5.88							*	*
Eukiefferiella cf. *similis* Goetghebuer, 1939	10.29							*	*
Eukiefferiella sp.	1.47						*		
Tanytarsus gr. *gregarius* Kieffer, 1909	32.35	11.76	36.36	*	*	*	*		*
Tanytarsus sp.	1.47				*				
Tvetenia calvescens (Edwards, 1929)	16.17			*			*	*	
Tvetenia sp.	1.47			*					
Dixidae									
Dixa sp.	2.94				*		*		
Limonidae									
Hexatoma sp.	1.47				*				
Dicranota sp.	30.88			*	*	*	*	*	*
Ormosia sp.	7.35				*	*	*		
Muscidae g. sp.	4.41				*	*	*		
Psychodidae g. sp.	1.47			*					
Pericoma sp.	1.47			*					
Tonnoiriella sp.	36.76	5.88	15.9	*	*	*	*	*	
Rhagionidae g. sp.	1.47			*					
Simuliidae g.sp.	26.47			*	*	*	*	*	
Eusimulium latipes (Meigen, 1818)	2.94			*					
Odagmia ornatum (Meigen, 1818)	1.47						*		
Prosimulium hertipes (Fries 1824)	1.47							*	
Simulium variegatum (Meigen, 1818)	1.47							*	
Wilhemia sp.	2.94				*				*
Tabanidae									
Tabanus sp.	10.29			*	*		*	*	*
Tipulidae									
Tipula sp.	10.29			*	*	*	*	*	*

The species content of the bottom invertebrates from Rilska River was compared with this of some Bulgarian mountain rivers. The faunistic similarity with another left tributary of the Struma – Blagoevgradska Bistrica River (after Kovatchev S., 1976, Nachev N., 1982, 1983a, Uzunov Y., N. Nachev, 1984, Sakelarieva et al., 2008, Stoichev, Chernev, 2001) was assessed as not too high – 40.92 %. The composition of the bottom invertebrates was also compared with some mountain streams – tributaries of the Yadenica River, studied in 1982–1983 (Uzunov et. al., 1986). In this case the species similarity was even lower – 25.965 %, probably because of the considerably lower species richness in these brooks (56 species), although 75 % of them were registered in Rilska River.

The similarity amongst the investigated sites was assessed by Jaccard similarity index (reporting only the presence of species) and Bray-Curtis coefficient (comparing species relative abundance between sites). According to the Jaccard similarity index sites 2–3 and 1–2 were with highest similarity of presented species – around 50 %. Expected, downstream along the river was observed decreasing of species similarity, compared with the upper sites, as around 30 % of the species were found common for the upper and lower river stretches (Table 3).

Table 3. A similarity index between the investigated river sites according to Jaccard (1908).

Sites	2	3	4	5	6
1	49,6	44,5	38,3	34,9	33,5
2		52,5	47,7	36,1	35,7
3			44,4	32,8	34,5
4				37	43,4
5					42,5

Cluster analysis and the coefficient of Bray-Curtis also distinguished the examined sites according to the similarity of their species composition and separated them into two groups (Fig. 1.)

One of the groups (further marked as foothill part of the river course, just before mouthing) includes sites 5 and 6, with high similarity among them – 60 %. The other group (further marked as mountain part of the river course) consists of sites 1–4 (headwater+gorge) with average degree of similarity 41.24%. Low faunistic similarity – 22.57 %, was established among the both groups, characterized with different complex of abiotic factors, correlating with the altitude, which influenced longitudinal changes in the macroivertebrate structure and content.

Fig. 1. Dendogram Classification of the examined river sites based on the macrozoobenthos species content (Site numbers as on Table 1).

Larvae of Diptera and Oligochaeta prevail in the riverine macrozoobenthos. The total number of established dipterian taxa was 39 which is 20.31 % of whole species content found during this study. Most of them belong to the family Chironomidae (15 species). The representatives of family Ceratopogonidae and the chironomid genus *Ch.* gr. *riparius, C.* gr. *defectus*, and other dipterians like *Dicranota* sp.*, Tipula sp*. were found in all six sites along the river course. Class Oligochaeta was also presented with 39 taxa, most of them (16 species) from Naididae. All over were found the species *St. heringianus, N. pseudobtusa* and representatives of Enchytraeidae g. sp. Another benthic group with high level of species richness was Trichoptera (Caddisflies) with 35 taxa (18.23%), three of them distributed all over the river – *Hydropsyche* sp., *Drusus* sp., *O. hellenicum.* Close to them were mayflies (Ephemeroptera) presented with 32 taxa (16.67 %), with predominant presence of species from Heptageniidae (14 species). Nine mayfly species were found in all studied sites – *B. alpinus, B. muticus*, *B. rhodani, E.* sp. gr. *helveticus, Ecdyonurus* sp., *E. sylvicola, Rh.* sp. gr. *hybrida, Rhitrogena* sp., *H. confusa*. Stoneflies (Plecoptera) were presented with 26 taxa (13.54 %), as only *L. pseudosignifera* was found at all sites along the river course. The other macroinvertebarate groups were presented with lower number of species.

Although the prevalence of dipterian and oligochaets species in the river, 3 taxa from Ephemeroptera order were defined as mass occurring in the river (*pF* > 50 %): *B. alpinus* (75 %), *B. rhodani* (74 %), *Ecdyonurus* sp. (59 %), according to the values of frequency of occurrence. The commonly occurring species (*pF* 20–50 %) were 27 and 70 species have had values of *pF* 5–20 %. Most of the species – 94, were defined as rarely occurring species with *pF* < 5% and 53 of them (27.6 % from the total number) were found once only during the whole studied period.

The number of species that dominated in the river macrozoobenthos was 15 (Table 2). The highest degree of dominance – 59.26 %, was determined for *G. balcanicus.* This species was found only in the foothill part of the river, where it dominated expressively and was highly abundant during the whole vegetation period. Two dipterian larvae – *T.* gr. *gregarius* (*DT* = 36.36 %) and *Ch.* gr. *riparius* (*DT* = 29.41 %), also had relatively high degree of dominance in the river. The first one dominated at the sites in the upper course of the river, while the second one prevailed in the middle part and once in the mouthing site (probably, due to the drift). With similar value of *DT* (30 %) was also the caddisfly *H. incognita* distributed only in the downstream part of the river, with not high frequency of occurrence.

The species *B. alpinus* and *Rhitrogena* sp., distributed all over the river have had values of *DT* = 15 %. The same degree of dominance have had also the dipterian *Tonnoiriella* sp. (Psychodidae) which was abundant only at the site of Iliina River. The other species have had low degree of dominance and 6 of them where registered as dominants only once.

It was observed variations in the bottom invertebrate fauna in dependence with seasonal temperatures, as the water temperature determines the basic processes in the river – physical/hydrological, chemical and biological. In this case, May was distinguished with relatively small number of species (Fig. 2) due to the still low water temperatures (in the upper course in the interval of biological winter) and also the high waters during this time of the year.

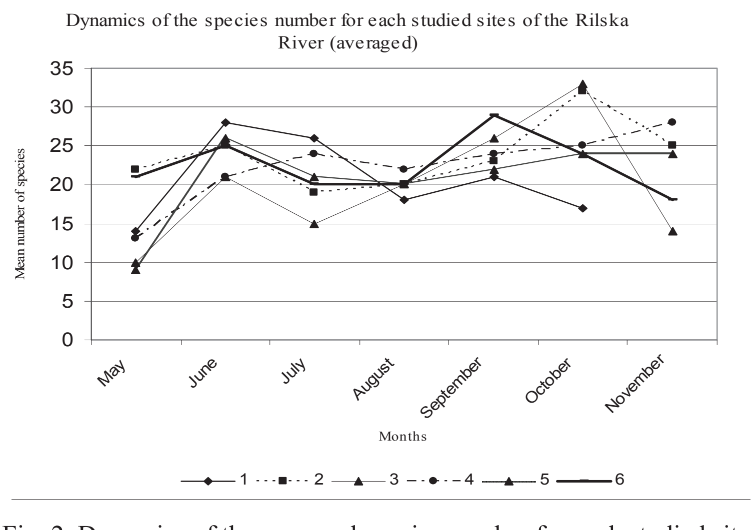

Fig. 2. Dynamics of the averaged species number for each studied sites of the Rilska River (Site numbers as on Table 1).

The seasonal fluctuations in number of bottom invertebrate species were characterized with two well expressed peaks in spring and autumnal period. The reduction in the number of species during the summer was due to the natural living cycle mostly of water insects (summer diapause).

During the year changes of the dominant species were observed due to the specific natural life cycles of the bottom invertebrates and the various conditions in the river through the seasons (Table 4).

Table 4. Distribution of the dominant bottom invertebrate species during the vegetation period (May-November).

Species	Spring			Summer			Autumn		
	pF %	*DF* %	*DT* %	*pF* %	*DF* %	*DT* %	*pF* %	*DF* %	*DT* %
1	2	3	4	5	6	7	8	9	10
Nais pseudoobtusa	27,8			50	4,17	8,33	30,8		
Gammarus balcanicus	33,3	16,7	50	33,3	25	75	50	30,77	61,54
Baetis alpinus	77,8	27,8	35,7	91,7	4,17	4,54	57,7	3,85	6,67
B. rhodani	77,8	11,1	14,3	79,2	8,33	10,5	80,8	7,7	9,53
E. sylvicola	11,1			16,7			84,6	3,85	4,55
Rhithrogena semicolorata	11,1			8,33	4,17	50	3,85		
Rhitrogena sp.	22,2			25	4,17	16,7	65,4	15,38	23,52
Leuctra fusca	5,55			25	4,17	16,7	3,85		
L. pseudosignifera	16,7			37,5			34,6	3,85	11,12
Hydropsyche incognita	5,55			20,8	8,33	40	11,5	3,85	33,33
H. tabacarui				12,5			23,1	7,7	33,33
Odontocerum hellenicum	27,8	5,55	20	12.5					
Chironomus gr. *riparius*	44,4	16,7	37,5	62,5	25	40	38,5	3,85	10,01
Cryptochironomus gr. *defectus*	38,9	11,1	28,6	37,5	4,17	11,1	34,6		
Tanytarsus gr. gregarius	22,2	11,1	50	41,7	20,83	50	34,6	3,85	11,12
Tonnoiriella sp.	22,2			25			61,5	15,38	25

During the spring, the species *B. alpinus, G. balcanicus* и *Ch.* gr. *riparius* were found with highest frequency of dominance (*DF*). In the same period highest degree of dominance (*DT*) has had again *G. balcanicus* and the chironomid *T.* gr. *gregarius* although it's low frequency of occurrence during the season.

During the summer the species with high frequency of occurrence as *B. alpinus, B. rhodani,* and *N. pseudoobtusa* have had relatively low degree of dominance. Dominant role in the community appeared the species *G. balcanicus* and *T.* gr. *gregarius* (although their local occurrence), and the mass occurring for the season species – *Ch.* gr. *riparius.*

During the autumn with highest value of *DF* was *G. balcanicus*, followed by the mass occurring for that season species – *Tonnoiriella* sp. and *Rhitrogena* sp., with considerable lower degree of dominance. The other dominant species during the autumn have had low *dF*-values, although their mass and commonly occurrence. Such fact could be explained by relatively stable species content of the bottom invertebrates which species diversity ensured the stability and resilience of the community.

CONCLUSIONS

Relatively high species richness was established in the present study of bottom invertebrate fauna of Rilska River. In total 190 taxa from 192 found in the river macrozoobenthos during the studied period are reported here for the first time. Along the river continuum there was observed significant difference in the faunistic similarity between the upper and lower stretches of the river. This result confirms the necessity of including the altitude as an obligatory element in determination the typology of riverine water bodies. The seasonal variations in the river (t^o, flow) resulted in within-year changes in the macroinvertebrate community structure and influenced seasonal replacement of species. The presence of sensitive to pollution taxons during the whole period of investigation, and at the all sites along the river was indicative for stable low-saprobic conditions in the river and its high self-purification capacity.

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ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“
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IMPACT OF ENVIRONMENTAL FACTORS ON THE PHYSICAL DEVELOPMENT OF GIRLS FROM RUSSE

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Abstract: The study object represents the dynamics of the main physical development parameters – growth and body mass (weight) of 433 girls aged 10 to 15 years from Russe. The investigated anthropometric characteristics were measured by the method of Martin & Saler (1957).

In view to the studied anthropological indexes it is established a regular increasing of height, weight and BMI according to age. The highest growth rate occurred in the age groups of 12 and 13 years (“puberty jump”). The values of the anthropometric parameters in the groups of 10 and 15 years have similar average values to those for Sofia and cities (highly urbanized areas). In respect to the other age groups, the average values of these parameters are similar to those, which are calculated for the poorly urbanized areas.

Key words: growth, body weight, BMI, physical development

The organism’s growth and development expresses the growth and development of various tissues, organs and systems as a result of increased total body size (height and weight) by changing the proportions of different parts. According to numerous studies the environment has major impact on the course of these processes and the period for their execution. In the frame of the anthropologic-ecologic approach the research of the influence of social environment on the development, growth and maturation of a human organism, is widely practiced. Urbanization poses for solving many social, environmental and health problems affecting the individual physical development (Mitova, 2005; Nacheva Lazarova, Yordanova, 2002; Янев, Генов, 1992; Ямполская, 2000).

MATERIAL AND METHODS

The study aims to determine the influence of a physical factors complex on the development of 433 girls aged – 10 to 15 years from Russe. The following anthropometric indexes – height and body mass based on a calculated body mass index (BMI), have been used. The investigated anthropometric characteristics were measured by the method of Martin & Saler (1957).

DISCUSSION

The data analysis shows, that growth increases in accordance with age. The average height for the 10 years old girls is 139.4 centimeters, where as for 15-year olds is 161.0 centimeters (Fig. 1). During this puberty investigation period (10–15) years, the average growth is increased by 21 cm. It is also established a rapid increasing of growth rate in the range of 10 to 13 years of age. During this period, the average growth has been increased by 17 cm, with a peak between 12 and 13 years of age (Fig. 1). After that peak the growth rate is slightly decreased. By the age of 13 this index has reached its highest value. During the period of 14–15 years, the growth has been increased by 3–4 cm. The weight alterations of girls from Russe in the age groups from 10 to 15 years have been presented in Fig. 3. The average value of body mass index in the group of 10-years is 34.5 kg, and in the group of 15-years – 55.4 kg. Weight increases according to age from 3 to 5 kg (Fig. 2). This trend is impaired in groups of 12–13 years, where the weight increasing rate is the greatest – approximately 7 kg. This period is characterized by a fast physical development. The received data about height and weight of girls from Russe have been compared with the average values of the same characters for groups of girls from Sofia and other cities, villages and for the all country. Both indexes have similar values to those for girls from Sofia and other Bulgarian cities in terms of groups of 10 and 15 years. In view to the other investigated age groups, the values of these parameters are similar to those from less urbanized areas (Янев, Б., 1992), (Fig. 3, 4). The most significant changes in BMI are reported between 12 and 13 years. The growth rate between the different age groups are turned out to be various (Fig. 5, 6). There were established higher growth rates between 12 and 13 years of age, this is the period of fastest growth and development. The most significant changes in BMI are also reported between 12 and 13 years. The growth pace of the investigated groups is not the same (Fig. 5, 6).

In view to the investigated anthropological indexes it has been established a regular increase of height, weight and BMI according to age. The highest growth rate occurred in the age groups of 12 and 13 („puberty jump").

The mean values of these anthropometric parameters in groups of 10 and 15 years are similar to those for girls from Sofia and other Bulgarian cities (highly urbanized areas). In view to the remaining studied age groups, the values of these parameters are similar to those for girls from poor urbanized areas.

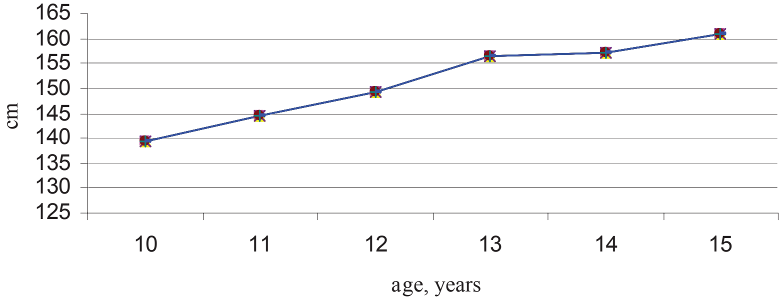


Fig. 1. The modify of the high recording to the age of the groups of city of Russe.

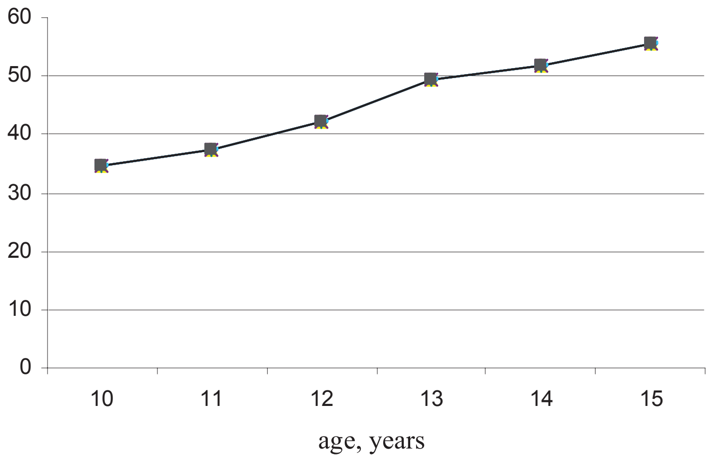


Fig. 2. The modify of the weight recording to the age of the groups of city of Russe.

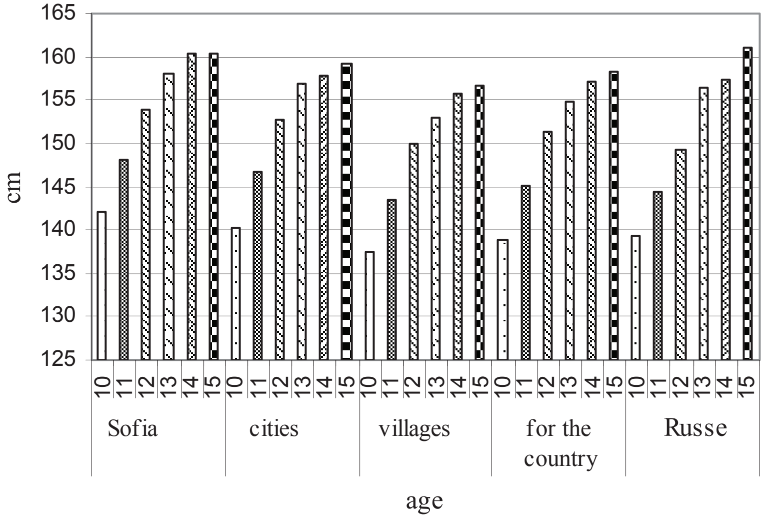


Fig. 3. The average value of the height in the groups.

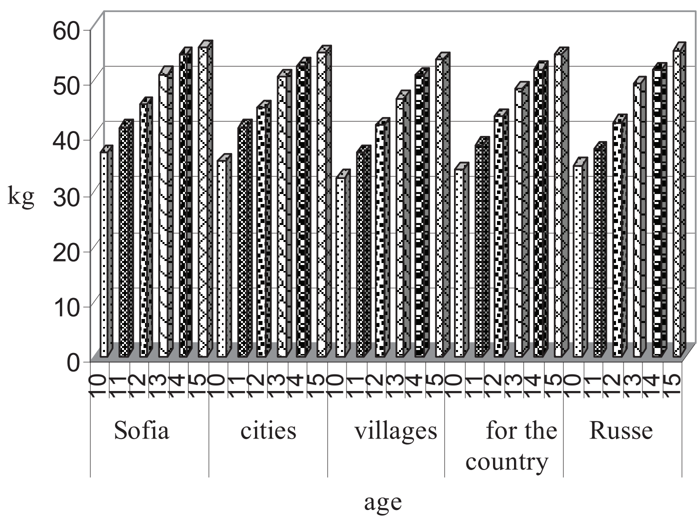
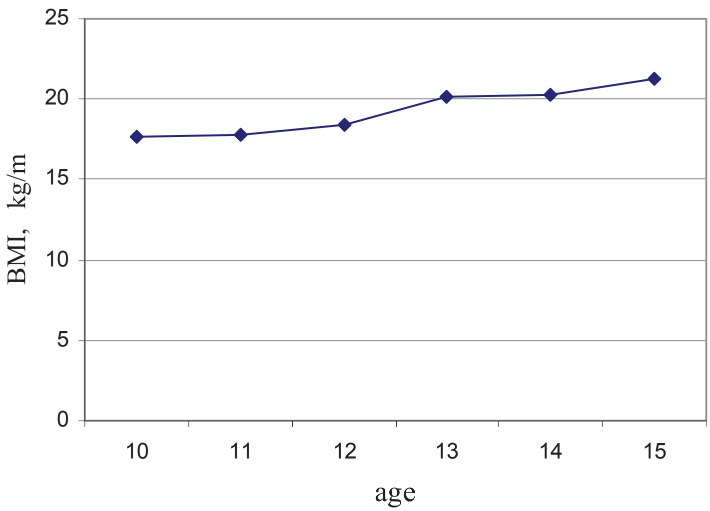


Fig. 4. The average value of the weight of the groups.



5. Fig. The change of BMI recording to the age.

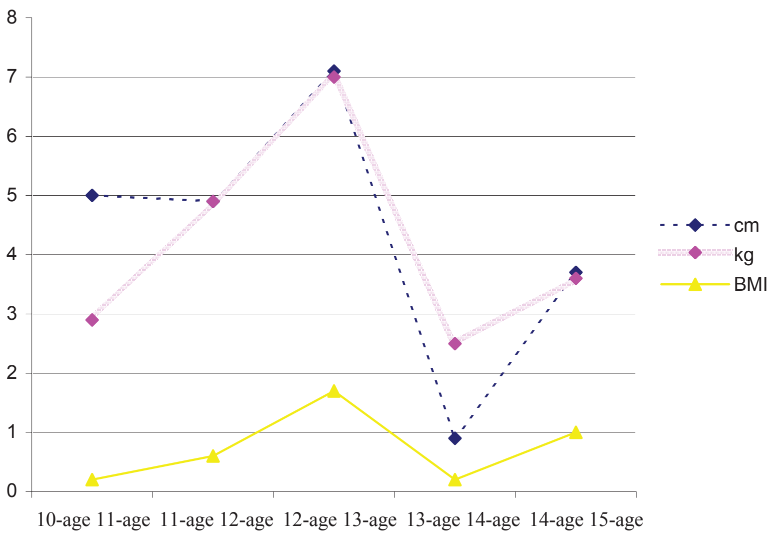


Fig. 6. The growth of the hight, weight and BMI of the group of the age 10–15.

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ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“
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IMPACT OF ENVIRONMENTAL FACTORS ON SEXUAL MATURATION OF GIRLS FROM THE CITY OF RUSSE END CITY OF THESSALONIKI

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Abstract. The first onset of menarche is the most important criteria for the state of puberty development in a girls. For the purpose of our study has been selected this indicator of sexual maturation of girls. 433 girls were examined from Russe and 204 girls from the city of Thessaloniki distributed in age groups from 10 to 15 years. The material has been collected by questionnaire method. Has been reported at the time and seasonal occurrence of menarche. The average age of onset of menarche in girls studied by the city of Russe is 11.99, while the group of Thessaloniki is 12.51. The study of seasonality in the onset of menarche for the sample from Russe demonstrated strong maximum during the winter season (typical of low urban populations), a group from the city of Thessaloniki is typical summer maximum (highly urbanized populations).

Key words: sexual development, menarche.

Sexual development (puberty period) is a key stage of human development during which the transformations take place as a result of that child’s body becomes a body of mature female or male. During the sexual maturation observed different periods rapidly changing body is under stress of a different nature. First onset of menarche is the most important criteria for the state of puberty development in girls. For the purpose of our study was selected this indicator of sexual maturation of girls. Influence on age of onset of menarche have: genetic factors (Kaprio et al., 1995), level of urbanization (Marrod et al., 2000), geographic location (Kapoor and Lahiri, 2003); altitude (Frédéric et al., 2001), social-economical conditions (Thomas et al 2001); health and lifestyle (Khanna, Kapoor, 2004); seasonality (Favaro, Santonastaso, 2009); feeding, physical activity (Merzenich et al., 1993); physical activity, obesity (Bayraktaroglu et al., 2007), hormones (Porcu et al., 2006).

MATERIAL AND METHODS

433 girls were examined from Russe and 204 girls from the city of Thessaloniki distributed in age groups from 10 to 15 years. The material was collected by questionnaire method. Was reported at the time and seasonal occurrence of menarche.

DISCUSSION

The results obtained during the occurrence of menarche, shows a clear dependence on the season. In late winter and early spring, along with the total exhaustion of the body and reduce physical activity, sharply reduced the cases of menarche, and again in autumn decreased probably under the influence of psychosocial stress in the new school year.

The highest values reported onset of menarche in the age group of 12 years in both groups tested (38.2% in girls from the city of Russe, 36.3% girls from the city of Thessaloniki (Fig. 1)). In the group of city Russe render an account large winter peak and 54.3% small increase during the summer 23.1%. Is almost identical distribution during the spring and autumn (11.6%; 11%) (Fig. 2). The group from Thessaloniki highest value recorded during the summer period, 30%, followed by spring and 26% identical distribution in autumn and winter (22%) (Fig. 3). The results obtained for the seasonal distribution of menarche from the survey confirms the literature data. It was found that for urban populations is very typical summer maximum of menarche, as seen in population of Thessaloniki (Fig. 3). For the less urbanized areas are typical two maximums: summer and winter such as a group of Russe. In the studied group from Thessaloniki summer maximum is not strong, suggesting that factors other than urbanization on the seasonality of menarche and affect climate.

The average age of onset of menarche in girls studied by the city of Russe is 11.99, while the group of Thessaloniki is 12.51. The study of seasonality in the onset of menarche for the sample from Russe demonstrates strong maximum during the winter season (typical of low urban populations), a group from the city of Thessaloniki is typical summer maximum (highly urbanized populations).

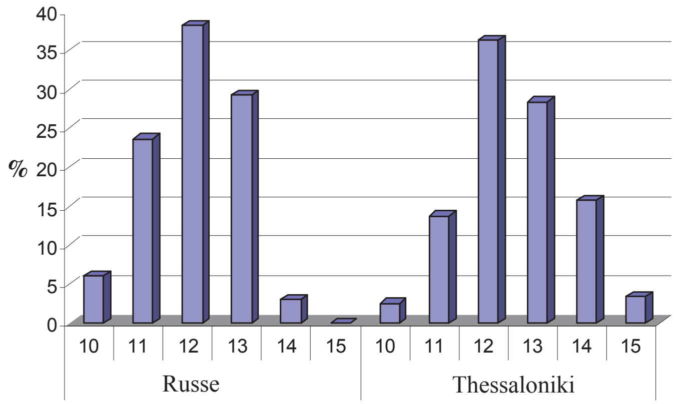

Fig. 1. Structure of onset of menarche in studied groups.

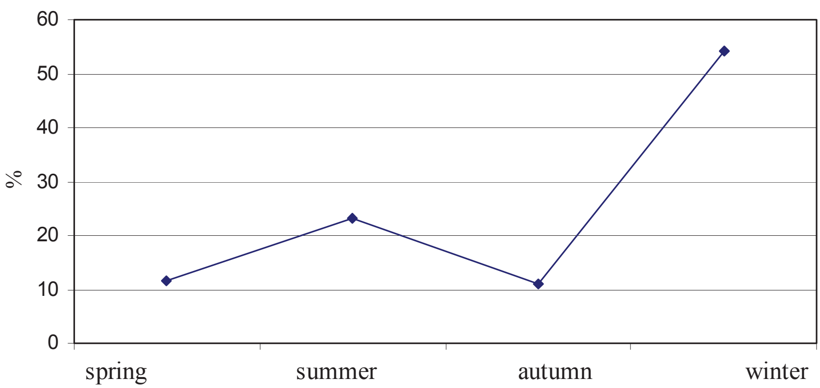

Fig. 2. Seasonal distribution of menarche by girl's groups of city of Russe.

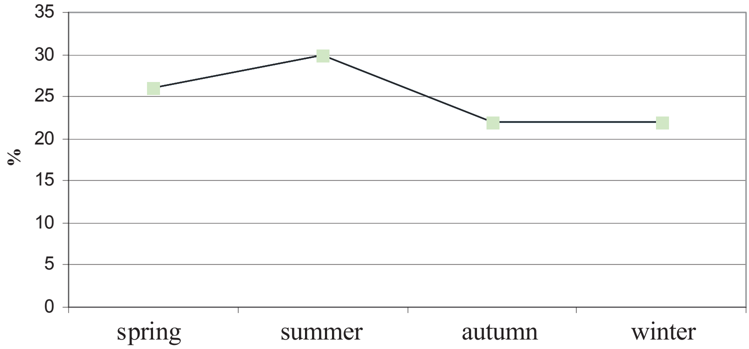

Fig. 3. Seasonal distribution of menarche by girl's groups of city of Thessaloniki.

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HISTOLOGICAL STUDY ON TESTIS OF ADULT MICE EXPERIMENTALLY INFECTED BY *YERSINIA PSEUDOTUBERCULOSIS*

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Abstract. An isogenous and one substrain of *Y. pseudotuberculosis* were used for infecting adult mice in order to observe histological changes in gonads. Significant histological changes in testes of mice infected by the substrain were established that confirms the fact that *Y. pseudotuberculosis* are microorganisms with psychrophilic characteristics and can be classified to the group of facultative psichrofils.

Key words: Y. pseudotuberculosis, testis, histological changes, mice

Pathogenic *Yersinia* are distributed in soil and water and possess high ecological plasticity. Pathogenic bacteria from the genus *Yersinia* are facultative inside the cells parasites – have the ability to adhere and invade host cells as well as to survive and reproduce in the cells (Yamamoto et al., 1996).

It is well known that *Y. pseudotuberculosis* can retain its ability to live and actively reproduce in the water and soil preferably at low temperatures. We have established in our previous studies (Orozova et al., 2007) histological changes in the stomach, inttsetines and liver of infected adult mice and also in the testes of mice infected by *Y. enterocolitica*.

In this study we have investigated the affect of Y. *pseudotuberculosis* in infected Balb/c mice.

Spermatogenesis is the process that takes place in the seminiferous tubules of the testis. It is well accepted that testes are organs with immunological privileges (Head et al, 1983). Despite of that there are data about suppression of the activity of spermatogenic tissue due to different inflammation processes (Adamopoulos et al, 1978; Cutolo et al, 1988; Buch and Havlovec, 1991).

MATERIAL AND METHODS

Bacterial strains. One strain of *Yersinia pseudotuberculosis* isolated from patients in Bulgaria and its substrain were examined. The substrain was isolated after 10 days of incubation in minimal growth medium supplemented with 10% NaCl and 2 mM glycin betain at 4 °C.

Experimental conditions. The test strains were subcultured onto TSA and incubated at 32°C overnight. A minimum of ten colonies were touched with a loop to inoculate 5 ml of Tryptic soy broth (TSA). The broth was incubated at 25 °C for 48 h. The cells were recovered by centrifugation and suspended and diluted in phosphate buffered saline to a density of about $1x10^6$ cells per ml. The density of these suspensions was verified by colony count. Totally 30 BALB/c mice were divided into 2 groups. Two individuals were leaved as controls. The animals from the first group were infected with bacterial suspension of the isogenic strain *Y. pseudotuberculosis*. Animals from the second group were infected with suspension of *Y. pseudotuberculosis* derivative strain. Each animal received 0, 2 ml of the respective cell suspension by intraperitoneal (i.p.) injection. All animals were held up to 15 days for observation. Each mouse that expired during the observation period was autopsied and pieces of testis were removed for histological observations.

Histological observations. The obtained samples were fixed by Becker's solution and after dehydration in ascending alcohols were embedded in paraffin wax. For staining Mayer's hematoxyline was applied on slides 5 µm thick. The samples were observed by "Amplival" microscope and photographed by "FM-Matic" camera.

RESULTS AND DISCUSSION

In 8 samples of mice testes infected with the isogenic strain of *Y. pseudotuberculosis* have not been established data about spermatogenic disorders (Fig. 2). In comparison with the control (Fig. 1) we have established a certain narrowing of the seminiferous tubules lumen but a normal distribution of the spermatogenic cells. There was a slight disruption of the wall in one section of the tubule that we consider as an artifact. Studying the samples of the other 7 mice we established changes in the spermatogenesis expressed by expelling of spermatogenic cells at different stages of development in the lumen of the tubules (Fig. 3). Normally in this region mature sperms are present.

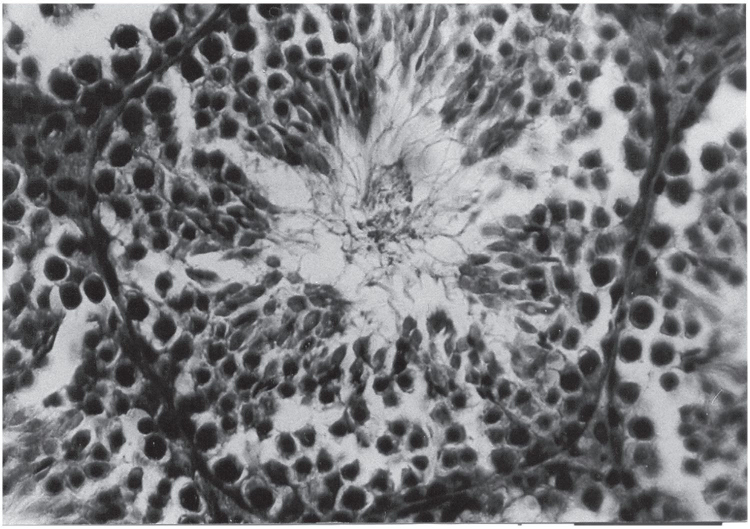

Fig.1. Cross section of a seminiferous tubule in a testis of non-treated mouse (control) 200 x.

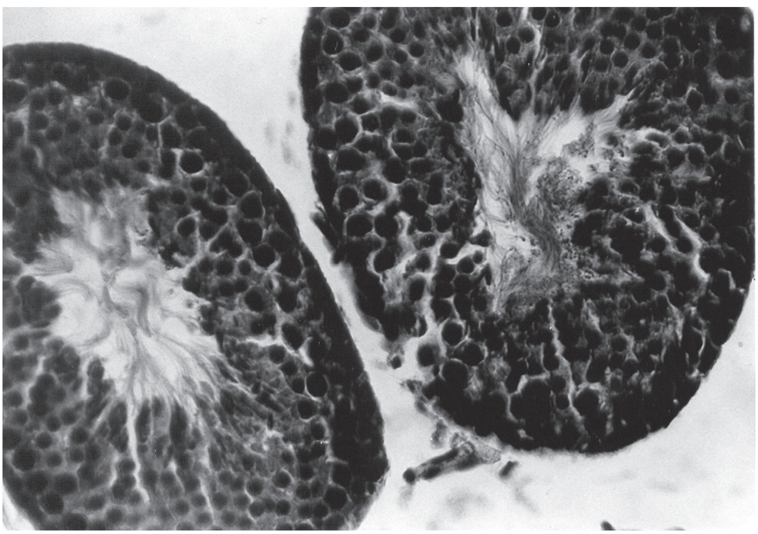

Fig. 2. Cross section of a seminiferous tubule in a testis of mouse treated with *Y. pseudotuberculosis* isogenous strain, 200 x.

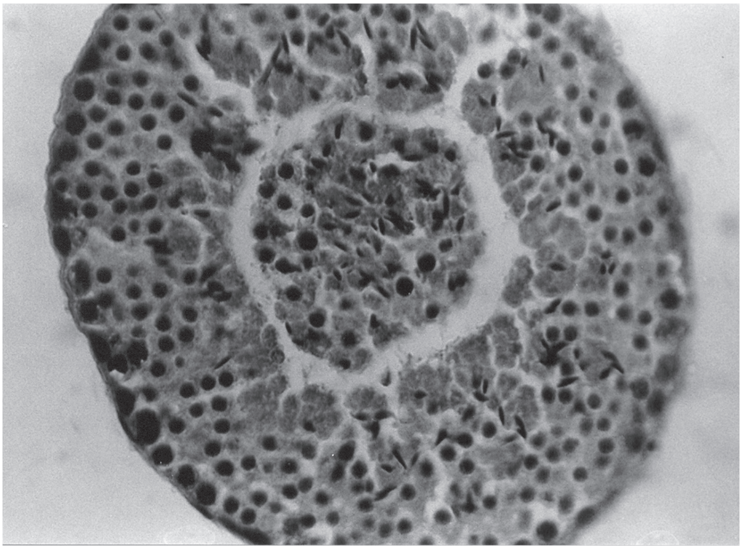

Fig. 3. Cross section of a seminiferous tubule in a testis of mouse with well expressed histological changes treated with *Y. pseudotuberculosis* substrain, 200 x.

In the testes of all mice treated by the substrain we have established destructive changes – interstitium disruptions, block of the spermatogenesis expressed by expelling of spermatogenic cells in the lumen of the seminiferous tubules (Fig. 4). The obtained results are relevant to result obtained by Liew et al. (2007). In their study male rat are injected with LPS (a component from the cell wall of the gram-negative bacteria) and the authors established a significant delay of meiosis for a 24 hours period followed (after 6 day period) by premature liberation of primary and round spermatides in the lumen. After 28th day the compensatory mechanisms start to act and the spermatogenic capacity of the testes is turned back to the pre-inflammation level. In our study mice were infected with both isogenic and substrain for a period of 15 days. It is possible in individuals without histological changes infected with the isogenic strain the acute phase to pass in the primary stage of infection. Since compensatory action depends a lot on the general physiological status of the individuals in some of the infected individuals on the 15th day there are expressed histological changes while in others they are not observed. The results from the infection with the substrain confirm that *Y. pseudotuberculosis* psychrophilic characteristics and can be classified to the group of facultative psychrofilic bacteria. In general we may conclude that the influence of *Y.pseudotuberculosis* on the testes is weaker than on the organs of gastrointestinal tract – stomach, intestines, liver.

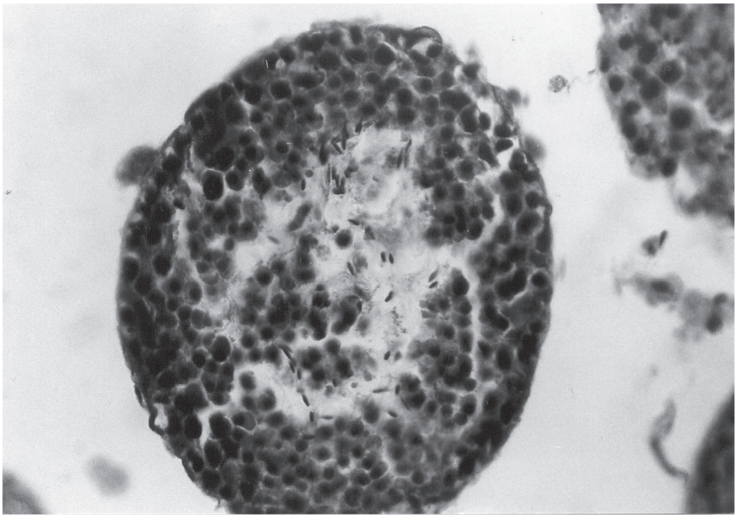

Fig. 4. Cross section of a seminiferous tubule in a testis of mouse treated with *Y. pseudotuberculosis*, 200 x.

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Abstract. A pair of *Yersinia enterocolitica* 0:3 1094 strains – isogenic and its substrain derivative isolated after 10 days of incubation in minimal growth medium supplemented with 10% NaCl and 2mM glycin betain at 4 °C were used to study the infection of BALB/c mice by intraperitoneal route. The isogenic strain and its substrain derivative caused histological changes in testes of infected adult mice.

Key words: Y. enterocolitica, histological changes, testis, spermatogenesis, mice

Strains of *Yersinia enterocolitica* are ubiquitous, being isolated frequently from soil, water, animals, and a variety of foods. They comprise a biochemically heterogeneous group that can grow at refrigeration temperatures (a strong argument for use of cold enrichment). Based on their biochemical heterogeneity and DNA relatedness, members of this group were separated into four species: *Y. enterocolitica*, *Y. intermedia*, *Y. frederiksenii*, and *Y. kristensenii* (Bercovier et al., 1980). Through additional revisions, the genus *Yersinia* has grown to include eleven species (Aleksic et al., 1987; Bercovier, 1980; Bercovier et al., 1984; Wauters et al., 1988), three of which are potentially pathogenic to humans: *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica*. Of these, *Y. enterocolitica* is most important as a cause of food-borne illness.

The association of human illness with consumption of *Y. enterocolitica*-contaminated food, animal wastes, and unchlorinated water is well documented (Aulisio et al., 1982; Aulisio, 1983). Refrigerated foods are potential vehicles because contamination is possible at the manufacturing site (Aulisio, 1982) or in the home (Aulisio, 1983). This organism may survive and grow during refrigerated storage.

Spermatogeneis in placental mammals is performed on the walls of seminiferous tubules in testes. Especially important for this process are androgenic hormones as well as Sertoli cell. The last besides supporting and protective function take part in maintaining the needed concentration of testosterone in the seminiferous tubules.

There are plenty of factors that influence the normal development of spermatogenesis – data exist (Adamopoulos et al., 1978; Cutolo et all., 1988; Buch and Havlovec, 1991) that show inhibition of normal testis function by system local infections and chronic inflammatory diseases.

According to references sublethal doses of lipopolysaccharide (LPS), a major component of the cell wall of gram-negative bacteria can disorganize spermatogenesis in adult rats (Tulassay et all., 1970; O'Bryan et al., 2000). In relation to our previous study on *Y. entrocolitica* influence on different internal organs (liver, stomach and intestines) we expand our investigation on histological study of adult testis.

MATERIAL AND METHODS

Bacterial strains. One strain of *Yersinia enterocolitica 0:3 1094* isolated from patients in Bulgaria and its substrain were examined. The substrain was isolated after 10 days of incubation in minimal growth medium supplemented with 10% NaCl and 2 mM glycin betain at 4 °C.

Experimental conditions. The test strains were subcultured onto TSA and incubated at 32 °C overnight. A minimum of ten colonies were touched with a loop to inoculate 5 ml of Tryptic soy broth (TSA). The broth was incubated at 25 °C for 48h. The cells were recovered by centrifugation and suspended and diluted in phosphate buffered saline to a density of about 1 x 10^6 cells per ml. The density of these suspensions was verified by colony count. Totally 30 BALB/c mice were divided into 2 groups. Two individuals were leaved as controls. The animals from the first group were infected with bacterial suspension of the isogenic strain *Y. enterocolitica 0:3 1094*. Animals from the second group were infected with suspension of *Y. enterocolitica 0:3 1094* derivative strain. Each animal received 0, 2 ml of the respective cell suspension by intraperitoneal (i.p.) injection. All animals were held up to 15 days for observation. Each mouse that expired during the observation period was autopsied and pieces of testis were removed for histological observations.

Histological observations. The obtained samples were fixed by Becker's solution and after dehydration in ascending alcohols were embedded in paraffin wax. For staining Mayer's hematoxyline was applied on slides 5 µm thick. The samples were observed by "Amplival" microscope and photographed by "FM-Matic" camera.

RESULTS AND DISCUSSION

We have established that infecting BALB/c male mice with isogenic strain of *Y. enterocolitica* as well as its derivative strains destructive changes in testes and destroying of normal spermatogenesis is observed. In control samples are observed well structured seminiferous tubules, normal interstitium, clear cycle of spermatogenous epithelium with well expressed spermiation in the lumen (Fig.1).

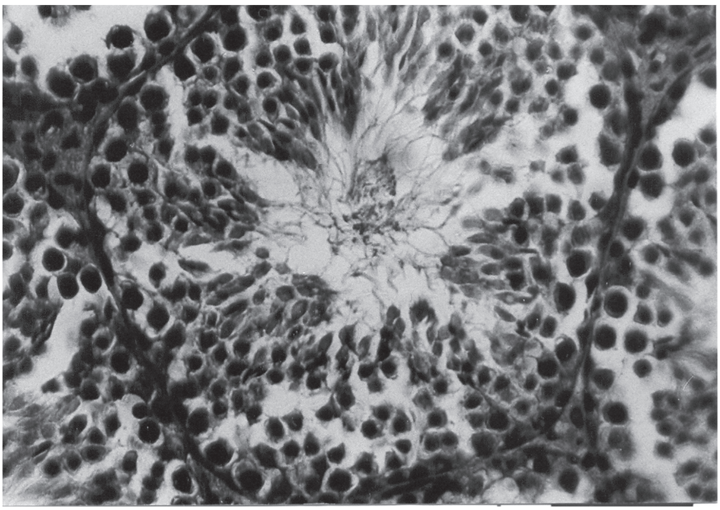

Fig. 1. Cross section of a seminiferous tubule in a testis of non-treated mouse (control) 200 x.

In the seminiferous tubules' lumens of all samples treated by *Y. enterocolitica* isogenous strain cells having morphological characteristics of primary spermatocytes, round spermatides and a few mature spermatozoa have been established (Fig. 2). These results claim undoubtedly about destruction of spermatogenesis of the infected animals. Moreover disruption of the interstitium integrity has been observed (Fig. 2).

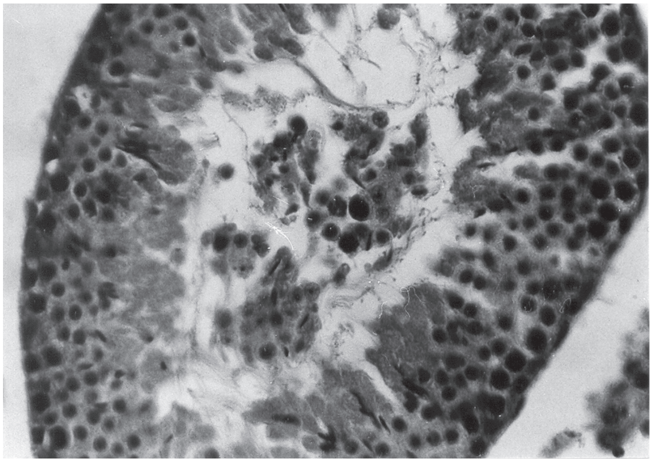

Fig. 2. Cross section of a seminiferous tubule in a testis of mouse treated with *Y. enterocolitica* 0:3 1094 isogenous strain, 200 x.

In the testes of 13 mice treated with *Y. enterocolitica* substrain well distinguished lumen of the seminiferous tubules is observed, while in the samples of two other individuals lumen has not been observed at all (Fig. 3).

In all individuals infected by the substrain of *Y. enterocolitica* blocking of the spermatogenesis at different stages has been established expressed by expelling of the spermatogenous cells in the lumen of the seminiferous tubules mainly of primary spermatocytes and round spermatides in 13 individuals (Fig. 4) and towards the center of the seminiferous tubules in 2 individuals (Fig. 3).

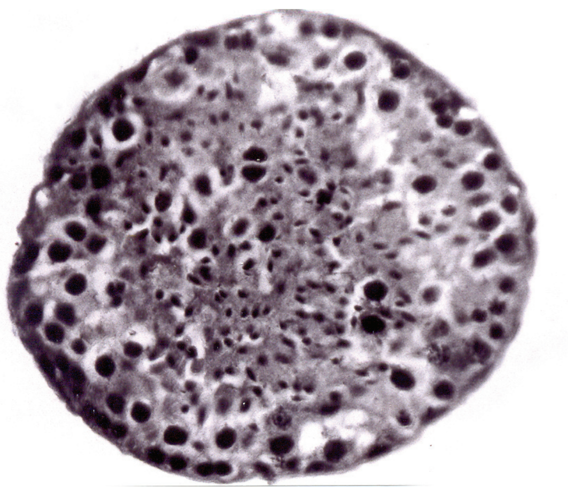

Fig. 3. Cross section of a seminiferous tubule in a testis of mouse treated with *Y. enterocolitica* 0:3 1094 substrain, 200 x.

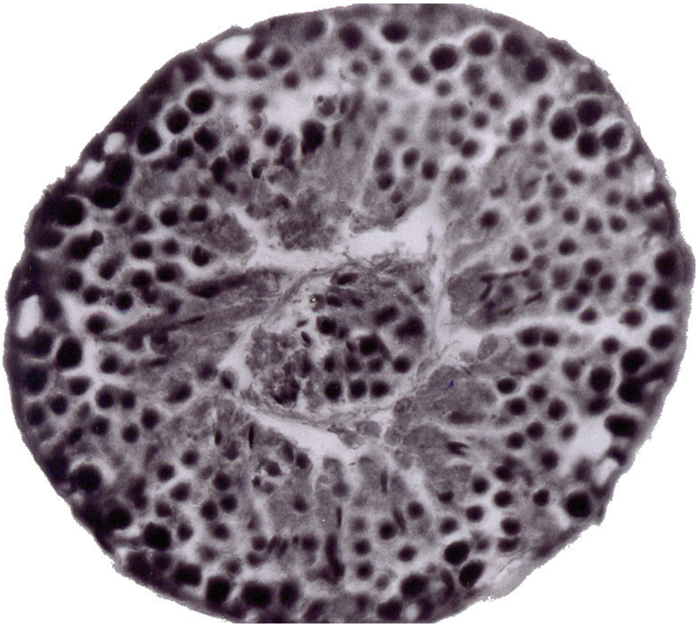

Fig. 4. Cross section of a seminiferous tubule with well-structured lumen in a testis of mouse treated with *Y. enterocolitica* 0:3 1094, 200 x.

The obtained results show though *Y. enterocolitica* is an enterobacterial species it affects the spermatogenesis too, causing the premature release of cells in the lumen of the seminiferous tubules. We do not study the mechanism of this process but we suggest that some components of *Yersinia* cells have a destructive action on the cell contacts which leads to their premature release in the lumen of the seminiferous tubules. A similar results but after treating rats by certain doses of LPS are obtained by (Bryan et al., 2000) where it has been observed that LPS from the cell wall of gram-negative bacteria leads the breaking of intercellular contacts between Sertoli cells and spermatocytes and spermatids and thus to their premature release in the lumen of the tubules.

A possible explanation of histological changes obtained in our study may be due to autotransporter proteins AT-2 secreted by gram-negative bacteria that play essential role in pathogenesis of bacterial infection and can modulate apoptosis of the host cells (Henderson et al., 2004).

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INFLUENCE OF MORPHOGENETIC MOVEMENTS ON THE DIFFERENTIATION OF LATERAL AND VENTRAL EXPLANTS FROM THE MARGINAL ZONE OF *TRITURUS* EARLY STAGE GASTRULA

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Abstract. The dependence between differentional abilities of the lateral and ventral sectors of the marginal zone of *Triturus* and the following morphogenetic cell movements has been studied. For this purpose the tangential tension has been increased in the lateral-ventral area of the presumptive mesoderm by preliminary separation of the dorsal lip of the blastopore. In these conditions the percentage of axial structures in lateral explants has been increased while in ventral explants this percentage remains unchanged. The results show that the regional differences in the differentiation potentials of the lateral and ventral sectors of the marginal zone depend to a great extent on their surface tension and the realized in them cell movements.

Key words: Triturus, marginal zone, morphogenetic movements, convergention, extension, intercalation

Formation of the mesoderm is the first induction process in the embryonic development in vertebrates. As a result of a mesoderm induction the marginal zone of the early blastula acquire the ability to develop in mesoderm tissue types.

The origin of the mesoderm is studied the most entirely in Amphibian embryos. By experiments carried out by means of a recombination of animal ectoderm and vegetative endoderm fragments has been established that epigenetically the mesoderm is formed from the ectodermic part of the blastula under the influence of induction signals from the endodermic half. This conclusion is valid both for Anura and Urodela species.

According to the three-signals-induction hypothesis the mesoderm origination in Amphibians is a three-stage process (Smith et al., 1985). In this respect the first two induction signals are emitted by dorsal-ventral and lateral-ventral vegetative cells and cause the origin of the dorsal and ventral mesoderm. The third induction signal is generated by the dorsal mesoderm. It is spread in an equator circus and reprograms the adjacent segment of the ventral mesoderm in lateral mesoderm. In consequence the dorsal mesoderm defines forming of the embryonic axis (chord and somites) and the rest mesoderm regions ensure forming of the lateral (muscle tissue and mesenchyme) and ventral (mesenchyme, pronephros and blood) mesoderm structures.

The basic event in the early embryonic development of the vertebrates is their axialization. That is because the axial organs have a crucial impact on the origin and situation of a series of other structures that do not belong to axial ones like placodes of sensory organs, excretory organs etc. The axialization in Amphibians is a highly coordinated process in dynamic respect, in which the cells from the embryonic marginal zone mutually take part. During the course of its coordinated movement the cells of the involuting marginal zone converge towards the dorsal-medial line and mutually intercalate that leads to anterior-posterior extension of the embryo.

Recently the basic part of our knowledge for convergention is obtained by studying of *Xenopus* leavis explants. By experiments with open-faced explants from the dorsal marginal zone of *Xenopus* has been established that the main forces for realizing a convergention and extension have been generated by the cells from the deeper cell population (Wilson & Keller, 1991). The analysis of the cell behavior in the involuting part of the explants has shown convergention and extension movements combined with the chord and somite formation. At that during the first stage of gastrulation involuting dorsal marginal zone becomes thin and slightly elongates without converging while during the second stage as a result of an active cell convergention involuting material significantly increases its length. Explants thinning and elongation in the first gastrulation stage is a result of a radial intercalation in which cells move along the radius of the embryo to form fewer layers of greater area. The processes of convergention and extension in the second stage are a result of medial-lateral intercalation in which cells move among one another along the medial-lateral to form a longer, and somewhat thicker array.

One of the unclear problems in the early embryonic development in vertebrates and in particular in amphibians is about a connection between differentiation potentials of the different marginal zone sectors and the morphogenetic cell movements performed in them. An experimental data exists that convergention-intercalation movements and the character of the differentiations in the suprablastoporal area of the early gastrula in *Xenopus* depend on tangential tension in embryos – the decreasing of the tangential tension suppress these movements and leads to anomaly in development of the axial organs (Belousov et al., 1990) and vice versa, the artificial stretching of the explanted supraplastoporal area in a direction perpendicular to the normal reoriented convergention-intercalation movement (Lakirev et al., 1988).

The main goal of our experiment was to study the impact of the increased surface tension on the differentiation abilities of the lateral and ventral sectors of the marginal zone in *Triturus* embryos. To do this we have stimulated banding of the lateral-ventral fragments in their dorsal end and thus we have increased their surface tension. In these conditions the percentage of the axial structures in the lateral fragments has increased while in the ventral ones has remained unchanged.

MATERIALS AND METHODS

The early gastrula stage – 8th stage according to Glaesner (Glaesner, 1925) of *Triturus* vulgaris embryos have been used in the experiments that have been carried out. The embryos have been collected from natural ponds during their reproductive period – March-April. Just fertilized ova have been chosen that have been moved in covered glasses with "normal amphibian medium" (NAM) for further development at temperature 10–15 ^{o}C. The stage early gastrula has been identified by morphologic features, namely, a concentration of pigment in the area of a future dorsal blastopore lip and by initial involution of the embryonic pre-chord material. The embryos have been decapsulated manually by means of sharp needles. The following experimental series have been carried out:

Series 1: The lateral and ventral segments from the marginal zone of intact embryos on the stage early gastrula stage. The aim of the operation is to study the self-differentiation of the isolated tissue fragments. The total number of the explants from this series is 32 and 28 respectively.

Series 2: The same sectors have been explanted 2 hours after operative explantation of the early gastrula suprablastoporal zone. The purpose of this operation is to enlarge the tangential tension in the lateral-ventral sectors of the marginal zone by banding of their dorsal ends. The number of the studied explants of this experimental series was 34 and 30, respectively.

After the operation tissue fragments from both series have been transferred and cultivated in vitro in a sterile "normal amphibian medium" (NAM) (Slack & Forman, 1980) for 72 hours. NAM consists of 110 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 0,5 mM Na NCO_3, 0,1 mM EDTA, 1 mM sodium phosphate pH 7,4, 100 units/ml penicillin, 60 units/ml streptomycin and 2 mg/l nystatin. In the end of the cultivation period the explants have been processed by Buen's fixative for 3 days, dehydrated in ascending alcohols and embedded in paraplast. The series slides thick 5 µm have been prepared and have been stained with Meyer's haematoxyline. The slides have been observed by light microscope.

The registered differentiations have been assessed by morphological criteria. The main characters of the chord tissue are highly vacuolized cells with star-shaped nuclei and well visualized nucleoli. Cross and longitudinal striation and large lipid-filled vacuoles are characteristic for muscle cells. The erythrocytes have been recognized according to their oval shape, transparent cytoplasm and highly condensed nuclei without nucleoli. The presence of fibrous layer has been accepted as a feature of a nervous tissue.

RESULTS AND DISCUSSION

Isolation and cultivation in vitro of the embryonic explants is the easiest way for assignment of their regional determination. By means of this method self-differentiation of tissue fragments from different sectors of marginal zone in Amphibians has been studied. It has been carried out for a series of Anura and Urodela species as *Xenopus* leavis (Holtfreter, 1938), *Rana fusca* and *Rana esculenta* and *Cynops (Triturus) pyrrhogaster* (Nakamura & Takasaki, 1970). The analysis of the results obtained shows that in dorsal-ventral direction in Amphibians three clearly defined conditions of determination of the presumptive mesoderm material exist. Explants from the marginal zone dorsal sector in vitro cultivation constantly form chord often with association with striated muscle tissue. In addition, prolific ectodermic derivatives are discovered – neural vesicles and blocks of neural-crest-type cartilage. Explants from the lateral marginal zone always form a large block of striated muscle surrounded by an epidermal covering. Often muscles are accompanied by notochord, neural structures, kidney tubules and loose mesenchyme. Ventral explants usually are differentiated with formation of concentric situated epidermis, loose mesenchyme, mesothelium and erythrocytes. In spite of the determination state differentiation abilities of the different sectors of the presumptive mesoderm are quite plastic and in a certain conditions they might be changed. For example, after operative removal of Xenopus dorsal lip of the blastopore part of the embryonic axial structures are formed by the lateral and ventral parts of the marginal zone (Cooke, 1973).

A special role in gastrulation and axialization of the early embryos in Amphibians plays the suprablastoporal (dorsal) area of the marginal zone. During the gastrulation, differences between this area from one hand and lateral-ventral from the other hand are rather significant both in morphogenetic and differentiation aspect. On the first place, the cells from the suprablastoporal area obtain abilities for morphogenetic and differentiation reformations depending on their new situation. For instance, the isolated fragments from suprablastoporal area on the stage middle gastrula show abilities for independent gastrula-like movements (involution and delamination) and for differentiation of their cells according to the place they take in the formed "minute gastrulas", while the explanted parts of the latero-ventral area can not realize such movements and differentiations connected with them. Second, during the gastrulation the suprablastoporal area is characterized with significantly higher morphogenetic cell activity in comparison with the rest regions of the marginal zone. Due to this reason the formation of the axial structures in vertebrate embryos and especially in Amphibians is accompanied with intensive convergention of the cells from the suprablastoporal area to dorsal-medial line. At this convergention the cells intercalate between each other that leads to sagital extension of the embryo. Similar processes but with significantly weaker intensiveness take place in the zones that are near to the ventral and lateral lips of the rounded blastopore.

The experimental data exists pointing out that convergention-intercalation movements in the different sectors of the marginal zone are stimulated by the tangential tension in the embryo and are important factor in differentiation of the axial structures (Belousov & Snetkova, 1994). It has been established for example, that blocking of involution in *Xenopus* suprablastoporal area by its covering with ectoderm from the embryos on later stages of development strongly decreases the percentage of the axial differentiations in the dorsal explants while the stimulation of the tangential tension in the lateral areas of the embryo increase the percentage of these structures in the isolated lateral fragments. These results show that the regional differences in differentiation potentials of the various parts of the marginal zone significantly depend on their tangential tension and on the cells movements taking place in them.

The comparative differentiation of the lateral explants in our experiment is shown on the Table 1. It is seen that the increase of the tangential tension in the explants by stimulating their bending in their dorsal end leads to rise of percentage of the dorsal (axial) structures in them. This rising is quite significant for the notochord and the nervous tissue as a whole though the percentage of the fore-brain differentiations is unchanged. At the same time the frequency of ventral derivatives (mesothelium and blood cells) in the explants decreases.

Differentiation of ventral explants in the two experimental series is shown on the Table 2. It is obvious from the results the increasing of tangential tension in the ventral explants does not change the frequency of the axial structures and does not influence the percentage of the ventral derivatives, too.

Table 1. Comparative differentiation of lateral mesoderm explants of intact early gastrula stage embryos (*1*) and of the same stage embryos with preliminary explanted dorsal blastoporal lip (*2*) after 72 hours cultivation period; * – number of explants.

Differentiation	1	2
Nervous tissue	34%(11)*	47%(16)*
Front brain structures	3%(1)	3%(1)
Notochord	34%(11)	62%(21)
Muscle	66%(21)	82%(28)
Blood	19%(6)	12%(4)
Mesothelium	28%(9)	21%(7)

Table 2. Comparative differentiation of ventral mesoderm explants of intact early gastrula stage embryos (*1*) and of embryos on the same stage with preliminary explanted dorsal blastopore lip (*2*), after 72 hours cultivation period; * – number of explants.

Differentiation	1	2
Nervous tissue	7% (2)	7% (2)
Front brain structures	–	–
Notochord	4% (1)	–
Muscle	18% (5)	10% (3)
Blood	68% (19)	67% (20)
Mesothelium	64% (18)	68% (19)

CONCLUSION

The obtained results can be interpreted in two directions. On the first place, the change in the frequency of differentiations registered shows that on the early gastrula stage determinative abilities of separate sectors in the marginal zone are still enough plastic. As a rule that means that the embryonic material from these sectors can form more broad range of differentiations compared with those that are realized in a normal development. Secondly, differentiation plasticity of the different regions in the marginal zone can be connected with the character of the morphogenetic processes that take place in them and their tangential tension. As it has been mentioned the block of involution and the tangential tension in the explants from the dorsal sector in Xenopus is combined with stoppage of convergation-intercalation movements and the decrease of axial differentiations percentage. Similarly, the rise of tangential tension by stimulating of involution in the lateral explants in Triturus activates the morphogenetic cell movements and increases the percentage of the axial structures. The fact that the stimulation of the involution and the increasing the tangential tension in the ventral explants does not change the frequency of the axial derivatives can be explained with the very low activity of the convergation-intercalation movements in this region and with that due to the changes in the tangential tension do not influenced this activity.

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VERTICAL DISTRIBUTION OF THE AMPHIBIANS AND REPTILES IN RILSKA RIVER BASIN (RILA MOUNTAINS, SOUTHWEST BULGARIA)

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Key words: batrachofauna, herpetofauna, altitudinal distribution, Rila Mts.

Vertical distribution of the amphibians and reptiles is a result of a complex impact of climatic and geographic characteristics and parameters of the environment. Environmental climatic factors impact on distribution of ectotherm animals is well documented. Air temperature seems to be the main one (Brattstrom, 1965; Krebs, 1994; Somero, 2005). In ectotherms the mechanisms of thermoregulation have to rely on selecting and shuttling between appropriate thermal microhabitats to acquire and maintain appropriate body temperatures (Brattstrom, 1965, 1970, 1979; Spellerberg, 1972a; Huey & Pianka, 1977; Hertz & Huey, 1981; Avery, 1982; Huey, 1982). They are capable of behaviorally regulating their body temperatures, their physical environments eventually limit the extent of how much they can manipulate (Huey, 1974; Huey & Stevenson, 1979).

Rila Mountain to which belongs the studied territory offer a typical example of a postglacial fauna changes and a good place for studying these processes. Rilska river valley is a typical postglacial river system. The strong impact of the glaciers is evident especially in the upper part of the valley.

Altitudinal split of Bulgarian batracho- and herpetofauna was summarized and generalized in Petrov (2007). Previously the species composition in the study region and species distribution in four altitudinal belts was given in Peshev et al. (2005). Additional distributional data for some species we found in Beshkov & Stoyanov (2002), Stoev (2003) and Veselinov (1993). Here we present a new records and species by species altitudinal data for all amphibians and reptiles encounted in Rilska river valley.

MATERIAL AND METHODS

The studied territory includes the Rilska river basin, 392 km^2 in total and cover a vertical diapason from 345 to 2500 m. Data were collected for a ten years period (1999-2009). Already published data from Beshkov & Stoyanov (2002), Stoev (2003) and Veselinov (1993) was also included. Species by species distribution was plotted in 100 m altitudinal belts. Each observed amphibian and reptile was identified at the species level and its exact position was marked using a hand-held GPS unit (Garmin Etrex Vista, manufacturer specified accuracy ± 5 m). Statistical procedures were performed with Microsoft Office Excel 2007. Data cordinates for some species localities are presented in Apendix I in format Lat/Lon hddd° mm' ss.s.

RESULTS AND DISCUSSION

Summarized data are present in Table 1. Species richness decrease clearly with altitude (Fig. 1) and it is strongly correlate to it ($R = 0.85$, $F_{(1.2)} = 51.1$, $p < 0.001$). The highest number of species was encounted between 600 and 700 m and 900–1000 m, respectively 22 and 23 species. Both sections fall in to the low mountainous altitudinal zone. They include as well as the highest records in the study region for some species (*Epidalea viridis, Bufo bufo, Hyla arborea, Pelophylax ridibundus, Rana graeca, Emys orbicularis, Eurotestudo hermanni, Testudo graeca, Ablepharus kitaibelii, Darevskia praticola, Lacerta viridis, Natrix natrix, Dolichophis caspius, Vipera ammodytes*) but also the second belt include the lowest altitudinal records for some of the high mountainous species (*Rana temporaria, Lacerta agilis bosnica, Vipera berus*). Species by species altitudinal distribution is present in Fig. 2. Some of the encounted species are present only in the lowest zone of the study area below 600 m (*Triturus karelinii, Lissotriton vulgaris, Podarcis erhardii, Podarcis tauricus, Platyceps najadum*). Detailed data about the minimal and maximal altitude of the species localities are present in Table 1.

The tailed amphibians show a clear altitudinal zonation in the Rilska river basin. Both *T. karelinii, L. vulgaris* inhabit only the pre-mountainous area. There absence in the upper part of the valley can be explained by the miss of suitable places for reproduction, taking into account the fact that in the other part of the country there uppermost distribution limit is quite higher, e.g. for *T. karelinii* near 1500 m (Dobrev, 2007a) and for *L. vulgaris* 1854 m (Tzankov et al., 2009). Third newt species *Ichthyosaura alpestris* inhabit only the upper part of the studied area. Localities point Ia-03 is the highermost for this species in Bulgaria (Veselinov, 1993). *Salamandra salamandra* inhabit mid-altitude belts between 600–1800 m (Fig. 2) that agree with a typical altitudinal diapason for this species in Bulgaria (Dobrev, 2007b).

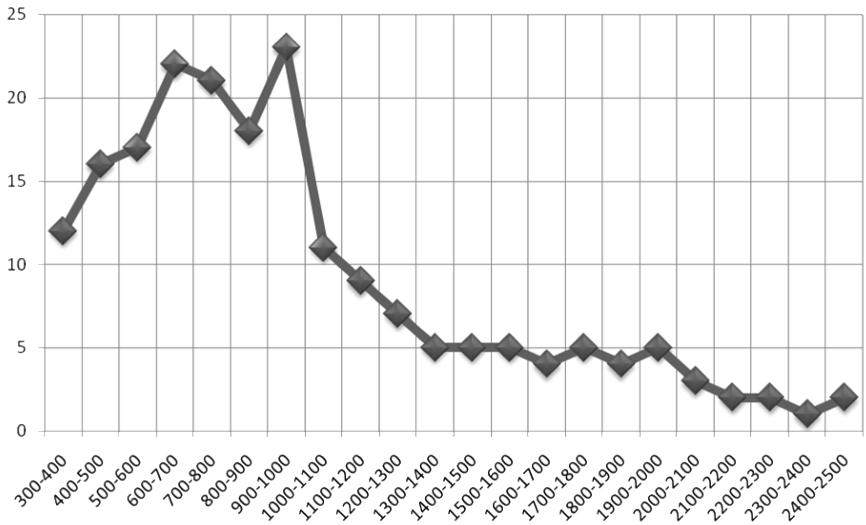

Fig. 1. Species richness vs. altitudinal belts in Rilska river basin.

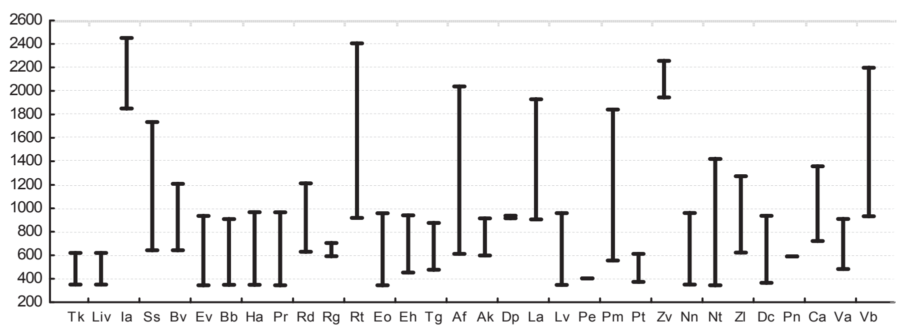

Fig. 2. Species by species altitudinal distribution in Rilska river basin.
For species abbreviations see Table 1.

Table 1. Minimal and maximal altitude records of the species along the Rilska river basin. Details on abbreviations for the conservation status are provided below the table.

Species	Abb.	min	max	Conservation status					
				BPA	RDB	92/43	BERN	IUCN	CITES
Triturus karelinii (Strauch, 1870)	Tk	350	620			II, IV	II	LC	
Lissotriton vulgaris (Linnaeus, 1758)	Liv	350	620						
Ichthyosaura alpestris (Laurenti, 1768)	Ia	1850	2450						
Salamandra salamandra (Linnaeus, 1758)	Ss	643	1734	III			III	LC	
Bombina variegata Linnaeus, 1758	Bv	643	1209	II, III		II, IV	II	LC	
Epidalea viridis (Laurenti, 1768)	Ev	345	935	III		IV	II	LC	
Bufo bufo Linnaeus, 1758	Bb	348	908	III			III	LC	
Hyla arborea Linnaeus, 1758	Ha	348	967	II, III		IV	II	LC	
Pelophylax ridibundus (Pallas, 1771)	Pr	345	966	IV		V	III	LC	
Rana dalmatina Fitzinger in Bonaparte, 1840	Rd	630	1212	II		IV	II	LC	
Rana graeca Boulenger, 1891	Rg	592	704	III		IV	III	LC	
Rana temporaria Linnaeus, 1758	Rt	918	2405						
Emys orbicularis (Linnaeus, 1758)	Eo	345	958	II, III		II, IV	II	NT	
Eurotestudo hermanni (Gmelin, 1789)	Eh	453	940	II, III		II, IV	II	NT	II
Testudo graeca Linnaeus, 1758	Tg	476	875	II, III		II, IV	II	VU	II
Anguis fragilis (Linnaeus, 1758)	Af	612	2038						
Ablepharus kitaibelii Bibiron&Bory de Saint-Vincent, 1833	Ak	598	914	III		IV	II	LC	
Darevskia praticola (Eversmann, 1834)	Dp	915	937						

Table 1. (cont.)

Lacerta agilis Linnaeus, 1758	La	905	1928					
Lacerta viridis (Laurenti, 1768)	Lv	347	959			IV	II	LC
Podarcis erhardii (Bedriaga, 1882)	Pe	403	403					
Podarcis muralis (Laurenti, 1768)	Pm	555	1841					
Podarcis tauricus (Pallas, 1814)	Pt	374	612			IV	II	LC
Zootoca vivipara (Von Jacquin, 1787)	Zv	1945	2256					
Natrix natrix (Linnaeus, 1758)	Nn	350	960				III	LC
Natrix tessellata (Laurenti, 1768)	Nt	345	1420			IV	II	
Zamenis longissimus (Laurenti, 1768)	Zm	623	1274	III	T	IV	II	
Dolichophis caspius (Gmelin, 1789)	Dc	365	936	III		IV	II	
Platyceps najadum (Eichwald, 1831)	Pn	590	590	III		IV	II	
Coronella austriaca Laurenti, 1768	Ca	722	1357					
Vipera ammodytes (Linnaeus, 1758)	Va	482	909	IV		IV	II	
Vipera berus (Linnaeus, 1758)	Vb	932	2198					

BPA – Biodiversity Protection Act of Bulgaria 2002. Annexes: II – species whose conservation requires the designation of special areas for habitat protection; III – species protected statewide; IV – species under protection and regulated use.

RDB – Red Data Book of Bulgaria 1985. T – threatened.

92/43 – Council Directive 92/43/EEC 1992. Annexes: II – species whose conservation requires the designation of special areas of conservation; IV – species in need of strict protection; V –species whose taking in the wild and exploitation may be subject to management measures.

BERN – Bern Convention on the Conservation of European Wildlife and Natural Habitats. Appendices: II – strictly protected fauna species; III – protected fauna species.

IUCN – International Union for Conservation of Nature. VU – vulnerable; NT – near threatened; LC – least concern.

CITES – Convention on International Trade in Endangered Species of Wild Fauna and Flora. Appendix II – species not necessarily now threatened with extinction but that may become so unless trade is closely controlled.

Anuran species in general reach a lower altitude in the study area than the other part of the species range. That's rule is supported by *Bombina variegata* (1209 m, in the other parts over 2000 m), *E. viridis* (935 m vs. over 2000 m), *B. bufo* (908 m vs. typically up to 1300 m) *H. arborea* (967 m vs. typically up to 1300 m), *P. ridibudus* (966 m vs. typically up to 1400 m) and R. graeca (704 m vs. typically up to 1200 m), all maximal altitudes are according to Stoyanov (2007). *Rana dalmatina* fall into the typical for this species altitudinal zone (Stoyanov, 2007). In the study area *Rana temporaria* reach one of the highest documented records (Table 2), but also one of the highest places for reproduction. Referent altitudinal limits are taken from Biserkov et al. (2007).

Vertical distribution of the turtle species encounted fall in to a already know typical altitudinal belts, as only for *Emys orbicularis* the highest record in this study (Table 1) is close to the higher limits of his distribution in Bulgaria (1100 m, Beshkov & Nanev, 2002). In the study area this species was found mainly in artificial ponds, and her uppermost records may be considered as man released. Tortoises, both species reach common altitude but from the region are present with single individuals in isolated localities Fig. 2.

Two lizard species are confined to the lowland and lower part of the study region, *P. erhardii* and *P. tauricus* (Fig. 2). Both *L. viridis* and *L. agilis* meet only in a narrow zone (Fig. 2) as for the first one this altitude is quite lower compared to the maximal for this species (1800 m, Tzankov, 2007). Four other lizards *Anguis fragilis*, *A. kitaibelii*, *Podarcis muralis* and *Zootoca vivipara* have a typical altitudinal distribution of species. With a very narrow altitudinal distribution *D. praticola* differ from the other species. Climatic factors, habitats choice and competition with the other species may give an explanation for it.

Encounted snakes as a whole reach a lower altitude than the maximal of species. *N. natrix* (960 m, vs. up to 2100 m), *D. caspius* (936 m, vs. up to 1500 m), *Zamenis longissimus* (1274 m, vs. up to 2000 m) *Coronella austriaca* (1357 m, vs. up to 2200 m) (maximal altitudes for these species are according to Naumov (2007), *V. ammodytes* (909 m vs. up to 1900 m (Slavyanka Mt. NMNH-S–III–1–46) *P. najadum* (590 m, vs. up to 900 m (Naumov et al. 2007)). For *Natrix tessellata* encounted altitude is the highest recorded in Bulgaria, 1420 m., previously highest record was 1100 m (Beshkov, 1961)). *Vipera berus* distribution cover typical for this species altitudinal range (Fig. 2).

A clearly well expressed zonation in amphibians and reptiles distribution was observed during the present study. The highest number of species, over 15 observed between 500 and 1000, and over 20 between 700 and 1000 clearly demonstrate the principle importance of those areas for species diversity protection. Amphibian species tend to be highly vulnerable due to the limited places for reproduction. Both altitudinal belts fall outside the protected area in Rila Montain covering by the national legislation, e.g. Rila Monastery Natural Park. But they are covered by the NATURA 2000 protected area "Rila buffer" BG0001188.

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