ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., 64 (3), 2012: 273-281

Comparative Studies of Water Invertebrate Fauna from Benthal and Hyporheic Habitats in Iskar River Catchment. I. Infauna: Free-Living Nematodes (Nematoda) and Chironomid Larvae (Diptera: Chironomidae)

***Lyubomir A. Kenderov*[1], *Stefan. A. Stoichev*[2], *Ivanka Y. Yaneva*[1]**

[1] Department of General and Applied Hydrobiology, Biology Faculty, Sofia University 8 Dragan Tsankov Blvd., Sofia 1164, Bulgaria; E-mail: lubomir.kenderov@gmail.com
[2] Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences 1 Tsar Osvoboditel Blvd., Sofia 1000, Bulgaria; E-mail: stefanstoichev@yahoo.com

Abstract: 14 species of free-living nematodes and 22 species (larval stage) of family Chironomidae (Diptera) from the riverine and hyporheic habitats of Iskar River catchment are reported in this study. The five chironomid species and two nemic species were new for the faunal list of this catchment. The most of the identified chironomid species were found in both compared biotopes; respectively the species similarity between them was high. Contrary to them, the free-living nematodes from both habitats were more different and have got the lower species similarity. The species number from both taxonomic groups varied greatly along the river course. In conditions of maximum pollution (below the city of Sofia), the chironomid larvae – inhabitants of river bottom, were well presented but in anthropogenically unaffected sites of the mountain river sector (above Samokov town) they were poorly represented. Contrary to them, the hyporheic chironomids and free-living nematodes were heavily depressed by pollution and almost disappear from the underground community in the area below Sofia.

Key words: taxonomic composition, density, similarity, ecological state, surface and groundwater fauna

Introduction

The studies of surface fauna (river) and underground (hyporheic) waters in Bulgaria had a relatively long history and they were among the pioneering in the world, especially in aspect of pollution and self-purification processes (Cvetkov 1965, Russev 1959, Cvetkov, Petrova 1987). But the targeted faunistic and ecological comparisons between the two biotopes were carried out rarely.

Recent studies on free-living nematodes and family Chironomidae (Diptera) in some Bulgarian rivers and lakes were done by Stoichev (1994, 1996a, 1996b, 1999).

Our studies (Kenderov, 2010) on the composition and distribution of benthic and hyporheic invertebrates in Iskar River catchment indicated that the groundwater communities were constructed by three ecological groups hydrobiontes: stigophilic and stigobiontic representatives of meyofauna (crustaceans and aquatic mites); infauna (free-living nematodes, oligochaetes, and larvae of family Chironomidae) and stigoxenes (adventitious invertebrates from benthal habitat, mainly larvae of aquatic insects and mollusks). It was found that the changes in the composition and quantity of hyporheic organisms from the first group (meyofauna) were determined in most from the ecological state of river and hyporheic ecosystems (Kenderov, Apostolov 2008).

The main objective of this study is to compare the composition, distribution and dynamics of some benthic and hyporheic representatives of the second group (infauna) under different environmental conditions established in surface and hyporheic biotopes of river.

Study Area

Iskar River is the longest Bulgarian river with its length of 368 km and largest Bulgarian Danube tributary with its watershed of 8647 km^2. The river springs from Rila Mountain (2500 m a.s.l.) and flows into Danube at 637 river kilometer, near to Baykal village (20 m altitude).

From the middle to the end of XX century, Iskar River was exposed to high amount of organic loading and industrial pollution from Sofia City vicinity. The saprobic state of river in this period was defined as a polysaprobity downstream the city (in 1964, the saprobic index of Rothschein was up to S_R=10.0) or iso-saprobity (in 1968, 1969, 1985 the macrozoobenthos was eliminated). The small degree of improvement, mainly to α-mesosaprobity was determined on the lower sites. Due to this fact, the benthal invertebrate fauna from the whole middle sector and the part of lower was strongly affected (Russev 1994).

Our preliminary investigations from the last years showed that the state of sector directly affected by anthropogenic impacts of Sofia did not change and the polysaprobity was retained. In contrast to the previous periods/studies, the significant self-purification processes and quick attainments of stable climax β-mesosaprobity state in the main part of middle sector (Svoge – Reselets) were observed. In this state the river flows into Danube (Kenderov, Yaneva 2009).

The ecological state of mountain area of Iskar catchment was stable and close to the natural for the last 50 years. The saprobic situation was determined as a xeno- and oligosaprobity (S_R= 67.01 – 85.51, Russev 1959, 1968). One exception was observed in our investigations – the local organic loading next to the town of Samokov (Kenderov *et al.* 2008).

The present study was carried out on 20 sampling sites located on Iskar River and its tributaries

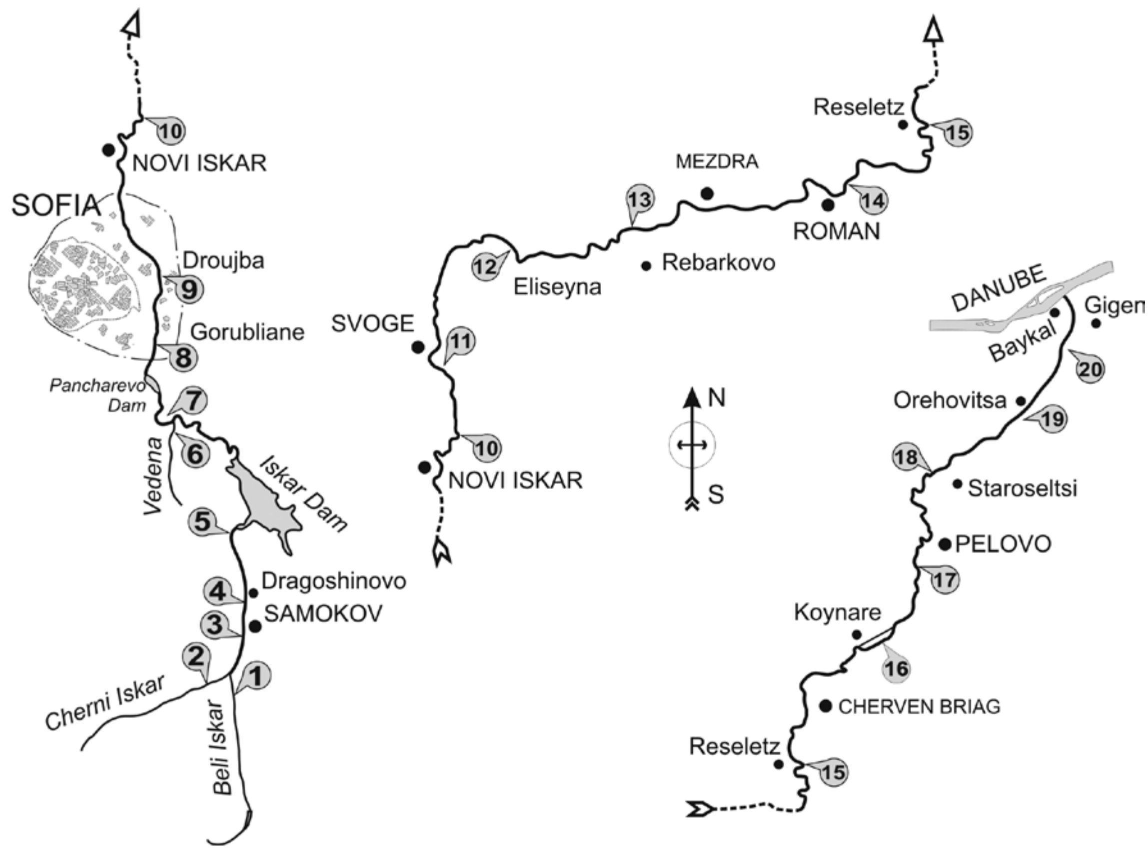

Fig. 1. Scheme of Iskar River. The river is presented by 3 different pictures with number of stations.

Beli Iskar,Cherni Iskar and Vedena River as following: st. 1-7: mountain area; st. 8-15: middle sector; st. 16-20: lower sector (Fig. 1). Mountain area starts from 1020 m (st. 1), ends at 638 m and is with a total length of 38 km. Middle sector extends from 579 m (st. 8) to 104 m (st. 15) and with length of 154 km. Lower sector starts from 75 m (st.16) and ends at 20 m (st. 20), length – 89 km.

Material and Methods

The study was carried out in 8 field investigations during the period 23 December 2003 – 25 August 2009 (Appendix 1). At any point 10 dm^3 samples were taken from the hyporheal (depth of 30/40 cm) through Bou Rouch method, using 90 µm mesh size net (Bou, Rouch 1967, Bou 1974). At the main part of sites (without st. 8, 9) were collected qualitative (hand-net) and quantitative (ISO 8265: 1988) macrozoobenthic samples, using 580 µm mesh size net. The data of the last sampling (21-25 August 2009) were used only in species list of benthic chironomids from sites No 7,10,13,14,19,20. Altogether were collected 117 hyporheic and 182 macrozoobenthic samples (96 qualitative and 86 quantitative).

The taxonomic similarity (QS) after Sörensen (1948) between the faunistic complexes from the two studied biotopes was determined. The analysis of dominance according to De Vries method (Kozhova, 1970) was done. The next scale was applied: very rare species (pF <5%); rare species (pF = 5-20%); frequent species (pF=20-50%); very frequent species (PF >50%).

Results and Discussion

Taxonomic composition and similarity

Nematode – complex

In the present study 14 species of free-living nematodes were identified. Two species of these – *A. obtusicaudatus* and *E. iners* have been reported firstly for the fauna of Iskar River, and the last species is new for all Bulgarian Danube tributaries (Appendix 2). The 11 and 7 species were obtained respectively for the benthic and hyporheic biotopes respectively. The species *A. obtusicaudatus*, *E. agilis*, *D. paradoxus* were found only in hyporheal but *D. stagnalis*, *M. centrocercus*, *M. stagnalis* and *T. steatopyga* were characteristic for both biotopes. The rest of the species were found only in benthic biotopes. Therefore, the species similarity between the two nematodes complexes in studied habitats was relatively not highly (QS= 44.4%).

Chironomidae- complex

22 different species taxa of chironomid larvae were identified (Application 3). For first time five species – *A. lucens, B. zabolotzkyi, D. vulneratus*, *M. chloris* and *M. pedellus* were here reported for the fauna of Iskar River catchment. In the hyporheic biotope, 14 species were found, including *E. tendens* and *E. similis* that were not registered for the benthal. The biotope of the river bed was inhabited by 21 chironomid taxa. The larger number of common species between two biotopes defined a high taxonomic similarity between them (QS = 74.3%).

Dominant analysis

Nematode – complex

The main part of benthic nematodes species from Iskar catchment were defined as very rare (pF< 5%) (Appendix 2). Only two species wewe defined as rare, *T. gracilis* (pF = 5,7%) and *D. stagnalis* (pF = 15,9%). None of the species was a dominant in benthic communities (Dt = 0%).

The importance of nematodes for hyporheic communities in Iskar River was a different. Three species were into the category of very rare (pF<5%): *A. obtusicaudatus*, *M. centrocercus* and *T. steatopyga*. Three other species were classified as rare (pF = 5 – 20%): *E. agilis*, *D. paradoxus* and *M. stagnalis*. The last species in a few cases was dominant (Dt = 14,0%). The only species that often occurred was *D. stagnalis* (pF = 62,9%). It was found almost at all sampling sites (not in st. 3, 5) and had a critical meaning for hyporheic community because in many cases it was a dominant (Dt = 38,3%).

Chironomidae – complex

The chironomids from the benthic communities of Iskar River were represented by 17 species (taxa) within the category of very rare (pF <5%), (Appendix 3). Of these, *D. tritomus*, *E. gracei*, *Cr. gr. algarum*, *Cr. algarum* and *G. gr. cauliginellus* dominated. The last species had a specific meaning as in the few cases where it was found, it had a relatively high degree of dominance (Dt = 50%). The rare species (pF = 5-20%) were *Cr. defectus*; *Cr. gr. defectus* and *T. gregarius*. As frequent (pF=20-50%) found species was determined only *Ch. plumosus* (incl. *Ch. gr. plumosus*). One taxon, defined as very frequent (PF = 60,2%) was *Ch. riparius* (*incl. Ch. gr. riparius*). It was essential for the benthic communities, because in most cases it was a dominant (Dt = 54,7%).

The importance of frequency and dominance of different hyporheic chironomids was almost identical to that of their benthic analogues. All very rarely found taxa in hyporheal (pF <5%) had the same frequency in surface habitats. These were a total of 10 species. Of these, *D. tritomus* was characteristic with a high degree of dominance (Dt = 69,9%). The rare species (pF = 50-20%) were 5. All of them were dominants. As frequent found taxa were determined *Ch. plumosus* (*incl. Ch. gr. plumosus*) (pF = 30,2%) and *Ch. riparius* (*incl. Ch. gr. riparius*) (pF = 32,8). The last taxon was important for hyporheic communities, as most cases it was a dominant (Dt = 42,3%).

Density

Nematode – complex

River benthal. According to data from quantitative benthic samples, providing a total of 119 individuals, the average density of free living nematodes in the benthal of Iskar River catchment was determined on 13 ind. m^{-2}.

Seasonal fluctuations in density (Fig.2a) were very typical: the lowest number was registered during the summer months of the three studied years (2004 – 5 ind. m^{-2}, 2005 – 3 ind. m^{-2}, 2006 – 6 ind. m^{-2}) and during the autumn months the highest number was detected (2004 – 25 ind. m^{-2}, 2005 – 18 ind. m^{-2}). The minimum density of nematode complex in summer 2005 was associated with negative impact of high current velocity and anomalous large water discharge established in this period.

The wide fluctuations in nematodes density was observed along the river length (Fig. 2b). This was determined mainly by the processes of river pollution and self-purification. In the mountain area (st. 1-3), where the anthropogenic impact on the river ecosystem was not identified (Kenderov *et al.* 2008), the density of nematodes was low (between 1 and 8 ind. m^{-2}). The presence of greater amounts of organic matter below the town of Samokov (st. 4) determined an increasing density of free-living nematodes (average 13 ind. m^{-2}). A high organic loading (Kenderov, Yaneva 2009) downstream Sofia (st. 10) generated an opposite trend in the quantitative parameters of bottom nematodes. The nematode complex was excluded from benthic communities. In this site only one species – *E. carteri*, represented with 14 individuals in one qualitative sample was found (spring 2004). Despite of the self-purification processes in the middle part of Iskar River, the density of nematodes complex was remained relatively low, which could be partly explained by toxic impact of heavy metals in this sector (Parvanov *et al.* 2008). Only in the lower river sector, where the ecological situation was improved, and the heavy metals concentrations were minimal, the highest densities of bottom nematodes were observed. At st. 16 the density reached its maximum – average 80 ind. m^{-2}. In the last sampling site (st. 20) the very low values were determined (only 2 ind. m^{-2}) but this was probably due to unstable hydrological regime from the effect of Danube River.

Hyporheal. In the present study a total of 1300 individuals of hyporheic nematodes were collected. This number corresponded to a density of 11 ind./sample. During the different studied periods the average density of hyporheic nematode complex varied less than the benthal nematode complex (Fig. 2c). In 2004 the density was determined between 8 ind./sample (summer) and 16 ind./sample (autumn). In 2005 the density was detected between 9 ind./sample (spring) and 16 ind./sample (autumn). The highest average density was found in summer 2006 – 21 ind./sample. As opposed to the nematodes inhabiting river benthal, during the period of extremely discharges (summer 2005), the density of hyporheic nematodes was not diminished. On the contrary, the increase in density compared to spring 2005 was observed. It could be assumed that part of the benthic nematodes penetrate more deeply into the river sediments in order to avoid negative conditions and to find more stable environment as the hyporheal represents.

Along the river length, the dynamics of hyporheic nematodes density almost repeated the quantitative changes in benthic nematode complex (Fig. 2d). Again in the mountain river sector (st. 1-3) the density (average 9 ind./sample for each station) was determined. In the presence of larger quantities of organics in hyporheal downstream the town of Samokov (st. 4) the density (24 ind./sample) was increased. The pollution found after Sofia (st. 10) (Kenderov, Yaneva 2009) reflected in sharp density decrease – 1 ind./sample. As with nematodes from the river benthal, the density of hyporheic nematodes was increased on the below-lying sites. It reached a

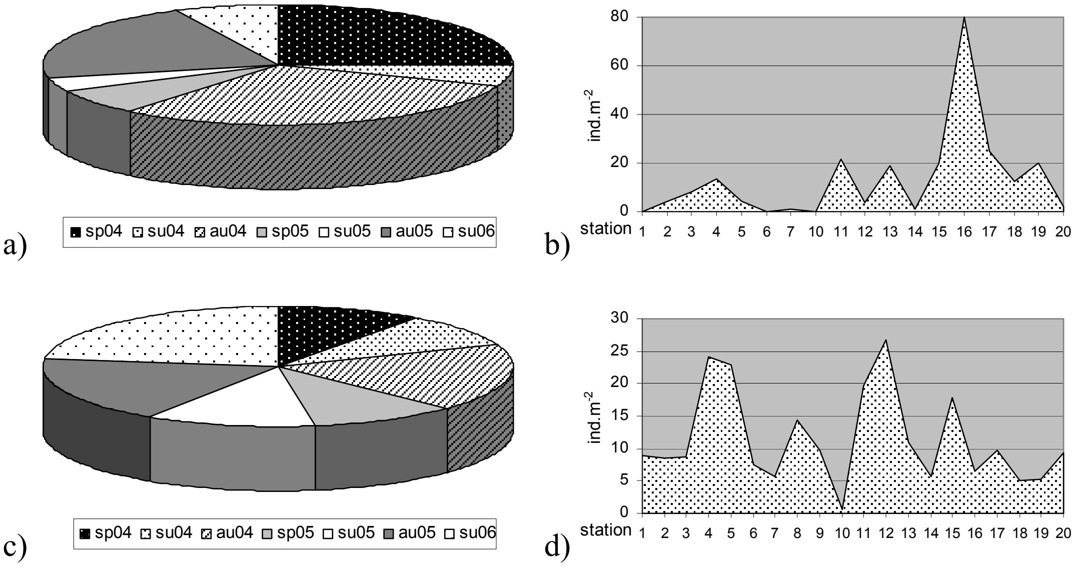

Fig. 2. Density of benthal and hyporheal nematodes in Iskar River catchment during the entire study period (2004-2006). Macrozoobenthic nematodes: a) during the different seasons b) along the river length. Hyporheic nematodes: c) during the different seasons d) along the river length. Legend: sp= spring; su= summer; au= autumn, 04= 2004, 05= 2005, 06=2006.

maximal value at st. 12 (27 ind./sample). This fact is interesting because at this point the high concentrations of some heavy metals were determined because river sediments accumulation from decades (Parvanov *et al*. 2008). Here, unlike all other sampling sites, the nematodes were the best performing group in quantitative aspect, although that in the entire catchment they were not as important as water mites; chironomids; oligochets, and Cyclopoida, that have several times higher density (Kenderov, Apostolov 2008). In the downstream sector of river the variations of quantitative parameters of the nematodes were smaller, average between 10 ind./sample (st. 17) and 5 ind./sample (st. 18, 19). Unlike the benthic nematodes, the density of this group in hyporheal increased at st. 20 (9 ind./sample). This fact is explained by the greater stability of the hyporheic biotope and the smaller fluctuations in its hydrological regime.

Chironomidae- complex

River benthal. For the entire study period a total of 7611 individuals of chironomid larvae were collected with quantitative benthal samples. The average density of the group was 485 ind. m^{-2}. During different studied periods the density varied significantly (Fig. 3a). For 2004, the lowest density was determined during summer (677 ind. m^{-2}) and the highest – during spring (813 ind. m^{-2}). For 2005 the highest density was registered in autumn (259 ind. m^{-2}) and the lowest in summer (97 ind. m^{-2}). During summer 2006 the highest density for all investigated periods was detected – 819 ind. m^{-2}. The observed minimal density during summer 2005 was explained (as with Nematoda) with the negative impacts of high flow velocity and anomalous large water quantities.

Along river length the large fluctuations in the number of chironomids were observed (Fig. 3b). At some stations, located in the mountain area, the low density (st. 3 – 171 ind. m^{-2}, st. 6 – 131 ind. m^{-2}) was detected. The highest density in this river sector was determined on st. 4 (946 ind. m^{-2}), where our previous studies showed local increase in organic loading (Kenderov *et al*. 2008, Kenderov, Yaneva 2009). The middle sector of river was characterized by the greatest dynamics of this parameter. At the site downstream Sofia (st. 10) where the organic loading was maximum, the highest density of chironomids was reported (2813 ind. m^{-2}). This high density was primarily due to abundance of *Ch. riparius*.

On the downstream-located sites, a significant reduction of density (st. 11-12) up to the complete disappearance (st. 13) of benthic chironomids was determined. The specific dynamics in this sector was due to two opposing processes, on the one hand – improving the environmental conditions, and – the potential depression of chironomid larvae from the toxic effects of high concentrations of some heavy metals. In this aspect, the chironomid larvae were proving much more sensitive than the representatives of Class Nematoda. On the following sites in the middle sector (st. 14-15) the number increased several times. This coincided with a gradual decrease downstream (dispersion, chemical bonding) of heavy metals concentrations (Parvanov *et al*. 2008). The values of density in most sites of the lower sector remained similar. An exception was observed at st. 17 where the substrate was sandy and at the area of the river mouth (st. 20), where the large fluctuations in the water regime happened. At these two sites one of the lowest values were detected (111 ind. m^{-2}, resp. 17 ind. m^{-2}).

Hyporheal. A total of 3357 individuals of chironomid larvae from hyporheic biotope were collected (average density of 29 ind./sample). The fluctuations in different seasons did not coincide with those established for their macrozoobenthic analogues (Fig. 3c). The lowest density was detected during spring seasons of 2004 and 2005 (9 ind./sample), and the highest – during autumn periods (73, resp. 27 ind./sample).

The dynamics of hyporheic chironomids density along the river was opposite to that established for benthic ones (Fig. 3d). For example, the largest average density was not registered in the most polluted river site but in anthropogenically unaffected upstream sector – maximum at st. 1 (137 ind./sample) and at st. 5 (95 ind./sample). Even relatively not so high anthropogenic loading downstream the town of Samokov (st. 4) caused a significant reduction in the density of hyporheic chironomids – 16 ind./sample (for st. 4) compared to 52 ind./sample for the upstream located at st. 3. The extremely deteriorating ecological state downstream Sofia City (st. 10) caused even greater reduction in density, as here the minimum value for the entire catchment was determined – 2 ind./sample. At other sites from the middle river sector, the hyporheic chironomid complex did not success to substantially recover its density. This was registered until for the latest sites downstream. The maximum density (21 ind./sample) was reached at the latest sampling site (st. 20), although the one of the lowest densities for benthic representatives was detected here. It could be assumed that this is possibly due to penetration of benthic representatives of the group deeply into the river sediments, where conditions are relatively more stable.

Discussion

Two investigated taxonomic complexes had different contribution to formation of benthic and hyporheic communities of Iskar catchment. The free-living nematodes were poorly presented in both qualitative and quantitative aspects. In contrast, the chironomids had a high frequency and dominant role in riverine and hyporheic communities.

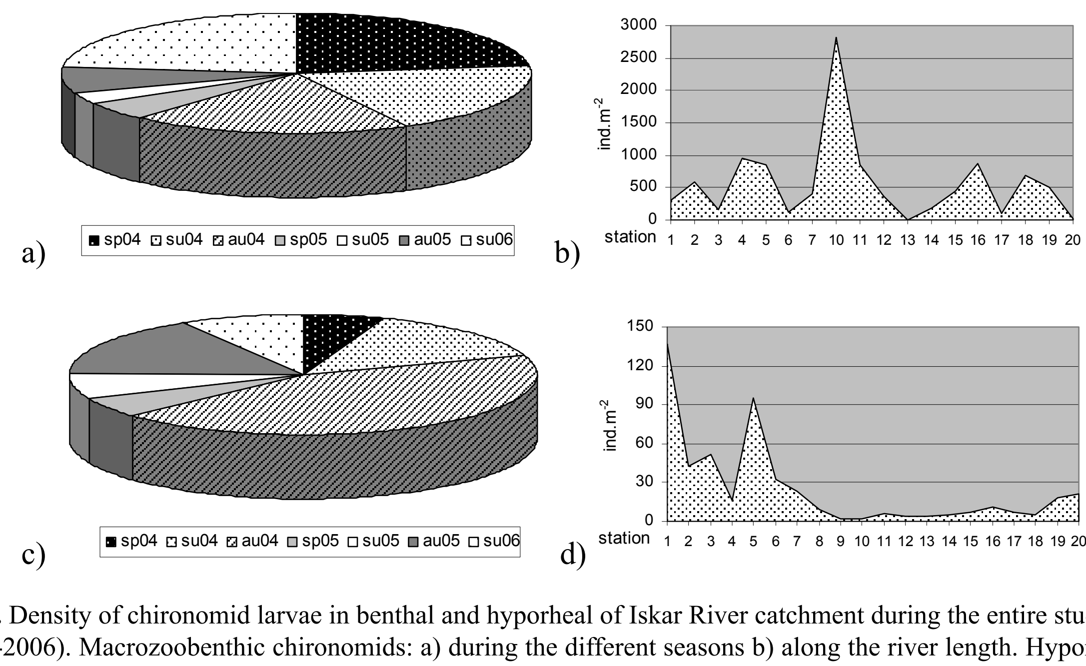

Fig. 3. Density of chironomid larvae in benthal and hyporheal of Iskar River catchment during the entire study period (2004-2006). Macrozoobenthic chironomids: a) during the different seasons b) along the river length. Hyporheic chironomids: c) during the different seasons d) along the river length. Legend: see Fig. 2.

The similarity analysis showed that the main part of species from family Chironomidae was found in both biotopes. The probable reason is that the chironomids belong to the transient hyporheos (hyporhéos temporaire) and have a need of relation with surface habitats. Contrary, the hyporheic nematodes are more detached and they are persistent habitants of hyporheal (hyporhéos permanent) without a need of direct contact with surface.

The stress factors as accidental floods (summer 2005) or dynamic hydrological regime (the last sampling sites above the river mouth in Danube) affect in opposite way of benthic either hyporheic complexes. The benthic nematodes and chironomids drastically decrease their density but the hyporheic ones increase their density. It may be a result of migration from surface to hyporheal, as suggested at similar situation from Griffith & Perry (1993).

The pollution and self-purification processes determined with a great significance the dynamics of quantitative parameters of studied complexes. In conditions of insufficient trophic sources in mountain river sector, the nematodes and the chironomids from both habitats had relatively low density. At maximal organic loading (downstream Sofia) the remarkable difference between the river and hyporheal was distinguished. The presence of unlimited trophic source in river ecosystem allowed augmentation of some eurybiontic chironomids in high densities. Contrary, in hyporheic biotopes of this river sector only the single individuals were found and the nematodes were completely eliminated. The probably reason may be the depletion of oxygen from organic decay.

Acknowledgements: The authors express their great appreciation for the help in field and laboratory work, and some valuable advices in preparing the manuscript of: assist. prof. Y. Todorova, PhD, D. Parvanov, PhD (Biology Faculty, Sofia University); M. Pavlova, PhD student, S. Kazakov, PhD student, and assist. prof. Y. Presolska (Institute of Biodiversity and Ecosystem Research, BAS). The authors thank the reviewer for providing useful comments and suggestions.

References

Cvetkov L. P. 1965. Groundwater Isopoda from Trace. – In: *Fauna of Trace*. **II**. Sofia, Bulgarian Academy of Sciences. 311-317. (In Bulgarian).

Cvetkov L. P., A. N. Petrova. 1987. Stygobiomonitoring. *Questionaries of fone ecological monitoring.* Second school of biological monitoring. 15-17. II. 1984. BAS, 165-170. (In Russian).

Kenderov L. 2010. Composition, distribution and transfer of hyporheic community from Iskar River catchment in relation of ecological state of the river. PhD Thesis, Sofia University 'St. Kliment Ohridski'. 258 p.

Kenderov L. A., A. M. Apostolov. 2008. Composition and structure of hyporheal crustacean complex of Iskar River Valley in different environmental conditions. – *Natura Montenegrina*, **7** (2): 145-167.

Kenderov L. A., I. Y. Yaneva and M. N. Pavlova. 2008. Ecological assessment of the upper stretch of the Iskar River based on selected biological parameters in conformity with the Water Frame Directive 2000/60/EU. – *Acta Zooogica Bulgarica*, Suppl. **2**: 255- 268.

Kenderov L. A., I. Y. Yaneva. 2009. Ecological characteristics of the Iskar River catchment. – *Biotechnology & Biotechnological Equipment*, **23**: 276- 280.

Kozhova, O. M. 1970. Formation of phytoplankton in Bratsko Reservoir. – In: Formation of Environmental Conditions by Bratsko Reservoir, 26-161. Moscow. (In Russian).

Parvanov D. A., Y. I. Topalova and L. A. Kenderov. 2008. Iskar River: heavy metals in waters, sediments and hyporheic zone. – *Ecological Engineering and Environmental Protection*, **4**: 4-11.

Russev B. 1959. Influence of industrial wastewaters on life in Arda River and Iskar River. – *Priroda*, **4**: 51-55. (In Bulgarian).

Russev B. 1968. Saprobiological assessment of some Bulgarian rivers. – *Bulletin of Zoological Institute with Museum*, BAS, **27**: 141-156. (In Bulgarian).

Russev B. (Ed.). 1994. Limnology of Bulgarian Danube tributaries. Sofia, Book Tiger, 255 p. (In Bulgarian, German summary).

Stoichev S. A. 1994. Contribution to the study of Chironomid fauna (Diptera, Chironomidae) from Bulgarian part of Danube River and its tributaries. Frequency of presence and dominant analysis on the founded species. – *Hydrobiology*, BAS, **39**: 91-101. (In Bulgarian).

Stoichev S. A. 1996a. On the Chironomid fauna from Bulgarian inland waters. – *Lauterbornia*, **25**: 117-123.

Stoichev S. A. 1996b. On the free-living nematode fauna from Bulgarian inland waters. – *Lauterbornia*, **25**: 23- 30.

Stoichev S. A. 1999. Contribution to the study of the free-living nematode fauna (Nematoda: Nematelminthes) from surface and groundwaters in the catchment area of Iskar River and Struma River. – *Acta Zoologica Bulgarica*, **51**: 25-33.

Received: 05.01.2012
Accepted: 06.06.2012

Appendix 1. Timetable of sampling periods and studied sites.

Field trip	Stations
23.XII.03 – 25.II.2004	1-7
20.IV – 23.VI.2004	1-12, 14-20
13.VII – 18.VIII.2004	1-12, 14-20
13.XI – 21.XI.2004	1-12, 14-20
19.IV – 17.V.2005	1-12, 14-20
16.VIII – 17.IX.2005	1-12, 14-20
11.XI – 26.XI.2005	1-12, 14-20
11.VIII- 19.VIII.2006	3, 4, 11-13, 15, 19
21.VIII- 25.VIII.2009	7, 10, 13, 14, 19, 20

Appendix 2. Species composition, frequency of occurrence (pF,%) and range of dominance (Dt,%) of Nematoda complex from benthal and hyporheal of Iskar River and tributaries. With (*) are marked first record for Iskar River fauna, with (**) are marked first record for Bulgarian Danube tributaries fauna.

NEMATODA	Benthal			Hyporheal		
Taxon	**Stations**	**pF,%**	**Dt,%**	**Stations**	**pF,%**	**Dt,%**
Aporcelaimidae						
* *Aporcelaimellus obtusicaudatus (*Bastian 1865)	not found			1619 20	2.6	-
Diplogastridae						
Diplogaster rivalis (Leydig 1854)	14 17	1.1	-	not found		
Dorylaimidae						
Epidorylaimus agilis (de Man 1880)	not found			1 2 18	6.9	-
** *Eudorylaimus iners* (Bastian 1865)	15	0.6	-	not found		
Dorylaimus montanus Stefanski 1923	4 7	1.7	-	not found		
Dorylaimus paradoxus Eliava 1967	not found			2 3 4 15	10	-
Dorylaimus stagnalis Dujardin 1845	1-5 11 14-19	15.9	-	1 2 4 6 – 20	62.9	38,3
Dorylaimus sp.	14			not found		
Mesodorylaimus centrocercus (de Man 1880)	1	0.6	-	4	0.9	-
Eudorylaimus carteri (Bastian 1865)	4 10	1.1	-	not found		
Monhysteridae						
Eumonhystera filiformis (Bastian 1865)	19 20	1.1	-	not found		
Monhystera sp.	2 5 16			not found		
Monhystera stagnalis Bastian 1865	2 5 15 17	2.8	-	2 4 7 11 12 18	8.6	14,0
Panagrolaimidae						
Panagrolaimus hydrophilus Bassen	16	1.1	-	not found		
Qudsianematidae						
Thornia steatopyga (Thorne & Swanger 1936)	12	1.7	-	11	1.7	-
Tobrilidae						
Tobrilus gracilis (Bastian 1865)	4 11 16 18 19	5.7	-	not found		
Tobrilus sp.	18			not found		

Appendix 3. Species composition, frequency of occurrence (pF,%) and range of dominance (Dt,%) of Chironomidae (Diptera) complex from benthal and hyporheal of Iskar River and tributaries. With (*) are marked new record for Iskar River fauna.

Chironomidae (Diptera)	Benthal			Hyporheal		
Taxon	**Stations**	**pF,%**	**Dt,%**	**Stations**	**pF,%**	**Dt,%**
* *Acricotopus lucens* (Zetterstedt 1850)	1 14 17 19	2.3	-	13	0.9	-
Acrycotopus sp.	14			not found		
* *Beckidia zabolotzkyi* (Goetghebuer 1938)	14	-		not found		
Chironomus (Chironomus) plumosus (L. 1758)	12 20	22.2	23.0	7-9 10-12 15-19	30.2	20.0
Chironomus (Ch.) gr. plumosus (L. 1758)	2 4-7 10-12 15 16 19 20	22.2	23.1	4 5 7 12 16-19	30.2	20.0
Chironomus (Ch.) riparius Meigen 1804	1-7 11 12 14-20	60.2	54.7	1-7 11 14-17	32.8	42.3
Chironomus (Ch.) gr. riparius Meigen1804	1-5 7 10 11 14-20	60.2	54.7	1 3 5 18	32.8	42.3
Chironomus sp.	3 4 15 18			6 15 17 19		
Cricotopus (Cricotopus) algarum (Kieff. 1911)	18	1.7	33.3	not found		
Cricotopus (Cr.) gr. algarum (Kieffer 1911)	3 6	1.7	33.3	not found		
Cricotopus (Isocladius) sylvestris (Fabr.1794)	1 4 6 14 18	4.6	-	not found		
Cricotopus (I.) gr. sylvestris (Fabr. 1794)	4 7 19	4.6	-	1 3	1.7	-
Cricotopus sp.	3 5 17 18			not found		
Cryptochironomus defectus (Kieffer 1913)	3 4 6 14 15 18 19	15.9	35.7	4 7 11	9.5	31.8
Cryptochironomus gr. defectus (Kieffer 1913)	2 4-7 11 14 16-18	15.9	35.7	2-5 16	9.5	31.8
Cryptochironomus sp.	7 11 18			not found		
* *Demicryptochironomus vulneratus* (Zett.1838)	3 5	1.1	-	not found		
Dicrotendipes gr. nervosus (Stæger 1839)	12 19	1.7	-	not found		
Dicrotendipes tritomus (Kieffer 1916)	14 16 18	2.8	20.0	3	0.9	69.9
Endochironomus tendens (Fabricius 1775)	not found			5 11 12 15	3.5	-
Eukiefferiella gracei (Edwards 1929)	1 4 15 18 20	4.6	25.0	2 5 6	7.8	7.8
Eukiefferiella similis Goetghebuer 1939	not found			6	3.5	-
Eukiefferiella cf. similis Goetghebuer 1939	1 2 3 4	4.6	-	1 4	3.5	-
Eukiefferiella sp.	1 18			not found		
Glyptotendipes (G.) cauliginellus (Kieff.1913)	11 12	1.1	50.0	19	0.9	-
Glyptotendipes glancus (Meigen)	16 19	0.6	-	2	0.9	-
Larsia curticalcar (Kieffer 1918)	3 13	1.1	-	1 2 14	2.6	23.3
Larsia gr. curticalcar (Kieffer 1918)	18	1.1	-	not found		
Metriocnemus (M.) hygropetricus (Holm. 1883)	3	0.6	-	not found		
* *Microtendipes gr. chlorus* (Meigen 1818)	20	-	-	not found		
* *Microtendipes pedellus*(De Geer 1776)	20	-	-	not found		
Prodiamesa olivacea (Meigen 1818)	4 19	1.7	-	not found		
Prodiamesa sp.	4	-		not found		
Tanytarsus gregarius Kieffer 1909	1-5 7 12 15-19	19.9	40.0	1 2 7 8 11 20	14.7	24.7
Tanytarsus gr. gregarius Kieffer 1909	19	-		1-3 11 15 16 19 20	14.7	24.7
Tanytarsus sp.	1 4 15 16	-		not found		
Tvetenia calvescens (Edwards 1929)	2 16	1.1	-	3	0.9	-
Tvetenia sp.	1	-		not found		