



## COMPOSITION AND STRUCTURE OF HIPORHEAL CRUSTACEAN COMPLEX OF ISKAR RIVER VALLEY IN DIFFERENT ENVIRONMENTAL CONDITIONS

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### Key words:

Crustacea,  
hyporheal,  
taxonomic composition,  
ecological state,  
Iskar river- Bulgaria

### SYNOPSIS

This work investigated the crustacean hyporheic complex of the Iskar River and its tributaries Beli Iskar, Cherni Iskar, Vedena River. During the study 43 different (sub)species taxa of crustaceans were found. Twenty-one (sub) species taxa were reported for the first time in the Iskar catchment. The orders *Harpacticoida* and *Cyclopoida* showed the most number of taxa – each of them with 14 (sub)species; while the class *Ostracoda* was presented with 8 species. The main part of them is stygoxenes or stygophiles and probably come from the meiobenthic complex of the river benthos.

The data for total abundance, number of individuals were reported and the dominance analysis was done according to DE VRIES, 1937. The faunal similarity with previous investigated phreatic crustacean in Iskar valley was compared according to SÖRENSEN, 1948.

The most species (and the highest abundance) were found in mountainous part of Iskar catchment, where environmental conditions were optimal. In the section of Iskar River behind the Sofia city, the high anthropogenic influence probably affected on crustacean abundance – the complex was presented with few species in a limited number. In the lower river sectors, the self-purification processes were observed and the hyporheic crustaceans tried to restore.

## INTRODUCTION

The stygobiological studies of underground waters in Bulgaria had been initialized in 1963 with a study on the Thracian fauna organized by the Institute of Zoology (CVETKOV and PETROVA, 1965). For a period of 20 years the stygobiological group achieved to characterize the main hydrogeological regions in the country with the emphasis to the phreatic waters (eustygal) (CVETKOV, 1972; CVETKOV et al., 1976, 1982; PETROVA et al., 1986). These studies referred to the fauna of the hyporheal waters although they were supporting and complementary. In spite of the sufficient factual material (CVETKOV, 1975) and the recent studies of this specific biotope (PANDOURSKI, 1994b, 2000; PANDOURSKI and APOSTOLOV, 2005) there is still lack of complete investigation of the catchments of a big river from its mountain part to its mouth, which to be supported with a study of the main environmental factors.

Our main objective during the last years (2004- 2006) is to study in deep the hyporheal biocenosis of Iskar River. This work presents some of the most important results on the hyporheal crustacean complex and the ecological factors in the hyporheal and the river.

## STUDY AREA

Iskar River is the longest Bulgarian river (401km). It is on third place according to catchment area (8647 km<sup>2</sup>). The river springs on northeast from Damga peak (Northwest Rila Mountain) at 2500 m altitude. Starting from the high alpine zone of Rila Mountain, the river passes consecutively through Valley of Samokov, Gorge of Pancherevo, Valley of Sofia, Gorge of Stara planina, Predbalkan heights and Danube plain. It flows into the Danube near to Gigen village at 25 m altitude.

The first studies of the underground fauna of Iskar river were performed in 1966-67 and 1973- 74 by CVETKOV et al. (1976). They investigated the underground waters in the middle and down stream of the river and the hyporheal biotope. Forty-one (sub)species crustacean, two species water mites and three species *Gastropoda* have been identified. The cyclopoids - 11 (sub)species, harpacticoids -14 (sub)species and the *Malacostraca*, especially genus *Niphargus* (*Amphipoda*) were predominant. The authors underlined the poor presence of class *Ostracoda*, presented with only 4 species. A zoogeographic characterization had been made on the basis of crustacean distribution through the river length. According to it, Iskar Valley had been classified to the west part of Eastern Balkans with some specific particularities. *Microcerberus phreaticus* Cvetkov 1963, *Parabathynella stygia* Chappuis 1926 and *Microcharon bureschi* Cvetkov 1976 are the species characterizing in best way the phreatic fauna. The last species is the most important, as it was not found out of Iskar Valley.

The recent studies of PANDOURSKI (1994a, 1994b, 2000 and unpubl. data, personal communication) on underground water *Cyclopoida* included Valley of

Sreden Iskar. Using the method of Karaman- Chappuis, the author studied the river banks of the main river and 9 tributaries. As a result, 19 (sub)species cyclopoids, 8 species of order *Harpacticoida*, some representatives of *Syncarida* (not det.), *Ostracoda* (not det.), *Niphargus sp.*, *Microcerberus phreaticus*, and representatives of macrozoobenthic complex have been identified.

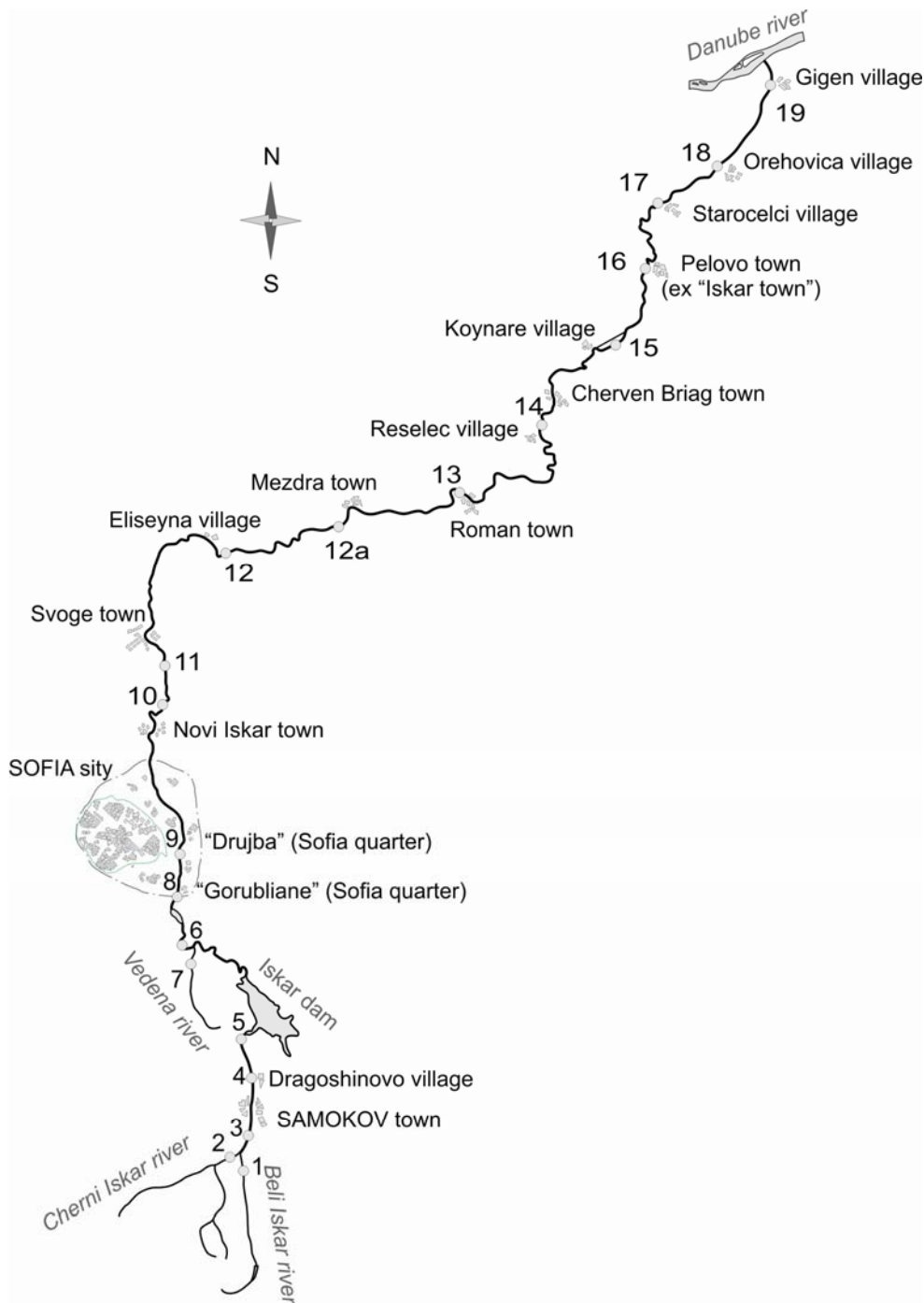


Fig.1: Map of sample sites.

The preliminary investigations of the composition and the distribution of hyporheal biocenosis of Iskar valley consisted of an analysis of the ecological (saprobiological) statement of the fluvial ecosystem (KENDEROV et al., in press – a, KENDEROV et al., in press – b). On the other side, a study of the pollution of Sreden Iskar with petroleum products, organic compounds, heavy metals, etc. (TOPALOVA et al., 2005b; PARVANOV et al., in press), as well as an analysis of the microbial complex in the water and the hyporheal sediments (TOPALOVA et al., 2005a;) and a characterization of sediments – granulometric composition, organic content, porosity etc. (KENDEROV, 2007) are also presented.

## MATERIAL AND METHODS

### 1. Sample schedule and sites

This work included 116 samples of the hyporheal of Iskar River and the tributaries Beli Iskar, Cherni Iskar and Vedena. In 99 of them crustacean were found. Samples were collected in 20 stations through the river length (Fig. 1) during the period 23.XII.2003 - 19.VIII.2006.

### 2. Hyporheal samples

The samples from hyporheic fauna were collected in three seasons (spring, summer, autumn-winter) of 2004 and 2005. Additionally, the samples from stations No 3, 4, 11, 12, 12a, 14, 18 were collected during the August, 2006. On stations 8 and 9 the sampling procedures were realized only in 2004.

Hyporheal fauna was collected with a Bou- Rouch pump (BOU and ROUCH, 1967, BOU, 1974) by depth from -30 to -40 cm (approximately). At each station 10 litre samples were taken and filtered through a 90 µm mesh net. They were fixed with a weak formalin solution. Then, in the laboratory each sample was washed, colored with eosin and inspected under magnification of 10x -20x.

### 3. Physical and hydrochemical samples

The main physical and hydrochemical parameters (temperature, oxygen content and pH) were measured “*in situ*” in each station and time point by calibrated portable instruments WTW, 330i-series. The measurements were performed immediately after the sample collection before the filtering through the mesh net.

The samples for heavy metal analyses were collected with a Bou- Rouch sampler after the pumping of first 10 liters. Each sample was fixed with 3 cm<sup>3</sup> HNO<sub>3</sub> per 1 dm<sup>3</sup> hyporheal water. The laboratory treatment and result measurement were performed with atomic absorption spectrometry AAS Perkin- Elmer 3030B. All samples were collected during May 2005, August - September 2005, November 2005, August 2006 at stations No. 3, 4, 5, 6, 10, 11, 12, 14, 18.

#### 4. Characterization of the hyporheal sediments

The samples were collected from stations No. 3, 4, 11, 12, 12a, 14, 18 in August, 2006. The core-sampler with diameter 60 mm was used. The sampler was dig mechanically and the core was obtained from the -30 -40 cm depth.

The granulometric characteristic of sediments was done by dry sieving method. The set of sieves with different pore size was selected according to the Udden-Wentworth scale for sediment classification (UDDEN, 1914; WENTWORTH, 1922).

The water contains and amount of organic matter were determined after OLSEN and TOWNSEND(2005), modifying method described in details in KENDEROV(2007).

#### 5. Hydrobiological methods

A complex of chosen hydrobiological methods was applied for assessing the basic biological parameters of hyporheic crustaceans in Iskar catchment: number of individuals (individuals/10 dm<sup>3</sup> = ind/sample), total abundance, number of taxa, occurrence and dominance after DE VRIES (1937), faunal similarity between the studied biotopes and sites after Sørensen (1948). The summarized results for saprobiological state of surface (river) waters, assessed by method of saprobic valences have been presented (ROTHSCHEIN, 1962; ZELINKA and MARVAN, 1961).

## RESULTS AND DISCUSSION

### I. Characterization of the ecological factors

#### 1. Main physical and hydrochemical indicators

##### 1.1. Temperature

The temperature of hyporheal waters varied according to the season changes in the air temperature. Along the length of the river it increased from the mountain regions to the mouth (Fig. 2). The minimal measured temperature was 0.2°C (Cherni Iskar, november 2005) and the maximal was 24.0°C (Staroselci, august 2004). Out these limit values, the variations were minimal. The average temperature during the springs was 14.5°C, during the summers- 18.1°C and autumns- 7.56°C.

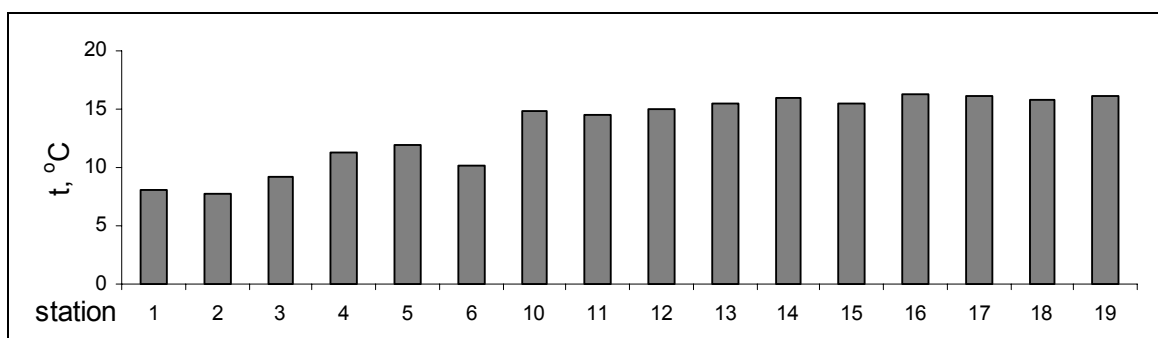


Figure 2. Temperature in the hyporheal waters (average values)

### 1.2. pH

This factor showed a significant stability (Fig. 3). There were some minimal variations during the seasons (average pH 7.4- 7.8) or on different river sectors (average pH 7.1- 7.8). Values under the neutral were measured only in station 4 (pH 6.7, may 2005) and in the region below Sofia, station 10 (pH 6.6, November 2005; pH6.7, august 2004). These data permit to define the hyporheal waters of Iskar River as neutral to poor alkaline.

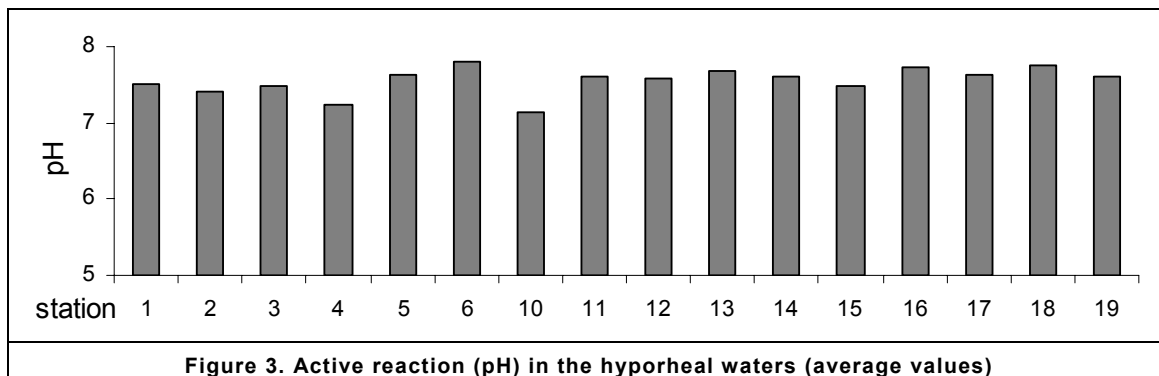


Figure 3. Active reaction (pH) in the hyporheal waters (average values)

### 1.3. Oxygen concentration

The concentration of oxygen showed significant variations in the different river sectors (Fig. 4). The stations situated above Samokov had the biggest average values for the investigated period (st.1- 8.8 mg.dm<sup>-3</sup>; st.2- 9.4 mg.dm<sup>-3</sup>; st.3- 8.3 mg.dm<sup>-3</sup>). Here, the biggest absolute value was also measured - 15.7 mg.dm<sup>-3</sup> (st.2, November 2005). After the town of Samokov, the oxygen concentration dropped suddenly (average 4.8 mg.dm<sup>-3</sup>). The studies of KENDEROV et al., (in press- a) and TOPALOVA et al., (2005a) showed the presence of a local anthropogenic influence in this region.

The lowest values of oxygen concentration were detected in the region below Sofia (st.10). The minimal value was 0.4 mg.dm<sup>-3</sup> (June 2004) but the other measurements in this station were similar (average value 1.1 mg.dm<sup>-3</sup>). These extremely low values are explained with the great organic loading below Sofia (KENDEROV et al., in press- b).

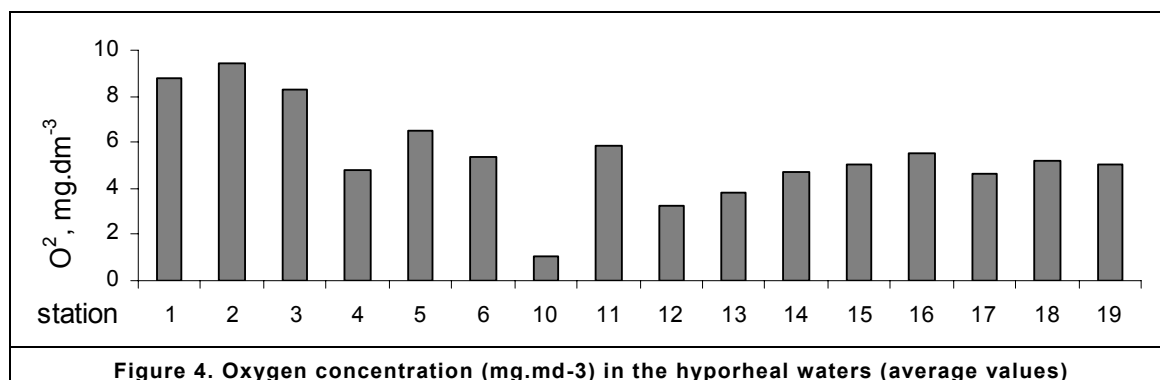


Figure 4. Oxygen concentration (mg.md-3) in the hyporheal waters (average values)

The oxygen concentration in the hyporheal waters through the stream (the length of the river) increased gradually. In spite of the presence of good self purifying processes in the fluvial system (KENDEROV et al., in press- b), this increase was not significant. In the down stream regions of the river (st.19) the average concentration was  $5.11 \text{ mg.dm}^{-3}$ . The slow speed of the current, the presence of more significant amount of fine sediments and the higher temperatures (especially during the summers) had a limiting role.

The season variations in oxygen concentration are not significant. In general, the lowest values were detected in all stations during the summers and the biggest – during the autumns. The average value for all samples, collected during the springs was  $5.6 \text{ mg.dm}^{-3}$ , during the summers –  $4.41 \text{ mg.dm}^{-3}$ , and during the autumns -  $6.41 \text{ mg.dm}^{-3}$ .

## 2. Heavy metals

The preliminary analyses on the heavy metal concentrations in the surface water, sediments and hyporheal water of Iskar River (PARVANOV et al., in press) showed a concentrated pollution in the region between Elisejna (st.12) and Reselec (st.14). The surface waters were mostly polluted below the mine complex near to Elisejna (st.12). The concentration of heavy metals in the hyporheal was several folds higher than in the river water and was detected in the lower regions (st.14).

With this work, the data for Pb, Zn and Cu concentrations are completed for two additional periods – November 2005 and August 2006 and for one additional point – Iskar River above Samokov. We present some data for another element – nickel (Ni). All results support the above conclusion. The average values (Fig. 5) for the concentration of these elements in the hyporheal water were highest in the region below Sofia (st.10 - 14). It is probably in cause of Sofia industry and the mine complex near to Elisejna. The zinc (Zn) had the highest concentration from the first stations (st.3 and 4). A maximal average value of  $292.27 \text{ mg.dm}^{-3}$  had been achieved in station 14. The concentration of Cu was also high – maximal  $97.59 \text{ mg.dm}^{-3}$  at station 12 and  $87.37 \text{ mg.dm}^{-3}$  at station 14. The content of Pb in the hyporheal waters was highest at st.10 (average  $52.37 \text{ mg.dm}^{-3}$ ). The nickel was the element with lowest concentration, varying between  $0.6\text{-}15.7 \text{ mg.dm}^{-3}$  in st.5 and 11.

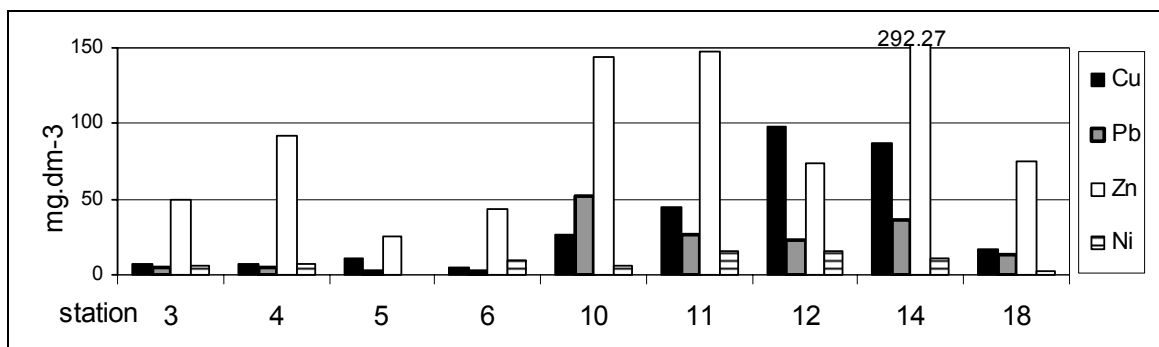


Figure 5. Heavy metal concentration (mg.dm-3) in the hyporheal waters (average values)

### 3. Characterization of the hyporheal sediments

#### 3.1. Granulometric composition and fine sediment content

The quantitative representation of different granulometric fractions of the hyporheal sediments in Iskar River was irregular. The coarse fractions (coarse and medium size gravel, 32- 16 and 16- 8 mm) were most abundant, while the fine fractions (sand 1- 0.5 mm) were rare. Their distribution along the river was different in response to the local conditions. For example, at station 3, the fraction 16-8 mm (medium size gravel) was best presented, while at station 4, where Iskar River enters Valley of Samokov and slows down its course, the fraction 1-0.5 mm (coarse sand) was well presented. The stations 14 and 18 had a similar distribution with best presented fractions – coarse sand (1-0.5 mm) and medium sand (0.5-0.25 mm). In spite of some existing variations, the average particle diameter showed a tendency to decrease along the river (Fig. 6). At first station (above Samokov) it was 3470.2  $\mu\text{m}$ , while at last station (near to Orehovica) – 2699.9  $\mu\text{m}$ .

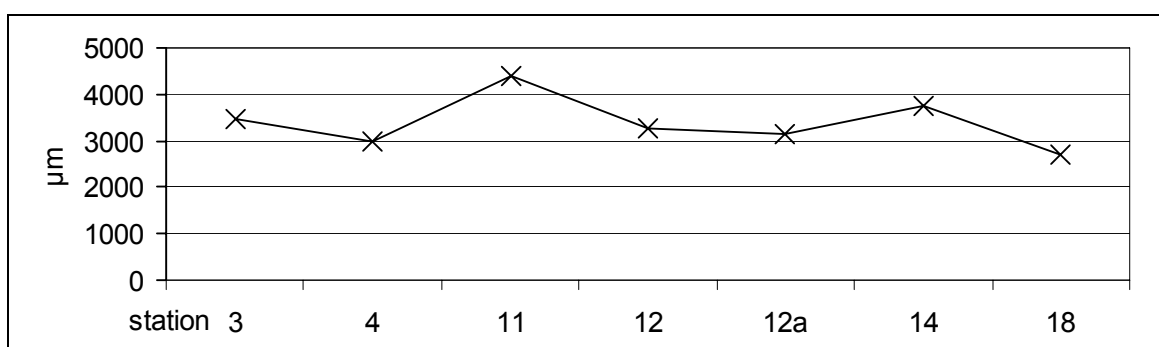


Figure 6. Average particle diameter of the hyporheal sediments in Iskar River

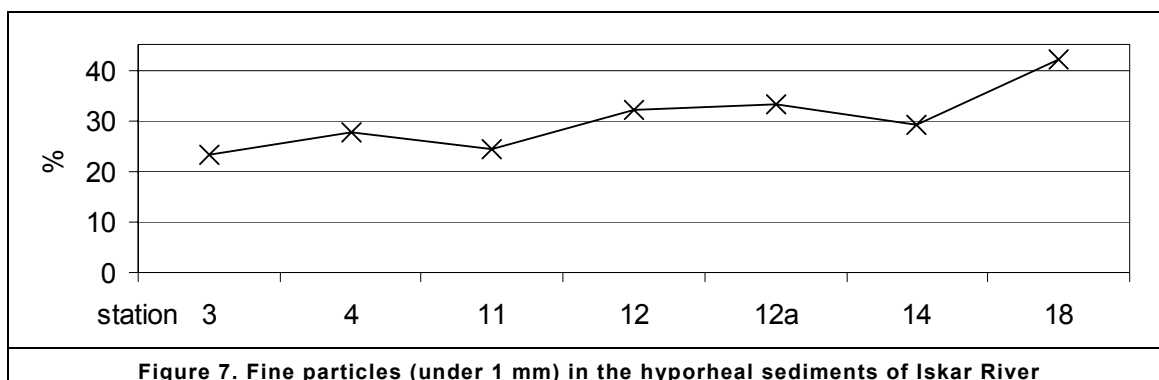


Figure 7. Fine particles (under 1 mm) in the hyporheal sediments of Iskar River

Our study showed gradually decrease in the particle diameter through the river length. The amount of fine particles (under 1 mm) increased twice (Fig. 7). At the station near Samokov, the particles with grain size < 1 mm were 23.35%, while near to Orehovica they were 42.21%. This gradual increase of the fine particles could be explained by the natural processes of fragmentation of coarse particles during their course downstream and by the higher sedimentation degree in the regions with slower stream speed (st.18).

### 3.2. Porosity

The pore space, measured indirectly by the water content in the sediments decreased through the river length (Fig. 8). It was highest in the sediments at the stations above Samokov (28.38%) and Dragoshinovo (28.75%) and was lowest near to Orehovica (21.63%). This decrease was in correlation with the increased content of fine sediments (under 1 mm).

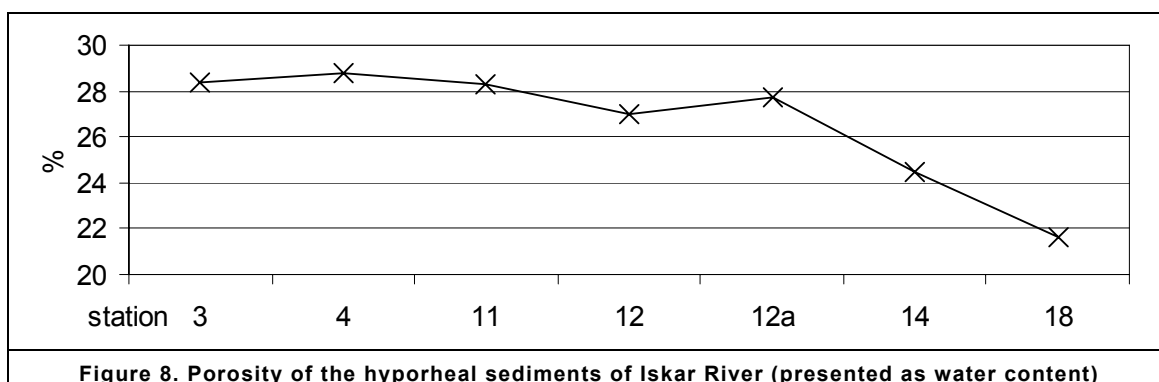


Figure 8. Porosity of the hyporheal sediments of Iskar River (presented as water content)

### 3.3. Organic compounds

The highest organic content was measured by the "ash and weight" method in the sediments of Middle Iskar (3.05- 3.63%) (Fig. 9). The reason for this is the big organic loading of the river, including the alluvial sediments (KENDEROV et al., in press- b, TOPALOVA et al., 2005a, 2005b). The lowest value was measured at station 3 (0.85%) and station 4 (0.70%). In spite of detected organic loading below the town

of Samokov (st.4), there was not significant collection of organic compounds maybe as a result of the high metabolic activity of microorganisms (TODOROVA and TOPALOVA, 2005).

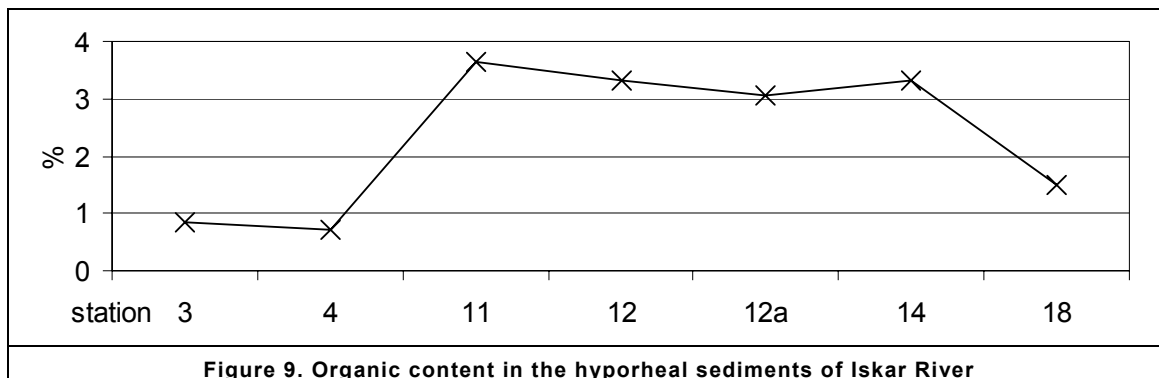


Figure 9. Organic content in the hyporheal sediments of Iskar River

#### 4. Ecological state of surface waters

The study of saprobiological (ecological) state of surface (stream) waters of Iskar catchment (details in KENDEROV et al., in press- a, KENDEROV et al., in press- b) was done simultaneously with the analysis of the hyporheal biotope. This gives a reason for doing a comparison between the river state and its influence on the situation in hyporheal.

The montaneous region of Iskar River was in the best ecological status (Fig. 10). As a tendency, the saprobic index of Rothschein was between oligosaprobity to  $\beta$ -mesosaprobity. A xenosaprobity was found in several cases only in springs samples (Sr = 83.23, may 2005, st. 3 etc.). A local disturbance in the saprobic status was detected below Samokov (st. 4), where a  $\beta$ - or  $\alpha$ -mesosaprobity was found (Sr = 31.99 – 51.99). In addition of the organic loading from the town, the slow speed of the stream in the Valley of Samokov and the unsuitable for the macrozoobentic cenosis bottom substrate – sand, exercised important influence. At the next station (st. 5), the self purification processes in the river restored the status to oligosaprobity or  $\beta$ -mesosaprobity.

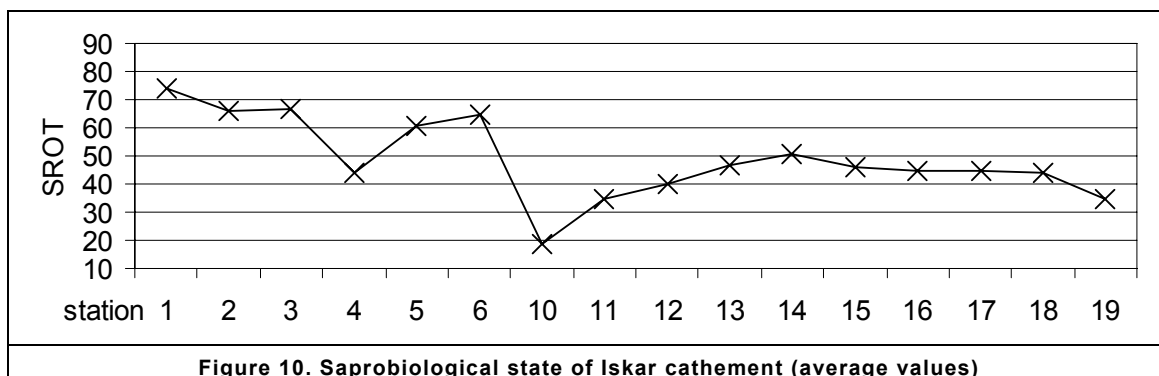


Figure 10. Saprobiological state of Iskar catchment (average values)

The city of Sofia had a negative influence on the ecological state of Iskar River. As a result of the significant organic loading, the saprobic index showed polysaprobity or  $\alpha$ -mesosaprobity ( $Sr = 16.25- 20.07$ ) downstream of Sofia city (st. 10). The self purification processes and the positive influence of the mountainous tributaries of Stara Planina contributed to improve the ecological state. Even at the next station (st. 11)  $\alpha$ -mesosaprobity was detected. At Elisejna (st. 12) the saprobic index was between  $\alpha$ - and  $\beta$ -mesosaprobity. At the next stations (st. 13 and 14) the river was in stable climax state of the benthic community. For the whole period, the saprobic index of this region was in the limits of  $\beta$ -mesosaprobity ( $Sr = 41.69$  to  $55.37$ ).

In its lower part Iskar River showed unstable ecological status. The saprobic index was varying between  $\beta$ - and  $\alpha$ -mesosaprobity with a tendency to decline to the last stations. Near the village of Gigen (st. 19) the saprobic index showed worse  $\beta$ -mesosaprobity and during the April 2004 and May 2005 it was at the limit between  $\alpha$ -mesosaprobity and polysaprobity ( $Sr = 20.00$  and  $21.71$ ). The slow speed of the stream, the high temperatures (especially during the summer months), the big depth and the unsuitable substrate (sand, gravel and silt) had a negative influence on the self-purification processes of Iskar River in its lower part.

#### **5. Iskar catchment according to the ecological factors**

The above data for the main physical and hydrochemical factors of the hyporheal water, the granulometric characterization, the content of organic compounds and the sediment porosity, together with the results over the ecological state of Iskar catchment, permit to divide Iskar catchment in several different regions:

##### **Upper stream of Iskar River (from the mountainous region to Sofia)**

Iskar River and its tributaries Cherni Iskar, Beli Iskar and Vedena are not anthropologically affected or are weakly affected. The granulometric composition of the hyporheal sediments is of coarse particles, especially of gravels. This suggests good porosity, and good circulation of the hyporheal water. The circulation assures enough oxygen for the hyporheal organisms and for the oxidation of the organic compounds. The sole exception is the region below Samokov, where the river is affected by the town. In Valley of Samokov the river slows its current rate, which results in the accumulation of finer sediments (mainly sands). However, the porosity remains higher and assures good oxygen content. The organic content of the sediments remains low, as a result of the intensive microbial activity and the presence of enough oxygen.

##### **Middle part of Iskar (from Sofia to Reselec)**

The strong organic pollution below the city of Sofia reflects in organic loading of the sediments. Maybe this is the reason for the intensive decomposition processes, which exhaust the oxygen content. The good porosity of the sediments in this region has a negative role – it allows the infiltration and the retention of coarse organic particles, which in addition deteriorates the conditions. The situation becomes worse

after Elisejna, where high content of heavy metals was detected. This state retained in the whole middle part of Iskar River independently of the intensive self-purification processes at the last stations in the sector.

### **Down stream of Iskar River (from Reselec to the flow in Danube)**

In spite of the slow current rate and the higher summer temperatures, self-purification processes were found in Iskar River after its leaving from Gorge of Stara Planina. The oxygen content in the hyporheal water and the degradation of the organic compounds are good. A potential limiting factor is the high content of fine sediments (under 1 mm), which are in negative correlation with the interstitial spaces (porosity). This limiting role does not affect water circulation in the sediments (since the oxygen is abundant and the organic compounds are not deposited) but affect the organisms in physical way (lack of interstitial space, instability of the substrate).

## **II. Characterization of the crustacean hyporheal complex**

### **1. Analysis of taxonomic composition**

#### **1.1. Taxonomic composition**

Forty three species and subspecies crustacean taxa were identified (Appendix 1). The orders *Harpacticoida* and *Cyclopoida* – 14 (sub)species and class *Ostracoda* – 8 (sub)species were best presented. Suborder *Cladocera* was presented with 3 species and the other groups with 1 species.

A significant number of the identified taxa are new for Iskar cathment. Twenty-one species and seven other taxa, determined up to genus are identified for first time in the Iskar river hyporheal. The main part of these new species are from order *Harpacticoida*- 8 (sub)species, class *Ostracoda* (7 species), subclass *Cladocera* (3 species), all are marked with “ \* ” in Appendix 1.

The group *Cladocera* is interesting as there is not published data, although some reports for their presence in hyporheal samples (PANDOURSKI, personal communication). The accidental findings of *Bosmina* (*B.*) *longirostris* should be considered as chance and as a result of the influence of upper reservoirs (Pancharevo dam).

The representatives of class *Ostracoda* have a great importance for the hyporheal fauna of Iskar River. They are presented with a high number of species – 8, the half of which are new for Bulgaria. The taxonomical identification to species for some of them was not possible but we can accept the presence of 14 different ostracode taxa.

Order *Harpacticoida* is well known from previous studies. However, 7 new (sub)species were identified. The finding of specific stygobionts of genus *Parastenocaris*, identified once at station 2 (Cherni Iskar) is of significant importance.

Order *Cyclopoida* is best studied. From the 14 (sub)species taxa, found in the river, only *Diacyclops bicuspidatus bicuspidatus* was a new, although the main

species (s. lat.) was already found in the hyporheal of Iskar River near to Dolni Pasarel (PANDOURSKI, 1994b).

The higher crustacean are presented with only two underground taxa - *Parabathynella stygia* and *Niphargus* sp., as well as the common for the river benthic *Gammarus balcanicus* and *Asellus aquaticus*. The finding of *Parabathynella stygia* is interesting and important, since this is one of the species that are characteristic for the zoogeographic determination of Iskar Valley (CVETKOV et al., 1976). Unfortunately, *Microcerberus phreaticus* and *Microcharon bureschi* (the later one was reported only in Iskar Valley) were not found. As these species are in relation with the phreatic underground water, it is possible that the ecological conditions in the shallow hyporheal water is not suitable for them.

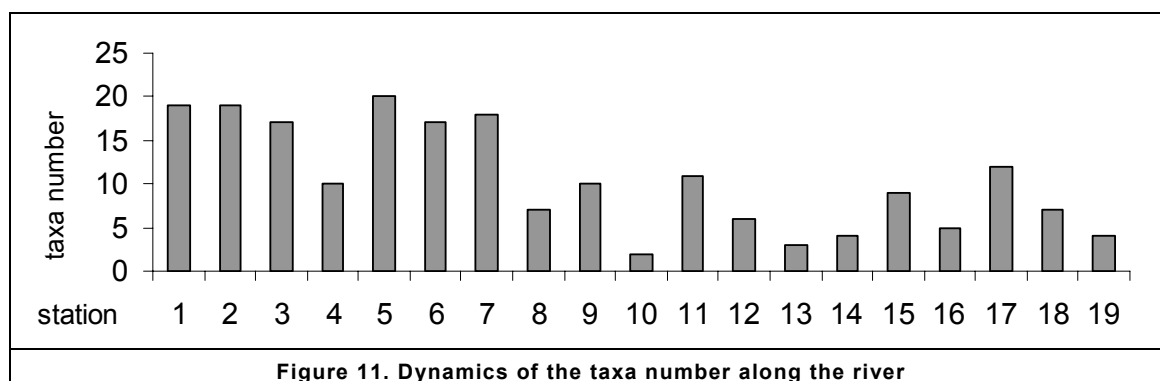
## 1.2. Distribution of taxa number in different river sectors

### Upper stream of Iskar River

The greatest number of taxa (species richness) was detected in mountainous part of Iskar catchment (Fig. 11 and App. 1). At stations located on Beli Iskar River and Cherni River Iskar were found 19 taxa in each. The main part of them was from order Harpacticoida, Cyclopoida and class Ostracoda. It should be marked the finding of specific underground genus *Parastenocaris* on st.2 (single find). On station above Samokov town, the 17 taxa were determined. This is one of the few stations with finding of high underground crustaceans - *Parabathynella stygia* and *Niphargus* sp.

On station below Samokov, the taxa number decreased twice – 10 taxa. This double decrease was a result of anthropogenic influence of Samokov and more fine sediments in hyporheal. The species richness on next 5 st. was commensurate with that of the first stations. 20 (sub)species have been determined here but it should be noted that the *Gammarus balcanicus* (1 ind.) is a typical macrozoobenthic organism and its founding is rather accidental.

The species richness of st. 6 and 7 was high. On station 7 the representatives of higher crustaceans were found - *Parabathynella stygia* and *Niphargus* sp. The low number of taxa in stations 8 and 9 was due partially to the smaller sample number (only 2004) and the possible influence of above situated Pancharevo Dam.



### **Middle Iskar**

On the station below Sofia city (10) the species richness was the lowest. Only two species were found – *Eudiaptomus gracilis* and *Asellus aquaticus*. The origin of the both organisms is accidental – *E. gracilis* follows from the near located river floods, and *A. aquaticus* is one of the few macrozoobenthic species tolerant to high organic pollution. It can be assumed that on this station, as a result of strong organic loading and other negative environmental factors the hyporheic biocenosis is not develop.

The founded taxa above Svoge town were 11. The great number of taxa is due more to the influence of feeding pure mountain waters of Ljaskovska River tributary than the improvement of ecological state in hyporheal. As above it was shown the ecological factors registered a significant pollution in main stream. The interesting result is the finding of typical stygobionts – *Parabathynella stygia* and *Niphargus sp.*, in addition of tolerant to organic pollution *Asellus aquaticus*.

On the next located stations the species richness retained low. The 6 taxa were determined at station 12, all belonging to the order *Cyclopoida*. The 3 taxa were determined at station 13, the 4 taxa from *Harpacticoida*, *Cyclopoida* and class *Ostracoda* – on st. 14.

The crustaceans from Middle Iskar were represented mainly with some (sub)species from orders *Harpacticoida* and *Cyclopoida*. The organisms from order Cladocera were not found, and class *Ostracoda* were represented with 2 taxa.

### **Down stream of Iskar River**

Still on the first station below the Gorge of Stara Planina (st. 15) the species richness was increasing twice. Only the 9 (sub)species were detected. On the next stations, the species richness even if some variations retained the higher than in Middle Iskar part. On station 16, the 5 taxa were determined, on station 17 – 12 taxa, on station 18 – 7 taxa. The lowest value was founded on last station (19) – only 4 taxa. It should be underlined the increased role of class *Ostracoda* in downstream part of Iskar River and poor representation of subclass Cladocera- only two taxa with one specimen.

### **1.3. Relationships of species to the underground environment**

The main part of the determined species are occasional inhabitants of the hyporheal –stygoxenes -15 (sub)species or stygophiles -17 (sub)species. The typical underground inhabitants (stygobionts) are only 11 (sub)species (*Cyclopoida* - 4, *Harpacticoida* - 2, *Ostracoda* - 4, *Syncarida* - 1), representing 25.6% of the crustacean hyporheic complex of Iskar catchment. The researches on phreatic fauna of Iskar Valley (CVETKOV et al., 1976) showed a much greater contribution of stygobiontic crustaceans - 56.0%. Finding the double-less stygobionts in our case should be considered as a normal, because of specificity of hyporheic zone like an ecotone between surface (river) and underground water (GIBERT et al. 1990), with specific population, as compose of both benthic and underground organisms. In this sense, the

finding of more stygoxens and stygophiles suggests that in the formation of hyporheic crustacean complex the greater role plays the river meiobenthos, although the specific studies on this community have not yet done.

## **2. Abundance of hyporheic crustacean**

### **2.1. Total abundance**

Total of 7734 organisms was collected in studied periods. The most abundant taxa were *Harpacticoida* (4728 ind.) and *Cyclopoida* (2133 ind.). The numbers of *Ostracoda* (430 ind.) and *Cladocera* (317 ind.) were high, too. The *Syncarida* (68 ind.), *Amphipoda* (25 ind.), *Isopoda* (22 ind.), and *Calanoida* (3 ind.) were with a low abundance.

### **2.2. Distribution of crustacean abundance along the river length**

The abundance distribution in different river sectors has been characterized by a strong heterogeneity – a result of the significant divergences in ecological state of river and hyporheic biotopes on sampling sites. The species numbers were the highest in mountain part of Iskar River and the lowest in the most polluted sector of Middle Iskar in all studied seasons (Fig. 12).

#### **Upper stream of Iskar River**

The highest abundance was measured on st. 1 – average 299 individuals in sample. On st. 2 the detected value was high, too - 202.4 ind./sample. The *Harpacticoida* and *Cyclopoida* crustacean were with the greatest contribution for these numbers. For example, the harpacticoides in one sample from Beli Iskar, august 2004 were represented with 594 organisms, referred to 5 (sub)species. On st. 3, the total number of crustacean was average 120 ind./sample. In addition of harpacticoides and cyclopoides, the ostracods had increasingly big role. The station below Samokov town (st. 4) had a sharply decrease in abundance. The hyporheic crustaceans were represented with 4.6 ind./sample, although the good species biodiversity. The 10 (sub)species determined on this station were presented with single organisms, and during the august of 2004, the crustaceans were not found. The abundance was restored on st. 5. The total of 97 ind./sample was detected. This increasing retained in the stations 6 and 7, respectively with 79.4 ind./sample and 156.4 ind./sample. The low abundance in st. 8 and 9 was due to the smaller sample number (only 2004) and the possible influence of above situated Pancharevo Dam.

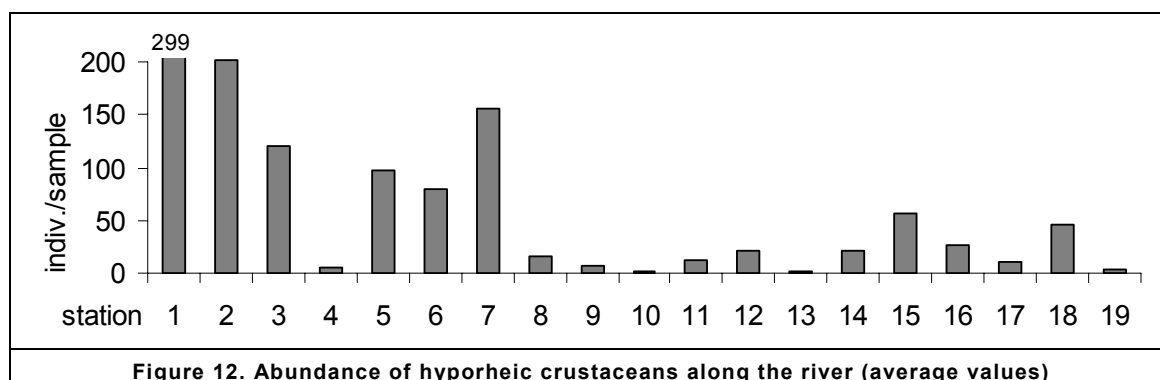


Figure 12. Abundance of hyporheic crustaceans along the river (average values)

### Middle Iskar River

The lowest abundance (1 ind./sample) was detected on station 10 below Sofia city. From 6 collected samples, the crustaceans were found only in two. In May 2004, 1 organism of *Asellus aquaticus* was found, and in November 2005 - 1 organism of *Eudiaptomus gracilis*. The disturbed ecological state did not permit the development of hyporheic fauna. On the next station 11 the total number remained low - 12 ind./sample despite the better species diversity (11 taxa). The determinate species were found usually in single number. Although the gradual improvement of ecological conditions in stream and hyporheic sediments in the next stations, the low abundance tendency was remained. On station 13, one of the lowest abundances was observed - 2 ind./sample. In the next sampling site (st.14), the increasing was registered 21.33 ind./sample. This increasing was largely due to one sample (august 2006) with 101 organisms of *Paracyclops fimbriatus fimbriatus*.

### Downstream of Iskar River

On sampling site 15 the abundance was the biggest in whole middle and downstream of Iskar River - 75 ind./sample. This increasing was coincided with enhanced taxa richness (9 taxa). On station 16 the abundance decreased - 26.3 ind./sample. The tendency was maintained on the next site with 11.4 ind./sample. Despite the increased species richness (the highest for the sector), the most species found at this station were represented by single individuals. In station 18 the abundance reached 46.8 ind./sample with the most contribution of *Harpacticoida*. On the last station, the studied parameter was reduced again - 4.33 ind./sample.

### 3. Faunal similarity

The comparison of hyporheal crustacean complex from Iskar catchment with past investigations on phreatic underground waters in Iskar Valley (CVETKOV et al., 1976), shows only 12 similar species (K= 28.57%) in two studies. From one side this is due to the longer period between the two works - about 30 years. But from the other side, the ecological conditions in hyporheal are different from the more distant phreatic waters. In hyporheic biotope, the oxygen concentrations were high and pH

was neutral to weakly alkaline while CVETKOV et al. (1976) had measured pH under neutral value and minimal oxygen concentration (0.5- 3.0 mg.dm<sup>-3</sup>).

The more significant similarity was found between the data for Cyclopoida and the last studies of PANDOURSKI (1994b, 2000). The similarity coefficient was K = 72.7%. This probably is due to the better studying of the group, the similar biotope, the identical locality and the shorter period between the two studies.

The faunal similarity of our data shows a great difference between the different river sectors. The value of this parameter for the comparison of upper stream and middle part of Iskar River is 43.8%. This is a result of significant change in ecological state along the river – from the anthropogenic unaffected mountain sector to the part with organic and industrial pollution below the Sofia city. The similarity between the middle and downstream part of Iskar River is low, too - 42.1%. The possible reason for this result is a realization of self-purification processes and corresponding change of species composition. But the self-purification is not enough for restoration of ecological state to the level in mountain river sector and the faunal similarity between down stream and upper stream of Iskar River is 42.4%.

#### 4. Dominance analyses (after De Vries)

The data for occurrence of species in all samples classify the taxa in three categories (Appendix 1).

The frequently found taxa (pF= 20- 50%) are the least – only three (sub)species. They all are stygobiontes. Here should be referred the copepodites stages of Harpacticoida and Cyclopoida that were found in each sample. The rarely found taxa (pF= 5- 20%) are 18 species and one ostracod *Shellencandona sp.* The very rare taxa (pF= <5%) are the most - 22 species and 10 taxa, determined to the level “genus”. Very frequently found species (pF>50%) were not found. The possible reason may be was the big difference of ecological state in different river sectors and the corresponding low faunal similarity. But from other side, the finding of significant percentage of very rare species may be is an indicator for stenobiotic nature of taxa.

From the all found taxa only 23 species (53.4%), 2 genera and copepodites stages of Harpacticoida and Cyclopoida are dominant. The following stygobiotic species were with the leading meaning (pF>20%, Dt>25%) *Bryocamptus (L.) dacicus*, *Diacyclops languidoides languidoides*, *Paracyclops fimbriatus fimbriatus* and the copepodites. In opposite, the species *Stenocypris bolieki*, *Eudiaptomus gracilis*, *Bryocamptus (Rh.) zschokkei tatrensis*, *Eucyclops serrulatus* *Canthocamptus (C.) staphylinus*, *Asellus aquaticus* were determined in single findings but usually in dominance. These species are stygoxenes and stygophiles with specific meaning. For example, the ecological tolerant *A. aquaticus* was found only in river part with the most significant pollution of surface waters and hyporheic sediments – st.10 and 11 and frequently this organism was the single representative of crustaceans.

## CONCLUSION

The dynamics of main environmental factors - physical and hydrochemical parameters of hyporheic water, granulometric characteristic of sediments and ecological status of surface waters determined largely the composition and structure of crustacean hyporheic complex of Iskar catchment.

The greatest species richness and the highest abundance were determined in the upper part of Iskar River. The crustaceans from Harpacticoida and Cyclopoida were with essential meaning. In this sector the environmental conditions were optimal – the surface waters were negligible anthropogenic affected; the sediments had a good porosity with sufficient oxygen levels for hyporheic organisms and for realization of effective decomposition of deposited organic substances.

In the section of Middle Iskar the lowest number of crustacean species was established. Typically, the few founded species were represented in the limited abundance – single organisms. This was attributed to the strong organic loading of Sofia city and the industrial pollution of the river. Despite the detected self-purification processes at below situated sampling sites, the species richness and abundance retained low.

In the downstream part of Iskar River the species richness increased, but this was not always correlated with an increase in the abundance. Probably the greater quantity of fine sediments was a limiting factor for the development of hyporheic crustaceans. This result partly explains the greater role in community of the ostracods.

This study shows the relationship of composition and structure of hyporheal crustacean complex with the varied environmental conditions in different sectors of Iskar River.

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## Appendix

### Appendix 1.

Species list of crustaceans from hyporheal of Iskar River with data for distribution on stations and dominance analyses. With " \* " are marked the new for Iskar River hyporheal (sub)species and taxa.

### **CLADOCERA**

#### **Bosminidae**

*Bosmina (Bosmina) longirostris* (O. F. Müller 1776)\* : stations 8, 9 ; pF= 2.02%.

#### **Daphniidae**

*Daphnia sp.* \* : station 8 ; pF= 1.01%

#### **Eurycercidae**

*Alona sp.* : station 8 ; pF= 1.01%

*Alona affinis* (Leydig 1860) \* : stations 1, 2, 3 ; pF= 3.03%

*Alona guttata* Sars 1862 \* : stations 1, 2, 3, 5, 15 ; pF= 13.13%

*Alona rectangula* Sars 1862 \* : stations 1, 2, 3, 4, 5, 6 ; pF= 9.09%

#### **Macrothricidae**

*Ilyocryptus sp.* \* : stations 5, 9, 17 ; pF= 3.03%

### **CALANOIDA**

Copepodites : station 8, pF= 1.01%

#### **Diaptomidae**

*Eudiaptomus gracilis* (Sars 1863) \* : stations 9, 10 ; pF= 2.02, Dt= 50%

### **CYCLOPOIDA**

Copepodites : stations 1, 2, 3, 5, 6, 7, 8, 9, 11, 12, 15, 16, 17, 18, 19 ; pF= 38.38%, Dt= 31.58%

#### **Cyclopidae**

*Acanthocyclops sp.* : station 2, pF= 1.1%, Dt= 100 %

*Acanthocyclops strimonis* (Pandourski 1994) : stations 2, 3 ; pF= 3.03%, Dt= 33.33%

*Acanthocyclops vernalis* (Fischer 1853) : stations 2, 3, 5, 8, 9, 11, 14 ; pF= 8.08%, Dt= 50.00%

*Acanthocyclops vernalis vernalis* (Fischer 1853) : stations 1, 6, 7, 11, 12, 15, 17 ; pF= 8.08%, Dt= 37.50%

*Cyclops sp.* : stations 6, 16 ; pF= 2.02%, Dt= 50.00%

*Diacyclops bicuspidatus bicuspidatus* (Claus 1857) \* : station 12 ; pF= 1.01%

*Diacyclops bisetosus* (Rehberg 1880) : station 7 ; pF= 1.01%

*Diacyclops clandestinus* (Kiefer 1926) : stations 1, 5, 11, 17 ; pF= 4.04%.

*Diacyclops languidoides* (Lilljeborg 1901) : stations 1, 2, 4, 5, 6, 7, 11, 12 ; pF= 17.17%, Dt= 52,94%

*Diacyclops languidoides languidoides* (Lilljeborg 1901) : stations 1, 2, 3, 4, 5, 6, 7, 9, 11, 12, 15, 17 ; pF= 26.26%, Dt= 34.62%

*Diacyclops languidus languidus* (Sars 1863) : stations 6, 9 ; pF= 4.04%

*Diacyclops* sp. : stations 1, 6, 7 ; pF= 4.04%

*Eucyclops serrulatus* (Fischer 1851) : stations 4, 6, 9, 18 ; pF= 4.04%, Dt= 50.00%

*Macrocyclus albidus* (Jurine 1820) : station 11 ; pF= 1.01%

*Megacyclops viridis* (Jurine 1820) : stations 2, 5, 6, 7 ; pF= 9.09%, Dt= 11.11%

*Paracyclops fimbriatus* (Fischer 1853) : stations 2, 12, 13, 15, 18 ; pF= 6.06%, Dt= 66.67%

*Paracyclops fimbriatus fimbriatus* (Fischer 1853) : stations 1, 2, 3, 4, 5, 7, 8, 9, 12, 14, 15, 17 ; pF= 20.20%, Dt= 30.00%

*Paracyclops* sp. : stations 7, 17, 18, 19 ; pF= 4.04%, Dt= 25.00%

## **HARPACTICOIDA**

Copepodites : stations 1, 2, 3, 4, 5, 7, 9, 13, 14, 15, 16, 17, 18 ; pF= 21.21%, Dt= 69.90%

### **Ameiridae**

*Nitokra hibernica* (Brady 1880) \* : stations 1, 15, 16, 17, 18 ; pF= 11.11%, Dt= 81.82%

### **Canthocamptidae**

*Attheyella* (*Attheyella*) *crassa* (Sars 1863) : stations 3, 4, 5, 6, 7, 9 ; pF= 18.18%, Dt= 55.56%

*Attheyella* (*Attheyella*) *wierzejskii* (Mrazek 1893) : stations 1, 2 ; pF= 2.02%

*Attheyella* (*Attheyella*) *wierzejskii crenophila* Damian 1955 \* : station 5 ; pF= 1.01%

*Bryocamptus* (*Limocamptus*) *dacicus* (Chappuis 1923) : stations 1, 2, 3, 4, 5, 6, 7, 8, 13, 17, 18, 19 ; pF= 27.27%, Dt= 55.56%

*Bryocamptus* (*Rheocamptus*) *pygmaeus* Sars 1863 \* : stations 3, 5, 16 ; pF= 3.03%

*Bryocamptus* (*Rheocamptus*) *zschokkei* (Schmeil 1893) \* : stations 1, 2, 3, 4, 5, 6, 7, 15, 16, 17, 19 ; pF= 19.19%, Dt= 78.95%

*Bryocamptus* (*Rheocamptus*) *zschokkei tatrensis* Minkiewicz 1916 : stations 2, 7 ; pF= 2.02%, Dt= 50.00%

*Bryocamptus* (*Rheocamptus*) *zschokkei zschokkei* (Schmeil 1893) \* : stations 1, 2, 3, 6, 7 ; pF= 9.09%, Dt= 66.67%

*Bryocamptus* sp. : station 15 ; pF= 1.01%, Dt= 100%

*Canthocamptus* (*Canthocamptus*) *staphylinus* (Jurine 1820) : stations 5, 6, 7, 14 ; pF= 5.05%, Dt= 60.00%

*Epactophanes richardi* Mrazek 1893 : stations 5, 11 ; pF= 2.02%

*Elaphoidella gracilis* (Sars 1863) \* : station 15 ; pF= 1.01%

*Paracamptus schmeili* (Mrazek 1893) \* : stations 1, 2, 3, 5, 6, 9 ; pF= 15.15%, Dt= 33.33%

### **Parastenocarididae**

*Parastenocaris* sp. : station 2 ; pF= 1.01%

## **OSTRACODA**

*Ostracoda* fam. g. sp. : stations 2, 3, 9 ; pF= 4.04%, Dt= 25.00%

### **Candonidae**

*Candona neglecta* Sars 1887 \* : stations 3, 11 ; pF= 6.06%

*Candoninae* g. sp. : stations 1, 5, 13, 19 ; pF= 4.04%, Dt= 25.00%

*Cryptocandona vavrai* Kaufmann 1900 \* : station 6, pF= 1.01%  
*Eucandona* sp."1" \* : station 6, pF= 2.02%  
*Eucandona* sp."2" \* : station 5 pF= 1.01%  
*Nannocandona faba* Ekman 1914 \*: station 1 ; pF= 1.01%  
*Trapezicandona transleithanica* (Löffler 1960) \* : stations 2, 3, 4, 6 ; pF= 5.05%, Dt= 20.00%  
*Typhlocypris* sp. \* : station 17 ; pF= 1.01%, Dt= 100%  
*Typhlocypris pratensis* (Hartwig 1901) \*: stations 1, 4, 5, 7 ; pF= 9.09%, Dt= 11.11%  
*Shellencandona* sp. \*: stations 14, 15, 16, 18 ; pF= 8.08%, Dt= 62.50%

#### **Cyprididae**

*Cyprididae* g. sp. : station 2 ; pF= 2.02%  
*Eucypris* sp. : station 17 ; pF= 1.01%  
*Potamocypris pallida* Alm 1914 \*: stations 1, 7 ; pF= 4.04%

#### **Herpetocypridinae**

*Stenocypris bolieki* Ferguson 1962 \*: station 18 ; pF= 1.01%, Dt= 100%

#### **Ilyocyprididae**

*Ilyocypris* sp. : station 18 ; pF= 1.01%

#### **Limnocytheridae**

*Kovalevskiella bulgarica* (Danielopol 1970) : stations 1, 7 ; pF= 2.02%

### **SYNCARIDA**

#### **Parabathynellidae**

*Parabathynella stygia* Chappuis 1926 : stations 3, 7, 11, 17 ; pF= 8.08%, Dt= 62.50%

### **AMPHIPODA**

#### **Gammaridae**

*Gammarus balcanicus* Schäferna 1922 : stations 1, 5, 7 ; pF= 4.04%

#### **Niphargidae**

*Niphargus* sp. : stations 3, 7, 11 ; pF= 3.03%

### **ISOPODA**

#### **Asellidae**

*Asellus aquaticus* (Linnaeus 1758) : stations 10, 11 ; pF= 5.05%, Dt= 100%

