

# The endohelminth fauna of barbel (*Barbus barbus*) correlates with water quality of the Danube River in Bulgaria

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## SUMMARY

Infection of barbel with 10 species of metazoan parasites including 3 trematodes, 3 acanthocephalans and 4 nematodes was observed in fish collected from 3 localities in the Bulgarian part of the river Danube between summer 2004 and summer 2007. The composition as well as the diversity characteristics of the parasite communities were analysed seasonally and showed a clear correlation with the composition of the invertebrate fauna and water quality. The most prevalent species was the acanthocephalan *Pomphorhynchus laevis*, which was also the dominant species of the intestinal component communities at all sampling sites. The second most frequent parasite at all Danube localities was *Rhabdochona hellichi*, which occurred in significantly higher numbers at the less polluted sites. Overall, the diversity of helminth communities increased with decreasing levels of nutrients and pollutants at all sampling sites. Therefore, the composition and diversity of parasite communities may be used to characterize ecosystem health and integrity.

Key words: *Barbus barbus*, *Eustrongylides* sp., diversity, water quality, pollution.

## INTRODUCTION

In recent years, fish parasites attain increasing interest from an environmental point of view (Sures, 2006, 2008). Many studies demonstrate the close relationship between parasitism and ecological conditions in a given environment and describe how parasites can be used to enlarge knowledge on ecosystem function and integrity (Hudson *et al.* 2006; Lafferty *et al.* 2008). Pollution with toxic substances such as metals or polychlorinated biphenyls (PCBs) as well as an enrichment of nutrients (eutrophication) may affect the occurrence and physiology of parasites. The effects of toxic pollutants and eutrophication on parasites can be direct (e.g. by reduction of the number of free living stages or intermediate host) or indirect (e.g. host immunosuppression) depending on the pollution type and parasite life cycle (Sures, 2008). Various studies demonstrate, for example, that toxic pollution reduces the diversity of heteroxenous parasites, whereas parasites with direct life cycles (monoxenous) are less affected. The latter are often ectoparasites which are in direct contact to the surrounding water and are thus adapted to changes in environmental conditions (Valtonen *et al.* 1997; MacKenzie, 1999; Perez-del Olmo *et al.* 2007). Concerning toxic pollutants it emerges that certain substances such as metals or PCBs cause immunosuppression in the fish host and

thus may increase parasitism by a reduced host defence (Hoole, 1997). The resulting numerical changes (increase or decrease of abundance and intensity) of aquatic parasites leading to changes in structure and diversity of parasite communities as a response to different forms of pollution may be used for bio-indication purposes (MacKenzie *et al.* 1995). Accordingly, the occurrence and diversity of parasites stand as a measure of ecosystem health even if the underlying functional chains are often unknown.

In order to use fish parasites as pollution indicators, the fish host must be widely distributed and easily sampled (Kennedy, 1997). Therefore, in the present study we focused on barbel (*Barbus barbus*) and its parasite communities at different sampling sites along 3 lower reaches of the Danube River. The barbel is the second largest native cyprinid fish species in Europe, being widespread in major European river systems. Although many studies on the parasite fauna of *B. barbus* have been published from selected localities of the Danube basin, data from east Europe and especially from the Balkan Peninsula and the Danube delta are scarce. Only few studies on parasites of barbel in the Danube River in Bulgaria (Kakacheva-Avramova, 1962, 1977, 1983; Margaritov, 1959, 1966; Nedeva *et al.* 2003) and in Romania (Roman, 1955) exist, whereas most information on barbel parasites is delivered from Central Europe (Michalovič, 1954; Moravec and Scholz, 1991; Moravec *et al.* 1997; Laimgruber *et al.* 2005). Until now, the complete endohelminth fauna of *B. barbus* reported for the Danube drainage

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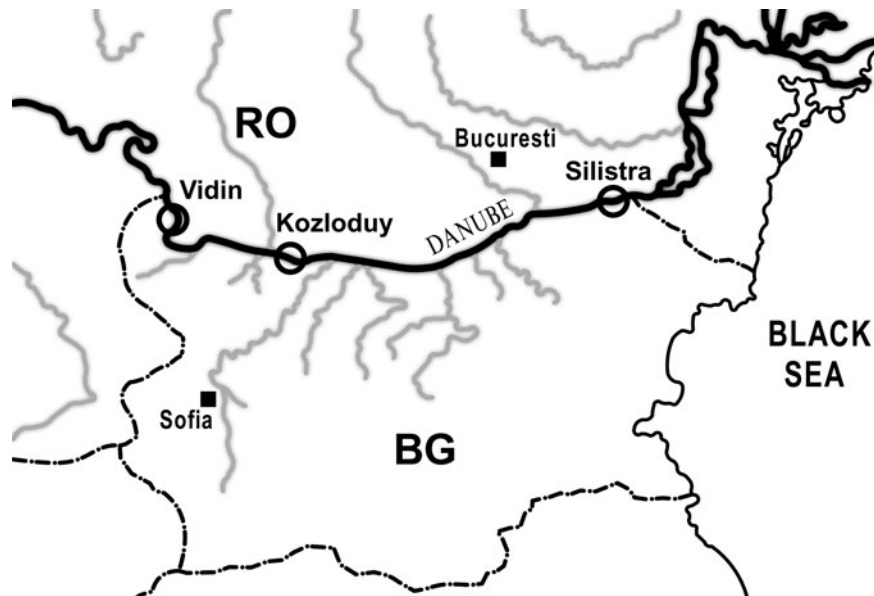


Fig. 1. Location of the sampling sites along the Danube River in Bulgaria. BG – Bulgaria; RO – Romania.

system in Central Europe consists of 43 species with 22 trematodes, 9 cestodes, 7 nematodes and 5 acanthocephalans (Moravec *et al.* 1997). In contrast, the list of barbel endohelminths in the Bulgarian section of the Danube River (Kakcheva-Avramova, 1977) includes only 6 species, but there are a few unpublished studies, which describe up to 11 species.

The aim of the present study was to obtain a more complete picture of the endohelminth fauna of *B. barbus* and to study the composition and diversity of parasite communities with respect to the environmental conditions of the habitats. It is expected that the structure and diversity of parasite communities over consecutive years at sites that differ in their degree of eutrophication and in their concentration of toxic metals reflect the ecological conditions.

## MATERIALS AND METHODS

### *Sampling sites*

The study was carried out in a seasonal manner (April, July and October) ranging from summer 2004 to summer 2007 at 3 different localities of the Bulgarian part the Danube River. The sampling sites (see Fig. 1) were selected on the basis of different degrees of eutrophication and toxic pollutants, as the main objective of the current research was to check whether parasite communities reflect the environmental conditions of their habitats. The first sampling site is located near Vidin (river kilometre 834), about 10–15 km away from the inflow of the river Timok (845 km), which is one of the biggest metal pollution sources downstream in the Danube. The second sampling site was selected near the town of Kozloduy (685 km), approximately 160 river kilometres downstream from Vidin. The third site was

on the border between Bulgaria and Romania near the town of Silistra (375 km) that represents the last Bulgarian locality in an eastward direction of the river. The sampling stretches covered approximately 5 river kilometres at each sampling site (Fig. 1).

### *Fish sampling*

In total 407 barbels were collected by fishermen, using drift nets. The number of individuals with a minimum total length of 20 cm varied between 10 and 35 fishes per sampling site and season (Table 1). During the whole sampling period a total of 165 fish were caught in Vidin and 193 in Kozloduy. Sampling continued for only 2 years in Silistra, where 49 barbels were sampled between 2006 and 2007. Additionally, spring sampling at all sites was performed only in the years 2006 and 2007. After catching, the fish were frozen at  $-15^{\circ}\text{C}$  and transported to the laboratory, where total length (TL), standard length (SL), body weight (BW), sex and age for each fish was determined. The condition factor (K) was calculated as follows  $K = 100 * BW * TL^{-3}$  (Schäperclaus, 1990). The fish were subsequently dissected and analysed for parasites using standard parasitological techniques. The skin, scales, fins, gills, eyes, gut, cavities and organs were examined using a stereomicroscope (magnification  $\times 8$  to  $\times 50$ ). Nematodes were fixed in 70% ethanol and mounted in glycerine for further identification whilst all other parasites could be identified directly.

### *Determination of helminth community structure and statistical treatment*

Parasitological parameters used followed those suggested by Bush *et al.* (1997) – prevalence (P, %),

Table 1. Morphological parameters and characteristics of collected fish material

| Sampling sites | Sampling time | No. of fishes | Weight (g)       |          | Total length (cm)  |           | Condition factor   |           |
|----------------|---------------|---------------|------------------|----------|--------------------|-----------|--------------------|-----------|
|                |               |               | Mean $\pm$ s.d.  | Range    | Mean $\pm$ s.d.    | Range     | Mean $\pm$ s.d.    | Range     |
| Vidin          | Spring        | 48            | 702 ( $\pm$ 577) | 108–3909 | 40.9 ( $\pm$ 8.3)  | 25.5–72   | 0.88 ( $\pm$ 0.10) | 0.65–1.05 |
|                | Summer        | 58            | 836 ( $\pm$ 604) | 207–2145 | 43.2 ( $\pm$ 10.4) | 28.7–63.7 | 0.85 ( $\pm$ 0.08) | 0.74–1.07 |
|                | Autumn        | 59            | 565 ( $\pm$ 486) | 81–2390  | 38.5 ( $\pm$ 9.2)  | 23.4–67.2 | 0.83 ( $\pm$ 0.15) | 0.30–1.28 |
|                | Total         | 165           | 649 ( $\pm$ 509) | 81–3909  | 39.9 ( $\pm$ 8.8)  | 23.4–72   | 0.87 ( $\pm$ 0.14) | 0.30–1.80 |
| Kozloduy       | Spring        | 37            | 599 ( $\pm$ 280) | 120–1125 | 39.6 ( $\pm$ 6.3)  | 26–52.3   | 0.89 ( $\pm$ 0.10) | 0.68–1.15 |
|                | Summer        | 86            | 587 ( $\pm$ 371) | 125–2208 | 38.9 ( $\pm$ 7.6)  | 24.5–60.5 | 0.88 ( $\pm$ 0.09) | 0.64–1.07 |
|                | Autumn        | 71            | 539 ( $\pm$ 370) | 140–1785 | 38.5 ( $\pm$ 7.9)  | 26.2–56.9 | 0.86 ( $\pm$ 0.16) | 0.38–1.49 |
|                | Total         | 193           | 573 ( $\pm$ 355) | 120–2208 | 38.9 ( $\pm$ 7.5)  | 24.5–60.5 | 0.88 ( $\pm$ 0.13) | 0.38–1.49 |
| Silistra       | Spring        | 10            | 801 ( $\pm$ 216) | 400–1050 | 43.2 ( $\pm$ 5.2)  | 34.8–50   | 0.99 ( $\pm$ 0.15) | 0.77–1.28 |
|                | Summer        | 27            | 948 ( $\pm$ 367) | 410–1785 | 44.7 ( $\pm$ 5.9)  | 34.1–54.8 | 1.01 ( $\pm$ 0.13) | 0.80–1.31 |
|                | Autumn        | 12            | 624 ( $\pm$ 168) | 420–900  | 39.7 ( $\pm$ 4.4)  | 34.3–47   | 0.99 ( $\pm$ 0.12) | 0.71–1.16 |
|                | Total         | 49            | 838 ( $\pm$ 327) | 400–1785 | 43.2 ( $\pm$ 5.7)  | 34.1–54.8 | 1.00 ( $\pm$ 0.13) | 0.71–1.31 |

intensity range (IR), abundance (A) and mean intensity (MI) of the infection. The following diversity indices were calculated to describe the richness and diversity of the parasite communities: Brillouin's index ( $H_B$ ), Shannon-Wiener index ( $H_S$ ), Shannon-Wiener evenness (E), Simpson's index (D) and Berger-Parker index (d) according to Magurran (1988) and Sures *et al.* (1999).

Correlations between intensity and fish weight were checked using Spearman's rank correlation coefficient. A one-way ANOVA was employed to determine significant differences in the diversity characteristics of the intestinal infra-community and to compare the number of each parasite species between sampling sites. For estimating differences in fish condition factors between sampling sites, the Mann-Whitney U-test was applied.

### Water quality

Water quality data for sites adjacent to our fish sampling sites (see Table 2) were obtained from the technical reports published by the Joint Danube Survey (ICPDR, 2002, 2008a, c) and annual reports and the database of TNMN (Trans National Monitoring Network, ICPDR, 2004, 2005, 2008b). These research programmes and activities are initialized by the International Commission for Protection of the Danube River (ICPDR). The available data were used as a basis to interpret the composition and richness of helminth communities at the same localities.

## RESULTS

### Total parasite fauna

In total 10 endohelminth parasite species were recovered, including 3 trematodes (*Diplostomum spathaceum* (metacercariae) in the eye lens, *Posthodiplostomum cuticola* (metacercariae) on the skin,

Table 2. Data on selected aqueous nutrient and pollution parameters according to ICPDR (2008b) for upper and lower sites of the Bulgarian part of the Danube River

| Parameters              | Year  | Vidin <sup>1</sup> | Kozloduy <sup>2</sup> | Silistra |
|-------------------------|-------|--------------------|-----------------------|----------|
| Ammonium (mg/L)         | 2003  | 0.185              | 0.265                 | 0.079    |
|                         | 2004  | 0.191              | 0.183                 | 0.075    |
|                         | 2005  | 0.207              | 0.288                 | 0.078    |
|                         | 2007* | 0.016              | 0                     | 0        |
| Nitrate (mg/L)          | 2003  | 1.203              | 0.661                 | 1.119    |
|                         | 2004  | 1.446              | 0.977                 | 1.435    |
|                         | 2005  | 1.41               | 0.829                 | 1.574    |
|                         | 2007* | 1.45               | 1.44                  | 1.56     |
| Nitrite (mg/L)          | 2003  | 0.033              | 0.022                 | 0.019    |
|                         | 2004  | 0.025              | 0.021                 | 0.02     |
|                         | 2005  | 0.032              | 0.022                 | 0.016    |
|                         | 2007* | 0.059              | 0.064                 | 0.016    |
| Orthophosphate (mg/L)   | 2003  | 0.054              | 0.053                 | 0.064    |
|                         | 2004  | 0.116              | 0.061                 | 0.071    |
|                         | 2005  | 0.12               | 0.068                 | 0.059    |
|                         | 2007  | 0.069              | 0.043                 | 0.041    |
| Total phosphorus (mg/L) | 2003  | 0.323              | 0.108                 | 0.119    |
|                         | 2004  | 0.184              | 0.103                 | 0.164    |
|                         | 2005  | 0.21               | 0.130                 | 0.149    |
|                         | 2007* | n/a                | n/a                   | n/a      |
| Cadmium ( $\mu$ g/L)    | 2003  | 1                  | 1.000                 | 1        |
|                         | 2004  | 1                  | 1.167                 | 1        |
|                         | 2005  | 1                  | 1.825                 | 1        |
|                         | 2007* | n/a                | n/a                   | n/a      |
| Copper ( $\mu$ g/L)     | 2003  | 14.9               | 9.083                 | 6        |
|                         | 2004  | 18.7               | 6.417                 | 2.5      |
|                         | 2005  | 17.5               | 5.158                 | 1        |
|                         | 2007* | n/a                | n/a                   | n/a      |
| Lead ( $\mu$ g/L)       | 2003  | 1.8                | 2.333                 | 2.8      |
|                         | 2004  | 2                  | 2.583                 | 1        |
|                         | 2005  | 1.8                | 2.767                 | 1        |
|                         | 2007* | n/a                | n/a                   | n/a      |

<sup>1</sup> Sampling site Novo Selo, 1 km away from Vidin.

<sup>2</sup> Sampling site Iskar-Baikal, 40 km away from Kozloduy.

\* Data delivered by 2nd Joint Danube Survey – Onboard results (ICPDR 2008a).

n/a, Data not available.

Table 3. Prevalence, mean intensity and mean abundance of the parasites of barbel from three sampling sites along the Danube River in Bulgaria

| Parasite species                       | Sampling site | Prevalence P (%) | Mean intensity MI ( $\pm$ s.d.) | Intensity range | Abundance |
|--|---------------|------------------|---------------------------------|-----------------|-----------|
| <i>Rhabdochona hellichi</i>            | Vidin         | 24.2             | 15.9 ( $\pm$ 35.6)              | 1–207           | 3.9       |
|  | Kozloduy      | 47.7             | 34 ( $\pm$ 99)                  | 1–759           | 16.2      |
|  | Silistra      | 46.9             | 72.9 ( $\pm$ 180.7)             | 1–761           | 34.2      |
| <i>Pseudocapillaria tomentosa</i>      | Vidin         | 4.8              | 1.4 ( $\pm$ 0.7)                | 1–3             | 0.07      |
|  | Kozloduy      | 4.1              | 2.3 ( $\pm$ 2.0)                | 1–7             | 0.09      |
|  | Silistra      | 10.2             | 2 ( $\pm$ 1.7)                  | 1–5             | 0.2       |
| <i>Eustrongylides</i> sp. larv.        | Vidin         | 24.2             | 10.1 ( $\pm$ 20.5)              | 1–93            | 2.5       |
|  | Kozloduy      | 17.1             | 9.1 ( $\pm$ 14.1)               | 1–68            | 1.6       |
|  | Silistra      | 14.3             | 2.1 ( $\pm$ 1.9)                | 1–6             | 0.3       |
| <i>Hysterothylacium</i> sp. larv.      | Vidin         | —                | —                               | —               | —         |
|  | Kozloduy      | 0.5              | 1                               | 1               | 0.01      |
|  | Silistra      | —                | —                               | —               | —         |
| <i>Pomphorhynchus laevis</i>           | Vidin         | 100              | 124.6 ( $\pm$ 122.5)            | 1–874           | 124.6     |
|  | Kozloduy      | 99               | 84.3 ( $\pm$ 77.7)              | 2–424           | 83.4      |
|  | Silistra      | 98               | 117.7 ( $\pm$ 107.5)            | 4–523           | 115.3     |
| <i>Acanthocephalus anguillae</i>       | Vidin         | 1.2              | 1                               | 1               | 0.01      |
|  | Kozloduy      | 0.5              | 2 ( $\pm$ 2)                    | 2               | 0.01      |
|  | Silistra      | —                | —                               | —               | —         |
| <i>Leptorhynchoides plagicephalus</i>  | Vidin         | 0.6              | 1                               | 1               | 0.006     |
|  | Kozloduy      | —                | —                               | —               | —         |
|  | Silistra      | —                | —                               | —               | —         |
| <i>Diplostomum spathaceum</i> larv.    | Vidin         | 7.3              | —                               | —               | —         |
|  | Kozloduy      | 8.8              | —                               | —               | —         |
|  | Silistra      | 6.1              | —                               | —               | —         |
| <i>Postodiplostomum cuticola</i> larv. | Vidin         | 16.4             | —                               | —               | —         |
|  | Kozloduy      | 17.1             | —                               | —               | —         |
|  | Silistra      | 38.8             | —                               | —               | —         |
| <i>Metagonimus yokogawai</i> larv.     | Vidin         | 12.7             | —                               | —               | —         |
|  | Kozloduy      | 15.5             | —                               | —               | —         |
|  | Silistra      | 10.2             | —                               | —               | —         |

*Metagonimus yokogawai* (metacercariae) on the scales), 3 acanthocephalans (*Pomphorhynchus laevis*, *Acanthocephalus anguillae*, *Leptorhynchoides plagicephalus* in the intestine) and 4 nematodes (*Rhabdochona hellichi*, *Pseudocapillaria tomentosa*, *Hysterothylacium* sp. (larvae) in the intestine and *Eustrongylides* sp. (larvae) in the body cavity) (Table 3). One acanthocephalan species (*L. plagicephalus*) and 2 nematodes (larvae of *Eustrongylides* sp. and *Hysterothylacium* sp.) were recorded for the first time for barbel. Only 1 fish from the sampling site Vidin was infected with a single adult male of *L. plagicephalus*, which is thus considered an accidental infection. Larvae of *Hysterothylacium* sp. were found in the gut of 1 barbel collected at the sampling site Kozloduy. *Eustrongylides* sp. occurred at all sampling sites during the entire period. This nematode, together with the nematode *R. hellichi*, was the second most widely distributed parasite species at the sampling site Vidin (P, 24.2%; MI, 10.1). Also at the sampling sites Silistra and Kozloduy it occurred with high prevalence and intensity (Kozloduy P: 17.1%; MI: 9.1; Silistra P: 14.3%; MI: 2.1). The pattern of infection presents a clear correlation between fish size,

prevalence and intensity of infection. The highest prevalence was found in barbels with a length between 40 and 60 cm. Infection intensity increased significantly (Spearman correlation,  $P < 0.05$ ) with body size (Vidin:  $r = 0.32$ ; Kozloduy:  $r = 0.39$ ; Silistra:  $r = 0.34$ ).

Total species richness ranged between 9 worms for Vidin and Kozloduy and 7 for Silistra. The most abundant parasite was the acanthocephalan *P. laevis*. At the sampling site Vidin 100% of the fishes were infected with this acanthocephalan and the mean intensity was 124.6 worms per fish. Only 2 fishes from Kozloduy (P, 99%; MI, 84.3) and 1 from Silistra (P, 98%; MI, 117.7) were not infected with *P. laevis*. The second most frequent species at all sampling sites was *R. hellichi*. The number of *R. hellichi* individuals showed significant differences between Vidin and Kozloduy ( $P = 0.029$ ,  $F = 4.795$ ), and Vidin and Silistra ( $P = 0.003$ ,  $F = 8.78$ ), whereas no differences were detected between Kozloduy and Silistra.

The trematodes were the third group in terms of prevalence. Metacercariae of *P. cuticola* were most frequently found, followed by *M. yokogawai* and

Table 4. Average diversity characteristics of the infra-community of helminths of barbel from the Danube River

| Sampling sites                                       | Vidin           | Kozloduy        | Silistra        |
|--|-----------------|-----------------|-----------------|
| No. of barbels                                       | 165             | 193             | 49              |
| Mean no. of helminth species per barbel $\pm$ s.d.   | 1.55 $\pm$ 0.61 | 1.68 $\pm$ 0.66 | 1.69 $\pm$ 0.77 |
| Maximum no. of helminth species per barbel           | 3               | 4               | 4               |
| Mean value of Brillouin's Index ( $H_B$ ) $\pm$ s.d. | 0.10 $\pm$ 0.16 | 0.15 $\pm$ 0.19 | 0.16 $\pm$ 0.22 |
| Maximum value of Brillouin's Index ( $H_B$ )         | 0.76            | 0.68            | 0.66            |

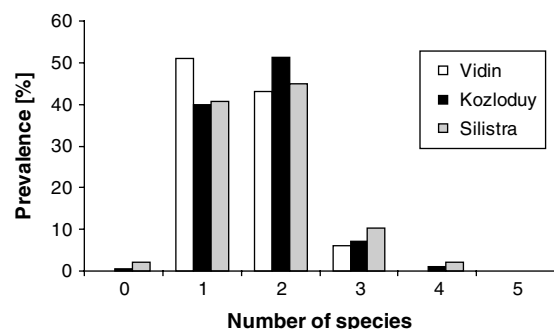


Fig. 2. Prevalence of coexistent helminth species of barbel from three sampling sites of the Danube River.

*D. spathaceum* at all sampling sites. There are no data available concerning the intensity of infection, since only the presence of metacercariae was recorded. The nematode *P. tomentosa* was present in all Danube sites during the whole sampling period. Whilst the prevalence was similar (4.8% and 4.1%) at the sampling sites Vidin and Kozloduy, it was more than 2 times higher in Silistra.

#### Diversity of helminth communities

Diversity and dominance indices were calculated without considering trematodes as they were not counted individually. Diversity characteristics of the infra-community are presented in Fig. 2 and Tables 4 and 5. Most of the fish were infected with either 1 or 2 parasite species simultaneously (Fig. 2). At Vidin more than 50% of all fish were infected with 1 species only, whereas at Silistra 10% of the barbels were co-infected with 3 species. A clear increase in average diversity in downstream direction is reflected by the Brillouin index, which showed the highest value at Silistra. Statistical analyses revealed significant differences for the Brillouin index between Vidin and Kozloduy ( $P=0.005$ ,  $F=8,101$ ) and Vidin and Silistra ( $P=0.038$ ,  $F=4,375$ ), whereas no difference was found between the sampling sites Kozloduy and Silistra ( $P=0.853$ ,  $F=0.034$ ). Concerning seasonal differences highest infracommunity diversity was found in spring and autumn for 2 sites, only Kozloduy showed a higher Brillouin index in summer than in autumn.

Similarly, component community diversity (Table 6) was also found to be higher downstream

(Silistra) than upstream (Vidin). This tendency is also reflected by the Berger-Parker dominance index, for which highest values were found in Vidin and lowest in Silistra. Kozloduy showed medium values compared to the other sampling sites. The highest seasonal diversity was found in spring in Vidin and Kozloduy and in summer in Silistra (Table 7).

#### Water quality classification

The mean values of nutrient and heavy metal concentrations for the period 2003–2005 adjacent to our sampling sites are summarized in Table 2. Nutrients such as ammonium-N, nitrite-N, ortho-phosphate and total phosphorus were lowest at the downstream site. Similarly, concentrations of copper (Cu) and lead (Pb) in the upper Danube sites were higher in the period 2004–2005 (ICPDR, 2008b), whereas no significant difference occurred for cadmium (Cd). Results obtained from the second Joint Danube Survey (JDS2) performed in autumn 2007 revealed the same pattern of pollution and eutrophication parameters between the sampling sites (ICPDR, 2008a). Accordingly, no significant change in nutrient and heavy metal levels occurred during our sampling period. Although no taxa lists are available for macrozoobenthos communities all sampling sites were categorized to class II according to the saprobic index (ICPDR, 2002).

#### DISCUSSION

The composition of endoparasite communities at the investigated Danube sites were principally similar but showed differences which can be attributed to the local ecological conditions. In general, 10 endohelminth species were identified, none of which is a barbel specialist. The dominant parasite species at all sampling sites was the acanthocephalan *P. laevis*. Similar results were obtained in the upstream part of the Danube River (Moravec *et al.* 1997; Schludermann *et al.* 2003; Laimgruber *et al.* 2005). However, the parasite list of *B. barbus* published by Margaritov (1966) and Kakacheva-Avramova (1977) for the Bulgarian section of the Danube River differs greatly from the parasite fauna detected in the present study. During our study period no cestodes were recovered, although Margaritov (1966) and

Table 5. Seasonal profile of the diversity characteristics of the infra-community

| Sampling sites   | Vidin          |             |             | Kozloduy    |             |             | Silistra    |             |             |
|--|----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
|  | Spring         | Summer      | Autumn      | Spring      | Summer      | Autumn      | Spring      | Summer      | Autumn      |
|  | No. of barbels | 48          | 58          | 59          | 37          | 86          | 71          | 10          | 27          |
| Mean no. of helminth species per barbel ± s.d.           | 1.71 ± 0.62    | 1.60 ± 0.65 | 1.37 ± 0.52 | 1.81 ± 0.66 | 1.67 ± 0.69 | 1.62 ± 0.62 | 1.40 ± 0.84 | 1.74 ± 0.81 | 1.59 ± 0.71 |
| Maximum no. of helminth species per barbel               | 3              | 3           | 3           | 3           | 4           | 3           | 3           | 4           | 3           |
| Mean value of Brillouin's Index (H <sub>B</sub> ) ± s.d. | 0.14 ± 0.19    | 0.07 ± 0.12 | 0.09 ± 0.17 | 0.19 ± 0.23 | 0.15 ± 0.20 | 0.13 ± 0.17 | 0.16 ± 0.26 | 0.11 ± 0.18 | 0.23 ± 0.27 |
| Maximum value of Brillouin's Index (H <sub>B</sub> )     | 0.68           | 0.76        | 0.71        | 0.68        | 0.66        | 0.67        | 0.65        | 0.66        | 0.65        |

Table 6. Comparison of the average richness and diversity characteristics of the total component community of helminths of barbel

| Sampling sites                         | Vidin (n = 165)  | Kozloduy (n = 193) | Silistra (n = 49) |
|--|------------------|--------------------|-------------------|
| No. of helminth species                | 6                | 6                  | 4                 |
| Shannon-Wiener Index (H <sub>S</sub> ) | 0.23             | 0.52               | 0.56              |
| Shannon-Wiener Evenness (E)            | 0.13             | 0.33               | 0.40              |
| Simpson's Index (D)                    | 1.10             | 1.42               | 1.56              |
| Berger-Parker Index (d)                | 0.95             | 0.82               | 0.77              |
| Dominant species                       | <i>P. laevis</i> | <i>P. laevis</i>   | <i>P. laevis</i>  |

Table 7. Seasonal profile of the diversity characteristics of the total component community

| Sampling sites |                | Vidin | Kozloduy | Silistra |
|----------------|----------------|-------|----------|----------|
| Spring         | H <sub>S</sub> | 0.34  | 0.60     | 0.32     |
|                | E              | 0.25  | 0.43     | 0.22     |
|                | D              | 1.21  | 1.61     | 1.20     |
|                | d              | 0.91  | 0.75     | 0.91     |
| Summer         | H <sub>S</sub> | 0.09  | 0.59     | 0.62     |
|                | E              | 0.06  | 0.37     | 0.44     |
|                | D              | 1.03  | 1.58     | 1.68     |
|                | d              | 0.98  | 0.76     | 0.72     |
| Autumn         | H <sub>S</sub> | 0.24  | 0.22     | 0.42     |
|                | E              | 0.17  | 0.16     | 0.38     |
|                | D              | 1.11  | 1.10     | 1.33     |
|                | d              | 0.95  | 0.95     | 0.86     |

Kakacheva-Avramova (1977) reported 3 cestode species (*Caryophyllaeus brachycollis*, *C. laticeps*, *C. fennica*) for barbel. The absence of cestodes during our sampling period could be explained with high *P. laevis* infection levels, which result from the barbel's preferred diet consisting of amphipods and small fishes. The feeding habits of barbel and its diet are influenced by the available local invertebrate fauna, which itself is determined by the water quality and habitat composition. A major characteristic of the principal invertebrate fauna in the Danube River is the high abundance of gammarids, from which some species are known to be appropriate intermediate hosts for *P. laevis* (Rumpus and Kennedy, 1974; Marshall, 1976; Moravec and Scholz, 1991; Dezfuli *et al.* 2000). Preferred feeding of fish on amphipods results in high abundance of *P. laevis*, which obviously reduces the diversity of parasite communities (Kennedy *et al.* 1986; Moravec *et al.* 1997).

The second most frequent parasite at all Danube localities, *R. hellichi*, occurred at the sampling site Vidin with a prevalence of 24.2%. The prevalence was about 2-fold lower compared to the data obtained from the other 2 sampling sites. According to Moravec and Scholz (1995) trichopteran larvae from the genus *Hydropsyche* serve as intermediate hosts

for the transmission of *R. hellichi* (see, for example, Moravec, 1995). Thus, the lower prevalence at Vidin can be explained with a lower abundance of the intermediate host, which could be correlated to a higher eutrophication and pollution level in this part of the river. The larvae of *Hydropsyche* sp. are well-established indicators which are used to assess the water quality (Moog, 1995). For example, the saprobic index of trichopteran larvae varies between 2.1 and 2.3 and corresponds to water quality class 2.

Moreover, the prevalence recorded for the nematode *Eustrongylides* sp. in Vidin was the highest at all sampling sites. This supports the pollution hypothesis, since the first intermediate host described for *Eustrongylides* sp. are aquatic oligochaetes such as *Lumbriculus variegatus* (Lumbriciidae), *Tubifex tubifex* and *Limnodrilus* sp. (Tubificidae) (Moravec, 1994). All these oligochaete species indicate disturbed aquatic habitats (saprobic index over 3, pollution with chemicals) where they are highly abundant.

The results of the present study correspond very well with data of Valtonen *et al.* (1997) who also correlated the occurrence of single parasite species in fish with the abundance of intermediate hosts from lakes with differences in trophic status and degree of pollution. For example the acanthocephalan *Acanthocephalus lucii* showed the highest prevalence in perch (*Perca fluviatilis*) from a eutrophic and polluted lake. The intermediate host of *A. lucii*, *Asellus aquaticus*, is known to be pollution tolerant and is highly abundant under contaminated conditions (Murphy and Learner, 1982). Not only the occurrence of a single parasite species can be related to environmental parameters but also the composition and diversity of whole parasite communities is determined by environmental conditions such as eutrophication, pollution and changes in substrate composition. These conditions can either directly affect the parasite (e.g. toxic effects on free-living stages) or indirectly by affecting the abundance and distribution of the respective intermediate and final hosts (Sures, 2004). Evidence from the field revealed the composition of fish helminth communities being largely dependent on the benthic invertebrate fauna, which itself is directly dependent on water quality and benthic habitats (Sures and Streit, 2001; Laimgruber *et al.* 2005; Thielen *et al.* 2007).

In the present study the lowest value for the Brillouin index and the Shannon-Wiener diversity was recorded for Vidin. As parasite diversity is considered a measure of ecosystem health (Hudson *et al.* 2006), the higher diversity at Silistra gives evidence for better environmental conditions in the lower river stretch. This is confirmed by hydrochemical data, which indicate a higher level of pollution and eutrophication at Vidin compared to Silistra. Eutrophication might favour the occurrence of intermediate hosts known to be tolerant against high nutrient concentrations such as annelids and crustaceans.

Additionally, the presence of toxic metals supports the occurrence of parasites transmitted by annelids or crustaceans, for example, by compromising the immune system of the definitive host. Thus, the combined effects of high nutrient and pollutant concentrations represent favourable ecological conditions especially for the dominant occurrence of *P. laevis*. This dominance also negatively affects in-fracommunity and component community diversity as it leads to lower values for the Shannon-Wiener and Simpson index. Our results therefore provide good evidence that aquatic ecosystem health could be assessed by investigating the composition and diversity of fish parasite communities which, also due to their position in food webs (Lafferty *et al.* 2008), represent an integrative measure of the overall ecological conditions.

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