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Effect of River Fragmentation on the Abundance and Size Structure of *Barbus petenyi* (Actinopterygii: Cyprinidae), Iskar River, Bulgaria

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Abstract: Habitat fragmentation affects the populations of many fish species, but little attention is paid to cyprinids. The black barbel, *Barbus petenyi* (Heckel 1852) is a characteristic species in the mountain and sub-mountain streams of the lower Danube tributaries. *B. petenyi* is of particular interest because of its value as a game fish and as well as an indicator species listed in Annex II of the European Commission's Habitats and Species Directive (92/43/EEC). The 19 km sector of the upper section of Iskar River was investigated during May-June, 2011. In the investigated zone, five flow-obstructing structures (weirs) were identified that have the potential to prevent or limit the upstream movement of *B. petenyi*. The abundance (ind.·100 m^{-2}), biomass (kg·100 m^{-2}) and size structure of *B. petenyi* were determined immediately downstream and upstream of each weir. It was observed that the abundance of *B. petenyi* declined dramatically in upstream direction due to the restricted re-colonization and migration processes. Barbel abundance did not depend on the distances between fragmentation structures. A significant difference was found in the body length frequencies of the barbel caught above the weir compared to those caught below. Several protection measures are discussed.

Key words: river fragmentation, *Barbus petenyi,* fragmentation, abundance, size structure, Iskar River

Introduction

Interruption of the river longitudinal connectivity through construction of barriers, such as hydroelectric power plants, dams, weirs, culverts, and bridges, is one of the most serious and widespread modifications of running water ecosystems (Ward, Stanford 1979, Dynesius, Nilsson 1994, Welcomme 1994, Jungwirth 1998, Nilsson *et al*. 2005). In many cases river fragmentation leads to significant habitat degradation as a result of changes in different physical and chemical parameters, such as water velocity, temperature regime, sedimentation processes, nutrition accumulation (Kingsford 2000). Obstructions restrict the free-flowing rivers and suppress drift detritus and invertebrates, i.e. physical barriers reduce the biological productivity of the ecosystem (Cortes *et al*. 2002). Particularly more sensitive to the effects of fragmentation are fishes performing significant migrations (Jungwirth 1998, Northcote 1984, Northcote 1998).It is considered that the adverse impact of physical barriers on fish is due not only to the disturbance in the seasonal breeding and migration, but also to limited access to preferred habitat and food resources, increasing the chances of occurrence of disease, reducing the gene flow between individuals constituting the population (Philippart *et al*. 1988, Aarts *et al.* 2003, Lucas, Baras 2001, Blanchet *et al.* 2010). The river impoundment are associated with significant changes in fish community structure, age-size structure of native species and dispersal of non-native fish species (Taylor *et al.* 2001, Miranda *et al.* 2005, Habit *et al.* 2007, Alexandre, Almeida 2010). For many years the attention was focused mainly on salmonid species and it was considered that the other fish species were less threatened in case of river fragmentation (Ward 1927, Johnson 1960, Morita, Yamamoto 2002). In the last few years this opinion changed mainly because of awareness that non-salmonid fishes also migrate in rivers, seeking shelter or food, and of the possibility that the upstream obstructions may be linked to the declining resident fish communities (Cowx 1998, Peter 1998). It is now known that many rheophilic cyprinids from the temperate zones perform short and medium distance migrations (Montgomery *et al.* 1983, Whelan 1983, Northcote 1984, L'Abée-Lund, Vøllestad 1987, Tyus 1985, Smith 1991, Lucas, Baras 2001). An example of such a species is Romanian barbel, *Barbus petenyi* (Heckel 1852, Teleostei, Cyprinidae) known in Bulgaria as black or stream barbel. *B. petenyi* belongs to genus *Barbus*, family Cyprinidae (Tsigenopoulos *et al.* 1999; Tsigenopoulos, Berrebi 2000), and is one of the four barbel species described in Bulgarian fresh waters (Berrebi *et al.* 1996, Kotlik, Berrebi 2002, Kottelat, Fryhof 2007). *B. petenyi* is widely distributed in the mountain and sub-mountain reaches of rivers flowing into the Danube River (Drenski 1921, Drenski 1951, Paspalev, Peshev 1955, Karapetkova 1994, Heckel, Kner 1858, Dikov *et al.* 1988, Dikov *et al.* 1994, Kotlik, Berrebi 2002, Trichkova *et al.* 2004). Representatives of the genus *Barbus* have been found to perform migration for foraging, shelter and spawning. For example, male and female *B. barbus* and *B. meridionalis* migrate during the spring to spawning grounds (Baras, Cherry 1990, Baras 1992, Lucas, Batley 1996, Lucas, Frear 1997, Vilizzi *et al.* 2006). Movements from several meters to 20 km in length have been reported (Baras 1992, Lucas, Frear 1997, Peňáz *et al.* 2002). In larger rivers such as Rhine and Danube, some of barbels tagged by Steinmann *et al.* (1937) were re-captured in a distance more than 300 km from their release site. It is considered that the main purpose of these movements is searching suitable spawning ground. Another part of the local migrations are performed by juvenile fish which are strong enough to return back in the upper reaches, where their feeding and breeding locations are. Sometimes in slow-current stretches of rivers some small immature individuals may be seen and it might be wrongly interpreted that there is suitable habitat for the species in standing water bodies. In fact, however, these fish have been transported downstream by the strong water flow usually during spring mounts.

As the black barbel is a rheophilous species, it is sensitive to the changes in the structural diversity of rivers and some local populations may be in risk of extinction as a result of river regulation (Stefanov 2007, Pavlova, Pehlivanov 2009). Therefore, the black barbel could be used as a functional describer or 'indicator species'. *B. petenyi* is listed in Annex II of the European Commission's Council Directive 92/43/EEC. The black barbel's habitats fall within 40 of the proposed 225 sites from the European ecological network NATURA 2000 in Bulgaria.

A key question when it comes to building water abstraction weirs or hydroelectric power plants is whether river modification is a potential limiting factor for the distribution of the black barbel and whether its populations in such regulated river in Bulgaria are at real risk. Adequate assessment of the actual status of the fish population should include the following criteria: fish abundance (density), size-age structure and distribution of the target species in individual rivers (Cowx *et al.* 2009).

The aim of this study was to assess the possible influence of river fragmentation by weirs on the basic population parameters of *B. petenyi* inhabiting the upper zone of the River Iskar. To this end, our specific objectives were: 1) to determine the abundance and biomass of the black barbel upstream and downstream of the river obstructing devices; 2) to determine the size structure of the barbel inhabiting areas upstream and downstream of each fragmentation. This information will contribute to future plans for management of this species and help assess the need for implementation of conservation measures.

Material and Methods

Study area

The study was conducted in the upper course of Iskar River, Bulgaria, during May-June, 2011. It is the longest Bulgarian river (about 360 km), with many reservoirs built on it. It is formed by the confluence of Cherni Iskar, Levi Iskar and Beli Iskar Rivers. The average slope of the catchment is 6.7‰ (a maximum value of 233‰ and a minimum of 0.75‰). The average rate of discharge in the main river for the period 2003–2004 was 4.55 L/s/km^2 (DRBD, 2004). In the upper reaches (Rila Mountains), the rate of discharge has the highest value (36.4 L/s/km^2, Cherni Iskar River), which gradually decreases downstream until it reaches Sofia Plain. In the Balkan Range, the rate of discharge again increases to 7.47 L/s/km^2, and after the confluence with Zlatna Panega River, its value decreases to 6.52 L/s/km^2. In the upper stream of Iskar River there are four reservoirs: 'Beli Iskar', 'Iskar', 'Pasarel' and 'Pancharevo'.

The study area was located along the upper Iskar River within a range of 20 km and at altitudes of 940-1020 m a.s.l. (Fig.1). In this zone, five flow-obstructing structures (weirs) with mean height of 3 m and two reservoirs ('Iskar' and 'Pasarel') were identified that have the potential to prevent or limit the upstream movement of fish species. For the purposes of this study we selected 12 sampling sites. The sites are grouped into three categories: directly downstream of the weirs (DW), directly uppstream of the weirs (UW), one site between 'Pasarel' and 'Iskar' reservoirs (P1) and one site at least 3 km away from the last upstream weir (S12). All investigated sites represent adequate river morphology and are characterized by alternating poo-–riffle sequences. The length of each study site is within 10-40 river widths (Cowx *et al.* 2009). The black barbel co-existing species were: *Squalius cephalus*, *Alburnoides bipunctatus, Gobio gobio, Phoxinus phoxinus, Romanogobio kessleri, Romanogobio uranoscopus and Sabanejewia balcanica.*

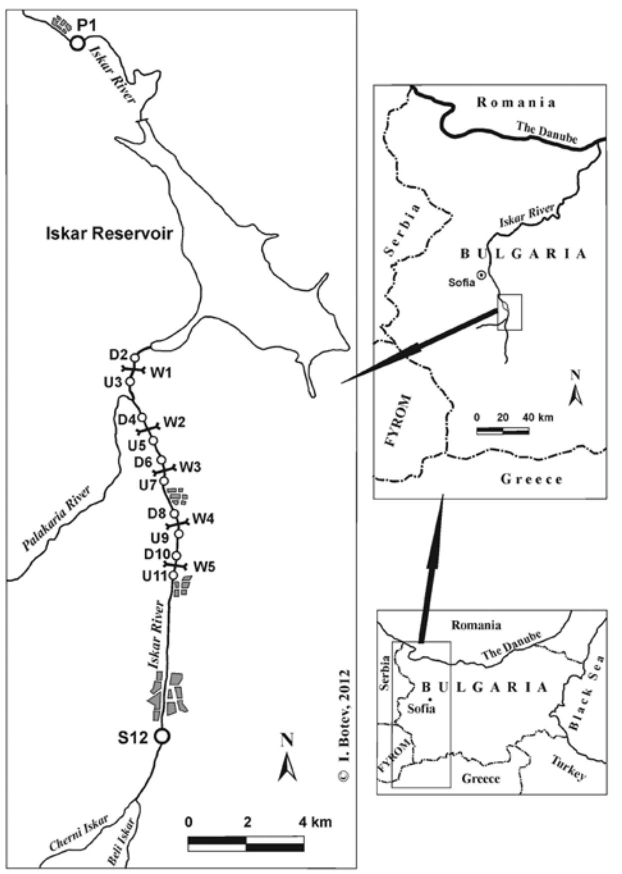

Fig. 1. Locations of weirs (W1-W5) and sampling sites (P1-S12) on section of Iskar River, Bulgaria.

Fish Sampling

Fish were collected in the period after peak of high water (May-June 2011) by electrical fishing (model Samus-725G) (DC, 30 cm diameter aluminium anode, medium voltage from 200 V to 350 V, current strength average of 3 A to 8 A, depending on water electroconductivity) and frequency of 45 pulses per second. Two consecutive electrofishing operations were carried out, with an interval of at least 60 min between the first and second catch (Seber, Le Cren 1967). Caught fish were anaesthetized with clove oil, counted and their total length (TL, mm) and body weight (W, g) were measured. After the second catch, all fish were released back into the river in the area of the catch.

Data analysis

Zippin maximum-likelihood method for two catches (Zippin 1958) was used for estimating population size (N). Capture probability (*p*) was estimated for each site and for each species. Species' abundance was expressed as number of fishes caught per 100 m^2. If Zippin method was not applicable, abundance was calculated by multiplying the total number of fish caught (Cs), by the N/C_s ratio estimated for a site where the lowest catch efficiency for the barbel was observed. Absolute estimates (total catch) were used for a population in which the total number of captured individuals in a given site did not exceed three fish in two catches. The equation for calculating the biomass (B) is $B=B_s N/C_s$, where B_s is the total weigh of fish caught, and C_s is the total number of fish caught (Agostinho, Penczak 1995).

The abundance and biomass in the sections locked between dams/weirs ('Passarel' – 'Iskar', 'Iskar'-W1, W1-W2, W3-W4, W4-W5, W5-S12) was obtained by aggregating data from stations located in the area between two fragmented structures: section Z1=P1; Z2 = (U3 + D4)/2; Z3 = (U5 + D6)/2; Z4 = (U5 + D6)/2; Z5 = (U7 + D8)/2, and Z6 = S12.

Fish were measured to the nearest millimeter (TL), and size-class distribution from upstream and downstream catches were compared graphically. Because of the non-normal distribution of the length-frequency data, non-parametric methods of analysis were used. The length – frequency distribution of fish caught below each weir was compared to those caught above, using Mann-Whitney *U*-test. Analysis of variance (one-way ANOVA, $P < 0.05$) was applied to evaluate the differences in the fish abundance and biomass in the sites located upstream and downstream of the weirs. Prior to analysis, all variables were evaluated for normality. Those that significantly departed from a reasonable normal distribution were log ($x + 1$). For statistical analysis, the programs Statistica 7.0 (© StatSoft Inc.) and XLStat (Addinsoft) were used.

Results

B. petenyi were found at all sampling sites located between Reservoir 'Pasarel' and the confluence of the rivers Beli Iskar and Cherni Iskar**.** The abundance of *B. petenyi* at sampling sites varied between 0.41·100 m^{-2} and 70.1·100 m^{-2} (Fig. 2). The mean abundance of *B. petenyi* for the whole investigated area was 17.8·100 m^{-2}. The lowest abundance was observed in two sites: the one above the last weir (S12) and the one under Iskar Dam (P1). At weirs W1, W2 and W5 the abundance was significantly higher in the downstream areas of the weir than that in the upstream sections ($P < 0.05$). At weirs W3 and W4 the downstream and upstream abundance did not differ significantly. The biomass of the black barbel varied also significantly between sites: from 7.95 g·100 m^{-2} to 288.4 g·100 m^{-2}. The mean biomass for the whole investigated section was 83 g·100 m^{-2}. Downstream of the weir sections the barbel biomass was significantly higher than that in the upstream zones ($P < 0.05$) (Fig. 3). When considering the abundance and biomass of black barbel in the areas bounded between fragmented structure (zones Z1–Z6), we observed a clear downward trend in these indicators with the distance from the first weir in the direction of the higher parts of the river. The zones with high level of abundance were those situated immediately upstream of Reservoir Iskar (Z2) and zone Z3 covering the area between weirs W1 and W2 (Fig. 4). Accordingly, the areas of highest biomass scored were directly above Iskar Reservoir and the zone limited between weir W1 and W2 (Fig. 5). We found no relation between fish abundance and length of river stretch locked between two weirs.

Similar to abundance, the length-size structure of the black barbel showed some specifics depending on the location of caught – downstream or upstream of the weir. The statistical analysis revealed significant differences between the length frequencies distribution of barbel caught above the weir compared to those caught below the weir W1 (Mann–Whitney

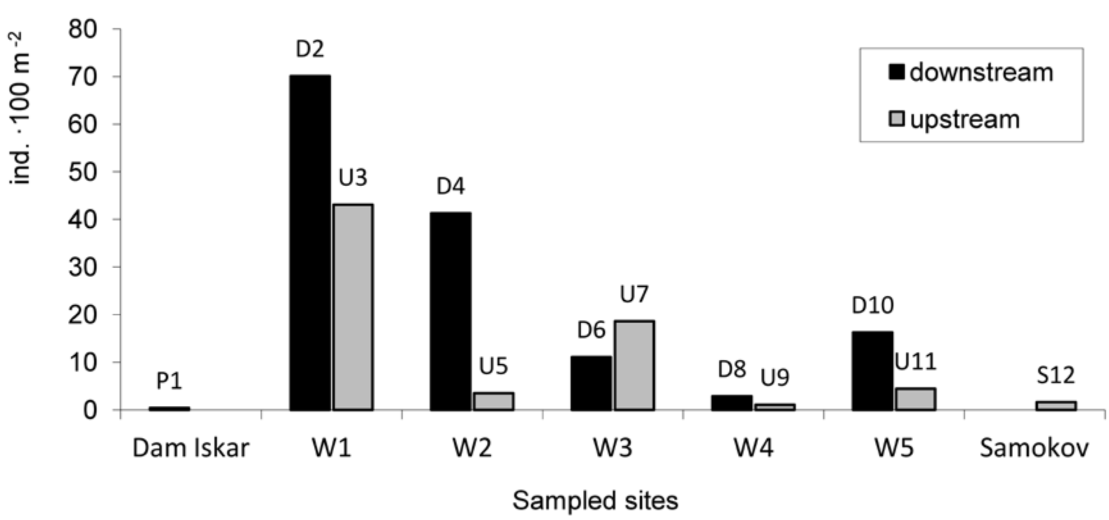

Fig. 2. Distribution of *B. petenyi* abundance (N of specimens per 100 m^2) at sites downstream (D) and upstream (U) of the weirs (W1 – W5) located in the upstream section of Iskar River, Bulgaria.

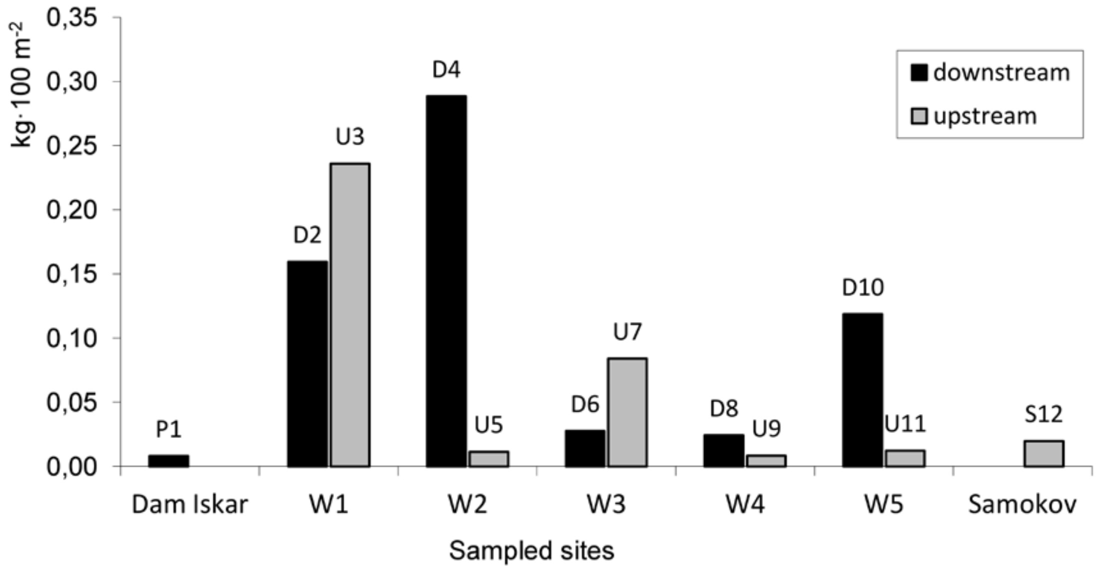

Fig. 3. Distribution of *B. petenyi* biomass (kg per 100 m^2) at sites downstream (D) and upstream (U) of the weirs (W1 – W5) located in the upstream section of the River.

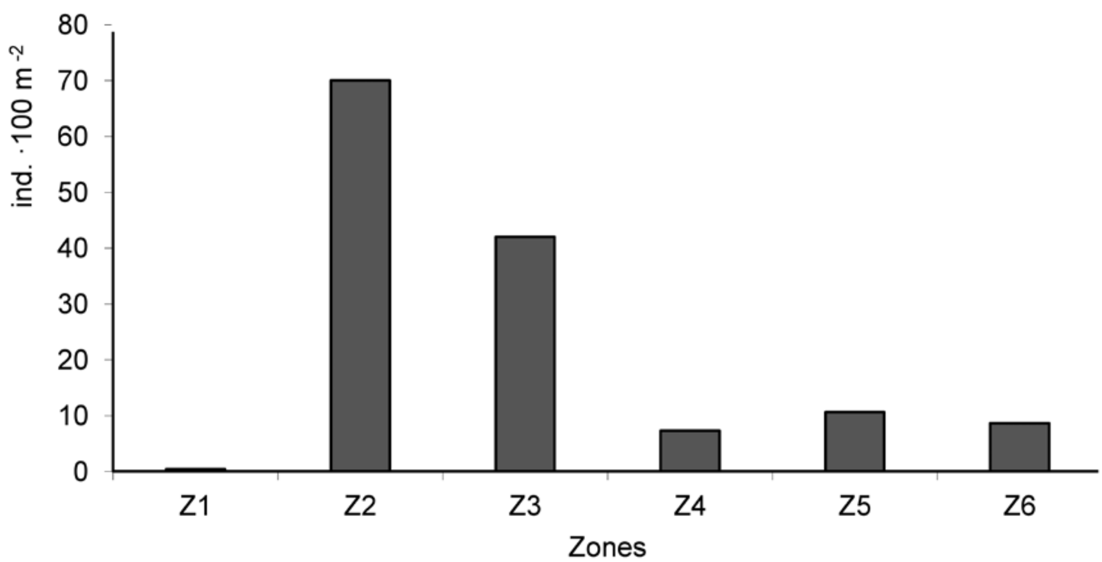

Fig. 4. Abundance of *B. petenyi* (N of specimens per 100 m^2) in zones (Z1 – Z6) limited between river – obstructing devices (weirs, dam), located in the upstream section.

U-test, $Z = -3.850$, $P = 0.001$). There was a greater proportion of small fish caught at the downstream area (Fig. 6). A significant differences was found in length – frequency of barbels caught above the weir W2 with those caught below the same weir (Mann–Whitney *U*-test, Z = 2.093, P = 0.036), however, smaller fish were caught above the weir than below (Fig. 7). At weir W3, the same tendency was observed: the fish caught upstream of the weir had smaller body length as compared to those caught downstream (Mann–Whitney U-test, Z =1.96, P = 0.05) (Fig. 8). At weirs W4 and W5 statistically significant differences were not observed either in the length-frequency distribution, or in the mean length.

Discussion

The observed irregular distribution of *B. petenyi* abundance at the investigated section of Iskar River may be associated with different factors with local negative influence such as impact pollution, urbanization, microhabitat unsuitability, interruption of longitudinal and/or lateral connectivity. The populations of the black barbel usually show patchy

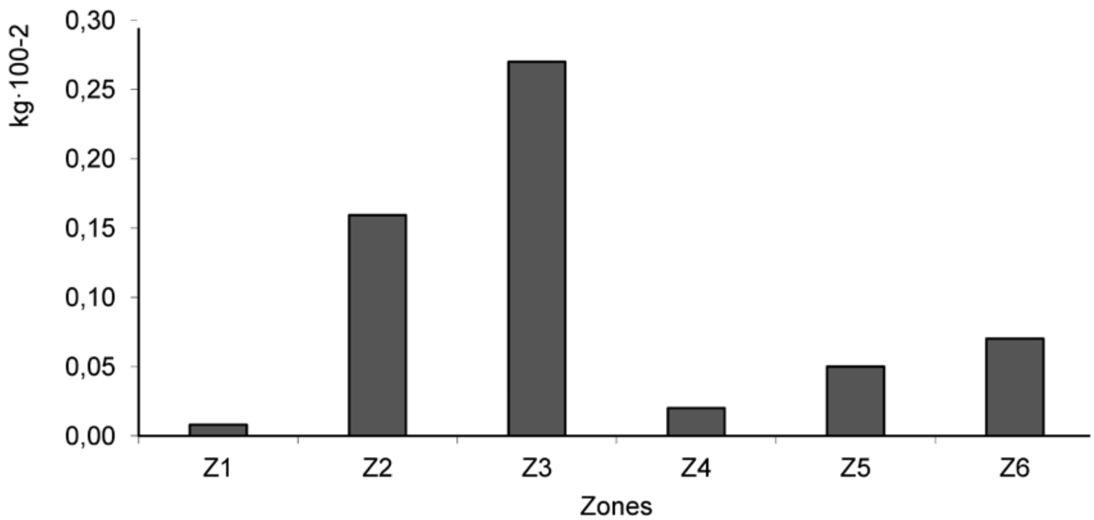

Fig. 5. Biomass of *B. petenyi* (kg per 100 m^2) in zones (Z1 – Z6) limited between river – obstructing devices (weirs, dam), located in the upstream section of Iskar River.

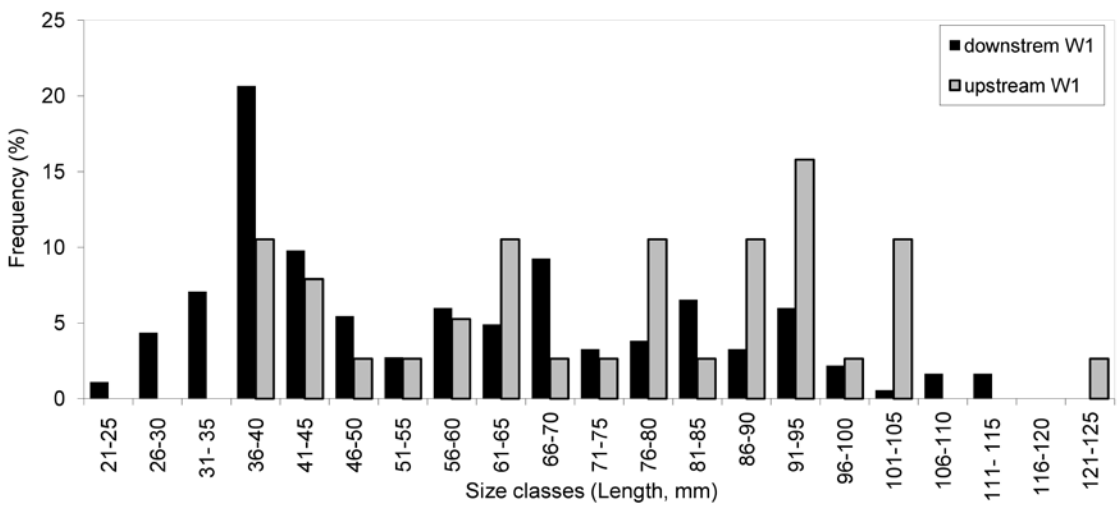

Fig. 6. Length-frequency comperison (TL,mm) between *Barbus petenyi* caught below the weir W1, and those caught at the uppstream areas of the same weir (N=222).

distribution, due to the fact that the specific hydrological and ecological conditions are not equally favourable for this species at all locations along the river length (Karapetkova, Dikov 1986, Dikov *et al.* 1988). However, the investigated river sector is only 20 km in length and is characterized with macro- and microhabitat conditions favourable for the black barbel and lack of other considerable negative factors except multiple fragmentation existing in this sector of the river. This could suggest that the observed tendency towards reduced the black barbel abundance upstream of weir W1, could most probably be a result of interrupted upstream passage of fish making their breeding and dispersal migrations impossible. Species belonging to the *Barbus* genus are known to perform upstream movements during the spring, followed by downward movements of females in summer but a tendency for males to remain upstream for several months (Baras *et al.* 1994, Lucas, Frear 1997, Vilizzi *et al.* 2006). Migration behaviour of the barbel could be also inferred from their seasonal occurrence in fish ladders. Radiotelemetric investigations show that the density of *Barbus barbus* during the breeding period increased significantly in areas located downstream of the obstructing devices (Peter 1998). Similar migratory behaviour of *B. petenyi* would explain the observed higher abundance downstream of the weirs, where the fish most probably concentrate in their attempt to move upstream. It has also been reported that, al-

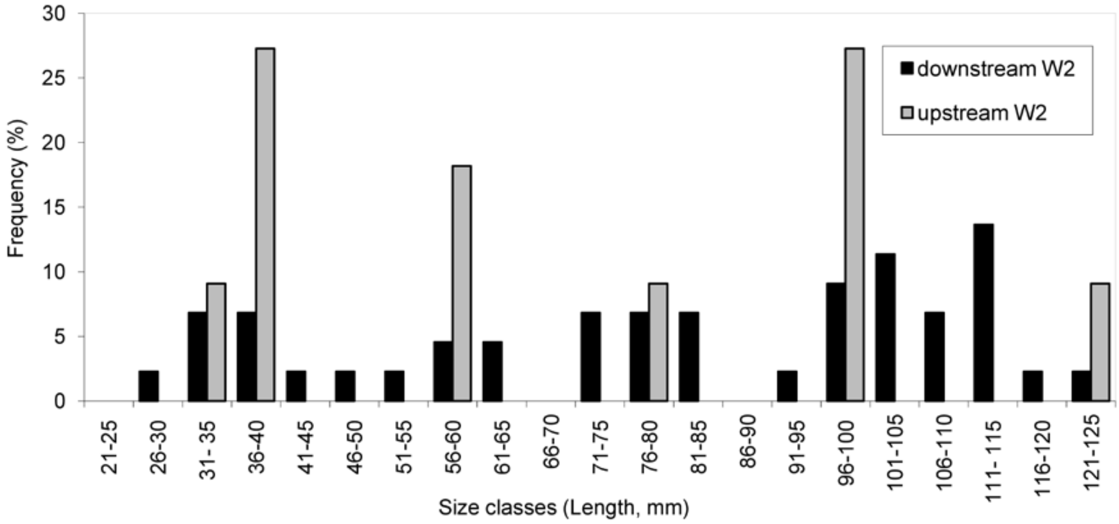

Fig. 7. Length-frequency comperison (TL,mm) between *Barbus petenyi* caught below the weir W2, and those caught at the uppstream areas of the same weir (N=55).

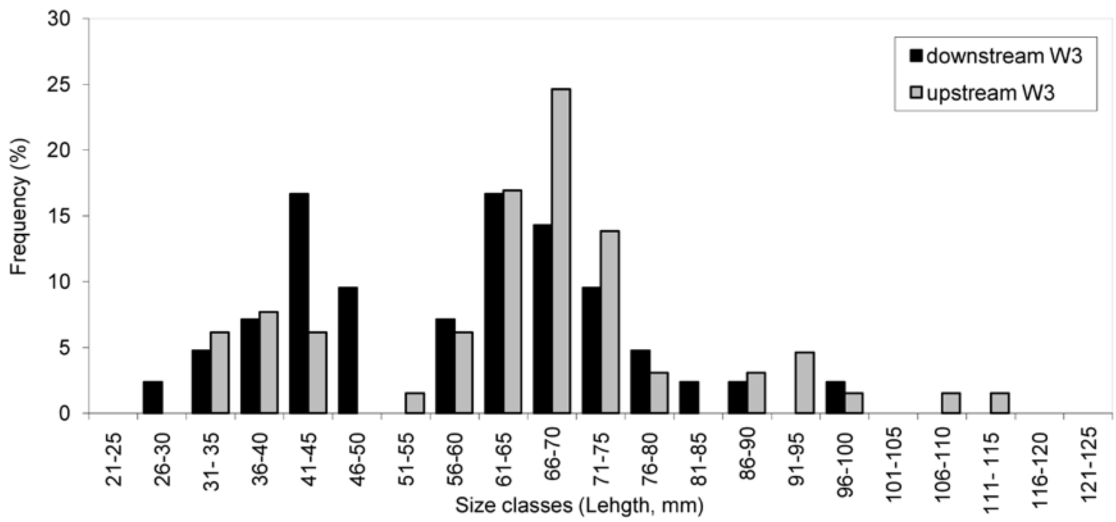

Fig. 8. Length-frequency comperison (TL,mm) between *Barbus petenyi* caught below the weir W3, and those caught at the uppstream areas of the same weir (N=107).

though barbels are good swimmers, they are unable to pass significant obstructions (Lucas, Frear 1997), and even obstructions of 0.4 m in height prove to be impassable, resulting in complete lack of the species in the areas upstream of weirs (Peter 1998). Unable to pass to the upper stretches of the river, the fish are most probably forced to seek favourable breeding conditions in the areas enclosed between the weirs. Therefore, the population of the black barbel, limited between two insurmountable barriers formed by fish which reproduce in this area plus replenishment with larvae and eggs drifted downstream by the water flow. Physical isolation of the barbels inhabiting areas between weirs, probably lead to the formation of small subpopulations. According to Gerking (1953), in streams with a well developed riffle-pool structure, each stream section separated by riffles can be considered as a more or less isolated unit containing a natural population of its own. The connectivity between these subpopulations plays an important role in the formation and functioning of metapopulation (Schmutz, Jungwirth 1999). Commonly, as a result of river fragmentation, a considerable part of the water inhabitants become no longer members of panmictic populations but rather of isolated populations between the weirs (Schmutz, Jungwirth 1999). Barbel movements restricted or impeded by the construction of dams, or weirs seems to be especially serious problem in those streams where natural habitat physical heterogeneity and riffle-pool development are in decline and the spawning migrations to remote sites are necessary (Peňáz *et al*. 2002). Prolonged isolation could have severe demographic and genetic consequences in the future (Jungwirth 1998, Peter 1998, Blanchet *et al.* 2010). River fragmentation and local pollution incidents have been identified as causal factors in population declines of lithophilous and rheophilous cyprinids in some rivers (Philippart *et al.* 1988, Baras *et al.* 1994).

Moving away from the first weir in the investigated area of Iskar River, two main tendencies in length distribution of the black barbel were observed: increase in the frequency of fish with larger size and difference in length frequency distribution between fish inhabited upstream and downstream areas of the weirs, as smaller fish were caught above the weir than below. This distribution of the size structure indicates that the locations downstream of the weirs are concentrated mainly with reproductive individuals which head towards the breeding sites further upstream along the river (Baras *et al*. 1994, Lucas, Frear 1997). The fish observed at the highest sites (U11) were predominantly 121–125 mm in length. According to Dikov *et al*. 1994, they belong to 3-4 age classes. These demographics could possibly reflect the fact that during the spring period of high waters, larvae and smaller fishes are drifted downstream and compensatory replenishment cannot come from other sections of the river (Peter 1998). The size-age structure of fish populations can serve as an indicator for the effectiveness of 'fish pass' structures (Harris *et al*. 1998).

The restoration of longitudinal connectivity of river beds, fragmented by the construction of different obstructions (reservoirs, weirs, etc.), is of preeminent importance in the conservation of the native barbel populations (Baras *et al*. 1994, Lucas, Batley 1996, Lucas, Frear 1997, Schmutz, Jungwirth 1999). The construction of fish passes is the main method for mitigating the effects of river obstruction. The highly uneven abundance and demographic structure distribution of the black barbel should be taken into account when choosing stations for monitoring of fish populations in fragmented rivers.

Conclusions

River fragmentation and disrupted longitudinal connectivity in upper part of Iskar River lead to lower abundance of the black barbel and to changes in its demographic structure. Isolation and formation of subpopulations would bring about disturbances in the genetic structure and drastic decline of the river stretch inhabited by the black barbel.

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