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Spatial Variation in the Abundance and Population Structure of Bullhead *Cottus gobio* L., 1758 (Actinopterygii: Cottidae) from the Iskar River Basin (Danube River Drainage, Bulgaria): Implications for Monitoring and Conservation

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Abstract: The bullhead, *Cottus gobio*, can be found in several Bulgarian rivers, and they most probably represent the southern boundary of species distribution in Europe. The bullhead population in the Iskar River basin is the most numerous and highly fragmented. However, relatively little is known about the current distribution and population status of *C. gobio* in this river basin. In the present study, in the period between 2011 and 2015, *C. gobio* was discovered in the upper part of the Iskar River and eight of its tributaries: Beli Iskar, Cherni Iskar, Levi Iskar, Lakatitsa, Lopushanska, Preka, Batulyiska, and Zlatna Panega. Since 2014, *C. gobio* has been reintroduced in the Palakaria River (left tributary of the Iskar River). All detected localities of *C. gobio* were examined for abundance, biomass, size structure, weight–length relationship, and condition. The abundance and biomass of bullhead ranged from 3.8 to 31.4 ind,·100 m^2 and from 0.01 to 0.32 kg·100 m^2 for biomass, respectively. The proportion of juveniles also varied significantly, not only between different streams but also between different sites at the same location. In five localities, the average proportion of young fish was more than 40%. The estimations for parameter *b* of the weight–length relationship ($W = aL^b$) ranged between 2.963 and 3.369. The condition (Kn) for all sites was above 1. For monitoring purposes, this study suggests using two main parameters, fish abundance (density) and proportion of young of the year and 1+ fish, as indicators of the state of populations of *C. gobio*. Densities lower than 10 ind, ·100 m^2 and a proportion of juveniles under 40% should be considered unfavourable states of populations. Based on these reference values, it can be concluded that the *C. gobio* inhabiting the Cherni Iskar River are in a favourable condition.

Key Words: *Cottus gobio,* distribution, abundance, condition factor, WLR, Iskar River basin

Introduction

About 15 species of the genus *Cottus* L., 1758 (Scorpaeniformes: Cottidae) are found in European waters (Freyhof et al. 2005). They are relatively small, bottom-dwelling fish that prefer mainly cold, well-oxygenated streams, medium-sized rivers, and, much more rarely, lakes, channels, and large rivers (e.g. Tomlinson & Perrow 2003, Kotusz et al. 2004, Freyhof et al. 2005). Two *Cottus* species have been described in Bulgarian freshwaters: the endemic species *Cottus haemusi* Marinov & Dikov, 1986 and the more common *Cottus gobio* Linnaeus, 1758. While *C. haemusi* can be found only in the Kostina River, a tributary of the Beli Vit River (Uzunova 2011), *C. gobio* is more widely spread and can be found in mountainous tributaries of the rivers Ogosta, Iskar and Yantra (Danube River drainage system). The main negative factors, which are considered to play a role in *C. gobio* distribution and population viability, are habitat destruction, river fragmentation and water pollution (e.g. Hanfling & Brandl 1998, Utzinger et al. 1998, Hanfling et al. 2002, Knaepkens et al. 2002, Uzunova 2011). In conservation biology, the concept of population viability has been formally defined as a high probability of persistence over a long period of time (Thompson 1991). For adequate conservation of *C. gobio*, implementation of a monitoring system that reflects the population status is crucial. According to Cowx et al. (2009), assessment of the status of fish population should include three main parameters: fish density, population demographic structure, and size of the distribution area. For assessing the population vitality of *C. gobio*, particular emphasis is placed on the presence of specimens of different sizes and ages, Perrow & Côté (1999) suggested that the proportion of young of the year (YOY) *C. gobio* should represent approximately 50% of the total density at the end of the growing season, and this can be considered a 'favourable condition'. A comparatively high proportion of the 0+ group is considered evidence of a successful reproduction of the species, Tomlinson & Perrow (2003) suggest reducing this requirement to 40%. The presence of large adults (> 75 mm fork length) is another proposed indicator of a healthy *C. gobio* population (Perrow & Côté 1999). Population abundance and biomass, either individually or in combination, are also used for assessment of the status of *C. gobio* stocks. According to most studies, *C. gobio* mean densities in streams are <1 individual / m^2 (e.g. Crisp et al. 1974, Welton et al. 1983, Copp 1992, Waterstraat 1992, Cowx & Harvey 2003). Jansen et al. (2000) and Utzinger et al. (1998) found densities in the range of 1.89 to 14.7 individuals per m^2. Similar differences in *C. gobio* abundance from site to site were also found in the Garonne stream system (Legalle et al. 2005). In those parts of Northern Europe, in which *C. gobio* populations are not threatened and the species is quite common, abundances of less than 0.2 individuals per m^2 for upland rivers and less than 0.5 individuals per m^2 for lowland rivers, respectively, indicate sites with unfavourable conditions (Copp et al. 1994, Cowx & Harvey 2003, Carter et al. 2004, Cowx et al. 2009). Some other parameters of the life history have also been found that could be used as indices of population viability and population status. The weight–length relationship (WLR) and Fulton's condition factor (K) are two main parameters used in fishery research (Anderson & Neumann 1996). The WLR has several applications, mainly in fish biology, physiology, ecology, and fishery assessment, Cone (1989) indicated that the relationship between fish weight and length is frequently used to compare the effect of biotic and abiotic factors on the health or well-being of a fish population. Fish condition assessment is commonly use as an indicator of physiological well-being and reflects the proximate body composition of individual fish (e.g. lipid content, protein content, caloric content). The value of *K* can be used to estimate changes in nutritional condition. Murphy (1991) stated that condition indices provide a comparative measure of fish plumpness. Plump fish may be indicators of favourable environmental conditions (e.g. habitat conditions, prey availability), whereas thin fish may indicate less favourable environmental conditions.Several other life-history parameters, such as the age at maturity, proportion of spawners in the spawning stock, and fecundity, may also be used as indices of population viability. However, the measurement of these indices requires sacrificing the fish.

A considerable decline in the *C. gobio* populations has been reported for some countries, such as Switzerland, Germany, and Austria (Utzinger et al. 1998, Fischer & Kummer 2000). Bullhead is included in Annex II of the European Commission's Council Directive 92/43/EEC on the conservation of natural habitats and wild fauna and flora (Habitats Directive). In the Bulgarian Red Data Book, bullhead is classified as critically endangered (Stefanov & Trichkova 2015).

The Iskar River provides one of the southernmost habitats where *C. gobio* lives in Europe. *C. gobio* was reported for the first time in the Iskar River by Chichkoff (1939). Later, it was discovered in different tributaries of the Iskar River by Drensky (1951) and Paspalev & Peshev (1955). *C. gobio* is mentioned for the rivers in the Vitosha Mountain by Bulgurkov (1958). Over the years, *C. gobio* has become extinct in some localities, like the Palakaria River (left tributary of the Iskar River). On the other hand, there is a chance that some localities of *C. gobio* in the Iskar River basin are still undiscovered.

There is no contemporary and detailed assessment of the distribution and population status of *C. gobio* in the Iskar River basin. To fill this knowledge gap, the aims of the present study are to find out all localities of bullhead in the Iskar River basin and to examine their population and biological characteristics, which can be used as indicators for the population status of the species. The present findings will contribute to the development of adequate monitoring and conservation programmes.

Material And Methods

Study area

Iskar River is the longest Bulgarian river with total length of 368 km. It is formed by the confluence of Cherni Iskar and Beli Iskar Rivers. It has 25 tributaries with length over 15 km. The density of the river network in the catchment is 1.1 km·km^{-2}. The average slope of the catchment is 6.7‰ (a maximum value of 233‰ and a minimum of 0.75‰). In the upper reaches in Rila Mountains, the rate of discharge has the highest value (36.4 l·s·km^{2} in Cherni Iskar River), which gradually decreases downstream until it reaches the Sofia Plain. One large reservoir has been constructed along its course and there are also numerous natural and man-made river fragmentations.

Batuliyska River has total length of 40.2 km and takes its source from Murgash peak in Western Stara Planina Mountain. After the village of Rebrovo it flows into Iskar River. The catchment area of the river is 256 km^{2}, which represents about 3% of the catchment area of Iskar River. Zlatna Panega River begins from the karst spring Glava Panega. The total length of the river is 50.3 km and its catchment area is 350.2 km^{2}. It flows to Iskar River near the town of Cherven Bryag. The investigations of *C. gobio* distribution cover Iskar River basin with a focus on upper reaches of the streams.

Fish sampling

Fish sampling was conducted during the low water level period (August - September, 2011-2015), and *C. gobio* locations were determined through Point Abundance Sampling by Electrofishing method (PASE according to Nelva et al. 1979, Copp 1989). SAMUS-725G device (12 cm diameter ring anode, average voltage of 200-350 V, operating at an average of 3 - 8 A depending on water conductivity) was used. The PASE was applied every 200-500 meters in order to establish the presence or absence of species in every surveyed river. In case of detection of *C. gobio*, the density of the points of electrofishing was significantly increased (each 10-50 m). This allows precise determination of the upper and lower boundaries of *C. gobio* distribution in the particular river.

For assessment of *C. gobio* abundance electrofishing have been applied following the CEN standard (CEN, 2003) with the same equipment except ring anode, which was replaced with a 30 cm one. A single electrofishing transect without block nets was applied in every river stretch. Sampling

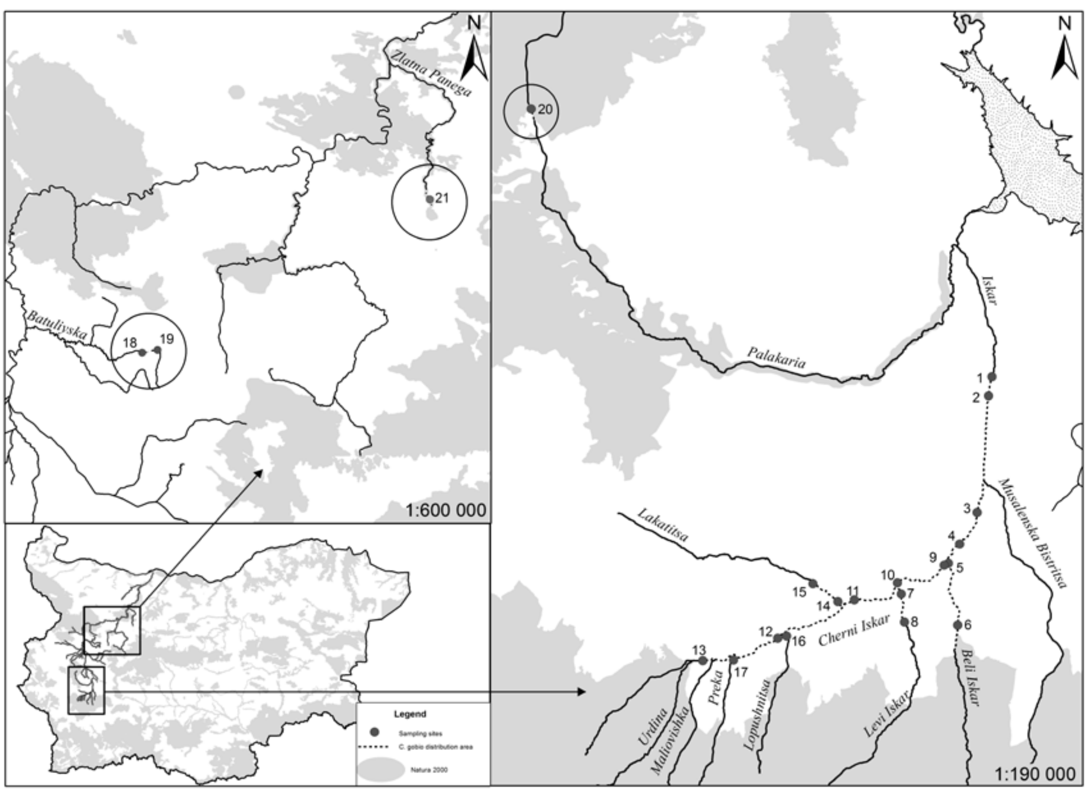
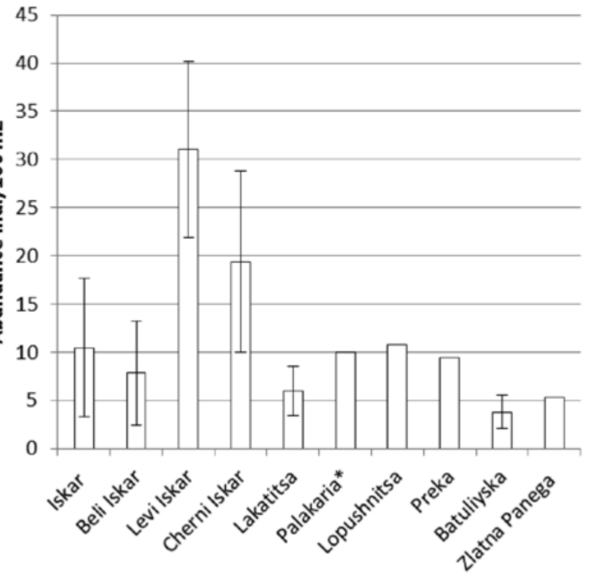
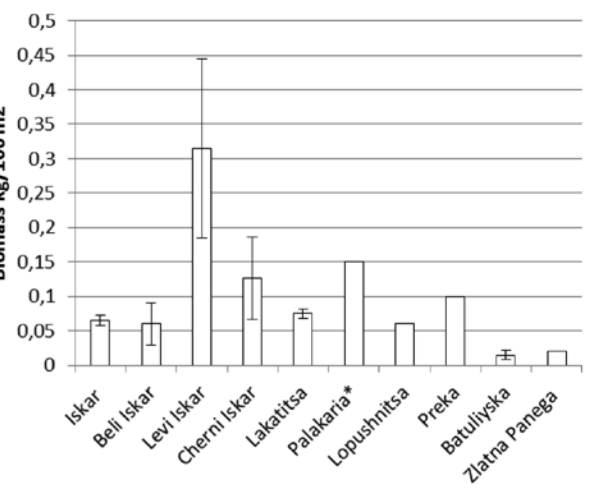


Fig. 1. Map of investigated area and sites where *Cottus gobio* was found



a



b

Fig. 2. Abundance (2a) and biomass (2b) of *Cottus gobio* in Iskar river basin. Error bars show the standard deviation

transects were between 30 to 165 m long, depending on river width (which varied from 3 to 18 m). The total fishing area per site varied between 287 to 1300 m^2. The electrofishing was conducted from one bank to the other over the entire river stretch. The collected fish were counted, measured (total length, TL in 0.1 cm and total weight, W in 0.1 g) *in situ* and then returned to the same river stretch.

More than 50 individuals of *C. gobio* have been re-introduced in the Palakaria River in 2014. Growth parameters of this fish were monitored through regular capture by electofishing.

All fish were returned after measuring to the locations of their capture.

Data analyses

The fish abundance was expressed as number of fishes caught per 100 m^2 and the biomass in kg per 100 m^2.

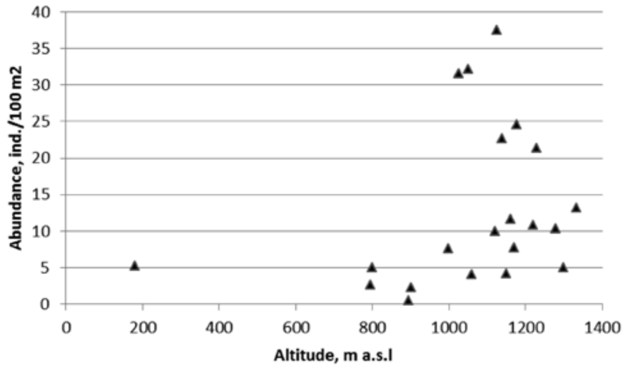


Fig. 3. Density of the populations of Cottus *gobio* located at different altitudes in the basin of river Iskar

The total length frequency was calculated with a 1 cm total length interval and it varied between 3 and 15 cm. The frequency formula is as follows:

TLi = Ni/ N × 100% (i = 3.1 – 4.0 cm, 4.1 – 5.0 cm, 14.1 – 15.0 cm). (1)

Where TLi is the frequency for a certain interval, Ni is the number of specimens in one total length interval, and N is the total number of specimens from one station.

The WLR was calculated using Eq, (2), where *a* and *b* are the coefficients, L is the total length (cm), and W is the wet weight (g):

$W = aL^b$ (2)

The 0+ fish and those smaller than 50 mm were excluded from the analysis to avoid possible differences in body form between juvenile and adult fish (Murphy 1991), *C. gobio* with a total size of up to 40 - 45 mm is defined as one summer old fish (0+), fish from 50 to 70 mm in length, corresponds to the 1+ age group, those with lengths from 80 to 90 mm constituted the 2+ group, and those with lengths from 100 to 110 mm formed the 3+ group (Cowx & Harvey 2003, Carter et al. 2004).

The statistical significance level of the coefficient of determination (r^2) and 95% confidence limits of *b* were estimated. Obvious outliers were identified and removed, according to the plot of the log W over log L (Froese 2006).

The relative condition factor (Kn) was calculated using the equation:

$Kn = M_o / M_E$ (3)

where M_o is the observed mass of each individual and M_E is the expected mass using the length–weight relationship ($M_E = aL^b$) (Le Cren 1951, Knaepkens et al. 2002).

To test for significant differences among parameter values, a one-way analysis of variance (ANOVA) and a post hoc Tukey test were carried out.

Table 1. Limits of distribution of bullhead in the upstream and downstream direction of the rivers of basin of the River Iskar

River	Upstream distribution end poin		Altitude m a.s.l.	Downstream distribution end point		Altitude m a.s. l.	Total area of distrubution (ha)	Area of distribution covered by "Natura 2000" (ha)	Factors constraining the spread of bullheaad	
	N	E		N	E				upstream	dawnstream
Iskar	42°17'19.04"	23°32'5.33"	1046	42°22'11.0"	23°33'23.9"	894	19.91	…	no	wiers, village area. pollution, agriculture
Beli Iskar	42°14'57.2"	23°32'29.8"	1225	42°17'19.04"	23°32'5.33"	1046	3.82	…	wier of water-abstraction	no
Levi Iskar	42°15'16.9"	23°30'46.1"	1196	42°16'32.23"	23°30'15.16"	1114	2.37	…	weir and low water level (HPP)	no
Cherni Iskar	42°14'15.1"	23°23'22.3"	1332	42°17'19.04"	23°32'5.33"	1046	16.11	…	increasing the slope of the river, natural barriers	no
Lakatitsa	42°16'26.4"	23°27'13.8"	1170	42°15'57.12"	23°28'30.46"	1149	1.08	…	no	no
Palakaria*	42°29'4.40"	23°16'10.20"	1125	42°28'28.60"	23°16'27.00"	1082	0.55	0.584	natural barriers. low water level	village area, pollution, agriculture
Lopushnitsa	42 15'05.2"	23 26'19.1"	1220	42°15'9.27"	23°26'22.96"	1212	0.10	…	increasing the slope of the river	no
Preka	42°14'16.7"	23°24'31.1"	1298	42°14'25.5"	23°24'25.6"	1278	0.17	…	increasing the slope of the river	no
Batuliyska	42°52'14.2"	23°37'13.8"	800	42°52'14.2"	23°37'05.2"	795	0.28	…	low water level in summer	village area, pollution, agriculture
Zlatna Panega	43°05'22.9"	24°09'40.3"	188	43°06'59.1"	24°09'09.4"	179	5.52	5.52	barrage, big artificial lake near the spring	village area, pollution, barrage
TOTAL	...	...	...	...	...	...	49.90	1.104		

* Bullhead was re-introduced in river during 2014-2015

Table 2. Descriptive statistics and W-L relationships (WLR) for 9 locations of *Cottus gobio* in River Iskar Basin (Danube drainage). N = sample size. standard length (cm) and weight (g). r^2: coefficient of determination. W: weight (g). min: minimum. max: maximum. L: length (cm). S.D.: standard deviation. S.E.: standard error. C.I.: confidence interval. b: slope

River/site	N	W [g]. mean (*Wmin- Wmax*)	SD	TL [cm]. mean (*Lmin- Lmax*)	SD	Parameters of the length–weight relations					r^2
						a	*b*	SE (*b*)	95% CI of *b*		
Iskar	178	7.34 (0.7 - 2.81)	5.19	8.15 (5.0 - 13.7)	1.77	0.0127	2.963	0.051	2.951	2.964	0.949**
Beli Iskar	57	8.87 (0.6 - 32.2)	7.89	8.11 (4.1-13.6	2.64	0.005	3.369	0.068	3.232	3.506	0.979**
Levi Iskar	180	11.06 (0.39 - 29.5)	6.28	8.99 (3.5- 13.2)	2.11	0.0131	3.001	0.058	2.887	3.115	0.942**
Cherni Iskar	259	8.35 (0.7 -27.6)	6.08	8.24 (3.0 - 13.0)	2.1	0.0087	3.149	0.042	3.061	3.238	0.954**
Lakatitsa	22	12.22 (0.9 - 31.2)	9.98	8.8 (3.9 - 13.50)	2.82	0.0083	3.195	0.109	2.966	3.424	0.987**
Palakaria*	12	26.43 (10.2 - 36.3)	8.56	12.23 (9.6 - 14.0)	1.33	0.0033	3.578	0.241	2.961	4.19	0.956**
Lopushnitsa	60	7.92 (2.6 - 25.9)	5.73	8.02 (4.0 -13.0)	1.75	0.0133	3.001	0.082	2.886	3.116	0.961**
Preka	34	9.03 (1.0 - 32.2)	9.67	7.89 (4.0 - 13.6)	3.04	0.0054	3.366	0.989	3.178	3.555	0.978**
Batuliyska	48	4.71 (0.7 - 21.6)	3.35	7.01 (3.2 - 11.4)	1.45	0.0073	3.243	0.031	3.059	3.426	0.967**
Zlatna Panega	12	4.58 (1.0 - 13.0)	5.09	6.04 (4.0 - 10.5)	2.59	0.215	2.773	0.0742	2.561	2.998	0.993**

* Bullhead was re-introduced in river during 2014-2015

** $P < 0.01$

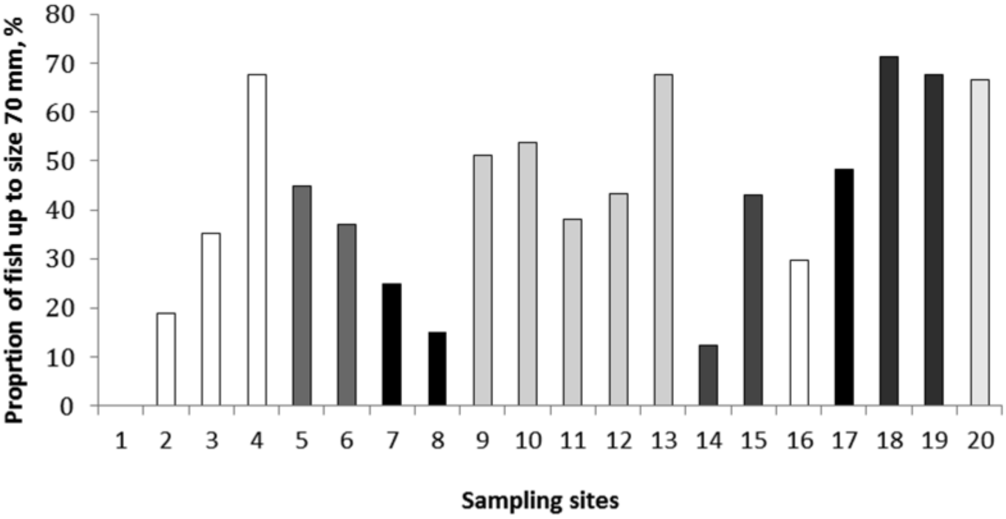


Fig. 4a. Proportion of *Cottus gobio* with body length up to 70 mm at each sampling site in the Iskar river basin

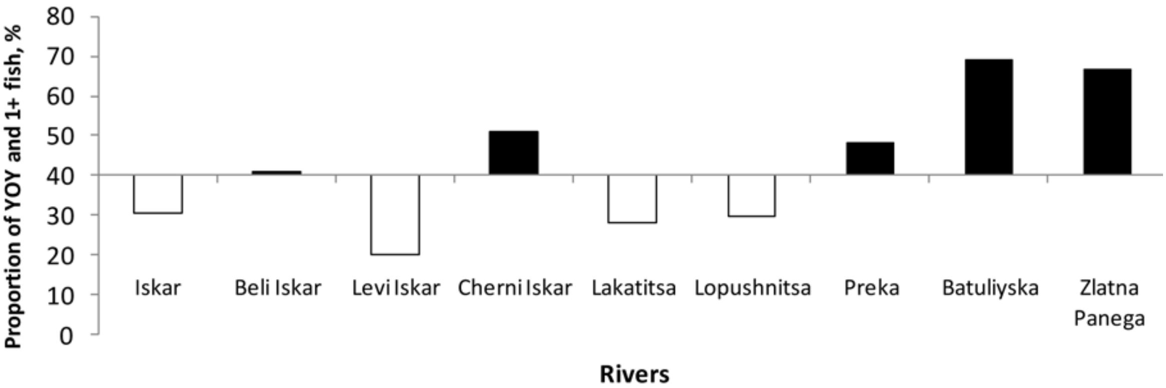


Fig. 4b. Proportion of *Cottus gobio* with body length up to 70 mm (YOY and 1+) for each of the rivers where the species is found

For statistical analysis the software SPSS 17.0 was used.

Results

Bullhead were found in the Iskar River and eight of its tributaries – Cherni Iskar, Lakatitsa, Beli Iskar, Levi Iskar, Lopushnitsa, Preka, Zlatna Panega, and Batuliyska River – in the altitudes between 179 and 1332 m a. s. l. (Fig. 1). The total area inhabited by the *C. gobio* population in the Iskar River basin is 49.9 ha (Table 1). In the individual rivers, bullhead occupies aquatories with a length ranging between 500 m (Batulyiska River and Preka River) and 20 km (Cherni Iskar River). The bullhead abundance varies from 4.7 ind. / 100 m^2 (registered in the Batulyiska River) to 31 ind. / 100 m^2 (Levi Iskar River), with a mean abundance for all bullhead localities of 13 ind. / 100 m^2 or 0.13 ind. / m^2. The highest density per single site, 37.5 ind. / 100 m^2, was registered for the Levi Iskar River (Fig. 2a). The density of *C. gobio* increases with increased altitude (Fig 3). The biomass values of *C. gobio* from different localities are presented in Figure 2b.

The highest individual and mean body lengths of *C. gobio* were recorded in the Levi Iskar River (Table 2). The proportion of juvenile *C. gobio* in each river is presented in Figure 4. For five rivers, the proportion of *C. gobio* with a total length up to 70 mm was more than 40%. The average value of this parameter for all localities was 42%.

The estimations of parameter *b* of the WLR ($W = aL^b$) ranged between 2.295 and 3.578, with a median of 2.987 (Table 2). The bullheads in the Iskar River and the Zlatna Panega River have *b* less than 3.00.

There is a relatively low variation in body condition (Kn) of *C. gobio* from different localities (Table 3). The mean Kn values varied from 0.992 (Palakaria River) to 1.147 (Beli Iskar River). The

Table 3. Mean. minimum (min.). maximum (max.) of relative condition factor (Kn) of *Cottus gobio* collected in the Iskar River Basin

River	Sample size	Kn. mean	min - max	±SD
Iskar	178	1.013	0.601 - 1.567	0.151
Beli Iskar	54	1.147	0.807 - 1.809	0.166
Levi Iskar	169	1.008	0.555 - 1.599	0.146
Cherni Iskar	237	1.009	0.682 - 1.727	0.159
Lakatitsa	21	1.012	0.623 - 1.273	0.153
Palakaria*	12	0.992	0.863 - 1.123	0.086
Lopushnitsa	54	1.005	0.788 - 1.665	0.13
Preka	31	1.092	0.742 - 1.702	0.198
Batuliyska	45	1.002	0.867 - 1.278	0.099
Zlatna Panega	12	1.007	0.876 - 1.064	0.097
* Bullhead was re-introduced in river during 2014 -2015				

Kn values of *C. gobio* in the Palakaria River were significantly different from the values in the other sites (P = 0.01. $F_{2.25}$ = 5.69, one-way ANOVA. Tukey procedure of post-hoc analysis).

Discussion

The bullhead's distribution in the Iskar River basin is represented by several completely isolated, relatively non-numerous populations inhabiting upper parts of the streams. Two populations were found and described for the first time in the Zlatna Panega River and the Batuliyska River. The analysis of the records from 1922 to 1985 and newly collected data during the period 2011–2015 showed a change of the area occupied by *C. gobio* in the Iskar River basin. The reasons for extinction of *C. gobio* in some locations, such as the lower sections of the Palakaria River, are probably intensive agricultural and industrial activities around the river valley, leading to deterioration of water quality and modification of the bottom substrate (Nikolova & Pehlivanov 2008). The observed isolation of *C. gobio* in the Iskar River basin is caused mainly by natural geological and climate processes. Limited ability to migrate and specific ecological requirements are additional factors for the prolonged isolation. Such isolation has occurred for many other bullhead populations throughout European rivers in the period after last glaciation (Riffel & Schreiber 1995, Hänfling & Brandel 1998, Englbrecht et al. 2000). It is believed that the territory of the Balkan, Iberian and Italian peninsulas, a part of the so-called 'refuges', were inhabited during the Pleistocene (Hewitt 1999). The bullheads were distributed from such 'nests' during the interglacial periods (Thieneman 1950, Banarescu 1992). Nowadays, the dispersal of *C. gobio* is limited by another factor: river fragmentation. Besides the natural barriers, it was observed that many artificial transverse structures occur in the riverbed (e.g. weirs, HPPs, dams, water abstractions) (Kanev & Uzunova 2015). Most of them are weirs with a height above 20 cm, which are insurmountable barriers for *C. gobio* movement, mainly in the upstream direction (Utzinger et al. 1998). Bullhead dispersal has also been limited by its specific ecological requirements (Fischer & Kummer 2000, Knaepkens et al. 2005, Gosselin et al. 2010). Another factor restricting the natural distribution of *C. gobio* is the extremely low water levels in some rivers during the summer–autumn period. Such a situation was observed in the Batulyiska River, where the water depth decreased by as much as 5 cm in August–September. The influence of this factor will probably increase in the near future due to the rise in temperatures caused by global climate changes.

Like many other fish species, the local *C. gobio* populations represent significant differences in abundance and biomass between regions, rivers, lowland and upland streams, and even different sites in the same river (e.g. Crisp 1963, Welton et al. 1983, Copp 1992, Waterstraat 1992, Cowx & Harvey 2003, Tomlinson & Perrow 2003). Most studies claim that the upland streams tend to support lower densities of *C. gobio* (Crisp et al. 1974, Mills & Mann 1983). For example, Utzinger et al. (1998) observed densities between 0.002 and 0.41 ind.·m^{-1} in extreme alpine conditions. In contrast, we observed an increase in number with increasing altitude. This is probably due to more favourable environmental conditions for *C. gobio* in these areas, which are usually outside settlements and urban zones. General lower values of *C. gobio* densities observed in the Iskar River basin are probably associated with the specifics in the hydrological regime of rivers in the region. It is characterised by periods of extremely high water levels and velocities during the snow melt and rain in the spring. Such conditions are unfavourable for 0+, and it is likely that a significant number of this fish are washed downstream and/or seriously injured. Therefore, taking into account the influence of climatic and hydrological factors, we consider that densities >10 ind.·100 m^2 can be considered as indicators for a favourable status of bullhead populations.

According to the literature, *C. gobio* with a total size of up to 40–45 mm is defined as one summer old fish (0+) (Cowx & Harvey 2003, Carter et al. 2004). We assumed that the next size group, fish from 50 to 70 mm in length, corresponds to the 1+ age group. We observed that in the Iskar River, *C. gobio* reach maturity at the end of the second year of life (unpublished data). Therefore, fish with a size of up to 70 mm could be considered juvenile. The older age classes were not clearly distinguished from the length frequency distribution analysis, but according to our findings, those with lengths from 70 to 90 mm constituted the 2+ group. and those with lengths from 90 to 110 mm formed the 3+ group. The relatively high abundance of juvenile fish is considered evidence of successful reproduction of the species, Cowx & Harvey (2003) assumed that to achieve a favourable conservation status, more than 40% of the bullhead population in a discrete section of a river should be in the 0+ age class. We assumed that the proportion of all juvenile fish (0+ and 1+) higher than 40% can be considered as a favourable status of the *C. gobio* population. The lower proportion of juvenile fish in this river basin is probably due to extreme hydrological conditions, especially in the spring months, In a deeper analysis of the distribution of juvenile fish in each river, it can be seen that their presence varies considerably between stations surveyed, even in the same river. The difference in the microhabitat preferences of the juvenile and adult fish is the main reason for the unequal distribution of bullheads from different age/size groups along the inhabited river sections (Mäki-Petäys 1997, Van Liefferinge et al. 2005). Whereas 0+ fish prefer shallower parts of the river with less current, adults appear to prefer higher water velocities and coarser bottom substrate, especially during the reproductive period (Cowx & Harvey 2003, Legalle et al. 2005, Gosselin et al. 2010). In some of the surveyed rivers, there are other factors that could dramatically decrease the number of 0+ fish, such as artificial changes in the hydrological regime due to a periodic increase or decrease of the water level downstream from hydropower plants. The existence of two small HPPs directly above the area inhabited by bullheads in the Levi Iskar River could explain the almost complete absence of small bullheads in this river stretch.

The WLR gives us information about the life history and morphological comparisons between different fish species or between different fish populations from various habitats (Petrakis & Stergiou 1995, Gonçalves et al. 1997, Santos et al. 2002). They can be used as an indicator of the environmental status of streams (Vila-Gispert & Moreno-Amich 2001). Slopes of less than 3.0 may indicate populations in a crowded or stunted condition (Carlander 1969).

All Kn values of *C. gobio* were >1, and this indicates that the specimen is in better condition than an average individual of the same L range. Only bullheads from the Palakaria River show values <1. We have to take into account the fact that fish in the Palakaria River were reintroduced relatively recently and are still in the process of acclimatisation (Uzunova et al. 2015). Therefore, according to the data from this index, fish from all localities are in good condition.

The variability of the life-history parameters reflects the different habitat conditions of the individual rivers, natural or modified. This makes it difficult to derive reference values of biological parameters applicable to all populations or subpopulations. Appropriate indicators for monitoring the populations of *C. gobio* that can be used are the fish abundance (density) and the proportion of juvenile fish. The measurement should be carried out in the same period each year, not earlier than August, when 0+ are already clearly visible. Due to the high observed variability of the biological parameters and derived indices, we recommend monitoring of less numerous populations to be held every year in at least three representative river sites, In parallel, we recommend conducting activities to eliminate the negative acting factors such as pollution, hydro peaking, and excavation of gravel and stones from the river bottom. Due to the inability to overcome barriers, reintroduction of individuals in some isolated river sectors would be the best way to facilitate the dispersal of the population. Since all detected populations in the Iskar River basin are located outside the coverage of the EU NATURA 2000 network of protected areas, activities to expand the network should be undertaken.

Conclusions

The bullhead distribution in the Iskar River basin is represented by four completely isolated and relatively non-numerous populations. With the present study, the distribution area of *C. gobio* in Bulgaria is considerably extended, as two new populations are described. The proposed population parameters and their reference values could contribute to the development of more effective monitoring programmes and adequate assessment of the bullhead populations.

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