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Assessment of the conservation status of endemic sculpin *Cottus haemusi* (Cottidae) in the river Vit (Danube Tributary), northwest Bulgaria

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ABSTRACT

Key-words: Cottus haemusi, *distribution, abundance, size, conservation measures*

Cottus haemusi (Marinov and Dikov, 1986. Acta Zool. Bulg. 3, 18–23) is an endemic fish species that is restricted to the upper tributaries of the river Vit, Northwest Bulgaria. After its discovery in 1986, no further investigation of the *C. haemusi* population has been conducted. The aims of the present study were to determine its current population status based on the distribution, abundance and size structure of the *C. haemusi* population and to analyse the main environmental parameters of its habitat. Five upland tributaries and the main river were examined in low-water periods in 2009 and 2010. Two-pass electrofishing surveys were performed at 14 sites to estimate species presence, abundance and size distribution. *C. haemusi* was only detected in two tributaries of the river Vit: Kostina and Toplja. The current investigation failed to find the species at previously recorded sites. The total distribution area of this species is estimated to be 16 200 m^2. The observed abundance of the Vit sculpin ranged from 5.6 to 8.4 individuals·100 m^{-2}, with a mean of 7.0 individuals·100 m^{-2}. Investigation of the size structure revealed the relatively low contribution of one-summer-old individuals. Microhabitat preferences did not differ significantly from other European cottidae species. Only a higher percentage of cobbles distinguished sites with sculpins from those without. The restricted distribution and loss of the bullhead population from some localities in the river Vit could be explained by human disturbance and the deterioration of natural habitat. Several protection measures are discussed.

RÉSUMÉ

Évaluation de l'état de conservation du chabot endémique *Cottus haemusi* (Cottidae) dans la rivière Vit (affluent du Danube), nord-ouest de la Bulgarie

Mots-clés : *Cottus haemusi*, distribution, abondance, taille, mesures de conservation

Cottus haemusi (Marinov et Dikov, 1986. Acta Zool. Bulg. 3, 18–23) est une espèce endémique de poisson limitée aux affluents supérieurs de la rivière Vit, nord-ouest de la Bulgarie. Après sa découverte en 1986, aucune investigation complémentaire de la population de *C. haemusi* n'a été menée. Les objectifs de la présente étude étaient de déterminer le statut actuel de la population basée sur la distribution, l'abondance et la structure en taille des populations de *C. haemusi* et à analyser les principaux paramètres de l'environnement de son habitat. Cinq affluents de

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montagne et le fleuve principal ont été examinés en période d'étiage en 2009 et 2010. Deux campagnes de pêche électrique ont été effectuées sur 14 sites pour estimer la présence de l'espèce, son abondance et sa distribution de taille. *C. haemusi* n'a été détecté que dans deux affluents de la rivière Vit : Kostina et Toplja. L'étude actuelle a échoué à trouver l'espèce dans des sites déjà signalés. L'aire de répartition totale de cette espèce est estimée à 16 200 m^2. L'abondance observée du chabot Vit variait de 5,6 à 8,4 individus·100 m^{-2}, avec une moyenne de 7,0 individus·100 m^{-2}. L'étude de la structure en taille a révélé la contribution relativement faible des individus âgés d'un été. Les préférences de microhabitat ne diffèrent pas significativement des autres espèces de Cottidae européennes. Seul un pourcentage plus élevé de galets caractérise des sites avec chabots de ceux sans chabots. La distribution réduite et la disparition de la population de certaines localités dans la rivière Vit pourrait être expliquée par des perturbations humaines et la détérioration des habitats naturels. Plusieurs mesures de protection sont discutées.

INTRODUCTION

The genus *Cottus* (Cottidae, Scorpaeniformes) consists of several relatively small, bottom-dwelling freshwater fish species that occur mainly in cold, well-oxygenated mountain streams, as well as in lakes and channels (Gaudin and Caillere, 1990; Bănărescu, 1992; Tomlinson and Perrow, 2003; Kotusz *et al*., 2004). Some species in this genus, such as *Cottus gobio*, are widespread with moderate to high abundance throughout Europe (*e.g.* Smily, 1957; Koli, 1969; Copp, 1992) whereas others have a very restricted distribution (*e.g. Cottus petiti*) (Persat *et al*., 1996; Kottelat and Freyhof, 2007). Some populations of *Cottus* species are becoming endangered as a result of water pollution and habitat deterioration (Pedroli *et al*., 1991; Utzinger *et al*., 1998; Fischer and Kummer, 2000; Knaepkens *et al*., 2005). The bullhead, *Cottus gobio*, was 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 the bullhead is classified as critically endangered (Stefanov and Trichkova, in press).

The habitat preferences of *Cottus* species have been described in several studies (*e.g.* Tomlinson and Perrow, 2003; Carter *et al*., 2004; Kotusz *et al*., 2004; Legalle *et al*., 2005; Legalle, 2008; Gosselin *et al*., 2010). Investigation of the abundance, age and growth rate of sculpins revealed variation not only between species, but also between populations from different locations (*e.g.* Mann, 1971; Crisp *et al*., 1974; Welton *et al*., 1983; Waterstraat, 1992; Perrow *et al*., 1997; Fischer and Kummer, 2000; Jansen et al., 2000; Cowx and Harvey, 2003; Carter *et al*., 2004; Kotusz *et al*., 2004; Legalle *et al*., 2005; Van Liefferinge *et al*., 2005; Abdoli *et al*., 2007). The spread of local populations of bullhead has been hampered by the low migratory ability of the species, its specific ecological requirements and river fragmentation (natural and man-made), which have prevented significant dispersal (Utzinger *et al*., 1998; Fischer and Kummer, 2000; Knaepkens *et al*., 2005). The current distribution of *Cottus* species across Europe is a result of colonization and re-colonization processes during interglacial periods (Riffel and Schreiber, 1995; Hänfling and Brandl, 1998; Englbrecht *et al*., 2000; Kontula and Väinölä, 2001; Volckaert *et al*., 2002; Šlechtová *et al*., 2004). After the most recent (Würm) glaciation the contact between bullhead populations inhabiting areas surrounded by mountain ridges or different drainage systems was interrupted and they became completely isolated. The freshwater ecosystems on the Balkan, Iberian and Italian Peninsulas are considered to be the most isolated zoogeographical units in Europe (Banarescu, 1992), and many endemic forms have developed as a result of this isolation (Hewitt, 1999). Based on molecular and morphological data 15 bullhead species can be distinguished in Europe (Freyhof *et al*., 2005; Kottelat and Freyhof, 2007).

In Bulgaria two *Cottus* species have a relatively restricted and fragmented distribution in rivers belonging to the river Danube drainage system. A few small *Cottus gobio* populations are found in upper streams of the rivers Ogosta and Iskar (our data), while the sculpin population in the Vit river has been defined as a new species, known as the Vit sculpin, *Cottus haemusi* (Marinov and Dikov, 1986). Morphological data indicate that the sculpin found in the river Beli Vit (the main affluent of the Vit river) has the smallest relative head length and more rays in the anal fin compared to all European populations (Marinov and Dikov, 1986). Since its discovery in 1986, no further investigations of the abundance and distribution of *Cottus haemusi* has been carried out (Karapetkova and Dikov, 1986).

The Vit river flows into the River Danube, near the village of Somovit. Large sections of the middle and lower river basin are designated as protected areas of the NATURA' 2000 National Network. The uppermost parts of some of the tributaries are located in reserves within the Central Balkan National Park, in which fishing is prohibited. According to Cowx *et al*. (2009), adequate assessment of the status of the fish population should include three criteria: fish density, population structure and the distribution of the target species in individual rivers. Assessment of these criteria is practical and cost-effective (Cowx *et al*., 2009).

The aim of this study was to assess the current status of *C. haemusi* in the river Vit basin on the basis of its distribution, abundance and size structure and to describe the habitat characteristics of the sites at which bullheads exist. 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 River Vit is situated in central North Bulgaria. The source of the river is in the Central Balkan mountain range, below Vezhen Peak, at an altitude of 2030 m a.s.l. The river has a watershed area of 3220 km^2, a total length of 189 km, and a mean river slope of 9.6‰. After the river leaves the mountain it crosses the Central Danube plain and flows into the river Danube near the town of Somovit. The main river Vit is formed by the fusion of two rivers – Beli Vit and Cherni Vit. Several small tributaries run into Beli Vit – Stara Ribaritza (12 km length), Cherna Reka (7 km), Zavodna (5 km) and Kostina (9 km). The mean annual discharge (*Q,* $m^3 \cdot s^{-1}$) of Vit river into the estuary is 19.18 $m^3 \cdot s^{-1}$. The annual discharge of Beli Vit (measured near to site BV2) is 4.88 $m^3 \cdot s^{-1}$ (min 1.806 and max 7.688). The streams' mean annual discharges and mean discharges for October (based on a 37-year period) were close to identical (Kostina – 0.59/0. 036 $m^3 \cdot s^{-1}$; Toplja – 0.966/0.073 $m^3 \cdot s^{-1}$; Stara Ribarica – 0.65/0. 039 $m^3 \cdot s^{-1}$; Cherna – 0.42/0.021 $m^3 \cdot s^{-1}$; Zavodna – 0.57/0.041 $m^3 \cdot s^{-1}$). The max discharge values were observed in April and May. In some years, values of up to 21 $m^3 \cdot s^{-1}$ were recorded. Average annual rainfall in the mountain area of Vit river is 950 mm.

Based on searches of the literature and preliminary *in situ* investigations, we selected five tributaries of the river Vit that were possible locations of Vit sculpin populations – Stara Ribaritsa, Cherna Reka, Zavodna, Kostina, Toplja and Beli Vit (Figure 1). Creeks and streams that dried up during the summer months were excluded from the present study. Fourteen sample sites were selected from the upper and middle reaches of each tributary (Table 1).

> FISH COLLECTION AND MEASUREMENT

Fish were collected in 2009 and 2010 by electrofishing (using a SAMUS-725G device) during low-flow periods (late September and early October), using the following procedure: stretches of river (from 50 to 100 m mean length) were blocked off with stop nets (3.0 mm mesh size, knot-to-knot) and two successive electrofishing passes (30-cm diameter aluminium ring anode, average voltage 200–350 V, operating at an average 3–8 A, depending on water conductivity) were performed to estimate the abundance of fish in that section. The electric current produced 45 pulses per second. Each fishing pass was carried out in an upstream direction. The time interval between the first and second pass was at least 60 minutes. All fish

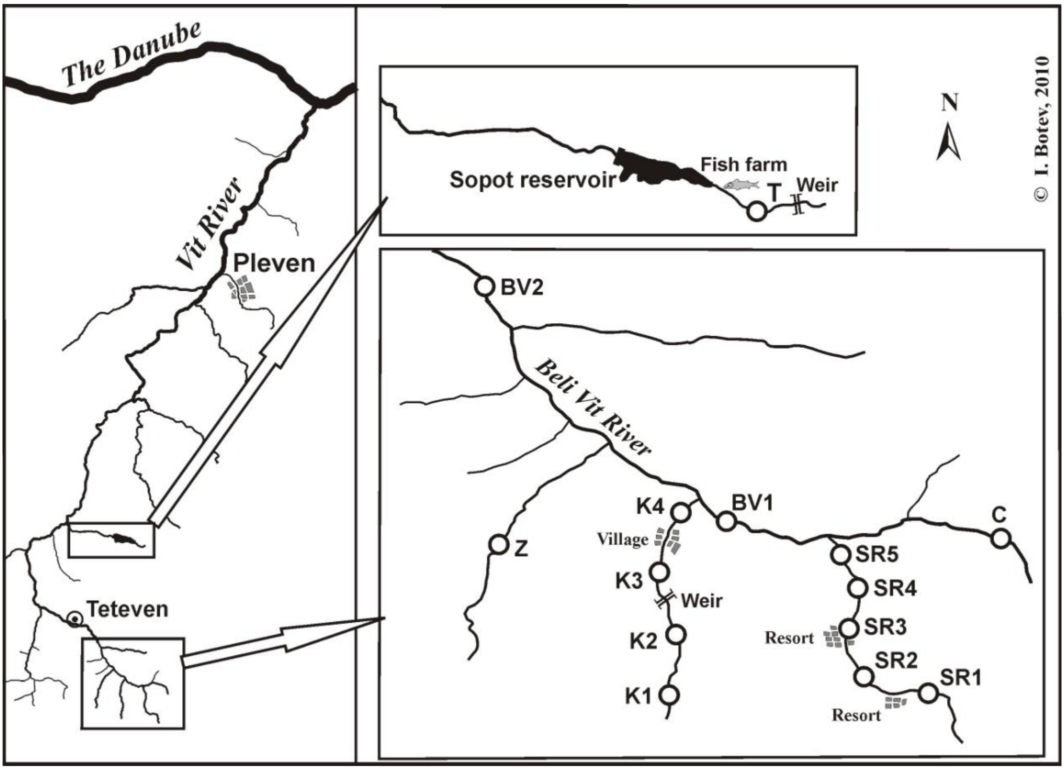

Figure 1
Map of the river Vit showing study areas and the 14 sample sites.

were anaesthetized with clove oil, identified, counted, measured total (*TL*, mm) and standard (*SL*, mm) length, and total weight (*W*, g)) and released after the second pass.

> HABITAT CHARACTERIZATION

At each study site, the physical and chemical stream features that might affect the status of bullhead populations were measured 1 h after electrofishing and removal of the block nets. Beginning at the downstream end of each site, transects were established perpendicular to the flow along the centre line of the stream, spaced at 10-m intervals. At each transect, we recorded the habitat and water parameters at four points situated close to the left and right riverbank and two in the middle of the river. The following parameters were estimated for all 14 river sections: mean water depth was classified as (1) shallow (10–30 cm), (2) moderate deep (30–50 cm), (3) deep (50–100 cm) or (4) very deep (>100 cm). Oxygen saturation (ppm), pH and water temperature (°C) were measured *in situ* using a WTW Oxi 330i and WTW pH 330i respectively. Water velocity on the bottom was measured by current velocity meter (Model 2100, Swoffer Instruments, Inc.) and classified as the following categories: (l) low velocity (0–5 cm·s^{-1}), (2) moderate (5–15 cm·s^{-1}) or (3) high velocity (>15 cm·s^{-1}). Substrate composition (in % of section area) was visually estimated in a 1-m-wide band centred across each transect and categorized as follows: sand (0.06–2 mm in diameter), gravel (2–2.0 cm), pebbles (2.0–5.0 cm), cobbles (5.0–25.0 cm), boulders (25–50 cm), rocks (above 50.0 cm), or silt (<0.06 mm). Emergent and submerged aquatic vegetation (% of section area) was classified as: (1) missing, (2) sparse, (3) intermediate, or (4) dense. Adjacent vegetation was described as: (1) dense coniferous, (2) mixed broad-leaved and coniferous, (3) sparse, or (4) grass. With the aim of describing land use and anthropogenic influence along the investigated river sections, five classes were identified: (1) recreational fishery, (2) fish farms, (3) resort complex, (4) village zone, or (5) no human activities or park zone (Table 2).

Table 1
Names, codes and location of sampled rivers.

River	Site code	Site number	Geographic coordinates	Altitude, m a.s.l.
Kostina	К1	1	N 42° 49. 868' E 024° 20.103'	666
Kostina	К2	2	N 42° 50. 039' E 024° 20.262'	652
Kostina	К3	3	N 42° 50.160' E 024° 20.383'	636
Kostina	К4	4	N 42° 50.899' E 024° 21.262'	582
Toplja	T	5	N 42° 57. 556' E 024° 29.317'	415
Stara Ribarica	SR1	6	N 42° 47. 267' E 024° 25.633'	886
Stara Ribarica	SR2	7	N 42° 47. 103' E 024° 25.071'	772
Stara Ribarica	SR3	8	N 42° 47. 967' E 024° 25.341'	722
Stara Ribarica	SR4	9	N 42° 48. 167' E 024° 25.693'	679
Stara Ribarica	SR5	10	N 42° 49. 633' E 024° 25.156'	649
Cherna	C	11	N 42° 49.822' E 024° 26.625'	726
Zavodna	Z	12	N 42° 49. 633' E 024° 20.001'	672
Beli Vit	BV1	13	N 42° 50. 985' E 024° 21.575'	562
Beli Vit	BV2	14	N 42° 56. 188' E 024° 12.513'	372

> DATA ANALYSIS

Population size or population density ($\dot{N}$) and probability of capture (p) were computed according to Seber and Le Cren (1967). $\dot{N}$ was calculated as $\dot{N} = C_1^2 / C_1 - C_2$, where C_1 is the number of fish removed in the first sample, and C_2 is the number of individuals in the 2nd capture event; p was calculated as $p = C_1 - C_2 / C_1$. The main assumptions of the model are: (1) a closed population, (2) equal capture probability for all individuals and (3) the constant probability of capture between samples (electrofishing passes). Capture probability was estimated for each site. Population size ($\dot{N}$) was recorded as individuals $100 \cdot m^{-2}$. The biomass or standing crop was estimated according to Mahon *et al*. (1979).

Differences in habitat and water parameters between sampling sites with and without sculpin were evaluated using the Mann-Whitney *U*-test. Spearman's correlation analyses were used

Table 2

Mean values of environmental parameters measured at 14 sites in the river Vit catchment (October 2009/2010). Site area (in square meters); mean site width (m), water depth (1 = 10–30 cm; 2 = 30–50 cm; 3 = 50–100 cm; 4 > 100 cm); bottom substrate %: sand (0.06–0.2 cm in diameter), gravel (0.2–2.0 cm), pebbles (2.0–5.0 cm), cobbles (5.0–25.0), boulders (>25 cm), rocks (>100.0 cm), silt (<0.06 cm); water velocity (1 = 0–10 cm s^{-1}*; 2 = 5–15 cm* s^{-1}*; 3 >15 cm* s^{-1}*); oxygen saturation (ppm); river and land use (1 = recreational fishery; 2 = fish farms; 3 = hotels; 4 = village zone; 5 = national park zone); costal vegetation (1 = dance; 2 = mixed broad-leaved and coniferous; 3 = sparse; 4 = grass); aquatic vegetation (1 = missing; 2 = sparse; 3 = intermediate; 4 = species rich).*

Site code	Study site area	Mean site width	Water depth	Silt %	Sand %	Gravel %	Pebbles %	Cobbles %	Boulders %	Rocks %	Water velocity	Oxygen	pH	T C	Human activities	Coastal vegetation	Aquatic vegetation
K1	250	4.2	2	0	0	9	4	6	33	48	3	107	7.8	8.2	5	1	1
K2	300	5.1	2	0	3.4	16.7	12.5	8.3	33.3	25.8	3	105	7.8	8.5	1	1	1
K3	400	5.5	2	0	5	32.5	16.9	30	11.3	4.4	2	102	7.8	8.5	4	1	1
K4	250	4.6	2	0	11	35	18	24	12	0	2	106	7.8	8.5	4	1	1
T	450	6	1	0	37.8	38.3	10.6	13.3	0	0	1	100	7.1	12	1	1	1
SR1	293	4.5	1	0	15	31.7	8.3	0	10	18.3	3	101	7.8	8.5	5	2	1
SR2	352	6.2	2	0	25.7	32.8	11.4	0	12.9	17.1	3	102	7.8	8.5	5	2	1
SR3	355	6.2	1	0	35.7	43.6	12.1	8.6	0	0	2	106	7.5	9.4	1	1	1
SR4	565	7.5	2	0	53.2	36.3	5.9	4.5	0	0	2	100.5	7.5	10.3	3	1	1
SR5	400	6.8	2	0	53.1	19.4	7.5	6.9	13.1	0	2	101	7.3	11.6	4	1	1
C	125	2.5	1	0	0	56	8	10	6	20	2	103	7.2	12	1	2	1
Z	260	3.8	2	0	28	42	9	6	15	0	2	103	7.8	10	1	4	1
BV1	660	8.5	3	0	43.1	32.3	12.7	5.4	6.5	0	2	100	7.5	13	4	3	1
BV2	590	7.2	3	20	43.3	21.1	12.5	2.9	0	0	1	95	7.3	14	4	3	2

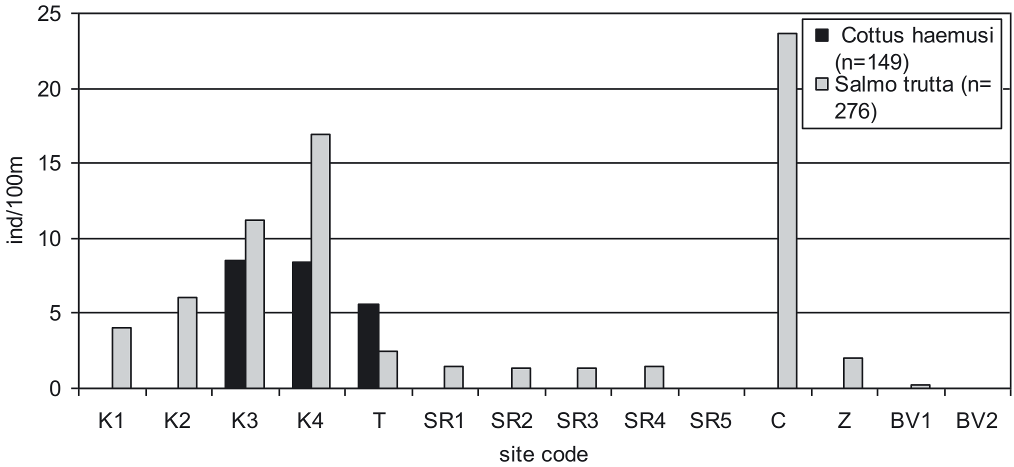

Figure 2
Mean relative abundance (fish number 100 m^{-2}) of Salmo trutta and Cottus haemusi at 14 sampling sites in the River Vit.

to compare the density of fish with the resource quality in different sections. All analyses were performed using XLSTAT software package. A probability level of <0.05 was considered significant.

RESULTS

> BULLHEAD DISTRIBUTION AND SIZE STRUCTURE

Sculpins were found at only three sampling sites located in two tributaries of the river Vit – Kostina (K3 and K4 sites) and Toplja (site T) (Table 1, Figure 1). The distribution of *C. haemusi* in the River Kostina was extremely limited, being restricted to a stretch of less than 1.2 km. Site K4 is located within a small village and site K3 is located at the upper end of the distribution area, bounded by a high semi-natural weir. The situation observed in the river Toplja was not much better – the bullhead population inhabited approximately 1.5 km of the river – from a cave from which the river takes it source to an impassable weir and small fish farm.

The length frequency data for bullheads from the river Kostina (site K3) revealed the prevalence of bullheads of 40 to 45 mm in length followed by those between 96 and 100 mm (Figure 2). At sampling site K4, the 96 to 105 mm size group prevailed. The mean total body length for bullheads at site K4 was 107.8 mm (*SE* = 3.06) and did not differ significantly from that measured at site K3 – 94.08 mm (*SE* = 5.5) ($P < 0.01$, one-way ANOVA). However, the largest bullhead in the river Kostina (140 mm) was caught at site K3. In the Toplja River, the most numerous bullheads were those with a length of 60 to 95 mm. In this stream less than 5% of the population was smaller than 40 to 45 mm. The largest fish captured was 128 mm long.

> BULLHEAD ABUNDANCE

The mean sculpin abundance in the river Kostina ranged from 8.4 to 8.5 individuals 100·m^{-2} and was substantially higher than that in the river Toplja (5.6 individuals 100·m^{-2}). The capture probabilities were relatively large and consistent (Table 3). The biomass of bullheads reached a mean of 130 g per 100·m^{2} in the river Kostina and only 54.5 g per 100·m^{2} in the river Toplja (Table 3).

Brown trout *Salmo trutta* L. coexisted with *C. haemusi* along the monitored sections of the river Vit. Brown trout were found at 12 different sites in five streams draining into the river. In fact,

Table 3
Number of captured bullheads (numerator) and their total weight (denominator) at three sampling sites in the river Vit for 2009 and 2010. C_1 *and* C_2 *are the catches from successive electrofishing passes,* N *and* B *(g) are the total number and weights of the fish,* $\dot{N}$ *is the population density,* B* *is the standing crop, and p is the capture probability.*

Site code	C_1	C_2	N	B	$\dot{N}$	SE $\dot{N}$	95% CI for $\dot{N}$	p	$\dot{N}$ (100·m^2)	B*	B* (100·m^2)
K4 (2009)	16	5	21	304.1	23.3	3	6.1	0.69	9.3	337.4	135
	250	54.1									
K4 (2010)	15	3	18	160	18.8	1.3	2.6	0.8	7.5	167.1	66.8
	132	28									
K3 (2009)	26	8	34	449	37.7	3.9	7.8	0.7	9.4	497	124.3
	350	99									
K3 (2010)	22	6	28	359	30.3	2.2	4.4	0.7	7.6	388.5	97.1
	310	49									
T (2009)	18	4	22	173	23.1	1.7	3.4	0.8	5.1	180.9	40.2
	153	20									
T (2010)	21	5	26	231	27.6	2.1	4.1	0.76	6.1	245.2	54.5
	204	27									

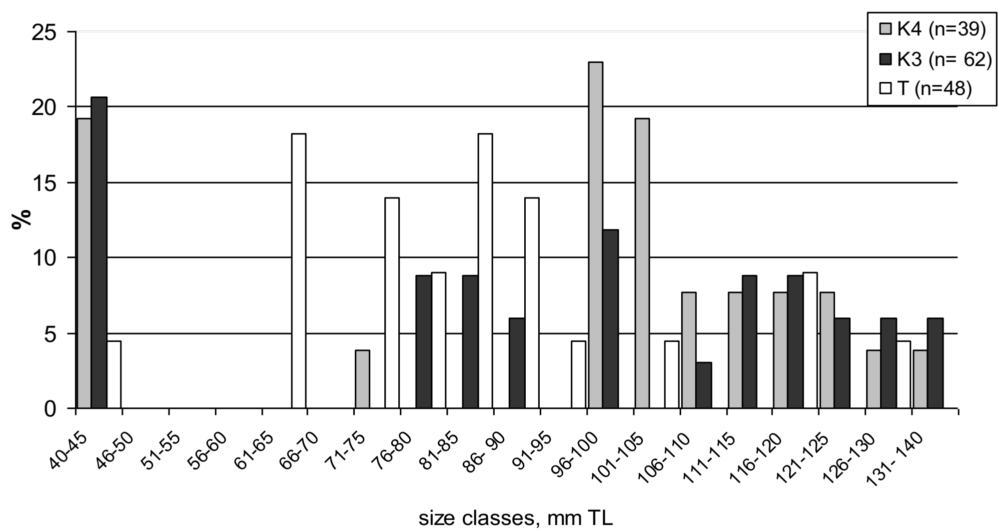

Figure 3
Length-frequency distributions of Vit sculpin C. haemusi at sites K4, K3 (river Kostina) and site T (river Toplja).

brown trout dominated at all sampling sites except T, SR5 and BV2 (Figure 3). Other species including *Barbus* sp. and *Phoxinus phoxinus* occasionally occurred at sites BV1 and BV2.

> HABITAT PARAMETERS

Correlation analysis revealed that *C. haemusi* prefers sites at which the river bottom is dominated by cobbles and pebbles (Table 4). There were no significant differences in habitat

Table 4
Correlation coefficient (Spearman r) between habitat parameters of sampling sites and the mean abundance of Cottus haemusi in the river Vit in October 2009 and 2010.

	Spearman *r*	*p*
Water depth	–0.103	0.726
Mud	–0.144	0.624
Sand	–0.181	0.537
Gravel	0.181	0.537
Pebbles	0.536	0.048
Cobbles	0.717	0.004
Boulders	–0.09	0.76
Rocks	–0.213	0.464
Water velocity	–0.351	0.219
Oxygen	0.062	0.835
pH	0.089	0.763
T C	–0.178	0.542
Human activities	0.088	0.764
Coastal vegetation	–0.43	0.125
Aquatic vegetation	–0.144	0.624

parameters between sites with and without sculpin. Only substratum covered by cobbles significantly differentiated sites at which Vit sculpins were found (Mann-Whitney *U*-test, Z = 1.96, *P* = 0.01).

DISCUSSION

The present assessment of the population status of *C. haemusi* was based on three main criteria: population demographic structure, abundance and species distribution as recommended by Cowx and Harvey (2003) and Cowx *et al*. (2009). The data from the length frequency analysis suggested that the investigated Vit sculpin populations contained more than three age classes. According to bibliographic sources, the 40 to 45 mm size group was defined as one summer old (0+) (Cowx and Harvey, 2003; Carter *et al*., 2004; Abdoli *et al*., 2007). We assumed that the next size group – fish of 50 to 90 mm in length – corresponds to the 1+ age group. The older age classes were not clearly distinguished in the length frequency distribution analysis, but those with lengths from 95 to 110 mm constituted the 3+ group and those with lengths from 110 to 125 mm formed the 4+ group. The relatively high abundance of the 0+ group in the river Kostina was evidence of the successful reproduction of this species. However, in the river Toplja the one-summer-old bullheads made a relatively low contribution to the population. The mean occurrence of the 0+ individuals was less than 5%. These results differed significantly from those reported for *Cottus gobio* in UK SAC rivers, where the one-summer-old individuals accounted for 95% of the population (Cowx and Harvey, 2003; Carter *et al*., 2004). It is considered that to achieve favourable conservation status, more than 40% of the bullhead population in a discrete section of a river should be in the 0+ age class (Cowx and Harvey, 2003). We hypothesized that spring high waters and floods contributed to the low survival rates of the early life stages of *C. haemusi*.

There was no significant difference between the samples from the two sites in the river Kostina in terms of abundance and mean TL. This was expected because the sampling sites K3 and K4 were situated only 1.2 km apart, with similar habitat conditions and no fragmentation between sites. The observed mean values of population density were identical to those reported for the river Stara Ribaritza (sites 6 to 10 in our study) in the first investigation of *C. haemusi* in 1986 (8.4–8.9 individuals 100 m^{-2}). In the same investigation the species was found in the Beli Vit River (sites 13 and 14 in our study) at a density of 0.2–0.3 individuals 100 m^{-2} (Karapetkova and Dikov, 1986). However, the current study did not find any sculpin in these river sections. The abundance of *C. haemusi* at all investigated sites was lower than that of *C. gobio* in similar habitats. Most studies have found mean *C. gobio* densities in streams of <1 individual·m^{-2} (*e.g.* Crisp *et al*., 1974; Welton *et al*., 1983; Copp, 1992; Waterstraat, 1992; Cowx and Harvey, 2003). In contrast, one population showed substantially higher densities of 75 individuals·m^{-2} (Mann, 1971). Jansen *et al*. (2000) and Utzinger *et al*. (1998) found densities in the range of 1.8–14.7 individuals·m^{-2}. Appreciable differences in bullhead 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 bullhead populations are not threatened and the species is quite common or even abundant, abundances of less than 0.2 individuals·m^{-2} and 0.5 individuals·m^{-2} for upland rivers and lowland rivers respectively indicate sites with unfavourable conditions (Copp *et al*., 1994; Carter *et al*., 2004; Cowx and Harvey, 2003; Cowx *et al*., 2009). There are several possible reasons for the relatively low abundance of *C. haemusi* in the river Vit tributaries. Firstly, the upper stretches of Bulgarian mountain rivers are characterized by a low abundance of macrozoobenthos and these zones are typically oligotrophic. Secondly, the water volume and velocity increase drastically almost every spring and significant amounts of newly born fish are probably killed or washed downstream. However, the lack of a significant difference in the densities measured 25 years ago and now suggests that the observed abundance of *C. haemusi* may be normal for this species in such conditions.

The sampling sites in the present study were situated in zones that differed in their nature protective status. No bullheads were found in protected areas in the Central Balkan National Park (SR1, SR2, K1, and K2) although we had expected to find significant numbers there. At five of the sites (SR1, SR2, K1, K2 and C) at which bullheads were absent, the habitat conditions were deemed optimal at the time of investigation and there were no apparent reasons for their absence. Probably, the construction activities along the river and missing water treatment facilities near these protected areas are two particular reasons for accidental alterations in water quality. Consistent with this hypothesis was the absence of any fish species downstream from the hotel complex situated between sites SR3 and SR2. Bullheads were not recorded in the rivers Zavodana and Beli Vit where conditions were considered sub-optimal. All these facts suggest the possible role of human intervention in the disappearance of bullheads from the river Vit system. It is likely that the present bullhead population in the river Kostina was transferred there from the river Stara Ribaritza a few years ago. It has been reported that bullheads are capable of rapid recolonization of rehabilitated watercourses (Pretty *et al*., 2003). By tagging fish with PIT markers Knaepkens *et al*. (2005) demonstrated that bullhead populations not only consist of stationary individuals but also of relatively mobile fish that cover distances of up to 270 m. However, bullheads have little ability to overcome natural or man-made obstructions, and anything with a height of 20 cm or more is impassable for bullheads moving upstream (Utzinger *et al*., 1998). The migration of bullheads above sampling site K3 is prevented by an artificial obstruction (weir) and numerous natural rock shoots. In the river Toplja there is a weir (1.6 m) that is impassable in periods of low water. These obstacles in combination with their low migratory ability determine the restricted distribution of bullheads observed in the Kostina and Toplja rivers.

There is no previous evidence on the interaction and/or negative influence of the co-existing species of salmonids on the presence or density of bullheads (Welton *et al*., 1983). Conversely, bullheads are known predators of trout eggs and compete for space with trout (Gaudin and Caillere, 1990). However, significant quantities of brown and rainbow trout are re-stocked annually in all rivers in the study region. It has also been suggested that salmonids have a negative impact as predators of sculpin eggs or larva (Tomlinson and Perrow, 2003).

The distribution of *Cottus* species in rivers is influenced by a variety of small-scale physical characteristics, such as substratum type, water depth, water velocity and temperature. In accordance with previously reported results we observed that the Vit sculpin has a preference for substrata dominated by cobbles and pebbles (*e.g.* Smily, 1957; Jansen *et al*., 2000; Carter *et al*., 2004; Van Liefferinge *et al*., 2005; Gosselin *et al*., 2010). *Cottus poecilopus* also prefers cobbles and boulders (Kotusz *et al*., 2004). The prevalence of cobbles and boulders, which provide natural refuges, can be used to predict bullhead occurrence (Knaepkens *et al*., 2002). The importance of cobbles for sculpins is due to the fact that cobbles not only serve as a shelter during the day but also as a spawning substrate. At the microhabitat scale, different combinations of water depth and stream velocity were preferred by sculpins. Legalle *et al*. (2005) found that bullheads were associated with shallow depths and high velocities. On the contrary, Perrow *et al*. (1997) and Gosselin *et al*. (2010) observed a positive correlation between bullheads and greater depths and slow velocity. In fact, there is an ontogenetic shift and seasonal changes in the preferences of sculpins for water depth, velocity and size of the substrata (Carter *et al*., 2004; Van Liefferinge *et al*., 2005) and the bullhead may be more flexible in its habitat requirements than generally assumed (Utzinger *et al*., 1998; Jansen *et al*., 2000). Most studies have found that bullheads avoid muddy bottoms and homogeneous areas and wider sections of the river (Legalle *et al*., 2005; Gosselin *et al*., 2010). *C. gobio* occurs within a relatively wide temperature gradient. Temperatures higher than 20 °C restricted the distribution of bullheads in lower stretches of stream systems (Volckaert *et al*., 2002). It was also established that bullhead occurrence at the macro scale is negatively correlated with the distance from the source, stream width and slope (Legalle *et al*., 2005). Thermal conditions also have a major influence on bullhead distribution and density (Legalle *et al*., 2005).

Our investigation of the population structure, abundance and distribution revealed that the sculpin population in the river Vit has been seriously damaged. Changes in bullhead distribution were mainly attributed to the destruction and alteration of stream habitats formerly inhabited by the Vit sculpin. The disappearance of bullheads from previously established locations and the low population densities indicate that the species is threatened with extinction in this region. It is well known that human activities such as pollution and channel calibration generally result in decreased habitat diversity, and many fish species have often been forced into small and isolated populations (*e.g.* Holcík, 2003). Habitat degradation and chronic water pollution are the main factors contributing to bullhead decline (Deufel *et al*., 1986; Legalle, 2008). Probably, the current population status of the Vit sculpin is a result of the complex influence of factors such as accidental water inflow (from hotels), disturbance during construction along the main river and streams (hotel building), poaching and river fragmentation (weirs).

The successful conservation of rare and endangered species requires competent management decisions directed at particular rivers or river sections. The development of adequate reference values for the population density and demographic structure of Vit sculpins should be based on a long-term monitoring programme with the aim of assessing the annual variability in these parameters. Building of mountain resorts in the upper Vit basin combined with natural water resource limitations in this region will threaten its aquatic biodiversity in the future. The following urgent conservation measures to improve the unfavourable population status of bullheads in the River Vit are recommended: (1) rehabilitation of habitat conditions and minimization of the impact of physical barriers on bullheads, (2) reintroduction of bullheads in areas in which the species has occurred in the past and establishment of new populations in potentially suitable environments, (3) special protection of areas with existing bullhead populations and limitation of recreational fishery in the area, (4) increase information and public awareness about rare and endangered populations, and the measures required to allow their preservation. Artificial propagation and re-stocking could be employed if these measures are unsuccessful.

Such restoration plans will only work efficiently with collaboration between local human communities, the national park authorities and scientists.

CONCLUSIONS

On the basis of the studied parameters it can be concluded that the conservation status of *C. haemusi* is not favourable in the river Vit system. The species is restricted to stretches of a few kilometres in two small upland tributaries, the Kostina and Toplja rivers. The species is absent from locations at which it was formerly present. Its abundance in the River Toplja is relatively low and the contribution of young fish is insufficient.

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