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Age and growth of *Sabanejewia balcanica* in the Rijeka River, central Croatia

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A b s t r a c t . Growth patterns of the golden loach, *Sabanejewia balcanica* were examined in the Rijeka River in Central Croatia on a total of 77 specimens collected in a single sampling session in June 2006. No significant difference was found in total body length between males and females. Age was determined from otoliths. Both males and females live 4 years, though due to the sampling period, no 0+ age fish were found in this study. The length-weight relationship was calculated as $W=2x10^{-6} TL^{3.3229}$ for males and $W=3x10^{-6} TL^{3.2811}$ for females, indicating positively allometric growth. According to the growth factor k, growth of males was three times faster than that of females.

Key words: Balcan spiny loach, length-weight relationship, otolith, von Bertalanffy growth function

Introduction

The Balcan spiny loach (*Sabanejewia balcanica*) is a bottom living fish widely distributed in the Danube Basin (Romania, Bulgaria, Bosnia and Herzegovina, Serbia, Slovenia, Slovakia, Czech Republic, Ukraine, Russia) (K o t t e l a t 1997, L u s k et al. 2000, P o v ž & Š u m e r 2000, K o š č o et al. 2008) and it has been reported in the Aegean watershed (Macedonia and Greece) (G e o r g i e v 2004, K o t t e l a t 1997). D e l i ć et al. (2003a) conducted the first assessment of *S. balcanica* (Karaman, 1922) in Croatia, and its distribution was established in eight rivers and streams in central Croatia, thereby expanding its known distribution in the Sava and Danube River basins. Although this species is not included on the 2006 IUCN Red List, it is internationally protected by Annex III of the Bern Convention and Annex II of the European Habitats Directive. Its IUCN status in Croatia is listed as vulnerable (M r a k o v č i ć et al. 2006).

To date, the biology of this species has been poorly studied. With the exception of a few studies on its distribution and morphology (R o l i k 1960, F r a n k i e w i c h 1985, W i t k o w s k i 1994, L u s k et al. 2000, P o v ž & Š u m e r 2000, D e l i ć et al. 2003b), there have been no studies to date on its age and growth. The objective of this study was to estimate the age and growth of *S. balcanica* and to present the first data on this life history trait for this species. This species, though present over a wide area, is rare and difficult to find. On this sampling occasion, 77 individuals were found, contrary to prior sampling sessions where no individuals were found in lowland sections of this river and, contrary to D e l i ć et al. (2003a), in which no *S. balcanica* were found in any lowland river sections in

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Croatia where severe anthropogenic impacts were present. As such, this sample is considered an adequate representation of the population at the sampling time.

Materials and Methods

Study area

The Rijeka River was selected for a study of the age and growth of this rare species. The river is a tributary of the Ilova River in the Danube River Basin and arises from the confluence of two streams: Krivaja and Šandrovac originating from Mt. Papuk and Mt. Bilogora respectively. Samples were collected 2.5 km upstream from the confluence of the Rijeka River into the Ilova River near the village Maslenjača. This is a lowland section of the river, under heavy anthropogenic influence due to intensive farming activities in the area. The riverbed substrate was sand and silt. The river banks are earthen, but are not overgrown with vegetation. Vegetation in the river course was poor, with *Apium nodiflorum* present, covering about 5–10% of the riverbed. Physico-chemical parameters of the sampling site were recorded as followed: water temperature = 17.1°; pH = 7.68; conductivity = 374μS/cm; dissolved oxygen = 7.6 mgO_2/L; oxygen saturation = 78.9%; flow = 0.5–0.7 m/s; depth = 50–70 cm.

In addition to *S. balcanica*, the following species were captured at the sampling site: *Eudontomyzon mariae*, *Barbatula barbatula*, *Leuciscus cephalus*, *Rhodeus amarus*, *Alburnoides bipunctatus* and *Gobio gobio*. This survey of fish fauna differs somewhat from the survey conducted by Delić et al. (2003a) which, in addition to these species, also recorded the presence of *Leuciscus leuciscus*, *Cobitis elongatoides* and *Perca fluvatilis* in the lower reaches of the Rijeka River.

Samples were captured by electrofishing with a 9.5 kW Briggs & Stratton electrofisher. Fish were collected in a single sampling session on 23 June 2006. In total, 77 fish were caught (36 males, 28 females and 13 juveniles). Upon collection, fish were frozen and all measurements conducted in the laboratory, including weighing to the nearest 0.01 g and measuring total length (TL) and standard length (SL) to the nearest 0.1 mm. For further analysis TL was used. Sex was determined by gonad inspection.

Otoliths were removed and cleaned in methanol prior to taking measurements under 200x magnification under a Carl Zeiss Axiovert 200 inverted microscope with Axiocam and the AxioVision imaging software. Growth was determined by back calculations from otolith measurements based on the proportional body hypothesis, assuming constant proportional deviation of the individual size of the otolith from the mean size throughout life (Francis 1990). The growth pattern for the population was calculated as described by the von Bertalanffy growth model as $L_t=L_{inf}$ (1-exp (-k(t-t_0))) where L_t is the total length (mm) at age t (years), L_{inf} is the asymptotic length in mm (Ricker 1971). Growth curve parameters (k, L_{inf}, t_0, SE) were estimated using the LFDA5 software package (Kirkwood et al. 2001). The growth performance index (Φ') was calculated using the following formula (Pauly & Munro 1984): $\Phi' = \log(k) + 2\log(L_{inf})$, where k and L_{inf} are parameters derived from the von Bertalanffy growth function.

Results and Discussion

Total fish length ranged from 55 to 87 mm for males and from 56 to 91 mm in females (Fig. 1) with smaller fish (< 50 mm) considered juvenile as sex was indeterminable from

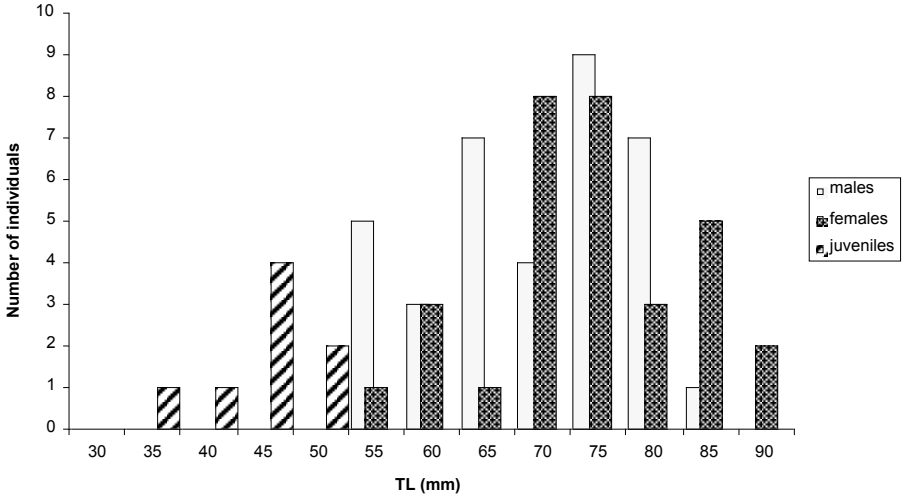

Fig. 1. Length frequency for single capture of *Sabanejewia balcanica* in the Rijeka River (n=77).

gonad examination. The total length of males and females was compared but no significant difference was found (t = -1.57, df= 62, p=0.12). This disagrees with data for related species of the genus *Cobitis*, in which males are typically smaller than females (Slavík & Ráb 1996, Przybylski & Vallladolid 2000, Kostrzewa et al. 2003, Zanella et al. 2003). However, like in *Cobitis* species, males dominated in the smaller length classes, while females dominated the larger classes (Robotham 1981, Kostrzewa et al. 2003, Zanella et al. 2003).

The sex ratio was 1.28:1 in favour of males, though this ratio was not statistically significant from the 1:1 ratio (χ^2=0.35, p=0.48). This result agrees with previous data for *S. balcanica* (ratio of 1:1.1 in Mišík (1958) and 1:1 in Witkowski et al. (1990) (both cited in Bohlen & Ritterbusch 2000)). The slightly greater share of males in the population disagrees with the majority of data for populations of related species of the *Cobitis* genus (Bohlen & Ritterbusch 2000, Erös 2000, Przybylski & Vallladolid 2000, Kostrzewa et al. 2003, Zanella et al. 2003), though a male-dominated sex ratio was found for a single population of *Cobitis narentana* in Croatia (Schneider et al. 2000). However, due to the small sample size, this sex ratio obtained here can in no way be considered definitive and further investigations on larger sample sizes and throughout the year are required.

The age of first sexual maturity for both males and female corresponds to the beginning of their second year, with males sexually mature at 55 m TL and females at 56 mm TL. All 2+ fish were found to be sexually mature, while no 1+ fish were mature. No 0+ fish were found in the study, due to the time of the sampling, which corresponds with what is thought to be the end of the spawning season, which in *S. balcanica* lasts from April to June (Mrakovčić et al. 2006).

The length-weight relationship was calculated for the entire population and separately for males and females (Table 1). The parameters a and b were estimated by linear regression on the transformed equation: log(w) = log (a) + $b$log(TL). The obtained coefficients were analysed and the slope (b) indicated positively allometric growth for both males (b=3.3229) and females (b=3.2811), though no significant difference was found in the slope b between

Table 1. Length-weight regression parameters ($W=aL^b$) for *S. balcanica*.

Sex	*a*	*b*	SE(*b*)	n	r^2
males	$3x10^{-6}$	3.3229	0.0785	36	0.860
females	$1x10^{-5}$	3.2811	0.0763	28	0.838

males and females (t=0.71, df=32, p=0.23). However, *b* for both males and females was found to significantly differ from *b*=0 (t=4.27, df=32, p<0.01). No length-weight data is available in the literature for other populations of *S. balcanica* or for any other *Sabanejewia* species. The slope *b* in species of *Cobitis* ranged from 2.97 to 3.64 in *Cobitis paludica* (Przybylski & Valladoid 2000), from 2.64 to 2.93 in *C. narentana* (Zanella et al. 2003), and was 3.00 in a population of *C. simplicispina* (Ekmekçi & Erk'akan 2003).

Age was determined by annuli readings. The otoliths of *S. balcanica* are somewhat elliptical or bean-shaped and rounded on the top surface. All otoliths were markedly smaller than 1 mm in diameter along the longest axis. This single catch of 77 specimens adequately represents the population. The population is represented by juveniles (1+) and 3 age groups for both females and males (2+, 3+, 4+). The only comparable study is that of Harka et al. (2002) for *S. balcanica* (under the name *S. aurata*) in the Tisza River in Hungary. The authors found only young fish, 0+, 1+ and 2+ (with the majority in the 0+ category). However, that was in fact a recovering population from a toxic spill to the river two years earlier. No other studies on age classes in *Sabanejewia* are available. However, these data differ from those on *Cobitis* species (Przybylski & Valladolid 2000, Ekmekçi & Erk'akan 2003, Kostrzewa et al. 2003, Zanella et al. 2003), which show that males have a shorter lifespan than females. The lengths obtained from back calculations of annuli readings (Table 2) showed good agreement.

The back-calculated lengths were used to fit the von Bertalanffy growth model (Table 3). The asymptotic length was larger than predicted by Taylor (Taylor 1958) in the formula $L_{inf} = L_{max}/0.95$, suggesting that the von Bertalanffy growth model somewhat overestimated L_{inf}. However, this difference in asymptotic body length (L_{inf}) between the sexes is less than that recorded for *Cobitis* species (Przybylski & Valladolid 2000, Zanella et al. 2003). The growth parameter k was three times higher in males than in females,

Table 2. Mean back-calculated lengths (TL in mm) of *S. balcanica* from otolith measurements.

Age group	Observed length			Back-calculated lengths at age			
	n	x	SD	I	II	III	IV
Males (n=36)							
0+	0						
1+	0						
2+	14	62.12	5.34	36.42	61.68		
3+	14	74.58	3.20	35.08	60.82	71.38	
4+	8	82.91	2.28	36.62	61.25	73.74	80.72
Females (n=28)							
0+	0						
1+	0						
2+	5	61.68	4.00	33.65	58.27		
3+	16	74.34	2.50	35.23	59.11	71.54	
4+	7	86.71	3.48	35.31	61.77	72.09	81.08

Table 3. Estimation of von Bertalanffy parameters for *S. balcanica* in the Rijeka River.

	Males	SE	Females	SE	**Population**	**SE**
$\mathbf{L_{inf}}$	101.43	5.77	111.43	6.62	85.00	5.8
k	0.36	0.04	0.10	0.04	0.41	0.11
$\mathbf{t_0}$	-0.93	0.02	-0.31	0.04	-0.32	0.04
n	36		28		77	
$\mathbf{r^2}$	0.898		0.952		0.934	

indicating a faster rate of growth for males towards achieving the asymptotic length. No data are available for other *Sabanejewia* species; however, this growth is substantially slower than that recorded for related *Cobitis* species: 0.69 for males and 0.24 for females in *C. paludica* (Przybylski & Valladolid 2000); 0.54 for males and 0.5 for females in *C. narentana* (Zanella et al. 2003), and 0.41 for males and 0.49 for females in *C. simplicispina* (Ekmekçi & Erk'akan 2003). The growth performance index was also calculated (males Φ' = 3.56; females Φ' = 3.09; population Φ' = 3.47), indicating a faster rate of growth for males than for females towards achieving L_{max}.

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