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Growth pattern of the endemic Neretvan roach, *Rutilus basak* (Heckel, 1843) in the Hutovo Blato wetlands

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Summary

The growth pattern of the endemic Neretvan roach, *Rutilus basak* from the Hutovo Blato wetlands was analyzed. A total of 441 specimens ranging from 6.0 to 21.7 cm TL were obtained by electro fishing between December 2007 and November 2008. The male:female ratio for all fish combined was 0.34 : 1.00 but changed across length classes. The spawning period extended from February to April, with a peak in the first half of March. Maximum observed age was 10 years. Predominance of age classes 1–5 in the catch (77.0% of individuals) was noted. The length–weight relationship showed a positive allometric growth ($b = 3.321$; $R^2 = 0.957$). Parameters of the von Bertalanffy equation were: $L_\infty = 20.201$ cm; $K = 0.257$ per year; $t_0 = -1.554$ year; $R^2 = 0.977$. This study revealed that *R. basak* invests energy in reproduction rather than in body size and thus has a relatively fast growth, short life span and precocious maturity. Moreover, elementary biological population parameters are provided that can be used for conservation purposes.

Introduction

The Neretvan roach, *Rutilus basak* (Heckel, 1843) (previously *Rutilus rubilio* ssp. *rubilio*), is an endemic species geographically restricted to the rivers and lakes of the Adriatic watersheds in Croatia and Bosnia-Herzegovina. In Croatia, this species is distributed in lakes and small rivers near Imotski and the Neretva River Delta, while in Bosnia-Herzegovina it is found in the lower Neretva River drainage basin (Neretva River and tributaries and the Hutovo Blato wetlands) (Mrakovčić et al., 2006; Kottelat and Freyhof, 2007). *Rutilus basak* is found over a wide range of mostly shallow water habitats, with moderate to slow currents, lakes, and artificial water bodies overgrown by vegetation. Spawning takes place close to shore, and with the eggs attached to submerged vegetation and hard substrates (Vuković, 1977; Mrakovčić et al., 2006; Kottelat and Freyhof, 2007).

Most important in the Neretva River Delta of Bosnia-Herzegovina is the Hutovo Blato wetlands, characterized by a highly diverse fish community composed of 22 species representing 20 genera and 12 families (Glamuzina et al., 2001). Recently, as a consequence of human activities, the hydrologic regime has been affected, presenting a great threat to *R. basak* as well as the entire autochthonous ichthyofauna. In addition, the non-migratory nature of the species requires conservation measures, as it is sensitive to environmental perturbation (Economidis, 2002). The Neretvan roach is classified as of least concern (LC) on the IUCN International Red List (Crivelli, 2006); in Croatian waters (Mrakovčić et al., 2006), however, it is considered as near threatened (NT). Although there are no major widespread threats, the species is endangered mainly by habitat loss and destruction (Crivelli, 2006), which partially describes the situation in the Hutovo Blato wetlands.

Although common and abundant in the lower reach of the Neretva River (Kosorić et al., 1983; Brigić et al., 2004) and Hutovo Blato wetlands (Kosorić, 1978; Glamuzina et al., 2001), scientific knowledge on this species is still sparse, scattered, and outdated. Publications contain mainly general biological and ecological data (Vuković et al., 1970; Vuković, 1977) and overall faunistic records (Kosorić, 1978; Kosorić et al., 1983). Several papers have also been published on systematic, genetic and serologic issues (Vuković and Vuković, 1968; Berberović et al., 1970; Vuković and Ivanović, 1970; Berberović and Sofradžija, 1972; Guzina and Vuković, 1978). Some old data on age structure are available from the Neretva River (Aganović and Kapetanović, 1978). However, the general characteristics useful for fisheries management such as age, growth and reproductive cycle have not been studied systematically.

The aim of this study was therefore to determine the population structure, age, and growth pattern of the Neretvan roach, *R. basak*, in the Hutovo Blato wetlands of Bosnia-Herzegovina to improve our knowledge on the general biology of this endemic fish species.

Materials and methods

Study area characteristics

The Hutovo Blato wetlands are a very important part of the Neretva River Delta in Bosnia-Herzegovina. The area was proclaimed as a natural park in 1995 and listed in the 2001 Ramsar Convention as an internationally protected wetland. The park area (74 km^2) is 20 km inland from the Adriatic Sea, along the Bosnia-Herzegovina and Croatia border. The altitudinal range of this area to the sea level is about 3 m. Hutovo Blato consists of two distinct areas separated by the Ostrovačko ridge: Gornje (Deransko) Blato, which is still untouched; and Donje (Svitavsko) Blato, which is now an artificial lake used as reservoir for hydropower production. Gornje Blato comprises several smaller water bodies characterized by different physical properties. For example, a series of small lakes (Jelim, Orah, Drijen and the smallest, Škrka Lake) are deep and rich with surface springs and underwater wells, which have led to the creation of unique ecosystem conditions with stabile temperatures of 13–13.5°C throughout the year (Tutman et al., 2008). Water from these small lakes

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form the Deran Lake, the largest among them. In contrast, this lake is shallow (0.5–1.5 m) with a muddy bottom and yearly temperature fluctuations ranging from 10 to 24°C (Glamuzina et al., 2001). Deran Lake drains into the Krupa River (9 km long), and represents the main waterway draining directly into the Neretva River (Fig. 1).

Data sampling and analysis

Sampling was carried out from December 2007 to November 2008, more intensively during the spawning migration and active spawning of fish in the Hutovo Blato wetlands. The fish were sampled by electro fishing by boat close to the shores of the lakes (Škrka, Jelim, Orah) and Londža stream (Fig. 1). During the sampling period temperatures were always recorded and were in the range between 13 and 15.2°C (Table 1).

A total of 491 specimens (430 adults and 61 juveniles) were caught throughout the sampling months. Total length (TL) (0.1 cm), total weight (TW) (0.1 g) and sex were determined for each individual. Gonads of sexually mature females and males were dissected and weighted. The gonado-somatic index was used to represent the relative reproductive state of the population.

Scales from 441 individuals (89.8%) were used for age and growth studies. For age determination, scales were removed from behind the pectoral fin, cleaned with distilled water, dried and mounted between microscope slides. Each scale was read by three different readers using a compound microscope (magnification 1.6 × 11.2) with a black background. When two of the three measurements did not agree the scale was discarded. The index of average percent error (IAPE) (Beamish and Fournier, 1981) as well as the mean coefficient of variation (CV) (Chang, 1982) was calculated to estimate the relative precision between readings. Low values of the indices indicated a good precision of age estimates. The annuli were interpreted according to Bagenal and Tesch (1978). Age of the Neretvan roach was determined from the number of scale annuli, while the time period of their formation was taken as the month when the distance of the outermost annulus to the scale's margin was minimal. False annuli were recognized by their irregularity and incomplete delineation, especially in the lateral area of the scale. The edge of the scale was treated as the last annuli and the area between the previous annuli and edge of the scale was treated as the annual growth increment. According to the period of the annuli appearance (April–June) and considering 1 March as the peak of spawning (Glamuzina, B., unpubl. data), each fish was assigned to an age class.

A pair-wise comparison of the mean lengths at age was used to test differences between males and females in same age classes. The Kolmogorov–Smirnov two-sample test was used for age frequency distribution analysis.

Table 1
Environmental characterization of water bodies, Hutovo Blato wetlands. Range data determined from monthly survey measurements

Site	Temperature range (°C)	Salinity (PPT)	Depth range (m)
Londža spring	13–13.5	0.2	1–2
Lake Orah	13–14.5	0.2–0.3	1–12
Lake Jelim	13–15.2	0.2–0.3	1–17
Lake Škrka	13–14.3	0.2–0.3	1–15

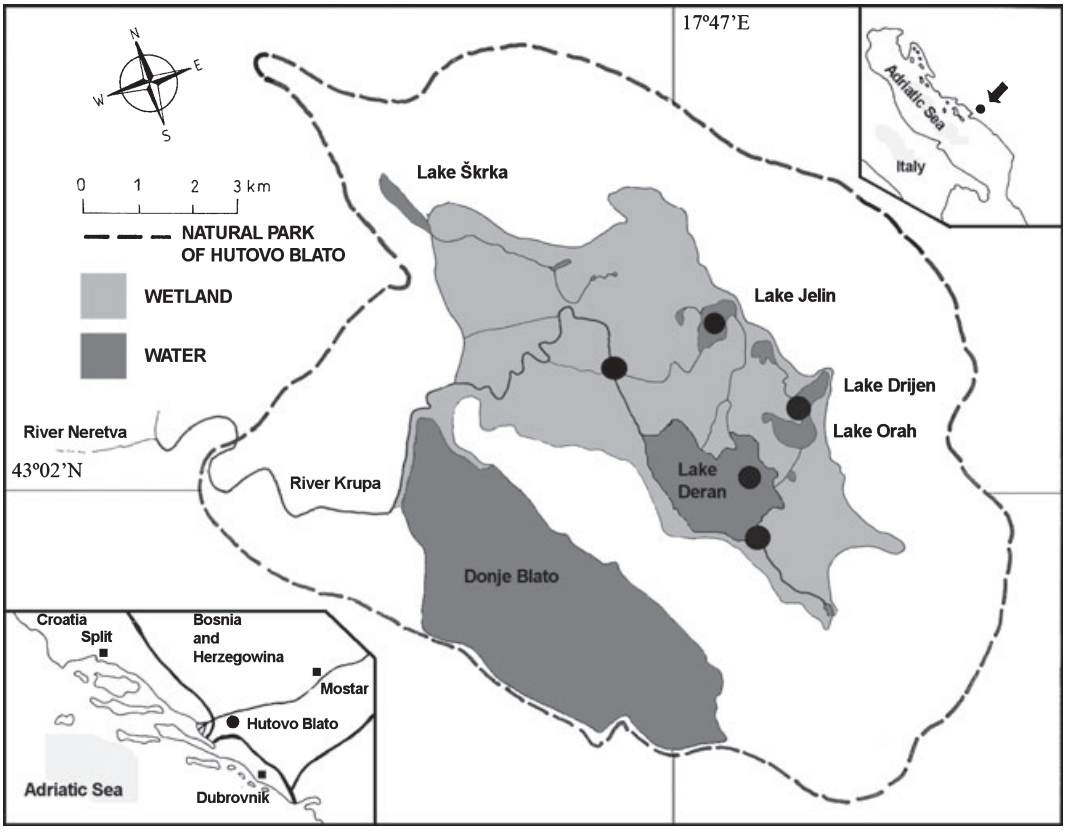

Fig. 1. Target area of Hutovo Blato wetlands (Bosnia-Herzegovina) indicating sampling locations at four study sites

The non-linear least squares regression procedure was used to estimate the growth parameters of the von Bertalanffy equation (VBGF) $TL = L_\infty(1 - e^{-k(t-t0)})$ where TL is total length at age t, L_∞ is asymptotic length, k is the body growth coefficient and t_0 is theoretical age at zero length (Beverton and Holt, 1957). The maximum likelihood test (Kimura, 1980) was used for comparison of growth parameters for males and females. In addition, the length–weight relationship was described by the equation $TW = aTL^b$ where TW is total weight (g) and TL is total length (cm).

Results

Of the 441 individuals of *R. basak* used for age determination, 96 (21.8%) were males, 284 (64.4%) females, and 61 (13.8%) immature individuals. Most adults were caught during the colder part of the year (November–April), whereas juveniles were caught in the summer period (July–September). The male:female ratio for all fish combined was 0.34 : 1.00. Although the ratio changed across length classes, females were dominant in all classes; however, this dominance was less expressed at the beginning of the spawning season (M : F = 0.39 : 1.00). The sampled fish ranged from 6.0 to 21.7 cm TL (mean 13.6 ± SD 3.2 cm) and from 1.7 to 82.0 g (mean 31.9 ± SD 20.3 g). Males ranged from 12.0 to 19.0 cm TL (mean 14.3 ± 1.2 cm) and females from 11.6 to 23.3 cm (mean TL 14.3 ± 1.1 cm). Mean lengths of males and females were not significantly different (Mann-Whitney test: $T = 45841.7$; $P = 0.358$). The length frequency distribution exhibited a mode at 15.0 cm (21.6%). The length–weight relationship for the entire sample was $TW = 0.0045 \times TL^{3.321}$ ($R^2 = 0.957$), indicating positive allometric growth.

The spawning period of *R. basak* began the end of February and finished in April, with a peak in the first half of March (Fig. 2). There was no spawning in January or February, while in March 17.6% and in April 63.2% of the fish were already spent. Gonad development commenced again in October and continued through January / February. The gonado-somatic index (GSI) indicated simultaneous spawning for both sexes, although males released sperm around 15 days before females began to spawn. GSI values were at a minimum in August and September. There was a high correlation between the gonado-somatic index and the percentage of ripe and running males and females throughout the study year. First mature individuals occurred in the 12.0 cm length class (Fig. 3), and all sampled specimens above 15.0 cm TL were fully mature. Total lengths at 50% maturity (Lm_{50}) were 14.0 cm for both males and females. At an estimated age of 2 years, 40.4% of males and 30.8% females were mature. In the third year all sampled individuals were fully mature.

Age was determined by counting scale annuli (Fig. 4). The index of average percent error (IAPE) of annuli counts for each reader did not differ greatly, but was slightly lower for the first investigator (2.23) compared to the second (2.52) and

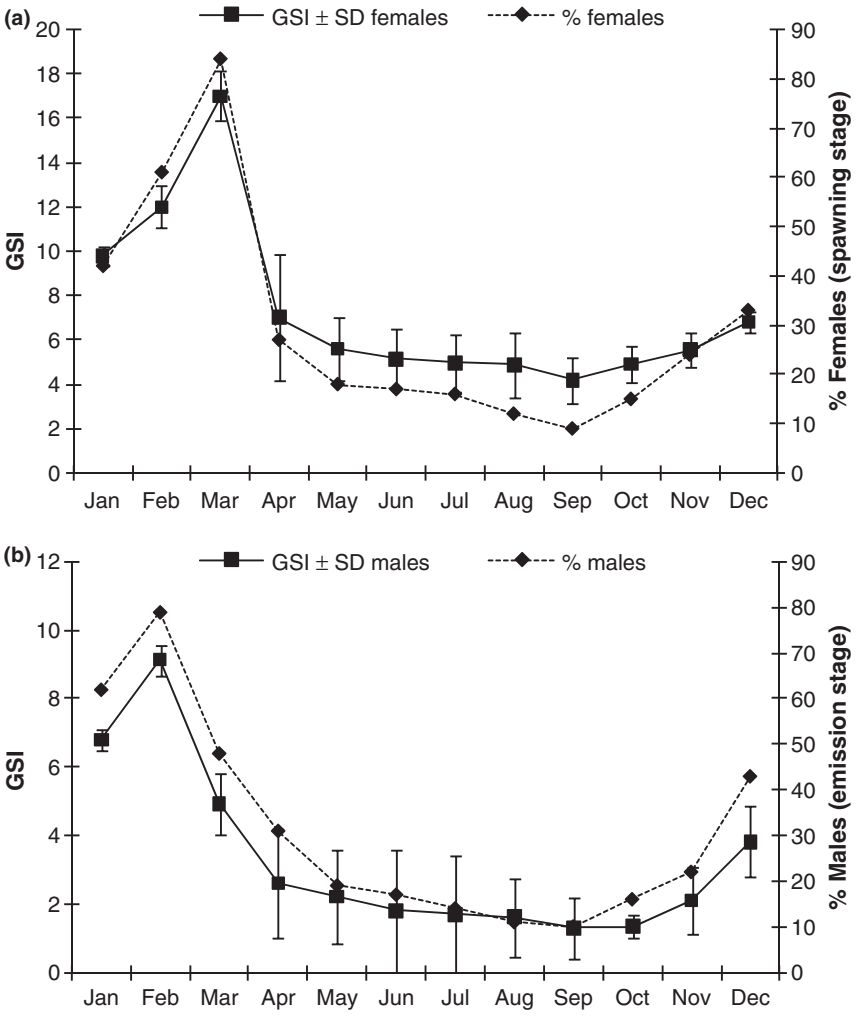

Fig. 2. Neretvan roach, *Rutilus basak*, gonad somatic index (mean value of GSI ± SD in bars) and percentage of spawning maturity stages (a) males and (b) females by month (Jan–Apr: N = 100; May–Dec: N = 30)

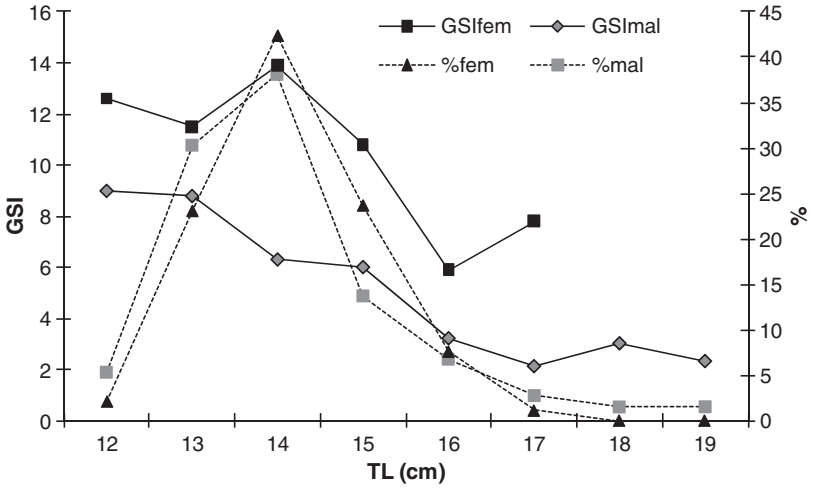

Fig. 3. Neretvan roach, *Rutilus basak*, gonad somatic index (GSI) and percentage of mature males (N = 96) and females (N = 284) by length classes

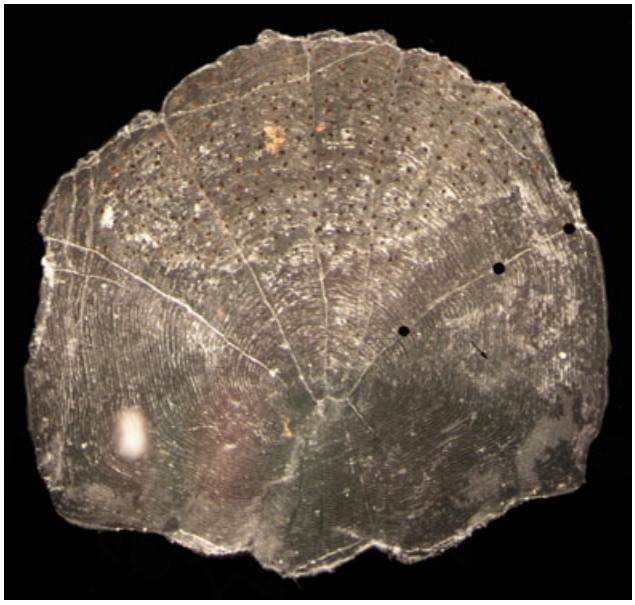

Fig. 4. Scale from 3-year-old *Rutilus basak*, Hutovo Blato wetlands. (14.0 cm; April) (magnification 1.6 × 11.2). Black dots: ages; black arrow: increments

third (2.84). The precision of the age estimates yielded a coefficient of variation of 1.3. In general, scales were hard to read, with identifiable increments unclear and the evident phenomenon of stacking of the growth zone towards the scale edge, especially in fish older than 6 years. The first annulus is formed very close to the scale focus and annuli appeared during the spring months. Ages ranged from 0 to 10 years (Fig. 5a), with age classes 1–5 dominating the catch (77.0%). The oldest specimens were estimated to be a 10-year-old female (21.7 cm) and 8-year-old (19.0 cm) male; 4-year-old individuals ranging from 14.7 to 15.7 cm TL (22.1%) dominated the sample. Evidently there were bimodal length distributions with the first mode at 10 cm (age 1+) and 15 cm (age 4+) (Fig. 5b).

A pair-wise comparison of the mean lengths at age of males and females showed that there were no significant differences for any of the age classes (*t*-test: $t_{0.05}$, $P > 0.05$). Overall, the age frequency distributions of males and females were not significantly different (Kolmogorov–Smirnov two sample test: $n_f = 294$, $n_m = 96$; $D = 0.528$, $P > 0.05$). The pooled length-at-age data for *R. basak* are presented in Table 2.

Parameters of the von Bertalanffy growth equation for the combined sample were: $L_\infty = 20.201$ cm (SE = 0.258); $K = 0.257$ per year (SE = 0.101); $t_0 = -1.554$ year (SE = 0.218); $R^2 = 0.977$ (Fig. 6). Non-linear least squares estimated parameters are given in Table 3. Growth parameters for males and females were not significantly different (max. likelihood test: $\Lambda = 3.58$, $\chi^2_{0.05,3} = 7.81$, $P > 0.05$). The calculated asymptotic length values agreed well with the maximum observed length. This study revealed that *R. basak* is a relatively fast-growing and long-lived species with a life span exceeding 10 years.

Discussion

Most *R. basak* specimens were concentrated in lakes and streams characterized by specific living conditions (temperatures between 13 and 15.2°C), which were a direct consequence of the numerous springs. Females dominated all length classes and the overall male : female ratio, but the dominance was less expressed at the beginning of the spawning season. Similarly, females were clearly more numerous in both populations of *R. arcassi* from the two contrasting tributaries of the River Duero (Spain) while the sex ratio was balanced only in younger individuals and those smaller than 65 mm (Rincon and Lobón-Cerviá, 1989). During the entire spawning period, mature *R. rubilio* females inhabiting Greek waters dominated the population proportionally, but with a less expressed dominance (Daoulas and Kattoulas, 1985).

Spawning migration of the investigated roach began in December with fish returning in April after spawning. Spawning in *R. basak* began earlier (end-February) than for other roaches, and duration of the spawning season (to mid-April, with a peak in first half of March) in the Hutovo Blato wetlands was similar to other areas for other species of the same genus.

Such winter gonad growth, in a period when somatic growth decreases, has been reported for other cyprinid species (Mann and Steinmetz, 1985; Rincon and Lobón-Cerviá, 1989). Spawning of *R. rubilio* in Lake Trichonis began somewhat later (beginning of March) and was completed by the end of April as evidenced by the sharply declining GSI (Daoulas and Kattoulas, 1985). Differences in the timing of roach spawning are reported even between tributaries of the same river (Rincon

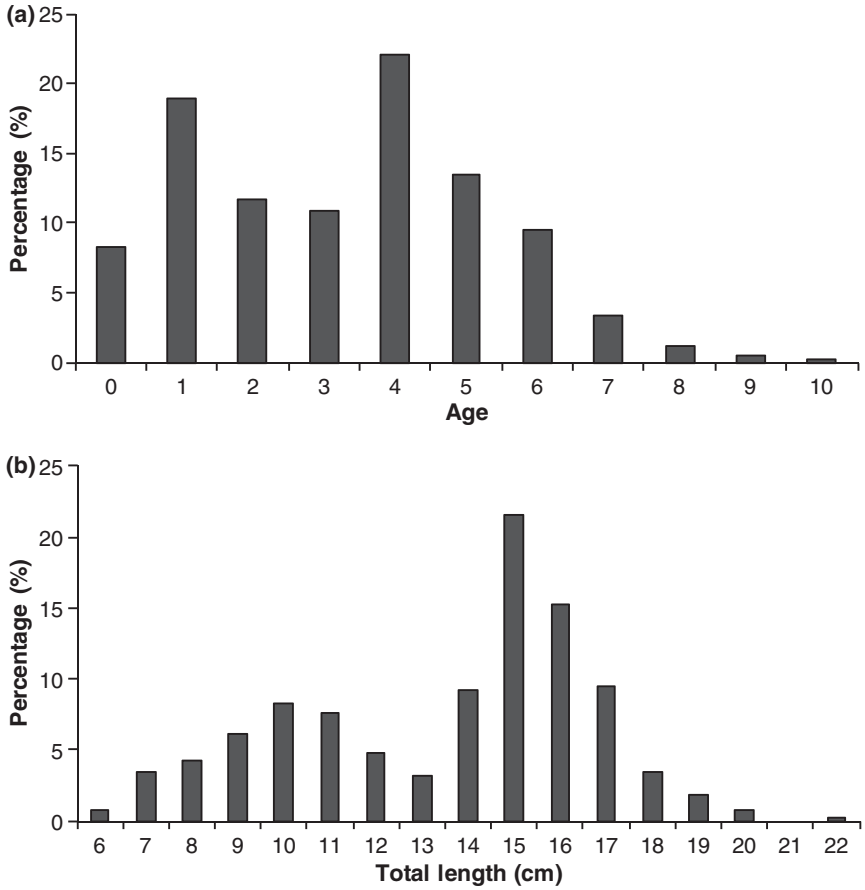

Fig. 5. (a) Age and (b) length population structure of Neretvan roach, *Rutilus basak*, Hutovo Blato wetlands

Table 2
Length-at-age data for Neretvan roach, *Rutilus basak* aged using scales readings

	Age											
Length intervals (cm)	0	1	2	3	4	5	6	7	8	9	10	Total
6	3											3
7	15											15
8	17	2										19
9	2	25										27
10		34	3									37
11		22	12									34
12		1	20									21
13			13	1								14
14			4	37								41
15				8	86							94
16					12	56						68
17						4	38					42
18							4	11				15
19								3	4			7
20									1	2		3
21												0
22											1	1
Total	37	84	52	46	98	60	42	14	5	2	1	441
%	8.4	19.0	11.8	10.4	22.2	13.6	9.5	3.2	1.1	0.5	0.2	100
Mean TL (cm)	7.6	10.0	12.1	14.3	15.2	16.1	17.2	18.3	19.3	20.4	21.7	
SD TL	0.81	0.79	0.94	0.36	0.32	0.27	0.28	0.28	0.33	0.14		
Mean TW (g)	3.9	9.6	19.7	34.0	39.4	45.5	52.4	66.8	87.4	100.8	131.9	
SD TW	1.75	2.94	6.02	4.45	5.98	5.94	22.76	9.66	5.32	1.77		

and Lobón-Cerviá, 1989). Knežević and Ivanović (1975) attributed a 2-month spawning duration of *R. rubilio* in the Montenegrin part of Lake Skadar (April–May) to partial spawning, while Daoulas and Kattoulas (1985) argued the case for differential maturity levels in the female population (the oldest females are the first to spawn). Cianficconi (1961) suggested that the prolonged spawning period could be the result of spawning interruption due to temperature

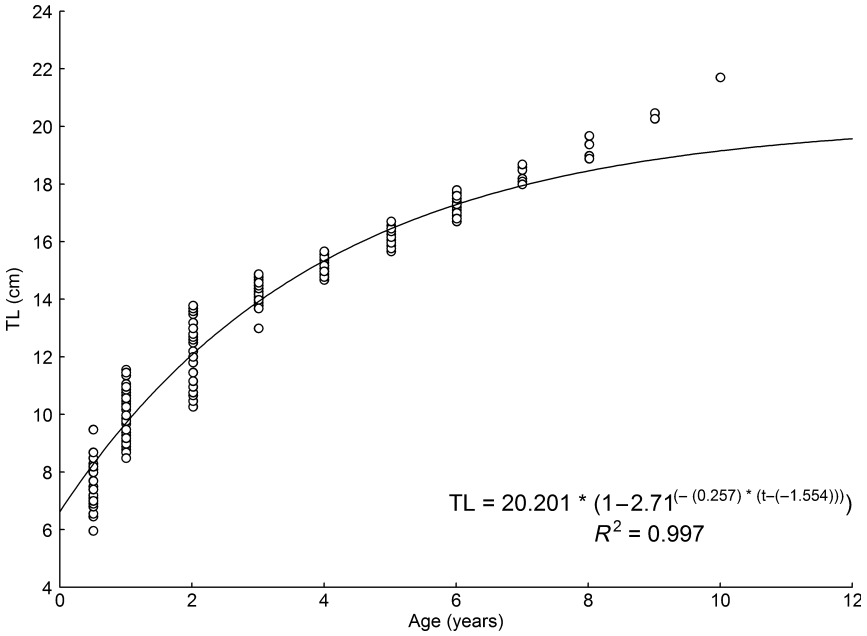

Fig. 6. WBGF growth curve of Neretvan roach, *Rutilus basak*

Table 3
VBGF growth parameters of *Rutilus basak*, Hutovo Blato wetlands, estimated by non-linear regression from scale readings for all data, males and females

Parameters	All data*	Males*	Females*
L_∞ (cm)	20.20 ± 0.258	20.17 ± 0.521	20.24 ± 0.343
K (per year)	0.257 ± 0.101	0.254 ± 0.126	0.257 ± 0.019
t_0 (year)	−1.554 ± 0.218	−1.534 ± 0.367	−1.558 ± 0.457

*All lengths in TL, cm ± SE.

fluctuations. In contrast, the present study showed that *R. basak* was not a partial spawner and that the water temperatures in Orah and Drijen lakes where the species spawn were almost constant throughout the year (13–14°C).

In general, scales were hard to read due to the existence of numerous annuli, which correspond with daily and monthly increments. The relatively constant water temperature probably influenced this phenomenon (Glamuzina et al., 2001). Also, much care was needed to avoid overlooking the first annulus that is formed very close to the scale focus; this same problem was also observed in the scale reading of *R. arcasii* (Rincon and Lobón-Cerviá, 1989). This is relatively often an important source of error in ageing cyprinids (Mann and Steinmetz, 1985).

The *R. basak* population in the Hutovo Blato wetlands contained specimens up to 10 years of age, with the fourth year class being most abundant. This was in agreement with the findings of Aganović and Kapetanović (1978) in the Neretvan roach population from the middle part of the Neretva River, with the 3-year-old age class providing the highest percentage of the population (max age 9 years). The Caspian roach, *R. rutilus caspicus* (Naddafi et al., 2005) also showed a very similar age structure. A relatively lower life span, however, was determined for *R. arcasii* (Rincon and Lobón-Cerviá, 1989).

This study revealed that *R. basak* is a relatively fast growing and long-lived species with a life span in excess of 10 years. The calculated asymptotic length of 20.2 cm for this species agrees well with the maximum observed length. Values of VBGF parameters for various roach species are given in Table 4. Growth slopes in the present study were similar to others, although asymptotic and maximum lengths differed significantly. Naddafi et al. (2005) suggested that the lower growth rate was caused by temperature effects and age on roach fecundity (significantly higher GSI). Moreover, the same authors concluded that growth opportunity and the cost of reproduction strongly influences the life history and allocation of energy resources. Horpilla et al. (2000) also suggested that a difference in sampling methods could have a considerable effect on the conclusions of growth differences in *R. rutilus* between two areas. Moreover, samples obtained with electro fishing could be characterized by the non-presence of older fish (Goffaux et al., 2005).

In conclusion, *R. basak* is characterized by small body size, fast growth rate, short life span and precocious maturity. How

Table 4
Values of VBGF parameters for different roach species

Species	Area	$L\infty$ (mm)	K	T_0	Source
Rutilus arcasii	River Moros, Spain	120.1	0.258	−0.116	Rincon and Lobón-Cerviá (1989)
R. arcasii	River Ucero, Spain	133.3	0.267	−0.007	Rincon and Lobón-Cerviá (1989)
R. rutilus caspicus	Anzali wetlands, North Iran	279.4 (m) 335.3 (f)	0.210 (m) 0.188 (f)	−0.7(m) −0.59 (f)	Naddafi et al. (2005)
R. rutilus caspicus	Gomishan wetlands, North Iran	315.0 (m) 342.4 (f)	0.225 (m) 0.194 (f)	−0.47 (m) −0.97 (f)	Naddafi et al. (2005)
R. basak	Hutovo Blato wetlands, B&H	202.0	0.257	−1.554	Present study

local selective pressures influence the demography and ecology of *R. basak* must be studied further. Also, problems arising in the Hutovo Blato, as in all wetlands, are (i) water pollution caused by urbanization and intensified agricultural land use, (ii) dramatically changed hydrologic regimes, and (iii) subsequent drying up of wetland areas, causing an overgrowth by marsh vegetation. These are serious threats not only in terms of reduced roach growth or shortened life-spans (Przybylski, 1996) but also in terms of habitat loss. Populations of *R. basak* and similar species are small and sensitive to loss of spawning and nursery areas, while genetic processes may influence diversity (Economidis, 2002). Populations are also threatened by restricted movements that prevent linkage of local populations to nearby habitats in support of the gene flow. To conserve these fragile populations appropriate management policies are necessary to target the specific needs of the populations (Economidis, 2002) before they reach the IUCN status of being threatened or endangered.

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