

# Age, growth, and population structure of endemic *Telestes karsticus* (Actinopterygii: Cyprinidae: Leuciscinae) from Sušik Creek, Croatia

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## Abstract

This study provides the first description of the age, growth, and population structure of the endemic karstic dace, *Telestes karsticus* Marčić et Mrakovčić, 2011, from Sušik Creek in Croatia. The oldest individual in the sample was a female of age 5<sup>+</sup> years and the oldest males were 4<sup>+</sup>. Females of the karstic dace have a higher maximum standard length than males (122.0 vs. 95.1 mm, respectively). In both sexes, the most numerous length category was 41–50 mm. The most numerous age group of karstic dace was 1<sup>+</sup>, with a gradual decline in the abundance of older age groups. In larger length categories, males were fewer, while females showed a bimodal distribution with a second maximum in the length category of 81–90 mm. The total ratio of males to females in the sample was 2.8:1, in favor of males.

## Keywords

age composition, endemic species, life history, population traits, size composition

## Introduction

The karstic dace, *Telestes karsticus* Marčić et Mrakovčić, 2011, is a relatively recently described species (Marčić et al. 2011), and the information on its ecology and population structure is scarce. Despite being a very old species, most likely originating at the Miocene–Pliocene boundary (5.3 million years ago) following separation from the common ancestor this species shared with *Telestes polylepis* Steindachner, 1866 (see Buj et al. 2017), it was not recognized as a separate species. Marčić et al. (2011) revealed cryptic diversity within *T. polylepis* at both the molecular and morphological levels, leading to description of *Telestes karsticus* as a new species. This

species is endemic to streams at only four locations on the north-western side of the Velika Kapela and Mala Kapela mountains in Croatia (Marčić 2013; Marčić et al. 2013; Čaleta et al. 2019). Such a small distribution range is very unusual for fish inhabiting the Black Sea basin, where rivers are large, with many tributaries and mostly interconnected. Its distribution is the consequence of the karst landscape of these watersheds, which lack surface connections with other rivers, draining instead towards the Danube River via underground connections.

The karstic dace is listed as endangered (EN) at the European level, i.e., in the Red List of Freshwater Fishes of Europe (Freyhof 2018), and also at the national level in the Red List of Croatian Freshwater Fishes with an

assessment of B1ab(i,ii,iii)+2ab(i,ii,iii) (ver. 12; Marčić et al. unpublished data). Though this species is perfectly adapted to karst environments, its extremely limited distribution range makes it particularly vulnerable. Any modifications to its small habitat could have irreversible effects on the population structure and viability. Furthermore, options for reintroduction or population augmentation are lacking, given the existence of only four populations. The distribution range of this species was somewhat larger in the past, but it has since vanished from several streams and has been severely reduced in the remaining localities. In the Stajničko Polje field, the karstic dace was last recorded in 2007 (with 12 individuals) in the Holjevačko Vrilo spring (Marčić 2013), despite visits to the spring at least once per year, while the population in the Drežničko Polje field is very small and includes just a few individuals within the spring itself.

Endemic species with very restricted distribution ranges, such as *T. karsticus*, are particularly vulnerable and typically have a reduced capacity to adapt to environmental change. The lack of knowledge about this species has also undermined its protection, and therefore, without an understanding of the biology of this species, it is not possible to ensure its protection and survival. To date, published studies on the biology of this species have examined its reproductive biology and seasonal diet profile (Marčić et al. 2017a, 2017b). However, an understanding of the population structure is crucial for adequate conservation plans, since biases from the normal age pyramid or population structure are indicative of pressures acting on the population. A balanced age structure provides a buffering capacity for the population, minimizing the effects of short-term environmental fluctuations on recruitment (Planque et al. 2010). In contrast, truncation of the age structure increases variability in the stock and its sensitivity to climate variation and other environmental modifications (Hidalgo et al. 2011; Rouyer et al. 2011). Moreover, since large, old, and experienced individuals have a higher reproductive value than young, small, and inexperienced ones, the age structure of the spawning population may be just as important as its biomass (Trippel et al. 1997; Berkeley et al. 2004). A skewed sex ratio is usually indicative of population disturbance. Thereafter, only populations with a normal age and sex distribution can be considered stable and viable. The aim of this study was to describe the age, growth, and population structure of this endangered endemic fish.

## Methods

Specimens of *T. karsticus* were collected at regular monthly intervals from July 2007 to July 2008 in Sušik Creek (45°44.13'N, 15°41.17'E). Sušik Creek is a sinking karst river that flows over about 5 km through a karst field at an elevation of 463 m. The creek width ranges from 2 to 10 m. Water levels are not constant through the year, with strong flows after rains or snowmelt raising depths to over 2 m in places, while in summer the

creek partially dries out and its depth elsewhere is less than 20 cm. Sampling was conducted using a backpack electrofishing device (Hans-Grassl, model IG-200-1) and captured specimens were anesthetized with an overdose of MS-222 and subsequently frozen. Fish were captured in a 200-m long transect in all habitats with the same effort during each sampling session. The use of a block net was impossible due to terrain configuration and the water depth and velocity. A total of 630 individuals (68 juveniles, 414 males, and 148 females) were analyzed. Standard length (SL) was measured using digital calipers with a precision of 0.1 mm and individuals were placed in standard length categories (each of 10 mm). Mass was determined with a digital scale with a precision of 0.1 g. Sex was determined by visual examination of the gonads. Population from Sušik Creek was assessed as the only location with a population sufficiently stable and large to ensure that sampling would not be detrimental for the species. Furthermore, the population was monitored in subsequent years and on last monitoring on June 2021, 800 adult individuals were recorded on the same locality in a 200 meter long transect making the current population at least 20000 strong (Marčić unpublished data).

The analysis of age and growth was performed on a subsample of 87 individuals. In the subsample, there were 47 males, 32 females, and 8 juveniles and the fish were selected to cover all standard length categories present in each monthly sample. Fish age was determined by direct readings of the scale rings, with independent readings performed by two different researchers (Busacker et al. 1990). Scales were removed from the dorsal part of the fish, under the dorsal fin, using histological needles, and cleaned of organic impurities in 3% KOH. Cleaned scales were placed on slides and observed under the microscope (Olympus BX51). Scales were photographed using the microscope Zeiss Axiovert 200 and measurements for back calculation taken using the program AxioVision Rel. 4.8. Each scale clearly showed the focus and concentric rings formed during growth. The scale radius and the radius of all rings on the scale were measured. The scale radius was measured as the largest length from the scale focus to the edge of the scale. Radii were measured along the same line. For the remainder of the sample, age was assessed using the length–weight relations for each month of sampling. Statistical significance of the number of males and females was determined with the  $\chi^2$  test. All mathematical calculations were performed in the Excel 2010 and Statistica 10.0 packages.

## Results

The range of standard length was examined for the entire sample of 630 individuals of the karstic dace from Sušik Creek. The length composition of the populations, particularly for males, females, and juveniles, is shown in Table 1 and expressed as the minimum, maximum, mean, and standard deviation.

**Table 1.** Length composition of total population, males, females, and juveniles of *Telestes karsticus* from Sušik Creek, Croatia, in period from July 2007 to July 2008.

Standard length [mm]	<i>n</i>	Min	Max	Mean ± SD
Total	630	15.0	122.0	49.7 ± 18.5
Males	414	18.5	95.1	48.5 ± 14.3
Females	148	20.2	122.0	63.0 ± 21.6
Juveniles	68	15.0	44.7	27.9 ± 6.7

*n* = number of specimens, SD = standard deviation.

The smallest male with developed testes had a standard length of only 18.5 mm, while the smallest female with developed ovaries had a standard length of 20.2 mm. The difference in the mean value of standard lengths between males and females was statistically significant ( $P < 0.0001$ ); females were larger on average. The largest reported individual was a female with a standard length of 122.0 mm. The largest individual without developed gonads had a standard length of 44.7 mm, which overlaps with mature individuals. The number of individuals by sex and length category is shown in Fig. 1.

Juvenile individuals were divided into four length categories, males in nine and females in ten length categories. The majority of juveniles (51.5%) fell within

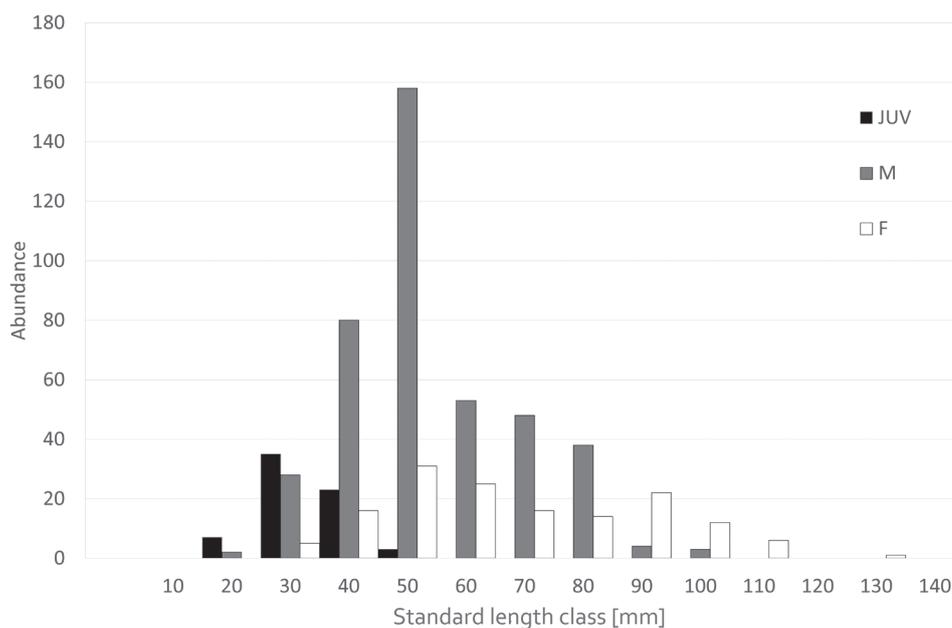
the 21–30 mm category. For both males and females, the highest abundance was found for the 41–50 mm category (M: 38.2%; F: 20.9%). Juveniles and males showed a unimodal distribution, while females showed a bimodal distribution, with a second peak in the 81–90 mm category.

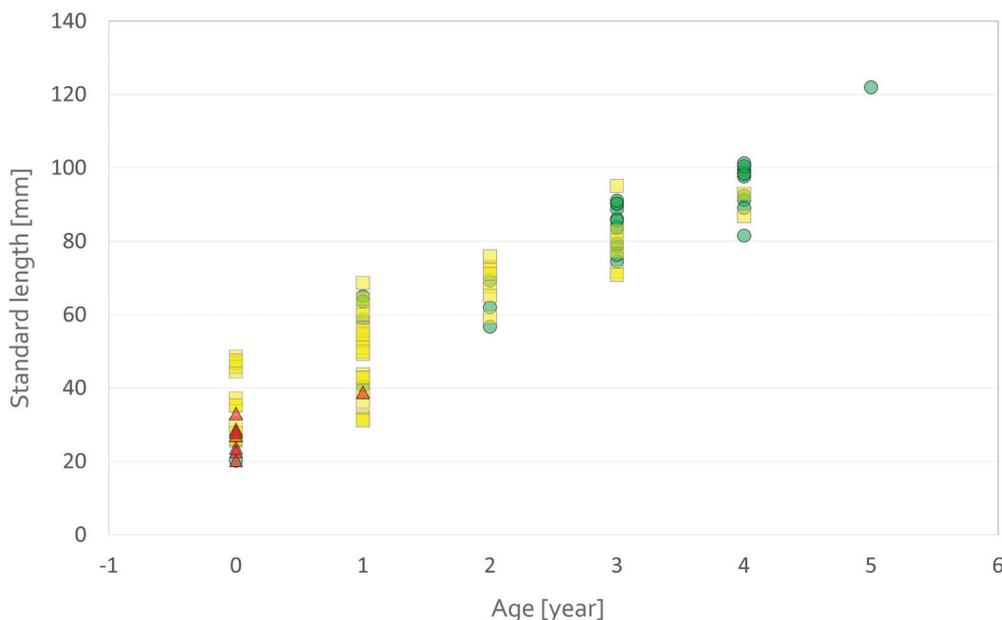
Scales were cycloid, normally developed, and small, covering the entire body. They overlapped on the entire body, except on the ventral side of the head, and in some individuals along the flank under the lateral line. Scales were round to oval in shape and varied in size, with a radius of 160.55  $\mu\text{m}$ , and the largest 1084.62  $\mu\text{m}$ , depending on their position on the body. Circuli were clearly visible and the annuli were relatively easy to view. The mean radius with standard deviation of sampled scales for each individual age group were:  $R_{0+} = 234 \pm 57 \mu\text{m}$ ,  $R_{1+} = 372 \pm 75.6 \mu\text{m}$ ,  $R_{2+} = 574 \pm 42.8 \mu\text{m}$ ,  $R_{3+} = 683 \pm 79.4 \mu\text{m}$ ,  $R_{4+} = 868 \pm 107.3 \mu\text{m}$ , and  $R_{5+} = 940 \mu\text{m}$ .

Six age groups were observed ( $0^+$  through  $5^+$ ). All juvenile individuals fell within the two age groups ( $0^+$  and  $1^+$ ). Males were found in five age groups (from  $0^+$  to  $4^+$ ), while females were found in all six (Fig. 2). Table 2 shows the number of the karstic dace individuals from the subsample in individual age groups, separated by sex, with mean values and standard deviations for standard length and mass.

**Table 2.** Individuals of *Telestes karsticus* from Sušik Creek, Croatia, in individual age categories with mean values and standard deviations of standard length (SL) and mass (*m*) by gender.

Sex	Juveniles			Males			Females			
	Age	<i>n</i>	SL [mm]	<i>m</i> [g]	<i>n</i>	SL [mm]	<i>m</i> [g]	<i>n</i>	SL [mm]	<i>m</i> [g]
	$0^+$	3	26.5 ± 5.7	0.3 ± 0.2	15	35.2 ± 9.4	0.9 ± 0.7	2	22.9 ± 3.8	0.3 ± 0.1
	$1^+$	—	—	—	21	48.4 ± 11.0	2.6 ± 1.6	4	57.0 ± 11.2	3.7 ± 1.9
	$2^+$	—	—	—	6	68.7 ± 6.0	5.9 ± 2.4	3	62.7 ± 6.3	4.5 ± 0.6
	$3^+$	—	—	—	7	78.6 ± 8.6	11.1 ± 4.8	12	84.1 ± 5.7	10.7 ± 2.3
	$4^+$	—	—	—	2	89.9 ± 4.3	11.7 ± 2.3	10	95.0 ± 6.3	16.7 ± 2.7
	$5^+$	—	—	—	—	—	—	1	122.0	36.7

**Figure 1.** Abundance of *Telestes karsticus* individuals in Sušik Creek, Croatia, by sex (F = female; M = male; JUV = juvenile) and standard length (SL) categories (each 10 mm).



**Figure 2.** Age categories of *Telestes karsticus* from Sušik Creek, Croatia, in relation to standard length (SL) for males (yellow squares), females (green dots), and juveniles (red triangles).

The Mann–Whitney U test showed that there were no statistically significant differences in the mean SL values between males and females in any age group ( $P > 0.05$ ), with the exception of the age group 0+ ( $P < 0.05$ ). The mean SL of juvenile individuals did not differ statistically from males or females in the age group 0+. The Mann–Whitney U test showed that there were no statistically significant differences in the mass of males and females in any age group ( $P > 0.05$ ). Table 3 shows the mass and length increments between age groups for males and females.

**Table 3.** Mass and length increments between consecutive age groups for males, females, and the total sample of *Telestes karsticus* from Sušik Creek, Croatia.

Sex	Age group	Mean mass increment [g]	Mean length increment [%]
Females	0 <sup>+</sup> –1 <sup>+</sup>	3.4 1133.3	34.1 148.9
	1 <sup>+</sup> –2 <sup>+</sup>	0.8 22.4	5.7 9.9
	2 <sup>+</sup> –3 <sup>+</sup>	6.1 135.3	21.4 34.1
	3 <sup>+</sup> –4 <sup>+</sup>	6.0 56.7	11.0 13.1
	4 <sup>+</sup> –5 <sup>+</sup>	20.0 119.8	27.0 28.4
Males	0 <sup>+</sup> –1 <sup>+</sup>	1.7 180.9	13.3 37.7
	1 <sup>+</sup> –2 <sup>+</sup>	3.2 121.6	20.3 41.9
	2 <sup>+</sup> –3 <sup>+</sup>	5.3 90.1	9.9 14.4
	3 <sup>+</sup> –4 <sup>+</sup>	0.6 5.2	11.3 14.4

The highest mass increment (1133.3%) and standard length increment (148.9%) for females were recorded between the 0<sup>+</sup> and 1<sup>+</sup> age groups. Males also showed the highest increase in mass (180.9%) in that time period, though the highest length increase (41.9%) was achieved between the age groups 1<sup>+</sup> and 2<sup>+</sup>. Females had the lowest mass increase (22.4%) and standard length increase (9.9%) between the age groups 1<sup>+</sup> and 2<sup>+</sup>, while males had

the lowest mass increase (5.2%) between the age groups 3<sup>+</sup> and 4<sup>+</sup>, and standard length increase (9.9%) between the age groups 2<sup>+</sup> and 3<sup>+</sup>.

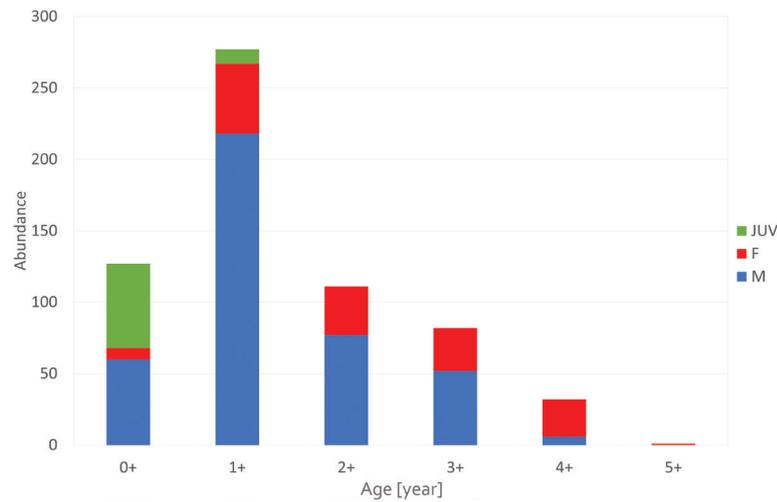
The mean SL values obtained through back calculations are shown in Table 4. Though these back-calculated SL values of standard length differed less than 7 mm from the measured mean standard length used in the determination of age for all age groups, the Mann–Whitney U test showed that those differences differed significantly ( $P < 0.05$ ), with the exception of the age groups 4<sup>+</sup> ( $P = 0.077$ ) and 5<sup>+</sup> for which there were too few individuals to determine statistical significance.

**Table 4.** Standard length of karstic dace, *Telestes karsticus*, from Sušik Creek, Croatia, obtained using back calculations by age group.

Age	n	Mean SL	Back calculated mean SL at age [mm]				
			SL 1 <sup>+</sup>	SL 2 <sup>+</sup>	SL 3 <sup>+</sup>	SL 4 <sup>+</sup>	SL 5 <sup>+</sup>
0 <sup>+</sup>	20	32.6					
1 <sup>+</sup>	25	49.8	45.4				
2 <sup>+</sup>	9	66.7	42.0	59.0			
3 <sup>+</sup>	19	82.0	42.9	61.7	76.3		
4 <sup>+</sup>	12	94.2	39.7	59.3	74.5	87.3	
5 <sup>+</sup>	1	122.0	47.4	63.0	86.2	101.8	115.8
Total back calculated mean SL at age			43.5	60.8	79.0	94.6	115.8

According to the Kruskal–Wallis test, standard length obtained using back calculations did not differ significantly between individuals of different age groups ( $P > 0.05$ ).

The population composition by sex and age is shown on the entire sample of 630 karstic dace from Sušik Creek (Fig. 3). Table 5 shows the calculated sex ratios by age category and the  $P$  values of the  $\chi^2$  test.



**Figure 3.** Age composition of the total sample of *Telestes karsticus* from Sušik Creek, Croatia (F = female, M = male, JUV = juvenile).

**Table 5.** Sex ratio of males (M) and females (F) of *Telestes karsticus* from Sušik Creek, Croatia, by age categories, with  $P$  values of the  $\chi^2$  test. Significant values ( $P < 0.05$ ) are shown in bold.

Age	Sex ratio M:F	$P$
0+	7.5:1	<b>2.9E-10</b>
1+	4.4:1	<b>4.5E-25</b>
2+	2.3:1	<b>4.5E-05</b>
3+	1.7:1	<b>0.0151</b>
4+	0.2:1	<b>0.0004</b>
5+	0:1	0.3173

The most abundant age category of the karstic dace was the 1<sup>+</sup> category (43.9%), with declining abundance in the older age classes. The oldest age category was 5<sup>+</sup> (0.1%), and consisted of a single female.

The total ratio of males to females in the sample was 2.8:1 in favor of males, which was statistically significant ( $P < 0.05$ ). Age categories from 0<sup>+</sup> to 3<sup>+</sup> were dominated by males, while females dominated in the 4<sup>+</sup> and 5<sup>+</sup> categories. The  $\chi^2$  test indicated significant differences in all age categories with the exception of the 5<sup>+</sup> category which included only a single female. Table 6 shows the calculated sex ratios by sampling date and  $P$  values of the  $\chi^2$  test.

**Table 6.** Sex ratio of males (M) and females (F) of *Telestes karsticus* from Sušik Creek, Croatia, by sampling date, with  $P$  values of the  $\chi^2$  test. Significant values ( $P < 0.05$ ) are shown in bold.

Sampling date	Sex ratio M:F	$P$
24.7.2007	1.5:1.0	0.1036
29.8.2007	2.6:1.0	<b>0.0004</b>
27.9.2007	0.9:1.0	0.8474
31.10.2007	4.0:1.0	<b>0.0000</b>
27.11.2007	15.0:1.0	<b>0.0000</b>
20.12.2007	6.8:1.0	<b>0.0000</b>
4.2.2008	4.3:1.0	<b>0.0046</b>
3.3.2008	4.3:1.0	<b>0.0000</b>
3.4.2008	1.9:1.0	0.0947
29.4.2008	1.0:0.0	<b>0.0000</b>
4.6.2008	6.8:1.0	<b>0.0000</b>
2.7.2008	0.9:1.0	0.7963
29.7.2008	2.3:1.0	0.2059

On the majority of sampling dates, the sex ratio was dominated by males, with the exception of 27 September 2007 and 2 July 2008 which were dominated by females. However, the  $\chi^2$  test showed that the majority of sampling dates had a statistically significant difference in the sex ratio.

## Discussion

The population structure of the karstic dace, *Telestes karsticus*, has hitherto been completely unknown. There are literature reports for the population composition of the related species *Telestes ukliva* (Heckel, 1843) (see Zanella, unpublished thesis; Zanella et al. 2009), *Telestes montenigrinus* (Vuković, 1963) (see Krivokapić 1992), and *Telestes souffia* (Risso, 1827) (see Vuković, unpublished thesis), and those results are compared with the results presented here. The maximum standard length of the karstic dace in Sušik Creek was 122 mm (total length 146 mm), confirming the assumption that it is a small bodied fish, and even smaller than other species in the genus *Telestes*. Zanella (unpublished thesis) reported the largest total length of *T. ukliva* at 150 mm, and Krivokapić (1992) reported the largest length category to 140 mm for *T. montenigrinus*. For *T. souffia*, Vuković (unpublished thesis) reported the largest length category to 180 mm from the Drina River and Vuković and Ivanović (1971) reported a maximum length of 250 mm for the same species. Kottelat and Freyhof (2007) listed maximum standard lengths of 120 mm for *Telestes beoticus* (Stephanidis, 1939), 165 mm for *Telestes croaticus* (Steindachner, 1866), 80 mm for *Telestes fontinalis* (Karaman, 1972), 100 mm for *Telestes metohiensis* (Steindachner, 1901), 160 mm for *T. montenigrinus*, 170 mm for *Telestes muticellus* (Bonaparte, 1837), 140 mm for *Telestes pleurobipunctatus* (Stephanidis, 1939), 150 mm for *T. polylepis*, 200 mm for *T. souffia*, 160 mm for *Telestes turskyi* (Heckel, 1843), and 100 mm for *T. ukliva*.

Females of the karstic dace from Sušik Creek, were generally larger than males, as was also found for *T. ukliva*

(see Zanella, unpublished thesis; Zanella et al. 2009) and *T. montenigrinus* (see Krivokapić 2002). Larger females are capable of producing greater numbers of eggs, therefore increasing reproductive success (Andersson 1994). The lower abundance within the age category 0<sup>+</sup> (20.1%) is likely a consequence of the sampling method, as electrofishing is known to underrepresent the 0<sup>+</sup> age category (Reynolds 1996). Such a population composition was also determined for *T. montenigrinus* (see Krivokapić 1992), while Zanella (unpublished thesis) found the highest abundance of *T. ukliva* in the 2<sup>+</sup> age category, and Vuković (unpublished thesis) found the highest abundance for *T. souffia* in the 3<sup>+</sup> and 4<sup>+</sup> age categories, depending on the season of sampling.

In the colder part of the year (November through March), when the water temperature is below 10°C, samples were comprised of individuals with an upper quartile of standard length less than 50 mm, and a median standard length of about 40 mm, suggesting that larger individuals overwintered at another, unknown location. One assumption is that larger individuals retract into subterranean areas where water temperatures are higher than in the surface stream, as suggested by Zanella (unpublished thesis) and Zanella et al. (2009), though such an assumption requires further study for the karstic dace.

The scale morphology of the karstic dace is similar to the scale morphology in other species in the genus *Telestes* (see Trgovčević 1905; Vuković, unpublished thesis). Clearly visible annuli on the scales enabled the assessment of individual age, as according to Busacker et al. (1990) the proportionality between most hard structures and fish size enables the determination of age, even for previous years of life. For the karstic dace, six age groups were found, with the oldest age group of 5<sup>+</sup>. This is less than the 7<sup>+</sup> that determined for *T. souffia* (see Vuković, unpublished thesis; Schwarz 1998) and *T. ukliva* (see Zanella, unpublished thesis), and 6<sup>+</sup> determined for *T. muticellus* (see Zerunian 2004) and *T. montenigrinus* (see Krivokapić 1998). However, this was higher than the 3<sup>+</sup> determined for *T. pleurobipunctatus* (see Barbieri et al. 2002). Both Zerunian (2004) and Vuković (unpublished thesis) also recorded only females in the oldest age group, which corresponds to the results presented here. The karstic dace and *T. pleurobipunctatus* are species that live in inconstant conditions, which is likely the reason for their lifespan being shorter than other members of this genus. Individuals of karstic dace in the aquarium survived longer than those ages recorded in nature (Marčić, unpublished data). This should be examined with further research on the remaining species of the genus *Telestes*.

The mass and length of males and females of the karstic dace have increased at a different pace. Males had a relatively high increase in mass between the age groups 0<sup>+</sup> and 1<sup>+</sup> (180%) which then declined with increasing age, to the minimum increase recorded between the age groups 3<sup>+</sup> and 4<sup>+</sup>, which was also the oldest recorded male in nature. Such a growth model indicates that males grow

faster and achieve their maximum increase in the age group 3<sup>+</sup>, after which time growth stagnates and the majority of individuals die. The length increase in males was similar to the mass increase. In females, the highest mass increase was also recorded between the age groups 0<sup>+</sup> and 1<sup>+</sup>, though this is likely the consequence of the small number (2) of females in the 0<sup>+</sup> category and the immaturity of their gonads. These results suggest that females stagnate in growth between the age categories 1<sup>+</sup> and 2<sup>+</sup> and then increase again between the age categories 2<sup>+</sup> and 3<sup>+</sup>. Such growth could be interpreted with the development of the reproduction potential in subsequent age groups, when energy is invested in gonad development, thereby increasing the mass of the individual. As expected, the mass increase dropped between the age groups 3<sup>+</sup> and 4<sup>+</sup>, and the large increase in mass increase between the age groups 4<sup>+</sup> and 5<sup>+</sup> should be viewed in light of the fact that there was only one female in the 5<sup>+</sup> age category, which was also the largest (SL 122 mm) and heaviest (36.7 g) individual of the entire sample. The length increases in females were similar to the mass increases. Vuković (unpublished thesis) determined for *T. souffia* from the Drina River that the mass increase rises while the length increase gradually decreases with advancing fish age. For *T. montenigrinus*, Krivokapić (1998) reported the highest mass increase between the age groups 2<sup>+</sup> and 3<sup>+</sup>, though she did not examine specimens separately by sex. The reasons for the variations in mass increases between the species may be explained as the result of the different ecological conditions in the distribution area, or the result of biological or genetic isolation.

Using back calculations, the standard lengths were determined for earlier years of the life of individuals. The standard length obtained using this method should correspond to the standard lengths of individuals of that age group, which was confirmed here. Though the mean values of SL obtained using back calculations differed from the mean SL values for the measured individuals by age groups, the differences were not statistically significant, with the exception of age group 4<sup>+</sup>. The back calculated SL values were generally smaller than the actual SL values measured by age groups, which corresponds to Lee's phenomenon (Lee 1912). The values obtained by back calculations are the values that the individual had at the time of formation of the annulus, i.e., in late February or early March. After this time, the individual continues its normal growth and a higher SL will be measured if the individual is caught in summer or later.

Sex ratio is an important demographic parameter crucial for population viability (Ospina-Alvarez and Piferrer 2008). According to the Fisher (1930) model, the sex ratio in animals should create offspring with an even sex ratio. In principle, populations with skewed sex ratios caused by environmental conditions can be considered disturbed or poorly adapted for the given conditions. As the reproductive potential of many fish species is determined by the number of females capable of producing eggs, a strong shift in sex ratio in favor of males can re-

duce the viability of vulnerable populations. The sex ratio of the entire sample of the karstic dace was statistically significant in favor of males, which was also reported for *T. ukliva* which had a ratio between 1.6:1 and 1.82:1 in favor of males (Zanella, unpublished thesis; Zanella et al. 2009). Male domination in the smaller length categories and females in larger categories were also seen for *T. montenigrinus* (see Krivokapić 2002). Zerunian (2004) also reported that the 7<sup>+</sup> age category for *T. muticellus* was comprised exclusively of females. However, the presently reported results differed from the even sex ratio for the species *T. montenigrinus* (see Krivokapić 1992) and *T. souffia* (see Vuković, unpublished thesis). Furthermore, the sex ratio of karstic dace was dominated by males in the majority of months, particularly in the colder months when only smaller specimens were captured. A stable sex ratio was confirmed during the spawning period (3 April 2008), when the sex ratio was also in favor of males, though this was not statistically significantly different from the theoretical ratio of 1:1. The sex ratio

was also even and not significantly different from 1:1 in July 2007, September 2007, and July 2008, when larger individuals were captured, and in July 2008 when very few individuals were caught (Table 6). The uneven sex ratio in karstic dace is unlikely to be due to a segregation of the sexes due to their varying microhabitat use, as sampling included all habitats and sampling effort was uniform. The dominance of females in the older age categories might be explained by a higher mortality rate among males; however, such an occurrence would not support the prominent dominance of males in younger categories.

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