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Evaluating bony structures for ageing and growth parameters of *Capoeta banarescui* inhabiting the lower Melet River (Ordu, Turkey)

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Summary

The aim of this study was to investigate the reliability of bony structures for age determination. A total of 247 *Capoeta banarescui* individuals were analyzed. Fish were caught on the lower Melet River between July 2010 and October 2012. Sex ratio was 2.5 : 1. The specimens ranged from 8.1 to 21 cm in total length and 5.07 to 108.93 g in weight. Age determined from vertebrae, scales, asteriscus and lapillus otoliths were variable. The highest percent agreement (PA) (69.2%) and the lowest average percent error (APE) (4.24%) and coefficient of variation (CV) (8.71%) were obtained for vertebrae. Hence, it was determined that the vertebra is the most reliable ageing structure for *C. banarescui* in the lower Melet River. Females were age I–V years and males I–V years. The von Bertalanffy growth equation was established for females, males, and all individuals, respectively: $L_t = 23.9 [1-e^{-0.22\ (t+1.89)}]$, $L_t = 21.8\ [1-e^{-0.26\ (t+1.58)}]$, $L_t = 26.7\ [1-e^{-0.17\ (t+2.21)}]$.

Introduction

Age determination of fish is important for estimating biological characteristics such as growth rate, age at first maturity, life history and population dynamics. Several hard parts or bony structures such as scales, vertebrae, otoliths, cleithra, fin rays, urohyal bone, and opercular bones have been used for age estimation (Chugunova, 1963; Das, 1994). Some of these structures are not equally reliable in all species for accurate and precise estimation of fish age (Bostancı et al., 2009).

Accurate fish age determination provides useful information for resource management and estimates on population dynamics (Campana and Thorrold, 2001). Several errors in determining age from bony structure can occur, which in turn can affect the estimates of several population parameters (Campana, 2001).

Many studies comparing the reliability of various ageing structures have been reported (Maceina and Sammons, 2006; Sylvester and Berry, 2006; Reid, 2007; Lozano et al., 2014) including species such as *C. c. umbla* (Ekingen and Polat, 1987), *C. trutta* (Polat, 1987) and *C. tinca* (Polat et al., 1992).

Capoeta banarescui Turan et al., 2006; was a common species in the lower Melet River; however, sufficient information on its biology is not yet available and therefore cannot be found in FishBase (Froese and Pauly, 2014). Age and growth characteristics are unknown. Furthermore, the use of various bony structures for ageing the fish has not yet been tested.

The objectives of this study were to determine the use of bony structures to reliably age the *Capoeta banarescui* sampled from the lower Melet River, by comparing age readings from otoliths, scales and vertebrae. Additionally, this paper provides the first basic information on the different bony structures for ageing and growth characteristics in the *C. banarescui*.

Materials and methods

Capoeta banarescui samples were obtained from the lower Melet River. A total of 247 specimens were used in the study. Some of the samples were captured between the lower Melet River and the Topçam Dam by electrofishing (SAMUS-725MP) and cover net (mesh size, 10–26 mm). Anglers between July 2010 and October 2012 provided most of the samples. Total length (TL) was measured for each fish to the nearest cm and total weight to the nearest 0.1 g. In addition, the differences between males and females were tested with a *t*-test. Sex compositions of fish samples were examined using the gonads in that fish were cut from the anus to the chest and the internal organs removed with sharp scissors. Whilst large samples were macroscopically examined, small samples were evaluated under the microscope. Sex composition was determined for 233 samples, providing the sex ratio of the population. Overall ratio of males to females was tested with the chi-square test.

Scales, vertebrae and otoliths were removed for age determination. Scales were removed above the lateral line and in front of the dorsal fin, dipped in 3% NaOH solution for circa 17 h, transferred to 96% ethyl alcohol for ca 30 min, then washed with distilled water and prepared on slides for microscopic analysis. The vertebrae (between 4th-10th) were removed and boiled for 5–10 min. The muscles and lipids were removed and dried at 105°C for 30 min. Asteriscus and lapillus otolith pairs were removed, cleaned and stored dry before the readings were made (Chugunova, 1963). Readings from the bony structures were taken three times by an experienced reader with no prior information regarding fish lengths, weight or sex, except for the collection dates and gonad stages of the samples. All readings were done independently. Scales were viewed under a stereo binocular microscope with transmitted light and magnification strength of 10X. Vertebra and otoliths were read under a stereo binocular microscope at 10× magnification with reflected light in a few drops of alcohol against a black background.

Accuracy of repetitive readings was evaluated by percentage agreement for assigned age amongst the first, second, and third readings by the experienced reader. In addition, the average percentage error (APE) was used to assess consistency in repeated age determinations. The APEs for all bony structures were estimated (Beamish and Fournier, 1981; Campana, 2001).

The coefficient of variation (CV) can be useful for assessing the consistency of repeated ageing (Chang, 1982); this coefficient determined the relative ease of age interpretation from different ageing structures (Beamish and Fournier, 1981). Certainly, the ageing structures with the lowest CV and APE are preferred as the more reliable ageing material (Campana, 2001). Mean CV and APE for each bony structure was therefore compared by ANOVA ($\alpha = 0.05$).

$$\mathrm{APE}_j = 100\% \times \frac{1}{R}\sum_{i=1}^{R}\frac{|\mathrm{x}_{ij} - \mathrm{x}_j|}{\mathrm{x}_j}$$

$$\mathrm{CV}_j = 100\% \times \frac{\sqrt{\sum_{i=1}^{R}\frac{(\mathrm{X}_{ij}-\mathrm{X}_j)^2}{\mathrm{R}-1}}}{\mathrm{X}_j}$$

The mean age (X_{kt}) was calculated using the formula for each bony structure (Baker and Timmons, 1991):

$$X_{kt} = \frac{\sum_i^n \sum_j^f X_{ijkt}}{nf}$$

Mean ages were calculated from reliable bony structure to compare with over- or underestimated age from other structures.

The von Bertalanffy growth equation was used for *C. banarescui:*

$$\mathrm{L}_t = \mathrm{L}_\infty[1 - e^{-k(t-t_0)}],$$

where Lt is the length-at-age t, L_∞ the maximum theoretical length, K the body growth coefficient synonymous to the rate at which L_∞ is attained, and t_0 is the age of zero length fish (Ricker, 1975). The values of L_∞, K and t_0 were calculated using the FISAT-II Program, and the growth equation was obtained.

Results

In this study, 67.6% females, 26.7% males, and 5.7% unsexed were determined for the examined *C. banarescui* samples: 167 females, 66 males and 14 unsexed. The female:male ratio was also determined as 2.5 : 1 within the population. Differences between sexes were statistically significant ($\chi^2 = 21.38$, SD = 1, $P < 0.001$). Specimens ranged from 8.1 to 21 cm TL and 5.07 to 108.93 g in weight for this population.

Percent agreement (PA) values that were a result of three readings on different bony structures were estimated. Highest agreement was 69.2% in the vertebrae; lowest was 42.4% in the scales. Age readings were evaluated and mean CV and APE for each ageing structure was calculated (Table 1). The CV (ANOVA; $F = 35.26$ $P < 0.001$) and APE (ANOVA; $F = 33.26$ $P < 0.001$) values were significantly different amongst the four structures. Lowest CV and APE recorded were 8.71 and 4.24 on the vertebrae, respectively. When the bony structures were arranged according to precision and ageing error, the arrangement formed for this species was:

for precision vertebra > lapillus > asteriscus > scale.
for CV and APE vertebra < lapillus < asteriscus < scale.

In this case, it is obvious that the vertebra has the highest percent agreement with the lowest ageing error (CV and APE). Thus, the vertebra is the most reliable bony structure for age determination of this species (Fig. 1). Mean age estimates were compared for each structure. The difference between the mean ages of each technique was approximately 1 year. This difference was found to be significant (ANOVA, $F = 86.69$ $P < 0.001$). In other words, ages of fish were underestimated by a minimum of 1 year using the scale, asteriscus and lapillus otoliths (Table 1). Thus, vertebra was concentrated for age composition in this population. Females and males were I–V years. While the female individuals were predominant with 24.7% in the second age group, the males predominated with 10.9% in the second age group according to vertebra age readings.

The vertebra, which is a reliable bony structure for ageing, was compared with other bony structures. During the age reading on the asteriscus (Fig. 2a), 82 of the individuals (38.5%) were determined to be the same age according to the vertebra. The asteriscus method underestimated the age using the vertebrae method on 86 specimens for age 1. In 32 specimens, asteriscus ages deviated by 2 years from vertebra ages. In two specimens the asteriscus method overestimated the age using the vertebra method by 1 year (Fig. 3). In total, 185 asteriscus-aged specimens were underestimated from vertebra ages (Fig. 3).

When age data of lapillus (Fig. 2b) were compared with the vertebra in 237 samples, both bony structures showed the same age in 88 (37.1%) individuals. In seven specimens the lapillus method overestimated the age by 1 year, but in 82 specimens, lapillus ages were underestimated by 1 year by comparison with the vertebra method. In the 5th stage, the lapillus method underestimated by 3 years the vertebrae method in 15 specimens (Fig. 4).

Table 1
Coefficient of variation (CV), average percent error (APE), percent agreement (PA) and mean age values using four bony structures of *Capoeta banarescui* to determine age

Bony structure	N	CV (%)	APE (%)	PA	Mean age
Vertebra	247	8.71	4.24	69.2	2.696
Scale	229	31.69	15.36	42.4	1.502
Asteriscus	213	24.41	11.91	44.6	1.756
Lapillus	237	19.89	9.73	48.1	1.793

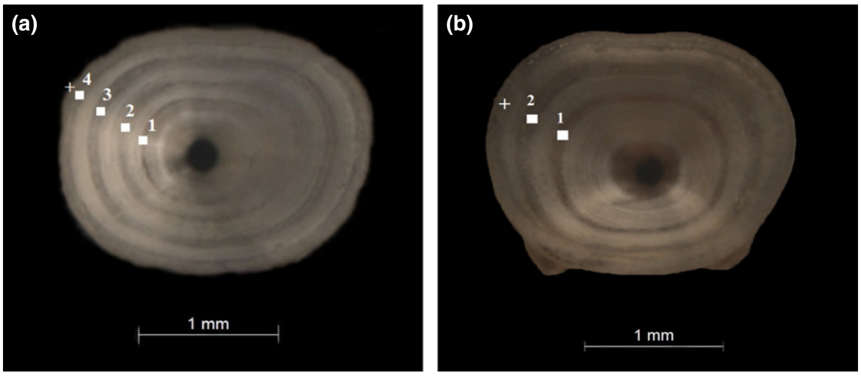

Fig. 1. Age 4+ (a) and age 2+ (b) vertebrae of *Capoeta banarescui*

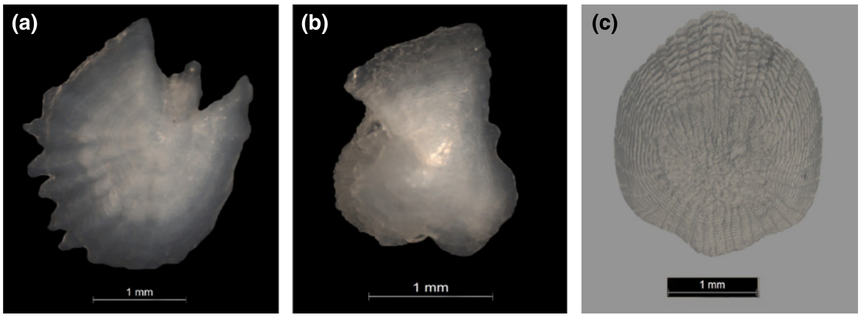

Fig. 2. Asteriscus (a), lapilllus (b) and regenerated scale (c) of *Capoeta banarescui*

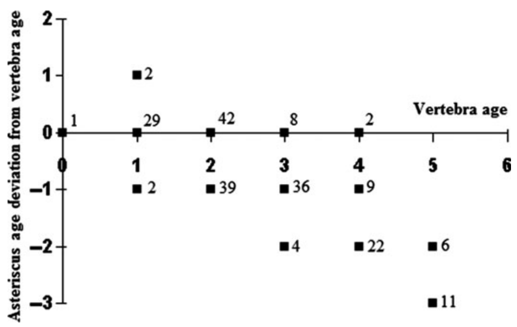

Fig. 3. Deviation of age determined by the asteriscus method from age determined by the vertebra method for *Capoeta banarescui*

When scale ages were compared with vertebra ages for this species, scale ages deviated from vertebra ages (Fig. 2c). The 229 samples were used to determine ageing from scales. According to the results of the scale method, 44 (19.2%) specimens were estimated to be in the same age range as the vertebrae method. In 117 specimens, scale ages deviated from vertebra ages for age 1. For age 5, the scale method underestimated by 3 years the vertebra method in 19 specimens (Fig. 5).

Growth parameters of *C. banarescui* were estimated using vertebra. The von Bertalanffy growth equation was calculated for each age group. Because of the differences ($P < 0.001$) shown between male and female length values, the growth equations were calculated for females, males, and all individuals separately (Table 2):

$$L_t = 23.9[1 - e^{-0.22(t+1.89)}]$$

$$L_t = 21.8[1 - e^{-0.26(t+1.58)}]$$

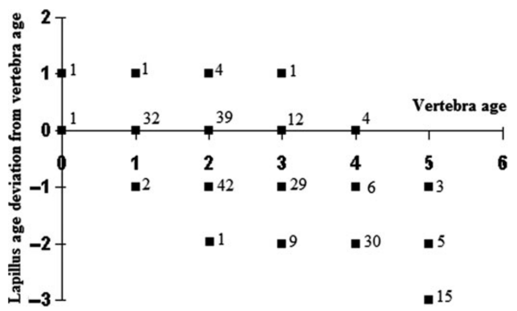

Fig. 4. Deviation of age determined by the lapillus method from age determined by the vertebra method for *Capoeta banarescui*

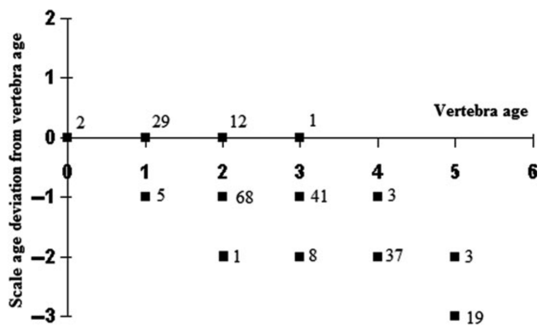

Fig. 5. Deviation of age determined by the scale method from age determined by the vertebra method for *Capoeta banarescui*

Table 2
Von Bertalanffy growth parameters for *Capoeta* species from different regions in Turkey

Species	Study area	Sex	Age	L_∞	K	t_0	Author
C. trutta	Karakaya	F	0–7	89.5	0.0571	−2.4138	Kalkan, 2008;
	Dam Lake	M	1–7	76.4	0.0604	−2.6514	
C. antalyensis	Aksu Stream	F+M	0–4	35.73	0.14	−1.48	Koca and Ölmez, 2010;
C. umbla	Hazar Lake	F+M	1–10	53.77	0.16	−1.84	Çoban et al., 2013;
C. erhani	Menzelet	F	0–6	33.83	0.964	−0.56	Ayyıldız et al., 2014
	Reservoir	M	0–6	32.02	0.843	−0.57	
		F+M	0–6	33.85	0.821	−0.48	
C. banarescui	Melet River	F	1–5	23.9	0.22	−1.89	This Study
		M	1–5	21.8	0.26	−1.58	
		F+M	0–5	26.7	0.17	−2.21	

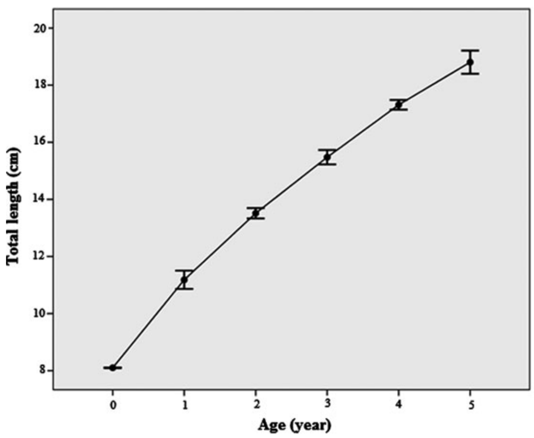

Fig. 6. Von Bertalanffy growth curve of *Capoeta banarescui* for all individuals. Data points represent means; bars are standard devations

$$L_t = 26.7[1 - e^{-0.17(t+2.21)}]$$

The growth curve was drawn using the total length values for all individuals (Fig. 6).

Discussion

The vertebra shows broad concentric opaque zones and narrow translucent zones around the nucleus. The clarity of annuli is clear. Marks on the vertebra are typically clear, consistent, and easy to interpret. Four hyaline rings (Fig. 1a) and two hyaline rings (Fig. 1b) were noted clearly on the vertebra. Determining the contrast between opaque and hyaline rings in vertebra and thereby ageing is relatively easier than with the other structures. False annulus or double rings do not appear and cause problems in age determination (Fig. 1a, b), thus making reliable vertebrae aging possible. Determining the location of the first ring and tracking the ensuing rings on all structures and identifying the shape of the edge of the bony structure are conveniently carried out in the vertebrae, and are the reason for the high agreement values shown in Table 1 by streamlining repeated age readings for the vertebrae. Similarly, CV and APE values indicating the margin of error were very low for vertebrae compared with other structures (Table 1).

The age rings, which are thin and brittle, are quite difficult to read in asteriscus of this species. Even removal and storage of the asterisci are highly problematic. The current study found that the asteriscus is inappropriate for age determination because the hyaline and opaque rings cannot be distinguished clearly. The centre of the otolith and the first age ring are difficult to distinguish and the circulus and annulus are confusing. Ergo, age determination cannot be made because of these issues (Fig. 2a). The first difficulty is the contrast determination between the opaque and hyaline rings; the second problem is following and interpreting the edges of the ring on the asterisci (Fig. 2a). The lower PA, and the higher APE and CV values for asteriscus otoliths support these observations (Table 1).

The lapillus is slightly concave and the posterior part thicker than the anterior part. Its nucleus is not clear and the growth rings in the middle half of the structure cannot be observed with any great clarity; therefore age readings were not carried out on the lapillus otolith (Fig. 2b).

In the scales, there are several studies on regeneration (Chilton and Bilton, 1986; Bereiter-Hahn and Zylberberg, 1993) and the absorption phenomena encountered. Determination of the first annual ring and tracking the following rings on the regenerated scales were difficult (Fig. 2c). Regenerated scales lead to underestimating fish age (Fig. 5). Scales were shown to be unreliable and less useful for age determination than were other structures. Among four hard structures of *C. banarescui*, we rejected the use of asteriscus, lapillus and scale in age determination.

Reliable age determination is an important parameter for biological activities such as age of sexual maturity and estimation of growth parameters in fish biology studies. Reliability of the bony structure as an instrument for age determination can vary from species to species, and may even vary amongst stocks of the same species. In further studies, it is suggested that the reliability of bony structures be again determined, because of the variation of reliable bony structures within the same species that live in different populations.

Chilton and Beamish (1982) noted that ageing of *Gadus macrocephalus* (Gadidae) should be done using scales and fin rays, and that the same family member of *Merluccius productus* otoliths should be used for ageing. Another example is *C. c. umbla*, which is a Cyprinidae family member. While the reliable bony structure for ageing *C. c. umbla* is the otolith (Ekingen and Polat, 1987), the dorsal fin ray section is the most reliable structure for ageing of *C. trutta* (Polat, 1987). Although these two species inhabit the same lake and belong to the same genus, the reliable structures used to determine age differ. Reliable age determination methods can differ, no matter how close the taxonomic relationship of the species. Thus, a reliable bony structure does not seem to be a generalization based on any taxa. In different studies from the same genus, vertebrae have been identified as the most reliable structure for ageing *C. tinca* inhabiting Altınkaya Dam Lake (Polat et al., 1992) and *C. banarescui* inhabiting the Melet River.

The von Bertalanffy growth functions calculated for *C. banarescui* in this study showed that the female theoretical maximal length value (L_∞ = 23.9 cm) was higher than in males (L_∞ = 21.8 cm). Growth parameters for all analysis material given in this paper were compared with the results of other authors for the *Capoeta* genus in Turkey (Table 2). The differences in the theoretical length value among regions can be attributed to the difference in the length range of individuals sampled in each area or in the differences among species.

Thus far, no studies on the determination of age and growth characteristics of *C. banarescui* have been reported. The current study results offer the first data for *C. banarescui,* which will permit comparative studies on variations of growth in different populations, thereby serving as a pilot study. Water pollution and fishing during the spawning period should be investigated and controlled whereas further studies should focus on direct or indirect age validation methods.

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