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AGE STRUCTURE AND BODY SIZE OF *Mertensiella caucasica* (WAGA, 1876) (CAUDATA: SALAMANDRIDAE) IN A POPULATION FROM TURKEY

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Here we present the age structure of a breeding Caucasian Salamander population from a high-altitude locality (Kazıkbeli Plateau, Kürtün, Gümüşhane; 2213 m a.s.l.). Age structure was analyzed by counting lines of arrested growth (LAG). Endosteal resorption raised difficulties of counting LAGs. Males were found to be average older than females. Range of age was from 5 to 11 years in males, 4 to 8 years in females and 1 to 3 years in juveniles. Snout-vent length (SVL) of each individual was used as body size and mean values were recorded as 64.24 mm in males, 58.45 mm in females, and 44.60 mm in juveniles. Difference of SVL between sexes was found statistically significant. Meanwhile, male-biased sexual dimorphism was calculated (SDI = –0.099). The SVL was correlated with age in both sexes as strongly positive.

Keywords: skeletochronology; longevity; growth; Caucasian Salamander.

INTRODUCTION

Molecular data allows us to distinguish the "true salamanders" (*Chioglossa*, *Mertensiella*, *Lyciasalamandra*, and *Salamandra*) from other taxons (newts) in Salamandridae family (Veith et al., 1998, Frost et al., 2006, Üzüm, 2009). Three genera of true salamanders clade distribution is limited to region around Mediterranean Sea while *Chioglossa* occurs only on Iberian Peninsula (Veith et al., 1998).

Mertensiella caucasica is a stream-dwelling salamander with a thin and elongated body (Baran et al., 2012). The male Caucasian salamanders have a fleshy protuberance at dorsal side of the tail base and are characterized with it (Üzüm, 2009). *Mertensiella* is represented by only one species and the sister taxon of *M. caucasica* is the Iberian *Chioglossa lusitanica* Bocage 1864 (Veith et al., 1998; Üzüm, 2009).

The range of *M. caucasica* includes the western part of the Lesser Caucasus Mountains, the southwest Georgia and the northeast Turkey. The distribution of *M. caucasica* in Turkey consists of Ordu, Giresun, Gümüşhane, Trabzon, and Artvin provinces (Baran and Atatür, 1998; Üzüm, 2009; Baran et al., 2012). It is a habitat specialist, mainly occur in habitats, including mixed, broad-leaved, and subalpine forests, and shrubs/grasslands above the timberline (Tarkhnishvili and Kaya, 2009). It spends a big part of life in shelters after metamorphosis. Optimal temperatures and also humidity are needed to start the breeding season for this animal (Tarkhnishvili and Serbinova, 1993). *M. caucasica* can be called low-tolerance species, and also listed in the IUCN Red List of Threatened Animals as Vulnerable (IUCN Red List).

Skeletochronology is the practical method for the revealing the ages of individuals and the estimating of some growth parameters in most vertebrates (Castanet and Smirina, 1990; Guarino, 2010; Üzüm et al., 2014; Altunışık et al., 2014; Makovicky et al., 2015). It allows us to estimate the age structure of amphibians in nature (Cogălniceanu and Miaud, 2003) and the age structure of a population completes the demographic parameters (Stearns and Koella, 1986). This method is being increasingly used on endangered species as it allows work on just some skeleton fragments without killing them (Castanet and Smirina, 1990; Guarino, 2010). On the other hand, skeletochronology also allows us to understand some environmental factors through comparing growth parameters within a short time (Caetano and Castanet, 1993, Bovero et al., 2006).

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Castanet and Smirina (1990) indicated that skeletochronology was an effective method to determine the age of animals, and Guarino and Erismis (2008) referred that phalangeal skeletochronology seems to be a reliable tool to obtain information on age and growth patterns of *R. holtzi*. Khonsue et al. (2010) addressed the using of the skeletochronology on protected and rare amphibian species. Briefly, this method has launched greatly used on amphibians in many years (Halliday and Varrel, 1988; Miaud et al., 1993; Üzüm, 2009; Üzüm and Olgun, 2009; Guarino, 2010; Üzüm et al., 2014)

The objective of this study is to enhance the data on some life history characteristics of *M. caucasica* and to understand efficiency of these characters by considering previous data (Tarkhnishvili and Gokhelashvili, 1994; Üzüm, 2009; Reinhard et al., 2015).

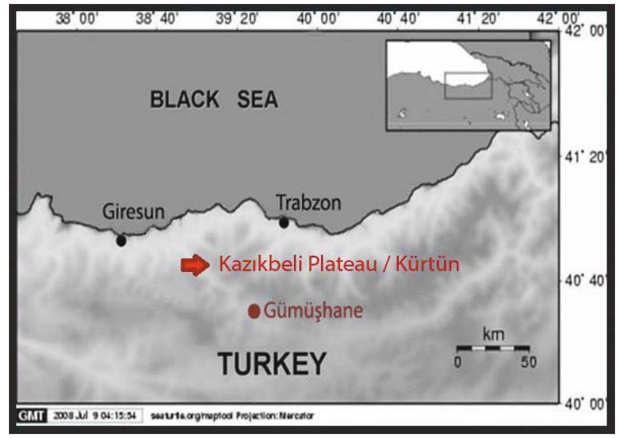

Fig. 1. Map shows the locality of studied population of *Mertensiella caucasica*.

MATERIAL AND METHODS

Study samples. We used 66 *M. caucasica* museum specimens (28 males, 30 females and 8 juveniles) in this study. The population was sampled from Kazıkbeli Plateau, Kürtün, Gümüşhane (40°32’ N 38°55’ E, altitude of 2213 m) (Fig. 1). Specimens found under stones in the shallow water of the stream were collected by hands. All material is deposited in the Zoology Laboratory of the Department of Biology at Science and Arts Faculty, Adnan Menderes University.

Body size. The salamanders were measured from snout to vent (SVL) by using a digital caliper with 0.02 mm of precision. We revealed the sex and maturity of each individual through external secondary characters. According to this method, males have a fleshy protuberance at the base of the tail, females have a prominent cloaca but without protuberance, and juveniles are differentiated from adult one in having smaller body length and lacking both the protuberance and the prominent cloaca (Baþoðlu et al., 1996; Baran and Atatür, 1998; Üzüm, 2009). We used Lovich and Gibbons (1992) sexual dimorphism index (SDI) to estimate sexual size dimorphism:

$$\text{SDI} = \frac{\text{mean length of the larger sex}}{\text{mean length of the smaller sex}} \pm 1,$$

+1 if males are larger or –1 if females are larger. It also defines as positive when females are larger than males and negative in the converse case.

Skeletochronology. Skeletochronological analysis were used to describe individual’s age (e.g., Castanet and Smirina, 1990; Olgun et al., 2005; Kyriakopoulou-Sklavounou et al., 2008; Üzüm et al., 2014). All phalanges stored in 70% ethanol solution to investigate. After cleaned from alcohol, the largest bone was washed in tap water then decalcified in 5% nitric acid for 1.5 – 2 h. The decalcified bones washed again with tap water for overnight. The diaphyseal region of each phalanx was cross-sectioned (18 μm) by using cryostat microtome at –25°C. Histological sections were stained for 5 min in Ehrlich’s hematoxylin. Afterwards, the sections transferred to microscope slide and placed in glycerin. Each individual’s sections were photographed at same selected magnifications in order to be observed as well. The age of each individual was determined by counting the number of LAGs (lines of arrested growth) from cross sections. We revealed the rate of endosteal resorption (e.r.) through sections from juveniles. The sections from adults and juveniles with the same magnifications were able to help us for comparing diameters of all resorbed LAGs, partly resorbed LAGs and non-resorbed LAGs.

Data analysis. The adult survive rate was calculated from the age structure. According to Robson and Chapman’s (1961, in Krebs, 1969) formula:

$$\text{Sr} = \frac{T}{\sum N + T - 1},$$

where Sr is average finite survival rate, i.e., a value of 0.75 means that on average 75% of the population survives from one year to the next; *T* is the sum of coded ages times their frequencies when age is found by setting the youngest included age-class to zero, the next age to one and so forth = $0N_x + 1N_{x+1} + 2N_{x+2} + ... + iN_{x+i}$; ΣN is the number of animals from age-class *x* to $x + i$ — $N_x + N_{x+1} + N_{x+2} + ... + N_{x+i}$; and N_x is the number of individuals in age-class *x*. Adult life expectancy (ESP), the expected total longevity

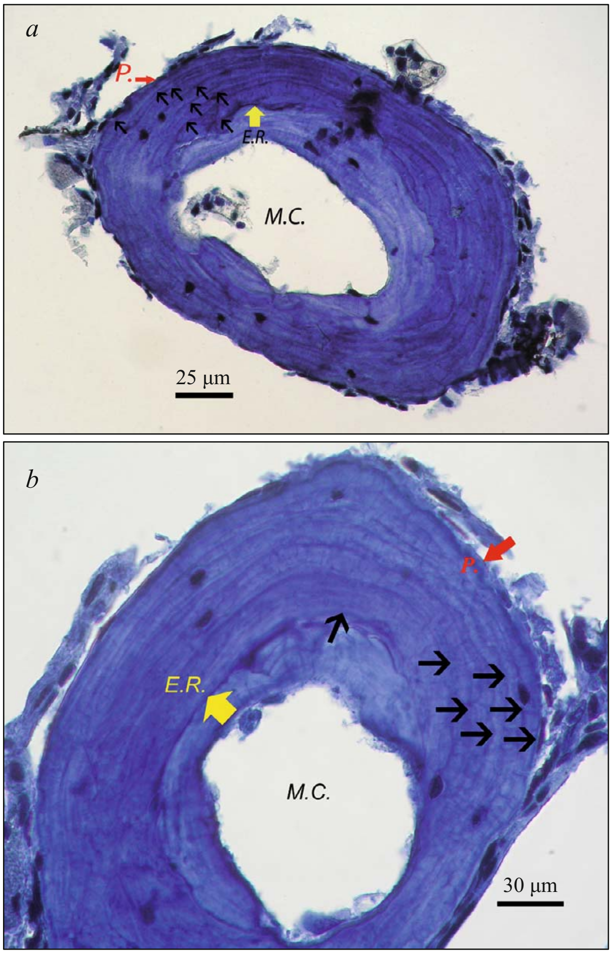

Fig. 2. The cross-sections from phalanges of male and female *M. caucasica*. *a*, 9 years old male section (first LAG was totally destroyed); *b*, 7 years old female section; *E.R.*, endosteal resorption; *M.C.*, medullary cavity; *P.*, peripheral; black arrows show LAGs.

of animals that have reached maturity, was calculated using Seber's (1973) formula:

$$\mathrm{ESP} = 0.5 + \frac{1}{1 - \mathrm{Sr}},$$

where Sr is the average finite survival rate of adults. All data was tested for normally distributed through descriptive statistics (including skewness and kurtosis values) and Kolmogorov – Smirnov *D*-test ($P > 0.05$). Because data of length showed normally distributed, we used parametric tests (*t*-test) to controlling for the differences of sexes by *t*-test. However data of age was not normally distributed. For this reason we used non-parametric tests (Mann – Whitney *U*-test) to reveal the situation of age. Spearman's correlation coefficient was calculated to verify the relationship between age and length. The best regression model was selected according to R^2 values. All of the statistic tests were processed with Statistica 7.0 (StatSoft Inc., USA) and Excel (Microsoft) at $\alpha = 0.05$.

RESULTS

All diaphysis bones in our study were showed narrow concentric lines after staining with hematoxylin. These lines show winter resting (hibernation) and termed as Lines of arrested growth (LAGs) (Castanet et al., 1977; Başkale et al., 2013; Üzüm et al., 2014). Lines of arrested growth (LAGs) were visible in the hematoxylin-stained cross-sections from all individuals (Fig. 2). As well as hibernation lines, aestivation lines were also observed in almost all cross sections from adult individuals. These lines were always light in color and located close to and through the hibernation lines. They were also not observed together with all winter resting lines. Endosteal bone formation that was deposited inside of the periosteal bone and separated by a resorption line was observed. Juveniles did not show any resorption, while endosteal resorption was observed almost in all cross sections from adults (96.56%). First line of arrested growth was partly destroyed in 19 males (67.85%) and 26 females (86.66%) and it was completely destroyed in 7 males (25%) and 3 females (10%). In 3 males and 3 females, second LAG was partly destroyed while in 2 males was destroyed totally.

Skeletochronological analysis allowed us to estimate age of all individuals in this study. The observed maximum longevity was 11 years for males and 8 years for females. The minimum age for adults was 5 years for males and 4 years for females (Fig. 3). According to this data, we found the age at maturity to be 4 – 5 years old after metamorphosis. Juveniles were found distributed from 1 to 3 years old. The average ages of males and females were found as 7.42 ± 1.37 and 5.80 ± 1.15 years, respectively (Table 1). The difference between average ages of males and females was found statistically significant (Mann – Whitney *U*: 169.00, $p < 0.001$).

Descriptive statistics for snout-vent length (SVL) are also shown in Table 1. Mean SVL of males and females were found to be statistically different ($t = –4.944$, $df = 56$, $p < 0.001$). SVL measurements in the same age groups between sexes were also compared in order to be able to put an interpretation on their growth strategy. Only 6, 7, and 8 age groups were chosen because of their individual numbers (Table 2). SVL differences between sexes in these age groups were statistically non-significant ($F_6 = 0645$, $p_6 = 0.433$; $F_7 = 0.128$, $p_7 = 0.726$; $F_8 = 1.111$, $p_8 = 0.327$). SDI was calculated as –0.099, indicating a male bias. The adult survival rate was estimated to be 0.71 for males and 0.65 for females. Adult life expectancy (ESP) was 3.94 for males and 3.35 for females.

The SVL was correlated with age in both sexes as strongly positive. Spearman's correlations were found as $r = 0.920$ ($p < 0.001$) and $r = 0.931$ ($p < 0.001$) for males and females, respectively. Linear model was selected as best regression model according to R^2 values. The regression equations were SVL = 45.007 + (2.615 × × age) for males and SVL = 35.559 + (3.948 × age) for females (Fig. 4).

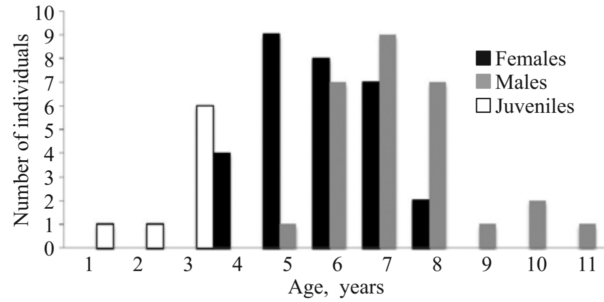

Fig. 3. Age distribution of all *Mertensiella caucasica* individuals.

DISCUSSION

The temperature is an environmental factor which effects are observable on cross-sections. The winter LAGs in the Caucasian salamander were clearly stained. Growth marks were also appertained to periods of aestivation during hotter months in some amphibians (Esteban et al., 2004). We also observed aestivation lines almost in all cross sections from our population. This was similar to be previous studies with amphibians such as *Triturus marmoratus* (Caetano and Castanet, 1993), *Chioglossa lusitanica* (Lima et al., 2000), and *Triturus karelinii* (Üzüm and Olgun, 2009). But, only one LAG was reported during each hibernating period for another population of *M. caucasica* from Kümbet Plateau (Üzüm, 2009). Having aestivation line shows some differences depending on the annual climatic conditions among the selected breeding sites (Üzüm and Olgun, 2009). Moreover, Kyriakopoulou-Sklavounou et al. (2008) reported that if the climatic conditions were not very dry and hot during summer, there would not be any aestivation lines in cross-sections of *Pelophylax ridibundus*.

TABLE 1. SVL and Age of *Mertensiella caucasica* in the Kazıkbeli Plateau, Kürtün Specimens [mean ± S.E. (range)]

	Males (*N* = 28)	Females (*N* = 30)	Juveniles (*N* = 8)
Age, years	7.42 ± 1.37 (5 – 11)	5.8 ± 1.15 (4 – 8)	2.63 ± 0.74 (1 – 3)
SVL, mm	64.24 ± 3.93 (57.4 – 74.5)	58.45 ± 4.88 (51.0 – 69.2)	44.60 ± 3.72 (37.8 – 49.2)

TABLE 2. Biometric Values of SVL (in mm) in All Adult Age Groups of *Mertensiella caucasica*

Age, years	*N*	Mean	Minimum	Maximum	S.E.	S.D.
			Males			
5	1	59.20				
6	7	60.34	57.4	62.7	0.79	2.1
7	9	63.28	61.0	65.6	0.47	1.45
8	7	66.37	64.8	68.1	0.47	1.25
9	1	67.69				
10	2	70.41	69.8	71.1	0.64	0.9
11	1	74.46				
			Females			
4	4	52.06	51.0	52.6	0.38	0.76
5	9	54.68	51.9	54.5	0.72	2.18
6	8	59.66	58.1	61.1	0.37	1.06
7	7	62.96	60.1	65.5	0.8	2.12
8	2	67.60	66.0	69.2	1.61	2.27

Notes. *N*, The number of specimens; S.E., standard error; S.D., standard deviation.

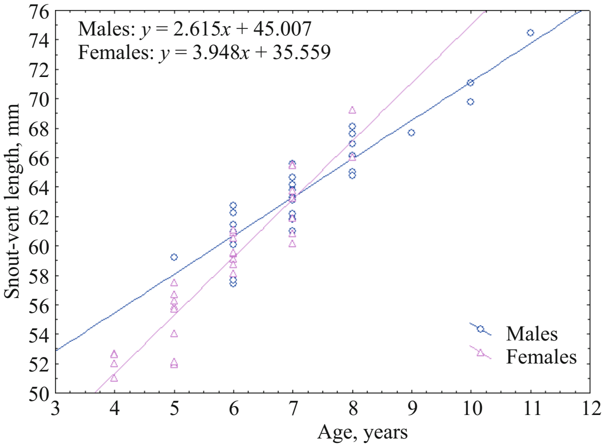

Fig. 4. Age – length distribution and regression equations of male and female *Mertensiella caucasica*.

Endosteal resorption was observed in 96.56% of adults in present study. Similar result was observed in previous study on *M. caucasica* population at an altitude of 1575 m above sea level by Üzüm (2009). It is suggested that resorption may be linked to environmental conditions (Smirina, 1972), e.g., less resorption for populations living in high altitudes than for lowland populations (Esteban et al., 1996, 1999) or the opposite (Caetano and Castanet, 1993; Üzüm, 2009).

Estimated age at maturity (5 years for males and 4 years for females) was similar with Kümbet population (Üzüm, 2009) (Fig. 3) while Reinhard et al. (2015) reported that minimum adult ages were found to be 6 and 7 years for males and females, respectively from Zigana Pass, Trabzon, Turkey. Tarkhnishvili and Gokhelashvili (1994) reported that after metamorphosis, at least 9 years passes (9 years for females and 13 years for males) before maturation for *M. caucasica*. Moreover, *Chioglossa lusitanica*, the sister taxon of *M. caucasica* from northern Portugal (Lima et al. 2000), has the same results with Kürtün and Kümbet populations. Kürtün, Kümbet and Zigana populations of *M. caucasica* are located closely in Turkey. The altitude of Kürtün is similar with Zigana's (2213 m a.s.l. for Kürtün, 2032 m a.s.l. for Zigana Pass), but age at maturity is the same as in Kümbet population (1575 m a.s.l.). So, it can be concluded that altitude differences do not effect age at maturity for *M. caucasica*, and the differences could be from their own ecological and biological characteristics of habitats as mentioned by Üzüm (2009).

The maximum age were recorded as 11 years for males and 8 years for females (Fig. 3). The recorded maximum age by Üzüm (2009) was 10 years for males and 9 years for females. These results were also found to be similar for its sister species, *C. lusitanica* where longevity was estimated at 10 years. Reinhard et al. (2015) estimated the maximum age as 18 years for males and 16 years for females while Tarkhnishvili and Gokhelashvili (1994) reported that adult *M. caucasica* could live as much as 26 years old. The differences in longevity could be based upon several factors such as variation of body size or different altitudes/latitudes of localities (Kyriakopoulou-Sklavounou et al., 2008). Consequently, according to these studies we cannot say that the altitude is a strongly contributor for life span of *M. caucasica*.

The mean ages were different in the sexes in our population indicating males were older than females like previously recorded by Üzüm (2009) and Reinhard et al. (2015). The individual numbers were higher in 6, 7, and 8 age groups for males and 5, 6, and 7 age groups for females (Table 2). Juveniles in our population were also found to have the same age distribution with the Kümbet population (1 – 3 years after metamorphosis) (Üzüm, 2009).

Males are larger than females in our population (see Table 1) as recorded in Kümbet (Üzüm, 2009). But, it is statistically significant only in Kürtün ($t = –4.944$, $df = 56$, $p < 0.001$). Difference in adult body size between the sexes is a result of growth difference between the sexes (Zhang and Lu, 2013). Ectothermic animal individuals may spend their energy in growth rather than reproduction early in their life, when productivity of mating success is strongly size-dependent (Kolarov et al., 2010). Mostly amphibian females are larger than their males (Üzüm, 2009; Han and Fu, 2013; Reinhard et al., 2015) and males which are larger than females can be explained by competing (Kupfer, 2007). According to Anderson (1994) and Zhang and Lu (2013), sexual selection favoring larger males to take a mating advantage and fecundity selection for larger females to increase reproductive capacity have been considered as the two major drivers of the evolution and maintain of SSD. Male-male combat regarding male-biased SSD (Shine, 1979) and female choice (Halliday and Varrel, 1986) are the evidence of sexual selection for larger males. Additionally, Reinhard et al. (2015) found the male-biased dimorphism in hind leg length and reported that highly significant arm length differences could be attributed to their courtship behavior. SDI was calculated as -0.099, indicating a male bias for our population. Similarly, SDI was reported as –0.06 for Kümbet population with male biased (Üzüm, 2009), and Reinhard et al. (2015) found as +0.0017 for Zigana population. There were no statistically significant differences in SVL measurement between sexes in both populations. In Ommatotriton ophryticus, males are also larger than females (Başkale et al., 2013) as in *M. caucasica*. On the contrary, females are larger than males in sister taxon *C. lusitanica* (Lima et al., 2000).

Adult ages are significantly correlated with size (Fig. 4) as seen in Üzüm (2009) ($r = 0.50$, $p < 0.05$). However, Reinhard et al. (2015) and Tarkhnishvili and Gokhelashvili (1994) found the opposite results.

With this study, we obtained new information about age, size and growth of *M. caucasica* from different habitat in Turkey. We also compared some life history characteristics of Kürtün population with the previous studies. These kinds of studies on the same and similar species allow us to understand environmental effects on populations' life history characteristics.

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