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Age Structure and Body Size of the Eastern Spadefoot Toad *Pelobates syriacus* Boettger, 1889 (Anura: Pelobatidae) from Afyonkarahisar, Turkey

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Abstract: This is the first study to report the average age of adulthood and sexual maturity in individuals of *Pelobates syriacus* Boettger, 1889 from Afyon, Turkey. The age of male and female individuals was found to vary between 3–6 years, wherein 6 years was the maximum age in both sexes. The mean age of males and females was 4.54 ±0.82 years and 4.37±0.92 years, respectively, and their age of sexual maturity ranged between two and three years. No significant difference was observed between sexes regarding age and snout-vent length (SVL). The survival rate (Sr) and life expectancy (ESP) were calculated to be 0.62 and 3.13 in males and 0.61 and 3.06 in females, respectively. The maximum SVL was 70.84 mm (ranging from 54.84 to 70.84 mm) in males and 69.76 mm (ranging from 57.00 to 69.76 mm) in females. Additionally, there was no significant difference in SVL between both sexes, but the mean value of SVL in males was larger than that of females. According to Spearman's Correlation Coefficient, no significant correlation was found between age and SVL in both males and females.

Key words: Age structure, body size, *Pelobates syriacus,* skeletochronology, Anatolia

Introduction

Life-history traits of amphibian species are comprised of complex patterns of growth, development, storage and reproduction. Understanding the longevity and the reproductive lifespan allows for comprehensive analysis of the life-history variation among amphibian populations (Sinsch 2015). Skeletochronology, one of the most commonly used techniques for determining an animal's lifespan, is performed by counting the lines of arrested growth (LAGs) in the bone tissue that forms during the period of an organism's hibernation (Nishikawa et al. 2017). This cost-effective and non-lethal technique has been validated for many amphibian (Kutrup et. al. 2011, Sun et al. 2016) and reptile (Bülbül et al. 2016, Eroğlu et al. 2017) species.

Body size generally shows congruency in pattern with the altitude and in ectotherms, it has an antagonistic relationship with temperature change. This is known as "Bergmann's rule" (Bergmann 1847), a commonly accepted rule applicable to some amphibian species (Morrison et al. 2004, Lai et al. 2005), while others do not show any correlation (Liao & Lu 2011; Ergül Kalayci et al. 2015). The reason for this inconsistency is still not clear.

The European spadefoot toads (with only one extant genus, *Pelobates* Wagler, 1830) include four species that are native to Europe, the Mediterranean, North-Western Africa and Western Asia. *Pelobates syriacus* Boettger, 1889 is the only species representing this genus in Turkey. The geographical range of this species includes North Macedonia, Bulgaria, Greece, Turkey, Israel, Syria and expands till the Transcaucasia. This species can be found from 0 up to 1935 m a.s.l. (Kuzmin 1999). Studies on *P. syriacus* in Turkey are limited. The literature survey suggests that various studies have been performed on *P.*

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syriacus: morphological differentiation (Uğurtaş et al. 2002), age structure (Cogălniceanu et al. 2014), ecological niches (Iosif et al. 2014), including the study of age structure in several populations (Eggert & Guyetant 1999, 2002, Rot-Nikčević et al. 2001, Andreone et al. 2004, Leclair et al. 2005, Guarino et al. 2011, Coğalniceanu et al. 2014). However, no skeletochronological studies have been carried out for clarifying the age structure of *P. syriacus* populations inhabiting Turkey. The life-history traits of populations of this species from Turkey are still unknown.

In this study, we aimed to identify the age of sexual maturity and the age structure of *P. syriacus* from Dereçine, the city of Afyonkarahisar, Turkey, using skeletochronology.

Materials and Methods

Permission for the collection of specimens was obtained from the local ethics committee (Recep Tayyip Erdoğan University 2016/51). Twenty-one specimens of *P. syriacus* (11 males, 8 females and 2 juvenile) were collected by N. Özdemir and S. Gül from Dereçine, the city of Afyonkarahisar (978 m a.s.l.) during the reproductive season and were analysed. Since this species is generally nocturnal, the fieldwork was conducted during the night. The animals were released back to their habitats after taking tissue sample, sex determination and measurement of snout-vent length (SVL).

Age was estimated using the skeletochronology method, following the procedure of Castanet & Smirina (1990). The longest toe of the forelimb was used and the skin and muscle tissues of each digit were removed. The remaining bones were decalcified in 5% nitric acid for approximately 2 h, after which they were washed with tap water. The cross-sections from the middle part of the diaphysis (with thickness of 17 μm) were obtained using freezing microtome (Shandon Cryostat, Germany) and were stained with Ehrlich haematoxylin for approximately 15 min. The number of LAGs was assessed by two independent authors (N. Özdemir and T. Ergül Kalaycı) and the images were captured with Olympus BX51. The endosteal resorption was evaluated by comparing the diameters of eroded and non-eroded marrow cavities between juveniles and adult specimens. Sexual maturity was marked when an obvious decrease in space between two subsequent LAGs was observed (Ryser 1988).

The annual adult survival rate (Sr) was calculated by the formula (Robson & Chapman 1961):

$Sr=T/(R+T-1)$,

where $T=N1+2N2+3N3+\ldots$, $R=\Sigma N_i$, and N_i = the number of individuals in the age group *i*.

The adult life expectancy (ESP) was calculated based on Seber's (1973) formula:

$ESP=0.5+1/(1-Sr)$

The snout-vent length (SVL) of the specimens was measured using digital calipers with 0.01 mm sensitivity. Sex was determined by identifying the adult males that have a glandular pad on the dorsal surface of the arm. The sexual dimorphism index (SDI) was inferred by applying the Lovich & Gibbons (1992) formula:

SDI = (mean length of the larger sex / mean length of the smaller sex) ±1,

We used +1 when males were larger than females and SDI was defined as negative or –1 when females were larger than males and SDI was defined as positive.

Normality was tested using the Shapiro-Wilk test. Normality was not rejected for SVL but was rejected for age ($P_{SVL}>0.05$, $P_{age}<0.05$). Statistical differences between the sexes were computed using the Independent Samples t-test (for SVL) and the Mann-Whitney U test (for age structure). Spearman's correlation was used to test the relationship between the variables. All statistical analyses were conducted with SPSS 21 (IBM SPSS Statistics for Windows).

The amount of annual precipitation and annual temperature in Afyonkarahisar were 33.52 kg/m^2 and 12.02°C, respectively (data were taken from the Turkish State Meteorological Service, 1950-2015).

Results

The lines of arrested growth were easily counted in most of the individuals (Fig. 1). Double LAGs (in 71% of the specimens) and endosteal resorption (in 62% of the specimens) were abundant but did not hamper age determination. Descriptive statistics of age and SVL are given in Table 1.

In both males and females, the age was found to vary between 3–6 years with the maximum age being 6 years (Fig. 2). The mean age was determined as 4.54 ±0.82 years in males and 4.37 ±0.92 years in females. We found no significant difference in the age of the two sexes (Mann Whitney U test, $P>0.05$, $U=38.00$).

In both males and females, the juvenile stage was between the first and second year, after which they attained sexual maturity at 2–3 years. Sr and ESP were calculated as 0.62 and 3.13 for males and 0.61 and 3.06 for females, respectively.

The maximum SVL was 70.84 mm (range 54.84 to 70.84 mm) in males and 69.76 mm (range 57.00 to 69.76 mm) in females. The SVL of juveniles ranged from 37.62 to 41.11 mm. There was no

significant difference between males and females in terms of SVL (P>0.05, t=0.64, df=17), but the mean SVL of males was larger than that of females (Table 1). SDI was determined as –0.024, which meant that there was a weak bias towards the males.

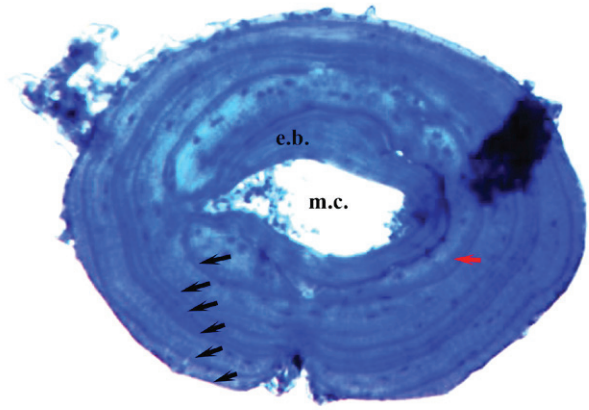

Fig. 1. Cross-sections of the phalange of *Pelobates syriacus* at 6 years. The black arrows show the number of LAGs and the red arrow shows the double line; e.b.,endosteal bone; mc, marrow cavity.

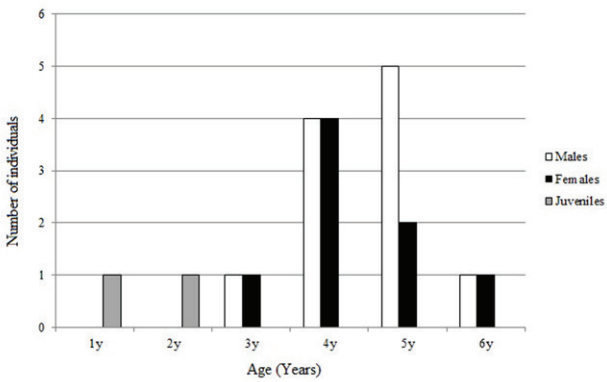

Fig. 2. Age frequency distributions of specimens of *P. syriacus*.

According to Spearman's Correlation Coefficient, no significant correlation was found between age and SVL in both males (r=0.30, P>0.05) and females (r=0.63, P>0.05).

Discussion

Although there have been some studies about the age structure of *Pelobates syriacus* from different environments, none of them have explored the age structure, survival rate and adult life expectancy of *Pelobates syriacus* inhabiting Turkey and to the best of our knowledge, this is the first study to document them.

The age structure and SVL's of species of the genus *Pelobates* have been reported in populations that experience various environmental conditions. Rot-Nikčević et al. (2001) found that the longevity of males and females in populations of *P. syriacus* from Serbia was 16 years and 6 years, respectively, and from Macedonia and Bulgaria it was 12 years and 15 years, respectively. Cogălniceanu et al. (2014) found for *P. syriacus* from Romania that the longevity of males was 10 years and of females 12 years. We found the longevity was 6 years for both sexes, which is the lowest among those observed for populations of *P. syriacus*. The life-history traits of animals across broad environmental ranges depend on their response capability and differential adaptation to local conditions, such as temperature, food supply, predation and competition. Our study area (Afyonkarahisar) is characterised by continental climate, which means that low temperature is common in winter. Also, while precipitation decreases the temperature increases during the year (precipitation increases in spring, but it decreases in summer within the year) (Bahadir 2012). This area is the

Table 1. Descriptive statistics for the age and SVL in specimens of *Pelobates syriacus*.

Sex	Number of individuals (years)	Mean SVL±S.D. (mm)	Range SVL (mm)	Mean age±S.D. (years)
Males	11 (3-6)	64.94±4.84	54.84-70.84	4.54±0.82
	1 (3)	54.84	54.84	-
	4 (4)	64.85±5.03	58.15-70.22	-
	5 (5)	67.42±2.35	64.84-70.84	-
	1 (6)	63.01	63.01	-
Female	8 (3-6)	63.44±5.29	57.00-69.76	4.37±0.92
	1 (3)	63.92	63.92	-
	4 (4)	59.75±4.48	57.00-66.45	-
	2 (5)	67.48±3.22	65.21-69.76	-
	1 (6)	69.58	69.58	-
Juvenile	2 (1-2)	39.36±2.47	37.62-41.11	1.5±0.71

most dried zone as compared to the other habitats of *Pelobates syriacus*. Features like overwinter survival, prey availability, etc. of species that specified by burrow and forage in terrestrial systems are affected by increased drought stress. Aridity might cause reduced soil moisture and associated plant and animal mortality (Lovich et al. 2017, Venturas et al. 2016).

In the genus *Pelobates*, the age of sexual maturity generally ranges between 2 and 3 years and varies between different geographical regions (Cogălniceanu et al. 2014). In our study, we found that the age of sexual maturity was similar to that of the other species of *Pelobates*. Individuals reached sexual maturity by 2–3 years in *P. syriacus*. The time required, from birth, to reach sexual maturity is an important life-history trait because it can influence the reproductive output by affecting the generation time (Farasat & Sharif 2015).

In amphibians, the annual survival rate may reflect their adaptations to different habitats. One study reported the adult survival rate and ESP for *Rana temporaria* as 0.82 and 6.1 years in males and 0.81 and 5.5 years in females, respectively (Miaud et al. 1999). In the present study, we calculated *Sr* and ESP as 0.62 and 3.13 in males and 0.61 and 3.06 in females, respectively.

Extreme climatic conditions (like dryness and hot periods) can result in double LAGs in amphibians and reptiles (Guarino & Erismis 2008). According to Rivas-Martinez et al. (2003), bioclimatic regions near Afyonkatahisar are classified as Mediterranean pluviseasonal-continental. Accordingly, we observed double lines in 71% of the individuals collected from this region.

Generally, it is believed that the individuals from north latitudes are older and larger than the ones from the south (Andreone et al. 2012). We have found that the maximum SVL of *P. syriacus* from Afyonkarahisar, Turkey, is 70.84 mm (54.84–70.84 mm range) in males and 69.76 mm (57.00 to 69.76 mm range) in females. Rot-Nikčević et al. (2001) found mean SVL of *P. syriacus,* from Macedonia and Bulgaria, to be 75.81 mm in males and 70.51 mm in females and Cogălniceanu et al. (2014) determined that in individuals from Romania the mean SVL of males was 76.5 mm and that of females was 74.4 mm.

Researchers have reported previously that in *P. syriacus,* either males or females are of similar size or the males are larger than the females (Rot-Nikčević et al. 2001, Uğurtaş et al. 2002). According to our data, although there were no significant differences between males and females from Dereçine, Afyon (978 m a.s.l.), mean SVL was larger in males (64.97 ±4.84 mm) than in females (63.43 ±5.29 mm). Cogălniceanu et al. (2014) did not find any differences in SVL and in age between both the sexes (Kruskal-Wallis, $P = 0.27$ and $P = 0.64$, respectively) of *P. syriacus* individuals from Romania. In this study, we determined SDI as 0.024, which meant that there was a weak bias towards males. Uğurtaş et al. (2002) determined SDI as 0.88 (Izmir) and 1.06 (Ivanovo) in the eastern spadefoot toads, suggesting a strong male bias.

According to our study, there was no significant correlation between age and SVL in either males or females. Rot-Nikčević et al. (2001) reported that in both sexes of *P. syriacus* and *P. fuscus* none of the morphometric characters showed a significant correlation with age. Body length is not always a good criterion for determining age. Nevertheless, in amphibians, age and body size are generally positively correlated but this relationship is usually quite weak. Additionally, there is a considerable variation in body size within a given age class (Halliday & Verrell 1988).

Our study highlights the importance of studying life-history traits of a species. More conclusive answers for *P. syriacus* can be obtained if more populations from different environmental conditions are studied. This study provides basic information about the population structure of *P. syriacus* from Afyonkarahisar, Turkey.

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