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AGE STRUCTURE AND BODY SIZE IN A TURKISH POPULATION OF THE GREEN LIZARD, *Lacerta viridis* (LAURENTI, 1768)

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The present study was designed to obtain information about life history traits of *Lacerta viridis* in the Belen population with an altitude of 292 m. In the adult sample (21 males and 15 females), the age ranged from 3 – 10 ($\bar{X}$ = = 6.97) years for both sexes collectively, 5 – 10 ($\bar{X}$ = 7.43) years in males, 3 – 8 ($\bar{X}$ = 6.33) years in females and not differed significantly between sexes. Age at sexual maturity was 2 – 3 years for both sexes. However, a confirmation is required with some sexual characters (e.g., the presence of eggs for female, developed hemipenes for male) for age at sexual maturity in both sexes. The mean body size (SVL) was not significantly different between sexes. There was no correlation between SVL and age for males while there was a positive correlation for females. A male-biased sexual size dimorphism (SSD = 0.07) was observed in the population of *L. viridis*.

Keywords: longevity; SVL; age at maturity; SSD; Belen.

INTRODUCTION

The Green Lizard is distributed in northeast Italy, eastern Germany, the Czech Republic, Slovakia, Hungary, eastern Austria, Slovenia, Romania, Moldova, southern Ukraine, the Balkan Peninsula in Croatia (including some Adriatic islands), Bosnia-Herzegovina, Serbia, Montenegro, Macedonia, Albania, and Greece (Sindaco and Jeremenko, 2008). In Turkey, the species is largely distributed in the area of Marmara region and along the Black Sea coastal region. It is classified as LC (Least Concern) in the IUCN Red List and the populations at the north-west edge of the species's range may be declining in that area (Isailovic et al., 2009). To understand population dynamics and life histories of the lizards, researches have been carried out (Guarino et al., 2010; Cabezas-Cartes et al., 2015; Kanat and Tok, 2015; Gül et al., 2017; Üzüm et al., 2018; Vergilov et al., 2018). Skeletochronology, in which the number of lines of arrested growth (LAGs) formed in bone is counted, is an effective method for age determination of wild animals (Kurita and Toda, 2013). It can be carried out with a sample of the endangered individuals from finger bones without detaching them from their habitat (Castanet and Smirina, 1990; Guarino et al., 2010).

The current knowledge of the life-history traits of *L. viridis* is based on the investigation of a population in France (Saint Girons et al., 1989). The present skeletochronological study was aimed to obtain comparative information from Turkish individuals of the Belen population of the species.

MATERIAL AND METHODS

A total of 36 specimens (21 males and 15 females) was caught from a population in Belen, Province of Zonguldak during the breeding season (capture permission No. 72784983-488.04-70541 issued by the Turkish Ministry of Agriculture and Forestry) The Belen population (41°08′23″ N 31°36′28″ E) is located at an altitude of 292 m a.s.l. The lizards were caught in a nut grove on May 12 – 14 of 2017 and sexed by examination of the secondary sexual characters (presence of light blue color at the ventral side of the head in adult males) and by checking for the presence or absence of hemipenis pockets. At the collecting site, the active period for lizards

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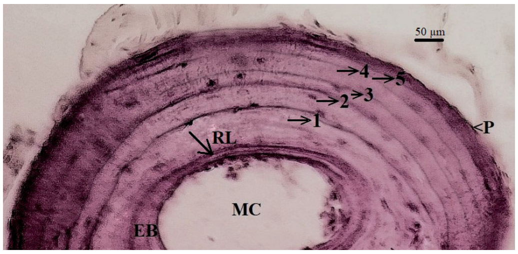

Fig. 1. Cross section (12 µm thick) of a toe bone of a five-year-old female (78.0 mm SVL) of *Lacerta viridis* from the Belen population in Turkey. The age was derived from the presence of five lines of arrested growth (marked with six > symbols in the upper portion of the picture) surrounding the resorption line (RL): MC, marrow cavity; EB, endosteal bone; RL, resorption line; P, periphery.

lasts from early April to lately October. The mean annual temperature and precipitation over the past 90 years in Belen were 14.7°C and 101.51 mm, respectively, according to data of 9th Meteorology Regional Directorate, Ankara. During the activity period of the lizards, the mean temperature and precipitation were 17.6°C and 84.56 mm, respectively in the study area.

Snout-vent length (SVL) was measured to the nearest 0.1 mm using a digital caliper. We quantified Sexual Size Dimorphism (SSD) with the Lovich & Gibbons (1992) index (SDI) according to the following formula: SDI = = (mean SVL of the larger sex/mean SVL of the smaller sex) ±1. The value +1 is used if males are larger than females and –1 if the opposite is true. The result is arbitrarily defined as positive if the females are larger and negative if the males are larger (Lovich and Gibbons, 1992).

From each lizard, the longest (4th) toe was clipped at the second phalange and preserved in 10% formalin solution for subsequent histological analyses. After toe-clipping, the lizards were released back into their original habitats. The specimens were treated in accordance with the guidelines of the local ethics committee of the Karadeniz Technical University (KTÜ.53488718-9/2016/60).

After ablation of the skin, the toes were put in 5% nitric acid solution for 2.5 h to decalcify the bone tissue. After the toe samples had passed a tissue processing system (Leica® Tissue processor), they were embedded in paraffin with a tissue embedding device (Thermo®). Deparaffinized cross-sections (10 µm, rotary microtome) of the phalanges were stained with hematoxylin using the procedure described by Bülbül et al. (2016), then mounted on microscope slides, closed using entellan® and observed under a light microscope.

Age was estimated using skeletochronological methods (Castanet and Smirina, 1990; Castanet, 1994). To minimize the subjective error margin, two of the authors (U. Bülbül and H. Koç) independently counted the LAGs on the cross-sections and their results were compared and harmonized. The observed double lines were counted as single lines as described by Guarino and Erişmiş (2008) and Eroğlu et al. (2018). The distance between two adjoining LAGs is a marker of individual growth in a given year (Kleinenberg and Smirina, 1969; Özdemir et al., 2012). Any apparent decrease in space between two subsequent LAGs was taken as a determiner for age of maturation (Ryser, 1998; Odabaş et al., 2019). We evaluated the occurrence of endosteal resorption by direct observation of the cross-sections under the microscope. Any serious problems depend on age estimation was not caused by the endosteal resorption. Uncountable cross-section samples were not incorporated into our study.

Since age classes and SVL measurements were normally distributed (One-Sample Kolmogorov – Smirnov Test, $P > 0.05$) within the sample, the parametric independent *t*-test was used for comparison of means and Spearman's Rank Correlation Test ($P < 0.01$). All statistic tests were processed with SPSS v. 21.0 for Windows.

RESULTS

A growth zone and thin hematoxylinophilic outer line corresponding to a winter line of arrested growth were present in cross sections of the phalanges in 100% ($n = 36$) of adult individuals (Fig. 1). In all cases, the first (innermost) LAG was not completely decomposed by endosteal resorption or the resorption zone did not even reach the first LAG. The resorption zone clearly seemed out of the endosteal bone in all cross-sections of *L. viridis* and never created difficulty for age determination. Endosteal resorption was observed in 6 (28.57%) males and 3 (20.0%) females. The rate of endosteal resorption was 25% for the Belen population. We observed double line in 9 (42.85%) males and 2 (13.33%) females. In total, 11 individuals (30.55%) of the Belen population had double lines in their cross-sections. The oldest females and males were 8 and 10 years old, respectively (Fig. 2). The age upon arrival at maturity was 2 for 10 males (47.61%) and 8 females (53.33%) while it was 3 for 11 males (52.38%) and 7 females (46.66%), respectively.

The means of SVL and age were, 88.5 ± 8.7 mm and 7.97 ± 1.63 years for all individuals of *L. viridis* (91.1 ± 8.4 mm and 7.43 ± 1.56 years in males and 84.9 ± 7.9 mm and 6.33 ± 1.54 years in females), respectively (Table 1).

Age ranged from 5 – 10 years in males and 3 – 8 years in females. The mean age of the specimens was not significantly different between the sexes (independent sample *t*-test; $t = 2.080$, $df = 34$, $P = 0.703$). Intersexual differences in body size (SVL) were slightly male-biased (SSD = –0.07). The mean SVL ($t = 2.229$, $df = 34$, $P = 0.758$) was not significantly different between sexes. There was no correlation between SVL and age for males (Spearman′s correlation coefficient ($r = 0.310$, $P = 0.172$) while there was a positive correlation for females ($r = 0.711$, $P = 0.003$).

DISCUSSION

The mean age, longevity and age at maturity can be influenced by genetic differentiation (among different species) or environmental factors (among different populations of the same species (Dubey et al., 2013; Gül et al., 2014; Bülbül et al., 2016; Sinsch and Dehling, 2017).

In the present study, we estimated life-history traits of *L. viridis* in a Turkish population. The current knowledge on the life-history traits of the species is based on the investigations of Saint Girons et al. (1989) and Bauwens and Diaz-Uriarte (1997) who performed their studies in France. Bauwens and Diaz-Uriarte (1997) studied only female individuals and reported adult lifespan as 38 months. A detailed study was performed by Saint Girons et al. (1989) and they studied two different populations (the southern population, Arcay and the northern population, Canal) from France and found the maximum longevity 57 months (almost 5 years) in the Arcay population and 5 – 8 years in the Canal population. In the Arcay population, the lizards live in a sandy environment and in a relatively warmer climate than in the Canal population. As they stated, the length of the activity season determines the life history traits and populations living in colder environments would exhibit higher mean age and longevity irrespective of altitude. We found higher longevity (8 years for females and 10 years for males) in Belen population. In accordance with this, the length of the activity season in Belen was slightly higher than Arcay and Canal populations. Moreover, the precipitation was significantly higher in Belen. There were not significant mean temperature differences among Belen, Arcay, and Canal sites. Higher longevity (11 years for males and 12 years for females) was also reported by Olsson and Shine (1996) in a population of another lacertid lizard species,

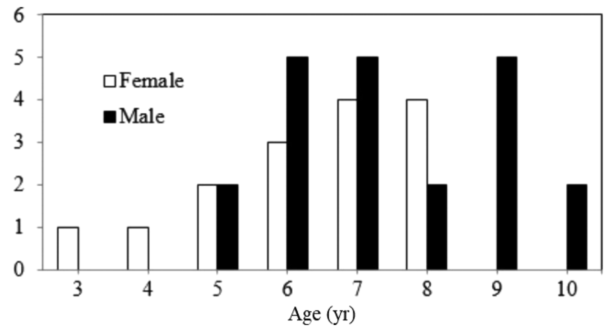

Fig. 2. Frequency distribution of the age in 21 male (black) and 15 female (white) *Lacerta viridis* from the Belen population (Turkey): ordinate, number of individuals.

Lacerta agilis (L., 1758) from Sweden. As a general rule, individuals from northern latitudes and high altitudes are longer than individuals who live in southern latitudes and low altitudes (Wapstra et al., 2001; Sears and Angilletta, 2004; Roitberg and Smirina, 2006; Guarino et al., 2010;).

The mean age was determined as 7.43 years in males and 6.33 years in females for *L. viridis* in the Belen population, while Saint Girons et al. (1989) found mostly 2 – 3 years old individuals of the species from France. The higher mean age of Belen population may also be associated with length of activity period and the higher precipitation in the active season of the lizards in the area. Other unknown effects (e.g., food availability or lack of predators) may be causes of the higher mean age of the studied population. In other lacertid species, the mean age was found low. Guarino et al. (2010) reported the mean age as 2.4 years in males and 2.5 years in females for *Lacerta agilis* while Roitberg and Smirina (2006) stated that the individuals of *L. strigata* (Eichwalt, 1831) were mostly 2 – 3 years old. These results show the effect of genetic differences on the mean age of lizards.

In our study, there was no significant difference in mean age for both sexes. Similar to this result, Guarino et al. (2010) reported that there was no significant difference in mean age for both sexes of *L. agilis*.

According to the results of the present study, the age when maturity was achieved was 2 – 3 years in both males and females of the Belen population. Although we determined the age of sexual maturity at 2 – 3 years in

TABLE 1. Descriptive Statistics of Snout-Vent-Length (SVL, mm) and Age (years) of the Belen Population of *Lacerta viridis*

Character	SVL	Age	SVL	Age
Sample size	21	21	15	15
Mean	91.1	7.43	84.9	6.33
Range	79.2 – 114.9	5 – 10	70.6 – 97.6	3 – 8
Standard error	1.83497	0.342	2.04346	0.398

both females and males by checking the decrease in space between two subsequent LAGs in the cross-sections, this decrease may be result of ecological factors, for example, estivation. Sexual maturity has to be confirmed in 2 – 3 years old individuals by the reproductive tract size and the state of the ovarian follicles and the presence of eggs in the females′ oviducts (Andrews, 1998; Akiki et al., 2015). In males sexual maturity may be confirmed by developed hemipenes, enlarged testes and, if possible, with highly convoluted epididymis which are typically associated with sperm production (Lozano, 2013). However, we did not catch any adult lizard under 3 years of age for female and 5 years of age for male. Therefore, we could not make a comparison to show whether the age of sexual maturity which we determined according to the cross sections is correct. Similar to our findings, Saint Girons et al. (1989) reported the age at maturity as 21 months in Arcay population and 33 months in Canal population of *L. viridis*. However, Bauwens and Diaz-Uriarte (1997) reported age at maturity as 1 year for females of *L. viridis* in a French population (Lorie-Atlantique, la Vendée). They did not study the age of the males. Our results are also close to an age of maturity which was previously reported as 22 – 23 months for *L. agilis* and *L. strigata* (Muskhelishvili, 1970; Khonyakina, 1970, 1972; Darevskij, 1984; Tertyshnikov, 2002), except the results of Shammakov (1981) showing that individuals of *L. strigata* from southwestern Turkmenistan reached maturity at 1 year. These results indicate that the age of sexual maturity in the lacertid lizards from different geographical regions may vary between 1 – 3 years old.

In Belen population of *L. viridis*, the mean SVL was found 91.1 mm in males (range; 79.2 – 114.9 mm) and 84.9 mm in females (range; 70.6 – 97.6 mm). When compared to our findings, Saint Girons et al. (1989) found lower SVL in males (78.6 – 100.0 mm) but higher SVL 85.1 – 114.3 mm) in females. Similarly, Bauwens and Diaz-Uriarte (1997) reported higher mean SVL (98.5 mm) in females of the species in the French population. Among reptiles the body length can widely vary among populations of the same species (Guarino et al., 2010). In ectotherms, the effects of climate and primary productivity on body size can be linked with thermoregulatory strategies, developmental dynamics, activity duration, and/or food availability. Several of these mechanisms are not mutually exclusive and might be acting jointly (Aragón and Fitze, 2014).

There was a positive correlation between SVL and age for females of the Belen population. The positive correlation between age and female body size may positively affect egg quality and clutch frequency. If this happens, the persistence of the population in Belen may be increased. A similar expectation was reported by Kurita and Toda (2013) for the Ryukyu Ground Gecko, *Goniurosaurus kuroiwae* (Namiye, 1912). However, no correlation was found in males. Conversely, Guarino et al. (2010) found a statistically significant relationship between age and SVL in both sexes of *L. agilis*. This situation can be explained by the interpretation in the study of Bauwens (1999) indicating lizards exhibit an uncertain growth in body size increasing with age.

Sexual size dimorphism (SSD) is an important characteristic distinguishing feature in the species of the Lacertid family (Arribas, 1996). SSD may evolve as a result of competition between the sexes for a limited resource, such as food (Best and Gennaro, 1984; Üzüm et al., 2014). On the other part, Vitt and Cooper (1995) and Hews (1990) stated that the primary cause of SSD is male-male competition to obtain possible mates. The Lacertid lizards generally have a male-biased sexual dimorphism (Kaliontzopoulou, et al., 2007). Similar to this expectation, male-biased SSD was found in the Belen population of *L. viridis*. However, the mean SVL was not significantly different between sexes of the Belen population.

The endosteal resorption rate may vary among individuals (Yakýn and Tok, 2014) and it is rather low in the members of *Lacerta* (Arakelyan and Danielyan, 2000). Moreover, endosteal resorption may be affected by environmental conditions and elevation may cause a change in the endosteal resorption (Smirina, 1972). Correspondingly, Caetano and Castanet (1993) observed less resorption in low-altitude populations. Similar to these findings, we found a low endosteal resorption rate (25%) for the Belen population of *L. viridis* which inhabits a lower elevation site.

The formation of double lines might be either due to a secondary period of arrested growth, like estivation, or a result of the occurrence of sudden climatic changes (Castanet et al., 1993; Castanet, 1994; Yakın and Tok, 2014) and unpredictable ecological factors (e.g., changes in food availability and occasional environmental changes) may be caused unexpected changes on double lines (Jakob et al., 2002; Guarino and Erişmiş, 2008). The Belen population is moderate in climate and rich in food resources for the lizards. So, we found a low rate (30.55%) of double line formation.

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