

Age Structure and Growth in a Turkish Population of the Italian Wall Lizard *Podarcis siculus* (Rafinesque-Schmaltz, 1810) (Reptilia: Lacertidae)

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Abstract: The life-history traits of *Podarcis siculus* were studied by skeletochronology in a population inhabiting an altitude of 28 m a.s.l. in Filyos, Turkey. The mean age was 6.8 years while the minimum and maximum age ranged from 4-12 years in males and 4-10 years in females, respectively. The mean age of the specimens was not significantly different between sexes. The age at sexual maturity was 2-3 years for both sexes. There was a significant positive correlation between snout-vent length (SVL) and age in males, while there was no correlation in females. A strong male-biased sexual size dimorphism (SSD) was observed in the population. For both sexes, the estimated asymptotic SVL was slightly lower than the maximum SVL. The recorded growth coefficient (k) was slightly lower in males than in females ($k \pm CI$, males: 0.76 ± 0.22 ; females: 0.94 ± 0.22). There was no growth rate difference between both sexes ($P = 0.594$).

Key words: Skeletochronology, LAG, SVL, von Bertalanffy, age at maturity

Introduction

The Italian wall lizard *Podarcis siculus* (Rafinesque-Schmaltz, 1810) is distributed in Italy, on the large islands of Sicily, Sardinia and Corsica, and along the northern part of the east Adriatic coast, as well as on many Adriatic islands. The species was also introduced into the Mediterranean region (Portugal, Spain, France, Montenegro, Turkey, Libya and Tunisia) and the USA (BEHLER & KING 1979, CONANT & COLLINS 1991). It has been classified as LC (Least Concern) in the IUCN Red List of Threatened Animals since 2009.

Age determination has been used by researchers to gain information based on life-history traits of reptilian species (CAETANO & CASTANET 1993, CASTANET 1994, ESTEBAN *et al.* 2004, ROITBERG & SMIRINA 2006, GUARINO *et al.* 2010). Skeletochronology is a very useful method in calculating age of lizards. It is based on counting of the lines of arrested growth (LAGs) in the phalangeal bones of the lizard species

in the hibernation periods (CASTILLA & CASTANET 1986, GIRONS *et al.* 1989, CASTANET 1994, NAYAK *et al.* 2008). Life-history traits, including age at maturity and longevity are associated with local environmental conditions, especially variation in the length of the active season for ectothermic animals at different altitudes and latitudes (PATRELLE *et al.* 2012). Recently, the age determination of reptiles in Turkey using skeletochronology has been carried out by many researchers (ARAKELYAN *et al.* 2013, ALTUNIŞIK *et al.* 2013, TOK *et al.* 2013, GÜL *et al.* 2014, 2015, ÜZÜM *et al.* 2014, 2015, YAKIN & TOK 2015, KANAT & TOK 2015).

Podarcis siculus inhabits a large area in Europe and Turkey. Only one study was performed on age and growth of the Italian wall lizard populations using skeletochronology from Italy (RAIA *et al.* 2010). However, there are no detailed studies based on life-history traits of the species from Turkey. The main

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aim of the present study was to analyse the age structure and some growth parameters of *P. siculus* inhabiting Turkey using skeletochronology.

Material and Methods

A total of 40 specimens (19 ♂♂, 21 ♀♀) were caught from a population in Filyos (Çaycuma-Zonguldak) during the breeding season (number of permission to capture: 72784983-488.04-94286). The Filyos population (41°33'89" N, 32°01'99" E) is located in a lowland area at an altitude of 28 m a.s.l. The lizards were captured by hand on the walls of buildings in the studied area and sexed by direct examination of the secondary sexual characters.

In Filyos, the active period for lizards is from early April to late October. The average air temperatures in daytime were 28°C during the sampling period (29-31 June 2015).

Snout-vent length (SVL) was measured to the nearest 0.01 mm using a digital calliper. We estimated sexual size dimorphism (SSD) according to the index as described in the study by LOVICH & GIBBONS (1992). For each lizard, the second phalange of the longest finger of the hind limb was clipped and preserved in 10% formalin solution for subsequent histological analyses. After recording of SVLs and toe-clipping, the lizards were released back into their natural habitats. The animals were treated in accordance with the guidelines of the local ethics committee (KTÜ.53488718-651/2014/56).

The clipped toes were treated for 2.5 hours with a 5% nitric acid solution for decalcification of bone tissue. Afterwards, the toes were loaded in a Leica tissue processing system for 16 hours with 80 minute periods (changing time of solution) using solutions of alcohol (eight times), xylene (two times) and paraffin (two times). Then, all tissue samples were embedded in paraffin with a tissue embedding device (Thermo brand). The cross-sections (15 µm) were obtained from embedded phalanges with a rotary microtome and they were stained using haematoxylin procedure. The stained cross-sections were closed using MERK entellan. Finally, the cross-sections were observed under a light microscope.

Age was estimated using skeletochronology analysis (CASTANET & SMIRINA 1990, SMIRINA 1994) based on the calculation of the lines of arrested growth (LAGs) in transverse sections of the middle part of phalangeal diaphysis using a portion of the second phalanx of the third toe (GÜL *et al.* 2014). However, the phalangeal LAGs could not be used to estimate the real age of individuals. Generally they underestimate the age by 1-2 years, especially in old-

er individuals. The LAGs in older individuals can be obscured by the replacement of the periosteal bone with the endosteal bone (HEMELAAR 1985, SAGOR *et al.* 1998). In phalanges in which the first LAG has been completely destroyed by endosteal resorption, the innermost visible LAG was actually deposited during the second, rather than the first (SAGOR *et al.* 1998). The number of LAGs was independently calculated by three observers (A. İ. EROĞLU, M. KURNAZ, U. BÜLBÜL) and the results were compared. The double lines in the cross-sections were not incorporated into age estimation. The age at sexual maturity was assumed where any obvious decrease in space between two subsequent LAGs was observed (RYSER 1988, YILMAZ *et al.* 2005, ÖZDEMİR *et al.* 2012).

As age classes and SVL measurements were normally distributed (One-Sample Kolmogorov-Smirnov Test, $P > 0.05$), we used a parametric test for comparison of means (Independent Sample T-Test) and for correlation (Spearman's Correlations Test, $P < 0.01$). All statistic tests were processed with SPSS 21.0 for Windows and the level of significance chosen was $P < 0.05$.

We used the von Bertalanffy's model to form the growth curves as described in the literature (JAMES 1991, WAPSTRA *et al.* 2001, ROITBERG & SMIRINA 2006, GUARINO *et al.* 2010). We followed the general form of the von Bertalanffy equation, $L_t = L_\infty (1 - e^{k(t-t_0)})$, where L_t is length at age t , L_∞ is a parameter indicating asymptotic maximum length, e is the base of the natural logarithm, k is a growth coefficient and t_0 is the age at hatching, which is the beginning point of the growth interval in the present study. Because data on the size of the studied population at hatching were lacking, we assumed size at hatching ($L_{t_0} = 25.1$ mm) as the mean value provided by In den BOSCH & BOUT (1998). The parameters L_∞ (asymptotic SVL) and k , and their asymptotic confidence intervals (CI), were estimated using a non-linear regression procedure using SPSS 21.0 software program. Then, the growth rates were calculated as $R = k (L_\infty - L_t)$. Growth curves were considered to be significantly different if the 95% CI did not overlap (JAMES 1991, WAPSTRA *et al.* 2001).

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% ($n = 40$) of adult individuals (Fig. 1). It was observed that the resorption zone did not reach the first LAG. The resorption zone clearly seemed out

of endosteal bone in all cross-sections for *P. siculus*, and never created difficulty for age determination. We observed double lines in 24 (60%) specimens. The oldest females and males were 10 and 12 years old, respectively (Fig. 2). The age at maturity was 2-3 years for both sexes, with 2 years (80%) in 32 specimens and 3 years in 8 (20%) specimens.

The means of SVL, age and growth rate values were 65.48 ± 0.86 mm, 6.80 ± 0.28 , 1.18 ± 0.48 for all individuals of *P. siculus* (68.48 ± 1.38 mm; 7.16 ± 0.49 ; 0.59 ± 0.31 in male specimens and 62.75 ± 0.65 mm; 6.48 ± 0.31 ; 0.37 ± 0.21 in female specimens), respectively (Table 1).

Age ranged from 4-12 years in males and 4-10 years in females. The mean age of the specimens was not significantly different between sexes (Independent Sample T-Test; $t = 1.213$, $df = 38$, $P = 0.233$). Intersexual differences in body size (length) were male-biased (SDI = -0.091). The mean SVL ($t = 3.749$, $df = 25.788$, $P = 0.001$) was significantly different between sexes. There was a significant positive correlation between SVL and age for males (Spearman's correlation coefficient $r = 0.746$, $P < 0.01$), while there was no correlation between SVL and age for females ($r = 0.365$, $P = 0.104$). The growth pattern estimated by von Bertalanffy's equation showed a good fit to the relation between age and SVL (Fig. 3). For both sexes, the estimated asymptotic SVL was slightly lower than the maximum SVL recorded ($SVL_{asym} + CI$, males: 69.89 ± 14.82 mm; females: 62.97 ± 13.91 mm). The growth coefficient was slightly lower in males than in females ($k + CI$, males: 0.76 ± 0.22 ; females: 0.94 ± 0.22). There was no growth rate difference between sexes (Independent Samples T-Test; $t = 0.547$, $df = 12$, $P = 0.594$).

Discussion

The present study provides data on the age structure and growth patterns of the Italian wall lizard from a Turkish population. Environmental factors (climate, altitude, latitude, predation and other conditions) may affect age structure of lizard species (ROITBERG & SMIRINA 2006). In the present study, the mean age of *P. siculus* was 6.8 years in Filyos, while it was 2.21 years for an island population and 1.52 years for a mainland population of the same species in the study of RAIÁ *et al.* (2010). The authors suggested that the older age and the higher mean SVL probably resulted from lower predation in the island population. The higher mean age for Filyos population in our study may be related to a lack of natural enemies in the habitat of the lizards or other unknown effects

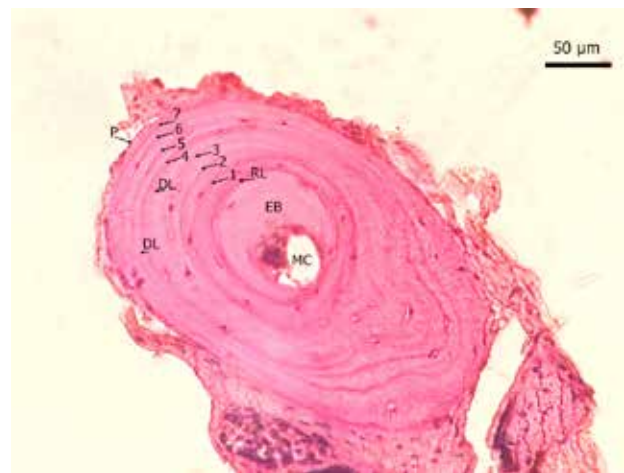


Fig. 1. A cross section (15 μ m thick) through phalange of a seven-year-old male (74.30 mm SVL) *Podarcis siculus* from Filyos population. For abbreviations, see text (MC: Marrow Cavity; EB: Endosteal bone; RL: Resorption Line; DL: Double Line; P: Bone outer margin)

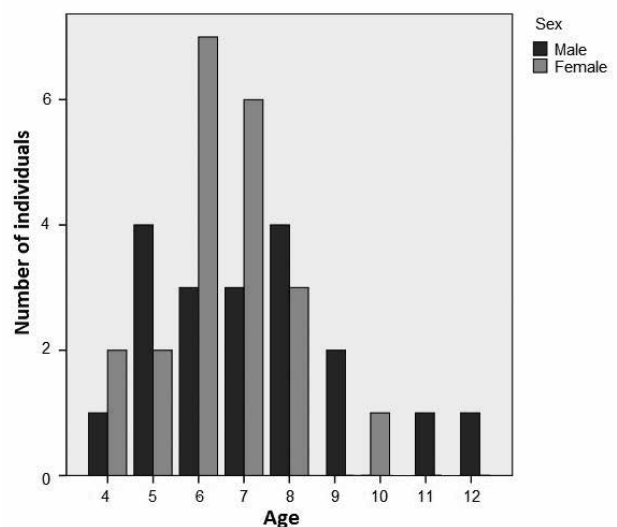


Fig. 2. Age distribution for both males and females of *Podarcis siculus* from the Filyos population

on the population. On the other hand, *P. siculus* is able to respond rapidly to changes in its environment and this may increase the survival of populations of the species (VERVUST *et al.* 2007).

The growth patterns of *P. siculus* indicated that there was an asymptotic growth up to sexual maturity; then growth continues at a decreased rate. Similar findings were found for the Balkan Mosor rock lizard, *Dinarolacerta mosorensis*, by KOLAROV *et al.* (2010). Most species of Lacertidae have a male-biased sexual dimorphism (KALIONTZOPOULOU *et al.* 2007). The exploitative competition causing the shifts observed in sexual dimorphism patterns in the two *Podarcis* species (*P. bocagei* and *P. carbonelli*)

Table 1. Descriptive statistics of age, SVL and growth rate of the Filyos population. For abbreviations, see text (n: number of samples; Range: maximum and minimum values; SE: standard error)

		♂♂	
Characters	SVL	Age	Growth Rate
n	19	19	8
Mean	68.48	7.16	0.59
Range	57.24-77.15	4-12	0.01-2.53
SE	1.38	0.49	0.31
		♀♀	
Characters	SVL	Age	Growth Rate
n	21	21	6
Mean	62.75	6.48	0.37
Range	57.15-67.54	4-10	0.00-1.36
SE	0.65	0.31	0.21

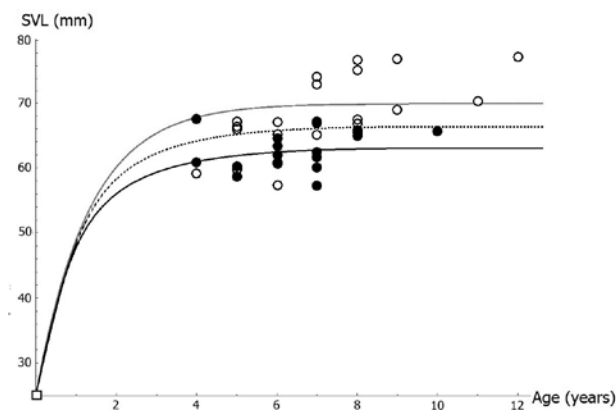


Fig. 3. The von Bertalanffy growth curves for males (open circle, grey line), females (solid circle, solid line) and all specimens (dot line) of *Podarcis siculus*. Open square shows SVL mean of the lizards at hatching (25.1 mm) as reported by IN DEN BOSCH & BOUTH (1998). Growth parameters are given in the text

was confirmed by KALIONTZOPOULOU *et al.* (2007). Their findings showed that the males of both species were larger than the females. Similarly to their data, our results showed that the mean SVL of the males of *P. siculus* was longer than the one for females. In addition, RAIA *et al.* (2010) also reported that males of *P. siculus* had longer SVL than females.

In the present study, there was a significant positive correlation between SVL and age for males, while there was no correlation for females. An opposite trend showing correlation between SVL and age for females in two lowland populations of the spiny tailed lizard, *Darevskia rudis*, was reported in the study of GÜL *et al.* (2014). On the other hand, ÜZÜM *et al.* (2014) found a significant positive correlation between age and SVL for both sexes. As indicated in

the studies of BEEBE & GRIFFITHS (2000) and OLSSON & MADSEN (2001), male lizards mature earlier than females in some species. Many factors, including age at maturity and longevity affect the adult body size (ÖZDEMİR *et al.* 2012). We found that age at maturity (2-3 years) was similar for both sexes, but longevity of the males (12 years) was higher than the one for females (10 years). The sexual difference in survivorship for Filyos population may be a reason of the male-biased SSD in SVL. The longevity is dependent on the active period, altitude, latitude and other climatic and environmental factors (BÜLBÜL *et al.* 2016). On the other hand, TARKHNISHVILI & GOKHELASHVILI (1996) suggested that longevity might be related to the type of the locality rather than climate. RAIA *et al.* (2010) found different longevity (4 years) for the specimens of *P. siculus* living in Italy. The Filyos site has suitable conditions (e.g. moderate climate, normal length of the active period and lack of intensive human activities with detrimental effects on the lizard) for lizards to attain higher longevity. High longevity results (9 years for males and 7 years for females) were also found in the study of ÜZÜM *et al.* (2014) in a lowland population (which has similar altitude of Filyos population) of the Bosc's fringe-toed lizard.

The level of SSD may vary between the populations and might be affected by climatic conditions. GÜL *et al.* (2014) reported a low level of male-biased SSD in a lowland population (700 m a.s.l.) of *D. rudis*, whereas highland population (2137 m a.s.l.) of this species exhibited a high level of SSD. This difference was explained with the colder environmental temperatures at the higher elevation site (GÜL *et al.* 2014). Although the population in our study inhabits lower altitude with milder climate, we found high-level SSD. On the other hand, longevity and age at first reproduction have been identified as the main determinants of SSD at an intra-specific level (LIAO & LU 2010, LYAPKOV *et al.* 2010, LIAO *et al.* 2013, 2015). Congruently, longevity of the males was higher than the females in our study. Moreover, SSD in many adult lizards is affected by sexual differences in the growth rates, and the fact that the larger sex grows faster than the smaller one (JOHN-ALDER & COX 2007, KOLAROV *et al.* 2010, ÜZÜM *et al.* 2014). Despite our findings showing strong male-biased SSD, we found no significant difference between growth rates for both sexes. Similar to our findings, RAIA *et al.* (2010) reported that growth rates were not significantly different between sexes in the mainland population of *P. siculus*, while males of the Island population grew significantly faster than insular females. RAIA *et al.* (2010) also stated that shifts in growth rate, body

size, age and size at maturity would depend on how these traits trade off with each other. We found that the mean age and age at sexual maturity were not significantly different between sexes, while there was a significant difference in body size. These results clearly show that the mean age and age at maturity were more effective on growth rate rather than body size in the studied population.

Some unsuitable ecological conditions (e. g. hot climate and dry period) are related to the higher percentage of double lines (JAKOB *et al.* 2002, GUARINO & ERİŞMİŞ 2008, ÖZDEMİR *et al.* 2012). Although Filyos site had a moderate climate, we observed a higher percentage (60%) of double lines. The high percentages of double lines may be a result of a lack of food sources or other probable unpredictable conditions.

Environmental conditions may also affect endosteal resorption (SMIRINA 1972). CAETANO & CASTANET (1993) reported fewer endosteal resorptions in lowland populations than in highland populations. Congruently, we did not find any endosteal

resorption in the studied population inhabiting lower elevation site. However, ARAKELYAN *et al.* (2013) and GÜL *et al.* (2014) reported contrasting results for other lizard species. On the other hand, daily and annual activities could have an effect on bone resorption of long bones in animals (HEMELAAR 1988, ESTEBAN 1990, LECLAIR 1990, AUGERT 1992, ESTEBAN *et al.* 1999).

In conclusion, our preliminary results showed similar growth rates between both sexes (having a similar age at sexual maturity but different longevity results). The relationship between age structure and growth in these lizards may occur in a different manner for other populations from different environmental and ecological conditions.

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