Demographic life history traits in a population of a critically endangered species, *Darevskia dryada* (Darevsky & Tuniyev, 1997)

Abdullah Altunışık* and Halit Eksilmez

Recep Tayyip Erdoğan University, Faculty of Arts and Sciences, Biology Department, Rize, Turkey

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Abstract

Knowing the age structure of endangered species is important in order to contribute to future conservation studies for such species. In this context, we investigated age structure, age at sexual maturity, potential reproductive lifespan and longevity in a population of the Charnali lizard, *Darevskia dryada*, an endangered species from Turkey. The results show that the Charnali lizard has a longer life span than other lizards of the genus *Darevskia* that live in the same region. We estimated that these lizards attain their sexual maturity at the age of one or two years and the potential reproductive life span for males and females is six and five years, respectively. As in many other lizards, the Charnali lizard exhibited a low-level male-biased sexual dimorphism in terms of increased size. We believe that this study, in which prior information related to the Charnali lizard is shared, will contribute to future conservation activities for this critically endangered species.

Keywords

Age structure; Charnali lizard; endangered; endemic species; life-history traits; skeletochronology

Introduction

Obtaining information on the life history traits and population dynamics of natural populations is very valuable for the conservation of endangered species (Andreone et al., 2005; Germano, 1992). For lizards, age at maturity, longevity, potential reproductive lifespan and age–size ratio are the main demographic life history traits that will shed light on conservation approaches in lizards.

* Corresponding author; e-mail: abdullah.altunisik@erdogan.edu.tr
Longevity and life history of lizards are affected by environmental factors such as elevation, drought, and climate in their habitats. The metabolism of such ectotherms is directly influenced by climatic conditions and its effects can be traced in the bone tissue of these animals in natural populations, which allows age estimation (Olgun et al., 2005). Skeletochronology is a widely preferred method for estimating ages of many ectothermic species since it is timesaving and prevents individuals from being sacrificed (e.g., *Furcifer pardalis*: Andreone et al., 2005; *Darevskia rudis*: Gül et al., 2014; *Phymaturus spectabilis*: Cabezas-Cartes et al., 2015; *Bufo andrewsi*: Liao et al., 2016; *Darevskia clarkorum*: Bülbül et al., 2016a. Moreover, this method is also very accurate and allows researchers in demographic and life-history studies to estimate the individuals’ age as closely as possible to their actual age (Matsuki & Matsui, 2009).

The objective of the present study was to provide more complete information regarding the life history of *D. dryada*, with particular attention to conservation. The Charnali lizard, *Darevskia dryada* (Darevsky & Tuniyev, 1997), is a species endemic to the Caucasus region; it is known from Hopa (Artvin province, northeastern Turkey). The vertical distribution of the species varies between 50 and 700 m. The IUCN Red List of Threatened animals has classified the species as CR (Critically Endangered) since 2009 because the area it occupies is probably less than 10 km², while its forest habitat has been severely reduced and continues to decline (IUCN 2017; Tuniyev et al., 2009). Therefore, our main purpose is to provide preliminary data on the age structure and body size of *D. dryada* as well as information about the species’ life span obtained by age estimates from skeletochronology.

**Material and methods**

**Study site and species**

The Charnali lizards were collected from a population near Hopa, Artvin province (Yoldere village: 104 m a.s.l.; 41°23′53″N, 41°28′29″E) during the breeding season of 2017. Like other parts of the Black Sea region, the study site is characterized by an oceanic climate with high and evenly distributed rainfall year round. According to the data from the meteorological station nearest to the study area, the annual average temperature is 12.3°C, and the annual total precipitation is 702 mm (https://mgm.gov.tr/). The habitat of this lizard, which is not very far from the forest road, consisted mainly of stone heaps, hidden among the higher vegetation of subtropical forest. The specimens were caught while they were sunbathing out on these stone heaps or hiding under the leaves. *D. dryada* lives in sympatry with *D. clarkorum* and *D. rudis* in this population. For surgical operations and morphological measurements, the animals were caught by hand and anesthetized by injecting 0.7% buffered MS-222 (tricaine methanesulfonate) into their intracoelomic cavity. The lizards were later released to their habitats after snout–vent length (SVL) was measured with a digital caliper (0.01 mm precision).
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Skeletochronological analyses

A total of 30 specimens (17 females and 13 males) were used for age determination. The age of individuals was determined by the skeletochronological method (Smirina, 1994) which is based on counting lines of arrested growth (LAGs) formed as a result of environmental factors, e.g. alternating favorable and unfavorable periods of bone growth. Unlike active growth seasons, in the winter season periosteal bone growth is reduced due to the influence of the cold climate and a difference occurs in growth rate, forming the LAGs. The skeletochronological procedure began by decalcifying the bones in 5% nitric acid solution for one hour and then rinsing them in tap water for over 12 hours. Cross sections (16 μm thickness) of the diaphysis of the phalange were prepared using a freezing microtome and then stained with Ehrlich’s haematoxylin for 15 min. The sections were mounted on a slide with a drop of water-based mounting medium and a cover slip was placed over the slide. For each individual at least three selected cross-sections (with small medullary cavity) were examined under a light microscope and recorded with a digital camera. We preferred sections with a small medullar cavity because the periosteal bone layer in these sections is wider. LAGs were counted and verified independently by both authors.

Statistical analyses

The total dataset was first tested for normality (Shapiro–Wilk test) and for homogeneity of variances (Levene test). Since the data were normally distributed (P > 0.05), statistical significance was analyzed by an independent-sample t-test. We used Pearson’s correlation coefficient to determine the magnitude and direction of the relationship between age and body size. For the mathematical expression of the relationship we performed regression analysis by the compound model, which has the highest $R^2$ value. All analyses were performed using SPSS 21 (IBM SPSS Statistics for Windows).

Sexual size dimorphism (SSD) was calculated as described in Ranta et al. (1994):

Sexual dimorphism index (SDI): (mean body length of the larger sex/mean body length of the smaller sex) – 1.

The distance between two LAGs is a good indicator of bone growth at a given age (Smirina, 1994), and the declining distance between the LAGs after a few years indicates that sexual maturity begins (Ryser, 1988). In the present study, we determined age at sexual maturity by considering the distance between the two LAGs in the periosteal bone. Potential reproductive lifespan was calculated as the difference between longevity and age at maturity.

The growth patterns were estimated according to the von Bertalanffy growth model, which has been used earlier in several studies on reptiles (Roitberg et al., 2006; Guarino et al., 2010; Bülbül et al., 2016b). The general form of the von Bertalanffy growth equation used was $L_t = L_\infty (1 - e^{-kt})$, where $L_t$ is length at age $t$, $L_\infty$ is a parameter representing asymptotic maximum size, $e$ is the base of the
natural logarithm, $k$ is a growth coefficient, and $t_0$ is the age at hatching, which is the starting point of the growth interval in the present study. Because data on the size at hatching were lacking for the studied population, we assumed size at hatching ($L_{t_0} = 27.6$ mm) as the mean value of *D. clarkorum*, the species closest to *D. dryada* (The Reptile Database, 2017), provided by In den Bosch & Bout (1998).

The parameters $L_\infty$ and $k$ were estimated using the program Microsoft Excel.

**Results**

Cross-sections at the diaphysis level of the phalange of a six-year-old *D. dryada* individual, which was used as control, showed endosteal and periosteal bone separated by a cementing resorption line (fig. 1) which did not reach the first LAG in all examined sections. Varying numbers of concentric LAGs were clearly marked and relatively easy to count in the periosteal layer.

The results of reading the age by direct observation under the microscope and in photographs on the computer showed that both males and females lived up to nine years. The age range was three to nine years (mean: 6.00 ± 0.53) in males and four to nine years (mean: 6.05 ± 0.33) in females (fig. 2). No significant difference was detected between males and females in terms of average age ($t = -0.099; df = 28; P > 0.05$). The results show that individuals from both sexes reach sexual maturity at an age of one to two years. Potential reproductive lifespan was six and five years in males and females, respectively.

The mean SVL of males was 58.70 ± 2.22 mm (range 46.09–71.32) and it was 56.15 ± 1.66 mm (range 45.47–68.58) in females (table 1). Males were not significantly larger than females ($t = 0.940, df = 28, P > 0.05$), but SDI was computed as
Table 1. Descriptive statistics of age and SVL for the studied population.

<table>
<thead>
<tr>
<th>Sex</th>
<th>n</th>
<th>Age (years)</th>
<th>SVL (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>Range</td>
</tr>
<tr>
<td>Females</td>
<td>17</td>
<td>6.05 ± 0.33</td>
<td>4–9</td>
</tr>
<tr>
<td>Males</td>
<td>13</td>
<td>6.00 ± 0.53</td>
<td>3–9</td>
</tr>
</tbody>
</table>

0.045, meaning weakly male-biased. For both sexes, the maximum SVL recorded was bigger than the estimated asymptotic SVL (SVL_asym, males: 61.70 mm; females: 63.44 mm). The growth rates of the males and females were not significantly different within the population ($t = 0.189$, df = 7, $P > 0.05$; $k$, males: 0.94; females: 0.76).

We found that the body size was positively correlated with age, which is a general life-history phenomenon in ectothermic species like lizards; a compound regression line was fitted between age and SVL in both females [$y = 40.295 + 1.055x$, fig. 3(A) ($n = 17, r = 0.58, P < 0.01$)] and males [$y = 42.213 + 1.055x$, fig. 3(B) ($n = 13, r = 0.73, P < 0.01$)].

Discussion

Our analysis of life-history traits of the studied population provides preliminary data on a critically endangered species, the Charnali lizard (*Darevskia dryada*). Among established methods of age determination, analyzing the bone structure using the skeletochronological method seems to be the most appropriate for assessing age and growth in amphibians and reptiles (Olgun et al., 2005; Bülbül et
Figure 3. Relationship between age and body size (SVL) in females (A) and males (B).
LAGs of these animals were considered to form in reaction to environmental changes such as fluctuations in climate or variations in the availability and quality of food (Guarino et al., 1998; Khonsue et al., 2000; Liao & Lu, 2010).

Like in many other ectotherms, changing environmental conditions affect the life-history features and longevity of lizards. Although the reasons for variations are manifold, food availability, risk of predation and climatic conditions are the most outstanding determinants (Roitberg et al., 2006). The longevity of lizards depends on these changes and varies widely across species: for instance, the lifespan of *Lacerta agilis* is about four years in the Italian Alps (Guarino et al., 2010), while in Russia’s low-altitude regions it can be up to six years (Roitberg et al., 2006). The lifespan of some species of the genus *Darevskia* whose demographic characteristics have been studied varies between 6 and 12 years. Among them, the species *D. rostombekowi*, *D. dahli* (Arakelyan & Danielyan, 2000), *D. sapphrina* (Arakelyan et al., 2013), *D. uzzeli* (Arakelyan et al., 2013) and *D. bithynica* (Gül et al., 2015) live for six years, *D. armeniaca* (Arakelyan et al., 2013), *D. unisexualis* (Arakelyan et al., 2013), *D. rudis* (Gül et al., 2014) and *D. parvula* (Bülbüll et al., 2016b) live up to eight years and *D. clarkorum* (Bülbüll et al., 2016a) lives up to 12 years. We found that the oldest individuals in the studied Hopa population are nine years old. When we take into consideration that Charnali lizards reach sexual maturity during their first or second year, we can say that they have a long time (at least five to six years) to reproduce and transmit their genes to future generations. The fact that the climate in the region is not very harsh, the low risk of predation and the abundance of food (personal comment) may have been effective in generating a relatively long lifespan in this endangered species.

Ectothermic vertebrates reach sexual maturity early or late according to the length of the activity period, which depends on the temperature. The temperature of the environment restricts the growth season for many lizards. Generally, lizards at low altitudes or low latitudes can grow and be active in much of the year, whereas the growth season is curtailed at high altitudes or high latitudes (Adolph & Porter, 1996). As indicated in many lizards (Galán, 1996; Roitberg et al., 2006; Tomašević Kolarov et al., 2010; Ramírez-Bautista et al., 2014) sexual maturity is associated with the attainment of a minimal body size and not a minimal age. In our study, the minimum size for reproductive males and females was 46.09 and 45.47 mm, respectively and age at maturity was found to be one to two years for both sexes. Since the studied area is in a relatively low-altitude region (104 m a.s.l.), ectothermic individuals living in such habitats are expected to reach sexual maturity at an earlier age for the reasons mentioned above. Similar results were reported for the other *Darevskia* species living in the same region. For instance, *D. clarkorum* and *D. parvula* individuals reach sexual maturity at the age of one to two years in a lowland population and two to three years in a highland population (Bülbüll et al., 2016a, 2016b).

Variation in sexual size dimorphism (SSD) may have significant effects on an organism’s life-history traits, ecology, population dynamics and behavior (Liao, 2013;
Altunışık, 2017). The adult body size depends on many factors including age at maturity and longevity (Özdemir et al., 2012). In the majority of lizards, it is common that males are larger than females (Fitch, 1981). SSD and some morphological characteristics are well known in lizards (Altunışık et al., 2013) and some authors have suggested that this phenomenon generally results from sexual selection, especially from mating competition between the males (Vitt & Cooper, 1985; Shine, 1989; Hewes, 1990; Vincent & Herrel, 2007). For instance, individuals might choose their mate based on size, which usually seems to result in male individuals having larger body size and a male-biased SSD. However, according to some other authors (Fitch, 1981; Best & Gennaro, 1984) the main reason for sexual dimorphism may be related to natural selection via competition for food among individuals (Fairbairn, 1997). Consistent with this trend, in this study and other studies conducted in the same region (Bülbül et al., 2016a, 2016b), a low level of male-biased sexual size dimorphism was observed for the relevant lizards. Contrary to these results, Gül et al. (2014) reported strong female-biased SSD in the highland population of D. rudis, whereas SSD appeared male-biased, although not statistically significant, in the lowland populations. According to these authors, SSD is associated with abiotic and biotic environmental factors and higher SSD must be expected in high altitude populations where the climate is colder. Temperature, precipitation and food availability are the main environmental factors constraining growth rate (Adolph & Porter, 1996). For instance, Iraeta et al. (2006) showed that lizards grew faster in sites with a higher total yearly precipitation (e.g., deciduous forests) and associated food abundance. As we mentioned before, the study region’s climate is not very harsh, there is a low risk of predation and an abundance of food, which depends on precipitation (annual total precipitation: 702 mm). Although we cannot reveal the effect of altitude due to the fact that we studied just a single population, we can suggest that life-history traits such as lifespan and age at sexual maturity together with these mentioned abiotic factors may have influenced SSD in this study. The fact that female and male individuals have the same longevity (nine years) and age at first reproduction (one or two years) and also a similar growth rate (k, females: 0.76; males: 0.94) may have been effective in generating a low level of male-biased size dimorphism.

The relationship between age and body size is an important life-history parameter that is frequently used in demographic surveys. We have also shown that, as in many lizards, the relationship between age and SVL is positive in D. dryada. Information on the life-history traits (e.g., age structure, growth) of endangered species may contribute to their effective conservation management. We suggest that the early age of first reproduction and the relatively long lifespan in the studied population are important data for this endangered lizard because these data can directly affect future generations of the population. In conclusion, demographic life history traits have been revealed in a population of endangered Charnalı lizards and basic information is provided for future conservation plans. We may have a chance to
learn more about *D. dryada* by analyzing populations living in different conditions, as the local conditions can directly affect the relevant life history characteristics.

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**References**


