Body size and age in three populations of the northern banded newt
*Ommatotriton ophryticus* (Berthold, 1846) from Turkey

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Abstract: We measured body size (snout–vent length, SVL) for three populations of *Ommatotriton ophryticus* newts in Turkey and estimated age using the skeletochronological method. Age was determined by counting the lines of arrested growth in cross-sections taken from phalanges. SVL was similar among populations and ranged between 58.41 and 85.78 mm in males and 50.33 and 71.56 mm in females. Males were significantly larger than females in all populations. Age at maturity was 4–6 years in males and 5–6 years in females. The average age of males and females did not differ significantly. Maximum age ranged from 9 to 12 years in males and from 9 to 11 years in females. Positive correlation was found between SVL and age for both sexes in all populations. A significant difference was observed among populations in terms of both age and body size (6-, 7-, and 8-year-old age classes). The demographic traits of the studied populations are the result of the interaction of multiple factors, including altitude, geographical location, and ecological factors.

Key words: *Ommatotriton ophryticus*, body length, age, relationship, Turkey

1. Introduction

Banded newts (genus *Ommatotriton* Gray, 1850) represent a well-defined lineage within the "modern Eurasian newts" (sensu Steinfartz et al., 2007). Within the genus, two species are generally recognized, the northern banded newt, *O. ophryticus*, and the southern banded newt, *O. vittatus* (Litvinchuk et al., 2005; Smith at al., 2008). These species can be diagnosed based on the trunk vertebrae number and allozyme and molecular data. *Ommatotriton ophryticus* ranges from the western Caucasus in southern Russia and Georgia, through northwestern Armenia and northern Turkey to the Bosphorus Strait (Olgun et al., 2009). According to Litvinchuk et al. (2005), two subspecies can be recognized in *O. ophryticus* on the basis of the number of trunk vertebrae, genome size, and protein electrophoretic analysis: *O. o. nesterovi* Litvinchuk, Zuiderwijk, Borkin & Rosanov, 2005 in the west and *O. o. ophryticus* (Berthold, 1846) in the east. They form one genetic clade with poor differentiation within, which might indicate relatively recent dispersal in the northern part of the range (Smith et al., 2008).

*O. ophryticus* is a medium-sized newt of up to 17–20 cm in total body length. It shows distinct sexual dimorphism in terms of size and secondary sexual characters (Baran et al., 2012): the male is larger than the female and during the breeding season it has an extremely high, deeply serrated crest with light and dark stripes. Males exhibit numerous dark spots on the flanks and tail and possess a silvery longitudinal band on the flanks that continues onto the tail. Females are rather drab and the ground color of the dorsum and flanks is uniformly olive green with fewer dark spots (Çiçek et al., 2011a).

*O. ophryticus* is found in coniferous, mixed, and deciduous forests up to subalpine meadows. In the breeding season, it inhabits lakes, ponds, large puddles, drainage canals, roadside ditches in meadows, and slow-flowing streams in open areas near or within forests (Olgun et al., 2009). Populations are declining due to habitat loss and fragmentation related to human activity, mainly along the Black Sea coast line, and also due to capture for the pet trade (Tarkhnishvili and Gokhelashvili, 1999; Mermer et al., 2008; Olgun et al., 2009; Çiçek et al., 2011a).

Life history characters such as body size, age at reaching maturity, longevity, and growth rate show variation among populations. Intraspecific variation in these characters results from differences in environmental variables that substantially affect the genetically fixed growth pattern (Özdemir et al., 2012). Arrested growth due to climatic conditions has been reported in many amphibians (Smirina, 1994; Castanet, 2002). The periodicity of arrested growth over the seasons, analogous to tree rings, also provides an opportunity to determine the age of...
individuals (Castanet and Smirina, 1990; Smirina, 1994). The skeletochronological method has been successfully used for age determination in many species of anurans (e.g., Miaud et al., 2007; Çiçek et al., 2011b, 2011c; Gül et al., 2011; Özdemir et al., 2012; Altunışık and Özdemir, 2013), as well as salamanders and newts (e.g., Miaud et al., 2000; Jakob et al., 2003; Olgun et al., 2005; Kutrup et al., 2005; Üzüm, 2009; Üzüm and Olgun, 2009a, 2009b; Üzüm et al., 2011).

It is widely assumed that amphibians and reptiles show indeterminate growth and therefore their body size and age are positively correlated (Duellman and Trueb, 1986; Halliday and Verrell, 1988). Variation in body size, age at maturation, and longevity across environmental gradients are some of the most intensively studied ecogeographic trends (Sinsch et al., 2007; Adams and Church, 2008; Hasumi, 2010; Altunışık and Özdemir, 2013). Few demographic studies (Kutrup et al., 2005; Mermer et al., 2008; Başkale et al., 2013) have been conducted for *O. ophryticus*. The aim of this paper is to present original data on body size and age structure (e.g., age of maturity, longevity) of *O. ophryticus* from three different localities and to reveal the relationship between these two life history characteristics.

2. Materials and methods

2.1. Sampling and body size determination

We used 169 (79 ♂, 90 ♀) *O. ophryticus* specimens from three different populations: Bahçeşultan (Bilecik Province), Tosya (Kastamonu Province), and Erbaa (Tokat Province) (Figure 1). Bahçeşultan samples were collected during the breeding season in 2011 under project number FEF-12022, and the animals were treated in accordance with the guidelines of the local ethics committee (B.30.2.ADÜ.0.00.00.00/050.04/2011/032). The other samples (from Tosya and Erbaa) were obtained from preserved samples kept in the herpetological collection of the Adnan Menderes University Biology Department.

Mean annual temperature and precipitation at Bilecik are 12.5 °C and 449.2 kg/m² (http://www.dmi.gov.tr/). Samples (20 ♂, 30 ♀) were collected from Bahçeşultan (40°02′N, 29°45′E; 1015 m a.s.l.) by dip-netting during the breeding season (29 April–1 May 2011). The population inhabits a temporary pond, which sometimes dries up during the summer and is located next to the road in the village.

Mean annual temperature and precipitation in Kastamonu are 9.8 °C and 478.5 kg/m² (http://www.dmi.gov.tr/). Samples (30 ♂, 30 ♀) were collected from the village of Çıban (41°11′N, 34°01′E; 1202 m a.s.l.) in May and June 2005 and April 2006. The population lives in a channel formed by snow melt, which widens in places and forms small puddles. The maximum depth of these puddles is about 70 cm and their area of surface is about 32 m².

Mean annual temperature and precipitation in Tokat are 12.5 °C and 435.1 kg/m² (http://www.dmi.gov.tr/). Samples (29 ♂, 30 ♀) were collected from Düden Lake (40°49′N, 36°35′E; 1239 m a.s.l.) in May 1990 and May 1991. The lake is approximately 150 m in length, 80 m in width, and 1.5 m in depth. It is poorly vegetated and its bottom is muddy.

The sex of each individual was determined based on external secondary sexual characters: males have a high dorsal crest and a prominent cloaca compared to females (Başoğlu et al., 1996; Baran et al., 2012). The snout–vent length (SVL) was measured as the distance between the tip of the snout and the posterior part of the vent by using a dial caliper with an accuracy of 0.02 mm. The longest toe of the forelimb (i.e. second or third) was cut and preserved in 70% ethanol for age determination by using the skeletochronological method.

Figure 1. Map showing the localities of studied samples of *O. ophryticus* (1. Bahçeşultan village/Bilecik; 2. Tosya/Kastamonu; 3. Erbaa/Tokat).
2.2. Age determination
Individual age was estimated by using skeletochronology. The standard skeletochronological method followed previous procedures (e.g., Miaud, 1991; Olgun et al., 2005) with some minor modifications. Preserved phalange samples were washed in tap water for approximately 24 h to purify the samples from the ethanol, decalcified in 6% nitric acid for 25–30 min, and then washed again in tap water for about 12 h. The cross-sections (18 µm thick) from the diaphyseal region of the phalange were obtained using a freezing microtome and were stained with Ehrlich’s hematoxylin for 5 min. The sections were placed in glycerin in order to be observed with a light microscope. Bone sections from each individual were photographed at the same magnification.

Age was determined by counting the lines of arrested growth (LAGs) in cross-sections. LAGs were clearly visible and independently counted by two authors. The maximum difference for LAGs counted was 1 year and, in this case, the age was estimated at ±1 year. The age at which a significant decrease in growth occurred, based on the thickness of the growth rings, was taken as the age of sexual maturity (Ryser, 1988). For identification of how many LAGs were destroyed by endosteal resorption, sections with small, nondestroyed marrow cavities were selected, and the perimeters of their inner LAGs were compared with the sections having endosteal resorption.

2.3. Statistical analysis
The age and body measurements (SVL) were normally distributed (Kolmogorov–Smirnov D test, all P > 0.05), and parametric tests were utilized to compare the variables. We used the Student t-test, a one-way analysis of variance (ANOVA), and post hoc LSD tests to compare the variations among sexes and populations. The Pearson correlation coefficient was computed to infer the pattern of relationships between SVL and age. The best regression model was selected according to R² values. All tests were processed with STATISTICA 7.0 (StatSoft Inc., USA) and Excel (Microsoft) and were interpreted at α = 0.05.

3. Results
3.1. Body size and age structure
Body size (SVL) was similar among populations and ranged between 58.41 and 85.78 mm in males and 50.33 and 71.56 mm in females. Descriptive statistics of body size and age for all populations in both sexes are given in the Table. The highest mean SVL for both sexes was found in Bahçesultan (SVL = 72.39 mm for males, 63.40 mm for females), while the lowest value was recorded in Tosya (SVL = 64.07 mm for males, 55.57 mm for females). The mean SVL of males was larger than the mean SVL of females, and it was significantly different between sexes in all populations (Student t-test; t = –9.659, df = 48, P < 0.001 for Bahçesultan; t = –10.580, df = 58, P < 0.001 for Tosya; and t = –9.945, df = 57, P < 0.001 for Erbaa).

In all phalangeal cross-sections, LAGs were well stained and relatively easy to count, as seen in Figure 2A. Endosteal resorption was frequently observed in the cross-sections from adults in all populations (Figure 2B). By comparing the diameters of eroded marrow cavities with the diameters of noneroded marrow cavities of cross-sections, we determined that endosteal resorption completely or partially destroyed only the first LAG. Endosteal resorption was observed in 90%, 98.33%, and 84.74% of the individuals from Bahçesultan, Tosya, and Erbaa, respectively. Endosteal bone deposition was also observed in some sections. However it was possible to estimate age for 100% of the available phalanges (n = 169).

The age distributions observed in the three populations are shown for the two sexes separately in Figure 3. The average age of males was 7.4 ± 0.28 years (range: 6–10 years) in Bahçesultan, 6.4 ± 0.19 years (range: 4–9 years) in Tosya, and 8.2 ± 0.25 years (range: 6–12 years) in Erbaa. The average age of females was 7.4 ± 0.20 years (range: 6–10 years) in Bahçesultan, 7.06 ± 0.17 years (range: 5–9 years) in Tosya, and 8.4 ± 0.24 years (range: 5–11 years) in Erbaa. While no significant differences were found between the age distributions of the two sexes in Bahçesultan (t = –0.048, df = 48, P > 0.05) and Erbaa (t = –0.398, df = 57, P > 0.05) and Tosya (t = –0.945, df = 57, P < 0.001 for Erbaa).

Table. Descriptive statistics on snout–vent length (SVL, mm) and age (years) of O. ophryticus from Bahçesultan, Tosya, and Erbaa (n: number of individuals; SE: standard error).

<table>
<thead>
<tr>
<th></th>
<th>Bahçesultan</th>
<th>Tosya</th>
<th>Erbaa</th>
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<tr>
<td>Males</td>
<td></td>
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<tr>
<td>SVL</td>
<td>20</td>
<td>72.39 ± 0.77</td>
<td>64.09–78.87</td>
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<tr>
<td>Age</td>
<td>20</td>
<td>7.4 ± 0.28</td>
<td>6–10</td>
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<td>Females</td>
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<tr>
<td>SVL</td>
<td>30</td>
<td>63.40 ± 0.55</td>
<td>57.64–70.72</td>
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<tr>
<td>Age</td>
<td>30</td>
<td>7.4 ± 0.20</td>
<td>6–10</td>
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P > 0.05), we found significant sex differences in age for Tosya (t = –0.337, df = 58, P < 0.05).

Males and females of Bahçesultan reached sexual maturity at the age of 6 while different age classes were determined for Tosya and Erbaa. The age of sexual maturity for males was estimated as 4 years in Tosya and 6 years in Erbaa. For females, this value was calculated as 5 years in both populations.

The age structure in both sexes varied significantly among the populations (F,76 = 15.825, P = 0.000 for males; F,76 = 9.158, P = 0.000 for females) with the oldest mean ages observed in Erbaa and the youngest mean ages in Tosya. All populations differed from each other significantly in the age classes of 6 (F,17 = 9.16, P = 0.001 for males, F,11 = 14.06, P = 0.000 for females), 7 (F,25 = 15.79, P = 0.000 for males, F,25 = 16.74, P = 0.000 for females), and
8 (F\(_{2,20} = 3.92, P = 0.036\) for males, F\(_{2,22} = 23.23, P = 0.000\) for females).

3.2. Relationship between body size and age
For all populations, a positive and significant correlation was found between age and SVL in females (Bahçesultan: \(r = 0.501, P = 0.004, y = 0.339x^2 - 3.951x + 73.54\); Tosya: \(r = 0.397, P = 0.029, y = 0.158x^2 - 0.996x + 54.56\); Erbaa: \(r = 0.501, P = 0.004, y = 34.53x^{0.263}\)), while there was no such statistically significant correlation in males, except for the Erbaa population (\(r = 0.398, P = 0.032, y = 0.349x^2 - 5.051x + 88.36\)).

4. Discussion
We determined the age of three different \(O.\ ophryticus\) populations living in Turkey. The mean age for breeding males ranged from 6 years in Tosya and 7 years in Bahçesultan to 8 years in Erbaa, and for breeding females from 7 years in Bahçesultan and Tosya to 8 years in Erbaa. The maximum ages or maximum longevity (12 and 11 years) were observed in Erbaa. No significant difference in maximum age was observed between sexes within a population (t-test, \(P > 0.05\)). Longevity differing between sexes is not common. However, according to Bruce (1993), the sex that reaches sexual maturity earlier gets larger and lives longer than the other sex.

Various age classes for sexual maturity were recorded for \(O.\ ophryticus\) in this study and also in the literature. Males were sexually mature at 4 years in Tosya and at 6 years in Bahçesultan and Erbaa, while females were sexually mature at 5 years in Tosya and Erbaa and at 6 years in Bahçesultan. Kutrup et al. (2005) reported that the age at sexual maturity was 4 years in a high-elevation population (1300 m) and 2–3 years in a low-elevation population (300 m). The age of sexual maturity was determined as 3–5 years by Kuzmin (1999) and 3–4 years by Tarkhnishvili and Gokhelashvili (1999) for different \(O.\ ophryticus\) populations. Olgun (2012) reported that the age of sexual maturity was 4 years for both sexes. These intraspecific differences can be explained by environmental variation and the length of the larval stage (Duellman and Trueb, 1986). Dolmen (1983) studied \(Lissotriton vulgaris\) (formerly \(Triturus vulgaris\)) and \(Triturus cristatus\) in Norway and reported different ages of sexual maturity for both species between different populations. Caetano and Castanet (1993), Diaz-Paniagua et al. (1996), and Jakob et al. (2003) recorded different sexual maturity ages for \(T.\ marmoratus\). Olgun et al. (2005) and Üzüm and Olgun (2009a, 2009b) obtained various age classes for maturity for different populations of \(T.\ karelinii\).

In this study, we obtained different results for the age at reaching sexual maturity for males and females of \(O.\ ophryticus\). Females and males reached sexual maturity at the same age in the Bahçesultan population, while the age of maturity was different for males and females in Tosya and Erbaa: males reached sexual maturity 1 year before females in Tosya. In contrast, males reached sexual maturity 1 year later than females in Erbaa. According to the literature, male salamanders often mature earlier and at a smaller size than females (e.g., Tilley, 1973; Caetano et al., 1985; Miaud et al., 1993; Rebelo and Caetano, 1995). Miaud (1991), studying three different newt species, emphasized that a large portion of male individuals reached sexual maturity 1 year before females. This variation between sexes could be explained by the fact that females allocate more energy to gonad and embryo development than do males (Miaud et al., 2001). On the other hand, the age of maturity and longevity is also influenced by local environmental conditions. For example, it is known that the age of reaching sexual maturity increased according to altitude in \(T.\ carnifex, T.\ cristatus,\) and \(T.\ marmoratus\) (Caetano and Castanet, 1993; Olgun et al., 2005). Furthermore, living in permanent or temporary habitats (breeding habitat, etc.) affects the age structure of salamander populations (Diaz-Paniagua et al., 1996).
Endosteal resorption was almost always observed in the cross-sections from adults in all populations (90%, 98.33%, and 84.74% of the individuals from the Bahçesultan, Tosya, and Erbaa populations, respectively). Resorption may be associated with environmental conditions (Smirina, 1972), and populations living at high altitudes show less resorption than lowland populations (Esteban et al., 1996, 1999), or the opposite (Caetano and Castanet, 1993). For example, endosteal resorption was observed in all cross-sections of adult Mertensiella caucasica (Üzüm, 2009) and 78.4% of adult Neurergus crocatus (Üzüm et al., 2011) from high altitudes, while Miaud et al. (2000) reported that only 38% of cross-sections showed endosteal resorption in a high-altitude population of Ichthyosaura alpestris.

Males of O. ophyticus were larger than females in our populations, and the difference was statistically significant. This situation was previously emphasized by Malmgren and Thollesson (1999). By studying another two populations of O. ophyticus from different altitudes, Kutrup et al. (2005) obtained the same results. Schmidtler and Schmidtl (1967) and Olgun and Baran (1993) also determined larger body measurements for males than females in different populations. Similarly, males having a larger average SVL than females was also recorded in, e.g., Lissotriton vulgaris (Halliday and Verrell, 1988) and M. caucasica (Üzüm, 2009). Males being larger than females can be attributed to factors such as male interactions in the breeding season (Shine, 1979; Halliday and Verrell, 1988; Malmgren and Thollesson, 1999) and sexual selection, i.e. larger males having better access to females than smaller males (Monnet and Cherry, 2002; Üzüm, 2009). Generally in amphibians, females grow larger than males and their body size is often correlated with clutch size (Duellman and Trueb, 1986; Malmgren and Thollesson, 1999). For example, Diaz-Paniagua and Mateo (1999) determined female-biased sexual size dimorphism in five different L. boscai populations. The same results were also recorded for T. marmoratus (Caetano and Castanet, 1993; Diaz-Paniagua et al., 1996), I. alpestris (Miaud et al., 2000), T. karelinii (Üzüm and Olgun, 2009a), and N. crocatus (Üzüm et al., 2011). Factors such as climatic conditions, trophic resources, interspecific competition, and predator–prey interactions may explain differences in body length and age among individuals and populations (Adolph and Porter, 1996).

In our study, a positive and significant (but not strong) relationship between age and body size (SVL) was found for all populations, except for Bahçeçelatan and Tosya males. Contrarily, Kutrup et al. (2005) found no significant relation between age and SVL for high- and lowland populations of O. ophyticus. However, there is no positive relationship between age and body size in some species, or this relationship may vary depending on the sex. For example, positive correlation was reported for only females in Bufo bufo (Högland and Säterberg, 1989), while this correlation was only reported for males in R. pipiens (Leclair and Castanet, 1987), Pseudacris crucifer (Lykens and Forester, 1987), and Amietophrynus pardinus (formerly Bufo pardinus) (Bastien and Leclair, 1992). Diaz-Paniagua and Mateo (1999) did not find any significant relationship between age and body size in five populations of L. boscai. In contrast, there is a positive and significant relationship between age and body size in M. caucasica (Üzüm, 2009), N. crocatus (Üzüm et al., 2011), and T. karelinii (Üzüm and Olgun, 2009a, 2009b), as well as in some anurans like B. bufo (Hemelaar, 1986), R. temporaria (Ryser, 1988), R. holtzi (Miaud et al., 2007), and P. ridibundus (Güll et al., 2011).

To conclude, in different populations of O. ophyticus from northern Turkey, the age of sexual maturity varied from 4 to 6 years, the lifespan was 9 to 12 years, and the age structure of both sexes significantly differed among the populations. Furthermore, a significant difference was found among populations of O. ophyticus in terms of age-dependent body size concerning 6-, 7-, and 8-year age classes. Because the altitude, mean annual temperature, and precipitation of the localities were not much different, it was not possible to attribute differences in age composition of the populations to those factors. The demographic traits of the studied populations are the result of the interaction of several factors, including altitude, geographical location, and ecological factors.

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