Life history traits in *Bufotes variabilis* (Pallas, 1769) from 2 different altitudes in Turkey

Abdullah ALTUNIŞIK*, Nurhayat ÖZDEMİR
Department of Biology, Faculty of Arts and Sciences, Recep Tayyip Erdoğan University, Rize, Turkey

Abstract: Life history characteristics (e.g., longevity, age at maturity, body size, and body mass) of the green toad, *Bufotes variabilis*, were investigated from 2 populations inhabiting different altitudes (Çankırı, 960 m a.s.l., n = 57; Adana, 13 m a.s.l., n = 52) in Turkey. Age was determined using skeletochronology. The oldest individual was 11 years old (range: 4–11 years) in the highland population, while the oldest individual was 6 years old in the lowland population (range: 2–6 years). Sexual maturity was estimated at 3–4 years in the highland population and 2–3 years in the lowland population by the skeletochronological pattern. Mean body length was found to be 70.41 and 69.42 mm and 54.18 and 56.11 mm for females and males of the highland and the lowland populations, respectively. While mean body mass of the females and males from the highland population was 44.83 and 42.73 g, it was 19.09 and 19.66 g in the lowland population, respectively. Mean age, SVL, and body mass in the highland population were found to be significantly higher than in the lowland population. Colder climate, sufficient food availability, less human impact, and lower predator density in the highland population may explain the differences between the 2 populations. In addition, a significant positive correlation was found between age and body measurements (SVL and body mass) in both males and females of the populations.

Key words: *Bufotes variabilis,* life history traits, age structure, altitude, body size, Turkey

1. Introduction

Life-history traits of an individual, e.g., longevity, age at maturity, body size, and growth rates, indicate important variation among populations along environmental and altitudinal gradients. Change in life-history traits among populations of the same species arises from differences in environmental variables that substantially affect the genetically fixed growth pattern (Özdemir et al., 2012). Ectothermic vertebrates such as amphibians and reptiles are more sensitive to this variation.

Body size, age at maturity, and longevity variation 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). The most well-known ecogeographic trend, known as Bergmann's (1847) rule, states that, in wide-ranging endothermic animal species, individuals living in a cold climate tend to be larger than individuals living in a warm climate. Amphibians usually do not conform to this rule (Laugen et al., 2005; Adams and Church, 2008; Ficetola et al., 2010). Some amphibians even follow the reverse of Bergmann's rule (decreasing body size with cooler climate) (Ashton, 2002; Ma et al., 2009).

Demography is the key to life history theory, because life histories include some demographic traits that have evolved in response to environmental factors (Stearns, 1992; Heino et al., 1997; Sinsch et al., 2007). The environmental factors that are likely to encourage variations in life history parameters of amphibians are temperature, food availability, and humidity, which may vary seasonally depending on the latitude and altitude of the site (Feder and Burggren, 1992). In temperate regions, including Turkey, the length of the cold period has been identified as one of the most important variables in differentiating growth patterns concomitantly with latitudes and altitudes (Caetano and Castanet, 1993). If we know the age structure of an ectothermic animal with indeterminate growth, we can understand its population's demographic parameters from an ecological, developmental, ontogenetic, and evolutionary angle (reviewed in Hasumi, 2010). The effect of climatic conditions on the metabolism of ectotherms is recorded in their bone growth, which is a good tool for determining the animals' individual ages in natural populations (Olgun et al., 2005).

The green toad, *Bufotes variabilis* (syn. *Pseudepidalea. variabilis* (Pallas, 1769), according to Frost, 2013), which is the subject of this study, belongs to the family *Bufonidae*, which is composed of 50 genera. *B. variabilis* has a widespread distribution, from the eastern Mediterranean countries to Central Asia (AmphibiaWeb, 2014). According

* Correspondence: abdullah.altunisik@erdogan.edu.tr

to Stöck et al. (2006), the species is distributed from Greece eastwards through Turkey; in a recent phylogenetics study (Özdemir et al., 2014), this species was reported to be found throughout Turkey. In the present study, we aimed to reveal life history characteristics (e.g., longevity, age at maturity, body size, and body mass) of 2 populations of *B. variabilis* inhabiting different altitudes by using the skelotochronological method.

2. Materials and methods

One hundred and nine (57 ♂♂ and 52 ♀♀) *B. variabilis* adults were collected as part of a broader study within the guidelines of the local ethics committee for animal experiments (Rize University, approval reference number: 2012/4) during the breeding season in 2012 from the vicinity of Adana (Yeşilbağlar district), with an altitude of 13 m (36°28′794″N, 36°13′835″E; 29 ♂♂, 23 ♀♀), and the village Seydiköy (Çankırı), with an altitude of 960 m (40°31′595″N, 33°29′578″E; 28 ♂♂, 29♀♀), in Turkey. Adana is located in the south of Turkey and has a typical Mediterranean climate with dry and hot summers. Çankırı is located in the north of Central Anatolia and has a continental climate with dry and hot summers. Since meteorological data were not available for Seydiköy (960 m), meteorological data from the vicinity of Çankırı (720 m a.s.l.) were used instead. However, it is possible that Seydiköy has lower temperatures than the vicinity of Çankırı. In addition, sufficient food, lower predator density, and less human impact were observed in Seydiköy. Mean temperature was 23 °C and 18 °C for Adana and Çankırı during the activity periods (8 months and 6 months), respectively (www.dmi.gov.tr).

For each individual, sex was determined by the examination of external secondary sexual characters. Individuals' body mass was weighed to the nearest 0.01 g using microbalances, and snout-vent length (SVL) was measured using a digital caliper with an accuracy of 0.01 mm. The longest toe was then clipped, including the first and second phalanges. Toe samples were fixed in 10% formalin, stored in 70% ethanol, and successively used in histological analysis. After sampling, specimens were released at the place of capture.

Age was investigated by using the skeletochronological method (Castanet and Smirina, 1990). After dissection and preparation, the phalanges were purified from the ethanol in running tap water for roughly 12 h, decalcified in 5% nitric acid for 1 h, and then washed again under running tap water for 12 h and embedded in cryomatrix (Thermo). Cross sections (18–20 μm) of the diaphyseal part of each phalanx were acquired using a freezing microtome and later stained in Ehrlich's hematoxylin. The sections were submerged in glycerol for ascertainment under a light microscope. Endosteal resorption of the

first lines of arrested growth (LAG) was assessed by contrasting the diameters of eroded marrow cavities with the diameters of noneroded marrow cavities in sections from the youngest specimens. The number of LAGs was assessed independently by 2 observers (A. Altunışık and N. Özdemir).

Since age classes and body measurements (SVL and body mass) were normally distributed (Kolmogorov–Smirnov test, P > 0.05), parametric tests were utilized to compare variables between sexes (independent samples t-test). Levene's tests were used to test for differences in variances, while Pearson's correlation coefficient was computed to establish the pattern of relationships among body size, body mass, and age. Regression analysis was applied to calculate the correlation equation between age and body measurements (SVL and body mass). Data analysis was performed using SPSS 21.

We quantified sexual size dimorphism (SSD) according to the Lovich and Gibbons (1992) index: sexual dimorphism index (SDI) = (size of larger sex/size of smaller sex) ± 1, where the result is arbitrarily defined as positive (–1) when females are larger and negative (+1) in the opposite case.

3. Results

Descriptive statistics of age, body length, and body mass are given in Table 1. In all phalangeal cross sections, LAGs were clearly marked and relatively easy to count, as can be seen in Figure 1. Endosteal resorption, the sign of partial erosion of the periosteal bone, was observed in 79% (n = 45) and 33% (n = 17) of the individuals from the Çankırı and Adana populations, respectively.

Although males from the lowland population were slightly larger than females, no significant difference was observed between the sexes (independent samples t-test, t = –1.099, df = 51, P = 0.277, sexual dimorphism index (SDI): –0.035). While no significant difference (independent samples t-test, t = 0.439, df = 55, P = 0.663) was found between the sexes in the highland population, the female was larger, and SDI was 0.014 in this population. Minimum body length was 51.88 mm (average 70.41 ± 1.40) and 55.84 mm (average 69.42 ± 7.55) for females and males of the highland population, respectively. The smallest reproductive female was 44.42 mm (average 54.18 ± 6.73) and the smallest male was 47.94 mm (mean: 56.11 ± 5.87) in the lowland population. While body mass of the females and males from the highland population was 16.91–80.63 g (mean: 44.83) and 15.83–69.84 g (mean: 42.73), it was 10.91–31.95 g (mean: 19.09) and 13.27–34.06 g (mean: 19.66) in the lowland population, respectively.

For both sexes, there were significant differences between the populations in terms of both mean SVL (independent samples t-test, for females: t = 6.986, df =

Table 1. Descriptive statistics on age (year), snout-vent length (SVL, mm), and body mass (g) of the *Bufotes variabilis* from the Seydiköy and Adana populations (n: number of individuals).

Çankırı (Seydiköy)				Adana (Yeşilbağlar)		
Parameters	N	Mean	Range	N	Mean	Range
Males						
Age	28	6.42 ± 1.50	4–10	29	3.51 ± 1.08	2–6
SVL	28	69.42 ± 7.55	55.84–82.79	29	56.11 ± 5.87	47.94–71.25
Mass	28	42.73 ± 14.23	15.83– 69.84	29	19.66 ± 5.03	13.27–34.06
Females						
Age	29	6.51 ± 1.78	4–11	23	4.08 ± 0.79	3–6
SVL	29	70.41 ± 1.40	51.88– 88.00	23	54.18 ± 6.73	44.42–71.61
Mass	29	44.83 ± 0.40	16.91– 80.63	23	19.09 ± 0.33	10.91–31.95
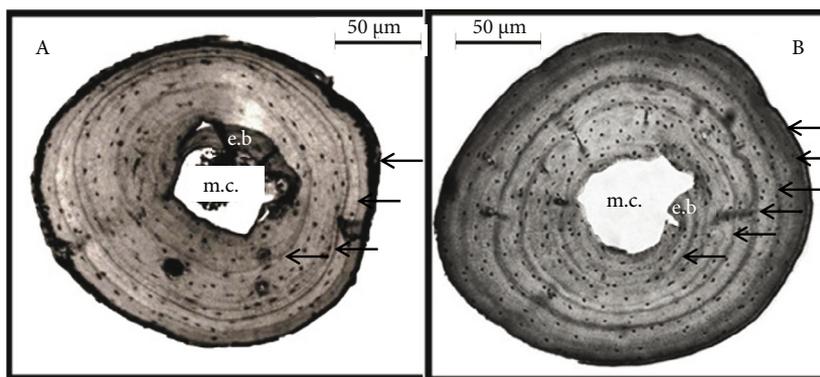
Figure 1. Cross-sections at the diaphysis level of a phalanx of a male (A) and a female (B) of *Bufotes variabilis* individuals (m.c. = marrow cavity, e.b. = endosteal bone). The LAGs are indicated by black arrows.

50, $P < 0.001$; for males: $t = 7.444$, $df = 55$, $P < 0.001$) and mean body mass (independent samples t-test, for females: $t = 6.595$, $df = 50$, $P < 0.001$, for males: $t = 8.215$, $df = 55$, $P < 0.001$), indicating that the Seydiköy individuals had larger values than the Adana ones.

In the highland population, the oldest age was 11 years, and age ranged between 4 and 11 years (mean: 6.51 ± 1.78) in females and 4 and 10 years (mean: 6.42 ± 1.50) in males (Figure 2). However, maximum lifespan was found to be 6 years in the lowland population, and age ranged between 3 and 6 years (mean: 4.08 ± 0.79) for females and 2 and 6 years (mean: 3.51 ± 1.08) for males. There was a significant difference in terms of average age between males and females (independent samples t-test, $t = 2.103$, $df = 50$, $P < 0.05$) for the lowland population. In contrast, no significant difference was found between the sexes in terms of mean age for the Seydiköy population ($P > 0.05$). Mean ages of both sexes in the highland population were

significantly higher than those of the lowland population (independent samples t-test, females: $t = 6.062$, $df = 50$, $P < 0.001$; males $t = 8.402$, $df = 55$, $P < 0.001$). Sexual maturity was estimated at 3–4 years in the highland population and 2–3 years in the lowland population by the skeletochronological pattern.

A significant positive correlation was found between age, SVL, and body mass in both males and females for the 2 populations (Table 2).

4. Discussion

Anurans in cool climates are limited in both their seasonal and daily activity periods by low temperatures (Mullally and Cunningham, 1956; Sinsch and Sherif, 1989). The body size of adults depending upon the timing of maturity is influenced by the effect of cold temperatures on amphibian larval and juvenile growth and development rates. It is possible that high-altitude populations are
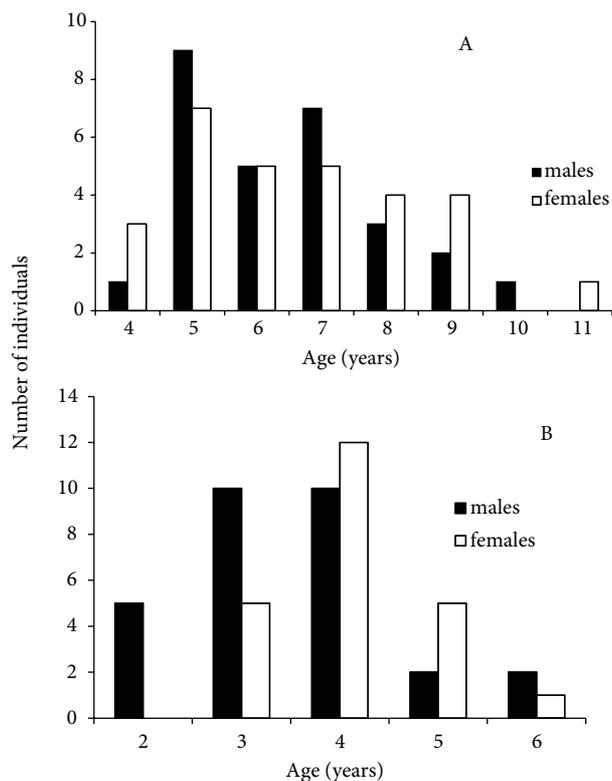
Figure 2. Age frequency distributions of the highland (A) and the lowland (B) populations.

exposed to cooler temperatures and shorter activity periods as compared with low-altitude populations. As a consequence of these effects, high-altitude amphibians commonly have a larger body size (Üzüm and Olgun, 2009).

The response of body size to temperature (the most commonly used environmental variable) is subject to the "developmental temperature–size rule" (Berrigan and Charnov, 1994). This rule is often associated with Bergmann's rule, in which populations or species in colder climates tend to have larger adult sizes. Since there are altitude and mean temperature differences in relation to elevation between the 2 populations conforming to this trend in the present study, it was found that the body lengths of females and males of the highland population were larger than those of the lowland population. However, this tendency is not always observed. Parameters such as food availability, habitat quality, and predation pressure on age at maturity and limited altitudinal gradients have effects on body size (Morrison and Hero, 2003). Colder climate, sufficient food availability, and lower human impact and predator density in the highland population may be an explanation for differences between the populations (personal communication).

The maximum observed longevity found in the present study was 11 years for females and 10 years for males. Similar to our results, in the studies by Sinsch et al. (2007; 60–100 m a.s.l.), the oldest females were reported as 11 years for *Bufo viridis*. Furthermore, Castellano et al. (1999) recorded the same result (11 years) only for males of this species in both Portuscoso (island; 50 m a.s.l.) and Zucchea (mainland; 350 m a.s.l.) populations. In a study conducted on *Bufotes variabilis* (referred to as *Bufo viridis*) in Georgia (Borjami Canyon, 900–1200 m a.s.l.), age ranged from 3 to 10 years for females and from 2 to 6 years for males (Gokhelashvili and Tarkhnishvili, 1994). Maximum lifespan was 5 and 8 years for Armenian and Iranian populations, respectively (Ledentsov and Melkumyan, 1986; Ashkavandi et al., 2012). The single demographic study (Kutrup et al., 2011) on *Bufotes variabilis* in Turkey was conducted on island (Giresun Island, 0–30 m a.s.l.) and mainland (Giresun Province, 20–100 m a.s.l.) populations from northeastern Turkey. The researchers reported age as ranging from 2 to 8 years for island and from 3 to 8 years for mainland populations. Since their study was performed at approximately sea level, no comparative data were available from a highland population of this species in Turkey. In the present study, we aimed to test the effects of elevation by choosing populations from 2 different altitudes.

The mean age of males and females respectively was calculated as 3.51 and 4.08 years in the Adana population, and 6.42 and 6.51 years in the Seydiköy population. In the lowland population, the age difference between males and females was found to be statistically significant, indicating that adult females were on average older than the males. Ashkavandi et al. (2012) also found that females had a significantly higher mean age than males. In contrast to these results, no significant difference was observed between the sexes in terms of age in either population in Kutrup et al.'s (2011) study and the highland population of our study. When focused on interpopulation differences in terms of age, our data showed highly significant variations. The mean ages of both sexes in the highland population were higher than those of the lowland population. In general, average age is expected to be higher in highland populations (Ledentsov and Melkumyan, 1986; Miaud et al., 2000, Kutrup et al., 2005; Altunışık and Özdemir, 2013), and our data also conform to this tendency. This result may be related to habitat quality, predator density, and climate variations. In contrast to this trend, in a study conducted on *Pelophylax ridibundus*, Gül et al. (2011) reported that the average age of the lowland population was higher than that of the highland population.

In general, amphibians attain sexual maturity after having reached a certain minimum size but a variable age (Gibbons and McCarty, 1984; Hemelaar, 1986; Ryser,

Table 2. Relationships among age, body size, and body mass of *Bufotes variabilis* populations.

	Çankırı (Seydiköy)		Adana (Yeşilbağlar)	
	Males	Females	Males	Females
Age–body size	$r = 0.947**$ $y = 28.40 + 7.18x - 0.02x^3$	$r = 0.942**$ $y = 4.90 + 16.67x - 1.18x^2 + 0.03x^3$	$r = 0.567*$ $y = 26.78 + 20.47x - 5x^2 + 0.44x^3$	$r = 0.717**$ $y = 49.67 - 0.66x^2 + 0.21x^3$
Age–body mass	$r = 0.836**$ $y = -31.21 + 13.26x - 0.04x^3$	$r = 0.854**$ $y = 66.11 - 37.12x + 8.15x^2 - 0.44x^3$	$r = 0.509*$ $y = -11.98 + 24.91x - 6.64x^2 + 0.60x^3$	$r = 0.658**$ $y = 16.46 - 1.45x + 0.11x^3$
Body size–body mass	$r = 0.867**$ $y = -60.49 + 1.41x + 1.49x^3$	$r = 0.921**$ $y = -67.99 + 1.44x$	$r = 0.903**$ $y = 33.01 - 0.71x$	$r = 0.927**$ $y = -35.20 + 1.09x - 2.74x^3$

$*$ $P < 0.01$, $**$ $P < 0.001$

1988). We observed that individuals from the highland population attained maturity later (3–4 years) than individuals from the lowland population (2–3 years). However, in the study by Kutrup et al. (2011), it was reported as 2–3 years for both populations. Similar to the findings for our high-altitude population, Gokhelashvili and Tarkhnishvili (1994) determined age at sexual maturity as 2–3 years for males and 3–4 years for females in a Georgian population (1200 m a.s.l.). Since anurans are often exposed to cold temperatures at high altitudes, both their seasonal and daily activity periods are limited by low temperatures. Thus, delayed maturity is generally expected to be the case.

The age of *B. variabilis* adults was significantly correlated with their size for both lowland and highland populations as expected. In addition, a significant positive correlation was found between both body size and mass, and age and body mass, except in the males of the Adana population. Both Castellano et al. (1999) and Kutrup et al. (2011) found significant differences between island and

mainland populations of *B. variabilis*, indicating that island adults were on average heavier than mainland adults. In the present study, the average body mass of the highland population was significantly higher than that of the lowland population. This circumstance may be explained by the abundance of food and nutrition-dependent body size in Seydiköy.

In conclusion, we observed an altitudinal variation pattern in mean age, longevity, body size, and body mass between the *B. variabilis* populations. Thus, observing our results on the variation of life history traits between 2 populations from different altitudes in relation to climatic data, we can suggest that differences in temperature, food availability, human impact, and predator density promote variations in age, body size, and mass.

Acknowledgments

References

Adams DC, Church JO (2008). Amphibians do not follow Bergmann's rule. Evolution 62: 413–420.

Altunışık A, Özdemir N (2013). Body size and age structure of a highland population of *Hyla orientalis* Bedriaga, 1890, in northern Turkey. Herpetozoa 26: 49–55.

AmphibiaWeb (2014). Information on amphibian biology and conservation. [web application]. Berkeley, California: AmphibiaWeb. Available at: http://amphibiaweb.org/. (Accessed: 2014).

Ashkavandi S, Gharzi A, Abbasi M (2012). A skeletochronological estimation of age structure in a population of the *Bufo viridis* (Anuran: Amphibia) in Central Zagros Iran. Asian J Exp Biol Sci 3: 99–104.

Ashton KG (2002). Do amphibians follow Bergmann's rule? Can J Zool 80: 708–716.

Berrigan D, Charnov EL (1994). Reaction norms for age and size at maturity in response to temperature: a puzzle for life-historians. Oikos 70: 474–478.

Caetano MH, Castanet J (1993). Variability and microevolutionary patterns in *Triturus marmoratus* from Portugal: age, size, longevity and individual growth. Amphibia-Reptilia 14: 117–129.

Castellano S, Rosso A, Doglio S, Giacoma C (1999). Body size and calling variation in the green toad (*Bufo viridis*). J Zool 248: 83–90.

Castanet J, Smirina EM (1990). Introduction to the skeletochronological method in amphibians and reptiles. Ann Sci Nat Zool 11: 191–196.

Feder ME, Burggren WW (1992). Environmental Physiology of the Amphibians. Chicago, IL, USA: University of Chicago Press.

Ficetola GF, Scali S, Denoël M, Montinaro G, Vukov TD, Zuffi MAL, Padoa-Schioppa E (2010). Ecogeographical variation of body size in the newt *Triturus carnifex*: comparing the hypotheses using an information-theoretic approach. Global Ecol Biogeogr 19: 485–495.

Frost DR (2013). Amphibian Species of the World: an Online Reference. Version 6.0 (17 February 2014). Electronic database accessible at: http://research.amnh.org/herpetology/amphibia/index.html.

Gibbons MM, McCarthy TK (1984). Growth, maturation and survival of frogs *Rana temporaria* L. Ecography 7: 419–427.

Gokhelashvili RK, Tarkhnishvili DN (1994). Age structure of six Georgian anuran populations and its dynamics during two consecutive years. Herpetozoa 7: 11–18.

Gül S, Özdemir N, Üzüm N, Olgun K, Kutrup B (2011). Body size and age structure of *Pelophylax ridibundus* populations from two different altitudes in Turkey. Amphibia-Reptilia 32: 287–292.

Hasumi M (2010). Age, body size, and sexual dimorphism in size and shape in *Salamandrella keyserlingii* (Caudata: Hynobiidae). Evol Biol 37: 38–48.

Heino M, Metz JAJ, Kaitala V (1997). Evolution of mixed maturation strategies in semelparous life histories: the crucial role of dimensionality of feedback environment. Phil Trans R Soc B 353: 1647–1655.

Hemelaar A (1986). Demographic study on *Bufo bufo* L. (Anura, Amphibia) from different climates, by means of skeletochronology. PhD, University of Nijmegen, Nijmegen, the Netherlands.

Kutrup B, Bulbul U, Yilmaz N (2005). Age structure in two populations of *Triturus vittatus ophryticus* at different altitudes. Amphibia-Reptilia 26: 49–54.

Kutrup B, Çakır E, Çolak Z, Bülbül U, Karaoğlu H (2011). Age and growth of the green toad, *Bufo viridis* (Laurenti, 1768) from an island and a mainland population in Giresun, Turkey. J Anim Vet Adv 10: 1469–1472.

Laugen AT, Laurila A, Jonsson KI, Soderman F, Merila J (2005). Do common frogs (*Rana temporaria*) follow Bergmann's rule? Evol Ecol Res 7: 717–731.

Ledentsov AV, Melkumyan LS (1986). On longevity and growth rate in amphibians and reptiles in Armenia. Proc Zool Inst Acad Sci USSR 158: 105–110.

Lovich JE, Gibbons JW (1992). A review of techniques for quantifying sexual size dimorphism. Growth Dev Aging 56: 269–281.

Ma X, Lu X, Merila J (2009). Altitudinal decline of body size in a Tibetan frog. J Zool 279: 364–371.

Miaud C, Guyetant R, Faber H (2000). Age, size and growth of the Alpine newt, *Triturus alpestris* (Urodela, Salamandridae), at high altitude and a review of life-history trait variation throughout its range. Herpetologica 56: 135–144.

Morrison C, Hero JM (2003). Geographic variation in life-history characteristics of amphibians: a review. J Anim Ecol 72: 270–279.

Mullally DP, Cunningham JP (1956). Aspects of the thermal ecology of the Yosemite toad. Herpetologica 12: 57–67.

Olgun K, Üzüm N, Avcı A, Miaud C (2005). Age, size and growth of the southern crested newt *Triturus karelinii* (Strauch 1870) in a population from Bozdağ (western Turkey). Amphibia-Reptilia 26: 223–230.

Özdemir N, Altunışık A, Ergül T, Gül S, Tosunoğlu M, Cadeddu G, Giacoma C (2012). Variation in body size and age structure among three Turkish populations of the treefrog *Hyla arborea*. Amphibia-Reptilia 33: 25–35.

Özdemir N, Gül S, Poyarkov NA, Kutrup B, Tosunoğlu M, Doglio S (2014). Molecular systematics and phylogeography of *Bufotes variabilis* (syn. *Pseudepidalea variabilis*) (Pallas, 1769) in Turkey. Turk J Zool 38: 412-420.

Ryser J (1988). Determination of growth and maturation in the common frog, *Rana temporaria*, by skeletochronology. J Zool 216: 673–685.

Sinsch U, Leskovar C, Drobig A, König A, Grosse W (2007). Life-history traits in green toad (*Bufo viridis*) populations: indicators of habitat quality. Can J Zool 85: 665–673.

Sinsch U, Sherif N (1989). Osmoregulation of natterjack toads (*Bufo calamita*) in tap water, urea and NaCl solutions. Verh Dtsch Zool Ges 82: 307–308.

Stearns SC (1992). The evolution of life histories. London, UK: Oxford University Press.

Stöck M, Moritz C, Hickerson M, Frynta D, Dujsebayeva T, Eremchenko V, Macey JR, Papenfuss TJ, Wake DB (2006). Evolution of mitochondrial relationships and biogeography of Palearctic green toads (*Bufo viridis* subgroup) with insights in their genomic plasticity. Mol Phylogenet Evol 41: 663–689.

Üzüm N, Olgun K (2009). Age, size and growth in two populations of the southern crested newt, *Triturus karelinii* (Strauch 1870) from different altitudes. Herpetologica 65: 373–383.