

The first demographic data and body size of the southern banded newt, *Ommatotriton vittatus* (Caudata: Salamandridae)

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Abstract. In this study, the age structure and some life history traits were revealed for the first time in a population of *Ommatotriton vittatus*, living in Tarsus (mid-south part of Turkey), at the western limit of the species' range. Maximum longevity was recorded to be eight years in both females and males and age at maturity was estimated as three years for males and four years for females. Although mean age did not differ significantly between sexes, males had significantly larger snout-vent length (SVL) than females. Age and body size were positively correlated with each other for both females and males. Since the populations of the Southern Banded Newt in Turkey are in decline, the present study that provides preliminary data on life history traits of this newt could be helpful for future biological studies.

Keywords. Amphibia, age, demography, skeletochronology, life history, newt.

INTRODUCTION

The study of growth in amphibians is important because it directly influences age at sexual maturity, timing of reproductive events, fecundity, and longevity (Hemelaar, 1988; Smirina, 1994; Miaud and Merilä, 2000; Navas, 2006). Therefore, knowing age structure of a population is essential for obtaining information about the life history of the species (Altunışık and Özdemir, 2015).

Although there are several methods (e.g., body size correlation, mark and recapture and skeletochronology) to estimate the age and growth of amphibians and reptiles, skeletochronology which is based on counting the lines of arrested growth (LAGs) visible in histological sections, has been the most preferred method by researchers in recent years (Carranza et al., 2014; Bionda et al. 2015; Sinsch et al., 2015; Altunışık et al., 2016; Bülbül et al., 2016), as it yields reliable results. As previously described in many amphibian species, periosteal bone growth depends on ecological factors, e.g., changing favorable and unfavorable

periods of bone growth. Unlike active growth seasons, in the winter season the periosteal bone growth is reduced due to the influence of the cold climate and a different growth rate occurs, which forms the LAGs.

The differences in body size between males and females of the same species is a key feature that leads to significant biological insights (Denoël et al., 2009) and several hypotheses have been put forward to explain these variations. It is well documented that the basic factors that elicit sexual dimorphism are generally sexual selection, natural selection and fecundity selection (Andersson, 1994; Fairbairn, 1997; Pincheira-Donoso et al., 2008; Liao et al., 2015; Altunışık, 2017). In addition, sexual differences in age and growth rate associated with life history hypothesis may also cause the emergence of sexual size dimorphism (SSD), which is a well-known population property (Young, 2005; Cadeddu et al., 2012; Liao et al., 2013).

A member of the cosmopolitan family Salamandridae, *Ommatotriton* genus has three species, *Ommatotri-*

ton nesterovi, *Ommatotriton ophryticus* (Northern Banded Newt) and *Ommatotriton vittatus* (Southern Banded Newt; van Riemsdijk et al., 2017). Here we focus on the Southern Banded Newt, which is a medium-sized newt of 90–110 mm in total length. During the breeding period it inhabits shallow, still or slowly flowing waters with a lot of vegetation, otherwise found in wooded areas or open places with loose rocks or stones not far from water.

O. vittatus ranges from mid-south of Turkey, through western Syrian Arab Republic, Lebanon and northwestern Jordan, south to Israel (Olgun et al., 2009; IUCN, 2017; Fig. 1). Mid-south of Turkey represents the western limit of the species' range. There are two subspecies of *O. vittatus*, namely *O. v. vittatus* and *O. v. ciliensis*. While the distribution area of the first subspecies is Turkey, Syria and Israel, the distribution area of the second one is within the borders of Turkey (Bogaerts et al., 2013).

So far, demographic investigations have been confined to Northern banded newt (Kuzmin, 1999; Tarkh-nishvili and Gokhelasvili, 1999; Kutrup et al., 2005; Özcan and Üzümlü, 2015) of *Ommatotriton* genus and no data are available for the Southern Banded Newt. The aim of this study is to determine age structure (longevity, age at sexual maturity, survivorship, adult life expectancy), mean body size and SSD of a population of *O. vittatus* in the Southeastern Turkey, at the western limit of the species' range, using skeletochronology.

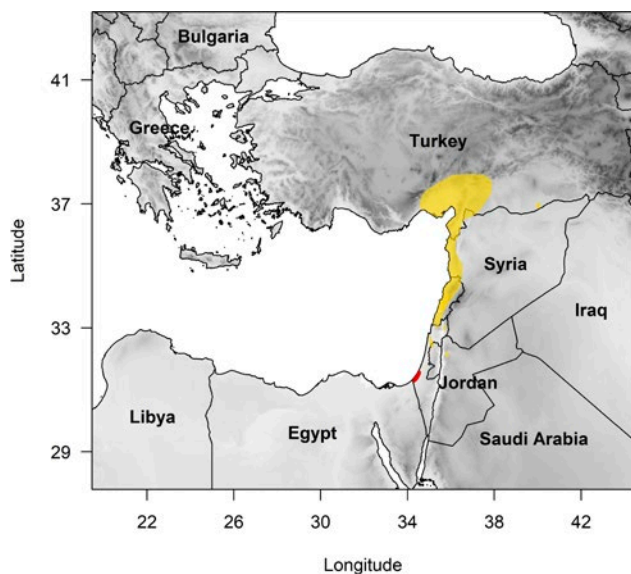


Fig. 1. Distribution map of *O. vittatus* (IUCN, 2017).

MATERIAL AND METHODS

Study area and species

The study site (25 m a.s.l.) is located in Tarsus, Mersin (36°54'N, 34°53'E) in the mid-south part of Turkey and has a climate that is Mediterranean and moderately continental. According to climatic data obtained from the meteorological station situated near the study site (Tarsus Meteorological Station) for the years 1950-2016, the average temperature during summer was 27.16°C, and 10.9°C in the winter (www.mgm.gov.tr).

A total of 40 adult *O. vittatus* individuals (23 males and 17 females) were caught by hand or using a dip net in the daytime during the breeding season in 2017. After anesthesia in MS-222, their snout-vent length (SVL) was measured using a digital vernier caliper (500-706-11, Mitutoyo, Tokyo, Japan) to the nearest 0.01 mm precision and the fourth toe of the left foot was clipped. In order to use toe samples in skeletochronological analysis, firstly they were fixed in 10% formalin and later preserved in 70% ethanol. After sampling, all newts were released back to their habitats. The sexes of the individuals were determined by externally visible secondary sexual characters (prominent cloaca and dorsal crest in males) (Bogaerts et al., 2013).

Skeletochronological analysis

We applied standard skeletochronology procedure that was described by Castanet and Smirina (1990) for laboratory protocols to assess the age structure. Firstly, we washed the phalanges kept in 70% ethanol in tap water then decalcified in nitric acid (5%) from 30 to 90 min depending on the bone structure. Using a freezing microtome (Shandon Cryostat, Germany), preferentially second phalange was cross-sectioned at 12-16 µm and stained with Harris hematoxylin for 10 min. The selected cross-sections from the narrow part of the phalanx diaphysis were placed in glycerin for scanning under a light microscope (Olympus BX51) at 200x and 400x magnifications and their photographs (1-10 for each cross-section) were taken by a camera (Pixera), which is connected to this microscope. The number of LAGs was assessed independently by two observers. The distance between two adjacent LAGs is a good indicator of individual growth in a given year (Kleinenberg and Smirina 1969). Therefore, in this study age at sexual maturity was determined using the distance between LAGs. Where we observed an obvious decrease in spacing between two subsequent LAGs, we took it to mark the age when sexual maturity was achieved (Ryser, 1988; Özdemir et al., 2012).

Data analysis

The normality of data was analyzed by Shapiro-Wilk test and the homogeneity of variances was analyzed by Levene test. After confirming that all data were normally distributed ($P > 0.05$), independent sample t-test was used to test if females and males differ in mean SVL or in mean age. SSD was quantified with Lovich and Gibbons (1992) sexual dimorphism index

SDI = (size of larger sex / size of smaller sex) \pm 1 (+1 if males are larger or -1 if females are larger), and arbitrarily defined as positive when females are larger than males and negative in the converse case.

Survivorship rate for adult individuals was calculated from the age structure according to following formula (Robson and Chapman, 1961): $S = T / (R + T - 1)$, where S is the finite annual survivorship rate estimate, $T = N_1 + 2N_2 + 3N_3 + 4N_4 + \dots + nN_n$, $R = \sum N_i$, and N_i is the number of individuals in the age group i. Adult life expectancy (ESP), the expected life span of the salamanders that have reached sexual maturity, was computed according to the formula of Seber (1973): $ESP = [0.5 + 1 / (1 - S)]$ where S represents the survivorship rate. Pearson's correlation coefficient was computed to measure the degree of relationship between SVL and age and in order to determine the correlation equation between the two variables; regression analysis was also performed. The growth patterns were estimated according to the von Bertalanffy growth model, which has been used earlier in several studies on amphibians (Ryser 1988; Miaud et al., 2000; Guarino et al., 2011). The general form of the von Bertalanffy growth equation used was $L_t = L_{\infty} (1 - e^{-k(t-t_0)})$, where L_t is length at age t, L_{∞} 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 metamorphosis, which is the starting point of the growth curve in the present study. Because data on the size at metamorphosis of the studied population were lacking, we used size at metamorphosis ($L_0 = 21.5$ mm), provided by Tarkhnishvili and Gokhelasvili (1999). The parameters L_{∞} and k were estimated using Microsoft Excel program. Data analysis was conducted using SPSS 17 statistical software package (IBM SPSS Statistics for Windows).

RESULTS

LAGs recorded in phalange bones were clearly visible in all cross-sections, as it can be seen in Fig. 2. According to the results of age reading obtained by direct observation in the microscope and photographs on the computer, the maximum lifespan was 8 years in both females and males. Females of *O. vittatus* were on average 5.82 ± 0.30 years old (range: 4-8) and males had an average age of 5.65 ± 0.29 years (range: 3-8; Table 1).

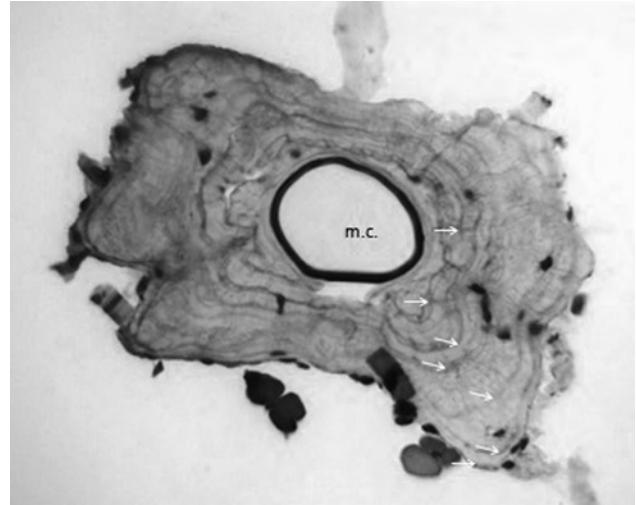


Fig. 2. Cross-section of a phalanx of an individual at the age of 7. White arrows show LAGs. m.c. = marrow cavity.

No significant difference was detected between the sexes with regard to mean age ($t = 0.407$, $df = 38$, $P > 0.05$) and age distribution (Kolmogorov-Smirnov test, $P > 0.05$). As it can be seen from age distribution of the population (Fig. 3), the age group with the highest frequency is six (14 individuals). Skeletochronological pattern showed that males and females attained sexual maturity at age of 3 and 4, respectively. Since age structure did not differ between the sexes, S and ESP calculations were combined and S was found to be 0.79 and ESP was 5.26 years.

SDI was -0.055, indicating a male biased size dimorphism. Independent sample t-test ($t = -2.327$, $df = 38$, $P < 0.05$) showed that males had significantly larger SVL than that of females. For both sexes, the maximum SVL recorded was higher than the estimated asymptotic SVL (SVL_{asym} : males, 48.74 mm; females, 47.28 mm). The growth coefficient was higher in males than in females (k: males, 1.80; females, 1.49).

Table 1. Descriptive statistics for the studied population.

Sex	N	Age (years)		SVL (mm)		ESP (years)	S
		Mean \pm SE	Range	Mean \pm SE	Range		
Females	17	5.82 ± 0.30	4-8	45.60 ± 0.79	40.38-52.30	5.26	0.79
Males	23	5.65 ± 0.29	3-8	48.12 ± 0.72	41.72- 54.15		

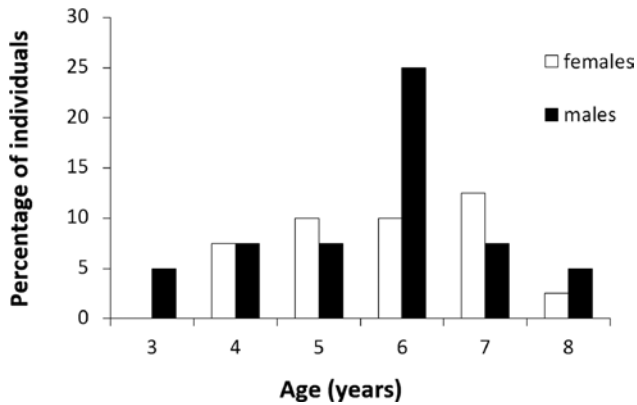


Fig. 3. Age distribution of *O. vittatus* from Tarsus population.

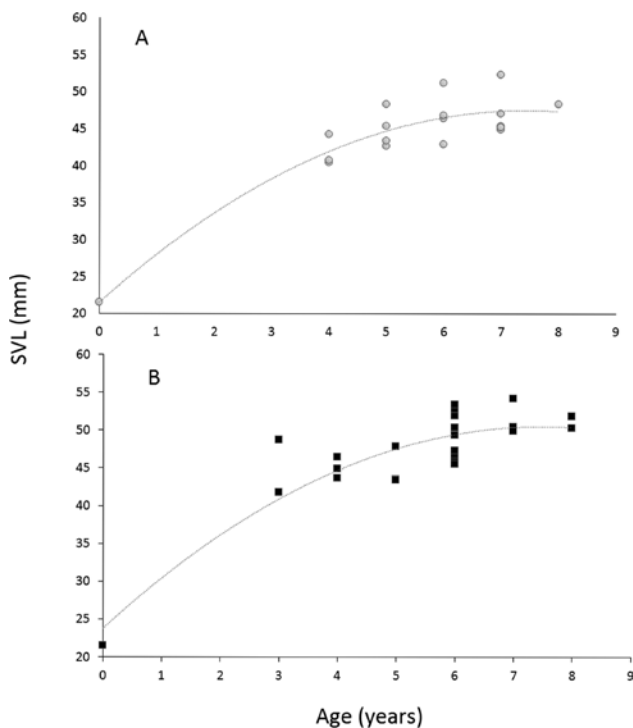


Fig. 4. Relationship between age and body size (SVL). Females (A) and males (B).

Body size was positively correlated with age in both females (Pearson's correlation $r = 0.589$, $P < 0.05$; Fig. 4A) and males ($r = 0.648$, $P < 0.01$; Fig. 4B).

DISCUSSION

The first demographic data on an *Ommatotriton vittatus* population was provided in this study. The results suggest that Southern Banded Newt has a lower lifespan than

the Northern Banded Newt. We found that maximum age could be reached at 8 years in both males and females in Tarsus population. In congeneric *O. ophryticus sensu lato*, maximum lifespan was recorded as 21 years for Georgian populations (Kuzmin, 1999; Tarkhnishvili and Gokhelasvili, 1999) and 10 and 16 years for the low and high altitude Turkish populations, respectively (Kutrup et al., 2005; Özcan and Üzümlü, 2015). In the present study, no significant difference was detected between females and males with regard to mean age. A similar result was recorded for Bahçesultan and Erbaa populations of *O. ophryticus sensu lato* (Özcan and Üzümlü, 2015).

Delayed maturity and longer lifespan are expected phenomena for high-elevation populations, because they are exposed to colder temperatures and shorter growth seasons in comparison with low-elevation populations. In line with this trend, the studied low-altitude population of *O. vittatus* had lower lifespan and age at maturity than that of high-altitude populations of *O. ophryticus sensu lato*, formerly referred to as *Triturus vittatus ophryticus*, (Kuzmin, 1999; Tarkhnishvili and Gokhelasvili, 1999; Kutrup et al., 2005; Özcan and Üzümlü, 2015).

It is expected that amphibians with indetermined growth should enhance not only their age at maturity but also division of resources between reproduction and growth over the years following sexual maturation (Kozłowski and Uchmanski, 1987). Generally, amphibians reach sexual maturity when they reaches a certain size, therefore sexual maturity may vary between different species and populations (Gibbons and McCarthy, 1984; Hemelaar, 1986; Ryser, 1988). The age at maturity, which is determined to be 3-4 years, is compatible with the two populations of *O. ophryticus* (Kutrup et al., 2005). However, in the Tosya and Bahçesultan populations of *O. nesterovi* and Erbaa populations of *O. ophryticus* located at higher altitudes, the age at maturity was determined as 4-6 years (Özcan and Üzümlü, 2015). Kuzmin (1999) stated that the sexual maturity was attained at 3-5 years in *O. ophryticus* for Caucasus populations. Similarly, Tarkhnishvili and Gokhelasvili (1999) established the earliest age at maturation in Georgian *O. ophryticus* populations from near Tbilisi as 4 years for females and 3 years for males (Table 2).

Sexual size dimorphism is common among amphibians. More specifically, about 90% of the anurans and 61% of the urodeles indicate a female-biased SSD (Shine, 1979; Kupfer, 2007; Reinhard et al., 2015). Interestingly, our results show that males of *O. vittatus* did not conform to this tendency but indicate male-biased dimorphism. Similar results were reported by Kutrup et al. (2005), Çiçek and Ayaz (2011), Başkale et al. (2013), Özcan and Üzümlü (2015) and Cvijanović et al. (2017) for dif-

Table 2. Demographic life history traits in *Ommatotriton* species throughout its geographical range.

Species	Location	Altitude (a.s.l.,m)	Sex	Mean age (years)	Age at maturity (years)	Longevity (years)	Mean SVL (mm)	References
<i>O. ophryticus</i>	Different parts of Georgia	-	Male	-	3-5	8-21	-	Kuzmin (1999)
			Female	-	3-5	8-21	-	
<i>O. ophryticus</i>	Akhdaldaba, Georgia	750	Male	5.53	3	8	-	Tarkhnishvili and Gokhelasvili (1999)
			Female	6.97	4	12	-	
<i>O. ophryticus</i>	Gürbulak, Trabzon, Turkey	300	Male	4	2-3	10	61.7	Kutrup et al. (2005)
			Female	4	2-3	10	53.3	
<i>O. ophryticus</i>	Hıdırnebi, Trabzon, Turkey	1300	Male	8.2	4	16	59.6	Kutrup et al. (2005)
			Female	9.2	4	16	51.0	
<i>O. nesterovi</i> *	Sakarya, Turkey	31	Male	4.92	3	8	90.79	Başkale et al. (2013)
			Female	4.90	2	6	86.06	
<i>O. nesterovi</i> *	Bahçesultan, Bilecik, Turkey	1015	Male	7.4	6	10	72.39	Özcan and Üzümlü, 2015
			Female	7.4	6	10	63.40	
<i>O. nesterovi</i> *	Tosya, Kastamonu, Turkey	1202	Male	6.4	4	9	64.07	Özcan and Üzümlü, 2015
			Female	7.06	5	9	55.57	
<i>O. ophryticus</i>	Erbaa, Tokat, Turkey	1239	Male	8.2	6	12	71.07	Özcan and Üzümlü, 2015
			Female	8.4	5	11	60.36	
<i>O. vittatus</i>	Tarsus, Mersin, Turkey	25	Male	5.65	3	8	48.12	<
			Female	5.82	4	8	45.60	

SVL, snout-vent length; *referred to as *O. ophryticus*

ferent populations of *O. ophryticus sensu lato*. Although rarely found in the literature, males of some urodele species (e.g., *Lissotriton vulgaris*, Halliday and Verrell, 1988; *Mertensiella caucasica*, Üzümlü, 2009; *Lyciasalamandra atifi*, reviewed in Reinhard et al., 2015) are larger than females in terms of mean body size. Most of the variation in size dimorphism has been considered to result from differences in age structure between sexes (Monnet and Cherry, 2002; Cadetdu et al., 2012; Liao et al., 2013).

The annual survival rate of this newt differs from the other congeneric species (*O. nesterovi*), because it was calculated as 0.35 for a long-term mark-recapture and skeletochronological study (Başkale et al., 2013). The fact that Southern banded newts have a higher annual survival rate than Northern Banded newt may lead to differences in the potential reproductive lifespan between these two newts. It is widely assumed that age and body size are positively correlated in amphibians (Halliday and Verrell, 1988). Although there is no positive correlation for some species, in some cases this correlation may vary depending on sex. Similar to *O. nesterovi*'s Tosya, Bahçesultan (Özcan and Üzümlü, 2015) and Sakarya (Başkale et al., 2013) and *O. ophryticus*'s Erbaa (Özcan and Üzümlü, 2015) populations, in the studied *O. vittatus* population, body size of both males and females showed positive significant correlations with age. Contrarily, no correlation was reported between body size and age in both popu-

lations of Northern banded newt (Kutrup et al., 2005). This discrepancy among the congeneric species may be explained by the decrease of growth rates after sexual maturity is achieved, as well as by the different interannual reproductive effort of individuals, resulting in different individual growth curves (Kutrup et al., 2005).

In conclusion, as mentioned before the relatively low longevity and age at maturity observed in Southern banded newt could result from local ecological conditions (e.g., altitude, temperature, length of the activity period). It is also necessary to investigate more populations exposed to various conditions in order to evaluate more precisely the interpopulational variation of these life-history characteristics in *O. vittatus*.

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