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ОРИГИНАЛЬНЫЕ СТАТЬИ

AGE DETERMINATION AND GROWTH PARAMETERS OF *TRITURUS ANATOLICUS* (CAUDATA: SALAMANDRIDAE)

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This study presents data on the demographic structure of *Triturus anatolicus* in Torkul Lake in the Province of Düzce, Turkey. Field studies were conducted during the peak of the reproductive season from 2017 to 2019. A total of 51 samples (phalangeal bones from 13 males, 35 females and three sub-adults) were collected to determine the demographic structure of the population by skeletochronology. Snout-vent length (SVL) was 72.03 ± 2.79 mm (min-max: 67–78 mm) in males and 73.12 ± 4.59 mm (min-max: 63–84 mm) in females, where the difference was not statistically significant. The sexual dimorphism index was 0.01 and it was found to be female-biased. The mean age of females was 5.94 ± 1.85 years and the mean age of males was 5.77 ± 1.79 years. The minimum reproductive age was three years in both sexes. The maximum observed age was ten years for females and nine years for males. Our results were compared with literature data, and *Triturus anatolicus* showed a similar pattern on age-structure with cognate species. We found that the high survival rates of the Torkul Lake population (female = 0.81; male = 0.77) may result from a low human interference and favourable climatic conditions.

Key words: Amphibia, Anatolia, growth rate, longevity, maturity age, survival rate

Introduction

In recent years, the taxonomy of *Triturus karelinii* (Strauch, 1870) was revised based on the presence of discrete nuclear DNA gene pools, deep genetic divergence of mitochondrial and nuclear DNA. The name of *Triturus ivanbureschi* Arntzen & Wielstra, 2013 was proposed for the central/western species that includes the populations of the south-eastern Balkan Peninsula and Turkey (Wielstra et al., 2013). Subsequently, Wielstra & Arntzen (2016) found that *T. ivanbureschi* had two different gene pools with a restricted gene flow in Turkey and suggested this taxon had better be treated as two distinct (albeit morphologically cryptic) species. As a result, Wielstra & Arntzen (2016) proposed the name of *Triturus anatolicus* Wielstra & Arntzen, 2016 for the eastern species with all populations in Turkey. The type locality of the newly described *Triturus anatolicus* is the Gölköy population (Kalecik, Ankara-Turkey), while the distribution area of this species is the Western and Central Black Sea Region of Turkey (Fig. 1). The distribution of *T. ivanbureschi* is limited to the Marmara Region and the central and northern Aegean region in Turkey. Also, a hybridisation zone of both species was documented in the western distribution area of *T. anatolicus* based on mtDNA and nuclear DNA data (Wielstra & Arntzen, 2016).

Skeletochronology uses physiological activity able to seasonal changing. It is an effective tool to evaluate population age structure models in amphibian species with the least damaging animals (Guarino et al., 1999; Esteban & Sanchiz, 2000). It is based on the count of incremental lines of growth in mineralised tissues (Castanet et al., 1977, 1993; Halliday & Verrell, 1988). The annual accumulated calcium layers in the finger bones were mostly used to determine the age of both anuran and urodelan species (e.g. Miaud et al., 1993, 2000; Cvetković et al., 1996; Guarino et al., 1999; Coğalniceanu & Miaud, 2003; Jakob et al., 2003; Olgun et al., 2005; Yılmaz et al., 2005; Başkale et al., 2018; Arısoy & Başkale, 2019).

The decline in amphibian populations in the world in recent years is currently well-documented (Wake, 1991; Alford & Richards, 1999; Houlahan et al., 2000; Gardner, 2001; Devitt et al., 2019). For this reason, the factors causing a population decline are important to understand for a better protection of amphibians and to safeguard their persistence. Therefore, a detailed accumulation of data is required, which includes many parameters such as ecosystems, age structure, reproductive and feeding data, life cycles, population structure and dynamics of the species. In this context, we aimed to determine the demographic structure of the Torkul population of *T. anatolicus* using skeletochronology.

Fig. 1. Distribution map of two species of southern crested newts. The green colour is the *Triturus anatolicus* distribution area. The red colour is the *Triturus ivanbureschi* distribution area. The dot refers to the type locality of *Triturus anatolicus* (Gölköy population), while the star indicates the current study site (Lake Torkul).

Material and Methods

Study site and field studies

Lake Torkul (40.679250 N, 31.173200 E; 1290 m a.s.l.) is located within the boundaries of the Uğurköy and Düzce provinces. It is a natural lake with an area of 0.05 km^2, positioned in a volcanic depression in the Torkul plateau (Fig. 1). The surroundings of Lake Torkul are covered with coniferous and deciduous angiosperm forest and the shoreline has a vegetation of moist meadows. Lake Torkul is rich in aquatic plant species. We found that Lake Torkul also hosts other amphibian species, namely *Ommatotriton ophryticus* (Berthold, 1846), *Bufo bufo* (Linnaeus, 1758), *Bufotes variabilis* (Pallas, 1769), *Hyla orientalis* Bedriaga, 1890 and *Pelophylax ridibundus* (Pallas, 1771). Although human-induced changes in habitat are low compared to other similar habitats, the presence of a picnic area, camping activities and hunting forestry activities, put natural life under pressure.

Individuals of *T. anatolicus* were captured with a dip net during the spring of the breeding seasons in 2017–2019. The snout-vent lengths (SVL) were measured with dial callipers at 0.02 mm accuracy. We determined the sex of individuals based on the secondary sexual characters: males have crests on their back and a tail during their breeding phase. According to the standard skeletochronological technique (Castanet & Smirina, 1990) to determine the age of an individual animal, the longest digit of the hind foot was cut and fixed in 70% ethanol until laboratory studies.

Laboratory studies

The fixed bone samples were processed following the routine skeletochronological procedure (Castanet & Smirina, 1990; Castanet et al., 1993; Miaud et al., 1993; Smirina, 1994; Olgun et al., 2005). The finger bones of each animal were cleaned from the tissues such as muscle and skin, washed in running water for 12–14 h, decalcified for 2–3 h in 5% nitric acid and then placed in distilled water overnight. The bones were dehydrated using a graded ethanol series and then cleared in xylene, before embedding in paraffin. We made 14–16 µm thick cross-sections from the central region of the diaphysis using a rotary microtome (LEICA RM 2145). The cross-sections were stained with Ehrlich's Haematoxylin and Eosin and analysed under an Olympus CX31 light microscope equipped with a digital camera (Kameram 5). The age of each amphibian was determined by both authors who independently counted the number of Lines of Arrested Growth (LAGs) in each of the bone cross-sections.

Statistical analysis

The age and SVL data were normally distributed (Kolmogorov-Smirnov D test, all $p > 0.05$), thus allowing comparisons using parametric tests.

We used an independent samples t test to compare morphometrically the sexes. All statistical analyses were conducted using SPSS v. 20.0 (SPSS, 2011). We assumed that the age at first reproduction (maturity age: AM) is the lowest age recorded among the breeding individuals.

The sexual dimorphism index (SDI) was calculated according to the formula:

$$SDI = \frac{\text{The mean length of the larger sex}}{\text{The mean length of the smaller sex}} \pm 1$$

This formula was generated by Lovich & Gibbons (1992). The plus-minus sign (±) gives +1, if males are larger than females, defining the result as negative, and -1, if females are larger than males, defining the result as positive.

Growth was estimated according to the von Bertalanffy equation (von Bertalanffy, 1938) used previously in several studies of amphibians (i.e. Miaud et al., 2001; Guarino et al., 2011; Gül et al., 2011; Üzüm et al., 2011; Erişmiş, 2018). The modified growth was calculated according to the formula:

$$SVL_t = SVL_{\max} - (SVL_{\max} - SVL_{met})e^{-k(t-t_{met})}$$

where SVL_t is the average body length at age *t*. SVL_{max} is the asymptotic maximum body length. SVL_{met} is the body length at metamorphosis that was used to measured newly metamorphosed individuals at late spring (25–30 May 2019) fixed to mean 28.3 ± 1.35 mm for the Torkul population. The indicator *k* is the body growth rate coefficient (units are yr-1) defining the curve shape. The indicator t_{met} is the age at metamorphosis (0.3). The parameters SVL_{max} and *k* and their asymptotic confidence intervals (CI) were estimated by nonlinear regression.

The annual growth rate (AGR) is the rate of the difference between the mean SVL of the individuals in each age group *i* and the mean SVL in each age group *i*-1. It is calculated according to the formula used previously for amphibians (Erişmiş, 2018; Arısoy & Başkale, 2019):

$$AGR = \frac{\text{The mean SVL of the Age}_{i} - \text{The mean SVL of the Age}_{i-1}}{\text{The mean SVL of the Age}_{i-1}}$$

The survival rates assume a constant survival rate across all age classes and sampling of individuals with respect to age. They were estimated according to Robson & Chapman (1961) using the following formula:

$$S = \frac{T}{R + T - 1}$$

where S is the finite annual survival rate estimate, T is $N_1+2N_2+3N_3+\ldots+N_i$, and R is ΣN_i, where N_i is the number of individuals in age group *i*.

Adult life expectancy (ESP) is the expected average age. It differs from the longevity value, which is the highest recorded age among individuals. ESP was derived from Seber (1973) and calculated according to the formula where S is the survival rate:

$$ESP = 0.5 + \frac{1}{1-S}.$$

Results

A total of 51 individuals (13♂, 35♀ and three sub-adults) were examined in this study. All examined bones in adult newts had well-defined LAGs and endosteal bones with visible layers. They appeared as thin and approximate concentric layers, more intensely stained than the rest of the cross-section. Endosteal resorption was observed in 33 of the total examined cross-sections (65%) while no double LAGs were found in any sample.

The mean SVL was 74.1 ± 4.59 mm (min-max = 63–83 mm) in females and 72.0 ± 2.79 mm (min-max = 68–78 mm) in males. The difference between them was not significant (Student t test: t = 1.539; SD = 46; p > 0.05). The sexual dimorphism index was calculated as 0.01, indicating a female bias that also supported the species exhibits sexual dimorphism in size. These data were supported by the sex specific asymptotic size of individuals in the population.

The inferred age was related to size (Table 1). Age, size and growth parameter variations within the Torkul population are given in Table 2. The age distribution was not significantly different between sexes in the Torkul population (Student t test: t = 0.292; SD = 46; p > 0.05). The age distribution of the Torkul population is demonstrated in Fig. 2. Our results showed a maturity age of three years in both sexes. The longevity of males was nine years whereas it was ten years in females.

The mean ESP of females and males was estimated as eight and six years respectively. Similarly, the mean survival rate of females and males was calculated as 0.81 and 0.77 respectively. In the Torkul population, growth patterns between males and females were compared according to the von Bertalanffy growth model (Fig. 3). We found that the AGR was higher in females than in males, while the peak of the growth rate was found in the age range of 1–3 years (Fig. 4). Then, this slowly decreased to the age range of 3–4 years in both sexes.

Table 1. Body size and age distribution of *Triturus anatolicus* depending on age classes and sex

Age, years	Sex	n	The mean SVL, mm	SD	min–max
0	Newly metamorphosed	14	28.30	1.35	25.8–31.2
1	Sub-adults	1	39.30	–	–
2	Sub-adults	2	51.80	3.54	49.3–54.3
3	Females	2	65.60	3.97	62.7–68.3
3	Males	1	67.80	–	–
4	Females	6	71.00	2.99	67.6–74.9
4	Males	3	69.50	1.63	68.1–71.3
5	Females	10	72.10	2.55	68.7–75.9
5	Males	2	71.20	1.62	70.0–72.3
6	Females	4	74.10	1.30	72.8–75.9
6	Males	2	72.80	1.46	71.8–73.9
7	Females	5	76.20	2.06	73.8–78.8
7	Males	3	73.20	0.32	72.9–73.6
8	Females	4	78.30	1.20	77.2–79.7
8	Males	1	74.60	–	–
9	Females	3	81.00	1.81	79.1–82.7
9	Males	1	77.80	–	–
10	Females	1	83.20	–	–

Note: SVL – snout-vent length, n – number of samples, SD – standard deviation, min – minimum value, max – maximum value.

Table 2. Comparison of age, body size and growth parameters for females and males of *Triturus anatolicus*

Parameters	Female	Male
Mean SVL ± SD, mm	73.12 ± 4.593	72.03 ± 2.793
Asymptotic SVL, mm	97.37	93.19
Growth coefficient	0.188	0.196
Survival Rate	0.81	0.77
ESP	8.01	6.25
Mean age ± SD, years	5.94 ± 1.846	5.77 ± 1.787
Age at maturity, years	3	3
Longevity, years	10	9
von Bertalanffy growth curve	$SVL_t = 97.37-(97.37 - 28.3)e^{-0.188(t - 0.3)}$	$SVL_t = 93.19-(93.19 - 28.3)e^{-0.196(t - 0.3)}$

Note: SD – standard deviation, SVL – snout-vent length, ESP – Adult life expectancy.

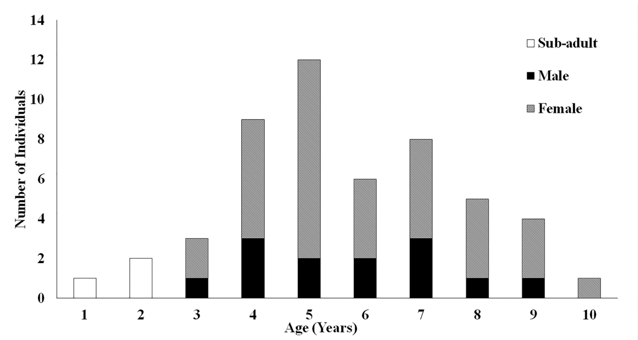

Fig. 2. Age distribution of sub-adult, male and female individuals of *Triturus anatolicus* in the studied Torkul population.

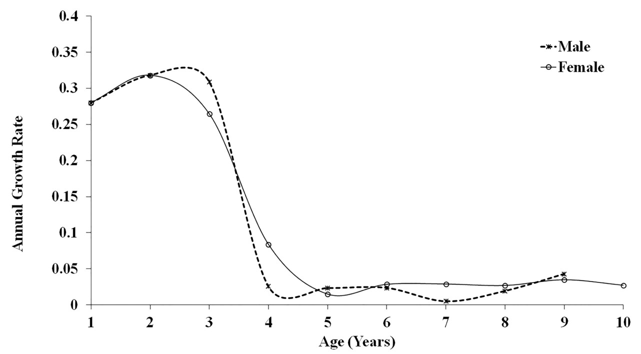

Fig. 4. The annual growth rate in male and females of *Triturus anatolicus*.

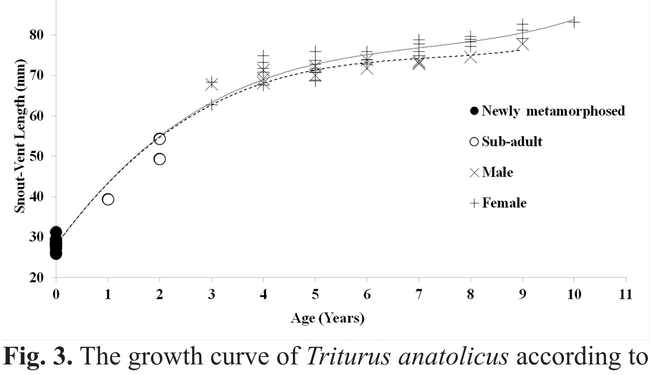

Fig. 3. The growth curve of *Triturus anatolicus* according to von Bertalanffy equation.

Discussion

Generally, females have a slightly higher SVL than males in urodelan species. At the same time the larger body size in females is related to fecundity, because there is a positive correlation between female body size and fecundity (Duellman & Trueb, 1994), while a larger body size in males is an advantage for competition among males (Shine, 1979). Wielstra & Arntzen (2016) reported that the SVL for *T. anatolicus* ranges from 50 mm to 63 mm in males and from 56 mm to 65 mm in females. In this study, we determined that the mean SVL for *T. anatolicus* is 72 ± 2.79 mm for males and 74 ± 4.59 mm for females.

We found a high frequency of endosteal resorption in the *T. anatolicus* Torkul population. Endosteal resorption is a case occurred commonly in amphibian long bones as in tetrapods (Castanet & Smirina, 1990). This phenomenon induces to wear out periosteal bone in bone marrow's side gap. Smirina (1972) suggested that endosteal resorption is related to environmental conditions. It is suggested that the frequency of endosteal resorption in a population of amphibians living in highland habitats is lower (Esteban et al., 1996, 1999) or higher (Castanet & Caetano, 1993) than in lowland populations. Interestingly, Hemelaar (1988) showed a relation between endosteal resorption severity and climatic conditions in a European population of *Bufo bufo*.

Growth lines, which are usually seasonally synchronised, are derived from a genetically controlled growth cycle (Esteban et al., 1996; Alcobendas & Castanet, 2000). Being variable among populations, the LAG storage structure is exposed to various environmental conditions. For example, the existence of LAGs is determined in both winter and summer in populations of *Triturus marmoratus* (Latreille, 1800) in the mountains in the north of Portugal (Caetano et al., 1985) and in populations near the Mediterranean coast in France (Jakob et al., 2002). On the other hand, only summer LAGs of *Triturus pygmaeus* (Wolterstorff, 1905) (referred as *T. marmoratus*) in Southern Portugal populations is monitored (Castanet & Caetano, 1993), while only winter LAGs is monitored in *T. marmoratus* populations in the lowlands in northern Portugal and in different geographic locations in northern regions (e.g. Western France) (Francillon-Vieillot et al., 1990). *Lyciasalamandra fazilea* (Başoğlu & Atatür, 1974) (referred as *Mertensiella luschani* (Steindachner, 1891)) in south-western Turkey displays growth lines composed in winter and summer seasons (Olgun et al., 2001). This study showed that the Torkul population of *T. anatolicus* does not have a double LAG. This means that the species is active in spring and summer months, while it goes into hibernation in hard winter months.

In *Triturus* species, the first age of maturity varies from two to four years, while the longevity is 8–15 years (Hagström, 1977; Dolmen, 1982, 1983; Francillon-Vieillot et al., 1990; Castanet & Caetano, 1993; Miaud et al., 1993; Cvetković et al., 1996; Tarkhnishvili & Gokhelashvili, 1999; Cogălniceanu & Miaud, 2003; Jakob et al., 2003; Sinsch et al., 2003; Maletzky et al., 2004; Olgun et al., 2005; Üzüm & Olgun, 2009; Lukanov & Tzankov, 2016), and the longest life time was found in the *T. cristatus* (Laurenti 1768) complex (Hagström, 1977; Francillon-Vieillot et al., 1990; Miaud et al., 1993; Sinsch et al., 2003). We found that the mean age of females is 5.94 years and the mean age of males is 5.77 years. In the Torkul Lake population of *T. anatolicus*, the age distribution was observed between 3–10 years in females and 3–9 years in males. The adult life expectancy was calculated for females and males as eight and six years respectively. The first reproduction age and longevity are being changed depending on local climatic conditions or elevation to latitude. Permanent or temporal habitats drying in the summer months are another factor affecting the age structure of a population. Diaz-Paniagua et al. (1996) claimed that amphibian species which have a large body size have a longer life span in permanent habitats. Olgun et al. (2005) emphasised that continental and arid climate habitat indicate the observed short life span and higher death risk of *T. karelinii* (= *ivanbureschi*). Similarly, a high mortality has been monitored in *T. dobrogicus* (Kiritzescu, 1903) (Coğalniceanu & Miaud, 2003) and *T. marmoratus* (Jakob et al., 2003). We have calculated that the survival rate of *T. anatolicus* was 0.81 in female and 0.77 in males. In other words, 81% of females and 77% of males survive during the study years.

Conclusions

We provided the first data on body size, growth rate, adult life expectancy, survival rate, maturity age and longevity of *T. anatolicus* from the Lake Torkul population using the skeletochronology method. The population showed high survival rates in both sexes. These high survival rates indicate that Lake Torkul is not considerably affected by human interference, and that climatic conditions are suitable for the survival of *T. anatolicus*, as well as, possibly, also for the other amphibian species living there.

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ОПРЕДЕЛЕНИЕ ВОЗРАСТА И ПАРАМЕТРЫ РОСТА *TRITURUS ANATOLICUS* (CAUDATA: SALAMANDRIDAE)

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В этом исследовании представлены данные о демографической структуре популяции *Triturus anatolicus* в озере Торкуль недалеко от провинции Дюздже (Турция). Полевые исследования проводились во время пика репродуктивного сезона в 2017–2019 гг. В общей сложности 51 образец (фаланговые кости от 13 самцов, 35 самок и трех особей предвзрослого возрастного состояния) был собран для определения демографической структуры популяции с помощью скелетохронологии. Длина тела от клоаки до кончика морды (SVL) составила 72.03 ± 2.79 мм (min-max: 67–78 мм) у самцов и 73.12 ± 4.59 мм (min-max: 63–84 мм) у самок, где статистически значимых различий не было выявлено между особями разного пола. Индекс полового диморфизма составил 0.01. Было обнаружено, что он смещен в сторону самок. Средний возраст самок составлял 5.94 ± 1.85 года, а средний возраст самцов – 5.77 ± 1.79 года. Минимальный репродуктивный возраст для обоих полов составил три года. Максимальный наблюдаемый возраст составлял десять лет для самок и девять лет для самцов. Наши результаты были сопоставлены с литературными данными, и *Triturus anatolicus* показал сходную закономерность в возрастной структуре с родственными видами. Мы обнаружили, что высокая выживаемость популяции озера Торкуль (самки = 0.81; самцы = 0.77) может быть результатом незначительного вмешательства человека и благоприятных климатических условий.

Ключевые слова: амфибии, Анатолия, долголетие, зрелый возраст, процент выживаемости, скорость роста