

AGE AND GROWTH OF THE SOUTHERN CRESTED NEWT,
TRITURUS KARELINII (STRAUCH 1870), IN A LOWLAND
POPULATION FROM NORTHWEST TURKEY

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We estimated the age, size, and growth patterns of the Southern Crested Newt, *Triturus karelinii*, from northwest Anatolia using skeletochronology. Lines of arrested growth (LAGs) recorded in phalanges allowed us to estimate the age of juveniles and adults. Results showed that age at maturity in this population was 3 years. The average ages of the males and females in this population were calculated as 5.07 ± 1.36 and 5.00 ± 1.63 years, respectively. Males attained a maximum age of 9 years, whereas females attained a maximum age of 8 years. No significant differences were found between the age distributions of the two sexes. Mean body length of males was significantly smaller than that of females. Growth of the Southern Crested Newt was well described by the von Bertalanffy model, and males and females did not differ in growth parameters. These growth parameters were compared with those obtained from other amphibians, especially salamandrids.

Key words: skeletochronology, age structure, growth, *Triturus karelinii*, Turkey

INTRODUCTION

Climatic conditions affect the metabolism of ectotherms and their influences are recorded in bone. The annual life cycle of amphibians can be deduced from the growth marks in the bone tissue (CASTANET & SMIRINA 1990, SMIRINA 1994). The formation of annual bone growth layers in amphibians has been confirmed by a number of studies (e.g. FRANCILLON 1980, HEMELAAR & VAN GELDER 1980, FRANCILLON & CASTANET 1985) and the counting of annual bone layers is a routine method of age determination of amphibians (CAETANO 1990, CASTANET & SMIRINA 1990, ESTEBAN 1990, SMIRINA 1994). Skeletochronology can be a reliable and powerful tool to assess individual mean longevity, growth rates and sexual maturity (CASTANET *et al.* 1993, SMIRINA 1994). Skeletochronology has been used to study a variety of anurans (PLYTYCZ *et al.* 1995, KUZMIN & ISHCENKO 1997) and urodeles (VERRELL & FRANCILLON 1986, LIMA *et al.* 2001).

The range of *T. karelinii* covers the south and west coast of the Black Sea region and the south and east coast of the Caspian Sea up to West Serbia (TARKH-NISHVILI 1996). In Turkey, it occurs across north western Anatolia, the central Black Sea region, the eastern Black Sea, Central Anatolia, European Turkey, the

Marmara region and the Aegean region (BARAN *et al.* 1992). The increasing number of skeletochronological studies available on the Southern Crested Newt, *Triturus karelinii* (OLGUN *et al.* 2005) allows for comparison of adult age, size and growth throughout its geographic range. We studied age structure of a population of *T. karelinii* from Arifiye, Adapazarı in northwestern Turkey in order to provide information on age structure and growth for this newt species. The altitudinal range of *T. karelinii* population is from sea level to 2100 m in Turkey (BAŞOĞLU *et al.* 1994, BARAN & ATATÜR 1998) and the focal population we studied was a low elevation population.

MATERIAL AND METHODS

The population examined was located in Arifiye (40°41'N, 30°22'E) at an altitude of 43 m above sea level. The animals breed in ditches that dry up in the summer and hold water during the autumn and winter. The ditches lay between the road and houses.

The newts used in the present study (30 males, 24 females and 20 juveniles) were caught by dipping a net in the water during the breeding seasons in April 2002 and March 2005. The sex of each individual was determined by examination of external secondary sexual characters. Males have a dorsal crest and prominent (swollen) cloaca. Juveniles, which are smaller in body length, lack the dorsal crest and prominent cloaca. Newts were measured from the tip of the snout to the posterior margin of the vent (SVL) by using a dial caliper with an accuracy of 0.02 mm. Length conversion are given using the linear equation as $Y = a + bX$ where a = intercept, b = slope. Sexual size dimorphism was estimated with the LOVICH and GIBBONS (1992) sexual dimorphism index (SDI):

$$SDI = \left(\frac{\text{size of larger sex}}{\text{size of smaller sex}} \right) \pm 1$$

+1 if males are larger or -1 if females are larger, and arbitrarily defined as positive when females are larger than males.

The longest finger of the forelimb was excised and preserved in 70% ethanol.

Age was determined by using skeletochronological analysis (CASTANET & SMIRINA 1990, OLGUN *et al.* 2005). The phalanges stored in 70% ethanol solution were dissected; the largest bone of the phalange was washed in running water for about 24 hours, decalcified in 5% nitric acid for 2 hours and then washed again in running water overnight. The mid-diaphyseal region of each phalanx was sectioned at 15–18 μm using a freezing microtome. The resulting sections were stained in Ehrlich's hematoxylin. The sections were mounted on slides and observed with a light microscope. Bone sections from each individual were photographed (at the same magnification) using a camera lucida, allowing for simultaneous comparison and facilitating the analysis of the bone growth pattern. Two different persons who have similar experience with the technique counted the annular rings of growth in each section.

We used non-parametric Mann-Whitney U -test to compare the age and SVL between the two sexes so variables showed non-normal distribution, the growth was estimated using VON BERTALANFFY'S (1938) growth equation:

$$SVL_t = SVL_{\max} - (SVL_{\max} - SVL_0)e^{-k(t-t_0)}$$

where SVL_t = average body size at age t , SVL_{max} = average maximum body size, SVL_0 = average body size at metamorphosis (t_0), t = number of growing season experienced (age), t_0 = proportion of the growing season until metamorphosis (age at metamorphosis), and K = growth coefficient (shape of the growth curve). The von Bertalanffy growth model was fitted to the average growth curve using the least square procedure. A chi-square (χ^2) test was used to detect differences between the observed and calculated length at age data. All tests were processed with STATISTICA 6.0 (Statsoft Inc., USA) and Excel (Microsoft) with interpreted at an $\alpha = 0.05$.

RESULTS

The average SVL of males (\pm standard error) was 63.50 ± 0.95 mm (range 55.44–77.4, $n = 30$) and 68.98 ± 1.29 mm (range 56.78–83.18, $n = 24$) in females. Females were significantly larger than males (t-test, $t = -3.49$, $df = 52$, $p < 0.0019$). The sexual dimorphism index was 0.09. The bulk of samples presented distinctive peaks at 60.00–64.99 mm in males and 65.00–69.99 mm in females (Fig. 1).

Because scientists report lengths of newts using one of a variety of measurements such as total body length (L) or body length (LCP), it is necessary to convert lengths for comparative purposes. In this context, we derived equations as in Table 1.

Lines of arrested growth were present in cross sections of juvenile and adult phalanges. They appeared as thin and approximately concentric layers, more intensely stained than the rest of the cross section (Fig. 2A–C). In juveniles, some cartilage remained in the marrow cavity, and the youngest individuals did not show

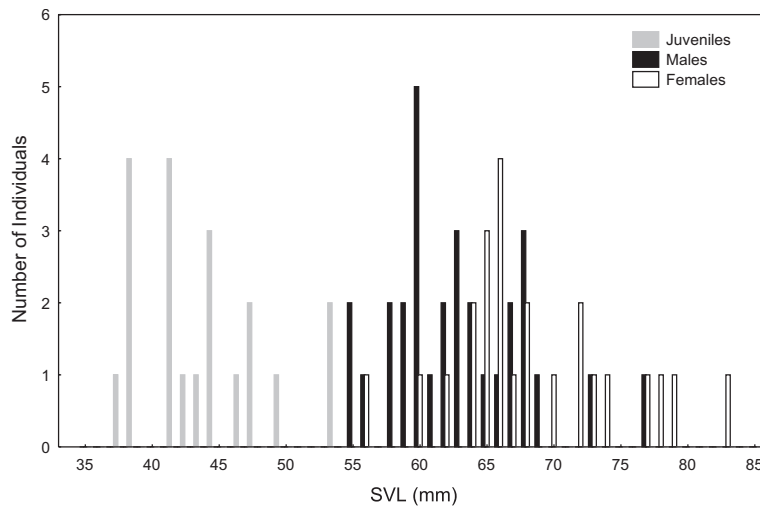


Fig. 1. Snout to vent length (SVL) of juvenile, male and female *Triturus karelinii* from Adapazarı in Northwest Anatolia (Turkey)

Table 1. Length–length regressions for *Triturus karelinii*

Males					Females				
Y	X	a	b	r ²	Y	X	a	b	r ²
L	SVL	3.4361	1.6842	0.81	L	SVL	-6.2019	1.8779	0.80
L	LCP	2.1462	1.9219	0.78	L	LCP	-2.5175	1.9797	0.88
SVL	L	10.3690	0.4814	0.81	SVL	L	16.221	0.4265	0.80
SVL	LCP	-0.1790	1.1307	0.94	SVL	LCP	4.7186	1.0120	0.95
LCP	L	11.7330	0.4039	0.78	LCP	L	8.6410	0.4447	0.88
LCP	SVL	3.5183	0.8315	0.94	LCP	SVL	-1.2703	0.9390	0.95

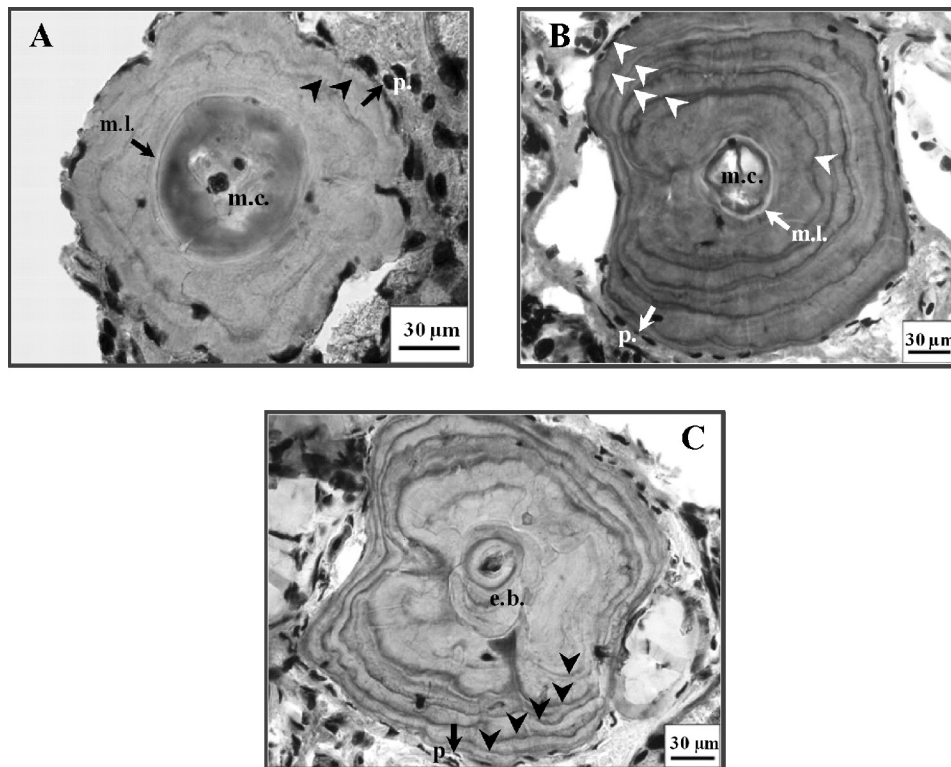


Fig. 2. Phalangeal cross section of *Triturus karelinii*. Arrows: metamorphosis line and periphery; Arrow-heads: lines of arrested growth (LAGs); e.b. = endosteal bone m.c.= marrow cavity, m.l.= metamorphosis line, p.= periphery. (A) Juvenile (SVL= 44.82 mm) caught in March. A metamorphosis line and two LAGs were observed in the periosteal bone. This individual was two years old. (B) Male (SVL= 73.94 mm). Six LAGs were observed in the periosteal bone. Note that a metamorphosis line is visible and endosteal resorption is not present. Age of this individual caught in March was 6 years old. (C) Female (SVL= 83.18 mm). Five LAGs were observed in the periosteal bone. The innermost LAG was eroded by endosteal resorption and this individual was 6 years old

endosteal resorption (Fig. 2A). Almost all juveniles ($N = 17$, 85%) and about half of adults ($N = 29$, 53.70%) exhibited a first thin line very closed to the edge of the marrow cavity (Fig. 2A–B). We interpreted this line as the metamorphosis line. In some juveniles and adults, endosteal resorption was present (Fig. 2C). Because this resorption can erode the first deposited LAGs, we compared the diameter of the smallest juvenile cross section (one year old without resorption) to the diameter of the resorption line in the oldest individuals. In all of them, the resorption eroded the first line, but did not eliminate the second one. We thus assumed that the number of LAGs is equivalent to the number of winters experienced by each individual (Fig. 2A–C), thus giving a direct estimation of individual age. The LAGs deposited in the cross sections can be divided in three main patterns: the first one (62.39%, $N = 43$) was a classical succession of single well-stained lines. The second pattern (11.59%, $N = 8$) was a succession of double lines, indicating two periods of arrested growth (aestivation and hibernation) per year. 26.09% ($N = 18$) of sections were exhibited both single and double LAGs.

Seventy-four bones (representing 20 juveniles, 30 males, and 24 females) were scored, 93.24% of which ($N = 69$) allowed for an estimation of the individual's age. Minimum age for adult males and females was determined as 3 years, maximum ages were determined as 9 and 8 years, respectively. The average age of the males and females were calculated as 5.07 ± 1.36 and 5.00 ± 1.63 years, respectively. The mean age of juveniles was 2.16 ± 0.50 years (range, 1–3 years). No significant differences were found between the age distributions of the two sexes (Mann-Whitney U-test = 288.00, $p = 0.70$). As the youngest females and males were 3 years old and the oldest juveniles were maximum 3 years old in our samples, age at maturity was estimated at 3 years old (Fig. 3).

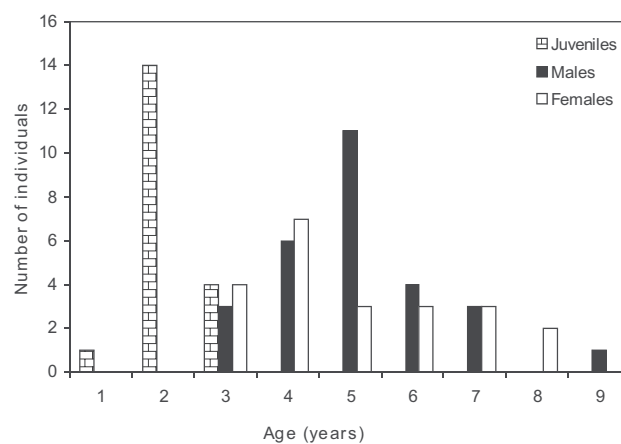


Fig. 3. Age distribution of *Triturus karelinii*

Table 2. Estimates of the von Bertalanffy growth parameters SVL_{max} , K and t_0 for males and females

	SVL_{max} (mm)	K (yr^{-1})	t_0 (years)
Males	70.954	0.345	-1.156
SE	4.196	0.115	0.718
CV	0.059	0.334	-0.621
Females	82.995	0.283	-1.057
SE	6.157	0.086	0.573
CV	0.074	0.303	-0.542

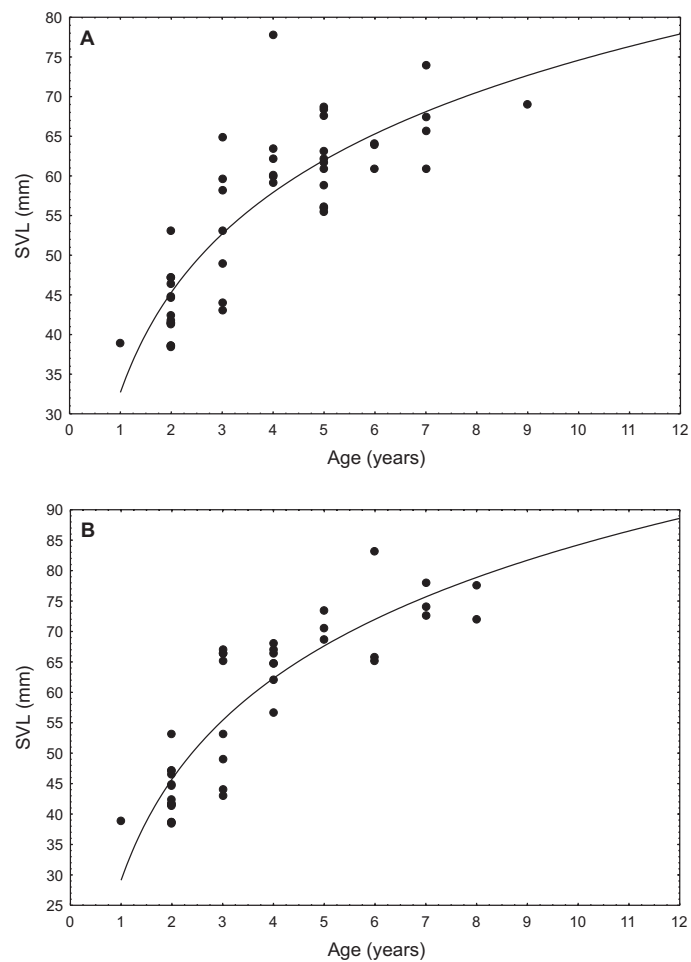
**Fig. 4.** Growth curves in male (A) and female (B) *Triturus karelinii*. Growth curves were fitted to von Bertalanffy's growth equation

Table 3. Observed and calculated mean lengths (SVL, mm) of *Triturus karelinii* for each age class of males and females

	Age groups (years)								
	1	2	3	4	5	6	7	8	9
Male									
SVL _{observed}	38.94	43.42	53.11	63.80	61.71	65.69	64.69	–	69.04
SVL _{calculated}	37.23	47.07	54.04	58.97	62.47	64.95	66.70	–	68.82
Female									
SVL _{observed}	38.94	43.42	56.77	64.26	70.87	71.39	74.92	74.85	–
SVL _{calculated}	36.63	48.05	56.67	63.16	68.05	71.73	74.51	76.60	–

The growth curve for *T. karelinii* fitted the von Bertalanffy growth model (Fig. 4). The estimated growth constants are presented in Table 2. There were no statistical differences between observed and calculated mean lengths ($\chi^2_{\text{male}} = 0.845$, $df = 7$; $\chi^2_{\text{female}} = 0.807$, $df = 7$, $p > 0.05$) of either sex (Table 3). The observed SVL of 4-year age class is larger not only than that of calculated SVL of the same age class but also that SVL (observed and calculated) of 5-year age class. This may be attributed to both the low number of individuals measured at 4-year age class and differences in individual growth rates.

The von Bertalanffy growth model (Fig. 4) fitted the relation between age and body size in both male ($r^2 = 0.95$) and female ($r^2 = 0.97$) *T. karelinii*. The value of K was not significantly different between the sexes ($K_{\text{male}} = 0.345 \pm 0.115$ and $K_{\text{female}} = 0.283 \pm 0.086$). On the other hand, SVL_{max} was significantly larger in females than males ($SVL_{\text{max(males)}} = 70.954 \pm 4.19$ and $SVL_{\text{max(females)}} = 82.995 \pm 6.157$).

DISCUSSION

As in other amphibians, *T. karelinii* shows bone growth marks, which correspond to period of inactivity due to climatic conditions (SMIRINA 1994, CASTANET 2002, OLGUN *et al.* 2005, YILMAZ *et al.* 2005).

In this study, *T. karelinii* exhibited a metamorphosis line and one or two growth arrests each year in adults (aestivation and hibernation). Some authors (e.g. CAETANO *et al.* 1985, CAETANO & CASTANET 1993, JAKOB *et al.* 2002) hypothesized that a growth arrest occurs at metamorphosis in a Mediterranean climate because metamorphs leave the aquatic environment for the terrestrial environment which could be less favourable (because the terrestrial environment changes temperatures more quickly and has much less productivity or prey availability than

water). The climate of Arifiye could similarly cause the expression of a line of metamorphosis in the bones of *T. karelinii*.

Another characteristic observed in the population of *T. karelinii* we studied, was the migration of juveniles to the water. The juveniles located in the aquatic sites during winter and spring can grow more than juveniles located in the terrestrial sites because of trophic resources (i.e. higher productivity or prey availability in the water than land). This situation can be observed in the bone growth, and these juveniles exhibit large skeletal growth. The presence of juveniles in the water is a frequent phenomenon for other large newts such as *T. marmoratus* and *T. cristatus*. OLGUN *et al.* (2005) also reported the same situation for another *T. karelinii* population (in Bozdag/İzmir) from a high elevation site.

It has been hypothesized that the growth marks visible in bones are the result of a genetically controlled growth cycle that is synchronized and reinforced by particular environmental conditions, such as seasonality (ESTEBAN *et al.* 1996, ALCOBENDAS & CASTANET 2000). At the low altitude of Arifiye, the active period is between March and October. So, some individuals only hibernate during the winter while some hibernate during winter and aestivate during the summer, depending on the annual climatic conditions.

The mean body sizes of males and females are shorter than those of examined *T. karelinii* population by OLGUN *et al.* (2005). These differences in body sizes may be explained by the different altitudes of the populations' ranges.

Body length and age for individuals in the *T. cristatus* complex were reported from populations experiencing a variety of environmental conditions (reviewed in COGALNICEANU & MIAUD 2003). The body length of individuals in the Arifiye population was in the range of related species. Females are often larger than males in *T. cristatus*, *T. marmoratus*, *T. carnifex* and *T. dobrogicus* as seen in our *T. karelinii* population (females were significantly larger than males). OLGUN *et al.* (2005) reported for another *T. karelinii* population that females' body length were larger than males, but did not differ significantly.

For more than a century, studies have shown that ectotherms usually grow to a larger size while living in cooler conditions (ATKINSON & SIBLY 1997). In 83% of 109 studies of ectotherms, larger size was observed at lower temperature (reviewed in ATKINSON 1994). The response of body size to temperature (the most commonly used/measured environmental variable) is subject to the "developmental temperature-size rule" (BERRIGAN & CHARNOV 1994), but genetically larger adults can be produced by selection at lower temperatures in *Drosophila* (PARTRIDGE *et al.* 1994). An increase in body size with altitude and latitude is observed in frogs, salamanders and newts (HOUCK 1982, LECLAIR & LAURIN 1996, MIAUD *et al.* 2000). A correlated increase in age at maturity and longevity is also observed

in salamanders and newts (HOUCK 1982, LECLAIR & LAURIN 1996, MIAUD *et al.* 2000, KUTRUP *et al.* 2005).

Age at maturity was estimated at 3 years old in the Arifiye population, which is similar to that observed in other population of *T. karelinii* (OLGUN *et al.* 2005) as well as other species of the *T. cristatus* complex (COGALNICEANU & MIAUD 2003). The maximum life span of *T. karelinii* was estimated as 9 years for males and 8 years for females, whereas the oldest individuals were 8 and 11 years old (males and females respectively) in a mountain population of *T. karelinii* (OLGUN *et al.* 2005). The relatively low longevity observed in our population could result from a higher mortality risk during the terrestrial phase caused by the arid climate of Arifiye. High mortality rates have been observed in floodplain and Mediterranean populations of *T. dobrogicus* and *T. marmoratus* (COGALNICEANU & MIAUD 2003, JACOB *et al.* 2003).

The variation of selective regimes (e. g. climate) can result in adaptation and differentiation among local populations (LINHART & GRANT, 1996). More inter-population and interspecific studies are thus needed on the proximate effect of various environments on newt life-history traits (fecundity is a good example of a lack of data) to test hypotheses about local adaptation versus phenotypic variation.

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