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Age and Growth of the Green Toad, *Bufo viridis* (Laurenti, 1768) from an Island and a Mainland Population in Giresun, Turkey

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Abstract: Age structure and some growth parameters of *Bufo viridis* from the Giresun Island and Giresun province in Turkey were studied by skeletochronology. We studied 56 specimens (41 female, 15 male) from island and 44 (5 female, 39 male) from mainland population. In both populations, age at sexual maturity was 2-3 years in both males and females. Age was ranged from 2-8 years for females and 3-6 years for males in island while 4-7 and 3-8 years in mainland population, respectively. Males and females did not differ in the mean age while SVL and weight were significantly different between males and females in two populations. In addition, we found positive correlations between age and SVL in both sexes in two populations.

Key words: Amphibia, longevity, skeletochronology, *Bufo viridis*, population, maturity

INTRODUCTION

Age determination of amphibians and reptiles is important in order to obtain information about mortality, longevity and other ecological factors (Yilmaz *et al*., 2005). The accurately assessed individual age permits us greater understanding of life history traits in long-lived animals such as amphibians. The method of skeletochronology is recently becoming more and more popular in accurately determining ages in amphibians (Kusano *et al*., 1995; Misawa and Matsui, 1999). This method is based on the presence of annual growth layers recorded in cross-sections of long bones (Halliday and Verrell, 1988). The formation of annual layers reflects the seasonal changes in the growth rate of an animal. The need for only a finger to observe the LAGs (Lines of Arrested Growth) in phalanges cross-sections is important in conservation biology, particularly with small and endangered populations because it avoids having to kill individuals (Eggert and Guyetant, 1999).

Bufo viridis is one of the most widespread amphibians in Turkey. Its distribution extends as far west as Morocco and eastern France and as far east as north-western China and Mongolia. Despite widespread in Turkey, there has not been any study on the age and growth of *Bufo viridis*. Data on age structure, age at sexual maturity, growth and longevity were provided by Gokhelashvili and Tarkhnishvili (1994) from a population which inhabits in Georgia, Ledentsov and Melkumyan (1986) from Armenia, Sinsch *et al*. (2007) studied four populations inhabits in Germany and also Castellano *et al*. (1999) who reported only longevity of males from island and mainland populations in Italy.

The purpose of this study is to compare the age, growth, age at maturity and longevity of an island and a mainland population of *Bufo viridis* from the Giresun island and Giresun province in Turkey.

MATERIALS AND METHODS

Samplings were made in the Giresun province (20-100 m a.s.l.) and Giresun Island (0-30 m a.s.l.) in June 2006. Giresun province is located on the coast of Black Sea region in the North-East of Turkey. There is only one island in the Black Sea region. It is 1.5 km far from the city of Giresun and it has 40 m^2 of areas. No human being lives in the island. A total number of 100 individual 56 (41 female, 15 male) from island and 44 (5 female, 39 male) from mainland population were captured by hand after 9 O'clock. Individuals were sexed, their body mass and Snout-Vent Length (SVL) were measured and then fourth toe of the right hind leg of each specimen was clipped and stored in 10% formalin solution. Finally, specimens were released in the capture sites.

For skeletochronological analysis, each toe was decalcified in 5% nitric acid solutions for 3-4 h and washed in running tap water for 24 h. After being embedded in paraffin, the second phalange of each toe was sectioned at 12-18 mm thick and stained with Hematoxylen. Ages were estimated by counting the

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number of LAGs present in the periosteal bone (Fig. 1). The analysis of growth marks was made by two different persons who had similar experience in the technique and who agreed on the identification criteria for LAGs and the final age estimation. The age at which a significant decrease in growth (based on the thickness of the growth rings) was detected was taken as the age of sexual maturity (Ryser, 1988). Growth was estimated using the Von Bertalanffy's equation:

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

Where:

SVL_t = The body size in age t
SVL_{max} = Asymptotic maximum body size
SVL_0 = The body size at metamorphosis
t = The age in years
t_o = Age at metamorphosis (in this study t_o is assumed to be 0)
k = The growth coefficient is the rate at which SVL_{max} is reached and determines the shape of the curve

The parameters SVL_{max}, k and their asymptotic Confidence Intervals (CI) were estimated by non-linear least-square regression.

Because age classes and body measurements (SVL and body mass) were normally distributed (the Kolmogorov-Smirnov D-test, $p>0.05$) we used parametric tests for comparison of means (Student's t-test) and for correlation (Pearson's correlations). All tests were processed with SPSS 10.00 for Windows and the level of significance chosen was $p<0.05$.

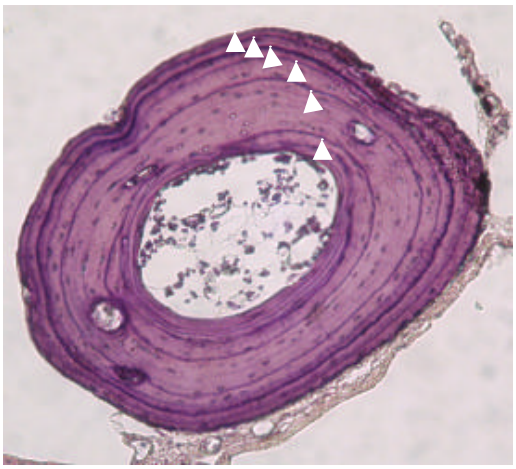

Fig. 1: Phalange bone cross-sections of *Bufo viridis*, female from mainland, 79, 6 mm SVL, 6 years old. Arrows = LAGs

RESULTS AND DISCUSSION

Age composition distribution of males and females for both populations is shown in Fig. 2. Descriptive statistics on age, body length and body mass are shown in Table 1.

Age, length and body mass between sexes in the island population: Age was ranged from 2-8 years for females and 3-6 years for males. Age of specimens from island population did not differ significantly between the sexes (Student's t-test; t = 0.109, df = 54, p = 0.913). Age at maturity determined from cross-sections was 2-3 years for both males and females. Both SVL (t = 1.240, df = 54, p = 0.220) and body mass (t = 1.875, df = 54, p = 0.066) did not differ significantly between sexes. There is a positive correlation between SVL and age for males (Pearson's correlation r = 0.535, $p<0.05$) and females (r = 0.730, $p<0.001$). Besides, there is positive correlation between body mass and age for females (r = 0.529, $p<0.001$) but not for males (r = -0.223, $p>0.05$).

Age, length and body mass between sexes in the mainland population: Age was ranged from 4-7 years for females and 3-8 years for males. Mean age of specimens from the mainland population did not differ significantly between the sexes (t = 0.667, df = 42, p = 0.509). Age at maturity determined from cross-sections was 2-3 years for both

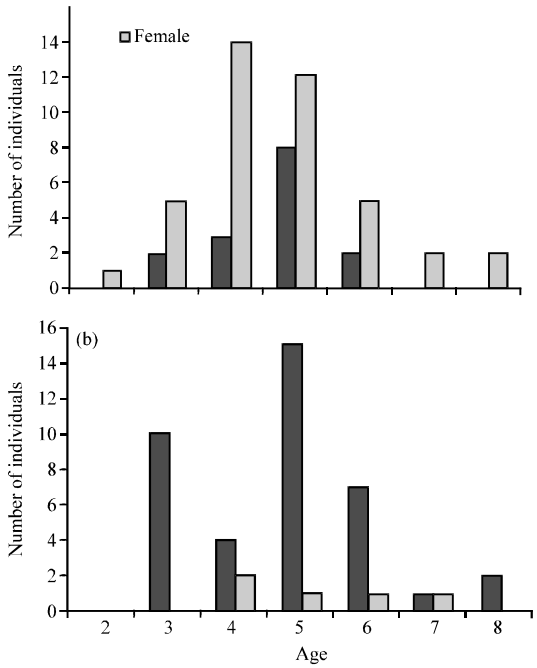

Fig. 2: Age composition distribution of males and females from: a) island and b) mainland

Table 1: Descriptive statistics on age (years), body length (mm) and body mass (g) of the *Bufo viridis* from island and mainland populations, SVL, snout-vent length (mm); n, number; range: minimum and maximum values; SD

	Island population				Mainland population			
Parameters	n	Mean	Range	SD	n	Mean	Range	SD
Males								
Age	15	4.66	3-6	0.89	39	4.76	3-8	1.36
SVL	15	77.11	64.5-85.9	5.02	39	66.12	55.1-77.8	5.49
Mass	15	43.36	27.5-66.1	8.49	39	24.04	13.1-41	6.75
Females								
Age	41	4.70	2-8	1.32	5	2.6	4-7	0.54
SVL	41	79.46	60-90	6.62	5	69.24	56.1-80.2	10.78
Mass	41	48.58	26.3-65.2	9.46	5	30.30	16.4-48.1	12.99

Table 2: Growth parameters and asymptotic standard error in *Bufo viridis* from island and mainland populations

Parameters	n	SVL_{max}	k	R^2
Island				
Male	15	82.01±3.17	0.59±0.13	0.388
Female	41	87.92±2.06	0.50±0.05	0.578
Mainland				
Male	39	72.90±1.76	0.49±0.05	0.524

males and females. Both SVL (t = 0.637, df = 42, p = 0.557) and body mass (t = 1.059, df = 42, p = 0.346) did not differ significantly between sexes. There are significant positive correlations between both SVL and age and body mass and age for males (Pearson's correlation r_{SVL} = 0.759, r_{BM} = 0.689, p<0.001) and females (r_{SVL} = 0.951, p<0.05, r_{BM} = 0.991, p<0.001).

Comparison of age, length and mass between populations: Males of the island populations were significantly bigger (t = 6.744, df = 52, p = 0.000) and heavier (t = 8.762, df = 52, p = 0.000) than males of the mainland populations while age did not differ significantly (t = -0.268, df = 52, p = 0.789). As in males, females of the island populations were bigger (t = 3.036, df = 44, p = 0.004) and heavier (t = 3.923, df = 44, p = 0.000) than females of the mainland populations. Age of females was also did not differ between two populations (t = -0.785, df = 44, p = 0.437).

Growth patterns: Growth curves of age versus SVL for both sexes of island and mainland populations except for female of mainland population are shown in Fig. 3 and their growth shown in Table 2. Island females had larger asymptotic sizes (SVL_{max}) than males of both populations and the asymptotic size of island males was larger than mainland males but this differences were not significant (t-test, p>0.05). The first 3 years males and females of island toads show the same growth rate and they grow faster than males of mainland toads.

The use of skeletochronological method allows the determination of individual variation of life history traits and provides data on growth and age at maturity. Although, skeletochronology has been applied to several amphibian species, no studies have been conducted in *Bufo viridis* in Turkey. In the study, age was ranged from 2-8 years for females and 3-6 years for males in island

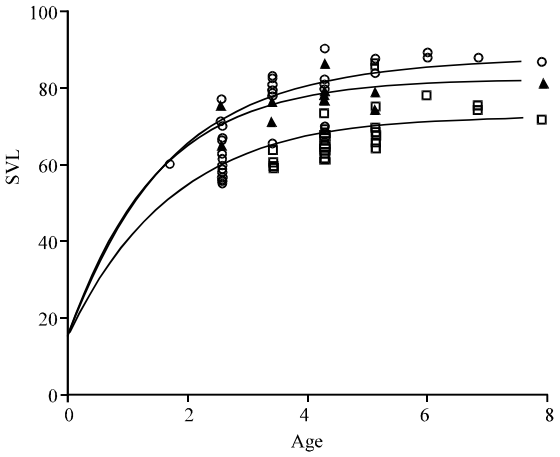

Fig. 3: The relationship between age and SVL in *Bufo viridis*. Curves show Von Bertalanffy's model. (o represents island females, ▲ represents island males and □ represents mainland males)

while 4-7 and 3-8 years in mainland population, respectively. Castellano *et al.* (1999) reported that age was ranged from 4-11 in both Portoscuso (island-50 m a.s.l.) and Zucchea (mainland-350 m a.s.l.) males of *Bufo viridis* but they did not report anything about females (Italy). In a study made in Georgia (Borjami Canyon, 900-1200 m a.s.l.) age was ranged from 2-6 for males and 3-10 for females of that population (Gokhelashvili and Tarkhnishvili, 1994). The oldest individuals collected in Armenia by Ledentsov and Melkumyan (1986) was 5 years old (Tarkhnishvili and Gokhelashvili, 1999). In Germany (60-100 m a.s.l.) longevity was found 11 years for females and 9 years in males (Sinsch *et al*., 2007). In general, there is a trend to increased age of highland populations of frogs and toads in comparison with lowland populations (Smirina, 1994; Yilmaz *et al*., 2005) but the results of the studies about *Bufo viridis* is not comply with this knowledge. So we do not say that the maximum life span was recorded 8 years old for two populations because we take the samples from ecologically different (island and mainland) but altitudes were similar areas.

In amphibians, maturity is generally attained after having reached a certain minimum size but a variable age (Gibbons and McCarthy, 1984; Hemelaar, 1986; Ryser, 1988). The males attain sexual maturity 1 year earlier than females or at the same age (Smirina, 1994). Similar to this, in Georgia, age at sexual maturity was found 2-3 years for males and 3-4 years for females (Gokhelashvili and Tarkhnishvili, 1994), 2-3 years for males and 2-4 years for females in Armenia (Tarkhnishvili and Gokhelashvili, 1999) and 1-3 years for males and 2-3 years for females in Germany (Sinsch *et al*., 2007). These studies have reported that age at maturation varied among populations.

Such differences at longevity and age at maturity between frogs from low and high elevations could result from the temperature and the length of active periods (Kara, 1994).

In the study, not only the altitude of sampling areas but also the active periods of toads are similar and we determine that age at maturity was 2-3 years in both males and females in two populations. In this study, the mean age of males and females did not differ between populations but both females and males of island toads are significantly bigger and heavier than those from mainland population. It means that these differences are not the result of an increased lifespan of island toads but of different growth rates.

Contrary to the results, Castellano reported that the mean age of their mainland toads are significantly bigger than island toads but like us they found that green toads of their island population are >30% longer and twice as heavy as mainland toads. The great majority of amphibians may continue to grow as long as they live and when they are fully adult they do not grow at the same rapid speed. The findings confirm this generalization. Researchers observed that island toads grow faster during their pre-adult stage than mainland toads as stated Castellano *et al*. (1999). While age at maturity can be partly genetically controlled (Berven, 1982; Bernardo, 1993), age at maturity and longevity of amphibians are mainly environmentally influenced and age structure may serve as an indicator of environmental variation along climatic (Bernardo, 1993) or pollution (Smirina, 1994) gradients (Cogalniceanu and Miaud, 2003). Mitochondrial 16S rRNA and 12S rRNA sequences were compared between these two populations and no differences were found.

CONCLUSION

In this study, it is founded that island toads are bigger and heavier because of environmental conditions, lack of predators and competition for food and many other factors.

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