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Age structure in two populations of *Triturus vittatus ophryticus* at different altitudes

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Abstract. We investigated age structure by skeletochronology in two breeding populations of *Triturus vittatus ophryticus* inhabiting altitudes ranging from 300 m (in Gurbulak) to 1300 m (in Hidirnebi) in northeastern Turkey. The mean age was 4 years (maximum 10) in Gurbulak population and 8 (maximum 16) in Hidirnebi population. In the lowland population, minimum age at maturation was two to three years while it was four years in the highland population. The mean snout-vent length of the populations was significantly different and we found no correlation between age and snout-vent length except for females of Gurbulak. Sexual dimorphism in body size and weight was detected in both populations.

Introduction

Using the skeletochronology method for age determination in amphibians has been the subject of many recent studies (e.g. Platz et al., 1997; Eggert and Guyétant, 1999). Some of them have been carried out for a variety of anuran species (Plytycz et al., 1995; Kuzmin and Ischenko, 1997). Also, this method has been frequently using in urodeles (Verrell and Francillon, 1986; Lima et al., 2001).

Differences in size and age at sexual maturation, longevity, and size variation were observed among populations inhabiting different altitudes in other amphibian species (Berven, 1982; Hemelaar, 1988; Caetano and Castanet, 1993; Marunouchi et al., 2000). Geographic variation in body size is frequently associated with differences in several life-history traits throughout the range of a species, such as fecundity, age at maturity and longevity. These, in turn, may be associated with variation in morphological characteristics, such as body size and growth rate (Diaz-Paniagua and Mateo, 1999).

Triturus vittatus ophryticus (Berthold, 1846) inhabits landscapes from dry forests in Eastern Georgia, to humid mixed and deciduous forests at the Black Sea Coast up to subalpine meadows (Kuzmin, 1999) and differs from the nominative form in: larger size, deeper intersections in the crest on the male's back, and differently distributed stripes on the crest (Ozeti and Yilmaz, 1994). Newts are most abundant in small lakes and ponds on grassland patches surrounded by forest, and they are also found in slowly running water bodies on subalpine meadows and (in areas with high humidity) in slowly running forest pools (Tarkhnishvili and Gokhelashvili, 1999).

Information about the age and growth parameters of *T. v. ophryticus* is rather scare (Kuzmin 1999; Tarkhnishvili and Gokhelashvili, 1999), pertaining to age at maturation and lifespan in Tbilisi and Caucasus. There has yet to be a report on the relation between size variation and altitude of habitats. The aim of our study was to estimate age of sexual maturity, longevity and the relation of body size to age for two breeding populations of this subspecies inhabiting different altitudes in Trabzon, norheastern Turkey.

Materials and Methods

Newts were collected from two breeding populations one of which is Gurbulak locality with an altitude of 300 m and the other is Hidirnebi with an altitude of 1300 m between April and November in 2001 (fig. 1). The site located in Hidirnebi is a channel which has approximately 30 m length, 1.50 m width and it is shallower (maximum depth: 0.35 m) than the

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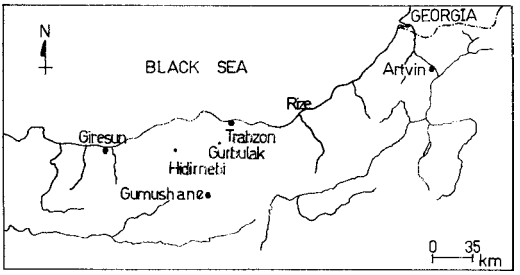

Figure 1. Map showing the localities of the studied samples.

other site. The site of Gurbulak is an artificial pond, square shaped, and its maximum depth is 2.68 m. In total, 91 adult animals were caught (42 males and 49 females), 40 (14 males and 26 females) from Gurbulak locality and 51 (28 males and 23 females) from Hidirnebi. Because we chose breeding populations for sampling, all newts were caught in water. In Gurbulak, there were still adult newts in water in November whereas there were none in Hidirnebi because of the cold weather. After November, they were not seen in water and we assumed they began hibernation in December.

Snout-vent lengths (SVL) of the newts were measured from the snout to the posterior end of the cloaca by a slide caliper in 0.5 mm units (Marunouchi et al., 2000). All newts were weighed to the nearest 0.01 g, fixed and then stored in 10% formalin.

Between the original and regenerating hind limbs from captive individuals, cross sections of the phalanges were similar in shapes, but those of femora had different shapes and large marrow cavities. In cross sections of samples after regeneration, femora can be distinguished as the regenerating bones because of their shapes, but phalanges cannot be distinguished and have a possibility to be used mistakenly for skeletochronology (Marunouchi et al., 2000). So we selected the femora for age determination.

Bones with skin and muscle were decalcified in 6% nitric acid for 40-60 minutes and washed in running tap water for 24 hours. Then the bones were sectioned at 15 μm by a freezing microtome. Cross sections were stained with Meyer's hematoxylin. The sections were then examined under a light microscope with an ocular micrometer and lines of arrested growth (LAG) present in the periosteal were counted to estimate the individual ages. Ages were established independently by two authors, after which they were compared with each other (Rebelo and Caetano, 1995). Age at first reproduction was inferred from the first sudden narrowing of the LAG, which may be related to reproduction (Caetano and Castanet, 1993; Rebelo and Caetano, 1997).

In order to determine whether age, sex and altitude affect SVL and weight of the newts, regression equations were constructed by the estimation of the Ordinary Least Squares (OLS) and from the equations, the best one was chosen for the analyse. Differences in body size and age distributions were analysed by means of ANOVA.

Results

The long bone diaphyses of *Triturus vittatus ophryticus* were similar to those of other urodeles of similar size (Castanet, 1975; Miaud, 1991; Rebelo and Caetano, 1995), consisting of avascular compact bone rarely crossed by radial blood vessels that reach the marrow cavity (Rebelo and Caetano, 1995). In almost all sections, endosteal bone was present (fig. 2). The mean age was 4 in Gurbulak population and 8 in Hidirnebi population (table 1). The oldest newt in the highland population was 16 years while the oldest one was 10 in the lowland population. Four years was the most common age at first reproduction in the two populations and in both sexes. Also, there were two-year-old and three-year-old male and female newts in the lowland population (Gurbulak) but there was no individual younger than four years old in the highland population (Hidirnebi).

The mean SVL of the populations was significantly different ($F_{89} = 6.68$, $P < 0.05$), the mean SVL of Gurbulak population was larger than Hidirnebi population for both sexes (table 1). Males in Gurbulak population were on average 3% larger than in Hidirnebi populations and females in Gurbulak population were on average 4% larger than in Hidirnebi populations.

The mean weight of Gurbulak population was higher than Hidirnebi population for both sexes (table 1). There was a significant difference between males and females in terms of mean weight in the populations ($F_{89} = 78.53$, $P < 0.01$). Males in Gurbulak population were on average 2% heavier than in Hidirnebi population and females in Gurbulak population were on average 11% heavier than in Hidirnebi population.

The results of the regression equations showed that there were not any siginificant relations between the age and SVL either in Gurbulak ($R^2 = 0.02$, $F_{38} = 0.97$, $P > 0.01$) or Hidirnebi populations ($R^2 = 0.00$, $F_{38} = 0.00$, $P > 0.01$). But the equation that has both age and sex as dependent variables was significant in both populations (Gurbulak: $R^2 = 0.46$,

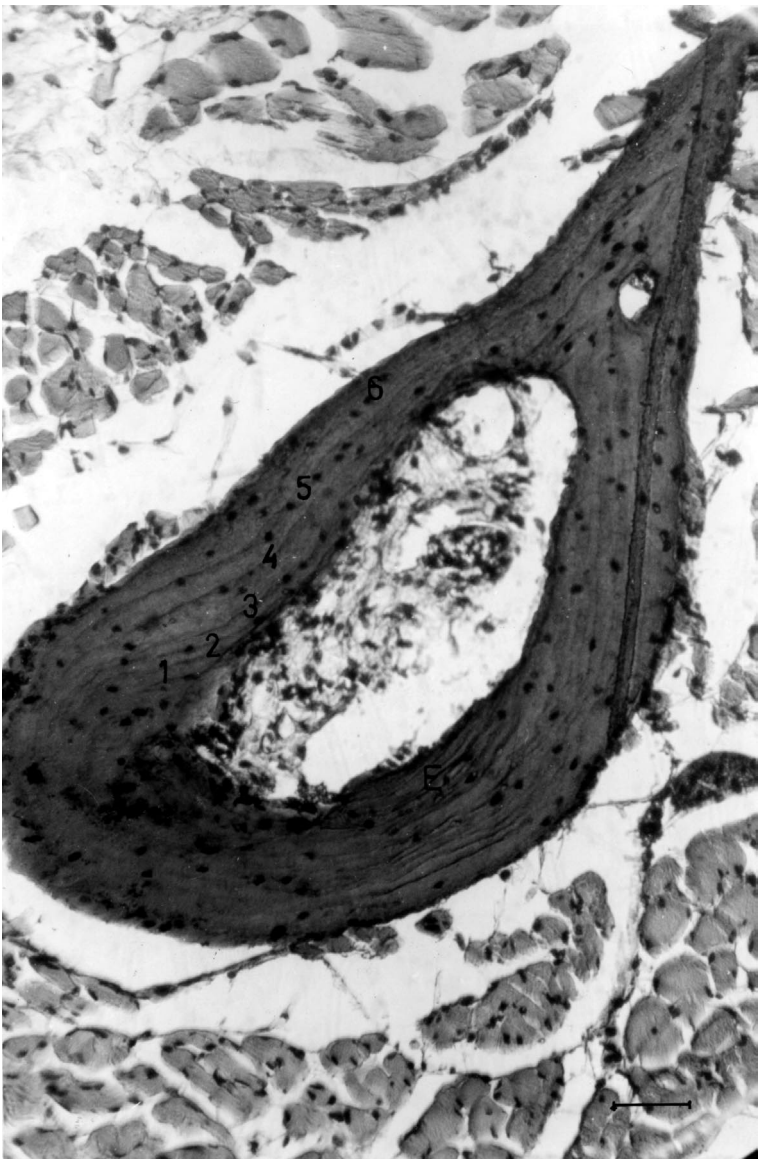

Figure 2. Cross-section of a femur of a 6 years old *Triturus vittatus ophryticus*. Numbers refer to Lines of Arrested Growth (LAG). E represent the endosteal bone. Scale bar = 50 μm.

$F_{37} = 15.78$, $P < 0.01$; Hidirnebi: $R^2 = 0.229$, $F_{48} = 7.168$, $P < 0.01$).

The regression analysis showed that there was no relation between age and body weight in both populations, separetely (Gurbulak: $R^2 = 0.04$, $F_{38} = 1.56$, $P > 0.01$; Hidirnebi: $R^2 = 0.00$, $F_{49} = 0.0$, $P > 0.01$). As in the analyses of independent variable SVL, there was a relation between the body weight and sex, in addition, weight was affected by only sex but not by age.

Discussion

Our study, conducted on two *Triturus vittatus ophryticus* populations from different altitudes (300 and 1300 m), shows significant variations in relative mean SVL and age. Age distribu-

Table 1. The mean age, the mean SVL and the mean weight in two populations of *T. vittatus* (Gurbulak: G, Hidirnebi: H, mean ± *s*).

Sex	Area	*n*	Age	SVL	Weight
Female	G	26	4.0 ± 1.67	53.3 ± 0.44	4.46 ± 1.59
Female	H	23	9.2 ± 2.54	51.0 ± 0.33	4.01 ± 1.00
Male	G	14	4.0 ± 2.43	61.7 ± 0.50	7.04 ± 2.00
Male	H	28	8.2 ± 2.60	59.6 ± 0.29	6.80 ± 1.15

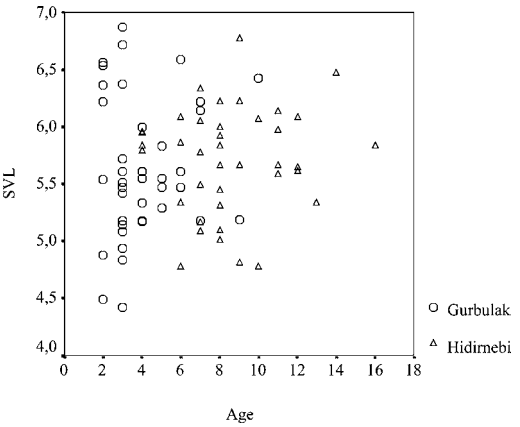

Figure 3. Relationship between SVL and age in Gurbulak and Hidirnebi populations.

tion of Gurbulak population ranged from 2 to 10 years while it ranged from 4 to 16 years in Hidirnebi population. The age distribution of adult *T. vittatus* from Akhaldaba Lake near Tbilisi ranged from 3 to 12 years (Tarkhnishvili and Gokhelashvili, 1999). The mean age of newts from this population was 5.53 years in males, and 6.97 years in females and in our study it was 4 years for Gurbulak and 8.2 for Hidirnebi in males while it was 4 and 9.2 years in females, respectively.

The maximum life span of *T. v. ophryticus* was estimated as 16 years old in our study whereas it is 8-21 years in different parts of the subspecific range (Kuzmin, 1999) and 21 years in western Caucasus (Tarkhnishvili and Gokhelashvili, 1999). Our results are in line with other estimates of longevity in natural conditions of other *Triturus* species: 16 years for *T. cristatus* in Sweden (Hagström, 1980), 15 years for *T. marmoratus* in Portugal (Caetano et al., 1985) and 14 years for the same species in France (Francillon-Vieillot et al., 1990). Also similar to our results, Marunouchi et al. (2000) found the maximum age 7 in lowland populations and 16 in highland populations for *Cynops pyrrhogaster*.

We found that the minimum age at maturation ranged from 2 to 3 years in the lowland population and 4 years in the highland population while Marunouchi et al. (2000) reported the age at maturation 3 years and from 4 to 7 years, respectively. Kuzmin (1999) reported that the sexual maturity was attained at 3-5 years in *T. v. ophryticus*. Similarly, Tarkhnishvili and Gokhelashvili (1999) established the earliest age at maturation in *T. vittatus* populations from near Tbilisi as 3 years for males and 4 years for females. Most males, however, mature after 5 hibernations, females after 6 hibernations. According to Tarkhnishvili and Gokhelashvili (1999) *T. vittatus* from the western Caucasus mature later: the oldest males are 12 years old, females are up to 21 years old. They also suggested that longevity might be related to the type of the locality rather than the climate (i.e. newts mature later in slowly running water). These findings are in contrast to our results for this subspecies. The present study showed that the age at maturation and lifespan tended to be higher at high altitudes than at lowland altitudes like in other urodelans (Hagström, 1980; Dolmen, 1983; Verrell and Francillon, 1986; Morunouchi et al., 2000). Caetano et al. (1985) compared the age composition of breeding parts of populations of *Triturus marmoratus* in lowland and in mountains (1500 m) of Portugal. Similar to our results, they reported that the newts in the lowlands attain sexual maturity earlier (at the age of 4-5 years old) than the newts in mountains (at the age of 5-6 years). In gen-

eral, there is a trend to increased longevity and a less clear trend to increased age of maturation in northern and mountain populations of amphibians in comparison with southern and lowland populations (Smirina, 1994). So, our findings for *T. vittatus ophryticus* are confirmed this tendency.

There are significant overlaps of the body length distributions between age classes even of immature animals, such features being common in mature individuals. The main reason of overlaps are the long breeding period and duration of metamorphosis, individual variations in the growth rate after maturation and ecological conditions as stated by Smirina (1994).

We found no significant relation between age and SVL for both populations. Thus body size of *Triturus vittatus ophryticus* cannot be considered as a reliable predictor of newt age, as observed in other urodelans (Halliday and Verrell, 1988; Diaz-Paniagua and Mateo, 1999). We even found negative tendencies with smaller individuals in the older age classes as reported in *T. boscai* (Diaz-Paniagua and Mateo, 1999). This may be explained by the drastic decrease of growth rates after sexual maturity is attained, as well as by the different interannual reproductive effort of individuals, resulting in different individual growth trajectories.

In contrast to our findings, Diaz-Paniagua et al. (1996) found that age and size were significantly related in *Triturus marmoratus pygmaeus*. Similarly, Verrell and Francillon (1986) and Cvetković et al. (1996) reported that age was significantly correlated with SVL, but only in males of *T. vulgaris* and *T. carnifex*. Also, Marunouchi et al. (2000) found significant correlations between SVL and age in six populations for both sexes in *Cynops pyrrhogaster* and they reported a significant correlation for only males in three populations and for only females in two populations.

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