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AGE DETERMINATION AND SOME GROWTH PARAMETERS OF A RANA RIDIBUNDA POPULATION IN TURKEY

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Age determination and some growth parameters were studied in a population of *Rana ridibunda* from Yıldızlı Stream, Trabzon, Turkey by skeletochronology. Age was determined for individual frogs by counting the number of resting lines (RL) in cross-sections taken from phalanges. The maximum longevity of females and males was 6 and 7 years, respectively. The minimum age at maturity was 2, at 55 mm snout-vent length (SVL), for females, and 2, at 46 mm, for males. Males were sexually mature after the third or fourth winter although some females mature after the fifth winter. SVL was ranged from 55 to 99 mm (74.64±13.37) in females and 38 to 83 mm (64.58±11.09) in males. Male SVL was significantly correlated with age, but no significant correlation was obtained between female SVL and age. Gonad weights show highly significant correlation with SVL in both sexes but no significant correlation with age.

Key words: skeletochronology, age determination, *Rana ridibunda*, growth, Turkey

INTRODUCTION

Age determination of amphibians and reptiles is important in order to obtain information about mortality, longevity, and other ecological factors. In most cases, it provides accurate estimations of individual ages and therefore overcomes a major difficulty in demographic and life history studies.

Among different indirect methods of age determination developed (HALLIDAY & VERRELL 1988), we used the most appropriate and reliable method namely skeletochronology (CASTANET *et al.* 1977), age determination by analysis of growth marks on bones. Since the growth layers are clearly visible in phalanges, age determination requires only the removal of a toe. On stained cross-sections of phalanges, growth marks appear as a concentric wide zones of periosteal bone, separated by narrow dark resting lines. In temperate zones, resting lines are formed annually during hibernation and they mark the size of the bone at that particular age (RYSER 1988).

Previous skeletochronological studies have been carried out for a variety of *Rana* species (GIBBONS & MCCARTHY 1983, LECLAIR & CASTANET 1987, RYSER 1988, ESTEBAN *et al*. 1996, PATON *et al*. 1997, PLATZ *et al*. 1997). In this study we examined the age, longevity, growth and maturation of a *Rana ridibunda* population from Trabzon, Turkey.

Rana ridibunda (water frog) is one of the most widespread amphibians in Turkey. Its distribution extends from central Europe in the west, to west Asia in the east and to North Africa in the south. Although widespread in Turkey, no study has been carried out on the age and growth of *Rana ridibunda*. Data on growth and maturation were provided by SHALDYBIN (1976), ALEKSANDROVSKAYA and KOTOVA (1986) and LEDENTSOV and MELKUMYAN (1986) from Russia and Armenia.

The purpose of this study is to determine the age structure and growth of *Rana ridibunda*. In Turkey, this species is collected and exported indiscriminately. Owing to this trade populations are dramatically decreasing and this species will thus soon become endangered (DEMIRSOY 1996).

We examined the age and size at maturity, the relationship of these demographic characters in males and females. Also the relationships between gonad, liver, stomach masses and age, and size were analysed. The aim of the study is to assess the individual age and growth rate of *Rana ridibunda* in a sample from Trabzon, north eastern Turkey.

MATERIALS AND METHODS

This study is based on 11 females and 38 males collected in 20–23 June 2000 from Yıldızlı Stream, Trabzon, on the north east coast of Turkey. Within 24 h of collection, the frogs were anesthesied by ether and killed in 70% alcohol. Individuals were sexed on the basis of the presence of well-developed external vocal sacs, and then their gonads were examined directly after dissection. Snout-vent length (SVL), from the tip of the snout to the posterior angle of the vent, was measured to the nearest 0.05 mm. Body mass was measured to the nearest 0.01 g as well as gonads, liver and stomach.

From the material collected, the distal two phalangeal elements of the fourth toe from the right hind foot were cut off, transferred to 10% formalin, and stored until sectioned. Skeletochronological studies followed the protocol of HEMELAAR and VAN GELDER (1980) and PLATZ (1997). Toes were decalcified in 5% nitric acid for 5 to 6 hours and left in water overnight. The mid-diaphyseal region of the distal phalangeal element of each toe was then sectioned at 15–20 µm using a LEICA CM model 1900 freeze microtome. The resulting sections were stained in Ehrlich's hematoxylin.

Medullary cavity perimeters, RL (resting lines) lengths and phalange perimeters of each individual was measured from the sections under microscope. From these data, we have calculated absolute bone thickness (µm) between successive RLs. These bone thicknesses were converted into bone growth rate by taking into account the length of corresponding growing period. For bone deposited between two successive RL's, a 6 month (=183 d) growing season was considered according to known phenology of *Rana ridibunda* in Black Sea Region (YILMAZ 1989). Bone beyond the last RL was obviously deposited during the time elapsed between arousal from hibernation (1 April) and date of capture. Regression analysis was performed to measure relations among the variables measured and the relationships between age and other variables.

RESULTS

In this population, females were slightly larger than males in terms of both SVL and body mass. Average SVL in females was 74.64±13.37 mm, compared to 64.58±11.09 mm in males; females weighed on the average 41.61±17.66 g and males 29.44±12.05 g. Significant differences were found between sexes, females being larger ($z = 2.14$, $p < 0.05$) and heavier ($z = 2.12$, $p < 0.05$) than males.

Age distribution was calculated for all the individuals and is shown in Fig. 1. As it was seen the oldest male in population was 7, and the oldest female was 6 years old. Average age in males was 3.90±1.37 yrs (range 1–7 yrs; n = 38), compared to 3.72±1.00 yr (range 2–6 yrs; n = 11) in females. The phalange cross-sections of 4-year-old individual are illustrated in Fig. 2.

The relationship between SVL and age is shown in Fig. 3. Male SVL was significantly correlated with age ($y = 77.04–32.53e^{-0.49(x-1)}$), but no significant correlation was obtained between female SVL and age. SVL was also significantly correlated with phalange size in males ($R = 0.567$, $p < 0.05$, Phalange size = 196.44+6.13 SVL), but not in females ($R = 0.382$, $p > 0.05$).

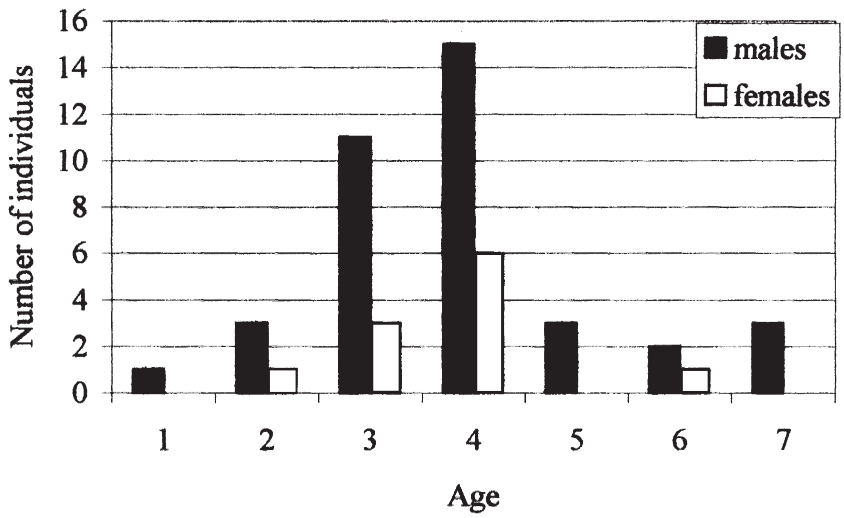

Fig. 1. Age distribution of the population

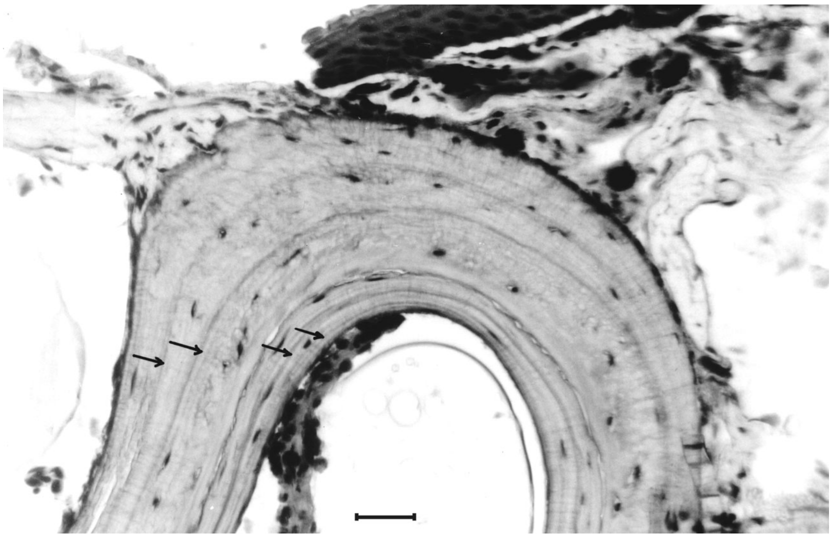

Fig. 2. Cross-section taken at the middle of the diaphysis of *Rana ridibunda*, a male, 58 mm SVL, 4 RL (scale: 108 µm)

In females, ovary mass was significantly correlated with SVL ($R = 0.816$, $p < 0.05$, ovary mass = –5.895+0.11 SVL) but we did not detect any significant correlation with age ($R = 0.159$, $p > 0.05$). In males, testes mass was significantly correlated with SVL ($R = 0.805$, $p < 0.01$, testes mass = –0.128+0.003 SVL) but not age ($R = 0.447$, $p < 0.05$, testes mass = 0.015+0.013 Age).

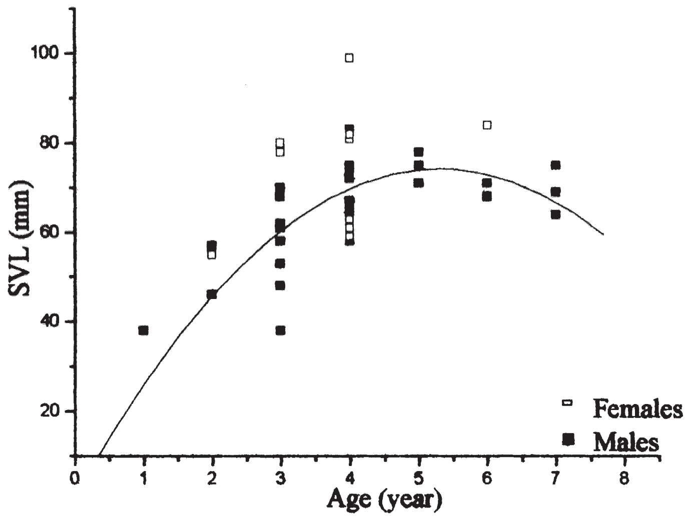

Fig. 3. The relationship between SVL and age (in males; $y = 77.04–32.53e^{-0.49(x-1)}$)

Liver mass was significantly correlated with SVL both in males and females (males: $R = 0.877$, $p < 0.01$, liver mass = –0.569+0.017 SVL; females: $R = 0.911$, $p < 0.01$, liver mass = –1.36+0.03 SVL). Liver weight was also significantly correlated with age in males ($R = 0.50$, $p < 0.05$, liver weight = 0.257+0.08 Age) but no significant correlation was detected among females ($R = 0.159$, $p > 0.05$, liver weight = 77.5+7.5 Age). And, there was a significant correlation between stomach weight (with food) and SVL in females ($R = 0.557$, $p > 0.05$, stomach weight = –2.50+0.07 SVL) contrary to males ($R = 0.433$, $p < 0.05$, stomach weight = –0.737+0.03 SVL). No correlation was obtained between stomach weight and age in both sexes (males: $R = 0.253$, $p > 0.05$; females: $R = 0.245$, $p > 0.05$).

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). The thickness of the growth rings of a one-year-old caught frog was 450 µm and medullary cavity 430 µm, it had a 20 µm bone thickness beyond RL-1. When converted into growth rate, these data give a value of 0.247 µm/day. The mean growth rates of 2, 3, 4, 5, 6 and 7 years old frogs were shown in Fig. 4.

Females were sexually mature after the third (22.20%), fourth (66.66%) or fifth (11.11%) winter and males after the third (38.46%) or fourth (61.53) winter. There were two subadult males (1-year-old, 5.64 g, 38 mm and 3-year-old, 5.83 g,

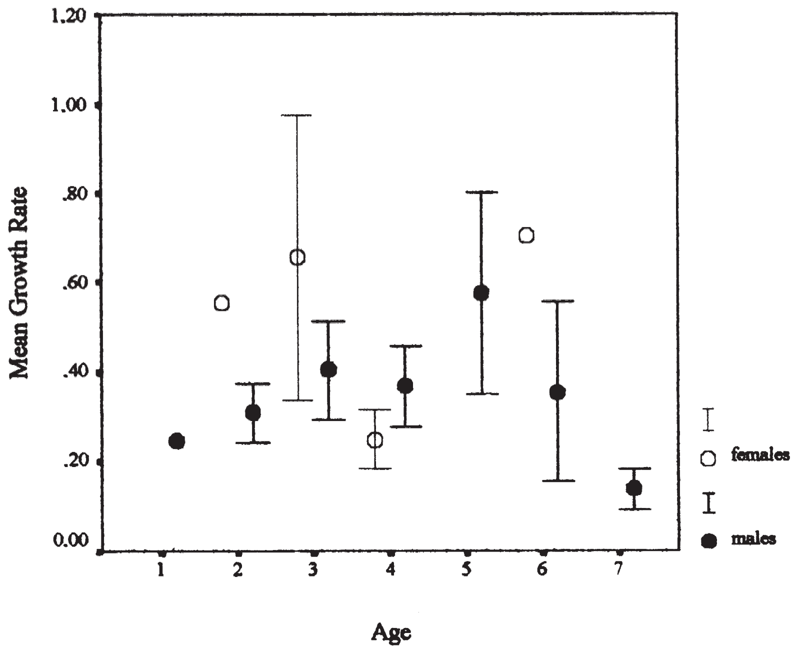

Fig. 4. The mean growth rates of males and females

38 mm) and a single female (2 years of age, 23.58 g, 55 mm) in population. The data suggest that sexual maturity in this population was reached by 3–4 years old while there were some reached by 2. The subadult males had a testes mass smaller than 0.001 g and the female had no ovary.

In amphibians, maturity is generally attained after having reached a certain minimum size, but a variable age (GIBBONS & MCCARTHY 1984, HEMELAAR 1986, RYSER 1988). Consequently, at first reproduction the body length have to exceeds a minimum size which in our study was 59 mm for females and 46 mm for males.

DISCUSSION

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 *R. ridibunda* in Turkey. The maximum life span recorded for our population from Yıldızlı Stream (36 m a.s.l.) was 7 years of age in females and 6 years in males, similar to results obtained in low altitude populations in Russia. The maximal life-span of this species is only 4 years in Tajikistan and 6 years in the Tulga region (central Russia). However, in the region of Volgo-Kamzky preserve (to the north east of Tula), it can reach an age of 11 years. Greater longevity is also registered for mountain populations of the water frog in Armenia, 9–10 years for population from Lake Sevan (1900 m a.s.l.). Age at maturity was 2 years for males from the Razdan valley and 3–4 years for males from Sevan and Chosrov. Females matured 1–2 years later (SMIRINA 1994). LEDENTSOV and MELKUMYAN (1986) also found that the maximal age of water frogs from another region of Armenia, located 2500 m a.s.l., was 11 years.

In our population, males and females first reproduced after their third or fourth winter, although some females delayed maturity until their fifth year. This intrapopulational variability can be attributed to the long breeding and metamorphic periods and, consequently, to the different sizes reached by individuals before their first winter. Our results of age at sexual maturity is similar to other estimates from Sevan and Chosrov populations. In general, there is a trend to increased age of northern and mountain populations of frogs and toads in comparison with southern and lowland populations (SMIRINA 1994). Such differences between frogs from low and high elevations could result from the temperature and the length of active periods as reported by KARA (1994). The age of sexual maturity may be influenced by differences in climatic conditions or by other aspects of life history, such as the tadpole period (DIAZ-PANIAGUA *et al.* 1996).

Body size and age are generally positively but weakly correlated in amphibians (HALLIDAY & VERRELL 1988), however, some do not have a positive correlation and it may be true for one sex and not the other. HEMELAAR (1986) reported positive correlation for *Bufo bufo* for both sexes as did RYSER (1988) for *R. temporaria.* HÖGLUND and SATERBERG (1989) found positive correlation between body size and age for female *B. bufo* but not for males. Mostly or only males have shown positive correlation in the studies of LECLAIR and CASTANET (1987) for *R. pipiens,* LYKENS and FORESTER for *Pseudacris crucifer* (1987), BASTIEN and LECLAIR (1992) for *Bufo pardalis.* In our study there is a positive correlation between age and size for both sexes but only significantly in males. So our findings are quite similar in this respect to previous studies except HÖGLUND and SATERBERG (1989).

From the measurements of bone thickness between RL's, it was seen that the growth pattern of *Rana ridibunda* is up to maturity, then continued growth at a decreased rate, and there is also variations in growth and hence age-specific sizes as well as in most other anurans (HEMELAAR 1986, RYSER 1988, GIBBONS & MCCARTHY 1983, 1984). Individual variation in growth may be caused by environmental factors (RYSER 1988).

As RYSER (1988), GIBBONS and MCCARTHY (1984) and HEMELAAR (1986) reported previously, in amphibians, maturity is generally attained after having reached a certain minimum size, but a variable age. This was also observed in our study.

The inter-sexual differences in growth and maturation pattern suggest that natural and/or sexual selection act differently on male and female. Body size is an important determinant of female fecundity, large females have larger ovary mass than smaller females (see also GIBBONS and MCCARTHY (1986), RYSER (1988)). Thus fecundity selection may one factor that may be explain why female water frogs reach a larger body size compared to males.

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