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NOTES ON STUDY OF AGE AND GROWTH OF THE HYRCANIAN FROG, *Rana pseudodalmatina* EISELT ET SCHMIDTLER, 1971 IN THE TALYSH MOUNTAINS

Artem A. Kidov,[1] Renat I. Zamaletdinov,[2] Kseniya A. Matushkina,[1] and Regina I. Mikhailova[3]

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The article presents the first results of a study of age and growth of the Hyrcanian frog, *Rana pseudodalmatina* in the Talysh Mountains. The animals were caught in the first Decade of March 2011 in Sym village in Astara District of Azerbaijan. The age was determined by a skeletochronological analysis of clipped phalanges of the third toe of hind limb. Preparations were made and examined with well-visible thick sections of 56 males and 7 females. Males in the sample were 1 – 5 years old and females were 2 – 4 years old. The average age of males was 3.2 years and 3.7 years of females. Most of studied frogs were specimens in the age of three and four years. The most intensive growth of the Hyrcanian frog occurs before reaching sexual maturity. A male at the age of one year had a body length equal to 309.8% of the average length of the metamorphs from the same reservoir, two-year-old males were 328.8 – 341.3%, three-year-olds were 271.7 – 385.9%, four-year-olds were 347.8 – 388.6%, five-year — 369.6 – 421.2%. The relative length of a two-year-old female was 342.4%, three-year-old females — 391.3 – 432.1, four-year-olds — 383.2 – 397.8%.

Keywords: the Hyrcanian frog; *Rana pseudodalmatina*; age; growth; skeletochronology.

INTRODUCTION

Brown frogs of the Southern Caspian region have been previously considered as *Rana macrocnemis* Boulenger, 1885 or *R. camerani* Boulenger, 1886 (Bannikov et al., 1977; Veliyeva, 1975; Alekperov, 1978). After description of the subspecies *R. macrocnemis pseudodalmatina* Eiselt et Schmidtler, 1971 (Figs. 1 and 2) from Iranian province of Mazandaran, question arose about taxonomic status of brown frogs of the Talysh and the Kopetdagh mountains (Bannikov et al., 1977; Borkin, 1977; Ananjeva et al., 1998; Kuzmin, 1999). Recently, using molecular genetic methods, it was considered that brown frogs from southeastern Azerbaijan should be referred to this taxon (Litvinchuk et al., 2008). Veith with co-authors (2003) considered the Hyrcanian frog a distinct species, on the basis of geographical isolation and significant molecular genetic differences from other brown frogs of Asia Minor and the Caucasus. This point of view was later supported by other authors (Frost, 2016; Kidov, 2016).

The Hyrcanian frog remains as compared to other brown frogs of the Western Palearctic, which are common in population and skeletochronological studies and studies (Ishchenko, 1996; 2005; Lyapkov et al., 2002; Matkovski et al., 2011; Lyapkov and Volontsevich, 2013). Such population characters as the age of maturity, the intensity of growth, and the maximum life span have not been established for this species yet.

The aim of this paper was to study these characters for the Hyrcanian frog from Azerbaijan using a skeletochronological analysis.

MATERIAL AND METHODS

Animals were caught in the first decade of March 2011 in dug flowing ponds, which are used by local people for fish farming, in Sym village of Astara District of

[1] Russian State Agrarian University — K. A. Timiryazev Moscow Agricultural Academy; 49 Timiryazevskaya ul., Moscow 127550, Russia; e-mail: kidov$a@mail.ru

[2] Kazan (Volga region) Federal University, 18, Kremliovskaya ul., Kazan, 420008, Russia; e-mail: i.ricinus@mail.ru

[3] N. É. Bauman Kazan State Academy of Veterinary Medicine, 35, Sibirsky trakt, Kazan, 420029, Russia.

Fig. 1. Adult female of the Hyrcanian frog from Sym village (Astara District, Azerbaijan).

Azerbaijan (38°29′ N 48°38′ E, 480 m a.s.l.; Fig. 3). The body length (*L*) was measured by a standard method (Bannikov et al., 1977) using a caliper with an error of 0.1 mm. Afterwards, the proximal phalanges of the third digit of the right hind limb was clipped. Used frogs were kept in artificial conditions for 3 – 5 days and after this released at places of capture. Subsequently, toe-clipped frogs were repeatedly found in surveyed ponds in January 2012 (Kidov, 2012) and January 2013.

The study of age structure of frogs was carried out using skeletochronological analysis according to a standard procedure (Smirina, 1989; Belyavsky et al., 2007). Studied bones were sectioned on a MK-25 freezing microtome.

In total, 63 frogs (56 males and 7 females) with well-visible thick sections were prepared and examined.

The process of determination of age of individuals was not limited a counting of thin lines of arrested growth (LAG) deposited in the periosteal bone during hibernation only. It is known that in most species of Palearctic amphibians there are no real osteons in skeletons. For this reason, during growth of animals, endosteal resorption occurs in tubular bones from a side of endosteal cavity. It is considered that the process is actively occurring before start of sexual maturity (Smirina, 1989).

The rate of endosteal resorption varies in different species and during life of individual. After start of sexual maturity, in connection with overall decreasing of growth the processes of resorption decrease or stop. Therefore, adhesion lines, which are not resorbed after start of sexual maturity and formed after it, evidently persist throughout a life of animal. After that, the rate of resorption decreases and almost stops. As a result of the resorp-

Fig. 2. Adult male of the Hyrcanian frog from Sym village (Astara District, Azerbaijan).

Fig. 3. The breeding site of the Hyrcanian frog in Sym village (Astara District, Azerbaijan).

tion process, destruction of the first LAGs is possible. To ensure accuracy of age determination it is necessary to analyze the rate of resorption (Smirina, 1989).

The most accessible method for determination of the rate of resorption is a comparison of the bone diameter in cross sections in to the diameter of bone marrow cavity and the bone diameter limited by the first visible complete line of adhesion in adult animals (Smirina and Makarov, 1987).

In a case of juveniles from laboratory breeding (Kidov et al., 2015), we measured the diameter or cross-sectional area of bone. The term "diameter" in this case means a mean value of measurements of large and small semiaxes. The term "area" in this case means a product of magnitudes of the large and small semiaxes. In case of adults, the bone marrow cavity and the bone diameter were measured, bounded by the first visible complete LAG. The obtained data were averaged, and bone diameters of yearlings were compared to the diameter of endostea of adults. It is considered that the error of this method does not exceed one year (Smirina and Makarov, 1987).

The age estimation was individually carried out for each individual of our data about rate of resorption in the species.

Our data about the average body length of metamorphs (18.4 ± 0.21 mm; Kidov, 2010) were used for study the growth dynamics of the Hyrcanian frog.

The Pearson test was used to analyze a relationship between age and body length of frogs.

RESULTS

Collected animals were mainly represented by males (88.9%). According to our data about peculiarities of biology of the Hyrcanian frog (Kidov, 2010; Kidov and Matushkina, 2013), males come to ponds in autumn and many of them overwinters in a water. Females mainly hibernate on land (Kidov, 2012). Males, which winter on land, are also migrating to reservoirs than favorable temperatures for reproduction will be established. Most of these males reachs at spawning sites until the end of a breeding season. Consequently, the number of males in a water body used for breeding is constantly increasing due to arrival of new individuals.

Females move to reservoirs only directly before spawning, one by one, and immediately after laying of eggs they tend to leave these water bodies. Probably, in many respects this form of behavior is caused by reproductive behavior of males. It has been repeatedly noted (Kidov, 2010; Kidov and Matushkina, 2013) that weak-

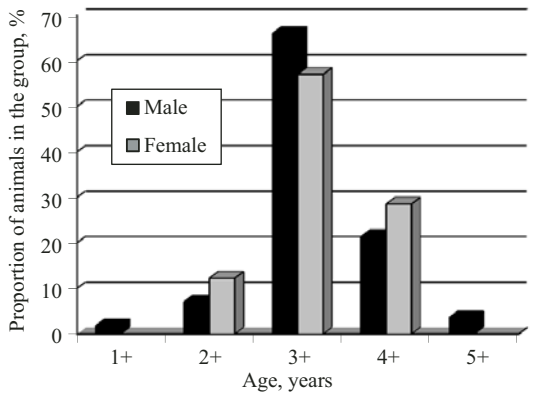

Fig. 4. The age structure of the Hyrcanian frog in the sample.

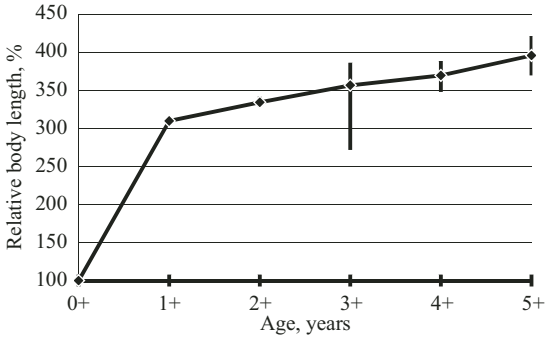

Fig. 5. The relative body length of males of the Hyrcanian frog in different age groups.

ened by spawning females that did not leave a breeding pond due to unfavorable weather conditions such as a frost or a snowfall, died due to constant attempts of males to mate with them. Similar cases have also been described for closely related long-legged wood frog in the North Caucasus (Popov, 1958).

In the process of measuring of the diameter of bone marrow cavity, it was established that among sexually mature individuals, two groups can be identified according to the rate of resorption of endosteal cavity (Table 1). This is typical for both sexes. Only the first LAG is resorbed (denoted respectively as ♀♀1 and ♂♂1) in the first group of individuals until a start of maturity. Individuals of the second group (designated respectively as ♀♀2 and ♂♂2) are characterized by resorption of the first two LAG. If we propose that has strong relationship between the rate of resorption and the age start of maturity, it is probably individuals from these two groups differ in terms of a maturation rate (Zamaletdinov et al., 2005).

According to data obtained, it can be assumed that during a growth process one or two LAGs are resorbed before start of maturity in both sexes.

The average age in the sample studied was 3.2 years for males and 3.7 years for females. The majority of animals studied consisted of specimens at age of three (66.1% for males and 57.1% for females) and four (21.4% and 28.6% respectively) years (Fig. 4).

The youngest male was one-year-old, and the youngest female was two-year-old. The oldest males were 5 years old, and the females were 4 years old.

We, like other researchers (Ishchenko, 1996; 2005; Lyapkov et al., 2002; Matkovsky et al., 2011; Lyapkov and Volontsevich, 2013), at the first believed that all frogs founded in a spawning pool during breeding season are potentially capable for reproduction, and at the second their minimum age can be considered as a lower age limit for achieving fertility.

In another closely related species, the long-legged wood frog, the age of the start of maturity varies from two to three years and depends from vertical distribution (Ishchenko, 1996; Gokhelashyili and Tarkhnishvili,

TABLE 1. The Diameter (mm) of the Bone Marrow Cavity (DMC) and Bone in the Middle of the Diaphysis of the Phalanx of the Fourth Finger of the Right Hind Limb (DB) $\frac{M \pm m}{\text{limits}(n)}$

Sex	DMC	DB			DB in the first year
		1 bonding line	2 bonding line	3 bonding line	
♀♀1	$\frac{0.215 \pm 0.012}{0.203 - 0.228(2)}$	$\frac{0.282 \pm 1.1}{18.3 - 20.5(2)}$	$\frac{0.389}{0.389(1)}$		$\frac{0.24 \pm 0.011}{0.2 - 0.27(5)}$
♀♀2	$\frac{0.289 \pm 0.012}{0.257 - 0.313(5)}$	$\frac{0.396 \pm 0.04}{0.394 - 0.407(5)}$	$\frac{0.483 \pm 0.031}{0.452 - 0.514(2)}$		
♂♂1	$\frac{0.228 \pm 0.004}{0.194 - 0.25(23)}$	$\frac{0.326 \pm 0.011}{0.246 - 0.458(22)}$	$\frac{0.384 \pm 0.012}{0.303 - 0.516(18)}$	$\frac{0.465 \pm 0.021}{0.398 - 0.522(5)}$	
♂♂2	$\frac{0.288 \pm 0.005}{0.252 - 0.361(33)}$	$\frac{0.394 \pm 0.09}{0.29 - 0.516(33)}$	$\frac{0.443 \pm 0.013}{0.376 - 0.574(14)}$	$\frac{0.445 \pm 0.014}{0.477 - 0.492(2)}$	

TABLE 2. Length of Body of the Hyrcanian frogs in Different Age Groups

Age group	Length of body, mm			
	male		female	
	n	mean ± *S.E.*(*S.D.*) / min – max	*n*	mean ± *S.E.*(*S.D.*) / min – max
1+	1	57.0	—	—
2+	4	61.5 ± 0.57(0.99) / 60.5 – 62.8	1	63.0
3+	37	65.6 ± 0.86(5.12) / 50.0 – 71.0	4	76.0 ± 1.78(3.08) / 72.0 – 79.5
4+	12	68.0 ± 1.08(3.58) / 64.0 – 71.5	2	71.9 / 70.5 – 73.2
5+	2	72.8 / 68.0 – 77.5	—	—
Total for sample	56	65.8 ± 0.70(4.94) / 50.0 – 77.5	7	73.0 ± 2.18(5.34) / 63.0 – 79.5

1994). The maximum age noted for *R. macrocnemis* from populations distributed on various altitudes in the North Caucasus was 5 – 12 years (Ishchenko, 2005).

A positive relationship ($r = 0.46, p \le 0.01$) was found between age of males studied and their body length (Table 2).

Apparently, like other Palaearctic brown frogs (Ishchenko, 2005), most intensive growth in the Hyrcanian frog occurs before reaching sexual maturity. Consequently, the male at the age of one year had the body length equal to 309.8% of the average length of metamorphs collected from this locality (Kidov, 2010), two-year-old males were 328.8 – 341.3% of the length, three-year-olds were 271.7 – 385.9%, four-year — 347.8 – 388.6%, and five-year — 369.6 – 421.2% (Fig. 5). The relative length of a two-year-old female was 342.4%, three-year-old females — 391.3 – 432.1%, four-year-olds — 383.2 – 397.8%.

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