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Age structure and growth in two Tunisian populations of green water frogs *Rana saharica*: a skeletochronological approach

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Age structure was determined by skeletochronology in Tunisian *Rana saharica* from two pond sites, one permanent, and the other temporary. Frogs matured at age 3 years; no males older than 5 were found, whereas some females had reached 7 years. Age for age, females were larger than males, but the differences were not significant. There was no obvious difference in age/size relationships between the two ponds. The high variability in juvenile sizes may relate to different life history patterns, as suggested for Moroccan *R. saharica*.

Key words: frog age-size relationships, skeletochronology, Tunisia

INTRODUCTION

In this paper, we report on age structure and size in two populations of *Rana saharica* from Tunisia. Meddeb & Cheniti (1998) earlier reported on the diversity of prey utilized by frogs in these two populations. The only previous report of age estimation in *R. saharica* is by Esteban et al. (1999), based on a Moroccan sample from the margins of the Sahara, a very different climate zone from that occupied by the Tunisian populations we have studied.

Although Steinwarz & Schneider's (1991) bioacoustic study indicated that there are two species of green water frog in Tunisia, *Rana perezi* (Seoane, 1885) and *R. ridibunda* (Pallas, 1771), Schleich et al.'s (1996) review of North African amphibians supported the conclusion that Steinwarz and Schneider's *R. perezi* is *R. saharica* (Boulenger, 1913), while admitting that the taxonomic situation of the North African water frogs is "confusing".

Arano et al. (1998) partially resolved the confusion using a combination of allozyme and morphometric characters. They showed that the Iberian Peninsula *R. perezi* is clearly distinct from the North African water frogs, confirmed as *R. saharica.* Furthermore, analysis of specimens from Morocco and Algeria provided evidence for a subspecies-level divergence, with the Moroccan population named as *R. saharica riodeoroi* and the Algerian as *R. saharica saharica*. Arano et al. (1998) noted that the precise boundaries between these two populations were yet to be determined, but that they did not precisely coincide with national borders. Arano et al. (1998) did not sample Tunisian populations of *R. saharica.*

We have used skeletochronology to estimate age. This method uses growth marks, especially "lines of arrested growth" (LAGs) deposited in growing bone, and has become widely used to estimate individual age, especially in reptiles and amphibians living in temperate climates where seasonal growth arrest occurs (see Castanet et al., 1993 and Halliday & Verrell, 1988 for methodology and review of uses). We sampled from two pond sites, one permanent, the other temporary, in an attempt to show whether habitat differences have significant effects on the ecology of these little-studied frogs.

MATERIALS AND METHODS

Study sites

Sampling was carried out at two sites, 90 km apart. The first site was a permanent pond in a disused quarry near Radès, 10 km from Tunis City (10°16'N, 36°45'E). The pond is about 3500 m^2 in area and is at an altitude of 20 m. Mean annual rainfall is 454 mm and mean temperature ranges from 7 °C in winter to 32 °C in summer. The second site was a temporary pond close to Menzel Bourguiba city (9°52'N, 37°45'E). The pond is about 5000 m^2 in area and is at an altitude of 10 m. Mean annual rainfall is 653 mm and mean temperature ranges from 8 °C in winter to 31 °C in summer. The pond normally dries out from June to August. Weather data were obtained from local meteorological stations at Radès and Menzel Bourguiba.

Frog samples

This study is based on 123 frogs, 79 from Radès and 44 from Menzel Bourguiba, captured in March and April 1995. This is the same sample as that used by Meddeb & Cheniti (1998) to report on food diversity. Frogs were caught during the day in the water, using nets, during the mating congregation. They were killed immediately, abdomens cut open to expose internal organs, and preserved in 10% formalin. Although it is possible to carry out age determinations without sacrificing frogs, by

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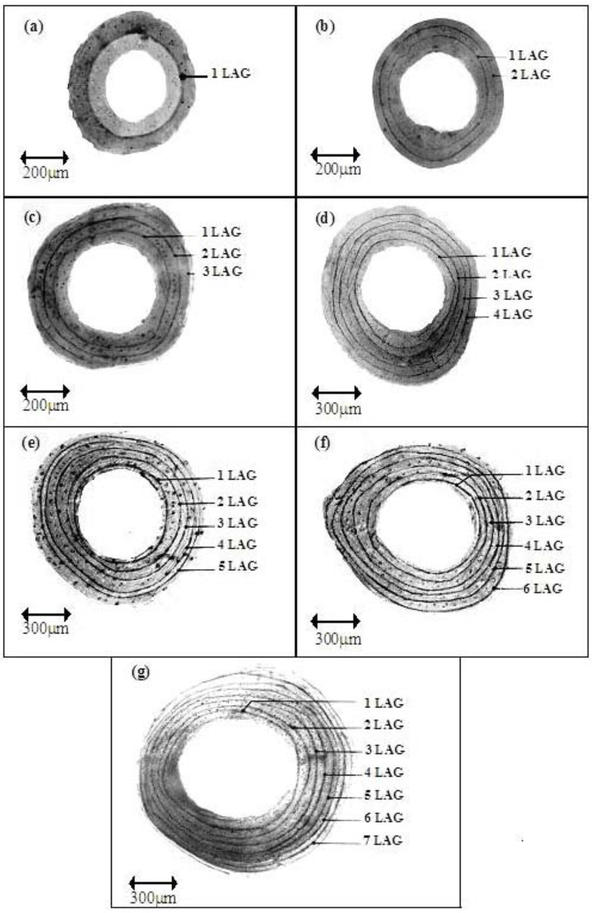

Fig. 1. Cross-sections of femoral diaphyses of *Rana saharica*, illustrating seven age classes. a) 1 year; b) 2 years; c) 3 years; d) 4 years; e) 5 years; f) 6 years; g) 7 years. LAG = line of arrested growth. Numbers refer to the year order of each LAG.

using digital phalanges for skeletochronology, we feel that the extra information gained on diet and reproductive status justifies the killing of this sample.

In the laboratory, sex was determined for each frog: adult males have black vocal sacs and black and brown nuptial pads on the first finger of the forelimb; internally, male and female reproductive systems are easily distinguishable. Ovaries were examined for the presence or absence of yolked oocytes as an indication of maturity. Snout–vent lengths (SVL) were measured using callipers to 0.1 mm.

Skeletochronology

Specimens were prepared for skeletochronological assessment using the standard methods for amphibians and reptiles reviewed by Castanet et al. (1993). Fixation in 10% formalin prevents demineralization of bones. Femurs were dissected out, measured using callipers to 0.1 mm and decalcified by immersion in 5% nitric acid for 48 h. They were then washed in running water for 36 h to remove all traces of formalin and nitric acid. Cross-sections were then made at 20 mm thickness through the diaphysis of each femur, using a freezing microtome set at -28°C.

Table 1. Age and sex distribution of *Rana saharica* from Radès and Menzel Bourguiba. Distribution given as percentage of the total sample from each site.

Age	Radès			Menzel Bourguiba		
(y)	No.	% male	% female	No.	% male	% female
1	2	2.5	0	5	6.7	4.4
2	17	11.4	10.1	13	15.6	13.3
3	27	16.5	17.7	19	17.8	24.4
4	22	21.5	6.3	2	4.4	0
5	9	7.6	3.8	5	6.7	4.4
6	1	0	1.3	0	0	0
7	1	0	1.3	1	0	2.2
Total	79	59.5	40.5	45	51.1	48.9

The sections were stained in Ehrlich's haematoxylin for 1.5 h, then rinsed in successive baths of tap water for 10 and 20 min, and mounted in Aquamount (Gurr).

Ten cross-sections from each femur were analysed independently by three experienced observers. Sections were code-numbered so that the observers did not know from which frog they were derived.

RESULTS

Age and sex distribution

Figure 1 shows cross-sections of femoral diaphyses of frogs estimated as aged 1–7 years. Our criterion was that a single line of arrested growth (LAG) represents a frog developed the previous year, with the LAG corresponding to the winter just past. Extra LAGs each represent an additional winter. LAGs were always distinct, and there was no sign of endosteal resorption eliminating the innermost LAG.

Table 1 shows the age and sex distribution of frogs sampled from the two sites. The three observers agreed on the ages of all frogs except one older female from Radès that was classed as 7 years old on majority verdict.

Juvenile frogs were under-represented in our samples. In both ponds, smaller frogs tended to be isolated from the main congregations of adults. Females at one and two years were immature, as deduced from the lack of yolked oocytes in their ovaries. On the basis of first appearance of secondary sexual characters (callosities on the first forelimb finger; black vocal sac) males also became mature at three years.

At both ponds, no males more than 5 years old were found, whereas small numbers of females reached 7 years. The sex ratio of our sample from Menzel Bourguiba was exactly 1:1; at Radès, it was skewed towards males, but not significantly so ($\chi^2 = 2.85$; $P>0.05$). There was no strong evidence that the ephemeral nature of the Menzel Bourguiba pond, compared to the permanence of the pond at Radès, had any effect on the age distribution of the frogs.

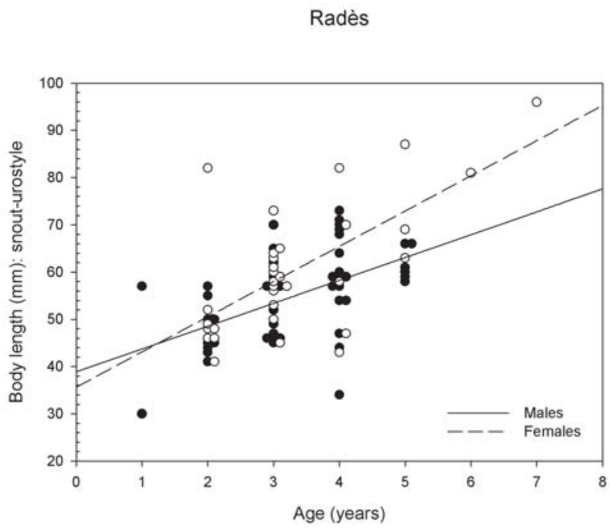

Fig. 2. Relationship between body length and age in male and female *Rana saharica* from Radès. Equations for regression lines are: males y=38.9+4.84x; females y=35.6+7.46x. Closed circles = males; open circles = females.

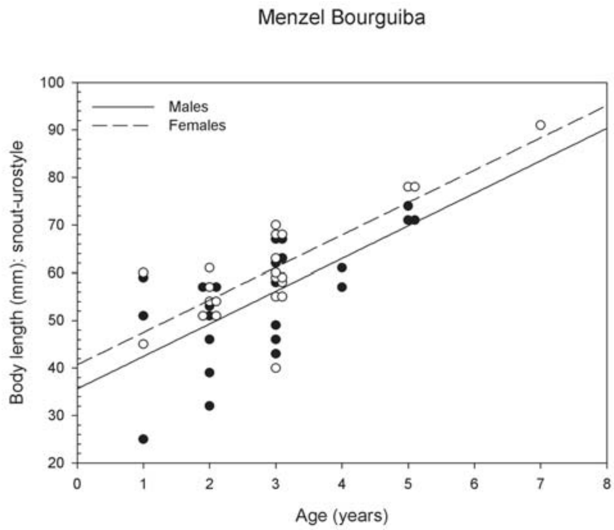

Fig. 3. Relationship between body length and age in male and female *Rana saharica* from Menzel Bourguiba. Equations for regression lines are: males y=35.60+6.84x; females y=40.7+6.86x. Closed circles = males; open circles = females.

Age and size relationships

Figures 2 and 3 show the relationships between age and snout–vent length in males and females for Menzel Bourguiba and Radès respectively. The relationships for males and females look a little different, with faster growth in females in both cases, but *t*-tests show that the lines are not significantly different in either case (Menzel Bourguiba: t=0.23, P>0.4; Radès: t=1.37, P>0.1). Similarly, the age/length relationships for the complete samples (males and females) at the two sites are not significantly different (not shown). The lack of difference between the sexes may be due to the high variability of the data: it is noticeable that in each age class, females are generally larger than males, and the female regression lines are above those for the males. There is considerable overlap in size between the age classes, with some individuals very small for their age and a few larger than expected from the slopes of the regression lines. Age and size are significantly correlated at Menzel Bourguiba for females (r=0.71, P< 0.001) and males (r=0.66, P<0.001); and at Radès for females (r=0.66, P<0.001) and males (r=0.51, P<0.001).

DISCUSSION

The results we report provide an interesting comparison with those of Esteban et al. (1999) from the same species. Their sample was from Erfoud in Morocco, about 660 km south and 1300 km west of our sites. Erfoud is at an altitude of 955 m, has a very low rainfall (70 mm per year), a thermal range of 2–41°C and a short (one month) annual cessation of plant growth. Esteban et al. (1999) were particularly interested in whether bone growth patterns in this hot dry climate would differ from their earlier findings from *R. perezi* in the Iberian Peninsula (Esteban et al., 1996).

For Moroccan *R. saharica* they found that older frogs had mostly well-defined but thin LAGs, corresponding to short winter periods of arrested growth. However, juveniles appeared to remain continuously active and growth marks were mainly annuli, denoting slower but not stopped growth.

Our two sampling sites (probably from a different subspecies) were from a very different climate (much wetter, less extreme temperature range, lower altitude) and latitude. LAGs were generally very distinct, suggesting a well-defined annual inactivity period. We saw no sign of a different pattern in juveniles. However, as noted earlier, we only sampled a small number in this class. Although activity patterns of *R. saharica* have not been studied in detail, personal observations (C.M.) suggest that they hibernate in mud from December to mid February; a summer aestivation has not been reported.

In our study, we assessed LAGs in cross-sections of femurs following Francillon (1979) and other authors. Esteban et al. (1999) used phalanges: some authors have preferred phalanges because they can be sampled without killing the frogs (not an issue in our case, because the frogs were killed in order to assess several aspects of their biology) or because they may suffer less from problems of LAG interpretation and endosteal resorption. Leclair et al. (2005) compared phalanges and femurs in a study of *Pelobates cultripes* and found no significant differences. Esteban et al.'s (1999) sample included some juvenile specimens that were not possible to age accurately because of a line interrupted as a "line of metamorphosis", usually close to another line. As noted, our sample included few juveniles, and we did not encounter closely-spaced lines according with Esteban et al.'s (1999) description.

Our sample of *R. saharica* appeared to mature a little older than those in Morocco (at three years old rather than two). Other features were similar: lack of clear sexual size dimorphism, maximum age of 6–7 years, maximum SVL of 80–90 mm. (The Tunisian populations we investigated had a few slightly older, larger individuals than in Morocco.)

Esteban et al. (1999) found high size variation within each age class, especially in the first year. When they took their sample, in April, ponds contained tadpoles at all stages, including metamorphosis, and the ovaries of females were at various stages in the oogenetic cycle. Schleich et al. (1996) summarized earlier reports that Moroccan water frogs may have two (or more) reproductive strategies, with short or extended mating seasons, and tadpoles metamorphosing before winter, or overwintering in pools to metamorphose at a larger size the following spring. Something of this sort also occurs in southern Spain in *R. perezi* (Esteban et al., 1996), complicating the age–size relationship for juveniles.

It is not known whether such flexibility of reproductive strategy occurs in Tunisian *R. saharica*, but it could be a possible explanation for the high variability we also found in age-size relationships, at least in younger frogs. We had expected to find some differences in age and size distribution between our two sites, related to their differing natures, Radès being a permanent pond and Menzel Bourguiba drying out in summer. The lack of significant differences may be due to sample variability, but further study of tadpoles and juveniles will help clarify whether life history variability is a factor in Tunisia.

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REFERENCES

Arano, B., Llorente, G.A., Montori, A., Buckley, D. & Herrero, P. (1998). Diversification in north-west African water frogs: molecular and morphological evidence. *Herpetological Journal* 8, 57–64.

Castanet, J., Francillon-Vieillot, H., Meunier, F.J. & de Ricqlès, A. (1993). Bone and individual aging. In *Bone Vol. 7. Bone growth – B*, 245–283. Hall, B.K. (ed.). Boca Raton, Florida: CRC Press.

Esteban, M., García-París, M. & Castanet, J. (1996). Use of bone histology in estimating the age of frogs (*Rana perezi*) from a warm temperate climate area. *Canadian Journal of Zoology* 74, 1914–1921.

Esteban, M., García-París, M., Buckley, D. & Castanet, J. (1999). Bone growth and age in *Rana saharica*, a water frog living in a desert environment. *Annales Zoologici Fennici* 36, 53–62.

Francillon, H. (1979). Étude expérimentale des marques de croissance sur les humérus et les fémurs de tritons crêtés (*Triturus cristatus cristatus* Laurenti) en relation avec la détermination de l'âge individuel. *Acta Zoologica* 60, 223–232.

Halliday, T.R. & Verrell, P.A. (1988). Body size and age in amphibians and reptiles. *Journal of Herpetology* 22, 253–265.

Leclair, M.H., Leclair Jr., R. & Gallant, J. (2005). Application of skeletochronology to a population of *Pelobates cultripes* (Anura: Pelobatidae) from Portugal. *Journal of Herpetology* 39, 199–207.

Meddeb, C. & Cheniti, T.L. (1998). Régime alimentaire de deux populations de grenouille verte de Tunisie *Rana saharica* Boulenger, 1913 (Amphibiens, Anoures, Ranidae). *Bulletin de la Societé Zoologique de France* 123, 3–14.

Schleich, H.H., Kastle, W. & Kabisch, K. (1996). *Amphibians and Reptiles of North Africa*. Koenigstein: Koeltz Scientific Books.

Steinwarz, D. & Schneider, H. (1991). Distribution and bioacoustics of *Rana perezi* Seoane, 1885 (Amphibians, Anura, Ranidae) in Tunisia. *Bonner Zoologische Beitrage* 42, 283–207.

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