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Maximum body size and age distribution in the Italian Stream Frog, *Rana italica* Dubois 1987 (Amphibia: Anura)

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Abstract. Data on mean longevity and age structure of *Rana italica* are very scanty. Skeletochronological data comparisons with other European brown frogs showed that life span of *R. italica* is similar to those of other congeneric species. In this study we report the age structure of a population of *R. italica* from Central Italy with a new record of species maximum size. The study population inhabits a perennial stream in a lowland oak wood. All the individuals were marked by means of toe clipping. Skeletochronological analyses showed that adult age ranged from 3 to 5 years in males and from 2 to 6 years in females with no intersexual difference. In our study, the oldest individual (estimated age was 6 years) was also the biggest (68 mm), representing the new body size record for the species. Previous skeletochronological studies on *R. italica* have shown that the mean longevity for this species is four years but the maximum age recorded is 5 and 8 years in low and high elevation populations, respectively. The ages recorded for the study population inhabiting a hilly area showed higher average and maximum values than those found in lowland populations.

Keywords. *Rana italica,* maximum size, age, skeletochronology.

Rana italica (Dubois 1987) is an anuran amphibian belonging to the Brown Frogs group, widely distributed within the Italian Peninsula, at elevations ranging between 30 and 1400 m a.s.l. (Picariello et al., 2006). Despite its abundance and distribution, the knowledge about the ecology of this species is scanty and is mostly the result of general observation rather than that of specific studies.

The Italian stream frog inhabits small streams within the wet, broad-leaf forests of the Apennines from central Liguria to Calabria (Picariello et al., 2006). It usually prefers fast flowing streams with a rocky substrate but it also occurs in small ponds, mountain peat bogs and in some man-made habitats such as water troughs. Adult males are smaller than adult females, with a maximum reported snout-to-vent length of 60 mm for males and of 65 mm for females (Lanza et al., 2009). In addition, males are brownish-grey while females are reddish-brown. Both males and females remain near stream pools for most of the year, using rocks on the bottom of deeper pools with permanent water as refuges against predators, as well as for mating and egg laying (Lanza, 1983; Picariello et al., 2006). Breeding occurs from late January to early May and oviposition peaks in February-March depending on the altitude (Guarino et al., 1993).

The only data on mean longevity and age structure of *Rana italica* derive from skeletochronological studies by Guarino et al. (1995a, b) performed on two populations of Southern Italy. Comparisons with skeletochronological data obtained from other European brown frogs show that life span of *R. italica* (8 yr) is similar to that of *Rana dalmatina* (7 yr, Guarino et al., 1995a; Sarasola-Puente et al., 2011), of *Rana iberica* (9 yr; Esteban and Sanchiz, 2000) but much lower than that of *Rana temporaria* (up

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to 17 yr; Ishchenko, 1996; Miaud et al., 1999; Guarino et al., 2008) and of *Rana arvalis* (11 yr; Ishchenko, 1996). However, it should be taken into consideration that often inter-specific and inter-populational differences in longevity and age at maturity occur depending on latitude and/or altitude (Guarino et al., 2003; 2008).

In this study we report a new maximum body size record for *R. italica* and data on frog age from a population living at the neighbouring of Rome (Central Italy) by using skeletochronological technique.

We collected 101 individuals (40 females, 25 males, and 36 juveniles) of *Rana italica* by dip netting at Cineto Romano (Latium, Central Italy: 42°02′52.39″N 12°56′39.35″E, about 400 m a.s.l.) during the breeding season (March-April) of 2011. Sex was determined on the basis of external morphology and the smallest frogs that did not show unequivocal secondary sexual characters were classified as juveniles. The study population inhabits an oak wood surrounding a small river in the Lucretili Mountains, immediately outside the Lucretili Regional Park boundaries. All the frogs collected were measured from snout-to-vent (SVL) and marked by means of toe-clipping, this technique apparently not affecting frog survival (Guarino et al., 2003). In order to assess the age of the frogs, the phalanxes from the third toe of the left hind leg from a representative sample of collected animals (18 males, 23 females, 11 juveniles) were fixed in 70% ethanol and analysed by means of the standard skeletochronological technique (Guarino et al., 1995a; 1998). Phalanxes were decalcified in 5% nitric acid for about 1h 15 min, rinsed with tap water and cross-sectioned at diaphyseal level using a cryostat. Phalangeal sections, 12 μm thick, were stained with Mayer's acid hemalum for about 25 min and mounted in synthetic resin mounting media (Hi-MO, Bio-Optica). In order to count the lines of arrested growth (LAGs) in the periosteal bone, for each phalanx, about twenty sections were observed under light microscope (Motic BA 340) equipped with digital camera (Nikon Coolpix 5000). We assumed that in the phalanx of this species possible LAGs were deposited annually according to what was observed for other temperate frogs (Paton et al., 1991; Guarino et al., 2003). All the slides were examined and the age was estimated independently by two researchers (VB and FMG). In case of contrasted estimation made by the two observers, the individual was discarded from the analyses. All the collected animals were released at the place of capture just after measuring and toe-clipping.

LAGs were identifiable in all examined phalanges. Although the distinctiveness of LAGs was different from one animal to the other and often within a single bone section, age estimation by LAGs counting was possible in all individuals examined. Age was assessed with an error rate of one year in 17% of the males (n = 3) and 26% of the females (n = 6) due to the presence of weakly stained lines interpreted by us as a LAG but that alternatively could be interpreted as supplementary line (*sensu* Castanet et al., 1993). In about 28% of males (n = 5) and 30% of females (n = 7), we encountered difficulties in distinguishing the most peripheral LAGs because too close to each other.

We used non-parametric Mann-Whitney U-test to compare body size among adult frogs (performed on both all sampled individuals and those investigated by means of skeletochronology), and the Spearman's Rank correlation to test the relationship between age and SVL. All the statistical analyses were carried out by using SPSS software (version 17) with alpha set at 0.05.

With regards to SVL, sexes did not show significant differences (mean ± standard deviation, females: 52.1 ± 0.46 mm; males: 51.5 ± 0.31 mm; Mann-Whitney U-test, U = 442.5, n = 65, P = 0.438; Fig. 1A). Mean SVL of juvenile was 29.5 ± 4.1 mm. Concerning the skeletochronological analyses, all the juveniles resulted to be one-year old. Adult age ranged from 2 to 6 years in females and from 3 to 5 years in males (Fig. 1B) and did not significantly differ between the sexes (U = 149, P = 0.131). In both sexes, SVL was highly correlated with age (Spearman's Rank correlation, males: $r_s = 0.67$, P = 0.004; females, $r_s = 0.79$, P < 0.001) but a wide overlapping between the sizes of individuals from different age classes was found (Fig. 2). Among the largest adults, three exceeded 60 mm, and were all females. Two of them measured 64.5 mm (4 and 5 years old, respectively) and the third 68.0 mm (6 years old; Fig. 3).

Most anurans show sexual size dimorphism (in which males are usually smaller than females) due to sexual selection and different life-history traits between genders (Monnet and Cherry, 2002). In our sample males were slightly older than females and this could explain the lack of size dimorphism found between sexes.

The maximum body length of *Rana italica* so far reported was 65 mm in females and 60 mm in males (Lanza et al., 2009). Therefore, the SVL of 68 mm measured on a female in this study represents a new record of maximum length for this species. It is also noteworthy that we observed several other big-sized individuals (two females with SVL of 64 mm and three males with SVL close to 60 mm). A possible explanation of our findings is that the study population is very long-lived for reasons yet to be ascertained. It is known that amphibians have indeterminate growth but their body size is not always a reliable indicator of age because a widespread extensive overlap in body size among age classes (Halliday and Ver-

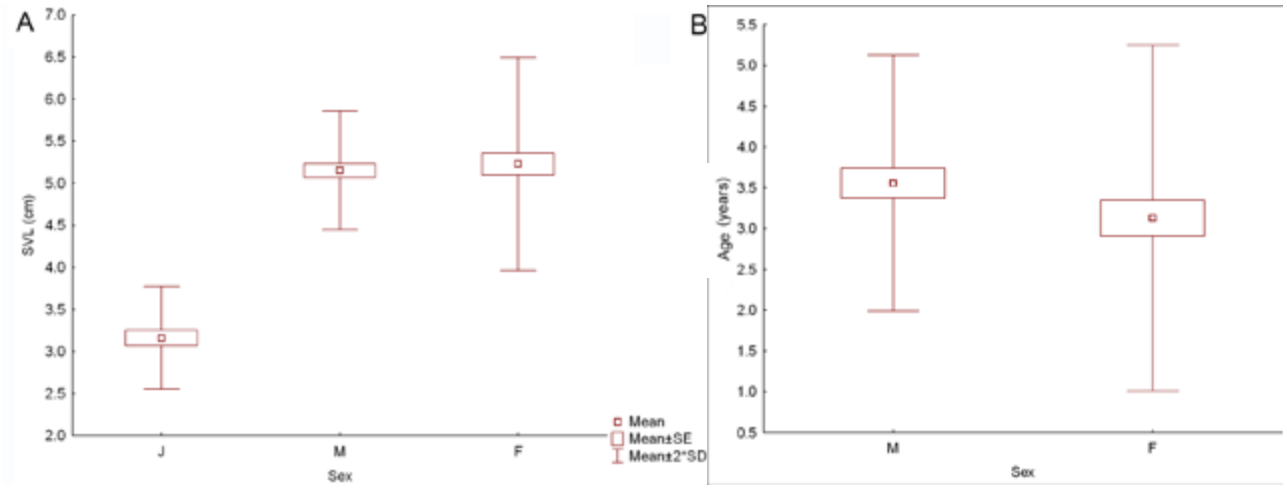

Fig. 1. Body size (A) and age (B) assessed by LAGs counting of *Rana italica* from the study site. Data on SVL refer to 52 individuals selected for skeletochronological analysis (juveniles: n = 11; males: n = 18; females: n = 23). Data on age refer to adult individuals only.

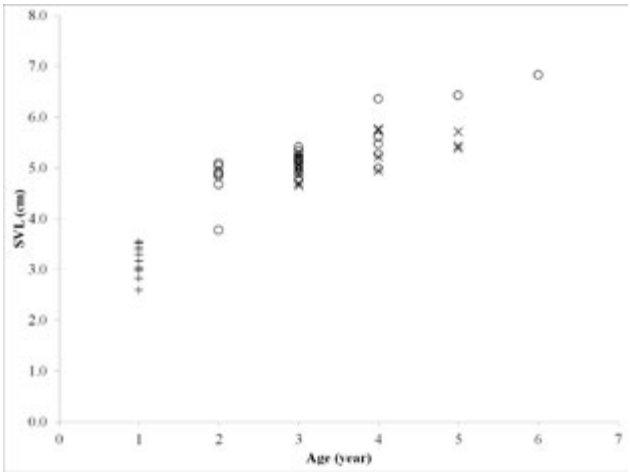

Fig. 2. Correlation between age classes and body size (SVL) in juvenile (plus), female (circles), and male (crosses) individuals of *Rana italica* from the studied population.

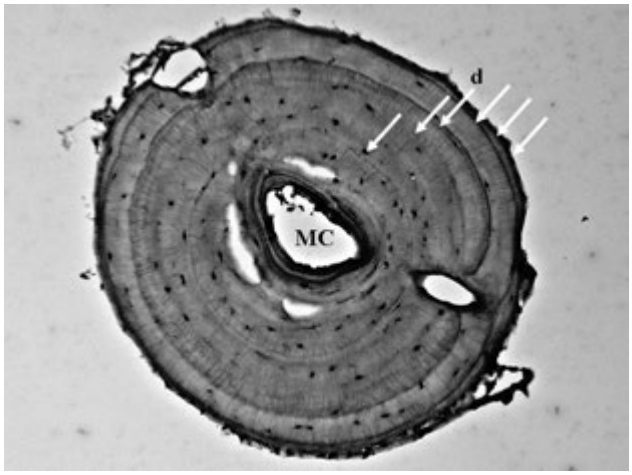

Fig. 3. Phalangeal cross-section of female of *Rana ialica*, 68.3 mm SVL. Arrows show lines of arrested of growth; d: double lines, MC: marrow cavity. Bar scale: 110 μm.

rel, 1988; Guarino and Erismis, 2008). On the contrary, skeletochronology has proven to be one of the most reliable techniques to assess individual age and growth in amphibians (Castanet et al., 1993) and it is well applicable also to *R. italica*, as confirmed in this as well as in previous studies (Guarino et al., 1995a, b). Previous skeletochronological studies on *R. italica* have shown that the mean longevity for this species was four years and the maximum age recorded was 5 and 8 years in low and high elevation populations, respectively (Guarino et al., 1995b). In our study, the oldest individual was also the biggest and its estimated age was 6 years. We did not recorded adult males younger than three years, this lack likely being due to research defect. The ages recorded for the study population inhabiting a hilly area, on average, are higher than those found for the species in lowland populations (Guarino et al., 1995b). For comparison, we analysed the body size reported for another amphibian species, *Salamandrina perspicillata*, well studied in its whole range from a biometric point of view (Romano and Ficetola, 2010), living syntopically with *R. italica* at the study site. The population of *S. perspicillata* from the study site differed in body size with respect to other 10 salamander populations from Central Italy sited at comparable elevation (range 240-500 m a.s.l.) and reported in Romano and Ficetola (2010) and Vignoli et al. (2011, 2012) (Cineto population: mean

SVL + SD = 38.6 ± 2.6 mm; other populations: mean SVL range = 30.9-41.7 mm). Interestingly, the population from Cineto showed a statistically significant larger size in eight comparisons (80%), smaller size in one comparison, and non-significant difference in one comparison, with no altitudinal trend (r_s = 0.261; n = 11; P = 0.465). The observed body size pattern (i.e., larger body size in comparison to other population at comparable elevation at the same latitude) shared by two amphibians would deserve further in depth analyses.

As a general rule, among anurans from temperate areas, intraspecific large variations in life history traits such as body size, age at maturity and mean longevity occurs depending on latitudes or altitudes, which cause different climatic influences (Hemelaar, 1988; Sagor et al., 1998; Miaud et al., 1999). However, large difference in body size, growth rates and age at maturity may also occurs between neighbouring populations living at similar altitudes and experiencing similar climatic conditions, as shown for *R. temporaria* (Augert and Joly, 1993). In this case, it was hypothesized that the inter-populational differences are related to a difference in the quality of the environment surrounding the water basins. Unfortunately, demographic information about this taxon is scanty and we do not know if other populations with large individuals occur in its areal. Furthermore, because few data are available for young frogs (i.e., no males were younger than 3 years), maybe due to a sampling bias, the mean age values obtained in the studied population could be biased towards higher life expectancy when compared with populations of *R. italica* of similar altitude and latitude. Further studies deepening the potential causal relationships between demographic patterns and ecological parameters are in progress to clarify this point.

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