Acta Herpetologica 1(2): 153-158, 2006

Aging *Salamandrina perspicillata* (Savi, 1821) by skeletochronology

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Abstract. We assessed age and first reproduction age in *Salamandrina perspicillata* females by means of skeletochronological analysis. As we examined sections from the third toe of the hind limbs, the tecnique herewith introduced is non-letal and compatible with ecological investigations. Females reached sexual maturity at four or five years; the oldest female was 12. SVL is a reliable body size index for assessing age (r^2 = 0.74).

Keywords. *Salamandrina perspicillata*, skeletochronology.

Salamandrina (Fitzinger, 1826) is a genus unique to peninsular Italy. Formerly, just *S. terdigitata* (Lacépède, 1788) belonged to this genus, but Mattoccia et al. (2005) recently proposed to split it into two species: *S. perspicillata* (Savi, 1821) occurs in central and northern Italy and *S. terdigitata* in southern Italy. In terrarium, *Salamandrina* can reach 12 years (Rimpp, 1978). Only indirect information is available on sexual maturity, based on minimum size of breeders of each population, but minimum body-size can vary strongly among populations (Vanni, 1980; Angelini et al., 2001, 2006; Della Rocca et al., 2005; Angelini, 2006). We assessed age and first reproduction age by means of skeletochronological analysis.

According to Halliday and Verrell (1988), skeletochronology is a reliable method to assess age in amphibians, mainly in temperate species. Using the skeletochronology method for age determination in amphibians has shown how different life-history traits in populations from different altitudes or environmental contexts may be marked by differences in longevity, in age and size at sexual maturity and in the relationships between body size and growth rate (Berven, 1982; Hemelaar, 1988; Caetano and Castanet, 1993; Diaz-Paniagua and Mateo, 1999; Kutrup et al., 2005). The earliest skeletochronological studies examined skull bones (e.g. Senning, 1940). Later, sections of humerus and/or femur were used (e.g. Smirina and Rocek, 1976; Francillon, 1979; Guarino et al., 1995), but in anurans and larger urodeles the phalanges are now used (e.g. Gittins et al., 1982; Gibbons and McCarthy 1983; Hemelaar, 1985; Acker et al., 1986; Reading, 1991; Flageole and Leclair, 1992;

Semlitsch et al. 1993; Guarino et al., 2003) precluding the need to sacrifice animals and thereby making the technique compatible with mark-recapture investigations. In recent years this non-letal technique has proved to be effective while operating on small or delicate urodeles like *Triturus helveticus*, *T. vulgaris* and *Euproctus platycephalus* (Guyetant et al., 1991; Marnell, 1997; Bovero et al., 2003).

From 2004 to 2005, we studied a population of *Salamandrina perspicillata* which breeds in a spring-fed trough in the Monti Lepini (Latium, central Italy) at 816 m a.s.l. As a mean of marking, we took a picture of the ventral pattern (Vanni et al., 1997) of 442 ovipositing females. We measured snout-vent length (SVL) of 277 females. Out of these, we also measured the total length (TL) of 215. Measures were taken by a ruler at the nearest mm.

We removed the third toe of the hind limb of 33 females, of which we recorded both SVL and TL. The removed digits were preserved with 70% alcohol and stored in labelled tubes. The females were realesed at the site. In the laboratory, we removed skin and muscles from the digits and isolated the medial phalangeal bone. This procedure was performed under a stereoscope, while maintaining the digit in a Petri-capsula filled with water. We decalcified the bones in 3% nitric acid for 40-50 minutes; after decalcification we put each of them in a glass with tap water. We changed water four times, after 15, 30, 45 and 60 minutes. Then the bones were sectioned at 13 μm by a freezing microtome. Cross sections were stained in Ehrlich's Hematoxylin for 2 minutes. We adopeted a 2 minutes staining time after observing an eccessively dark coloration in the slides stanned for longer times in our rather old Hematoxylin preparation.

We washed the stained sections with tap water. Since the phalanx of *S. perspicillata* is a very short bone, we could utilise only five to eight sections for the analysis. The best sec-

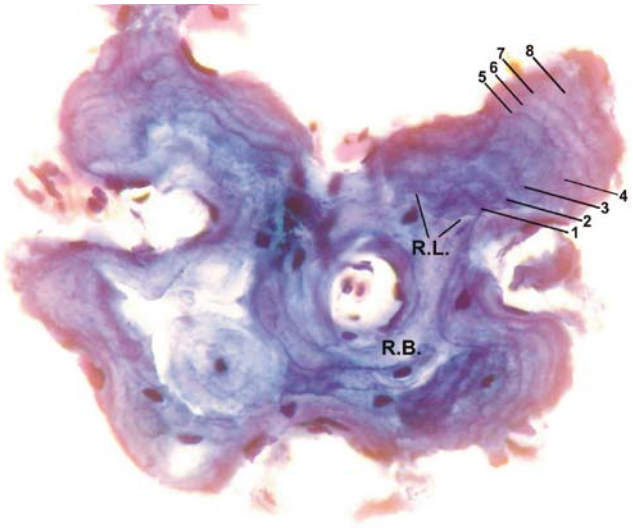

Fig. 1. Section of medial phalanx of an 8 years old female *S. perspicillata* showing Lines of Arrested Growth (numbered from 1 to 8). This female reached the sexual maturity at the age of four years. The first LAG was partially eroded. R.B.: Redeposited bone; R.L.: Resorption line.

tions were mounted on slides by Aquamount (GURR). The sections were then examined under a light microscope and lines of arrested growth (LAGs) present in the periosteal were counted in order to estimate the individual ages (Fig. 1). Since in central Italy, *S. perspicillata* may be active from late September to early May (Angelini et al., 2001; Utzeri et al., 2004; Angelini, 2006), while probably estivates in the rest of the year, we assume that each LAG represent a one-year growth period. Age at the first reproduction was inferred from the first sudden narrowing of the LAGs, according to Caetano and Castanet (1993). We compared the slides of humerus, femur and phalanx of five individuals we had found dead: the bones of each individual showed the same number of LAGs. In the phalanx sections the first LAG often appeareds partially eroded by endosteal remodelling. We did not observe any false or double lines. In six out of the 33 females we were unable to read the sections, and in one we could not assess the age at first reproduction.

In the field, mean SVL (± 1 SE) of ovipositing females was 42.0 ± 0.2 mm (n = 277; range 35-50) and mean TL was 107.6 ± 0.6 mm (n = 215; range 85–134). SVL size frequency was as Fig. 2.

Measures and age of the 27 females are in Table 1. The oldest female (12 years) was 47 mm in SVL and 119 mm in TL. The longest female, SVL = 50 mm and TL = 133 mm, was 10 years old. Using SVL as predictor of age, the regression equation for sexual mature females is *AGE = SVL(0.5598) – 15.932*.

Females (n = 26) reached sexual maturity at the age of four (80.8%) or five (19.2%) years. We captured two newly breeders: one was four years old and 37 mm in SVL, the other was five years old and 36 mm SVL. In our sample, females of the same age which bred the first time at four years were larger than those which bred the first time at five, although the difference is not significant (ANCOVA $F_{1,23} = 3.3$, $P > 0.08$).

A few populations of *Salamandrina perspicillata* have been studied with regards to individual body size (Vanni, 1980; Angelini et al., 2001, 2006; Della Rocca et al., 2005; Angelini, 2006). Single population mean body size (SVL) of adult females ranges from

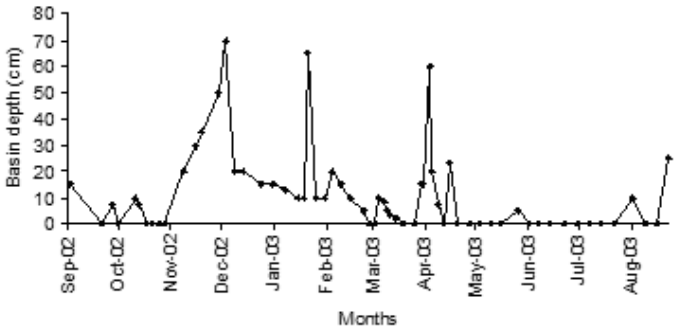

Fig. 2. Size frequency (SVL) of breeder female population at SMA.

Table 1. Age, SVL and TL of 27 females at SMA. Pearson's correlation between age and biometric features are in the last column.

	Range	Mean ± SE	Age Vs
Age (ys)	4-12	8 ± 0.4	
SVL (mm)	36-50	42.8 ± 0.7	$r_P = 0.86$ $P < 0.001$
TL (mm)	92-133	106.1 ± 1.9	$r_P = 0.75$ $P < 0.001$

33.6 ± 0.2 to 42.1 ± 0.2 mm (Angelini, 2006), and females are larger than males (Vanni, 1980). We think that the use of skeletochronology should be useful to understand such both inter-population and inter-sexual variability.

ACKNOWLEDGEMENTS.

We thank Prof. Cristina Giacoma (Dipartimento di Biologia Animale e dell'Uomo, Università di Torino) for laboratory support.

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