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Age and growth of the sand lizards (*Lacerta agilis*) from a high Alpine population of north-western Italy

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Abstract. We studied growth and longevity of *Lacerta agilis* from a sample (34 adults and 2 small-sized juveniles) of a population living at high altitude in north-western Italy using skeletochronological method. Snout vent length (SVL) mean of males did not significantly differ from that of females although the latter were in average bigger (SVL ± SD, males: 69.3 ± 7.1 mm, n = 11; females: 73.9 ± 9.7 mm, n = 22; Mann-Whitney *U*-test, $U = 1.76$, $P = 0.077$). Age ranged from 2 to 4 years (mean age ± SD = 2.3 ± 0.2) in males and from 2 to 3 years in females (mean age ± SD = 2.59 ± 0.5 years). Age mean did not significantly differ between the sexes (Mann-Whitney *U*-test, $U = 1.35$, $P = 0.174$). The two juveniles were 30 and 32 mm in SVL and both were 1-2 months old. In both sexes, a significant positive correlation between SVL and age was recorded although weakly significant for males (Spearman's correlation coefficient, males: $r_s = 0.70$, $P = 0.05$; females: $r_s = 0.75$, $P < 0.001$). Von Bertalanffy growth curves well fitted to the relationships between age and SVL and showed a different profile between males (asymptotics size, $SVL_{max =}$ 81.9 mm; growth coefficient, k = 0.63) and females (SVL_{max} = 100 mm; $k = 0.40$). Results indicate that individuals of *L. agilis* studied by us are short-living when compared with other populations of the same species.

Keywords. Growth, longevity, skeletochronology, *Lacerta agilis*, Italy, Alps.

INTRODUCTION

Lacerta agilis is one of the most widely distributed Palaearctic lizards, being found from the Pyrenees, central France and England in the west to Central Asia in the east (Sindaco and Jeremčenko, 2008). In Italy, the species seems to be quite rare; in fact, so far it has been found only in small areas of south-western Piedmont and north-eastern Friuli-Venezia-Giulia (Lapini and Sindaco, 2006).

Populations living in high altitude habitats in the south-western Alps, between Italy and France, are isolated from the main range of the species, where lizards live mostly at much lower altitudes.

Unfortunately, data on the biology of sand lizard in Italy are still largely incomplete (Di Già and Sindaco, 2004; Lapini and dall'Asta, 2004) and this represents a serious gap in terms of conservation of the species which appears as severely declining in a number of countries at the north-western part of its range (Edgar and Bird, 2006).

This study aimed to know longevity and growth rates of *L. agilis* from a south-western Alpine population using skeletochronological method.

MATERIALS AND METHODS

Animals

The sample investigated consisted of 36 animals (11 males, 23 females and 2 small-sized juveniles) captured by hand in July-early September 2001. In the field, each lizard was measured for snout-vent length (SVL, to the nearest 1 mm) and toe clipped at level of the second phalanx of the fourth, third or second toe of the right forelimb to mark individually the specimens. The cut toe was immediately fixed in 70° ethanol and stored until the time of skeletochronological analysis. After measurements and toe-clipping, each lizard was photographed and released in the same place where captured. No lizards were sacrificed for this study.

Study area

The studied population is located in the surroundings of the small village of Ferrere (Argentera municipality, Cuneo Province, Piedmont Region), between 1790 and 1890 m a.s.l. As in the other known localities of western Italian Alps, the vegetation is a typical alpine pasture habitat on calcareous substrate, on terraced slopes mostly facing either east or south, with only a few scattered small trees or bushes. At the study site *L. agilis* prefers the edge of stone heaps and dry-stone walls, hidden among the residual patches of higher vegetation of rather overgrazed pastures, in particular *Urtica dioica* and *Nepeta nepetella* (Di Già and Sindaco, 2004).

Laboratory technique

Skeletochronology was performed according to a standard protocol used for other species of reptiles (Andreone and Guarino, 2003; Guarino et al., 2004). After removal of soft tissues, phalanx was decalcified with 3% nitric acid for a time ranging between 1 and 2 hours depending on bone size, rinsed in tap water and cross sectioned using a cryostat. Diaphyseal cross sections was stained with Mayer's Haemalum (25 min) and mounted in aqueous resin. Periosteal lines of arrested growth (LAGs) were independently counted by two researchers using a light microscope equipped with an image analyser. Two representative sections with the smallest medullar cavity were acquired and transformed as digital photo. Images were optimized with respect to contrast and brightness using Adobe Photoshop version 6.0 to facilitate the distinction of the periosteal LAGs from the surrounding bone matrix. Possible under estimation of LAG number due to endosteal resorption, which may cause the complete loss of the inner (perimedullar) LAGs, was evaluated by osteometric analysis (Guarino et al., 2003, 2004). The perimeter of reversal line (RL), which is the boundary between periosteal and endosteal bone, and that of the bone outer margin (BOM) of the small sized individuals, was measured and compared with the perimeter of RL and BOM of animals with one or more LAG. Since we cut the second phalanx of the third toe from the forelimb of the two small sized lizard, the comparisons was possible only with males and females from which was cut the same type of skeletal element.

Growth patterns were estimated according to Von Bertalanffy's model, which previous studies have shown to be the model that better fits the relation between body size and age for most saurians (James, 1991; Wapstra et al., 2001; Roitberg and Smirina, 2006). The general form of the von Bertalanffy equation used is: $L_{t\,=}\ a \times (1\text{-}e^{\text{-k (t-t0)}})$ where L_t is the SVL (mm) at time t, a is the estimated average maximum SVL that can be reached (or mean asymptotic SVL), *e* is the base of the natural logarithm, k is the growth rate (the speed at which asymptotic SVL is attained), and t_0 is the age at hatching, which is the starting point of the growth interval under study. We assumed as size at hatching (L_{t0}) the mean value provided by In den Bosh and Bout (1998) because of the lack of incontrovertible data on the size at hatching of the studied population, due to the very low number of young collected during the field study. The parameters a (asymptotic SVL) and k, and their asymptotic confidence intervals, were estimated using a non-linear regression procedure by means of the Growth II software (Henderson and Seaby, 2006). Growth curves were considered to be significantly different if the 95% confidence intervals did not overlap (James, 1991; Wapstra et al., 2001).

All numerical data were analyzed by non parametric tests which have been proved to be effective in the case of small samples of data. In particular, Mann-Whitney U test was used for the comparison of means. Spearman's rank order correlation coefficient was used for estimating the relationship between age and SVL. A probability level of $P < 0.05$ was considered significant.

RESULTS

Females were bigger in average and maximum SVL than males but SVL mean did not significantly differ between the sexes (SVL ± SD, males: 69.3 ± 7.1 mm, n = 11, range: 60-81 mm; females: 73.9 ± 9.7 mm, n = 22, range: 50-90; Mann-Whitney *U*-test, $U = 81$, $P = 0.101$). The two juveniles were 30 and 32 mm long. All phalangeal cross sections showed well-defined lines of arrested growth (LAGs) (Fig. 1A-C). As in other species of temperate areas, we assumed that only one LAG is formed during each hibernating period, that for the analyzed lizards ranges from late September to advanced May. Therefore we considered the number of observed LAGs, plus that of completely reabsorbed LAGs, if any, equivalent to the individual's age. Both juveniles showed one visible hematoxynophilic line (Fig. 1A), very close to the marrow cavity and partially destroyed, here considered as hatching line Therefore, the two juveniles were estimated as few months old. Based on osteometric analysis, in two males (18.2%) and 4 females (13.6%), respectively, the innermost LAGs were estimated to be completely removed by endosteal remodelling. Maximum age was 4 years for males and 3 years for females (Fig. 2). The mean age ± SD was 2.4 ± 0.7 years in males (n = 11; range: 2-4 years) and 2.5 ± 0.5 years in females (n = 22 range, 2-3 years). Age mean did not significantly differ between the sexes (Mann-Whitney *U*-test, $U = 80$, $P = 0.161$). In both sexes, a positive correlation between SVL and age was recorded, but it was not significant for males (Spearman's correlation coefficient, males, $r_s = 0.59$, $P = 0.06$; females, $r_s = 0.65$, $P < 0.001$).

Growth pattern estimated by Von Bertalanffy's showed a well fit to the relation between age and SVL (Fig. 2). For both sexes, the estimated asymptotic SVL was slightly

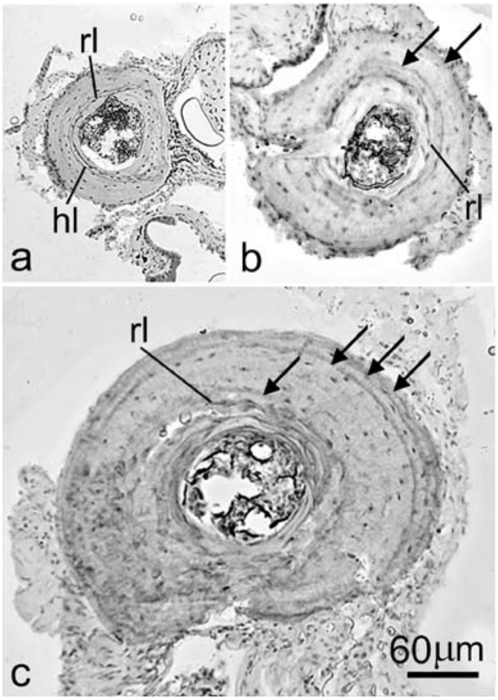

Fig. 1. Representative cross-sections of phalanges of *Lacerta agilis*. (a) Individual 32 mm SVL with no line of arrested growth (LAG). (b) Female 73 mm SVL with 1 visible LAG plus one situated at the outer margin of the periosteal bone. (c) Male 81 SVL with 4 LAGs. Arrows indicate LAGs. Abbreviations: hl = hatching line; rl = reversal line. All figures are at the same magnification.

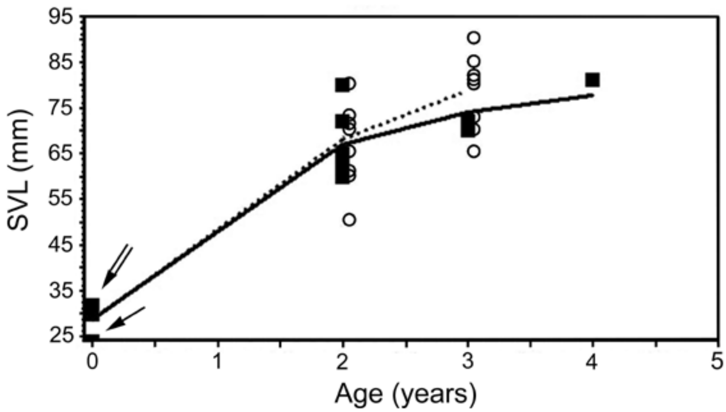

Fig. 2. The Von Bertalanffy growth curves for males (solid square, solid line) and females (open circle, dot line) of *L. agilis*. Arrow shows SVL mean of the lizards at hatching as reported by In den Bosch and (1988). Double arrow shows SVL of the two juveniles sampled in this study. Growth parameters are given in the text.

higher than the maximum SVL recorded (SVL_{asym} ± CI, males: 81.9 ± 1.65 mm; females: 100 ± 4.9 mm). Growth coefficient was higher in males than in females (k ± CI, males: 0.63 ± 0.04; females: 0.40 ± 0.03). Growth curve of males was significantly different from that of females.

DISCUSSION

Among reptiles body length, growth rates, age at maturity and longevity can widely vary between populations of the same species. As a rule, individuals from high-elevation sites and northern latitude live longer than those from low-elevations sites and southern latitudes (Wapstra et al., 2001; Roitberg and Smirina, 2006). Thermal constraints may provide a possible explanation for this trend. In fact, the shorter activity period in cooler climate, due to unfavourable conditions of temperature and food availability, should reduce the risk of predation (Seurs, 2005; Roitberg and Smirina, 2006). In addition, some authors also suggested that in cooler areas the lower predation pressure could also be due to the presence of a reduced number of predator species (Roitberg and Smirina, 2006). Therefore, it is interesting that longevity of *L. agilis* recorded in this study (4 years for males and 3 for females) is lower than those reported for other populations of the same species. In fact, in *L. agilis* living in Daghestan (Russia), at different altitudes, maximum longevity ascertained was 6 years for males and 5 years for females in the population from lowland and submontane regions (until 600 m. a.s.l.); 7 years for males and 6 years for females in the population from highlands (starting from 960 m. a.s.l) (Roitberg and Smirina, 2006). In *L. agilis* living along the western coast of Sweden, maximum longevity was 11 years for males and 12 for females (Olsson and Shine, 1996).

It is surprising that the studied population is so short living and the reason for this is not yet well understood. Although data about predation on the lizards under study are not available, predators nor potential competitors were not observed at high concentrations during the two years of field survey. The only lizard specialist is the smooth snake (*Coronella austriaca*), found only once during the study; an occasional predator is also the asp viper (*Vipera aspis*), rare in the area. Among birds, observed predators were the kestrel (*Falco tinnunculus*) and the red-backed shrike (*Lanius collurio*), both very widespread on the whole range of *L. agilis* and apparently not more numerous at the study site than in other similar places of the Western Italian Alps. Studies on other western Alpine populations of *L. agilis* are needed to understand if the short longevity of the studied sample is due to a local conditions or is the rule in these isolated populations.

The two small sized juveniles (30 and 32 mm in SVL) examined in this study were younger than 1 year (0 year old). This is highly plausible taking into account that: a) in this species, the mean SVL ± SD at hatching is 24.6 ± 5.1 according to In den Bosch and Bout (1998); b) at the sampling site the hatchings usually occur between advanced July-early August; c) activity period during which the lizards may forage and growth is very short (late May to end of August-early September); d) the two juveniles were sampled at the end of August 2001.

As in other saurian species (Galan, 1999; Wapstra et al., 2001), also in *L. agilis* growth rates are very high before the attainment of sexual maturity and decline with age. A recurrent adaptive explanation of this growth pattern is that the animals use energy mainly for somatic growth before sexual maturity. In males of *L. agilis* studied by us a marked increase in body length is observed between the first and the second hibernation (second year of life) and in most of females between the second and third hibernation (third year of life). These findings would suggest that males of *L. agilis* from north-western Italy attain maturity after the second year of life, while females after the third year of life, like in other mountain population of this species (Roitberg and Smirina, 2006).

The growth trajectories are differ between sexes. The lower value of k in the von Bertalanffy equation in females suggests that they attain the asymptotic body length slower than males. This finding is similar to that from Swedish populations but different from that from Russian ones. The diversity of growth pattern of the different populations of *L. agilis* could be due to different selective pressure in the different populations so far studied.

To conclude, the studied population is very peculiar because of its short-living individuals: this is maybe symptomatic of a ecological problem in the studied area, although causes are still unknown. If the study of other close ranging populations will confirm than other western Alpine *L. agilis* are short lived too, then the explanation will be found in some ecological adaptations, if not it will be searched on man induced problems. In the latter case, it will be very important to identify and remove the causes: in fact, in Italy *L. agilis* is a very rare reptile of national conservation concern, at the edge of its range, and the studied area was established as a Site of the Natura 2000 network of European protected areas also owing to its presence.

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