Population size, age structure and life expectancy in a Lacerta agilis (Squamata; Lacertidae) population from northwest Italian Alps

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Abstract. We studied population size and age structure of the sand lizard Lacerta agilis Linnaeus 1758, from northwest Italian Alps. Twenty-nine (9 males, 15 females, 5 juveniles) and 19 (8 males, 5 females, 6 juveniles) lizards were captured in 2011 and 2012, respectively. Adult population size, estimated by capture-mark-recapture, was 54 individuals in 2011 and 39 in 2012. Mean SVL did not significantly differ between males sampled in the two years of the study (mean ± SD, 2011: 67.1 ± 6.6 mm; 2012: 61 ± 8.7 mm). On the contrary, females sampled in 2011 were significantly larger (73 ± 7.2 mm) than females of 2012 (61 ± 7.4 mm). Adult age, assessed by skeletochronology, ranged 2-5 years both in males and females. All juveniles were one-year old. In both years of sampling there was no significant difference in mean age between the sexes. Males were represented mainly by three and four-year old individuals in 2011 and by two-year old individuals in 2012. Females were represented mainly by three-year old individuals in 2011 and by two-year old individuals in 2012. However, age distribution of adults did not differ for each sex as well as between the sexes both in 2011 and 2012. Although our results showed that the examined population of L. agilis has a stable demographic structure, it should be considered seriously vulnerable, because it is relatively isolated from other neighboring populations, has small absolute size and, therefore, may be highly exposed to negative effects of habitat degradation.

Key words: population size, age structure, skeletochronology, Lacerta agilis, northwest Italian Alps.

Introduction

The sand lizard Lacerta agilis Linnaeus, 1758, is a lacertid widely distributed in the Palaearctic Region, from the Pyrenees to the Central Asia, including large parts of Europe, Anatolia, Caucasus, northern Kazakhstan, Mongolia and north-western China (Bischoff 1984, Sindaco & Jeremenko 2008). The species is also present in Italy in two limited and distinctly separated areas of the Alps, respectively in the south-western Piedmont (Di Già & Sindaco 2004) and Friuli Venezia Giulia (Lapini & Sindaco 2006, Venchi & Sindaco 2011).

The demographic traits and, more in general, the ecology of Italian populations of L. agilis are still poorly known, although some aspects of age structure and diet have been recently investigated (Guarino et al. 2010, Crovetto & Salvidio 2013). Knowledge of population size, age structure and life expectancy is very useful for formulating appropriate management strategies for populations of different geographic areas (Eaton et al. 2005, Erismis 2011). Skeletochronology is one of the most reliable methods to estimate the age of the individuals and their growth rates in reptiles, as well as in other ectothermal vertebrates (Castanet et al. 1993). A recent skeletochronological study performed on a northwestern Italian population of L. agilis showed that individuals do not exceed 5 years of age (Guarino et al. 2010). This finding is interesting in view of the fact that in other parts of its range this species appears to be more long-lived, reaching 7 years in mountain populations of Dagestan (Russia) (Roitberg & Smirina 2006), and 12 years in coastal populations of Sweden (Olsson & Shine 1996). Since the population studied by Guarino et al. (2010) lives in an alpine environment, one would expect longevity values close to the species maximum. In fact, it is known that in saurians the age of the individuals within populations of the same species living in different geographic areas generally increases with altitude and this trend can be related to proximal factors such as the short length of activity season and/or low predatory pressure in highland sites (Wapstra et al. 2001, Roitberg & Smirina 2006).

The aim of this paper is to study the longevity and age structure of Lacerta agilis from another northwestern Italian mountain population living about 4 km away from that analyzed by Guarino et al. (2010), always using skeletochronological method. We also provide data on the absolute size
of this marginal population which lives relatively isolated from any other conspecific population. Finally, we used our results to investigate how this population is potentially threatened.

Materials and methods

Study site and field work
The studied population was sampled in NW Italy, in the Alps of Piedmont along the Stura Valley, at about 1625 m a.s.l. (GPS coordinates: 32° 33’00” m E; 49°17’30” m N, datum WGS 84). This population inhabits a relatively isolated area, extending only 4 ha, along the right side of the Stura stream. The site is highly frequented during summer by hikers and campers. Along the bare stony bed of the stream, few individuals of the common wall lizard (*Podarcis muralis*) are also found, while sand lizards inhabit more vegetated areas with sparse willow bushes (*Salix elaeagnos* and *Salix purpurea*) interspersed with trees, such as larches (*Larix decidua*), Scots pines (*Pinus sylvestris*) and birches (*Betula alba*). Therefore, there was apparently a clear spatial segregation between the two lizard populations. In the more forested parts of the plot, two snakes, potential lizard predators, such as the asp viper (*Vipera aspis*) and the smooth snake (*Coronella austriaca*), were occasionally observed.

Field research was conducted in 2011 and 2012, from the 20 of May to mid-August in 2011 and from 25 May to mid-August in 2012. These periods correspond to the locality season of the species (*Lapini & Dall’Asta 2004*). Animals were sampled by hand or noosing between 9:00 A.M and 2:00 P.M. After capture, each lizard was kept in plastic boxes until faeces were obtained for analysis of dietary habits (Crovetto & Salvìdio 2013). Before being released at the site of capture, lizards were marked individually with non-toxic paint to allow visual recognition.

Population size estimation
The population size was estimated by capture-mark-recapture (CMR) separately for 2011 (12 field sessions) and 2012 (14 field sessions). Subadult or newborn individuals were never recaptured, and therefore only the adult population size was estimated. In both years we pooled capture events in four even-spaced sessions that were analyzed by means of Capture software (White et al. 1982). In Capture different models, all assuming a closed adult population size was estimated. In both years we used our results to investigate how this population is potentially threatened.

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Results

Population size
A total of 29 lizards (9 males, 15 females, 5 juveniles) were captured in 2011, and of 19 lizards (8 males, 5 females, 6 juveniles) in 2012. The results of the CMR analysis are reported in Table 1. In goodness-of-fit procedures and between-model tests (Williams et al. 2001). In addition, a closure test was calculated to confirm the main assumption of the models.

Skeletochronological analysis
Individual age was estimated by phalangopal skeletochronology according to a routine protocol already widely experienced on long bones of other reptilian species (Guarino et al. 2004, 2010). A total of 48 phalanges (29 from lizards collected in 2011, and 19 from lizards collected in 2012) were analyzed. Skin and muscle tissue were removed from each clipped toe and the remaining phalanges was decalcified in 5% nitric acid for about 1 h and 30 min. Then, the phalanges was washed in running tap water (overnight) and transversely sectioned at diaphyseal level using a Reichert Jung - Cryocut 1800 cryostat. Serial sections, 10 µm thick, were stained with Mayer’s acid hemalum for about 25 min, mounted in resin mounting media, and observed under light microscope (Motic BA 340) equipped with digital camera (Nikon Coolpix 5000). For each toe, at least five sections were selected to count the lines of arrested growth (LAGs) in the periosteal bone. We assumed that in the phalanges of this species possible LAGs are formed each year according to what has been demonstrated for other temperate lizards (Castanet 1985) and taking into account the growth parameters of the sampling site, where the lizards regularly overwintered from early September to the beginning of May.

Age was determined by two observers who counted LAG independently to prevent any bias. In accordance with other researchers (e.g. Driscoll 1999), in order to estimate the number of LAG eventually destroyed due to endosteal resorption we considered the width of the marrow cavity, any signs of remodeling in the contact zone between endosteal bone (when present), and the possible presence of Kastschenko’s line. In the adult phalanges a small marrow cavity bone together with the presence of scarce remodeling phenomena were assumed as the condition in which there was not a total resorption of the LAG. On the contrary, it was not possible to estimate the complete loss of one or more innermost LAG (perimeatal LAGs) by means of osteometric analysis (see Castanet et al. 1993, Guarino et al. 2010), because we remove phalanges of different fingers, with diaphyseal diameter not comparable, in order to increase individual recognition of the lizards.

Mann-Whitney U-test was used to analyze the differences in mean of SVL and of age between the sexes or between the juveniles. The significant level was set at α = 0.05.
Table 1. Population size, estimated by Capture software, for Lacerta agilis living in northwestern Italian Alps.

<table>
<thead>
<tr>
<th>Year</th>
<th>Selected CMR Model</th>
<th>Capture probability</th>
<th>Population estimate (SE)</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>M(h)</td>
<td>0.13</td>
<td>54 (8.85)</td>
<td>42 – 76</td>
</tr>
<tr>
<td>2012</td>
<td>M(0)</td>
<td>0.12</td>
<td>39 (17.49)</td>
<td>21 – 134</td>
</tr>
</tbody>
</table>

Figure 1. Representative cross sections of Mayer's acid hemalum-stained phalanges from Lacerta agilis. A) Juvenile, 41 mm in SVL, with 1 visible LAG. B) Male, 75 mm in SVL, with 5 LAG. C) Male, 72 mm in SVL, with 5 visible LAGs of which one dubious; note an elongated vessel on the left. D) Male, 70 mm in SVL; with 3 or 4 LAGs; note the extensive vascularization in the periosteal bone which causes difficulties in the LAG counting. Arrows indicate lines of arrested growth. f: false line; RL: reversal line. Scale bar: 55 µm in A) and B); 70 µm in C) and D).

In both years the population could be considered closed (Z = -0.918, P = 0.18; Z = -1.549, P = 0.06 for 2011 and 2012, respectively) and thus the population size could be successfully estimated. In 2011, the selected model was M(h), in which capture probabilities vary among individuals, while in 2012 the more parsimonious model was M(0), that assumes constant probability of capture among individual and occasions. In both years the probabilities of capture were similar, although relatively low (Table 1). In any case, the small sample of 2012 produced large 95% confidence limits. For this reason, the adult lizard population in 2012 was smaller than the one estimated in 2011, but not significantly different due to a relatively large error (Table 1).

Skeletocronology
In the periosteal bone of all examined phalanges hematoxyphilic lines with the characteristics of
LAGs were observed although they were different in appearance and intensity of staining (Fig. 1). Only a part of the phalanges showed a clear-cut reversal line (Fig. 2A-B) and/or a marked distinction between endosteal and periosteal bone. Extensive remodeling phenomena were observed also in the periosteal bone at diaphyseal level (Fig. 1C-D). Most phalangeal sections were characterized by a small marrow cavity.

Number of LAGs was determined with an error rate of one in about 25% of the males (n=4) and 26% of the females (n=5) and with an error rate of two in only one adult (male). Finally one female sampled in 2011, had poorly expressed LAG and consequently it was excluded from successive analysis. All juveniles showed one LAG. On the basis of histological criteria described in the methods, the complete destruction of LAG due to endosteal resorption was excluded, except for 1 individual, for which consequently one LAG was added to estimate its actual number of LAGs.

Data on SVL and age assessed by LAG counting are reported in Table 2. Mean SVL did not significantly differ between males sampled in the two years of study, although males sampled in 2011 were slightly larger (mean ± SD : 67.1 ± 6.6 mm, see Table 2) than males sampled in 2012 (mean ± SD : 61 ± 8.7 mm). On the contrary, females sampled in 2011 were significantly larger (mean ± SD : 73 ± 7.2 mm). In both years of sampling there was no significant difference in mean SVL as well as mean age between the sexes. Mean SVL and age did not differ between juveniles of the two years.

Age structure of male and female adults is shown separately in Fig.2. For males, modal age was 3 and 4 years in 2011, and 2 years in 2012. For females, modal age was 3 years in 2011 and 2 years in 2012. There was no significant differences in age distribution between males (Kolgomorov-Smirnov test, D = 0.43, P = 0.31) as well as between females (Kolgomorov-Smirnov test, D = 0.58, P = 0.09) sampled in the two years of sampling. Also the age distribution between the sexes did not differ in both years (Kolgomorov-Smirnov test males vs females, 2011: D = 0.24, P = 0.84; 2012: D = 0.22, P = 0.99).

Discussion

In both years of monitoring, the adult population size of L. agilis here studied was estimated to be below 60 individuals, and the small decrease of the population observed between 2011 and 2012 was not significant. The probabilities of capture were low but adequate to estimate size and, moreover, the closure test gave no indication that the closure assumptions were violated (White et al. 1982). Therefore, we are confident that the estimated lizard densities were relatively accurate. On the bases of these results we consider the lizard population to be constant, at least during the study period.

In the study plot, the estimated adult population densities varied from 10 to 14 individuals per ha in 2011 and 2012, respectively. These values are much lower than those reported by Corbett (1988) and Corbet & Tamarind (1979) for populations inhabiting optimal habitats (i.e. sand dunes) in Britain, where 210-300 adult lizards per ha are estimated.

The density of the Italian population is even inferior to those reported for sand lizard populations living in suboptimal habitat types (e.g. heather dominated sand dunes and forested heath lands), in the Netherlands (Nicholson & Spellerberg 1989: 50 lizards per ha) or Czech Republic (Gvodzic 2000: about 70 adults and subadults per ha). On the other hand, the Italian sand lizard population densities are very similar to the populations living in highly isolated and unfavourable conditions.
Table 2. SVL (mm) and age (assessed by LAG counting) of Lacerta agilis studied in the present work. For each parameters mean ± standard deviation, extreme values (between brackets) and total number of examined animals (n) are reported. * P < 0.05.

<table>
<thead>
<tr>
<th></th>
<th>2011</th>
<th>2012</th>
<th>U Mann-Whitney test</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>SVL</td>
<td>Age</td>
<td>SVL</td>
</tr>
<tr>
<td>Males</td>
<td>67.1 ± 6.6</td>
<td>3.3 ± 1</td>
<td>61 ± 8.7</td>
</tr>
<tr>
<td></td>
<td>(56-75)</td>
<td>(2.5)</td>
<td>(52-74)</td>
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<tr>
<td></td>
<td>n = 9</td>
<td></td>
<td>n = 8</td>
</tr>
<tr>
<td>Females</td>
<td>73 ± 7.2</td>
<td>3.1 ± 0.9</td>
<td>61 ± 7.4</td>
</tr>
<tr>
<td></td>
<td>(54-86)</td>
<td>(2.5)</td>
<td>(55-71)</td>
</tr>
<tr>
<td></td>
<td>n = 15</td>
<td></td>
<td>n = 5</td>
</tr>
<tr>
<td>Juveniles</td>
<td>41.2 ± 3.7</td>
<td>1.2 ± 0.6</td>
<td>35.1 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>(38-47)</td>
<td>(1-2)</td>
<td>(28-41)</td>
</tr>
<tr>
<td></td>
<td>n = 5</td>
<td></td>
<td>n = 6</td>
</tr>
<tr>
<td></td>
<td>U Mann-Whitney test</td>
<td>males vs females</td>
<td>36</td>
</tr>
</tbody>
</table>

habitats, such as a high elevation rocky site in Sweden (Olsson 1988: 8-10 lizards per ha) or a small Danish island (van Bree et al. 2006: 13 lizards per ha). Therefore, the analysis of population density data clearly suggests that the Italian alpine population of L. agilis lives in a less favourable habitat type. It is known that several factors affect lizard population abundance, such as availability of food resources, presence of competitors and predators and degree of human disturbance (Perez-Mellado et al. 2008). At the study site human presence and predator pressure exerted by lizard eating snakes (Vipera aspis and Coronella austriaca) and predatory birds, such as Falco tinnunculus, Lanius collurio and Circaetus gallicus, may contribute to habitat disturbance and thus negatively influence the survivorship and age structure of the focal lizard population.

In the present study, two main difficulties were encountered to define the actual age by means of LAG number: i) the not always clear distinction between endosteal and periosteal bone due to the absence of a clear-cut line reversal and/or to a poorly diversified matrix through the cortical bone; ii) the presence in the diaphyseal cortex of extensive remodeling phenomena that in several phalangeal sections made the LAG incomplete, with irregular course and consequently of dubious interpretation. These difficulties coupled with variability of staining of the LAG have certainly affected the accuracy of our skeletochronological study as also demonstrated by the high percentage of LAG estimates with an error rate of one year. However, even if the accuracy of the data for the 48 animals examined in this study is somewhat less than in other skeletochronological studies performed on lizards (for bibliography see Castanet 1994) we are confident that the overall results are reliable. Moreover, our data are in accordance with those of Guarino et al. (2010) in showing that the Alpine populations of L. agilis show a lower life expectancy than those inhabiting other parts of the species range.

By combining recapture data with the skeletochronological observations it is evident that the growth of individuals is rapid before reaching sexual maturity, as already reported by Dudzik et al. (2015) in Poland. For example, a juvenile 1-year old, was captured in 2011, when it was 38 mm SVL and recaptured in 2012, when it was 55 mm SVL and with visible secondary sexual characters. A three-year old female, captured in 2011 with a SVL of 74 mm, was recaptured in 2012 with a SVL of 77 mm. Therefore at time of attainment of sexual maturity, individuals have a body length over the 50% of the maximum size reached by the species.

Lacerta agilis is listed on Appendix II of the Bern Convention, and on Annex IV of the Habitats Directive (Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora). In Italy, its IUCN status is categorized as Not Applicable (NA), because the species is only marginally present within the national boundaries (Rondinini et al. 2013). Although our results show that the demography and size of the focal population of L. agilis seems stable, this population should be considered seriously vulnerable, because it is highly isolated from the central European populations living on the northern
side of the Alps, has small population size and a shorter life expectancy in comparison with other populations living near the species core range. Therefore, the long-term viability of this population seems highly exposed to demographic stochasticity and negative effects such as habitat degradation due to human disturbance.

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References


