Age structure and growth in a population of *Pelobates varaldii* (Anura, Pelobatidae) from northwestern Morocco

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Abstract. Age structure and growth in the Moroccan spadefoot toad, *Pelobates varaldii* were estimated by skeletochronology and reported for the first time for a population living in NW Morocco. Snout vent length and body mass did not significantly differ between the sexes, although females appeared larger and heavier than males. Age ranged 2-7 years in males (mean age $\pm$ SD: $4.5 \pm 1.2, n = 66$) and 2-10 years in females (4.7 $\pm$ 2.4, $n = 20$). The difference in age was not significant between the sexes. The modal age was 5 years for males and 3 years for females. The age distributions significantly differed between the sexes. Von Bertalanffy growth curves showed a similar profile between the sexes although growth coefficient was higher in males than in females. Longevity and growth rates of *P. varaldii* were compared with those of other species of the genus *Pelobates*.

Keywords: Amphibia, demographic traits, longevity, Moroccan spadefoot toad, skeletochronology.

The Moroccan spadefoot toad (*Pelobates varaldii*) belongs to the family Pelobatidae which includes only three other species (*P. cultripes*, *P. fuscus*, and *P. syriacus*) with a wide distribution from Europe to Central Asia and Morocco (Busack, Maxson and Wilson, 1985).

*Pelobates varaldii* is known only from a few fragmented areas on the coastal plains of NW Morocco and it has been assessed as Endangered (EN) according to IUCN Red List of Threatened Species (IUCN, 2010). Furthermore, the Evolutionary Distinctive and Globally Endangered (EDGE) program of the Zoological Society of London recently listed *P. varaldii* on place 36 of their EDGE global amphibian top 100 based on the species Evolutionary Distinctiveness and Global Endangerment scores (Isaac et al., 2007). Similarly to the other spadefoot species, *Pelobates varaldii* is fossorial and largely nocturnal for most of the activity period that occurs in autumn-winter (Schleich, Kästle and Kabisch, 1996). Although ecology and habitat preference of this anuran begun to be recently investigated (de Pous et al., submitted), its demographic characteristics still remains largely unknown. On the other hand, the knowledge of individual life span and population age structure represents a priority to develop appropriate management strategies of threatened species.

In this study, we aimed to: (1) determine individual age and growth, and age structure of *P. varaldii* using phalangeal skeletochronology from a population in the Mamora Forest (northwestern Morocco), (2) compare the demographic traits of *P. varaldii* with those obtained from skeletochronological studies on related pelobatid species. Skeletochronology proved to be one of the most reliable methods for assessing individual age and growth rates of many vertebrates, including amphibians (Castañet et al., 1993). In addition, performing skeletochronology on phalanges is relevant in con-
servation biology because it avoids the sacrifice of animals, also allowing demographic studies over long time intervals.

Eighty-six adults (66 males, 20 females) and five newly metamorphosed of *P. varal dii* were collected by dip net on 24 January 2009 and 7 April 2009, respectively, at a temporary pond sited in the Mamora cork oak forest (34°11’48.6N, 06°21’27.0W; 57 m a.s.l.; pond size 966 m²). The forest is located on the sandy soils of the coastal plains north east from Rabat. Ponds are generally filled for a period of 5-7 months during the rainy season. The area has a Mediterranean climate with an average annual rainfall ranging from 450-600 mm and temperatures ranging from 11°C in winter to 34°C in summer (Aaf et al., 2005).

Males were distinguished from females by the presence of swollen humeral glands, which are typically developed during the breeding period. From each individual, snout to vent (SVL, to the nearest 0.1 mm) and body mass (in g) were recorded. Then the 4th toe of the right hind limb was clipped at the level of the penultimate phalanx and fixed in 70% ethanol until being processed for skeletochronological analysis. After biometric measurements and toe-clipping the animals were released into the sampling site.

We assessed the individual age using standard skeletochronological technique from phalangeal sections (for details see Guarino et al., 2003; Guarino, Di Già and Sindaco, 2008). The number of lines of arrested growth (LAGs) detected in the periosteal bone was independently counted by two observers; in case of discordance, the bone section was reanalyzed until consensus was reached. According to other authors (e.g., Leclair, Leclair Jr and Gallant, 2005), incomplete and faint lines were considered false LAG when in close proximity to a more strongly hematoxynophilic line (= true LAG). The number of LAGs potentially lost owing to endosteal resorption was evaluated according to Guarino et al. (2003), Guarino, Di Già and Sindaco (2008). Age at maturity was estimated from the rapprochement pattern of LAGs as performed by other authors (Francillon-Vieillot et al., 1990; Leclair, Leclair Jr and Gallant, 2005).

Growth models were estimated according to V on Bertalanffy’s equation as performed in other studies on amphibians (e.g., Hemelaar, 1988; Lima, Arntzen and Ferrand, 2000; Erismis et al., 2009). We used the following equation: $SVL(t) = SVL_{\text{max}} \times (1 - e^{-kt-t_0})$, where $SVL(t)$ is the body length at age $t$; $SVL_{\text{max}}$ is the estimated maximum body length; $e$ is the base of the natural logarithm; $k$ is the growth coefficient that defines the shape of the curve; $t_0$ is age at metamorphosis (0.3 year) which is the starting point of the growth interval under study. We considered as size at metamorphosis the mean value (28 mm) calculated by bibliographical data (Buchholz and Hayes, 2002). $SVL_{\text{max}}, k$, and their asymptotic confidence intervals (CI) intervals, were estimated using a non-linear regression procedure by means of the Growth II software (Henderson and Seaby, 2006).

Hematoxynophilic lines interpreted as lines of arrested growth (LAGs) were clearly identifiable in the periosteal bone of all adults whereas neo-metamorphosed did not show LAGs nor metamorphosis lines (fig. 1). Optical sharpness and distinctiveness of LAGs was different from one animal to the other and within a single bone section. In most individuals, LAGs were thin and well defined (fig. 1D, E, F), while in some individuals they appeared as annuli (fig. 1C). False lines were rare and usually encountered between the first and the second LAGs (fig. 1B, F). Double lines (sensu Castanet et al., 1993) were rare and without regular fashion (fig. 1D, E); in some individuals, such very close lines joined up for a small part of their trajectory appearing as a thick LAG (fig. 1E).

Age estimation by LAG counting was possible in all individuals examined except in one female. Age was assessed with an error rate of one year in 34% of the males ($n = 22$) and 35% of the females ($n = 7$) due to the difficulties in distinguishing the most peripheral LAGs mainly in the oldest individuals or to reliably interpret weakly contrasted LAGs. The perimeter of the reversal line (from 360 to 560 μm) did never exceed that of the innermost visible LAG (greater than 580 μm) of the smallest individuals thus excluding complete destruction of LAG due to endosteal resorption, except for three individuals (3.5% of total sample), where consequently one year was added to estimate their age.

Data on SVL, body mass, longevity and age at maturity are reported in table 1. Mean SVL and body mass did not significantly differ between sexes although the largest and heaviest individuals were females. Age ranged from 2 to 7 years in males (mean age: 4.5) and from 2 to 10 years in females (mean age: 4.7) and did not significantly differ between sexes. Most of the males (81.2%, $n = 52$) and the females (73.6%, $n = 14$) showed the first marked rapprochement between the 2nd and 3rd LAGs; accordingly it seemed plausible to conclude that sexual maturity is attained after 2 yr for both sexes. A small fraction of individuals (about 18.8%, $n = 12$, of the males and 26.4%, $n = 5$, of the females) did not show a clear inter-LAG interval reduction in their phalangeal cross-section.
Figure 1. Representative cryostat cross-sections of phalanges of *Pelobates varaldii*, stained with Mayer’s acid hemalum. (A) Newly metamorphosed individual 6J, with 0 LAGs and SVL not measured; (B) Female 9H, with 3 LAGs, SVL = 47.9 mm; (C) Male 5G, with 3 LAGs, SVL = 49.4 mm; (D) Female 8A, with 4 LAGs, SVL = 50.4 mm; (E) Male 9C, with likely 5 LAGs, SVL = 55 mm; (F) Male 2F, with 7 LAGs, SVL = 55.3 mm; (G) Female 1C, with likely 10 LAGs, SVL = 56.4 mm. Legend: a = annulus; f = false line; d = double line. Arrows indicate line of arrested growth. All figures are at the same magnification. Scale bar: 95 μm.
**Table 1.** SVL (mm), body mass (g) and age (yr) assessed by LAG counting. Data are indicated as mean ± standard deviation. For each parameter extreme values (between brackets) and total number of examined individuals (\(n\)) are also indicated. Concerning age at sexual maturity the percentage of individuals for the specified age class is reported.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Males</th>
<th>Females</th>
<th>Welch’s t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVL</td>
<td>51.7 ± 3.3</td>
<td>52.8 ± 5.9</td>
<td>(P = 0.43)</td>
</tr>
<tr>
<td>(43.9-60.6)</td>
<td>(45.0-64.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n)</td>
<td>66</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Body mass</td>
<td>15.8 ± 3.0</td>
<td>17.0 ± 5.9</td>
<td>(P = 0.38)</td>
</tr>
<tr>
<td>(10-24)</td>
<td>(11-27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n)</td>
<td>66</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>4.5 ± 1.2</td>
<td>4.7 ± 2.4</td>
<td>(P = 0.72)</td>
</tr>
<tr>
<td>(2-7)</td>
<td>(2-10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n)</td>
<td>64</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Age at maturity</td>
<td>2 (81.3%)</td>
<td>2 (73.6%)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Plot of age versus snout vent length (SVL) and the Von Bertalanffy’s growth curves fitted to the data. Grey square: males; open triangle: females.

Age class distribution was significantly different between sexes (Kolgomorov-Smirnov test: \(D = 0.34, P < 0.05\)). Modal age was 5 years for males (about 31% of the sample) and 3 years for females (about 32%) (fig. 2). In both sexes, age class distribution was positively skewed but much more in females (skewness 0.84) than in males (skewness 0.09).

SVL was significantly correlated with age on both sexes (Spearman’s correlation, \(r = 0.70, P < 0.05\) for males; \(r = 0.75, P < 0.01\) for females). However, for both sexes variation in body size at comparable ages was large, so that wide overlapping between the size of individuals from different age classes was observed (fig. 2).

Growth curve estimated by Von Bertalanffy’s model showed a similar shape in males and females (fig. 2) but growth coefficient was higher in males than in females (\(k \pm CI, \) males: 0.49 ± 0.025; females: 0.33 ± 0.018), thus indicating that the former reach the asymptotic length more rapidly than the latter. For both sexes a marked decrease in the growth rate was observed from the 2nd to the 3rd age, which is after the presumed attainment of sexual maturity. For both sexes, the estimated asymptotic SVL was slightly lower than the maximum SVL recorded in this study (SVLasym ± CI, males: 55.94 ± 0.43 mm; females: 61.8 ± 0.74 mm).

There is an extensive literature showing the applicability of skeletochronology for most of the amphibians, especially for species inhabiting the temperate areas. In general, a skeletochronological study produces reliable results when it is possible: (a) to distinguish clearly the LAGs from the other aperiodic bone marks (e.g., false lines and double lines); (b) to demonstrate the annual periodicity of LAGs; (c) to verify that the visible LAGs actually correspond to the LAGs formed during the individual growth and there are not completely destroyed LAGs due to bone remodeling.

As in many amphibian species (Guarino, Andreone and Angelini, 1998; Esteban et al., 1999; Khonsue, Matsui and Misawa, 2000; Leclair, Leclair Jr and Gallant, 2005), in the studied population of *P. varaldii* variability in the optical appearance of LAGs was observed. It is argued that the different distinctiveness of LAGs reflects the amplitude of growth cycles and the severity of quiescence period (hibernation and/or aestivation) both at individual and population levels (Leclair, Leclair Jr and Gallant, 2005). Our findings suggest that the variability in the distinctiveness of LAGs of *P. varaldii* is essentially an individual phenomenon since it was recorded also in toads with the same age, although they were likely subjected to the same climatological conditions, thus having identical length and intensity of torpor period. Another difficulty in accurately interpret-
Table 2. SVL (range in mm), age at sexual maturity (year), and maximum longevity in the different species of *Pelobates* studied by skeletochronology. NS, not specified.

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality and elevation a.s.l.</th>
<th>Sex</th>
<th>SVL</th>
<th>Age at maturity</th>
<th>Longevity</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fuscus</em></td>
<td>Sarre River, 255 m (NE France)</td>
<td>M</td>
<td>NS 2</td>
<td>7</td>
<td>Eggert and Guyetant (1999)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>2</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lorraine, NS (NE France)</td>
<td>M</td>
<td>NS 2</td>
<td>7</td>
<td>Eggert and Guyetant (2000)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>2-3</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. fuscus</em></td>
<td>Novara, 150 m (NW Italy)</td>
<td>M</td>
<td>38-47 2</td>
<td>5</td>
<td>Andreone et al. (2004)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>46-59 3</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. cultripes</em></td>
<td>Serra da Arriça, 300 m (SC Portugal)</td>
<td>M</td>
<td>43-49 2</td>
<td>8</td>
<td>Leclair, Leclair Jr and Gallant (2005)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>51-58 3</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. varaldii</em></td>
<td>Mamora Forest, 57 m (NE Morocco)</td>
<td>M</td>
<td>44-61 2</td>
<td>7</td>
<td>Present work</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>45-64 2</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In bone growth marks might be associated with bone microstructure. For example, woven bone or parallel fibered bone are better suitable than lamellar bone for LAG identification because in the latter LAGs can be confused with bone lamellae (Castanet, Francillon-Vieillot and Bruce, 1996). In *P. varaldii*, periosteal bone of phalanx is formed of parallel-fibered bone tissue and the LAGs were counted without ambiguity. In addition, false and double LAGs were not a problem for the accurate age determination in this species because they were rare and easily identifiable. However, the presence of false LAG mainly within the second LAG would indicate that partial arrest or slowing of the individual growth could occur more frequently during the juvenile phase.

Although we do not have experimental data to ascertain the periodicity of LAG formation, we assume that in *P. varaldii* each LAG is deposited yearly, taking into account the biological rhythms of the species and the climate of the sampling area that is characterized by marked seasonal variation. Indeed, this species breeds from late October to at least the end of January depending on the rainfall timing and stays active until May-June, whereas it enters into a phase of quiescence during the rest of the year burrowed in the ground (Schleich, Kästle and Kabisch, 1996). Therefore, the summer-early autumn warm months are most likely the period when the LAGs are laid down. As expected, the newly metamorphosed toads captured at the early of April of 2009 displayed no LAGs.

The endosteal resorption represents a further source of possible error in the skeletochronological assessment of individual age because it can cause the complete destruction of the innermost periosteal LAGs (Castanet and Smirina, 1990). In the studied *P. varaldii* population, osteometric analysis enlightened that complete resorption of LAGs almost never occurs. Consequently, it was possible to assess the individual age and the age structure of the population by counting the number of visible LAGs.

In this study, intersexual differences in maximum longevity (7 years for males and 10 years for females) were found, whilst the age at maturity was the same between the sexes. Maximum longevity and age at maturity appear similar to those of other *Pelobates* species (table 2). Greater longevity for females than males has been previously reported for other species of anurans (Cherry and Francillon-Vieillot, 1992; Leclair and Laurin, 1996) and it is generally interpreted as a consequence of the delayed sexual maturity (Kyriakopoulou-Sklavounou, Stylianou and Tsiora, 2008) or minor predation (Cherry and Francillon-Vieillot, 1992) of females compared with males. Concerning *P. varaldii*, the first interpretation appears to be unlikely in light of the fact that age at maturity did not differ between the sexes. The assumption that adult males of *P. varaldii*...
should have higher mortality rates remains to ascertain.

In the studied population of *P. varaldii*, age structure was significantly different between males and females. Among males the most numerous age classes were middle-aged individuals (4 and 5 years), whereas among females young breeders individuals (3 years) were predominant although females may have a life expectancy of 10 years. Interestingly, this pattern of age class distribution was very different from that observed for *P. fuscus* (Eggert and Guyetant, 1999) and *P. cultripes* (Leclair et al., 2005). However, several studies have shown that in anurans age structure for both sexes may markedly vary between the years in the same population (Augert and Joly, 1993; Driscoll, 1999) whereas our data were based on a single year. Therefore, long-term studies are needed for better revealing the dynamics of age structure in populations of *P. varaldii*.

Like many amphibian species (Gibbons and McCarthy, 1984; Erismis et al., 2009) in both sexes of *P. varaldii* growth is rapid prior to sexual maturity and decreases markedly thereafter. It is widely accepted that this growth pattern is related to reproduction costs, including gonadal maturation (Jorgensen, 1992). In addition, Von Bertalanffy model showed that there are few differences in growth rates of immature *P. varaldii* whereas the growth curves distinctly diverge between sexes from three years of age. This growth trajectory, where males and females mature at the same age and at similar size but one sex continues to grow more than the other, is similar to that showed by *P. cultripes* (Leclair, Leclair Jr and Gallant, 2005); also it seems consistent with “Model 4” described by Hasumi (2010) in his categorization of the growth patterns in indeterminate growing vertebrates. In Model 4, the difference between the sexes on post-maturational growth can result in sexual size dimorphism. Nevertheless, unlike *P. cultripes*, in *P. varaldii* no significant evidence of sexual size dimorphism was found although females tend to be larger than males. Therefore, it might be hypothesized that unlike *P. cultripes* inter sexual difference on post-maturational growth in *P. varaldii* are not such to cause significant sexual size dimorphism.

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