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# Bone growth and age in *Rana saharica*, a water frog living in a desert environment

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The structure and the histological expression of annual bone growth marks of Moroccan water frogs (*Rana saharica*) from an arid climate region (northern edge of the Sahara desert) differ from the pattern observed in water frogs from temperate climate regions. At early ages, when growing rates are high, the osteogenic activity of the froglets never stops completely, and during the resting period the histological marks formed in the bone are mostly annuli. However, growth marks formed in older individuals are mostly well defined thin LAGs, which correspond to a complete stop in of osteogenesis. Males and females mature when they are two years old. The oldest males and females were six years old. One-year-old froglets showed a wide range in body size (22.4 to 40.9 mm) associated with an extended period of metamorphosis. Considering LAG diameter as the diameter of the phalange at a given age, we obtained successive phalange diameters for each individual corresponding to each year of its life. The diameter of the LAG1 of adults of *Rana saharica* is significantly larger than the diameter of the LAG1 of one year old juveniles. We found similar results studying the tibiofibulae of the sister taxon of *R. saharica*, *R. perezi*. Larger froglets were thus more successful than smaller ones in generating the adult samples suggesting the existence of size mediated selection. Extended breeding periods, which allow various metamorphosis peaks along the year, would be apparently disadvantageous for the species, since only froglets of a few subcohorts would be able to survive. However, this strategy could be of importance in Mediterranean and desert areas where local conditions change dramatically from year to year.

## 1. Introduction

Physiological activity in animals of indeterminate growth, including amphibians, is strongly influenced by seasonal climatic variations. Bone growth in amphibians is affected by these seasonal variations determining, during the resting periods, the formation of special bone histological marks: lines of arrested growth (LAGs) when growth stops and annuli (*sensu* Peabody 1961) when growth only decreases. These marks appear alternating with wide layers of bone deposited during the active period (Castanet *et al.* 1993). Skeletochronological studies on individuals of known age (Schroeder & Baskett 1968, Gibbons & McCarthy 1983) and the use of vital fluorescent labeling (Smirina 1972, Francillon & Castanet 1985) have proven the annual periodicity of LAGs, reinforcing their value as a tool in determining directly individual ages (Castanet 1982). However, the histological expression and spatial organization of bone growth marks are largely dependent on local conditions of growth, and therefore, largely influenced by the external living conditions of the organism (Esteban *et al.* 1996).

Recent examples of the effect of climate on the histological expression of growth marks in a single species, the Iberian water frog, *Rana perezi*, have shown that populations exposed to long annual periods of inactivity, develop well marked LAGs (Juarranz & Patón 1990, Patón *et al*. 1991). Conversely, cyclical growth marks can be absent or very reduced and diffuse in populations living in less continental climates, where resting periods may not occur (Hernández & Seva, 1985, Esteban *et al.* 1996). These data, complemented with additional information on other species with wide geographic ranges, as *Rana temporaria* (Esteban 1990), support the generalized idea of the necessity of a period of general inactivity to arrest bone growth (Castanet *et al.* 1993). The intensity and duration of such a period of general inactivity sufficient to stop bone growth, and therefore to produce LAGs, have received very little attention. Smirina *et al.* (1986) showed that LAGs formation is evident in *R. temporaria* after two to five weeks of low temperatures in lab conditions.

Hot desert environments are especially relevant in testing hypotheses about bone growth and LAG formation, since the duration of the cold period is very reduced and seasonal climate variation is extreme. Anurans living in extreme arid climates of the pre-Saharan region only remain inactive for short periods due to low temperatures (Le Berre 1989), and the expression of growth marks in these species may be affected by such short but nonetheless marked periods of inactivity in different ways. In this study we have chosen *Rana saharica*, a Northwest-African water frog, which reaches the northern limits of the Sahara desert (Bons & Géniez 1996) and in contrast to other amphibians from desert areas does not seem to experience an inactive resting period during the summer (Schleich et al. 1996). *Rana saharica* inhabits a large variety of environments where fresh water is usually available throughout the year. Despite its ubiquity, there is very little information about its life history (Le Berre 1989, Bons & Géniez 1996, Schleich *et al.* 1996). Published data mainly deal with morphological aspects or population genetics and systematics (Arano *et al.* 1998, Buckley *et al.* 1994, 1996, Llorente *et al.* 1996). We believe that *R. saharica* is an adequate species for studying the influence of climate on the histological expression of growth marks. It is able to colonize environments ranging from pre-desert (Sahara) to alpine (High Atlas), and it is the sister taxon of the Iberian *R. perezi* (Uzzell 1982), which shows a high degree of plasticity in the histological expression of growth marks (Esteban *et al.* 1996).

Our goals in this study were (1) to describe the histological expression of bone growth marks in relation to extreme climate seasonality in areas with a very short period of winter inactivity, (2) to analyze comparatively, from skeletochronological data, the life history traits of frogs from desert environments with traits of closely related frogs living in warm temperate regions, and (3) to test for the existence of size mediated selection in froglets in a species with multiple annual reproductive cohorts, and its implications in life history theory.

## 2. Material and methods

Seventy-nine post-metamorphic individuals of *Rana saharica* were caught during the second week of April 1993 in

the surroundings of Erfoud (Morocco) (31°28´N, 4°10´W, 955 m elevation). The area is included in the "upper-desertic" ecoclimatic region of the North-Saharian edge with an average rainfall of 70 mm per year (Le Houerou 1989), an annual average temperature of 21.4°C, a thermal range of 39°C (maximum temperature of 41°C and minimum temperature of 2°C), and a short cold season, with a winter stop in vegetation growth during one month, and 2°C as the average minimum temperature of the coldest month (Le Houerou 1989). Annual evapo-transpiration for the region has been calculated at 1 476 mm (Le Houerou 1989). Frogs were found in small, low flowing, streams and ponds with a maximum water depth of one meter, surrounded by dense riparian vegetation. They were captured by hand or deep-netting during day time. Sampling was conducted during the spring season since representatives of all age classes are likely to be active during day time, as documented in its sister taxon *R. perezi* (García-París *et al.*, 1989). All specimens captured were used in previous morphological and genetic studies (Buckley *et al.* 1994, 1996, Llorente *et al.* 1996, Arano *et al.* 1998).

Snout-vent length (SVL) was measured using dial calipers. Individual sex was determined through gonadal development and external secondary sexual traits. Samples of different tissues were removed in the field for genetic analysis. Vouchers were deposited in the herpetological collections of Museo Nacional de Ciencias Naturales and Universidad de Barcelona. The second phalange of the second outer toe of the right foreleg was clipped and preserved in 70% alcohol for the skeletochronological study. Toes were rinsed during three hours in distilled water and decalcified in 3% nitric acid for three hours, and subsequently rinsed and soaked overnight in running tap water. Frozen sections of 15 µm from the midshaft of the diaphysis of the second phalange were obtained on a cryostat freezing microtome, stained in Ehrlich's haematoxylin for 15 min and rinsed for 30 min in tap water. Out of 10 to 20 transverse sections, the ones with the smallest medullar cavity and the thickest cortical bone, were selected and mounted in aqueous synthetic resin (Aquamont, Gurr) on a glass microscope slide. Bone sections were examined with a light microscope and photographed at a constant magnification.

Second phalange, tibiofibula and femur of a series of metamorphic larvae of *R. saharica* ($n = 7$), captured at the same time and in the same locality as the post-metamorphic specimens, were also studied to check for the presence of growth marks. Individual development stages were assigned according to Gosner's table (Gosner 1960).

Phalange and LAG diameters were measured on the photographs using a curvimeter. The relationship between body size (SVL mm) and phalange diameter (ØP µm) was determined by linear regression (males: SVL = 8.06 + 61.03(ØP); $r^2 = 0.651$; $n = 24$; females: SVL = –2.62 + 72.75(ØP); $r^2 = 0.922$; $n = 18$). Considering LAG diameter as the diameter of the phalange at a given age, we obtained successive phalange diameters for each individual corresponding to each year of its life. Using these as predictors of the size of a single individual throughout its life, we generated an extended data set that considerably increases sample sizes (i.e., every LAG diameter is a data point corresponding to the size of the specimen at a given age) when analyzing growth trajectories. Tibiofibulae of 103 post metamorphic individuals of *R. perezi* from a warm temperate region, previously studied in Esteban *et al.* (1996), were used to generate an additional data set for comparative analyses.

All statistical analyses were performed with the programs Statview IV (BrainPower Inc., Calabasas, CA) on an Apple Macintosh computer. We selected $p = 0.05$ level for statistical significance.

## 3. Results

### 3.1. Bone histological characteristics and growth marks

Stained sections of the phalanges are nearly circular in shape. The bone matrix is formed essentially of parallel-fibered bone tissue, although the outer cortex in older individuals becomes sublamellar or lamellar. A layer of endosteal lamellar bone is present at the edge of the medullar cavity in most adults and some juveniles. This bone is separated from the periostical bone by a highly stained resorption line. The medullar cavity is filled with cartilage in some juveniles (Fig. 1A).

LAGs are present in all the phalanges analyzed (Fig. 1), except in those of two froglets, which also lack a line of metamorphosis (Fig. 1A). We believe that these two specimens hibernated before the ossification process was started and therefore LAGs were not deposited. Seven froglets (no gonads differentiated), (SVL = 31.15 ± 2.10 mm) had two LAGs (Fig.1C). According to Hemelaar (1985), we may consider the inner LAG as a line of metamorphosis and the second line as the LAG of the first winter (= LAG1). However, the first LAG could also be the LAG1 formed during the larval period and, thus, the second LAG would be the line of metamorphosis. In any of these cases one line does not have chronological value. These specimens have not been used in analyses including age-specific phalangeal diameters.

The optical sharpness of growth marks is variable (Table 1). Twenty three percent of the winter marks are strongly marked LAGs, 39% are distinct LAGs but weakly expressed and 37% are annuli. Variability is mostly an intra-individual phenomenon. The first winter mark is expressed

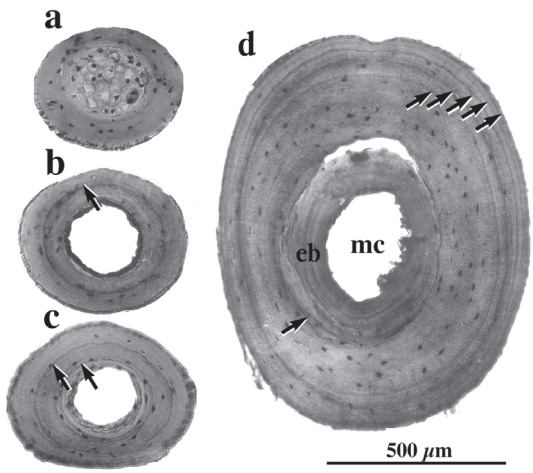


**Fig. 1.** Cross sections at the middle of the diaphysis of phalanges for *Rana saharica*. — A: Juvenile, 23.5 mm SVL, without marks of arrested growth. Medullar cavity is filled with cartilage. — B: Juvenile, 26.2 mm SVL, 1LAG. — C: Juvenile, 25.0 mm SVL, LM + 1LAG or 1LAG + LM. — D: Male, 54.2 mm SVL, 6 LAGs. Arrows: LAGs; EB: endosteal bone; MC = medullar cavity

as annuli (54%), or strong LAGs (34%), while later marks are almost always thin but distinctive LAGs.

Endosteal resorption at the periphery of the medullar cavity affects three adults (two males and one female). The diameter of the inner LAG (0.66 ± 0.04 mm) and the diameter of the medullar cavity (0.48 ± 0.08 mm) in those individuals are larger than the maximum diameter of the first LAG in juveniles (0.44 mm). We assume those individuals have lost LAG1.

### 3.2. Age, size and sexual maturity

Among the 79 post-metamorphic individuals studied there are juvenile froglets with undifferentiated gonads (SVL = 29.1 ± 3.6 mm; $n$ = 37), as well as males and females, with different degrees of gonadal development (SVL = 57.5 ± 9.7 mm; $n$ = 24 males; and 49.9 ± 11.3 mm; $n$ = 18 females). The maximum SVL observed was a female at 79 mm. Males were larger than females in our sample (Mann-Whitney $U$-test: $U$ = 8.80, $p$ = 0.028)

Estimated age in relation to snout-vent length is shown in Fig. 2. Juveniles have one LAG or none. The modal age is two years. The age of males and females range between one and six years. Age class distributions are not different between sexes (Kolmogorov-Smirnov $\chi^2$-test: $\chi^2$ = 4.96, $p$ = 0.167), although males are older than females (males = 3.63 ± 1.43 years; females = 2.91 ± 1.37; $U$ = 115.5, $p$ = 0.01).

There is a larger size variation in one-year old specimens (range between 24.6 and 40.9 mm, SVL = 31.4 ± 4.8 mm) than in any other cohort. This age class ranges from recently metamorphosed to submature individuals. The minimum size observed for adult males is 37.2 mm, and 34.8 mm for females. The oldest males (SVL = 71.0 mm, $n$ = 2, range = 70–72 mm) and females (SVL = 79.0 mm) are also the largest. Age and size are significantly correlated for males ($r$ = 0.849, $p < 0.0001$) and for females ($r$ = 0.911, $p <$ 0.0001).

Sexual dimorphism in size was calculated for each age class using the diameter of LAGs as body size estimator (see material and methods). There

**Table 1.** Optical expression of lines of arrested growth. $S$ = Number of strongly marked lines; $W$ = Number of weakly marked lines; $A$ = Number of annuli.

| | LAGs | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | | 6 | | |
| | *S* | *W* | *A* | *S* | *W* | *A* | *S* | *W* | *A* | *S* | *W* | *A* | *S* | *W* | *A* | *S* | *W* | *A* |
| Juveniles | 12 | 4 | 18 | | | | | | | | | | | | | | | |
| Males | 7 | 4 | 10 | 3 | 14 | 5 | 2 | 10 | 3 | 1 | 8 | 1 | 0 | 7 | 1 | 0 | 2 | 0 |
| Females | 6 | 1 | 11 | 4 | 3 | 4 | 0 | 4 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 |
| Total (%) | 34 | 12 | 54 | 21 | 52 | 27 | 10 | 70 | 20 | 8 | 75 | 17 | 0 | 80 | 20 | 0 | 67 | 33 |

are no significant differences between sexes within each age class (Table 2). Males and females were pooled together for subsequent comparisons among age classes. There are significant differences in the diameter of phalanges among age-classes (one-way ANOVA: $F_{(5, 147)} = 235$, $p < 0.001$). *A posteriori* analyses (Fisher multiple comparisons) do not show significant differences between the fourth and the fifth, and between the fifth and the sixth age-classes. Fig. 3 shows the average age-specific diameter of LAGs and hence phalangeal growth from first hibernation to adulthood. The increment in mean phalangeal diameter is small from the second winter, when all individuals are sexually mature.

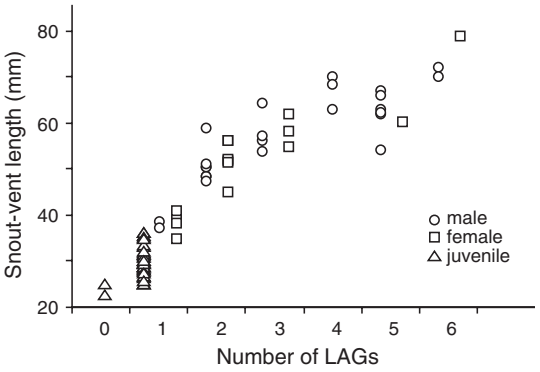


**Fig. 2.** Plot of age (LAG number) versus snout-vent length (SVL) of the sample studied for *Rana saharica*.

## 4. Discussion

### 4.1. Influence of climate on bone histology

Growth marks have been considered by Castanet (1982) to be the result of a genetically controlled cycle of growth, strongly synchronized and reinforced by seasonality. In accordance with this hypothesis, the presence of well expressed growth marks has been confirmed in most of the anuran populations located at high altitude or latitude, where climatic conditions impose a cessation in activity (Juarranz & Patón 1990, Docampo & Milagrosa-Vega 1991, Patón *et al.* 1991). Conversely, a weak expression of growth marks has been found in frogs from areas with a less continental climate (Hernández & Seva 1985, Esteban *et al.* 1996). Under such climatic conditions, absence of growth marks would suggest that internal cycles have a very limited influence or no influence at all on the formation of growth marks, and therefore, a general occurrence of an endogenous (genetic) control of growth mark expression has been already discussed (Esteban *et al.* 1996).

Anurans living in Moroccan sub-saharian climates only remain inactive for short periods due to low temperatures (Schleich *et al.* 1996). Castanet's (1982) hypothesis predicts the presence of growth marks for these desert amphibians, but their presence would be conditioned by the time available for their formation (Smirina *et al.* 1986) during such short, but nonetheless marked, peri-

**Table 2.** Mean and standard deviation of LAG's diameter for males and females. Mann-Whitney *U*-test for differences between sexes.

| | | Number of LAGs | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| Males | *n* | 22 | 22 | 15 | 11 | 8 | 3 |
| | $x \pm$ SD (μm) | 408 ± 58 | 636 ± 92 | 755 ± 80 | 848 ± 67 | 886 ± 0.92 | 901 ± 32 |
| | range | 334–531 | 461–798 | 640–878 | 717–933 | 823–1 098 | 879–923 |
| Females | *n* | 17 | 11 | 5 | 2 | 2 | 1 |
| | $x \pm$ SD (μm) | 421 ± 57 | 667 ± 62 | 797 ± 60 | 838 ± 140 | 901 ± 149 | 1 032 |
| | range | 337–560 | 557–805 | 700–842 | 738–937 | 796–1 006 | 1 032 |
| *U*-test (sexes) | | $U = 156.5$ $p = 0.387$ | $U = 105.5$ $p = 0.554$ | $U = 29.0$ $p = 0.4576$ | | | |

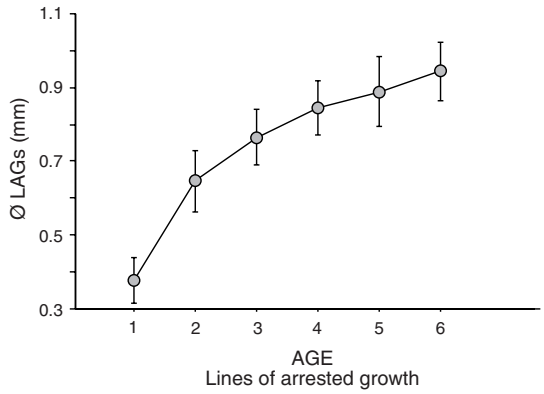


**Fig. 3.** Relationship between phalanges diameter and age in adults (males + females) for *Rana saharica.*

ods of inactivity. Growth marks may also correspond to the period of aestivation during warmer months (Tejedo *et al.*, 1997; Leskovar *et al.*, 1998), however *R. saharica* does not seem to experience a resting period during the summer (Schleich *et al.* 1996).

Our field data in bone growth of pre-Saharan frogs are consistent with the hypothesis of Castanet (1982) since growth marks formed in adult frogs are mostly well defined LAGs. These subdesert populations of *R. saharica* show a peculiar growth pattern, different from the pattern shown by frogs in extreme and mild temperate regions. At early ages, when growing rates are high, the osteogenic activity of the froglets does not stop completely, and the growing marks formed are mostly annuli. Growth marks formed in older individuals are mostly well defined, but thin LAGs, which correspond to a complete but short stop in bone growth. Although the length and intensity of the cold season is identical for adults and juveniles in a single locality, there is a marked ontogenetic shift in its effect on patterns of bone growth. These data are not in conflict with Castanet's hypothesis (1982), though they add an ontogenetic dimension to it. This age-dependent pattern of expression of growth marks in *R. saharica*, is consistent with the thermal ecology of Mediterranean frogs in the *R. perezi* group, in which juveniles may be active during cold periods even when adults usually are not (Alvarez & Salvador 1984).

Presence of a line of metamorphosis seems to be an individual phenomenon documented in species in which larval development is completed in a single year (Hemelaar, 1985). The presence of lines of metamorphosis in species with overwintering tadpoles has been reported (Esteban *et al.*, 1996), but not discussed. Overwintering tadpoles may go through a period of winter inactivity. This period would result in the formation of LAGs when limbs show an advanced stage in the ossification process. The presence of a wintering line in the second phalange, tibiofibula and femur of one tadpole confirms that LAGs may be formed in larvae. In the sample of seven froglets with two LAGs, it is not possible to differentiate between metamorphosis and wintering lines, since tadpoles may develop a wintering line before metamorphosis. The proximity of these two lines allow us to conclude that they represent in fact a metamorphosis line and a wintering line, and not two wintering lines.

One of the main difficulties in skeletochronological studies is the identification of the processes of bone resorption and reconstruction. This phenomenon is considered as the result of mechanical constraints and physiological demands throughout life (Castanet *et al.* 1993), and its intensity depends on different factors. Phalanges and tibiofibulae in anurans show the smallest resorption rates of all the long bones (Leclair 1990), and due to their reliability they are widely used for skeletochronology. Daily and annual activity and climate conditions probably have a strong effect on the intensity of bone resorption of anuran long bones; species from high altitudes/latitudes have a lower level of resorption than those that experience shorter resting periods (Esteban 1990, Hemelaar 1988, Leclair 1990, Augert 1992). These variations in the intensity of bone resorption occur also within a single species. Populations of *R. perezi* living in warm Mediterranean climates (Hernández & Seva 1985, Esteban *et al.* 1996) show a high degree of endosteal resorption. In northern colder regions no LAGs are destroyed (Patón *et al.* 1991). Nevertheless, in European populations of *Bufo bufo* intensity of endosteal resorption in phalanges is not related to climate conditions (Hemelaar 1985).

Endosteal resorption in *R. saharica*, where resting periods are short, affects only 9% of the adult frog sample, in which the first line is destroyed. The almost continuous reproductive pe-

riod of *R. saharica* may also play an important role in the degree of resorption since the investment of limited resources in reproduction may be especially high (Esteban *et al.* 1996).

### 4.2. Age, size and sexual maturity

In water frogs from temperate climates the age when reproduction first occurs is usually two years, although males may reach sexual maturity one year before females (*R. perezi*: Esteban *et al.* 1996, Patón *et al.* 1991, Arez & Caetano 1992; *R. esculenta* and *R. lessonae*: Neveu 1992). However, exceptions occur in *R. perezi* in Northern Spain where some females mature during their first year, while males mature one year later (Docampo & Milagrosa Vega 1991). In *R. saharica* the first breeding period takes place during the second year but some individuals, males and females, are sexually mature in their first year, with a minimum size of about 40 mm for both sexes. Atkinsons (1996) indicates that organisms living at risk (desiccation, loss of habitat by evaporation, predation by other ectotherms, etc.) can reduce the duration of the juvenile period favoring an acceleration in their development towards adulthood, even at the expense of size at the time of first reproduction.

We did not find sexual size dimorphism within each age class. Shine (1979) reported that in 90% of the anuran species studied, females are larger than males. Sexual differences in body size can arise by processes acting during the juvenile stage or after maturity. Age at sexual maturity (Halliday & Verrell 1988), survival rates (Howard 1981), growth rate, or longevity (Hemelaar 1988) can determine the existence of sexual dimorphism regarding in size at the adult phase. Halliday and Tejedo (1995) indicate that there is no sexual size dimorphism in those species in which both age at maturity and asymptotic size are the same for males and females. Age at maturity does not differ between sexes in *R. saharica* and, although our sample is not large enough to determine asymptotic size, the absence of significant differences in size at post-maturity ages may suggest similar final maximal sizes.

Males and females live for up to six years. The oldest individuals are the largest in the sample. Closely related species from temperate climates have similar longevity (Docampo & Milagrosa Vega 1991, Patón *et al.* 1991, Polls-Pélaz 1991, Arez & Caetano 1992, Neveu 1992, Esteban *et al.* 1987), although the oldest males occur in *R. saharica*.

### 4.3. Growth and the effect of a continuous breeding period on size

Body size and age are positively correlated in both males and females of *R. saharica*. The growth pattern in *R. saharica* is characteristic of water frogs from temperate climates. It is marked by a high growth rate during the juvenile phase, but reduced after maturity (*R. perezi*: Docampo & Milagrosa-Vega 1991, Esteban *et al.* 1996, Patón *et al.* 1991; *R. lessonae* and *R. esculenta*: Neveu 1992). This general pattern is combined in *R. saharica* with a high individual variability, which explains the overlap in size distributions among age classes.

The variation in size per age class is high, especially within the first one (Fig. 2). This wide range of size and sexual development may be explained as a consequence of the length of the metamorphic period, which in *R. saharica* is extended during most of the year, as it is in the case of Mediterranean populations of *R. perezi* (Esteban *et al.* 1996). Published data on the biology of *R. saharica* suggest the existence of differentiated reproductive cohorts (Schleich *et al.* 1996). Our sample taken in at single locality revealed tadpoles in all stages of development, including metamorphic individuals, and females with oocytes in various stages of maturity, both suggesting an extended breeding season.

The smaller froglets in the first age class probably had metamorphosed just before winter. Larger froglets and submature males and females in this age class correspond to the broad metamorphic period from spring (overwintering tadpoles) to fall. The length of the metamorphic period and individual differences in the time available to increase size before winter explain the coexistence of very different sized animals.

Halliday and Verrell (1988) suggest that growth before maturity will be the first factor responsible in determining adult size. Since meta-

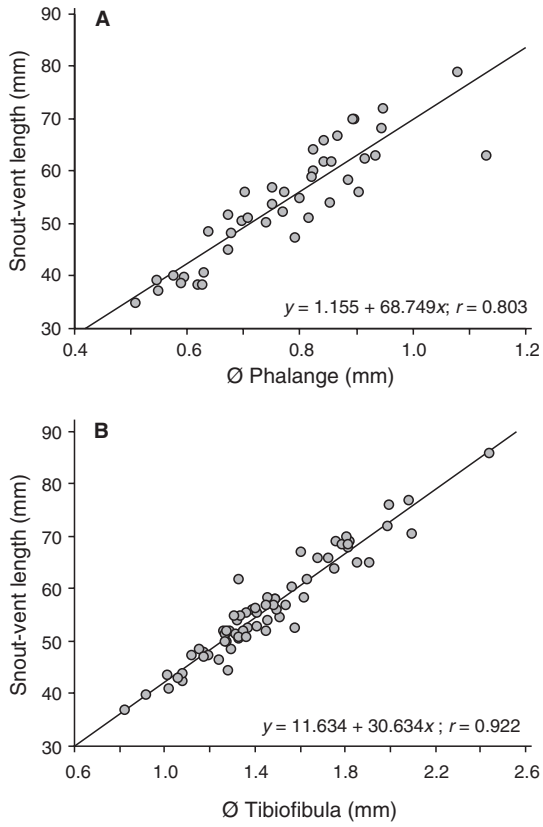


**Fig. 4.** Relationship between adult snout-vent length (SVL) and the diameters of (A) *Rana saharica* phalanges, and (B) *Rana perezi* tibiofibulae.

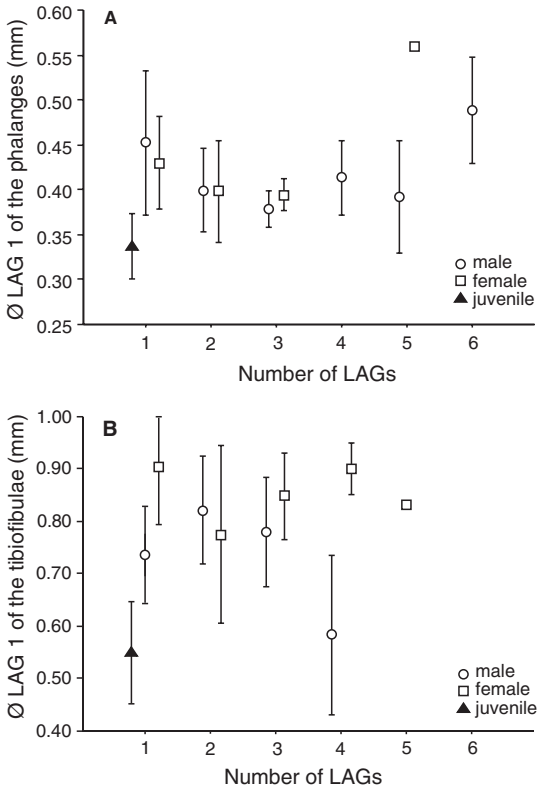


**Fig. 5.** Plot of age (LAG number) versus diameter of the first winter LAG in juveniles and adult males and females of (A) phalanges for *Rana saharica*, and (B) tibiofibulae for *Rana perezi*.

morphic froglets show very different sizes during their first winter, the existence of differential mortality among them can be expected. In such a case, adult individuals would be a sample of the first year froglet survivors. To investigate if froglets of any particular size (small or large) are differentially eliminated during the first winter, we can compare the size of first year froglets with the estimated size that adult frogs have during their first year of life. As an estimator of size at a given age, we use the diameter of the phalange, and the equation: SVL = 1.15 + 68.75PD ($r^2 = 0.803$, $p < 0.0001$) (Fig. 4A) which describes the relationship between phalange diameter (PD) and SVL. Our null hypothesis is that the mean diameter of LAG1 of first year froglets does not differ from the average diameter of LAG1 in current adults. The diameter of the LAG1 in adults of *Rana saharica* (0.414 ± 0.057 mm, range = 0.334–0.560 mm) is significantly larger ($t = 6.761$, $p < 0.0001$) than the diameter of the LAG1 in one year old juveniles (0.337 ± 0.036 mm, range = 0.271–0.439 mm). The null hypothesis is thus rejected and we can conclude that differential survival rates occur among first year froglets. Larger froglets were more successful than smaller in generating our adult sample (Fig. 5A).

However, such a conclusion may not be general since it is likely to be affected by climate differences between years, which in turn may shift the survival value of each metamorphic cohort. We reanalyzed previous data on the tibiofibulae of the Mediterranean species *R. perezi* (Esteban *et al.* 1996) to further test this hypothesis, using a different long bone, the tibiofibula, and frogs collected on a different time frame and under different climate conditions. We tested the same null hypothesis, using the diameter of the tibiofibula as an estimator of size at a given age. The equation: SVL = 11.63 + 30.63TD, describes the re-

lationship between tibiofibulae diameter and SVL ($r^2 = 0.922$, $p < 0.0001$) (Fig. 4B). The mean diameter of the LAG1 from the adult sample (0.804 ± 0.118 mm, range = 0.477–1.012 mm) is, as in the *R. saharica* example, larger ($t = 9.672$; $p < 0.0001$) than the mean diameter of the LAG1 in one year old juveniles (0.552 ± 0.096 mm, range = 0.427–0.735 mm). We reject the null hypothesis and we conclude that larger froglets are also more successful than smaller ones in generating our adult sample of *R. perezi*. These data support a size-dependent selection in the one-year-old froglets.

Extended breeding periods, allowing different metamorphosis peaks along the year, are apparently disadvantageous for the species since only froglets of a few subcohorts are able to survive. However, this strategy could be advantageous in areas where local conditions change dramatically from year to year. In years of summer drought, deep winter freezing, or during population peaks of aquatic predator insects, typical phenomena in Mediterranean ponds (Margalef 1983, Jeffries & Mills 1990), individuals from suboptimal cohorts, hatched after harsh conditions have disappeared, will have a better chance of survival, even at the expense of size. To test such a hypothesis further long term and controlled studies are necessary, as well as comparisons with frogs from different climate areas.

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