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Age, body size and growth pattern of *Argenteohyla siemersi pedersenii* (Anura: Hylidae) in northeastern Argentina

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In this study we analysed the body size, age structure, age at maturity, longevity and growth pattern of *Argenteohyla siemersi pedersenii* in north-eastern Argentina using skeletochronological methods. Body size was sexually dimorphic; females were significantly larger and heavier than males, regardless of age. As *A. s. pedersenii* is an explosive breeder with only one reproductive episode per year, we assumed that each Line of Arrested Growth (LAG) observed was equivalent to one period of decreasing growth, LAGs reflecting a direct estimation of individual age. The minimum number of LAGs counted was two for males and three for females, but there was no sexual difference in longevity. The von Bertalanffy's growth coefficients (K) were higher in males than in females for both size and body mass. Females had a lower growth rate than males, took longer to reach the minimum maturation size, and were older and larger at reproduction.

Keywords: *Argenteohyla siemersi pedersenii*; age; body size; growth; sexual size dimorphism; skeletochronology

Introduction

The genus of hylid frogs *Argenteohyla* is included in the monophyletic group known as “Casque-headed frogs” (Faivovich et al. 2005) and comprises a single species, *Argenteohyla siemersi* (Mertens 1937), with two allopatric subspecies: *A. s. siemersi*, the nominal southern form, and *A. s. pedersenii* (Williams and Bosso 1994) the northern form (Figure 1). In Argentina, *A. s. siemersi* inhabits the Entre Rios and Buenos Aires provinces (Mertens 1937; Gallardo 1964; Lutz and Barrio 1966), while *A. s. pedersenii* inhabits the Corrientes and Santa Fe provinces (Williams and Bosso 1994; Céspedes et al. 1995; Diminich and Zaracho 2008; Lajmanovich et al. 2012).

The main contributions to the biology and natural history of *A. siemersi* have been related to taxonomy (Barrio 1966; Trueb 1970; De Sá 1983; Williams and Bosso 1994; Céspedes 2000), advertisement call (Barrio 1966; Zaracho and Areta 2008) and to egg and clutch size (Diminich and Zaracho 2008). During the past two years, we have been studying the reproductive biology of *A. s. pedersenii* populations (Figure 1), providing a detailed description of the reproductive activity pattern, advertisement and encounter calls, reproductive behaviour, egg clutches and a redescription of the tadpole (Cajade

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Figure 1. Some aspects of the biology of *Argenteohyla siemersi pedersenii*. Upper left: female taking refuge in a leaf of the bromeliad *Aechmea distichantha*. Upper right: male emitting its advertisement call from the water surface. Lower left: female migrating to pond attracted by calling male. Lower right: amplexus resting out of water before female moved to the water to spawn.

et al. 2010). In the same study, the pattern of sexual size dimorphism showed significant differences when we analysed amplexed individuals, where females were larger than males.

Sexual dimorphism in body size is the most common sexual dimorphism among amphibians. Generally, females are larger than males (Shine 1979) and this variation could be attributed to either differential age at maturity (Monnet and Cherry 2002), growth rate, or both (Halliday and Tejedo 1995). Amphibians exhibit an indeterminate growth pattern with an exponential period that decreases considerably after the onset of reproduction, with the attainment of an asymptotic size (Hemelaar 1988; Halliday and Tejedo 1995). Hence, the analysis of the variation in terrestrial growth rates, age at maturity and longevity, is essential in the study of amphibian sexual size dimorphism (Monnet and Cherry 2002; Hasumi 2010; Sinsch et al. 2010; Marangoni et al. 2011).

One practical tool to discriminate variations in sexual age of maturity, longevity or sexual divergence in growth rates is skeletochronology. These variables are putative determinants that allow us to explain the actual pattern of sexual size dimorphism in amphibians. Skeletochronology is based on the presence of cyclic and annular bone growth, which can be visualized in cross-sections of bone (Castanet 1982; Castanet and Smirina 1990). This method of counting the number of lines of arrested growth (LAGs) in cross-sections of phalanges obtained by toe clipping, is the most commonly and successfully used tool for evaluating the age of many species of amphibians and reptiles, providing an age estimate through non-lethal means (Castanet and Smirina, 1990; Marangoni et al. 2009, 2011).

This study examines the determinants of the pattern of sexual size dimorphism in *A. s. pederseni*, by using skeletochronology, which provides data on age structure, age at maturity, longevity and growth pattern. We discuss and contextualize the formation and chronology of the growth markers with the reproductive biology provided by Cajade et al. (2010).

Material and methods

Study site, collection methods and individual measurements

Field work was carried out in a semipermanent pond located in a modified forest of *Schinopsis balansae* “quebracho”, at “El Perichon”, 10 km northeast from the city of Corrientes (27°25'55.6" S, 58°44'47.8" W). A detailed description of the study area – including climate, vegetation, pond and amphibian community – was given by Cajade et al. (2010). From 29 September to the 1 October 2008, corresponding to the second period of reproductive activity of *A. s. pederseni* sampled by Cajade et al. (2010), we marked as many individuals as possible by toe-clipping the third phalange. A total of 87 individuals (59 males and 28 females) of *A. s. pederseni* were marked, of which 50 were in amplexus (24 amplexant pairs were observed on the night of 29–30 September, and one pair was found on the night of 30 September–1 October). New individuals (unmarked) were not registered on the third night, suggesting that most individuals of that reproductive peak had been toe-clipped, and that this species has a typical explosive breeding behaviour. Toes were preserved to perform the skeletochronological analysis. Frogs were measured for their snout–vent length (SVL) with calipers (0.01-mm scale), body mass (BM) was measured with a pesola spring balance (0.1-g scale) and frogs were immediately released at the point of capture. In addition, because of the lack of recently metamorphosed individuals in the spring of 2008, we used SVL data of eight laboratory-reared metamorphosed individuals, at stage 46 (Gosner 1960), to make a more precise adjustment to the von Bertalanffy (1938) growth model (see below). These individuals were collected as tadpoles (stage 40) on 30 October 2010 in a pond near the locality of San Luis del Palmar, Corrientes Province, located approximately 20 km from the pond studied in 2008 (27°33'46,60" S; 58°34'55,66" W). These tadpoles were kept in the laboratory at a roughly constant room temperature (range 24–26°C) and the photoperiod followed a daily cycle of 16 : 8 h light : dark. Tadpoles were kept together in a plastic container filled with water from the collection pond and fed with boiled lettuce and commercial fish food *ad libitum*, until they reached metamorphosis (approximately 5 days later). Metamorphs were anaesthetized with lidocaine 10%, fixed in formalin 10%, preserved in alcohol 70%, and then housed in the herpetological Collection of the Museum of La

Plata, Argentina (Voucher specimens: MLP.A.5133–MLP.A.5140). Metamorphs were toe-clipped and measured in the same way as the adults (see above).

Skeletochronology

Laboratory protocols followed the standard methods of skeletochronology (e.g. Smirina 1972). Clipped digits were washed in water for 30 minutes and then decalcified in 5% nitric acid for 30–45 minutes. The digit samples were then dehydrated, paraffin-embedded, sectioned (14–16 μm , Arcano[®], Model RMT-30, rotation microtome, Instrumental Pasteur, Pasteur 788 (C1028AAP) Buenos Aires, Argentina) and stained using the Harris eosin & haematoxylin method. We took digital images, using a high-resolution camera SONY[®] SSC-DC50AP attached to an Olympus BX50 microscope, from the diaphysis where the size of the medullar cavity was at its minimum and the perimeter of bone was at its maximum. Cross-sections were observed and measured using the computer package IMAGE-PRO PLUS Version 4.5 (Media Cybernetics 1993–2001; Silver Spring, MD, USA) and calibrated using a standard micrometer. First, we recorded the presence/absence of the line of metamorphosis and the LAGs. Age was determined by two observers (FM and EG) who counted LAGs independently to prevent any bias. In those frogs with no remnant of the line of metamorphosis we estimated the degree of resorption by osteometrical analysis (Sagor et al. 1998; Tomašević et al. 2008). Second, we discriminated annual growth marks (LAGs *sensu stricto*) from non-annual ones (irregular interruptions during periods of inactivity), using the method described in Sinsch et al. (2007). The age of maturity was defined as the lowest age recorded in a reproductive frog found in amplexus from this population. We measured the longest and shortest perpendicular axes of bone perimeter (hereafter, BP, to the nearest μm) of two diaphyseal sections per specimen, following the methods of Hemelaar (1985). We determined the average diameter of bone perimeter by taking the square root of the product of the two axis measurements. Finally, we also measured the area of periosteal bone (hereafter, BA, to the nearest μm^2) to obtain a two-dimensional estimate for magnitude of three-dimensional bone growth (Sinsch et al. 2007), using the computer package UTHSCSA IMAGE TOOL (Wilcox et al. 2002), and calibrated using a standard micrometer.

Demographic life history traits

Five life history variables were measured for each sex (*sensu* Leskovar et al. 2006): (1) age at maturity: the minimum number of LAGs counted in breeding individuals; (2) longevity: the maximum number of LAGs counted in breeding individuals; (3) potential reproductive lifespan: the difference between longevity and age at maturity; (4) median lifespan: median of age distribution; (5) size at maturity: the average SVL of all first breeders with the minimum number of LAGs.

Growth estimation

We estimated growth by applying the von Bertalanffy (1938) growth equation:

$$\left(S_t - S_m - (S_m - S_0) e^{-K(t-t_0)} \right),$$

where t = number of growing seasons experienced (age); t_0 = age at metamorphosis (proportion of the growing season already elapsed at metamorphosis); S_t = average body size after having experienced t growing seasons; S_m = average maximal body size; S_0 = average body size at metamorphosis; K = growth coefficient, defining the shape of the growth curve.

Statistical analysis

All variables were log-transformed to meet the normality and homoscedasticity assumptions. We used multivariate and univariate analyses of variance to test for differences in body size, body mass, diameter of bone perimeter and area of bone, between the sexes. We used all individuals (amplected and non-amplected) to perform the analysis of sexual size dimorphism in SVL and BM, whereas in the previous study by Cajade et al. (2010) only amplected individuals were used. We carried out analyses of covariance (ANCOVA) using age as a covariate to account for the effect of age on SVL and BM. We also estimated the sexual size dimorphism with the Lovich and Gibbons (1992) sexual dimorphism index (SDI): where $SDI = (\text{size of larger sex} / \text{size of smaller sex}) \pm 1$ (+1 if males are larger or -1 if females are larger), and arbitrarily defined as positive when females are larger than males and negative in the contrary. We also analysed differences in body size and age between amplected and non-amplected males, to test whether increased male body size is an important determinant of male mating success in *A. s. pedersenii* (Shine 1979; Monnet and Cherry 2002). We used linear regressions to test the association between body size (SVL and BM) and age, and diameter of bone perimeter. The von Bertalanffy growth model was fitted to the average growth curve using the least squares procedure. All analyses were carried out with type III general linear models using the STATISTICA 6.0 statistical package (StatSoft Inc. 2001), and a significance level of $\alpha = 0.05$. All data were expressed as mean \pm 1 SD and mean \pm 1 SE for S_m and K , the von Bertalanffy growth curve parameters.

Results

A multiple analysis of variance (MANOVA) showed significant effects of sex on SVL, BM, BP and BA (Wilk's $\lambda = 0.591$, $F_{4,37} = 6.133$, $p < 0.001$). Univariate ANOVAs showed significant effects of sex on SVL and BM, but no significant effects on BP and BA (SVL: $F_{1,85} = 33.371$, $p < 0.0001$; BM: $F_{1,85} = 33.866$, $p < 0.0001$; BP: $F_{1,41} = 0.089$, $p = 0.767$; BA: $F_{1,41} = 1.281$, $p = 0.264$). Hence, body size was sexually dimorphic, with females being on average, significantly longer and heavier than males (Table 1). ANCOVAs show that, regardless of age, females are longer and heavier than males (SVL: $F_{1,53} = 22.720$, $p < 0.0001$; BM: $F_{1,53} = 20.491$, $p < 0.0001$). Males showed larger bones than females, although this difference was not significant (BP: $F_{1,41} = 0.088$, $p = 0.767$; BA: $F_{1,41} = 1.281$, $p = 0.264$) (Table 1).

The SDI in body size was 0.072 (SVL) and 0.31 (BM). In age classes 3 to 5, where sample size allowed estimation (age = number of LAGs observed, see Discussion), variation in SDI was: (age class/SDI) 3/0.146, 4/0.082, 5/0.113 for SVL, and 3/0.206, 4/0.348, 5/0.366 for BM. Females were larger in their average SVL and BM than males in all age classes. The mean body size of metamorphs was 32.48 ± 1.61 mm, $n = 8$ (SVL), and 2.29 ± 0.24 g, $n = 8$ (BM). There were no significant differences

Table 1. Basic statistics for body size: snout–vent length (SVL) and body mass (BM), and bone size: diameter of bone perimeter (BP, to the nearest μm) and bone area (BA, to the nearest μm^2), of males and females of *Argenteohyla siemersi pedersenii*.

Sex/trait	Statistics				
	<i>n</i>	Mean	Min	Max	SD
Males					
SVL	59	69.17	60	77	3.56
BM	59	18.8	11	28	3.51
BP	29	325.16	217.51	464.1	59.25
BA	29	102723.4	41991.2	194919.9	37290.5
Females					
SVL	28	74.19	68.48	83	4.14
BM	28	24.64	16.5	44	5.77
BP	14	319.19	202.02	456.51	58
BA	14	88740.5	52708.4	157640.2	27162.4
Metamorphs					
SVL	8	32.48	30.46	35.51	1.61
BM	8	2.29	1.9	2.81	0.24
BP	8	153.57	106.8	198.65	26.83
BA	8	18730.5	8094.5	32016.7	7127.4

between amplexed and non-amplexed males in their average SVL, BM and age (Wilk's $\lambda = 0.984$, $F_{3,36} = 1.198$, $p = 0.897$. ANOVAs, SVL: $F_{1,57} = 0.197$, $p = 0.658$; BM: $F_{1,57} = 0.733$, $p = 0.395$; Age: $F_{1,38} = 0.0008$, $p = 0.977$), although those males that were caught in amplexus showed smaller body size and were the oldest on average.

Out of 87 processed samples, 64 sections (40 males, 16 females, 8 metamorphs) showed recognizable bone structures that allowed age determination. In these sections, well-defined LAGs were found in the periosteal bone and were relatively easy to count to assess individual age (Figure 2). The line of metamorphosis was visible in 80% ($n = 51$) of the samples. Moreover, even in those specimens where the metamorphosis line had been replaced during the formation of the endosteal bone, the first LAG was never completely removed. Therefore, endosteal resorption never prevented age estimation. Annuli (*sensu* Peabody 1958) were easily distinguishable from actual LAGs. They always stained more faintly and were often broader, as previously described by Leclair et al. (2005) and Sinsch et al. (2007) in temperate species.

The demographic life history traits of *A. s. pedersenii* are summarized in Table 2 and the age structure of adults in Figure 3. Females were older and heavier at maturity than males, but the differences in body size at maturity between sexes were not significant (SVL: Mann–Whitney U -test = 7, $p = 0.881$; BM: Mann–Whitney U -test = 4.5, $p = 0.368$). The minimum number of LAGs (= age) counted in reproductive individuals was 2 for males and 3 for females (Table 2). We did not find sexual differences in longevity (5 years in both sexes). Hence, the difference in Potential Reproductive Lifespan between sexes was 1 year. The mean age was higher in females than males (Table 2), but it did not differ significantly (Mann–Whitney U -test = 242.5, $p = 0.132$).

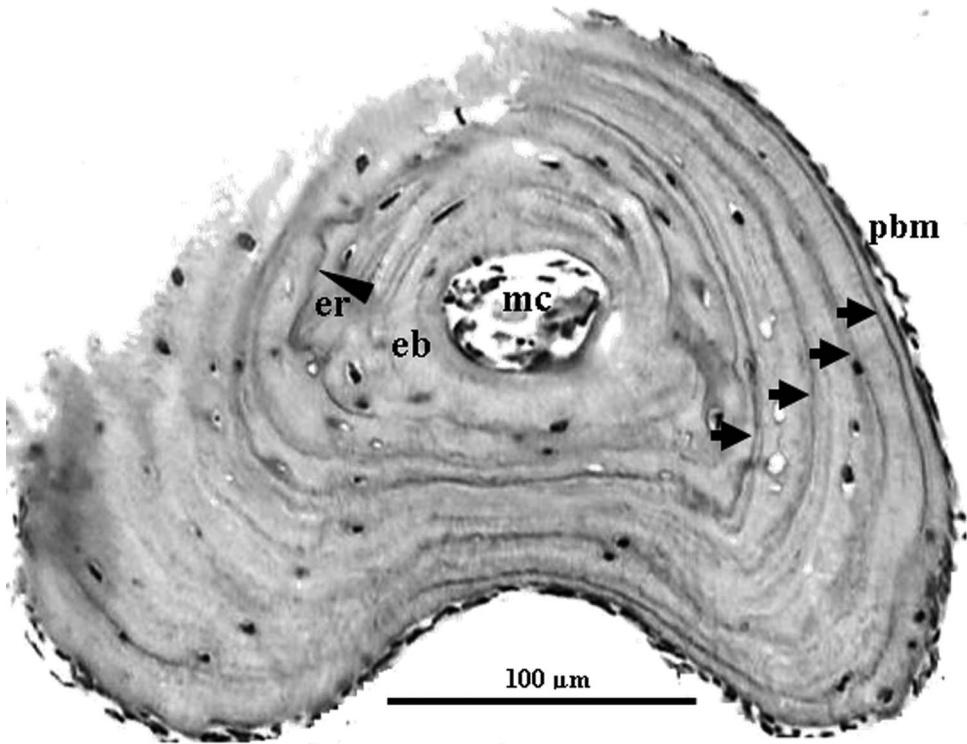


Figure 2. Cross-sections of phalanges stained with Ehrlich's haematoxylin of adult male 67.67 mm snout–vent length, from the El Perichón population. Four lines of arrested growth (LAGs) are visible in the periosteal bone (indicated by arrows). The first LAG was partially destroyed by the endosteal resorption (er). This individual was 5 years old. Arrowhead: endosteal resorption, mc: medullar cavity, eb: endosteal bone, pbm: periosteal bone margin.

The body size of *A. s. pederseni* was poorly correlated with age and bone size. A positive but not significant correlation was observed between female size (SVL and BM) and age. BM was strongly positively correlated with SVL in both sexes. A significant positive correlation between SVL and bone perimeter (BP) was only recorded in females (Table 3).

Growth fitted well to a von Bertalanffy model [R^2 -values, female: $R^2 = 0.978$, male: $R^2 = 0.949$, for SVL, and female: $R^2 = 0.948$, male: $R^2 = 0.897$ for BM, $p < 0.0001$ for both variables and sexes; and observed asymptotic SE of the estimated parameters (S_m and K), see below]. Average body size at metamorphosis S_0 was taken from eight recently metamorphosed individuals, as described above. The predicted maximal body size (S_m) was always larger than measured average values. Females had larger average maximal body size (S_m) than males (female: 87.06 ± 6.88 mm; male: 71.48 ± 1.57 mm for SVL, and female: 34.21 ± 8.20 g; male: 19.9 ± 0.97 g for BM). The growth coefficient (K), which determines how fast the body size approaches its maximum value, was higher in males than in females, for both size and body mass (SVL, females: 0.37 ± 0.10 mm; males: 0.82 ± 0.15 mm; BM, females: 0.30 ± 0.15 g; males: 0.87 ± 0.23 g) (Figure 4).

Table 2. Features of population life history traits of *Argenteohyla siemersi pederseni*.

Sex	N	Mean ± SE (LAGs)	Mode (Frequency)	Median lifespan (years)	AM (LAGs)	Longevity (LAGs)	PRLS (years)	Size at AM mean ± SE (mm)	Maximum size (age) (mm)
Males	40	3.52 ± 0.13	3 (19)	3	2	5	3	72.1 ± 1.43 (n = 3)	75.03 (at 3 LAGs)
Females	16	3.87 ± 0.17	4 (8)	4	3	5	2	72.9 ± 1.52 (n = 5)	64.04 (at 3 LAGs)

AM, age at maturity of the youngest first breeders; LAG, line of arrested growth; PRLS, potential reproductive lifespan; size, snout–vent length.

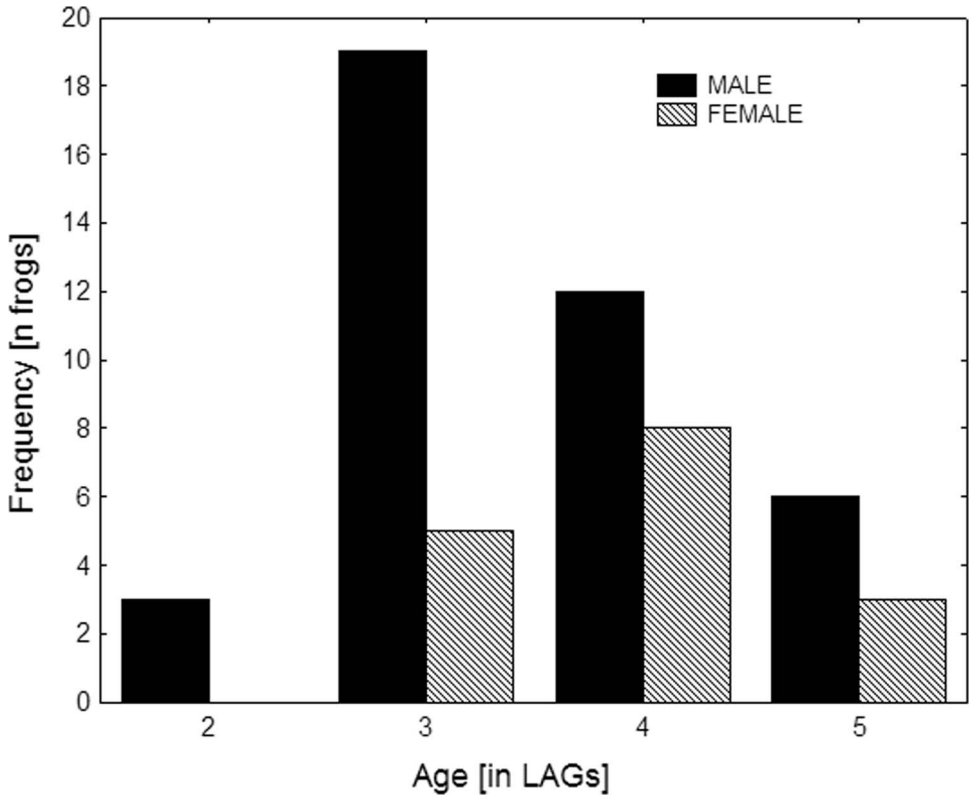


Figure 3. Age distribution of *Argenteohyla siemersi pedersenii*.

Table 3. Relationships between body size (SVL and BM), age, and diameter of bone (bone size) in both sexes of *Argenteohyla siemersi pedersenii*.

	Females			Males		
	<i>n</i>	Equation	<i>r</i> ²	<i>n</i>	Equation	<i>r</i> ²
SVL vs BM	28	$y = -3.27 + 1.005x$	0.637**	59	$y = -0.749 + 0.079x$	0.264**
<i>Body size versus age</i>						
SVL	16	$y = 4.217 + 0.022x$	0.094 ns	40	$y = 4.288 - 0.014x$	0.071 ns
BM	16	$y = 0.945 + 0.029x$	0.102 ns	40	$y = 0.984 - 0.001x$	0.0007 ns
<i>Body size versus bone size</i>						
SVL versus BP	15	$y = -2.526 + 1.992x$	0.346*	37	$y = 4.145 + 0.392x$	0.006 ns
BM versus BP	15	$y = 4.737 + 0.947x$	0.126 ns	37	$y = 4.92 + 0.917x$	0.052 ns

SVL, snout-vent length; BP, bone perimeter.

All variables were log-transformed. *p*-values: **p* < 0.05, ***p* < 0.0001.

Discussion

Body size, age and growth pattern

Argenteohyla s. pedersenii shows sexual size dimorphism in SVL, with females being, on average, longer than males (Cajade et al. 2010; this study). This pattern was also

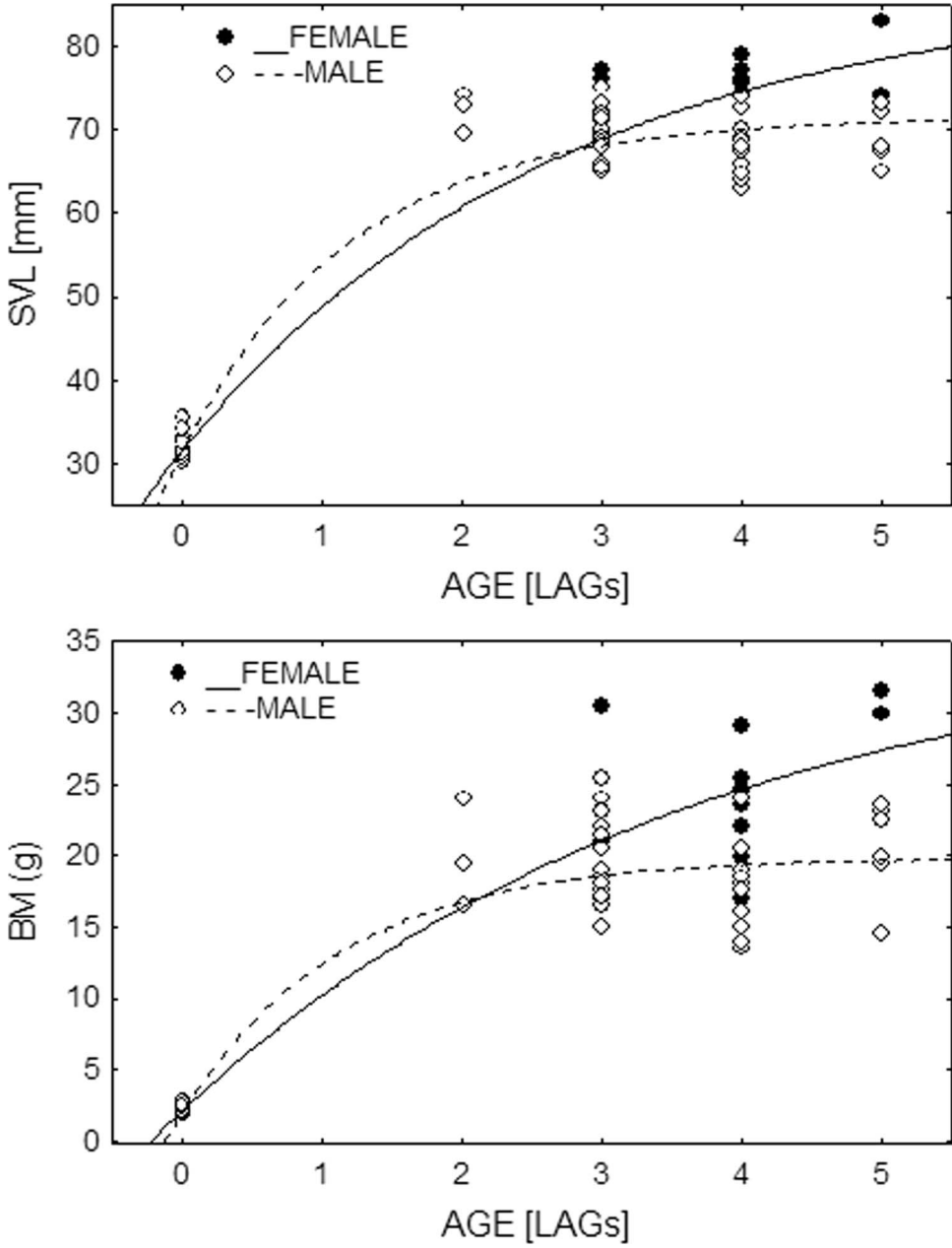


Figure 4. Growth curves for snout-vent length in males and females of *Argenteohyla siemersi pedersenii*. Growth curves were fitted to von Bertalanffy's growth equation: $[S_t - S_m - (S_m - S_0) e^{-k(t-t_0)}]$.

maintained when we analysed the body mass, although this dimorphism could be induced by differences in clutch mass, because the females measured in amplexus could have been gravid. This sexual dimorphism is common among amphibians, being

present in about 90% of anuran species (Shine 1979; Kupfer 2007), and several non-mutually exclusive factors can contribute to it, such as the capacity of larger females to produce larger clutches and larger eggs (Crump 1974); sex-specific differences in mortality rate caused by differential predation pressure between sexes (Howard 1981); restrictions on the growth of males because of the energy demand linked to reproductive activity (Woolbright 1989); or faster growth rates and delayed reproduction in females (Monnet and Cherry 2002; Marangoni 2006; Marangoni et al. 2008, 2011). In *A. s. pederseni*, we found that females reached maturity at 3 years of age, 1 year later than males, but they were also bigger at that age. In addition, the growth coefficient (K), which determines how fast the body size approaches its maximum value, was lower in females than in males, for both SVL and BM. Hence, the females of *A. s. pederseni* took longer to reach the minimum size at which maturation may occur (Hemelaar 1988) and were older and bigger at first reproduction than males, but with 1 year shorter Potential Reproductive Lifespan than males. We suggest that the differences found in the age of sexual maturation and growth rate may determine the sexual size dimorphism observed in *A. s. pederseni*. The sexual size dimorphism could also be accounted for sex-specific differences in longevity (Slatkin 1984); however, our results did not support this hypothesis, because both sexes had the same longevity (5 years). Alternatively, the sexual dimorphism in size could be determined by evolutionary (taxonomic) relationships, more so than by reproductive variables, because there is no significant correlation between fecundity and sexual dimorphism for size in amphibians (Crump 1974).

It is widely agreed that sexual selection in males is a major evolutionary force that selects for larger body size in many organisms (Blanckernhorn 2000). In amphibians, there is also ample empirical evidence for greater mating and reproductive success of large males, either related to male–male competition or female choice (Shine 1989). Based on this evidence, we expected to find that males of *A. s. pederseni* captured in amplexus would be larger than non-amplected males; however, there were no significant differences between amplected and non-amplected males in average body size and age, although amplected males had a slight tendency to have smaller body size and to be older. We suggest two possible explanations for this absence of differences in body size in males of *A. s. pederseni*. First, the absence of differences caused by a limited opportunity for effective female choice, as happens in explosive breeders (Emlen and Oring 1977; Wells 1977; Sullivan 1982). *Argenteohyla s. pederseni* is an explosive breeder with very short breeding periods, it breeds only once per year over a 3-day period, immediately after the first heavy rains in the middle of September (Cajade et al. 2010). A second alternative explanation, based on the results of this study, is that older males have an advantage in reproductive success over younger males (possibly as a result of reproductive experience), but they are not necessarily the largest. This is in agreement with previous studies that cast doubt on the hypothesis that size and age are significantly and positively related in anuran amphibians (reviewed by Halliday and Verrel 1988; Cherry and Francillon-Vieillot 1992; Leary et al. 2005).

Reproductive activity pattern and formation and periodicity of LAGs

As described above, *A. s. pederseni* is an explosive breeder with a very short breeding season. This population bred in a semi-permanent pond, which was completely dry from May to August and refilled with water during the wet seasons from September to

May of both years studied (Cajade et al. 2010). Consequently, this pond's hydroperiod determines a well-marked pattern of activity of *A. s. pederseni*. This pattern is characterized by a dry season (autumn–winter) during which this species has a period of inactivity where it is difficult to find adults moving through the study area, or even hiding in the bromeliads they use as refuge during the wet/spring season (Figure 1). Breeding activity occurs immediately after the first heavy rains in the middle of September, at the beginning of each wet season (spring) (Figure 1) (Cajade et al. 2010). For this reason, the well-differentiated activity/hibernation pattern has implications for the formation and periodicity of growth-marks in *A. s. pederseni*.

LAG formation is ultimately caused by a genetically based, circannual rhythm, which under natural conditions becomes synchronized with, and reinforced by, the seasonal cycle (Castanet et al. 1993; Smirina 1994; Morrison et al. 2004; Marangoni et al. 2009). Therefore, both intrinsic and extrinsic factors may cause LAG formation and periodicity. We found well-defined LAGs in 74% of the analysed clipped digit samples that were relatively easy to count to assess the individual age of *A. s. pederseni*. The present skeletochronological study is one of the few studies that confirms the presence of well-defined LAGs in amphibians from tropical or subtropical regions (see also, Kumbar and Pancharatna 2002; Marangoni et al. 2009; Jovanovic and Vences 2010; Andreone et al. 2011), and it reinforces the hypothesis that in amphibians of these regions, where growth seems to be less constrained by temperature and subjected to periodicity through wet/dry seasons, well-defined growth marks are formed. Hence, both intrinsic cycles and extrinsic factors may be having an influence on the rhythm of osteogenesis throughout the year in *A. s. pederseni*.

Finally, the periodicity of growth mark formation, one LAG per year, which is equivalent to the number of hibernations or aestivations of each individual, represents the most common observed pattern of periodicity of LAG formation in palaeartic, tropical and subtropical amphibian species (Castanet et al. 1993; Smirina 1994; Morrison et al. 2004; Marangoni et al. 2009, 2011; Andreone et al. 2011). However, exceptions to the “one LAG per year” rule (e.g. double LAGs), which represent informative histological correlates of local environmental impacts on life history, have been observed occasionally (Sinsch et al. 2007; Olgun et al. 2001, 2005). We found a strong correspondence between the local environment and the periodicity of LAG formation in *A. s. pederseni*. All individuals of this study were collected in the only reproductive peak of 2008, occurring immediately after a long dry period. The histological observation showed that the outermost LAG and the perimeter of the phalange were very close together with an extremely thin thickness of bone tissue between them, and had become almost indistinguishable (Figure 2), suggesting a recent period of decreasing growth and the consequent formation of a LAG. In addition, supplementary non-annual LAGs have never been observed. So, based on our skeletochronological results, and as *A. s. pederseni* reproduce once per year, we assumed that the number of LAGs observed is equivalent to only one period of decreasing growth that corresponds to the dry period (May–August), giving a direct estimation of individual age.

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References

- Andreone F, Giacoma C, Guarino FM, Mercurio V, Tessa G. 2011. Age profile in nine *Mantella* poison frogs from Madagascar, as revealed by skeletochronological analyses. *Alytes* 27(3):73–84.
- Barrio A. 1966. Descripción del alotipo macho y del canto nupcial de *Trachycephalus siemersi* (Mertens) (Anura, Hylidae). *Physis* 26(71):225–228.
- Blanckernhorn WU. 2000. The evolution of body size: what keeps organisms small? *Quart Rev Biol.* 75(4): 385–407.
- Cajade R, Schaefer EF, Duré MI, Kehr AI, Marangoni F. 2010. Reproductive biology of *Argenteohyla siemersi pederseni* Williams and Bosso, 1994 (Anura: Hylidae) in northeastern Argentina. *J Nat Hist.* 44(31):1953–1978.
- Castanet J. 1982. Recherches sur la croissance du tissu osseux des reptiles. Application: la méthode squeletochronologique [unpublished PhD dissertation]. Université Paris 7, Paris.
- Castanet J, Francillon-Vieillot H, Meunier FJ, De Ricqlès A. 1993. Bone Growth. Boca Raton: B. K. Hall, CRC Press. Vol. 7, Bone and individual aging; p. 245–283.
- Castanet J, Smirina E. 1990. Introduction to the skeletochronological method in amphibian and reptiles. *Ann Sci Nat Zool Biol Anim Paris. Sér* 13. 11:191–196.
- Céspedes JA. 2000. Historia natural de la rana de Pedersen *Argenteohyla siemersi pederseni* (Anura: Hylidae), y descripción de su larva. *Bol Asoc Herpetól Esp.* 11(2):75–80.
- Céspedes JA, Aguirre RH, Alvarez BB. 1995. Composición y Distribución de la Anfibiafauna de la Provincia de Corrientes (Argentina). *Facena.* 11:25–49.
- Cherry MI, Francillon Vieillot H. 1992. Body size, age and reproduction in the leopard toad, *Bufo pardalis*. *J Zool.* 228(1):41–52.
- Crump ML. 1974. Reproductive strategies in a tropical anuran community. *Misc Pub Mus Nat Hist Univ Kansas.* 61:1–68.
- De Sá R. 1983. Descripción de la larva de *Argenteohyla siemersi* (Mertens, 1937), (Anura: Hylidae). *Resum Comun J Cienc Nat Montevideo (Uruguay).* 3:40–41.
- Diminich MC, Zaracho VH. 2008. *Argenteohyla siemersi pederseni*. Reproduction. *Natural History Note. Herpetol Rev.* 39(1):74–75.
- Emlen ST, Oring LW. 1977. Ecology, sexual selection, and the evolution of the mating system. *Science* 197(4300):215–223.
- Faivovich J, Haddad CFB, Garcia PCA, Frost DR, Campbell JA, Wheeler WC. 2005. Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. *Bull Am Mus Nat Hist.* 294:1–240.
- Gallardo JM. 1964. Los anfibios de la provincia de Entre Rios, Argentina, y algunas notas sobre su distribución geográfica y ecología. *Neotropica* 10(31):23–28.
- Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16(3):183–190.
- Halliday TR, Tejedo M. 1995. Amphibian Biology, Social Behaviour. Chipping Norton: Heatwole, H., Sullivan, B.K. Vol. 2, Intrasexual selection and alternative mating behavior; p. 419–468.
- Halliday TR, Verrell PA. 1988. Body size and age in amphibians and reptiles. *J Herpetol.* 22(3):253–265.
- Hasumi M. 2010. Age, body size, and sexual dimorphism in size and shape in *Salamandrella keyserlingii* (Caudata: Hynobiidae). *Evol Biol.* 37(1):38–48.

- Hemelaar ASM. 1985. An improved method to estimate the number of year rings resorbed in phalanges of *Bufo bufo* (L.) and its application to population from different latitudes and altitudes. *Amphib-Rept.* 6(4):323–343.
- Hemelaar ASM. 1988. Age, growth and other population characteristics of *Bufo bufo* from different latitudes and altitudes. *J Herpetol.* 22(1988):369–388.
- Howard RD. 1981. Sexual dimorphism in bullfrogs. *Ecology* 62(2):303–310.
- Jovanovic O, Vences M. 2010. Skeletochronological analysis of age structure in populations of four species of Malagasy poisonous frogs, genus *Mantella*. *Amphib-Rept.* 31(4): 553–557.
- Kumbar SM, Pancharatna K. 2002. Annual growth layers in the phalanges of the Indian Skipper Frog *Rana cyanophlyctis* (Schn.). *Copeia* 2002:870–872.
- Kupfer A. 2007. Chapter 5; Sexual size dimorphism in amphibians: an overview. In: Fairbairn D, Blanckenhorn W, Szekeley T, editors *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*. Cary (NC): Oxford University Press. p. 50–59.
- Lajmanovich RC, Peltzer PP, Attademo AM, Cabagna MC, Celina M, Junges CM, Basso A. 2012. Amphibia, Anura, Hylidae, *Argenteohyla siemersi pedersenii* (Williams and Bosso, 1994): first record and some hematological data in Santa Fe Province, Argentina. *Check List.* 8(4): 790–791.
- Leary CJ, Fox DG, Shepard DB, Garcia AM. 2005. Body size, age, growth and alternative mating tactics in toads: satellite males are smaller but not younger than calling males. *Anim Behav.* 70(3):663–671.
- Leclair MH, Leclair Jr. R, Gallant J. 2005. Application of skeletochronology to a population of *Pelobates cultripes* (Anura: Pelobatidae) from Portugal. *J Herpetol.* 39(2): 199–207.
- Leskovar C, Oromi N, Sanuy D, Sinsch U. 2006. Demographic life history traits of reproductive natterjack toads (*Bufo calamita*) vary between northern and southern latitudes. *Amphib-Rept.* 27(3):365–375.
- Lovich JE, Gibbons JW. 1992. A review of techniques for quantifying sexual size dimorphism. *Growth Dev Aging.* 56(4):269–281.
- Lutz B, Barrio A. 1966. Notas batracológicas, II. Sobre la supuesta existencia del género *Flectonotus* Miranda-Ribeiro en la Argentina. II. Observaciones etoecológicas sobre *Trachycephalus siemersi* (Mertens) (Anura: Hylidae). *Physis.* 26(71):107–108.
- Marangoni F. 2006. Variación clinal en el tamaño del cuerpo a escala microgeográfica en dos especies de anuros (*Pelobates cultripes* y *Bufo calamita*). [PhD thesis]. [Sevilla, España]: Universidad de Sevilla.
- Marangoni F, Barraso DA, Cajade R, Agostini G. 2011. Body size, age and growth pattern of *Physalaemus fernandezae* (Anura: Leiuperidae) of Argentina. *NW J Zool.* 8(1):63–71.
- Marangoni F, Schaefer EF, Cajade R, Tejedo M. 2009. Growth marks formation and chronology of two neotropical anuran species. *J Herpetol.* 43(3):446–450.
- Marangoni F, Tejedo M, Gomez-Mestre I. 2008. Extreme reduction in body size and reproductive output associated with sandy substrates in two anuran species. *Amphib-Rept.* 29(4):541–553.
- MediaCybernetics, (1993–2001): Image-Pro Plus. Version 4.5.0.29. Silver Spring (MD): Media Cybernetics.
- Mertens R. 1937. Ein neuer Laubfrosch aus Argentinien. *Senckenbergiana* 19(1–2):12–14.
- Monnet MJ, Cherry MI. 2002. Sexual size dimorphism in anurans. *Proc Roy Soc Lond B, Biol Sci.* 269:2301–2307.
- Morrison C, J-M Hero, Browning J. 2004. Altitudinal variation in the age at maturity longevity, and reproductive lifespan of anurans in subtropical Queensland. *Herpetologica* 60(1):34–44.
- Olgun K, Miaud C, Gautier P. 2001. Age, size and growth of the terrestrial salamander *Mertensiella luschani* in an arid environment. *Can J Zool.* 79(9):1559–1567.

- Olgun K, Uzum N, Avci A, Miaud C. 2005. Age, size and growth of the southern crested newt *Triturus karelinii* (Strauch 1870) in a population from Bozdag (western Turkey). *Amphib-Rept.* 26(2):223–230.
- Peabody CE. 1958. A Kansas drought recorded in growth zones of a bullsnake. *Copeia* 1958: 91–94.
- Sagor ES, Ouellet M, Barten E, Green DM. 1998. Skeletochronology and geographic variation in age structure in the wood frog, *Rana sylvatica*. *J Herpetol.* 32(4):469–474.
- Shine R. 1979. Sexual selection and sexual dimorphism in the amphibians. *Copeia* 1979:297–306.
- Shine R. 1989. Ecological causes for the evolution of sexual size dimorphism: a review of the evidence. *Quart Rev Biol.* 64(4):419–461.
- Sinsch U, Marangoni F, Oromi N, Leskovar C, Sanuy D, Tejedo M. 2010. Proximate mechanisms determining size variability in natterjack toads. *J Zool.* 281(4):272–281.
- Sinsch U, Oromi N, Sanuy D. 2007. Growth marks in natterjack toad (*Bufo calamita*) bones: histological correlates of hibernation and aestivation periods. *Herpetol J.* 17(2):129–137.
- Slatkin M. 1984. Ecological causes of sexual dimorphism. *Evolution* 38(3):622–630.
- Smirina EM. 1972. Annual layers in bones of *Rana temporaria*. *Zool Z.* 51(10):1529–1534.
- Smirina EM. 1994. Age determination and longevity in amphibians. *Gerontology* 40(2–4):133–146.
- StatSoft. 2001. Statistica (Data analysis software system). Version 6. Tulsa (OK): StatSoft.
- Sullivan BK. 1982. Male mating behaviour in the Great Plains toad (*Bufo cognatus*). *Anim Behav.* 30(3):939–940.
- Tomašević N, Cvetković D, Miaud C, Aleksić I, Crnobrnja-Isailović J. 2008. Interannual variation in life history traits between neighbouring populations of the widespread amphibian *Bufo bufo*. *R d'Ecologie: Terre Vie.* 63:73–83.
- Trueb L. 1970. The generic status of *Hyla siemersi* Mertens. *Herpetologica* 26(2):254–267.
- Von Bertalanffy L. 1938. A quantitative theory of organic growth. *H Biol.* 10(2):181–213.
- Wells KD. 1977. The social behaviour of anurans amphibians. *Anim Behav.* 25(3):666–693.
- Wilcox D, Dove B, McDavid D, Greer D. 2002. UTHSCSA image tool, v. 3. [Internet]. San Antonio (Texas): University of Texas Health Science Center. Available from: <http://compdent.uthscsa.edu/dig/itdesc.html>.
- Williams JD, Bosso A. 1994. Estatus sistemático y distribución geográfica de *Argenteohyla siemersi* (Mertens, 1937) en la República Argentina (Anura: Hylidae). *Cuad Herpetol (AHA).* 8(1):57–62.
- Woolbright LL. 1989. Sexual dimorphism in *Eleutherodactylus coqui*: selection pressures and growth rates. *Herpetologica* 45(1):68–74.
- Zaracho VH, Areta JI. 2008. The advertisement call of *Argenteohyla siemersi pedersenii* (Amphibia, Anura, Hylidae), and comments on its taxonomic status. *Facena* 24:49–57.