
SEXUAL SIZE DIMORPHISM IN RELATION TO AGE AND GROWTH IN *HYPYSIBOAS CORDOBAE* (ANURA: HYLIDAE) FROM CÓRDOBA, ARGENTINA

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Abstract.—Most anurans exhibit sexual size dimorphism (SSD). This morphological variation between the sexes may be attributed to different ages at maturity, growth rate, or longevity. We present the first data on age structure and growth pattern of *Hypsiboas cordobae* using skeletochronology, and analyze SSD considering age-specific differences in body size based on 71 individuals. Snout-vent length (SVL) and body mass (BM) were significantly different between the sexes. Females were larger and heavier than males, but mean age between adult males and females was not significantly different. Minimum age at sexual maturity, longevity, and potential reproductive lifespan did not differ between the sexes. Female SVL and BM at sexual maturity were significantly larger than those of males. ANCOVA revealed significant sexual differences in body size and weight. The sexual dimorphism index in body size was 0.0679 (SVL) and 0.3621 (BM). Growth curves using the von Bertalanffy model showed a similar profile in both sexes, but the growth coefficient was higher in males. Age determination and the von Bertalanffy growth model for *H. cordobae* allowed us to determine SSD and our results may provide baseline data on the demography of *H. cordobae*.

Key Words.—age distribution; demography; longevity; skeletochronology; sexual selection

INTRODUCTION

Sexual size dimorphism (SSD) is a difference in body length or mass of sexually mature organisms and has been demonstrated in a variety of animals (Kupfer 2007). Differences in body size or shape between sexes within a population might be produced under sexual selection (Shine 1988) or from ecological differences between sexes (Slatkin 1984; Shine 1989). Amphibians are often sexually dimorphic in body size and shape, color, morphology, ornaments, and physiological characteristics (Kupfer 2007; Bell and Zamudio 2012). Several hypotheses aim to explain the existence of SSD in amphibian species. Male-biased SSD is promoted when males are territorial and involved in male-male combat (Tsuji and Matsui 2002; Kupfer 2007; Liao et al. 2015). Female-biased SSD is promoted by fecundity selection and high male mortality rates (Shine 1988; Liao et al. 2015). SSD is a widespread phenomenon in numerous anuran species in which females (Reinhard et al. 2015) or males (Taborsky et al. 2009) have a larger body size than the opposite sex. This variation can also be attributed to different age at maturity, growth rate, or longevity (Üzüm and Olgun 2009; Sinsch et al. 2010;

Casale et al. 2011); therefore, consideration of these factors is essential in the study of anuran SSD.

Determining the age of individual amphibians provides crucial information on demographic parameters, such as longevity, growth rate, and size at sexual maturity of a population (Duellman and Trueb 1994). Also, skeletochronology is considered a reliable method for age estimation and growth rates of many vertebrates, including amphibians (Sinsch et al. 2001; Marangoni et al. 2012). This method is based on the observation of annual growth rings of bones (commonly known as lines of arrested growth, LAGs). These rings are formed during hibernation and reflect the seasonal changes in growth rate (Smirina 1994; Bionda et al. 2015). However, age estimation may be underestimated or overestimated if a correct interpretation of LAGs is not performed (Sinsch 2015).

The genus *Hypsiboas* contains 93 species, most of them included in seven species groups (Frost, D.R. 2016. Amphibian Species of the World: An Online Reference. Version 6.0. Electronic Database accessible at <http://research.amnh.org/herpetology/amphibia/index.html>. [Accessed 26 August 2016]). The *Hypsiboas pulchellus* group currently has 37 species (Faivovich et al. 2005;

Köhler et al. 2010; Batista et al. 2015), including *H. cordobae* (Barrio 1965). This species is distributed in the hills of Córdoba and San Luis provinces, Argentina (Barrio 1965; Baraquet et al. 2015). Available literature about *H. cordobae* is mainly related to cytogenetics (Barale et al. 1991; Baraquet et al. 2013b), bioacoustics (Baraquet et al. 2013c, 2015), and hematology (Baraquet et al. 2013a, 2014).

Although morphometric analyses for *H. cordobae* were performed by Cei (1980) and Baraquet et al. (2012), none of these studies considered age-related differences in body size. The aim of this study is to present the first data on the growth pattern, age at maturity, and longevity of *H. cordobae*. Furthermore, we determine whether SSD, considering age-related differences in body size, exists in *H. cordobae*, and evaluate it in relation to demographic parameters.

MATERIALS AND METHODS

Study area.—Sampling site was located in central part of the Sierra de Comechingones, Córdoba, Argentina (32°50'34"S, 64°79'30"W, 1,200 m elevation). This region is characterized by a semi-humid to semi-dry climate with a deficit of water in the winter. This area is enclosed within the 19° C isotherm. Mean annual rainfall is approximately 901 mm (Oggero and Arana 2012).

Field data collection.—We collected 71 *H. cordobae* September 2013 to March 2014 (10 froglets, one juvenile, 39 adult males, and 21 adult females). We hand-captured individuals during surveys on the shores of streams and ponds, and newly metamorphosed froglets with tails not completely reabsorbed while emerging from ponds. We determined the sex of adults using external secondary sexual characters (black vocal sacs in males, and eggs readily visible through the abdomen wall in females). We measured the snout-vent length (SVL) of each individual using a digital caliper (0.01 mm) and body mass (BM) using a digital balance (0.01 g). We clipped the longest right phalanx of each frog and preserved it in 70% ethanol. We released all individuals at their capture site.

Age determination.—We performed laboratory protocols following standard skeletochronology methods (Bionda et al. 2015; Otero et al. in press): (1) fixation in 4% formalin (at least 12 h), (2) decalcification of bones (5–10%) formic acid (24 h), (3) paraffin embedding, (4) cross sectioning of the diaphysis at 10–12 μ m using a rotary microtome, (5) staining with Ehrlich's haematoxylin (3 min), (6) light microscopic count of the number of lines of arrested

growth (LAG) at 100 \times , and (7) documenting the most informative cross sections with photographs taken with a digital camera. We identified and interpreted LAGs following Sinsch (2015) for neotropical species, and LAGs were independently counted by two researchers. We identified double and false lines following Sinsch et al. (2007), and we assessed endosteal resorption by observing the presence of the Kastschenko line (KL; the interface between the endosteal and periosteal zones; Rozenblut and Ogielska 2005). Furthermore, we assessed the complete resorption of the innermost LAG by comparing the diameter of the smallest juvenile cross-section with the diameter of the resorption line of adults (Li et al. 2013). We estimated demographic variables following Bionda et al. (2015) and Otero et al. (in press): (1) age at maturity: the minimum number of LAGs counted in breeding individuals; (2) longevity: the maximum number of LAGs counted in reproductive individuals; (3) potential reproductive lifespan: the difference between longevity and age at maturity; (4) size at maturity: the average SVL of all first-time breeding adults with the minimum number of LAGs; and (5) modal lifespan: mode of age distribution.

We constructed a growth model following the von Bertalanffy (1938) equation, previously used in several studies of amphibians (Cogălniceanu and Miaud 2002; Guarino et al. 2011; Bionda et al. 2015). We used the following equation: $SVL_t = SVL_{max} - (SVL_{max} - SVL_{met}) e^{-K(t-t_{met})}$, where SVL_t = average SVL at age t , SVL_{max} = maximum (asymptotic) SVL, SVL_{met} = average SVL at metamorphosis, t = number of growing seasons experienced (age), t_{met} = age at metamorphosis, fixed to 0.4, and K = growth coefficient (shape of the growth curve). In the von Bertalanffy growth model, body length is considered as a function of age, and we fit length to the average growth curve using the least squares procedure.

Data analysis.—We used parametric tests when the data met the assumptions (normality and homogeneity of variance) and non-parametric Kruskal-Wallis tests otherwise. We used an analysis of covariance (ANCOVA) with age as a covariate to compare SVL and BM between sexes. Pearson's correlation coefficient was computed to assess the relationships between SVL-BM and age. We also estimated SSD using the Lovich and Gibbons (1992) sexual dimorphism index (SDI): $SDI = (\text{size of larger sex} / \text{size of smaller sex}) \pm 1$; +1 if males are larger or -1 if females are larger, and the result arbitrarily defined as positive when females are larger than males and negative in the contrary. We performed all tests using the Statistica 6.0/W software package (Statsoft Inc., USA) with $P \leq 0.05$ considered significant.

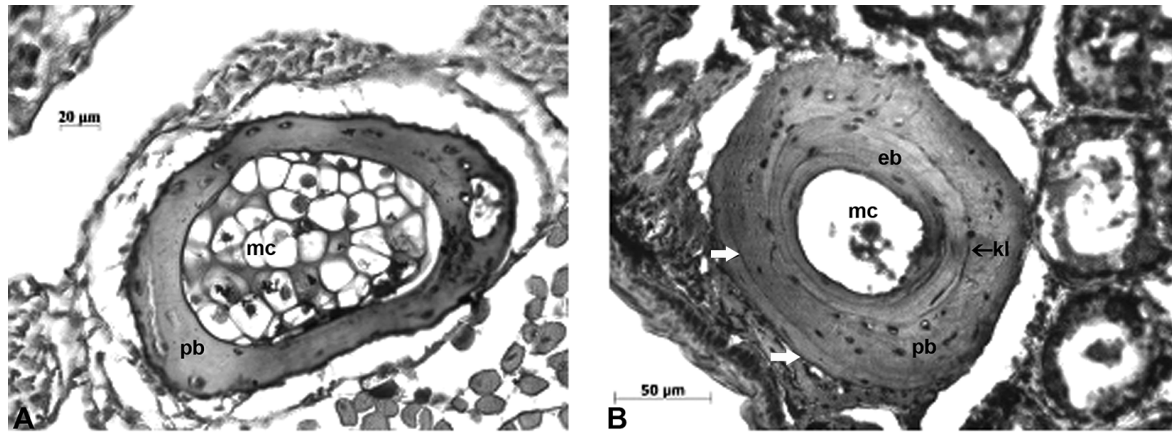


FIGURE 1. Examples of phalangeal cross-sections (10 μ m thick) of *Hypsiboas cordobae* (mc = medullar cavity; pb = periosteal bone; eb = endosteal bone; kl = Kastschenko line). (A) Newly metamorphosed individual (SVL = 31.9) without lines of arrested growth (LAGs), and (B) male; SVL: 45.1 mm; 2 LAGs (white arrows).

RESULTS

Bone histology.—All individuals studied showed recognizable bone structures that allowed age determination. In newly metamorphosed individuals, sections had a large medullar cavity and no LAGs (Fig. 1A). In adults, well-defined LAGs were found in the periosteal bone (Fig. 1B). Endosteal resorption was present in 24 individuals (39% of the total) and well-defined Kastschenko lines were easily observed, but the resorption did not hamper age determination because the first LAG was never completely reabsorbed (Fig. 1B). In many cases, the outer most lines were closely adjacent, but at the insertion site of the phalangeal ligament, it was possible to discern the peripheral LAGs and to reliably count them. We observed double LAGs and false LAGs in two males and one female, and we account for these in age estimation.

Body length, weight and age.—Female SVL averaged $51.27 \pm$ (SD) 5.06 mm and was significantly larger than male SVL, which averaged 48.01 ± 4.9 mm ($F_{1,58} = 5.85$, $P = 0.018$). Average female weight (12.3 ± 2.53 g) was also significantly greater than in males (9.03 ± 2.44 g; $F_{1,58} = 24.81$, $P < 0.001$). Mean age was not significantly different between adult males and females ($H = 1.32$, $P = 0.205$; Table 1).

Life-history traits of *H. cordobae* showed little variation between sexes (Table 2). We found no sexual differences in longevity. Modal age was similar between sexes; 3 y in both males (58.9%) and females (47.3%). Potential reproductive lifespan was similar between sexes. Minimum age at sexual maturity was close to 2 y in both sexes, and minimum SVL at sexual maturity was 38.9 mm in males and 41.35 mm in females (Fig. 2). Mean SVL at sexual maturity was slightly larger in females than in males, but this difference was not statistically significant ($H = 2.82$, $P = 0.092$); whereas, weight at sexual maturity was significantly greater in females than in males ($H = 4.53$, $P = 0.044$).

Sexual size dimorphism.—SVL and BM were significantly and positively correlated with age in both sexes (SVL males: $n = 39$, $r = 0.36$, $P \leq 0.05$; SVL females: $n = 21$, $r = 0.78$, $P \leq 0.05$; BM males: $n = 39$, $r = 0.39$, $P \leq 0.05$; BM females: $n = 21$, $r = 0.68$, $P \leq 0.05$). When the effect of age was controlled, SVL differed significantly between sexes ($F_{1,57} = 25.41$, $P < 0.001$) as did BM ($F_{1,57} = 26.07$, $P < 0.001$). However, in both sexes, variation in SVL and BM at comparable ages were large; consequently, we observed large overlaps between body size and weight of individuals from different age classes. The sexual dimorphism index (SDI) in body size was 0.0679 for SVL and 0.3621 for

TABLE 1. Snout-vent length (SVL), weight, and age of *Hypsiboas cordobae* collected south of the Sierra de Comechingones, Córdoba, Argentina. Mean values \pm SE and ranges (in parentheses) are given.

Parameters	Tadpoles (n = 10)	Juvenile (n = 1)	Males (n = 39)	Females (n = 21)
SVL (mm)	28.94 ± 1.93 (25.8–31.9)	35.1	48.01 ± 4.99 (38–56.26)	51.27 ± 5.06 (41.15–58)
Weight (g)	1.91 ± 0.32 (1.38–2.35)	4.01	9.03 ± 2.44 (4.5–15.1)	12.3 ± 2.53 (8.2–17.4)
Age (years)	0.4	1	3.18 ± 0.64 (2–5)	3.47 ± 0.87 (2–5)

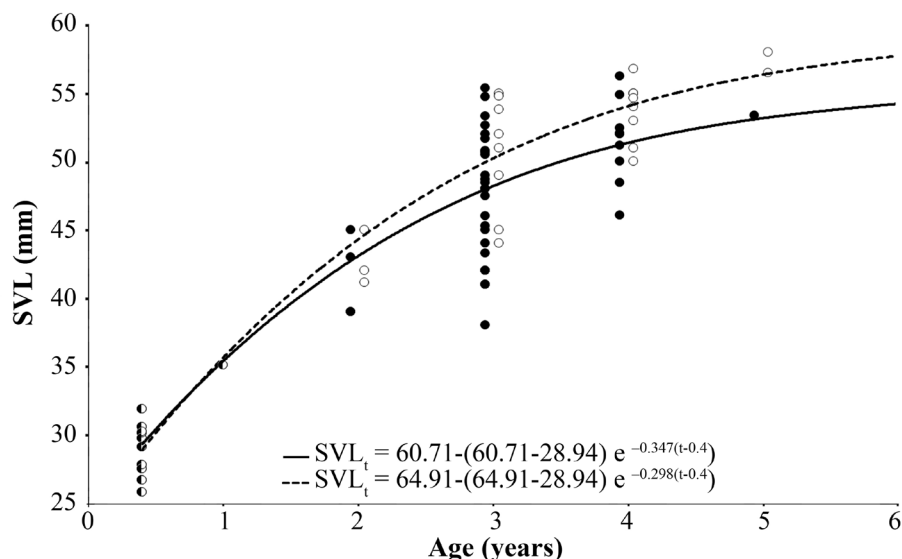


FIGURE 2. Relationship between body length (SVL) and age classes in *Hypsiboas cordobae*. Lines represent the von Bertalanffy’s growth model; males: solid line; females: dotted line.

BM. Variation in SDI (age class/SDI) was: 2/0.0293, 3/0.0634, 4/0.0427, 5/0.0721 for SVL, and 2/0.3136, 3/0.3059, 4/0.4294, 5/0.0357 for BM. Females had larger average SVL and BM than males in all age classes.

The von Bertalanffy growth model adequately fitted the age/body-length data with $r^2 = 0.832$ in males and $r^2 = 0.939$ in females. Growth models showed a similar shape in males and females (Fig. 2), but the growth coefficient was slightly higher in males than in females ($K \pm CI$, males: 0.347 ± 0.093 ; females: 0.298 ± 0.078). We observed a decrease in growth rate for both sexes from the 2nd to the 3rd year, which follows sexual maturity. The estimated asymptotic SVL for both sexes (males: 60.71 ± 4.64 mm; females: 64.91 ± 5.81 mm) was larger than the maximum SVL recorded in this study.

DISCUSSION

This is the first study to use skeletochronology to estimate the age and demographic parameters to corroborate SSD in *H. cordobae*. Formation of LAGs is genetically controlled based on a circannual rhythm (Marangoni et al. 2012; Sinsch 2015). The formation of bone growth layers of *H. cordobae* may be associated with the marked seasonality of the temperate region. In these regions, the length of the cold period has been

identified as one of the most important variables in differentiating growth patterns (Caetano and Castanet 1993). The validity of these patterns has been confirmed in several species: European Common Toads (*Bufo bufo*; Hemelaar 1981), Natterjack Toad (*Bufo calamita*; Sinsch et al. 2007), European Tree Frog (*Hyla arborea*; Ozdemir et al. 2012), and South American Common Toads (*Rhinella arenarum*; Bionda et al. 2015).

The histo-morphological structure of the diaphysis of *H. cordobae* phalange was relatively uniform, with a parallel-fibered bone matrix. Stained lines of arrested growth (LAGs) were clearly visible between zones of thicker layers of bone deposited during growth periods (Miaud et al. 2007). Several studies have demonstrated that bone resorption never, or only partially, reaches the innermost LAG (Sinsch et al. 2001; Iturra-Cid et al. 2010). These observations are consistent with our observations of Kastschenko lines in the bones of *H. cordobae*. We occasionally observed double and false lines, but they were unclear or incomplete and distinguishable from the LAGs. The age of individuals was more accurately estimated by considering bone resorption in cross sections of phalanges and detection of false LAGs.

We found a positive significant correlation between body size (SVL-BM) and age for both sexes. This observation, explained by the indeterminate growth

TABLE 2. Demographic life history traits of *Hypsiboas cordobae* collected south of the Sierra de Comechingones, Córdoba, Argentina. Mean \pm SE and ranges (in parentheses) are given. Abbreviations are n = sample size, A = age mode (frequency); AM = age at sexual maturity (years); L = longevity (years); PRLS = potential reproductive lifespan (years).

Sex	n	A	AM	L	PRLS	SVL at AM	Weight (g) at AM
Male	39	3 (58.9%)	2	5	3	41.5 ± 2.8 (38.9–44.6)	6.8 ± 1.71 (5.1–8.9)
Female	21	3 (47.3%)	2	5	3	43.42 ± 1.9 (41.35–45)	8.9 ± 0.81 (8–9.6)

typical of ectothermic animals, confirms the results of multiple studies (Kyriakopoulou-Sklavounou and Grumiro 2002; Özdemir et al. 2012; Cajade et al. 2013). The results of our study confirmed sexual size dimorphism on both SVL and BM for *H. cordobae*, with females being, on average, larger and heavier than males in all age classes, in concordance with other hylid species (Özdemir et al. 2012; Cajade et al. 2013). Many amphibians display sexual size dimorphism (Shine 1979; Monnet and Cherry 2002; Li et al. 2010), mostly because natural selection favors large females when female body length is positively correlated with fecundity (Gibbons and McCarthy 1986; Shine 1988; Liao et al. 2015). A positive correlation between body length and fecundity has been well documented in anurans (Davies and Halliday 1977; Liao et al. 2015), presumably because females need more stored energy to increase egg production, either to produce larger eggs, or to lay more eggs (Shine 1979; Halliday and Verrell 1988).

In many amphibian species, males reach sexual maturity one year earlier than females (Kyriakopoulou-Sklavounou and Grumiro 2002; Liao et al. 2010). However, age at sexual maturity was estimated at two years for both males and females *H. cordobae*. In this species, SVL did not differ significantly between sexes at sexual maturity, although the SVL in older individuals was significantly different between sexes, with females being larger. Therefore, greater length in adult females does not appear to be caused by either different growth rates in pre-reproductive age or to delayed sexual maturity in females, both patterns observed in several species of frogs (Liao and Lu 2010; Liao and Lu 2012).

Differential mortality between sexes has been reported for frogs, in which males have higher mortality than females possibly for being more active during the breeding season (Shirose et al. 1993; Kyriakopoulou-Sklavounou et al. 2008). However, in *H. cordobae*, both longevity and age structure were similar between sexes. Our results indicate that females are not larger than males when they reach sexual maturity, but that females grow larger than males after they are sexually mature, when more resources are allocated to reproduction (Halliday and Verrell 1988). Therefore, a reproductive behavior that is energetically costly for older males (Woolbright 1989) and females devoting more energy to somatic growth, could produce the sexual size dimorphism in *H. cordobae*.

Our von Bertalanffy growth models showed few differences in growth rates of immature *H. cordobae*, whereas, the growth curves distinctly diverge between sexes from three years of age onwards. Sexual size dimorphism may depend on the relative importance of age and growth rate parameters in the sexes (Özdemir et al. 2012; Liao et al. 2013). In *H. cordobae*, although

females showed a slow arrival at the estimated maximum SVL (as indicated by the lower K) they exhibited a greater average and post-maturity growth rate. Therefore, growth rate and growth duration were the main contributors to sexual size dimorphism.

In conclusion, age assessment and subsequent modeling of data using the von Bertalanffy growth model allowed us to determine SSD in *H. cordobae*, with females being larger and heavier than males. Sexual dimorphism in body size and shape are apparently related to continuous growth of females after sexual maturity; thus, females reach larger body sizes and increase their reproductive output and clutch size. Our results indicate the importance of age determination for morphometric studies involving intersex comparisons. In addition, our study provides baseline data on the demography of *H. cordobae* for use in future conservation studies, because it can be used to estimate basic ecological parameters of a population.

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