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Coping with Aridity: Life History of *Chacophrys pierottii*, a Fossorial Anuran of Gran Chaco

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Abstract. We studied size, age, and growth in *Chacophrys pierottii,* a fossorial amphibian inhabiting the arid region of Gran Chaco, in northern Argentina. We provide the first detailed demographic data for this cryptic species using skeletochronology. We observed female-biased sexual size dimorphism but no differences in age parameters between the two sexes. *Chacophrys pierottii* has a short life span of up to 5 a, both males and females reaching sexual maturity after 1 a. Our results suggest that the rapid growth to maturity followed by a relatively short reproductive lifespan is a response to the constraints posed by arid environments. The life history parameters in the studied population of *C. pierottii* are in line with the observed patterns of other anurans inhabiting xeric habitats. Our study provides conservation managers with basic data required for the accurate assessment of the species status and the adoption of proper conservation strategies.

Keywords. Age; Amphibia; Ceratophryidae; Sexual dimorphism; Skeletochronology.

INTRODUCTION

The Neotropics have a high amphibian species diversity, housing almost half the known species (Bolaños et al., 2008), but our knowledge of this group is at best mediocre (Duellman, 1999). Amphibians are the most threatened group of terrestrial vertebrates (Baillie et al., 2010; Blaustein et al., 2010; Hof et al., 2011), with 39% of the species in the Neotropics under threat, representing 60% of all globally threatened amphibians (Bolaños et al., 2008). Life history traits, like body size, mean lifespan, longevity, and age at first reproduction are among the most important parameters for the study of amphibian populations (Allen et al., 2017; Campbell et al., 2017) and a prerequisite for designing effective conservation strategies (Biek et al., 2002; Vukov et al., 2015; Greenberg et al., 2017). Because age- and size-related parameters determine the fitness of individuals, they are under strong selective pressure (Stearns, 1992) and, as such, are finely tuned to environmental conditions (Caruso et al., 2014; Cayuela et al., 2016; Liao et al., 2016). In most amphibian species, obtaining data on body size is usually straightforward, but the reliable estimation of age by means of capture-mark-recapture techniques is extremely labour intensive (Sinsch, 2015). As an alternative, skeletochronology is frequently used for age estimation in amphibians and reptiles (Sinsch, 2015) and has recently been used increasingly in tropical species (Sinsch and Dehling, 2017). In addition to providing reliable age estimates, skeletochronology has the advantage of not requiring euthanasia of the studied animals, since a single phalanx is sufficient (Castanet and Smirina, 1990).

Amphibians are particularly sensitive to both high temperatures and low humidity (Navas et al., 2008), so in order to colonize and survive in arid habitats, a fossorial life-style has evolved several times in different anuran families (Jorgensen and Reilly, 2013). Amphibians inhabiting arid habitats are under higher threat due to climate-change induced aridization and increasing temperatures (Duarte et al., 2012), so knowledge of their life history adaptations is required for adequate conservation measures.

Chacophrys pierottii (Vellard, 1948), the *"escuercito chaqueño"* or "Chaco horned frog," is a poorly known fossorial anuran of the family Ceratophryidae distributed across the arid and semi-arid sub-regions of the Gran Chaco ecoregion (northern Argentina), western Paraguay, and southern Bolivia (Frost, 2016). The Gran Chaco (Olson et al., 2001) is the second-largest ecoregion in South America, after Amazonia, and includes the largest seasonally dry forests in the Neotropics (Bucher, 1982). This environment is characterized by a strongly seasonal rainfall regime, with more than 80% of precipitation concentrated between October and April (Bucher, 1980) when the highest anuran activity occurs (Perotti, 1997).

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One of the highest rates of global deforestation occurs in the Gran Chaco (Hoyos et al., 2013), comparable to Amazonia (Hansen et al., 2013), to generate land suitable for monoculture (mainly soy) or dedicated pastures for livestock, thus causing the loss of a large number of species (Nori et al., 2016). Since *Chacophrys pierottii* leads a secretive, nocturnal, fossorial lifestyle with short, explosive reproductive events in ephemeral ponds (Bucher, 1982), little is known about its natural history. The information currently available is limited to larval stages (e.g., Faivovich and Carrizo 1992; Wild, 1999; Quinzio et al., 2006; Fabrezi, 2011; Fabrezi et al., 2016), diet and foraging (Pueta and Perotti, 2013; Schalk et al., 2014), occasional predation events (Pereyra and Akmentins, 2013), male vocalization (Lescano, 2011), geographic distribution (e.g., Rosset, 2001; Sanabria et al., 2012), and phylogeny (e.g., Fabrezi and Quinzio, 2008; Faivovich et al., 2014; Fabrezi et al., 2016). The goal of this study was to provide fundamental demographic data on body size- and age-related parameters in adult *C. pierottii* in order to better understand the life history adaptations that allow amphibians to cope with arid environments, and thus support their conservation.

MATERIALS AND METHODS

We performed nocturnal field surveys on 6–8 December 2013 on a 10 km dirt road between the localities Fuerte Esperanza and Misión Nueva Pompeya, Chaco province, Argentina (Ruta Provincial 61, between 24°56′27.99″S, 61°29′26.69″W and 25°1′41.53″S, 61°31′25.55″W; 150 m above sea level), following a heavy storm. A more detailed description of the study area was presented in Stănescu et al. (2016). We captured a total of 26 *Chacophrys pierottii* adult individuals (7 males and 19 females) on the road, between 21:00–01:00 h, during a reproductive migration event towards the temporary ponds formed on the sides of the road. Males were recognized by the presence of either an enlarged vocal sac or vocal slits in the floor of the mouth. For each individual, we measured snout–vent length (SVL) and head width (HW) using a digital caliper (0.1 mm precision) and body mass (BM) using an electronic scale (0.01 g precision). We clipped the third digit of the right forearm and preserved it individually in 70% ethanol for age determination by skeletochronology. Of the 26 individuals captured, 19 were preserved and deposited in the collection of Instituto de Biología Subtropical (Universidad Nacional de Misiones, Consejo Nacional de Investigaciones Científicas y Técnicas; LGE) under voucher numbers LGE 7709–7727, while the remaining 7 were released at the capture site. We assessed sexual size dimorphism (SSD) for each body measurement since it is a key aspect in our understanding of the evolution of life history traits and mating systems (Kupfer, 2007). We computed the sexual dimorphism index (SDI) following Lovich and Gibbons (1992): SDI = mean $size_{larger\ sex}$/mean $size_{smaller\ sex}$, with the result arbitrarily defined as positive when females were larger than males, and negative when males were larger than females.

Skeletochronology is a non-lethal, widely-used method to estimate age in amphibians by counting the number of lines of arrested growth (LAGs) in cross sections of phalanges (Sinsch et al., 2015). The growth periods appear as broad bands of tissue separated by narrower lines, or annuli, that mark periods of reduced growth (Halliday and Verrel, 1988). We followed the standard methods in skeletochronology (e.g., Smirina, 1972; Castanet and Smirina, 1990), with minor modifications proposed by Marangoni (2006). Clipped digits were washed in water for 30 min, decalcified in 5% nitric acid for 1–3 h, dehydrated, paraffin-embedded, sectioned using a rotation microtome (Arcano RMT-30) at 14–16 μm, and stained with Harris haematoxylin. For each studied individual we selected 5–10 cross-sections with the smallest diameter of the medullar cavity and the thickest periosteal bone between the line of medullar cavity and the outer line of periosteum (Rozenblut and Ogielska, 2005) and mounted them on microscope slides with a 50% glycerin and 50% alcohol solution. Afterwards, we took digital images of these cross-sections using a high-resolution camera (Sony SSC-DC50AP) attached to a microscope (Olympus BX50) and the two best digital images per individual were selected for further analysis. Cross-sections were observed and measured using Image-Pro Plus version 4.5 (Media Cybernetics 1993–2001), and calibrated using a standard ocular micrometre. Two independent observers (FM and AC) recorded the presence/absence of the line of metamorphosis (LM) and counted the LAGs. LM is a fine line within the first-year growth zone, separating larval tissue (usually stained darker) from the post-metamorphic bone, as indicated by Rozenblut and Ogielska (2005) and Sinsch and Dehling (2017). The presence of LM indicates that no resorption occurred. In those individuals with no remnant LM, we estimated the degree of resorption by osteometrical analysis following Sagor et al. (1998). Thus, we computed a frequency distribution with the diameters of the innermost and second LAGs and, when the diameter of the innermost LAGs exceeded the group mean by 2 SD or more, we considered that the first LAG was eroded. We distinguished annual growth marks (i.e., LAGs sensu stricto) from non-annual ones (i.e., irregular interruptions during short periods of inactivity) using the method described by Sinsch et al. (2007). Annuli (sensu Peabody, 1958) were easily distinguished from actual LAGs because they always stained more weakly than true LAGs and were often broader, as previously described by Leclair et al. (2005) and Sinsch et al. (2007) in temperate species.

We computed the following age-related parameters: mean lifespan (i.e., mean of age distribution), age of sexu-

Table 1. Descriptive statistics (mean ± SD) of the variables measured: body mass (BM), snout-vent length (SVL), head width (HW), and bone diameter (BD), of male and female *Chacophrys pierottii* from the Arid Chaco of Argentina. The differences between males and females were compared using ANOVA, *P* values indicated. The sexual dimorphism index (SDI) was computed according to Lovich and Gibbons (1992); *n* = sample size.

Size variables measured	Females (*n* = 19)	Males (*n* = 7)	ANOVA	SDI
Body mass (g)	26.37 ± 7.18	16.29 ± 2.15	$P < 0.01$	1.62
	12.33–41.26	14.16–19.47		
Snout-vent length (mm)	59.14 ± 4.16	51.44 ± 2.33	$P < 0.001$	1.15
	48.96–65.57	47.89–55.07		
Head width (mm)	24.04 ± 1.32	20.67 ± 1.44	$P < 0.0001$	1.16
	21.94–26.40	19.16–23.11		
Bone diameter (µm)	280.88 ± 48.88	251.03 ± 66.44	$P < 0.01$	1.12
	211.44–380.86	163.41–364.74		

al maturity (i.e., minimum age), longevity (i.e., maximum age) and potential reproductive lifespan (i.e., the difference between maximum and minimum age). We considered the distance between two LAGs to be an indicator of individual growth at a given age, and a pattern of decreasing intervals between LAGs after a few years is thought to indicate the onset of sexual maturity, with resources being reallocated from growth to reproduction (Smirina, 1994). Therefore, we additionally inferred the age of sexual maturity by observing the bone growth pattern in the cross-sections. We measured the longest and shortest perpendicular axes of bone diameter (BD) to the nearest µm from two diaphyseal sections per individual, following the method of Hemelaar (1985). We determined the average diameter of the diaphysis by computing the square root of the product of the two axis measurements.

We first tested the data for normality and homoscedasticity using Shapiro-Wilk and Levene tests respectively and then chose statistical tests accordingly. We used a significance level of $\alpha = 0.05$. All tests were performed using the statistical package Statistica 6.0 (Statsoft Inc., 2001). We computed von Bertalanffy's growth model (Bertalanffy, 1938) following Beverton and Holt (1957): $SVL_t = SVL_{max} \times (1\text{-}e^{-k} \times {}^{(t-t_0)})$, where SVL_t is the expected or average SVL at time (or age) t, SVL_{max} is the asymptotic average SVL, k is the growth rate coefficient and t_0 is the time or age when the average SVL was zero. We considered SVL at metamorphosis to be 35 mm from Quinzio et al. (2006). We fitted von Bertalanffy growth model by nonlinear least squares regression. This analysis was performed in R version 3.0.3 (R Core Team, 2014) with the packages FSA (Ogle, 2016) and nlstools (Baty et al., 2015).

RESULTS

Males and females showed significant differences regarding SVL, BM, HW and BD (MANOVA Wilk's $\lambda = 0.236$, $F_{4,21} = 8.924$, $P < 0.01$; Table 1). Univariate ANOVAs showed that females were significantly larger than males in all measured body size parameters, and SDI showed the highest value for body mass (Table 1). All processed samples corresponding to 26 individuals (7 males and 19 females) showed well-defined LAGs allowing for age determination (Fig. 1). The line of metamorphosis was visible in 90% ($n = 24$) of the samples. Endosteal resorption was observed only in four cases but did not prevent age estimation. Females were younger than males, but no significant difference in mean lifespan was found between sexes (ANOVA, $F_{1,24} = 1.751$, $P = 0.198$; Table 2). The minimum age observed in the sample was 2 a in females and 3 a in males, while longevity was 5 a in both sexes (Fig. 1). We successfully inferred the age of sexual maturity from the bone growth pattern in 65% of the individuals (12 females, 5 males). Thus, the growth pattern indicated that sexual maturity was attained after the first year of life in both males in females. Based on the minimum age observed in the sample, the potential reproductive lifespan was three years in females and two years in males, but according to the growth pattern observed in the sections it was four years in both sexes. The relation between age and SVL fitted von Bertalanffy's growth model only in females (SVL_{max} = 62.15 mm ± 3.38 SE, CI 95% = 54.98–69.33; k = 0.57 ± 0.26 SE, CI 95% = 0.02–1.14; Fig. 2). The predicted asymptotic average SVL (SVL_{max}) was slightly larger than the measured average values but smaller than the SVL of the largest female. The model could not be applied in males due to the small sample size.

Table 2. Age related parameters (reported in years) in the studied population of *Chacophrys pierottii*. PRL 1 = potential reproductive lifespan according to the minimum age observed in the samples; PRL 2 = potential reproductive lifespan according to the inferred age of sexual maturity from the bone growth pattern.

Age related parameters (a)	Females (*n* = 19)	Males (*n* = 7)
Mean age ± SD	3.74 ± 0.93	4.29 ± 0.95
Minimum age	2	3
Inferred age of sexual maturity	1	1
Maximum age	5	5
PRL 1	3	2
PRL 2	4	4

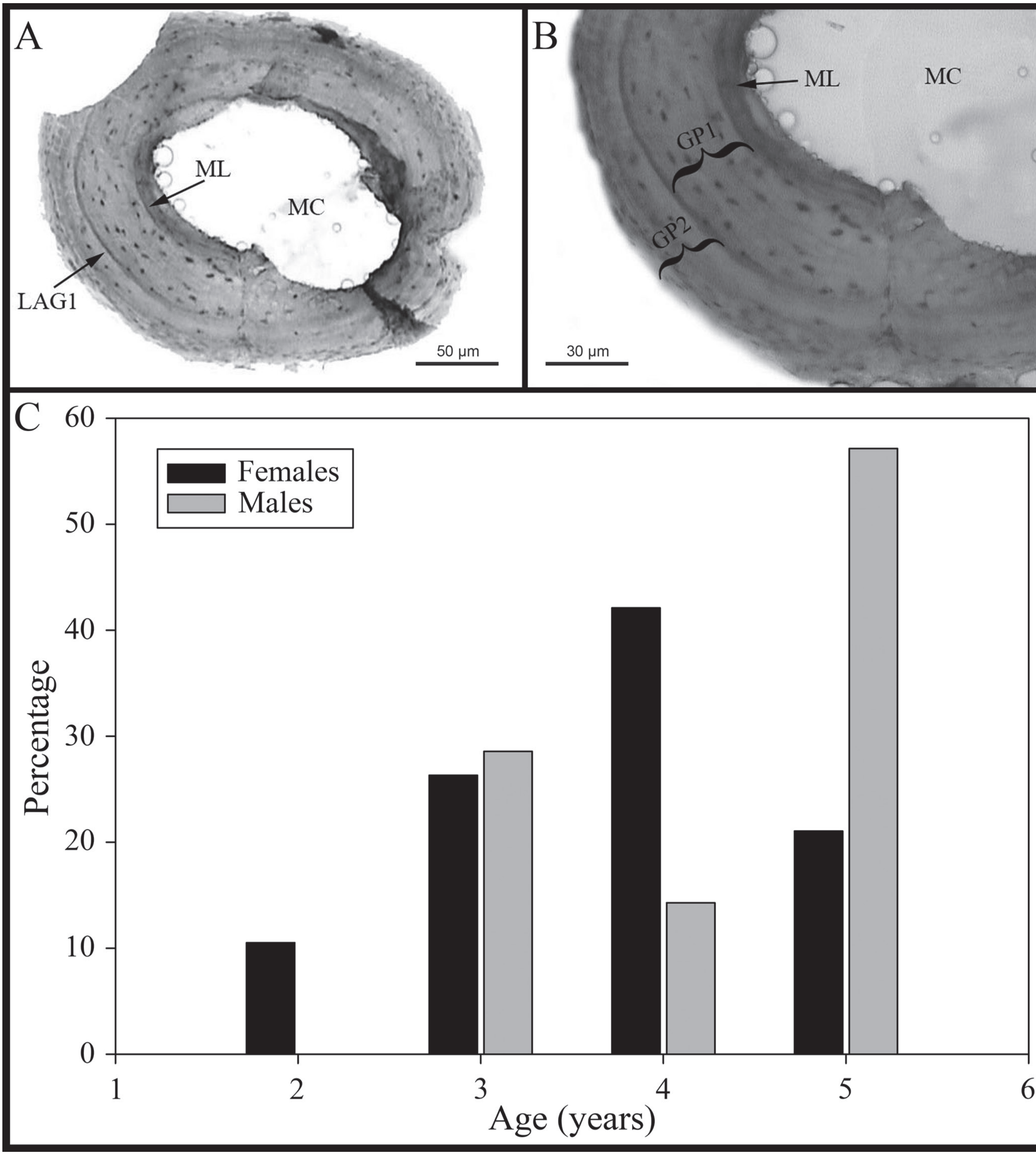

Figure 1. Cross-section through the phalanx diaphysis and age distribution of *Chacophrys pierottii*. **(A)** One line of arrested growth (LAG1) is visible in the periosteal bone (indicated by arrow), the medullar cavity (MC), metamorphosis line (ML). **(B)** Two distinct growth periods (GP) are visible, one between metamorphosis and the first LAG (GP1), and another between the first period of arrested growth and the moment of capture (GP2). **(C)** Age distribution in the studied population of *C. pierottii* from Arid Chaco, Argentina.

DISCUSSION

Our study provides the first demographic data including size and age-related parameters in adult *Chacophrys pierottii*. We found a strong sexual size dimorphism, females being significantly larger than males. This is the most common sexual dimorphism among amphibians, being reported in about 90% of anuran species (Shine,

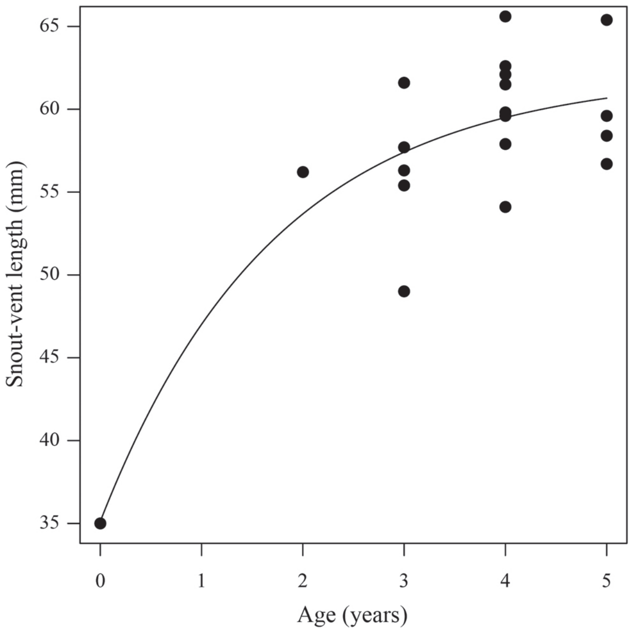

Figure 2. Growth model (Bertalanffy, 1938) of *Chacophrys pierottii* females (n = 19), considering snout–vent length and time to reach that length (age in years).

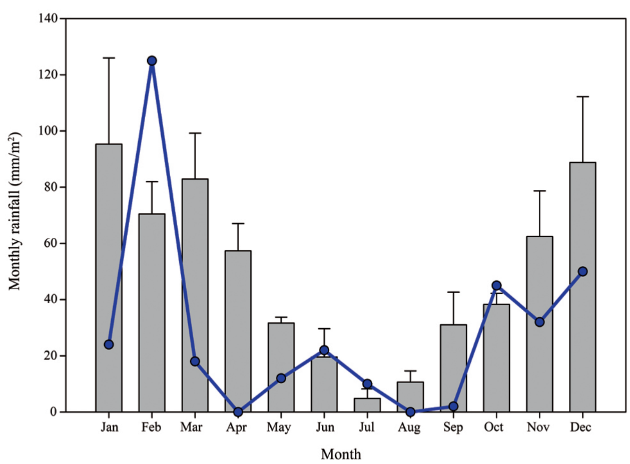

Figure 3. Average monthly rainfall 1967–2016 (grey bars) versus monthly rainfall in 2013 (blue line with dots) recorded at "Estación Experimental Agropecuaria Ingeniero Juárez," Instituto Nacional de Tecnología Agropecuaria, Argentina.

1979; Kupfer, 2007). There are several proposed causal factors (Halliday and Tejedo, 1995; Monnet and Cherry, 2002), such as the capacity of larger females to produce larger clutches and larger eggs (Crump, 1974; Kuramoto, 1978), restrictions on the growth of males because of the energy demand linked to reproductive activity (e.g., demands of acoustic advertisement, parental care and/or not feed while calling; Woolbright, 1989), and sex-specific differences in mortality rate caused by differential predation pressure between sexes (Howard, 1981).

The presence of well-expressed growth marks in the bone tissue of *Chacophrys pierottii,* which is subjected to a highly seasonal subtropical climate in the Arid Chaco, supports the hypothesis that LAG formation has a general intrinsic (i.e., genetic) control which is synchronized with, and reinforced by the natural seasonal cycles (e.g., Sinsch et al., 2007; Beşer et al., 2017; Kumbar and Lad, 2017; López et al., 2017; Sinsch and Dehling, 2017). The presence of LAGs in this species was previously reported by Fabrezi and Quinzio (2008) in two males. We successfully applied the skeletochronological method for the first time in this enigmatic species at the population level, which allowed us to provide new insights into its life history. Thus, we found that *C. pierottii* from the Arid Chaco are relatively short-lived (i.e., 5 a), females and males having a maximum of four reproductive events during adulthood. The age of sexual maturity inferred from the bone growth pattern indicates that both males and females are able to breed after their first year of life. However, the youngest males and females in our sample were respectively three and two years old. An explanation for the lack of one-year-old individuals in our sample, apart from the small sample size in males, could be that individuals might skip breeding during extremely arid years when resources are scarce, as shown by Muths et al. (2010, 2013) in temperate toads inhabiting harsh environments with limited resources and short growth periods. On the other hand, lack of reproductive success (i.e., failure to metamorphose and/or mass mortality of juveniles) during the previous reproductive period, could have led to the same result. Both hypotheses are supported by rainfall data obtained from "Estación Experimental Agropecuaria Ingeniero Juárez", Instituto Nacional de Tecnología Agropecuaria (INTA), located about 70 km north from our study site. The data showed that annual rainfall was only 340 mm/a in 2013, compared to 810 mm/a in 2012; the average annual rainfall in this area during the last 43 a (1967–2016) was 641 mm/a (min–max: 340–1,010 mm/a), being 2013 the most arid year during this given period of time (Fig. 3). The total rainfall during the activity season corresponding to October–December 2012 and January–April 2013, when the 2013 cohort of juveniles should have hatched and metamorphosed, was only 310 mm compared to 625.5 mm during the previous activity season, October–December 2011 and January–April 2012.

Similar life history patterns, with rapidly attained sexual maturity and short lifespan, were reported for other anurans inhabiting xeric environments which impose harsh constraints in terms of limited water availability and short breeding and growth seasons: *Sclerophrys pentoni* (Anderson, 1893) (Francillon et al., 1984), *Pelophylax saharicus* (Boulenger in Hartert, 1913) (Esteban et al., 1999; Bellakhal et al., 2008), *Incilius alvarius* (Girard in Baird, 1859), *Anaxyrus cognatus* (Say in James, 1823), *Anaxyrus punctatus* (Baird and Girard, 1852), and *Scaphiopus couchii* Baird, 1854 (Sullivan and Fernandez, 1999), *Mantella expectata* Busse and Böhme, 1992 and *Scaphiophryne gottle-*

bei Busse and Böhme, 1992 (Guarino et al., 2010), *Dermatonotus muelleri* (Boettger, 1885) (Stănescu et al., 2016), and *Ceratophrys stolzmanni* Steindachner, 1882 (Székely et al., 2018). Previous studies emphasize additional adaptations to xeric environments in *Chacophrys pierottii:* rapid larval development of 15–18 days and accelerated growth rates to metamorphosis, similar to *Lepidobatrachus laevis* Budgett, 1899 and *L. llanensis* Reig and Cei, 1963, other two inhabitants of the Arid Chaco (Fabrezi and Quinzio, 2008; Fabrezi, 2011; Zeng et al., 2014), or to *Ceratophrys stolzmanni,* an inhabitant of the Pacific dry forests of Ecuador (Székely et al., 2017). Quinzio et al. (2006) reported an average SVL at metamorphosis of 35 mm at the end of January, while Pueta and Perotti (2013) found that juveniles had an average SVL of 37.1 mm in February; according to these independent observations, an average growth rate of 2 mm/month can be inferred during the activity season in juvenile individuals. Thus, provided that a minimum average growth rate of 2 mm/month can be maintained during the activity season following metamorphosis (i.e., March–April and October–December), juveniles should be able to attain a minimum average SVL of 47 and 49 mm in December and January, respectively, when breeding events were observed. According to the observed bone growth patterns in cross sections, most of the growth occurs during the first year of life, decreasing abruptly after this age, when presumably sexual maturity occurs. This is consistent with the minimum SVL of sexually mature individuals in our sample—47.9 mm.

Although *Chacophrys pierottii* is categorized as "Least Concern" according to IUCN (Aquino et al., 2004) and "Not Threatened" according to the categorization of Argentinean amphibian fauna (Vaira et al., 2012), the species is threatened by the illegal pet-trade, being collected during explosive breeding events (Aquino et al., 2004), and habitat destruction, which is mainly caused by deforestation (Aquino et al., 2004; Hoyos et al., 2013). The short reproductive lifespan of the species has important conservation implications, because persistent droughts with low recruitment over consecutive years would endanger the survival of whole populations (Marsh and Trenham, 2001). A similar situation was reported for another Neotropical fossorial species, *Ceratophrys stolzmanni,* which inhabits seasonally dry forests in Ecuador (Székely et al., 2018). Thus, our study provides much-needed basic data that will support more accurate assessments regarding the conservation status of this species and implicitly, appropriate conservation strategies.

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