



Life history traits of a Neotropical microhylid (*Dermatonotus muelleri*, Boettger 1885) from the Arid Chaco, Argentina

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We explored age-related parameters and growth patterns in a population of *Dermatonotus muelleri* inhabiting the Arid Chaco, at the southern limit of the species' distribution range using skeletochronology. In addition, we studied sexual size dimorphism and female reproductive investment. Males and females attained sexual maturity at a similar age (2 years) and both had a low reproductive lifespan (3 years). Females were significantly larger than males and had a higher reproductive investment compared to other anurans. The growth rate coefficient (k) was similar in males (1.14) and females (1.07), indicating that the sexual size dimorphism in this species is caused by differentiated growth before sexual maturity. Overall, we suggest that the short reproductive lifespan justifies the high reproductive investment in the studied population.

Key words: age, explosive breeder, growth, reproductive investment, sexual size dimorphism

INTRODUCTION

Amphibians are suffering a severe worldwide decline, with nearly one-third (32.5%) of the species threatened (Stuart et al., 2004), mostly due to habitat loss and fragmentation (Gibbons et al., 2000; Cushman, 2006; Gardner et al., 2007). Almost half of all known amphibian species occur in the Neotropical realm, of which 38% are considered to be globally threatened (Bolaños et al., 2008). While the Neotropics is considered the global epicentre of catastrophic decline for amphibians, there is still a high rate of species descriptions from this area, which tends only to add new species to those already under severe decline. Sound conservation measures in this region are hampered by the limited knowledge on the life history and habitat requirements of the species of concern.

Demographic life-history traits are essential for understanding population dynamics and plasticity in response to environmental variability (Caswell, 1983). Age-related parameters (e.g., age at first reproduction, reproductive lifespan), fecundity and reproductive effort are some of the major life-history traits tackled by the life history theory (Stearns, 2000) and they are important components of anuran reproductive strategies (Duellman & Trueb, 1994). Skeletochronology, along with capture-mark-recapture studies, has generally proved to be a useful non-lethal method to estimate age and growth-related parameters in amphibians (Halliday

& Verrell, 1988; Smirina, 1994; Sinsch, 2015). Age is estimated from bone crosscuts, based on the presence of lines of arrested growth (LAGs), cyclic and annular bone growth mainly caused by the seasonal variations in temperature and/or rainfall (Smirina, 1994; Sinsch et al., 2007). Although most of the skeletochronological studies focused on amphibians from temperate areas, there is an increasing interest towards amphibians from tropical and subtropical regions, where growth is subjected to periodicity through wet/dry seasons (e.g., Guarino, et al., 1998; Khonsue et al., 2000; Kumbar & Pancharatna, 2001; Kumbar et al., 2002; Lai et al., 2005; Lindquist et al., 2012; Cajade et al., 2013). Furthermore, there is evidence that well-expressed growth marks may occur in tropical and subtropical amphibians in which growth is not constrained by environmental conditions, supporting the hypothesis that LAG formation is ultimately caused by a general genetic control (e.g., Castanet et al., 1993; Marangoni et al., 2009; 2012).

The Great Chaco ecoregion, the second largest in South America after Amazonia, includes the largest seasonally dry forests on the continent (Bucher, 1982). It is mainly a wooded region, strongly affected by extensive livestock raising, extractive forestry and poorly planned agricultural expansion (Nature Conservancy et al., 2005; Nori et al., 2013). Deforestation rates in this region are among the highest in the world (Hoyos, 2013), and Chaco is considered one of the highest diversity regions for herpetofauna in Argentina (Vaira et al., 2012).

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Dermatonotus muelleri is a neotropical microhylid widely distributed in central and southern Chaco, from Brazil (Maranhão to São Paulo) through south-eastern Bolivia, north-western Paraguay and northern Argentina, with natural populations threatened by habitat destruction and pet trade in Argentina and Paraguay (Colli et al., 2004). It is a nocturnal anuran, highly specialised to burrowing and preying on termites; it aestivates in subterranean chambers and emerges during the wet season, to feed and reproduce (Nomura et al., 2009; Nomura & Rossa-Feres, 2011). Although breeding is explosive and several reproductive events may occur during the wet season, females mate only once, while males are polygynous (Nomura, 2003). Larval development is fast and metamorphosis is completed within 22–26 days from egg deposition (Fabrezi et al., 2012).

The aim of our study was to explore life history traits of *D. muelleri*. To the best of our knowledge, the present study is the first to investigate age and growth traits in this species (see however Marangoni et al., 2009). We determined (i) age structure parameters, (ii) growth patterns, (iii) sexual size dimorphism and (iv) female reproductive investment in a population at the southern limit of the species' distribution range.

MATERIALS AND METHODS

Study area and sampling

The study area is located within the dry forests (Arid Chaco) of the western Great Chaco ecoregion, between the localities Fuerte Esperanza and Misión Nueva Pompeya (Chaco Province), northern Argentina. Natural habitats are represented by xerophytic forests of *Schinopsis quebracho-colorado*, associated with *Aspidosperma quebracho-blanco* and *Prosopis spp.*, alternating with open grasslands and cacti, including tree-cacti (Cabrera & Willink, 1980). The climate is characterised by extreme seasonal temperature variations, ranging from 40°C in summer to occasional winter frost; annual mean temperatures range from 24 to 25.5°C. The precipitation regime is strongly seasonal, with over 80% of the rainfall concentrated between October and March, while the driest months are July and August (Bucher, 1980; Savaria-Toledo, 1993).

We performed nocturnal field surveys on a 10 km dirt-road segment (Ruta Provincial no. 61: 24°56'27.99"S, 61°29'26.69"W; 25° 1'41.53"S, 61°31'25.55"W; 150 m.a.s.l), between 6–8 December 2013, following a heavy storm. In addition to *D. muelleri*, we recorded the presence of the following 21 species in the study area: Bufonidae (1 sp.) - *Rhinella schneideri*; Ceratophryidae (4 sp.) - *Ceratophrys cranwelli*, *Chacophrys pierottii*, *Lepidobatrachus laevis*, *L. llanensis*; Hylidae (9 sp.) - *Dendropsophus nanus*, *D. sanborni*, *Hypsiboas raniceps*, *Phyllomedusa sauvagii*, *Pseudis platensis*, *Scinax acuminatus*, *S. fuscovarius*, *S. nasicus*, *Trachycephalus typhonius*; Leiuperidae (1 sp.) - *Physalaemus biligonigerus*; Leptodactylidae (5 sp.) - *Leptodactylus bufonius*, *L. fuscus*, *L. laticeps*, *L. latinasus*, *L. mystacinus*; Microhylidae (1 sp.) - *Elachistocleis bicolor*.

We sampled 43 adult individuals (25 males, 18 females) of *D. muelleri* from temporary ponds along the road, during one explosive reproductive event. We determined sex based on the presence of the nuptial pads and dark vocal sac in males, or egg masses that could be visualised through the skin of females. We measured snout-vent length (SVL) and head-width (HW) with digital callipers at 0.1 mm precision and body mass (BM) with a portable electronic balance at 0.01 g precision. We clipped and stored the longest toe of the right forearm in 70% alcohol for age assessment. Ten females and 11 males were preserved for genetics and morphological studies and deposited in the herpetological Collection of Instituto de Biología Subtropical (National Research Council CONICET and Universidad Nacional de Misiones), Posadas, Misiones province, under acronyms LGE 7688–7708. The remaining 22 individuals were released at the capture site. The sampling procedures complied with all relevant regulations and the necessary permits were obtained.

Skeletochronology

We followed the skeletochronological method of Castanet & Smirina (1990) with minor modifications. We decalcified the penultimate phalanges for 8 hours in 5% nitric acid, washed and kept them in distilled water overnight. We cut 14 µm thick cross-sections using a Tehsys3000CR cryotome, stained them for 20–30 minutes in Ehrlich's haematoxylin and then washed with distilled water for 1 hour. Sections with the smallest marrow cavity and the thickest cortical bone were permanently mounted on slides using Aquatex® (aqueous mounting agent for microscopy, Merk Milipore) and photographed using an Olympus® E-620 microscope-mounted camera (Olympus CX® 31 microscope with Quick Photo Micro v. 2.3 software). Three independent observers (FS, FM and DC) counted the lines of arrested growth (LAGs) in 2–3 sections per individual.

Reproductive investment

We determined the ovarian mass (OM) as the difference between the body mass before and after ovary removal, in nine of the preserved females (acronyms LGE 7689, 7691–7693, 7696–699, 7705). The ovarian complement (OC) represents the total number of mature ova from each gravid female and is considered a measure of their fertility or reproductive potential (Crump, 1974; Basso, 1990). We removed and weighed approximately 10% of each ovary and counted the mature ova under a Nikon C-DS magnifying glass. Mature ova had well-defined black and yellow poles and pronounced larger size, consistent with the post-vitellogenesis class (Crump, 1974). We photographed a random sample of about 200 ova from each ovary with a digital Nikon Coolpix S10 camera, mounted on a Nikon C-DS magnifying glass. We measured the longest and shortest perpendicular axes of 100 ova per sample to the nearest 0.01 mm using Image-Pro Plus v. 1.1 (Media Cybernetics, 1993–94). We determined mature ovum size (OS) by square rooting the product of the two axis measurements.

Data analysis

All statistical analyses were performed in R environment, v. 3.0.3 (R Core Team, 2014). We used the following packages: stats (R Core Team, 2014), car (Fox & Weisberg, 2011), psych (Revelle, 2014), FSA (Ogle, 2014) and nlstools (Baty et al., 2014). We tested all data for normality and homoscedasticity using Shapiro-Wilk and Levene tests and chose the statistic tests accordingly.

Age-related parameters

We computed the following age-related parameters: mean lifespan (i.e. mean of age distribution), longevity (i.e. maximum age) and potential reproductive lifespan (i.e. the time span between the moment of sexual maturity and the maximum age observed in the sample). Since growth rate decreases after reaching sexual maturity, it was possible to determine the age of maturation by observing this pattern in a given individual (Smirina, 1994).

We used a two-sample Kolmogorov-Smirnov and Mann-Whitney test to check for differences in the shape of age distribution and median age between males and females. We used Spearman correlations to analyse the associations between age and size parameters.

Growth patterns

We computed von Bertalanffy growth model (von Bertalanffy, 1938) following Beverton & Holt (1957): $SVL_t = SVL_{max} \times (1 - e^{-k \times (t - t_0)})$, 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 used measurements of SVL at metamorphosis and from 1-year juveniles (19.11 and 52.84 mm respectively) provided by Marangoni et al. (2009). We fitted a von Bertalanffy growth model and estimated growth parameters (VBGPs) by nonlinear least squares regression. Two estimated VBGPs were considered significantly different at the 0.95 level when their confidence intervals (CI 95%) did not overlap.

Sexual size dimorphism

We checked for significant differences in size parameters (i.e. SVL, BM, HW) between sexes using Student's t -test and alternatively, Welch's t -test. We used Pearson product-moment correlation coefficient adjusted for small sample sizes (r_{adj}) to analyse the associations between these parameters.

We computed a sexual dimorphism index (SDI) with the results arbitrarily defined as positive when females are larger than males and negative in the converse situation (Lovich & Gibbons, 1992):

$$SDI = \text{mean size}_{\text{larger sex}} / \text{mean size}_{\text{smaller sex}}$$

Female reproductive investment

We estimated the ovarian size factor (OSF) which correlates the number and size of mature ova to body length, following Duellman & Crump (1974): $OSF = (OC \times OS) / SVL$. Finally, we estimated the reproductive effort (RE) following Prado & Haddad (2000): $RE = (OM / BM) \times 100$. We used Spearman (ρ) and Pearson product-moment correlation coefficient adjusted for small sample sizes (r_{adj}) to analyse the associations between size and reproductive parameters.

RESULTS

Age-related parameters

We were able to estimate age in all individuals and they all showed various degrees of endosteal resorption and double LAGs (Fig. 1). We were able to infer age at sexual maturity from the periosteal growth pattern in 61% of the females at 2 years, and in 44% and 12% of the males at 2 and 3 years respectively. The youngest age class estimated in our sample was 2 years in females (6%) and 3 years in males (60%). We thus considered that both sexes attain sexual maturity at the same age: 2 years. Mean lifespan did not differ significantly between males (mean \pm SD = 3.48 \pm 0.65 years) and females (mean \pm SD = 3.39 \pm 0.69 years) (Mann-Whitney $U=213$, $p=0.75$). Longevity and the potential reproductive lifespan (i.e. considering the estimated age at sexual maturity of 2 years) were 5 and 3 years respectively, in both sexes. There were no significant differences in the shape of age distribution between sexes (Kolmogorov-Smirnov $Z=0.180$, $p=1$) (Fig. 2). The most frequent observed age class was represented by 3 years old individuals, indicating that most of the active animals were in their second reproductive year. Age was not significantly correlated to size (i.e. SVL, BM, HW) in males or females.

Growth patterns

The relation between age and SVL fitted von Bertalanffy's growth model in both sexes (Fig. 3). The asymptotic average snout-vent length was significantly higher in females, while the growth rate coefficient was similar in both sexes (Table 1).

Sexual size dimorphism

Females were significantly larger than males in all three parameters measured: SVL Student's t -test: $t_{41}=5.549$, $p<0.001$; BM Welch's t -test: $t_{23.4}=11.390$, $p<0.001$; HW Student's t -test: $t_{41}=3.916$, $p<0.001$. The sexual dimorphism index was 1.08 for SVL, 1.07 for HW, 1.60 for BM. The sexual dimorphism index was much higher for BM since we used data from females captured

Table 1. von Bertalanffy growth pattern statistics in *D. muelleri* males ($n=25$) and females ($n=18$).

Sex	Parameter	Estimate	SD	CI 95%
Males	SVL_{max}	71.37	5.25	69.62–73.83
	k	1.14	0.98	0.83–1.60
Females	SVL_{max}	77.64	7.68	74.69–82.01
	k	1.07	0.96	0.73–1.60

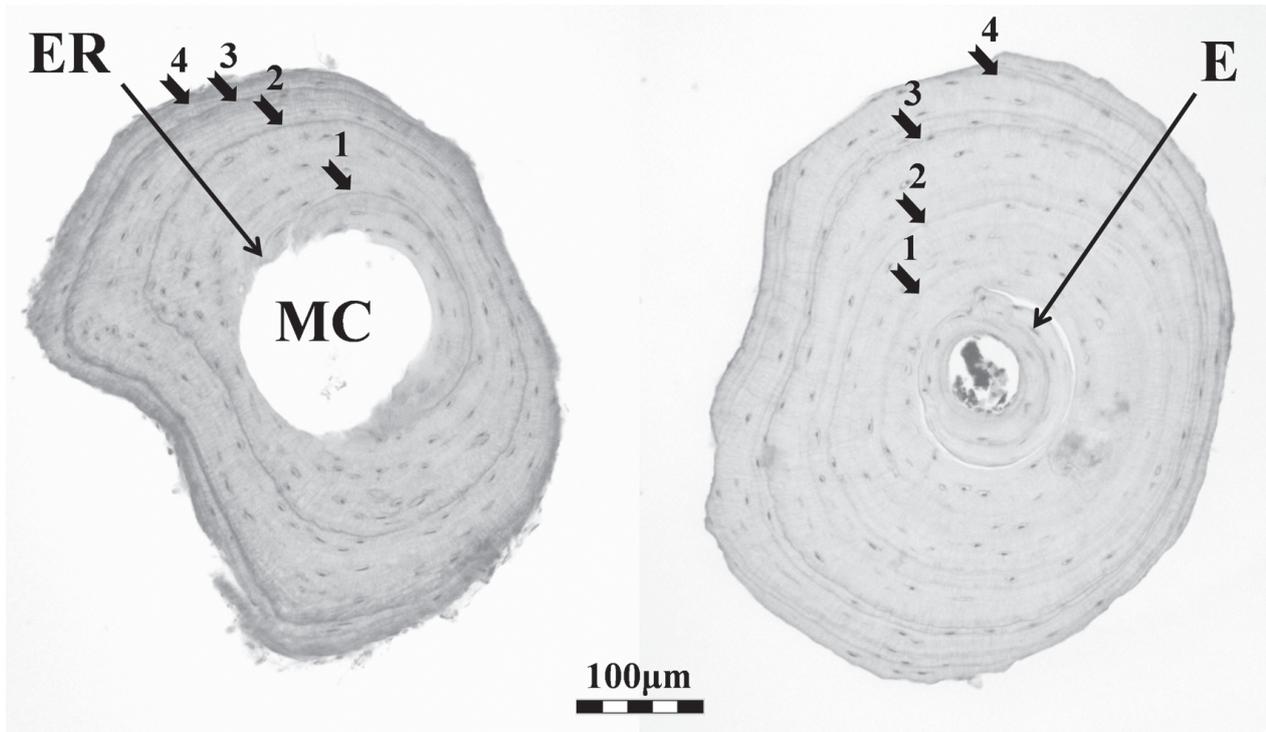


Fig. 1. Phalanx cross-sections in *D. muelleri*: 4-year old male (left) and female (right). LAGs are indicated by black arrows and numbers. MC - marrow cavity, ER - endosteal resorption, E - endosteum.

Table 2. Descriptive statistics of size measurements in *D. muelleri* males ($n=25$) and females ($n=18$): body mass (BM), snout-vent length (SVL) and head width (HW).

Sex	Parameter	Mean	SD	Min	Max
Males	BM (g)	32.55	3.41	25.94	41.79
	SVL (mm)	70.20	2.92	64.61	74.96
	HW (mm)	14.49	0.67	13.47	16.03
Females	BM (g)	52.06	6.67	38.91	62.79
	SVL (mm)	75.86	3.78	66.08	81.94
	HW (mm)	15.41	0.86	13.81	17.35

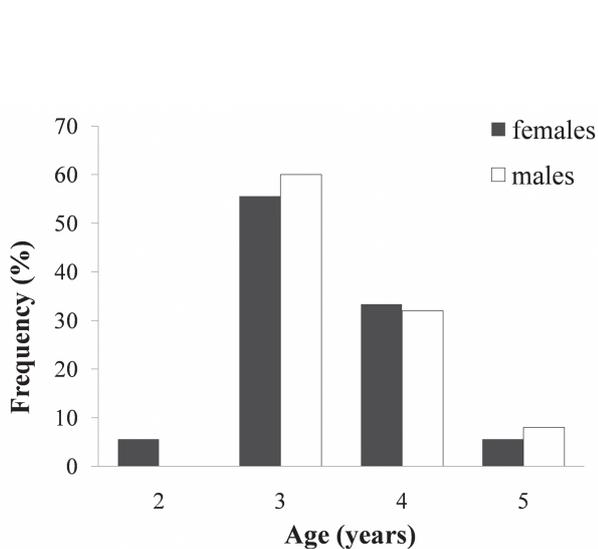


Fig. 2. Age structure of the studied *D. muelleri* population.

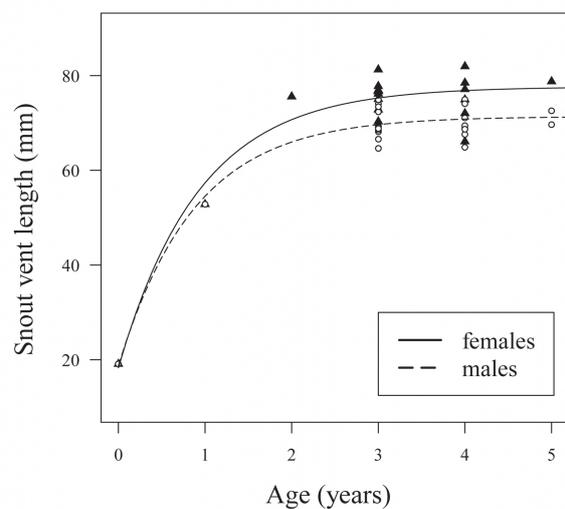


Fig. 3. Growth described by von Bertalanffy's model in *D. muelleri* males (white circles) and females (black triangles).

just before oviposition. Descriptive statistics of body size parameters are summarized in Table 2. We found significant associations between SVL and BM ($r_{adj}=0.508$, $p<0.01$), SVL and HW ($r_{adj}=0.570$, $p<0.01$), and BM and HW ($r_{adj}=0.250$, $p<0.05$) in females. We found significant associations only between SVL and BM ($r_{adj}=0.138$, $p<0.05$) in males.

Female reproductive investment

We computed the reproductive parameters in nine of the ten collected females (mean±SD): 15.85±1.94 g (OM), 10611±1784 ova (OC), 1.36±0.06 mm (OS), 218.8±23.5 (OSF) and 30.18±3.18 % (RE). OC was negatively correlated with OS (Spearman $\rho=-0.683$, $p<0.05$), RE was negatively correlated to SVL ($r_{adj}=0.450$, $p<0.05$), while OM was positively correlated to SVL ($r_{adj}=0.492$, $p<0.05$).

DISCUSSION

The studied population of *D. muelleri* from Arid Chaco showed a female-biased SSD, despite similar age-related parameters (i.e. mean lifespan, age at sexual maturity, reproductive lifespan and longevity) and growth rates. Because of the relatively short reproductive lifespan, females invest up to a third of their body mass in reproduction. The age and growth parameters are similar to those found in desert anurans (i.e. early sexual maturity and short lifespan, Esteban et al., 1999; Sullivan & Fernandez, 1999) as adaptive responses to the harsh environmental conditions. However, the estimated

growth parameters should be considered with caution considering the relatively small sample size.

Female-biased SSD is the most common pattern recorded in amphibians and the main proximate determinants proposed to explain this pattern are sexual selection, differences in age related parameters, growth and survival rates (Shine, 1979; Halliday & Verell, 1988; Hemelaar, 1988; Shine, 1990; Monnet & Cherry, 2002; Kupfer, 2007; Hasumi, 2010; Sinsch et al., 2010). Our results suit best the hypothesis that SSD might stem from differences in growth before sexual maturity (Halliday & Verell, 1988; Shine, 1990). Larger females had larger ovarian masses that were positively correlated to body size. Negative correlations were found between the number and the size of mature ova, and between the reproductive effort and body size. The only study providing some information related to the reproductive investment in *D. muelleri* was done by Perotti (1997), who found a similar ovarian complement and a high ovarian size factor in two females from Salta province, Argentina (Table 3). Previous studies suggest that the reproductive effort is correlated to female size both within and between taxa (Crump, 1974; Perotti, 1997; Prado & Haddad, 2005). However, *D. muelleri* females showed a high reproductive effort compared to other species within or even below its size class (Table 3).

The high reproductive investment (i.e. ovarian complement, reproductive effort and ovarian size factor) observed in *D. muelleri* can also be explained in the terms of r-selection (Pianka, 1970), where in a variable

Table 3. Reproductive investment in representatives from four anuran families in the Neotropics: Bufonidae (*Rhinella marina*), Hylidae (*Hypsiboas raniceps*, *Phrynohyas venulosa*, *Pseudis paradoxa*), Microhylidae (*Chiasmocleis bassleri*, *C. mehelyi*, *C. ventrimaculata*, *Dermatonotus muelleri*, *Elachistocleis cf. bicolor*, *Hamptophryne boliviana*) and Leptodactylidae (*Leptodactylus chaquensis*). RE: reproductive effort; OC: ovarian complement expressed as the total number of mature ovarian ova; OSF: the ovarian size factor. Values are expressed as mean±SD. *Minimum and maximum sample sizes used by authors when measuring the variables.

Species (n)	SVL (mm)	RE (%)	OC	OSF	Data source
<i>R. marina</i> (5)	132.6	-	8598	97.26	Crump, 1974
<i>H. raniceps</i> (6–34)*	60.2±4.4	11.9±6.0	1991±533	-	Prado & Haddad, 2005
<i>P. venulosa</i> (1)	83.2	53	10985	101.33	Perotti, 1994
<i>P. venulosa</i> (3–10)*	77.4±6.0	8.2±1.3	3981±271	-	Prado & Haddad, 2005
<i>P. paradoxa</i> (7–17)*	57.5±8.0	5.5±3.1	1834±1	-	Prado & Haddad, 2005
<i>C. bassleri</i> (3)	27.3	-	212	7.77	Crump, 1974
<i>C. mehelyi</i> (3)	23.8±0.6	-	217±33	-	Prado & Haddad, 2005
<i>C. ventrimaculata</i> (1)	22.0	-	210	9.55	Crump, 1974
<i>D. muelleri</i> (2)	74.2	-	10991	109.68	Perotti, 1997
<i>D. muelleri</i> (9)	65.7±4.2	30.18±3.18	10611±1784	219±24	Present study
<i>E. cf. bicolor</i> (3–13)*	26.1±1.5	18.0±4.5	478±279	-	Prado & Haddad, 2005
<i>H. boliviana</i> (2)	42.0	-	1788	42.33	Crump, 1974
<i>L. chaquensis</i> (3)	76.4	20.89	14649	116.21	Perotti, 1994
<i>L. chaquensis</i> (26–50)*	71.3±4.5	16.0±2.9	4936±1720	-	Prado & Haddad, 2005

and unpredictable environment with mortality not depending on the population density and at a low intra- and interspecies competition, the selection is directed to the production of larger number of smaller offspring. Overall, we suggest that the short reproductive lifespan justifies the high reproductive investment in *D. muelleri* and this trade-off represents an adaptive response to the specific environmental conditions from the Arid Chaco.

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REFERENCES

- Basso, N.G. (1990). Estrategias adaptativas en una comunidad subtropical de anuros. *Cuadernos de Herpetología Serie Monografías* 1, 1–70.
- Baty, F., Ritz, C., Charles, S., Brutsche, M., et al. (2014). *A Toolbox for Nonlinear Regression in R: The Package nlstools*. Available from: <<http://cran.at.r-project.org/web/packages/nlstools/>>.
- Beverton, R.J.H. & Holt, S.J. (1957). *On the dynamics of exploited fish populations*. Fisheries Investigation Series 2. London: Her Majesty's Stationery Office.
- Bolaños, F., Castro, F., Cortez, C., De la Riva, I., et al. (2008). Amphibians of the Neotropical Realm. In: *Threatened amphibians of the world*, 92–99. Stuart, S.N., Hoffmann, M., Chanson, J.S., Cox, N.A., Berridge, R., Ramani, P., Young B.E. (eds). Barcelona: Lynx Ediciones.
- Bucher, E.H. (1980). Ecología de la fauna chaqueña. Una revisión. *Ecosur* 7, 111–159.
- Bucher, E.H. (1982). Chaco and Caatinga - South American arid savannas, woodlands and thickets. In: *Ecology of Tropical Savannas*, 48–79. Huntley, B.J. & Walker, B.H. (eds). Berlin: Springer.
- Cabrera, A. & Willink, A. (1980). *Biogeografía de América Latina (Latin America Biogeography)*. Washington: Secretaría General de la Organización de los Estados Americanos, Programa Regional de Desarrollo Científico y Tecnológico.
- Cajade, R., Marangoni, F. & Gangenova, E. (2013). Age, body size and growth pattern of *Argenteohyla siemersi pederseni* (Anura: Hylidae) in northeastern Argentina. *Journal of Natural History* 47, 237–251.
- Castanet, J. & Smirina, E. (1990). Introduction to the skeletochronological method in amphibians and reptiles. *Annales Des Sciences Naturelles* 11, 191–196.
- Castanet, J., Francillon-Vieillot, H., Meunier, F.J., De Ricqlès, A. (1993). Bone and individual aging. In *Bone Growth–B*, 245–283. Hall, B.K. (ed). Boca Raton: CRC Press.
- Caswell, H. (1983). Phenotypic plasticity in life-history traits: demographic effects and evolutionary consequences. *American Zoologist* 23, 35–46.
- Colli, G., Reichle, S., Silvano, D. & Faivovich, J. (2004). *Dermatonotus muelleri*. In: *IUCN 2014. IUCN Red List of Threatened Species*, Version 2014.3. www.iucnredlist.org. Downloaded on 05 September 2014.
- Crump, M.L. (1974). Reproductive strategies in a tropical anuran community. *Miscellaneous publication - University of Kansas, Museum of Natural History* 61, 1–68.
- Cushman, S.A. (2006). Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation* 128, 231–240.
- Duellman, W.E. & Crump, M.L. (1974). Speciation in frogs of the *Hyla parviceps* group in the upper Amazon Basin (Monograph). *Occasional papers of the Museum of Natural History, the University of Kansas* 23, 1–40.
- Duellman, W.E. & Trueb, L. (1994). *Biology of Amphibians*. Baltimore: The Johns Hopkins University Press.
- Esteban, M., García-París, M., Buckley, D. & Castanet, J. (1999). Bone growth and age in *Rana saharica*, a water frog living in a desert environment. *Annales Zoologici Fennici* 36, 53–62.
- Fabrezi, M., Quinzio, S., Goldberg, J. & De Sá, R.O. (2012). The development of *Dermatonotus muelleri* (Anura: Microhylidae: Gastrophryinae). *Journal of Herpetology* 46, 363–380.
- Fox, J. & Weisberg, S. (2011). *An R Companion to Applied Regression*. Thousand Oaks, Sage Publications. Available from: <<http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>>.
- Gardner, T.A., Barlow, J. & Peres, C.A. (2007). Paradox, presumption and pitfalls in conservation biology: the importance of habitat change for amphibians and reptiles. *Biological Conservation* 138, 166–179.
- Gibbons, J.W., Scott, D.E., Ryan, T.J., Buhlmann, K.A., et al. (2000). The global decline of reptiles, déjà vu amphibians. *BioScience* 50, 653–661.
- Guarino, F.M., Andreone, F. & Angelini, F. (1998). Growth and longevity by skeletochronological analysis in *Mantidactylus microtypanum*, a rain-forest anuran from southern Madagascar. *Copeia* 1998, 194–198.
- Halliday, T.R. & Verrell, P.A. (1988). Body size and age in amphibians and reptiles. *Journal of Herpetology* 22, 253–265.
- Hasumi, M. (2010). Age, body size, and sexual dimorphism in size and shape in *Salamandrella keyserlingii* (Caudata: Hynobiidae). *Evolutionary Biology* 37, 38–48.
- Hemelaar, A. (1988). Age, growth and other population characteristics of *Bufo bufo* from different latitudes and altitudes. *Journal of Herpetology* 22, 369–388.
- Hoyos, L.E., Cingolani, A.M., Zak, M.R., Vaieretti, M.V., et al.

- (2013). Deforestation and precipitation patterns in the arid Chaco forests of central Argentina. *Applied Vegetation Science* 16, 260–271.
- Khonsue, W., Matsui, M. & Misawa, Y. (2000). Age determination by skeletochronology of *Rana nigrovittata*, a frog from tropical forest of Thailand. *Zoological Sciences* 17, 253–257.
- Kumbar, S.M. & Pancharatna, K. (2001). Occurrence of growth marks in the cross section of phalanges and long bones of limbs in tropical anurans. *Herpetological Review* 32, 165–166.
- Kumbar, S.M., Pancharatna, K. & Gatten, R.E. (2002). Annual growth layers in the phalanges of the Indian Skipper Frog *Rana cyanophlyctis* (Schn). *Copeia* 2002, 870–872.
- Kupfer, A. (2007). Sexual size dimorphism in amphibians: an overview. In: *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*, 50–59. Fairbairn, D.J., Blanckenhorn, W.U. & Székely, T. (eds). New York: Oxford University Press.
- Lai, Y.C., Lee, T.H. & Kam, Y.C. (2005). A skeletochronological study on a subtropical, riparian ranid (*Rana swinhoana*) from different elevations in Taiwan. *Zoological Sciences* 22, 653–658.
- Lindquist, E., Redmer, M. & Brantner, E. (2012). Annular bone growth in phalanges of five Neotropical Harlequin Frogs (Anura: Bufonidae: *Atelopus*). *Phyllomedusa* 11, 117–124.
- Lovich, J.E. & Gibbons, J.W. (1992). A review of techniques for quantifying sexual size dimorphism. *Growth Development & Aging* 56, 269–281.
- Marangoni, F., Schaefer, E., Cajade, R. & Tejedo, M. (2009). Growth-mark formation and chronology of two neotropical anuran species. *Journal of Herpetology* 43, 546–550.
- Marangoni, F., Barrasso, D.A., Cajade, R. & Agostini, G. (2012). Body size, age and growth pattern of *Physalaemus fernandezae* (Anura: Leiuperidae) of Argentina. *North-Western Journal of Zoology* 8, 63–71.
- Monnet, J.M. & Cherry, M.I. (2002). Sexual size dimorphism in anurans. *Proceedings of the Royal Society of London. Series B. Biological Sciences* 269, 2301–2307.
- Nature Conservancy, Fundación Vida Silvestre Argentina, Fundación para el Desarrollo Sustentable del Chaco, Wildlife Conservation Society Bolivia. (2005). *Evaluación Ecorregional del Gran Chaco Americano (Great American Chaco Ecoregional Assessment)*. Buenos Aires: Imprenta Kurz.
- Nomura, F. (2003). Ecología reproductiva e comportamiento de forrajeo e escavação de *Dermatonotus muelleri* (Boettger, 1885) (Anura, Microhylidae) (Reproductive ecology, foraging and burrowing behavior of *Dermatonotus muelleri* (Boettger, 1885) (Anura, Microhylidae)). Dissertation, Universidade Estadual Paulista, São José do Rio Preto, BR. 95 pp.
- Nomura, F. & Rossa-Feres, D.C. (2011). The frog *Dermatonotus muelleri* (Boettger 1885) (Anura Microhylidae) shifts its search tactics in response to two different prey distributions. *Ethology Ecology & Evolution* 23, 318–328.
- Nomura, F., Rossa-Feres, D.C. & Langeani, F. (2009). Burrowing behavior of *Dermatonotus muelleri* (Anura, Microhylidae) with reference to the origin of the burrowing behavior of Anura. *Journal of Ethology* 27, 195–201.
- Nori, J., Lescano, J.N., Illoldi-Rangel, P., Frutos, N., Cabrera, M.R. & Leynaud, G.C. (2013). The conflict between agricultural expansion and priority conservation areas: making the right decisions before it is too late. *Biological Conservation* 159, 507–513.
- Ogle, D.H. (2014). *FSA: Fisheries Stock Analysis. R package 0.4.22*. Available from: <<http://www.rforge.net/FSA/>>.
- Perotti, M.G. (1994). Aportes preliminares sobre la reproducción en una comunidad de anuros chaqueños en Argentina (Preliminary contribution on reproduction in a community of anurans from the Argentinean Chaco). *Cuadernos de Herpetología* 8, 39–50.
- Perotti, M.G. (1997). Modos reproductivos y variables reproductivas cuantitativas de un ensamble de anuros del Chaco semiárido, Salta, Argentina (Reproductive modes and quantitative reproductive parameters of an anuran assemblage from the semiarid Chaco, Salta, Argentina). *Revista Chilena de Historia Natural* 70, 277–288.
- Pianka, E.R. (1970). On r- and K-selection. *American Naturalist* 104, 592–597.
- Prado, C.P.A. & Haddad, C.F.B. (2005). Size-fecundity relationships and reproductive investment in female frogs in the Pantanal, South-Western Brazil. *Herpetological Journal* 15, 181–189.
- R Core Team. (2014). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Version 3.0.3. Available from: <<http://www.R-project.org/>>.
- Revelle, W. (2014). *psych: Procedures for Personality and Psychological Research*. Northwestern University, Evanston, Illinois, USA. R package 1.4.8. Available from: <<http://CRAN.R-project.org/package=psych>>.
- Savaria-Toledo, C. (1993). The Chaco savannah lands of South America with particular reference to the process of degradation in their pastoral and forestry resources, p. 86–90. In: *Grasslands for our World*. Proceedings of the XVIIth International Grassland Congress, Baker, M.J. (ed). Wellington: SIR Publishing.
- Shine, R. (1979). Sexual selection and sexual dimorphism in the amphibia. *Copeia* 1979, 297–306.
- Shine, R. (1990). Proximate determinants of sexual differences in adult body size. *American Naturalist* 135, 278–283.
- Sinsch, U. (2015). Review: Skeletochronological assessment of demographic life-history traits in amphibians. *Herpetological Journal* 25, 5–13.
- Sinsch, U., Oromi, N. & Sanuy, D. (2007). Growth marks in natterjack toad (*Bufo calamita*) bones: histological correlates of hibernation and aestivation periods. *Herpetological Journal* 17, 129–137.
- Sinsch, U., Marangoni, F., Oromi, N., Leskovar, C., et al. (2010). Proximate mechanisms determining size variability in natterjack toads. *Journal of Zoology* 281, 272–281.
- Smirina, E.M. (1994). Age determination and longevity in amphibians. *Gerontology* 40, 133–146.
- Stearns, S.C. (2000). Life history evolution: successes, limitations, and prospects. *Naturwissenschaften* 87, 476–486.
- Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., et al. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science* 306, 1783–1786.
- Sullivan, B.K. & Fernandez, P.J. (1999). Breeding activity, estimated age-structure, and growth in Sonoran Desert

anurans. *Herpetologica* 55, 334–343.

Vaira, M., Akmentins, M., Attademo, A., Baldo, D., et al. (2012). Categorización del estado de conservación de los anfibios de la República Argentina (Categorizing the conservation state of amphibians in Argentina). *Cuadernos de Herpetología* 26, 131–159.

von Bertalanffy, L. (1938). A quantitative theory of organic growth (inquiries on growth laws II). *Human Biology* 10, 181–213.

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