Body Size, Age and Growth Pattern of the most represented anurans in Inselbergs of northeastern Argentina

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ABSTRACT

The decline of biodiversity is an urgent concern that raises significant alarm today. Several species vanish without their biology being understood, or sometimes, even without awareness of their existence. Due to the significance that the Inselbergs has to biodiversity and nature, as they have been recognized as genuine evolutionary laboratories, they present a unique opportunity to enhance our understanding of biologically unexplored species. Our study presents the first information regarding the body size, age and growth pattern of *Scinax fuscovarius*, *Odontophrynus asper*, and *Melanophryniscus atroluteus*, the three most frequently encountered anuran species in the Inselbergs outcrops of northeastern Argentina. We have demonstrated that the variations in body size cannot be attributed to the effect of the specific hill where the species inhabit. We discovered that sexual dimorphism in age and the analyzed morphological variables is expressed differently in the three examined species. We also demonstrated that the observed differences in body size between populations of *M. atroluteus* of Inselbergs and the Atlantic Forest of Argentina can be attributed to differences in age at sexual maturity and growth patterns. However, we believe that other proximate mechanisms should be taken into account, such as the effects of the environment and inter- and intra-specific competition experienced during the larval stages, for a better understanding of the population differences in adult body size and age. Our findings will assist in the evaluation of species management and conservation strategies within their habitat.

Key words: Inselbergs, Paraje Tres Cerros, Body size, Age, Anurans.

RESUMEN

El declive de la biodiversidad es una preocupación urgente que suscita gran alarma hoy en día. Varias especies desaparecen sin que se comprenda su biología o, a veces, incluso sin que se sepa de su existencia. Debido a la importancia que los Inselbergs tienen para la biodiversidad y la naturaleza, ya que han sido reconocidas como auténticos laboratorios evolutivos, presentan una oportunidad única para mejorar nuestra comprensión de especies biológicamente inexploradas. Nuestro estudio presenta la primera información relativa al tamaño corporal, edad y patrón de crecimiento de *Scinax fuscovarius*, *Odontophrynus asper*, and *Melanophryniscus atroluteus*, las tres especies de anuros más frecuentemente encontradas en los afloramientos de los Inselbergs del noreste de Argentina. Hemos demostrado que las variaciones en el tamaño corporal no pueden atribuirse al efecto del cerro específico donde habitan las especies. Descubrimos que el dimorfismo sexual en edad y en las variables morfológicas analizadas se expresa de manera diferente en las tres especies examinadas. También demostramos que las diferencias observadas en el tamaño corporal entre las poblaciones de *M. atroluteus* de los Inselbergs y del Bosque Atlántico de Argentina pueden atribuirse a diferencias en la edad a la madurez sexual y en los patrones de crecimiento. Sin embargo, creemos que deben tenerse en cuenta otros mecanismos próximos, como los efectos del ambiente y la competencia inter e intraespecífica experimentada durante las fases larvarias, para comprender mejor las diferencias poblacionales en el tamaño corporal y la edad de los adultos. Nuestros hallazgos ayudarán a evaluar las estrategias de gestión y conservación de las especies dentro de su hábitat.

Palabras Clave: Inselbergs, Paraje Tres Cerros, Tamaño corporal, edad, Anuros.

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Introduction

Body size is perhaps the most important trait with the greatest implications throughout their life cycle (Atkinson, 1994; Woodward *et al.*, 2005; White *et al.*, 2007). This is because body size is related to the fitness of an animal, due to its close association with longevity, fecundity, metabolic rate and tolerance to environmental stress, among others (Calder, 1984; Schmidt-Nielsen, 1984). Anurans show an indeterminate growth pattern, with continuous growth, which asymptotizes once sexual maturity is reached (Hemelaar, 1998; Smirina, 1994; Halliday and Tejedo, 1995). Consequently, differences found in adult size of anurans are, in most cases, dependent on environmental and/or genetic factors that cause variations in the growth pattern between the post-metamorphic phase and reproductive maturity (e.g. Halliday and Verrell, 1988; Esteban and Sanchiz, 2000). Thus, one of the main objectives for evolutionary and systematic biologists is the detection and description of these patterns of inter and intra-specific variation, and the inference of processes that help to understand them (Cracraft, 1989). In addition, the need to increase our knowledge of these processes becomes even more relevant if we consider that in a given isolate ecosystem, these patterns of variation may lead to the evolution of endemic species, or even to the formation of new species (Endler, 1977; Foster and Endler, 1999).

*Inselberg*s (from the Germanic "island-mountain") are a type of isolated ecosystem on the mainland, found in many parts of the world, constituted by a diverse set of isolated rock habitats and recognized as biogeographic islands (Bornhardt, 1900; Porembski and Barthlott, 2000; Fitzsimons and Michael, 2017). In these ecosystems, the distinctive combination of environmental conditions (such as microclimate and soil) that sharply contrast with those in the surrounding matrix often gives rise to ecologically unique habitat features (Coor *et al.*, 1993; Porembski and Barthlott, 2000; Burke, 2003). Many authors agree that these isolated ecosystems represent centers of biodiversity concentration, refuges for threatened flora and fauna, as well as favorable scenarios for endemic phenomena and conservation of relictual populations (Porembski *et al.*, 1998; Porembski and Barthlott, 2000; Fredericksen *et al.*, 2003; Cajade *et al.*, 2013a). In addition, due to their isolation and particular environmental characteristics, these ecosystems represent true evolutionary laboratories where species experience evolution in isolation (Endler, 1977; Foster and Endler, 1999; Barthlott and Porembski, 2000; Porembski, 2007; Cajade *et al.*, 2013a; Argoitia *et al.*, 2021; Piñeiro *et al.*, 2021; Piñeiro, 2022).

In northern Argentina, three isolated Inselbergs located in the east-central region of Corrientes province (Fig. 1) have been explored through an interdisciplinary program of several projects to study their biodiversity (Meregalli, 1998; Ravenna, 2003, 2009; Cajade *et al.*, 2013a,b; Odriozola, 2014; Isler, 2016; Ojanguren-Afilastro *et al.*, 2017; Gervazoni, 2017; Fandiño *et al.*, 2017; Nadal *et al.*, 2018; Oliva and Panizza, 2019; Ellis *et al.*, 2020; Argoitia *et al.*, 2021; Piñeiro *et al.*, 2021; Courtis *et al.*, 2022). Recently, Piñeiro *et al.* (2021) described the herpetofauna diversity that inhabit there, where recorded 19 rare species, seven threatened species and two endemic species, and discussed the role of the Inselbergs in amphibians and reptiles conservation, contributing to its valorization. In addition, they emphasizing the need for further study help evaluate species and conservation strategies in the natural reserve where they live.

In order to increasing the knowledge of the life history traits of the amphibians that live there, and contribute to their conservation, we studied the body size, age structure and whether Sexual Size Dimorphism (SSD) existed, in the three most represented anurans in Inselbergs outcrops of northeastern Argentina (Piñeiro, 2022). We also analyzed if this dimorphism could be explained by proximate mechanisms such as differences in growth patterns. This will increase our knowledge of the life history traits of these amphibians, which help evaluate species management and conservation strategies in the natural reserve where they live. In addition, we tested whether there were differences in body size and age between the *Melanophryniscus atroluteus* populations of the Inselberg and the Atlantic Forest of Argentina. Finally, we discussed the possible causes that might determine it.

Materials and methods

Study site and Species
We carried out fieldwork in the Inselbergs Nazareno (29° 0.6′ 26.51" S, 56° 55′ 56.90" W, 179 m a.s.l., 83 ha) and Chico (20° 0.6′ 45.74" S, 56° 55′ 7.78" W, 148 m a.s.l., 34 ha), two out of the three hills located in the east-central region of Corrientes province,
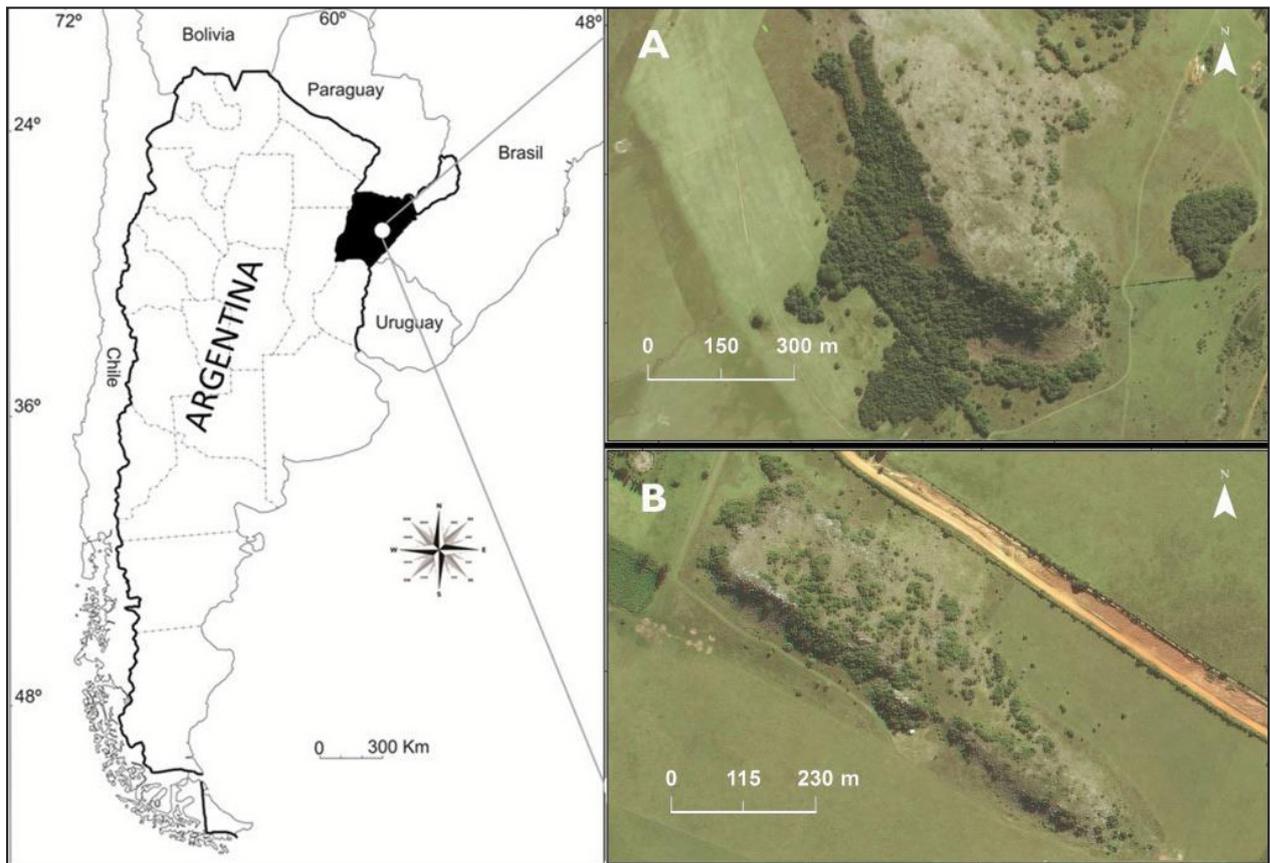
Figure 1. Isolated rocky outcrops of Paraje Tres Cerros, Corrientes, Argentina. View of the Nazareno (a) and Chico (b) hills.

Argentina (Fig. 1). The study area is located within the Espinal district, one of the subdivisions of the Pampeana province, corresponds to the Neotropical biogeographic region (Arana *et al.,* 2021). The climate and topography of the study site is detailed in Piñeiro *et al.,* (2021). The three most represented anuran species in the Inselbergs (Piñeiro, 2022), analyzed in the present study, were: Scinax fuscovarius, "Snouted-tree frog" is a species of frog of the Hylidae family, very common in arboreal and peri-domestic habitats. During the breeding season it is found in permanent water bodies such as streams, cutwaters and temporary ponds. The males of this species vocalize on the ground or on rocks at the water's edge, perched on herbaceous and arbustive vegetation, as well as on tree branches or fallen trunks. It´s distributed in various ecoregions in Argentina, Bolivia, Brazil, Paraguay, Uruguay (Zaracho *et al.*, 2012). *Odontophrynus asper*, "Common Lesser Escuerzo" is a species of toad of the Odontophrynidae family. This species has terrestrial and fossorial habits. It lives in forests and grassland, temporary and semi-permanent vegetated lagoons, in caves and urban canals. They bury themselves

and remain hidden most of the year. Males vocalize from the water among the vegetation. It´s distributed in Atlantic forest and humid Chaco ecoregions in Argentina, Brazil, Paraguay, Uruguay (Zaracho *et al.*, 2012). *Melanophryniscus atroluteus*, "Uruguay Redbelly Toad" is a small-sized toad species belonging to the Bufonidae family. This terrestrial species exhibits breeding activity that takes place from mid-autumn (April) and extends until mid-spring (late September-October), characterized by explosive events triggered by rainfall over the course of two or three consecutive days (Marangoni and Baldo, 2023). It inhabits grasslands between patches of forest. It has a defense behavior that consists of displaying the soles of the feet and the palms of the hands before a potential predator (*unken reflex*). It´s distributed in a humind Chaco ecoregions in Argentina, Brazil, Paraguay, Uruguay (Zaracho *et al.*, 2012).

Body size and Sexual size dimorphism
Sampling methods
We analyzed de body size and sexual size dimorphism using individuals obtained of two different sampling methods. On the one hand, we analyzed

132 individuals (*S. fuscovarius* n = 82, *O. asper* n = 37 and *M. atroluteus* n = 13), caught using pitfall traps and coverboards during a previous study conducted from April 2015 to April 2017 in Nazareno and Chico Hills (see Piñeiro, 2022, for a detailed description). On the other hand, in addition to the individuals caught using pitfall traps, we also measured 55 individuals of *M. atroluteus* collected by hand in a reproductive event on August 27 2017 (Table 1). This event occurred in a temporary pond (10 to 20 cm deep) located between the Nazareno and Chico Hills (29º 0.6' 28.8" S, 56º 55' 22.8" W), filled immediately after heavy rains. In this reproductive event of *M. atroluteus*, we collected either calling males or pairs in amplexus, during chorusing mornings between 10:00 and 12:00 h, to ensure that all individuals were mature.

Measurements and tissue samples.
The procedures described below were carried out on all individuals of the three species, regardless of the capture method used. Individuals were firstly sexed taking into account secondary sexual characteristics. Then, the following measurements were taken in the sample place of the capture according to Duellman (1970). We measured the snout-vent length (SVL), head width (HW) and tibia-fibula length (TFL), using a digital calliper (0.1 mm precision). Also, we measured body mass (BM) using an electronic balance MH-500g/0.01g. Moreover, we individually-marked all individuals by using toe-clipping codes (Donnelly and Guyer, 1994), and the toes stored in 70% ethanol at room temperature for age estimation through skeletochronology (see below). In addition, this marks also allowed us to test the existence of migrations between populations. Finally, individuals were released back into their habitats. The sampling followed the general guidelines proposed by the Dirección de Recursos Naturales of the Corrientes province, Argentina, under project code PI 12F007 of the Laboratorio de Herpetología, of the Universidad Nacional del Nordeste.

Skeletochronology
We used clipped toes from 121 mature individuals for age estimation through skeletochronology (*S. fuscovarius* n = 43, *O. asper* n = 25 and *M. atroluteus* n = 53). We preferably use the third toe of the right leg in case it has been cut off during individual marking. Otherwise, we use any toe that has been clipped to avoid additional injuries to the individuals. We

followed the standard methods in skeletochronology (e.g., Smirina, 1972; Halliday and Verrel, 1988, Castanet and Smirina, 1990; Sinch *et al.,* 2015), with minor modifications proposed by Marangoni (2006). Clipped toes were washed in water for 30 minutes, decalcified in 5% nitric acid for 1–3 hours, afterward dehydrated, paraffin-embedded, sectioned using a rotation microtome (Arcano RMT-30) at 14–16 μm, and stained with Harris hematoxylin. We took digital images of those cross-sections where the size of the medullar cavity was at its minimum and the periosteum was at its maximum, using a microscope (Leica Model DM500) and the software Leica LEAD Technologies Inc.V1.01. Cross-sections were observed and measured using the computer package Image-Pro Plus version 4.5 (Media Cybernetics 1993–1994, Silver Spring, Maryland, USA) and calibrated using a standard micrometer. Two independent observers (JMP and FM) recorded the presence/absence of the line of metamorphosis and counted the LAGs. In those individuals 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). 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 in Sinsch *et al.* (2007). We computed the bone size following the methods of Hemelaar (1985): the longest and shortest perpendicular axes of each LAG were measured, in each of two diaphyseal sections per specimen examined. Afterwards, axis measurements were multiplied together and the square root of the product calculated (average diameter of each LAG). This procedure was done for the LAGs in each of two diaphyseal sections per specimen.

Age-related parameters
We computed the following age-related parameters for each sex (*sensu* Leskovar *et al.*, 2006): (1) age at maturity was estimated as: a) the minimum number of LAGs counted in those breeding individuals from reproductive events (calling males or pairs in amplexus of *M. atroluteus*); or b) we inferred the age of sexual maturity by observing the bone growth pattern in the cross-sections in individuals from pitfall traps and coverboards (individuals of *S. fuscovarius* and *O. asper*). 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). (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 snout-vent length of all first breeders with the minimum number of LAGs.

Growth patterns
We used nonlinear estimation in STATISTICA 8 statistical package (StatSoft Inc. 2007, Tulsa, USA) to compute von Bertalanffy growth equation (von Bertalanffy, 1938): $S_t = S_m - (S_m - S_0) e^{-k(t-t0)}$

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 slope of the growth curve, measuring the exponential rate of approach to S_m. In order to obtain the size at metamorphosis (S_0, se above) and thus to fit the growth model (von Bertalanffy, 1938), were collected clutches of *S. fuscovarius*, *O. asper* and *M. atroluteus* in temporary pools formed after heavy rains in the study area. The clutches (in stages below 20 according to Gosner, 1960) were maintained at a constant density and temperature and a 12:12 photoperiod until the reabsorption of the tail (45-46 Gosner stage), when were measured and weighed. We measured the SVL and weight from 24 metamorphs random selected of *S. fuscovarius*, 10 of *O. asper* and 36 *M. atroluteus*, which were used to fit the von Bertalanffy growth model and estimated growth parameters (VBgPs) by nonlinear least squares regression. The metamorphs were housed in the Laboratorio de Investigación en Diversidad, Ecología y Conservación de Vertebrados (LABIDECOV, Universidad Nacional del Nordeste), Corrientes province, Argentina, under acronym LABIDECOV-16 (*S. fuscovarius*), LABIDECOV-15 (*O. asper*) and LABIDECOV-14 (*M. atroluteus*).

Statistical analyses
All variables were log-transformed in order to achieve normality. We tested all data for normality and homoscedasticity using ShapiroWilk and Levene tests and chose the statistic tests accordingly. We did not find differences in either body size

measurements between *M. atroluteus* from pitfalls and reproductive events (*P* value found = 0.896), and we therefore subsequently pooled the data sets from both capture methods to analyze the body size. We used multi- and univariate analyses of variance to test for differences in the body size variables and age-related parameters measured between hills and sexes within species. Means were compared using post-hoc Scheffé multiple comparison test, at α = 0.05 (Scheffé, 1953). We also assessed the sexual size dimorphism (SSD) for each body measurement using 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 are larger than males, and negative when males are larger. We used linear regressions to test the association between body size and age. We used a two-sample Mann-Whitney test to check for differences in mean age between males and females. We used data of body traits, age and growth included in a previous study by Marangoni and Baldo (2023), to test whether there were differences in body size between the *M. atroluteus* populations of the Inselberg (present study) and the Atlantic Forest of Argentina (Marangoni and Baldo, 2023). All statistical analyses were performed using the STATISTICA 8.0 statistical package (StatSoft Inc. 2007, Tulsa, USA).

Results

Body size and sexual size dimorphism
A MANOVA on SVL, BM, TFL and HW in the three species studied, revealed that there was no significant effect of the Hills where the individuals were collected (*S. fuscovarius*: Wilk's λ = 0.884, $F_{4,69}$ = 2.259, *P* = 0.072; *O. asper*: Wilk's λ = 0.663, $F_{4,23}$ = 66.574, *P* < 0.05 and *M. atroluteus*: Wilk's λ = 0.585, $F_{4,8}$ = 1.419, *P* = 0.312). We therefore subsequently pooled the data sets from both Hills to analyze the body size and sexual size dimorphism. The descriptive statistics of body traits measured and SDI in the three species are presented in Table 1.

Univariate ANOVAs showed significant effects of sex on SVL, TFL and BM for *M. atroluteus* (Table 1). We did not observed a regular pattern in SDI among species. This showed negative values in some cases, indicating that the males were larger than the females in certain variables. However, in other cases, the values were positive, indicating the reverse situation, with the females being larger than the males (Table 1). The body size showed signifi-

Table 1. Body traits: snout-to-vent length (SVL), head width (HW), tibio-fibula length (TFL), body mass (BM) and bone size (BS) of male and female of the three species studied. SDI = sexual dimorphism index. Δ = Individual from pitfall traps pooled with those from reproductive event. ∞ The sample sizes are those in which skeletochronology was applied (see Table 3). The asterisks compare sexual size dimorphism (SSD) within species: * = $P<0.01$, ** = $P<0.001$, ns = not significant. All values are means ± 1 SD.

Species/ Body Traits	Scinax fuscovarius (n = 82)				Odontophrynus asper (37)				Melanophryniscus atroluteus (68Δ)		
	Male (n=31)	Female (n=23)	Juvenile (28)	SDI	Male (n=11)	Female (n=16)	Juvenile (10)	SDI	Male (n=41Δ)	Female (n=27Δ)	SDI
SVL	38.96 ± 4.85	37.78 ± 4.31 ns	30.93 ± 4.24	-1.03	40.58 ± 4.31	39.68 ± 3.04 ns	34.55 ± 4.75	-1.02	21.91 ± 1.89Δ	22.49 ± 1.33*Δ	1.03
HW	13.03 ± 1.61	12.54 ± 1.37 ns	10.20 ± 1.30	-1.25	17.89 ± 1.22	18.16 ± 1.29 ns	15.63 ± 2.13	1.01	6.83 ± 0.52Δ	6.66 ± 0.41 NSΔ	-1.02
TFL	18.87 ± 2.46	18.22 ± 2.36 ns	14.79 ± 2.39	-1.04	11.31 ± 1.24	11.08 ± 1.10 ns	10.16 ± 1.80	-1.02	6.67 ± 0.60Δ	5.86 ± 0.26**Δ	-1.14
BM	4.59 ± 1.81	4.01 ± 1.36 ns	2.12 ± 0.91	-1.14	9.09 ± 2.49	9.11 ± 2.19 ns	5.89 ± 2.40	1.00	1.27 ± 0.37Δ	1.70 ± 0.65*Δ	1.34
BS∞	278.51 ± 48.08∞	275.33 ± 54.42∞ ns		-1.01	279.33 ± 46.76	313.54 ± 41.57 ns		1.12	202.68 ± 19.84	200.19 ± 21.01 ns	-1.02

Table 2. Relationships between body size: snout-to-vent length (SVL), body mass (BM), bone size (BS) and age, in the three species studied. All variables were log-transformed.

Specie/sex	Regression equation	r2	p	Regression equation	r2	p
Scinax fuscovarius	Male (n = 23)			Female (n = 20)		
SVL:BS	y = 7.967 - 0.646*x	0.206	**0.029**	y = 6.175 - 0.160*x	0.008	0.708
BM:BS	y = 5.861 - 0.172*x	0.159	0.059	y = 5.570 + 0.020*x	0.001	0.887
SVL:Age	y = 2.577 - 0.371*x	0.032	0.414	y = 1.974 - 0.288*x	0.006	0.745
BM:Age	y = 1.274 - 0.034*x	0.003	0.806	y = 1.033 - 0.076*x	0.004	0.797
Odontophrynus asper	Male (n = 11)			Female (n = 14)		
SVL:BS	y = 3.788 + 0.495*x	0.092	0.366	y = 4.234 + 0.409*x	0.415	0.056
BM:BS	y = 5.083 + 0.247*x	0.199	0.446	y = 5.247 + 0.223*x	0.142	0.184
SVL:Age	y = -7.185 + 2.281*x	0.296	0.084	y = -6.223 + 1.975*x	0.095	0.284
BM:Age	y = 1.051 + 0.091*x	0.004	0.851	y = 0.099 + 0.425*x	0.038	0.505
Melanophryniscus atroluteus	Male (n = 30)			Female (n = 23)		
SVL:BS	y = 5.256 + 0.002*x	0.001	0.852	y = 5.045 + 0.011*x	0.019	0.521
BM:BS	y = 5.287 + 0.059*x	0.028	0.371	y = 5.246 + 0.094*x	0.032	0.405
SVL:Age	y = -4.465 + 1.871*x	0.188	**0.002**	y = -3.912 + 1.698*x	0.147	**0.011**
BM:Age	y = 1.3456 - 0.0788*x	0.008	0.555	y = 1.6733 - 0.5504*x	0.178	**0.005**

cant positive relationship with bone size only in *S. fuscovarius* (Table 2).

Age-related parameters
All sections showed well-defined lines of arrested growth (LAGs) in the periosteal bone and were relatively easy to count to assess individual age (Fig. 2). Endosteal resorption never prevented the age estimation. The line of metamorphosis was visible in 78.8% of the total samples, considering the three

Figure 2. Cross sections of phalanges stained with Ehrlich's hematoxylin of (A) *Scinax fuscovarius* (male, 35.82 mm, 4.32 g, 5 years), (B) *Odontophrynus asper* (female, 40.34 mm, 8.55 g, 5 years) and (C) (female, 20.72 mm, 1.27 g, 4 years). An arrowhead indicates the lines of arrested growth (LAGs), medullar cavity (mc), endosteal bone (eb), periosteal bone margin (pbm).

species. But even, in those specimens in which the resorption line was visible (25.2%), and the line of metamorphosis was replaced during the endosteal bone modeling, the inner LAG was never completely removed. Annuli (Peabody, 1958) were easily distinguishable from actual LAGs, because they always stained more faintly and were often broader than true LAgs. The demographic age-related parameters of the three species studied are summarized in Table 3, and the age structure of adults in Fig. 2. Males of *S. fuscovarius* and *O. asper* were older than females, conversely females tended to be older in *M. atroluteus* (Table 3), although significant dimorphism in mean age was only found in *S. fuscovarius* (Mann-Whitney, U = 138,5; P = 0.025). There were not intra and inter-specific differences in age at maturity (1-2 LAGs) and longevity (5-6 LAGs) (Table 3). The most frequent observed age class was represented by 3-4 years old individuals, indicating that most of the active animals were in their second or third reproductive year (Table 3 and Fig. 3). Age was significantly correlated to SVL in males and females and to BM in females of *M. atroluteus* (Table 2).

Growth patterns
The patterns of growth for body size fits well with a von Bertalanffy's growth model (Table 4 and Fig. 3). The asymptotic average snout-vent length (SVL_{max}) was slightly higher in males of *S. fuscovarius* and *O. asper,* conversely were estimated bigger females in *M. atroluteus* (Table 4). The growth coefficient (*k*) value was higher in females of *M. atroluteus* and *O. asper*, whereas it was higher in males of *S. fuscovarius* (Table 4). However, no difference was *significant* in either SVL_{max} or *k* between sexes (lowest value obtained being P = 0.135).

Inselbers (ISB) vs. Atlantic Forest of Argentina (AFA) populations
M. atroluteus from the ISB were smaller and youngers than AFA ones (Fig. 4) and, they reached the age at maturity one year before in average (Table 3 and Fig. 5, see also Appendix A, to compare data from Marangoni and Baldo, 2023). The patterns of growth for body size fits well with a von Bertalanffy's growth model (r^2 = 0.987, n = 89 and r^2 = 0.979, n = 48, ISB and AFA respectively; Fig. 5). The asymptotic average snout-vent length (SVL_{max}) and growth rate coefficient (k) were lower in ISB than AFA population.

Table 3. Features of populations life history traits of male and female of the three species studied. AM = age at maturity: Δ = estimated by bone growth pattern of individuals from pitfall traps and coverboards; * = lowest age class recorded in reproductive events (calling males or pairs in amplexus); PRLS = potential reproductive lifespan; SVL = snout-vent length; BM = body mass.

Species/Sex (N)	Mean± SD [LAGs]	Mode [Frequency]	Median Lifespam [yr]	AM [LAGs]	Longevity [LAGs]	PRLS [yr]	SVL at AM X ± SD mm [n]	BM at AM X ± SD mm [n]	Maximum Size SVL-BM mm/g [at age in LAGs]
Scinax fuscovarius									
Male (23)	3.52 ± 0.95	3 (12)	3	2Δ	5	3	39.54 ± 0.23 (n = 2)	4.17 ± 0.37 (n = 2)	47.31 - 7.59 (3 - 3)
Female (20)	2.80 ± 1.24	2-3 (7)	2	1Δ	6	5	36.69 ± 5.42 (n = 2)	3.88 ± 2.24 (n = 2)	43.47 - 6.72 (4 - 2)
Odontophrynus asper									
Male (11)	3.73 ± 0.33	4 (6)	4	1Δ	5	4	34.88 (n =1)	8.35 (n = 1)	48.53 - 13.17 (4 - 5)
Female (14)	3.14 ± 0.35	3-4 (4)	3	1Δ	5	4	36.84 ± 1.02 (n = 2)	6.96 ± 0.61 (n = 2)	44.76 - 12.94 (4 - 2)
Melanophryniscus atroluteus									
Male (30)	3.0 ± 1	3 (3)	3	2*	6	4	20.87 ± 1.82 (n = 3)	1.21 ± 0.08 (n=3)	25.83 - 2.1 (3-4)
Female (23)	3.87 ± 1.01	4 (9)	4	2*	6	4	24.36 (n = 1)	1.97 (n=1)	25.92 - 2.45 (3-3)

Discussion

The slow pace of species description in relation to extinction rates means that several species disappear without their biology being known or without even having been aware of their existence (Mora *et al.*, 2011). In the last 20 years, several studies have demonstrated the importance of inselbergs in biodiversity and nature conservation (Fitzsimons and Michael, 2017; Michael and Lindenmayer, 2018). These are a unique opportunity to increase knowledge about biologically unexplored species, especially considering that inselbergs are regarded as true evolutionary laboratories with a wide variety of unique microhabitats, where species can undergo evolutionary processes in isolation (Barthlott and Porembski, 2000; Porembski, 2007; Cajade *et al.*, 2013a). Our study provides the first information about the body size and age structure of the three most commonly found anuran species in the Inselbergs outcrops of northeastern Argentina. We demonstrated that differences in body size cannot be attributed to an effect of the hill where the species inhabit. We found that sexual dimorphism in

age and the morphological variables analyzed, is expressed differently in the three species examined. We also demonstrated that the observed differences in body size between populations of *M. atroluteus* of Inselberg and the Atlantic Forest of Argentina can be attributed to differences in age at sexual maturity and growth patterns. Thus, our results will contribute to a better understanding of the life history characteristics of these amphibians, which in turn can aid in the assessment of species management and conservation strategies within their natural reserve habitat. The loss of biodiversity is a pressing issue that generates significant concern today.

Body size and sexual size dimorphism
The body size of different species inhabiting isolated environments, such as islands or island-like systems (e.g. Inselbergs), can be affected by a combination of ecological and genetic factors (Van Valen, 1973; Baeckens, 2020; Lomolino, 2005), which has been demonstrated in many taxa including amphibians (Roth, 1990; Raia and Meiri 2006; Herridge and Lister, 2012; Montesinos, 2012; Rebouças *et al.*, 2018). The effect of resource availability may determine for example a smaller body size (insular dwarfism), due

Table 4. Estimated parameters from von Bertalanffy's equation, for body growth (SVL) of the three species studied. SVL_{max} = average maximal body size (mm), k = growth coefficient, defining the shape of the growth curve, r^2 = model fit. All values are means ±1 SE (CI 95%).traps and coverboards; * = lowest age class recorded in reproductive events (calling males or pairs in amplexus); PRLS = potential reproductive lifespan; SVL = snout-vent length; BM = body mass.

Estimated parameters/ species	SVL_{max} ± SE	k	r^2
Scinax fuscovarius			
Male (n = 23)	38.54 ± 0.79 (37 – 40.12)	2.14 ± 0.52 (0.72 – 2.51)	0.954
Female (n=11)	37.05 ± 0.76 (35.52 – 38.57)	1.23 ± 0.35 0.32 – 2.14	0.958
Odontophrynus asper			
Male (n = 11)	41.51 ± 1.14 (39.11 – 43.91)	1.16 ± 0.43 0.26 – 2.08	0.974
Female (n=14)	40.16 ± 0.77 (38.56 – 41.75)	2.08 ± 0.67 (0.69 – 4.48)	0.979
Melanophryniscus atroluteus			
Male (n = 30)	22.41 ± 0.38 (21.65 – 23.17)	0.96 ± 0.15 (0.65 – 1.27)	0.990
Female (n=23)	23.18 ± 0.37 (22.44 – 23.93)	1.13 ± 0.35 (0.44 – 1.86)	0.993

to competition for limited access to food resources, among other causes. In these isolated environments natural selection may act more intensively and rapidly. This can result in specific adaptations to body size to make the best use of resources and environmental conditions (Anderson and Handley Jr., 2002; Keogh *et al.*, 2005). Conversely, without predation pressure, due to the lack of predators in isolated environments, individuals may grow larger and reach a larger size (island gigantism or insular gigantism) (Lomolino, 1985; Barahona *et al.*, 2009; Herczeg *et al.*, 2009). In addition, in small, isolated populations, genetic drift may play an important role. Changes in body size may occur due to random mutations and lack of genetic exchange with larger populations (Pardo *et al.*, 2005; Wang *et al.*, 2014). One of the aims of our study was to test whether the Inselbergs outcrops of northeastern Argentina, which form an island-like system (see below), have an impact on the life history traits of the three most

abundant anuran species in these environments. Our results revealed that the hills did not have a significant effect on the analyzed morphometric variables, indicating that differences in body size cannot be attributed to the specific hill where the species inhabit. However, we observed a reduction in body size in the populations from the Inselbergs when compared to the populations of *S. fuscovarius* studied by Goldber *et al.* (2018). These authors found that the populations of *S. fuscovarius* varied from east to west and less from south to north, with frogs being largest in the northwestern populations. Interestingly, the authors also found that individuals of both sexes from the three studied regions of *S. fuscovarius* have a larger body size than the populations from the Inselbergs. Goldber *et al.* (2018) suggest that factors primarily related to the life history are mainly driving the geographical variation observed in *S. fuscovarius*. Furthermore, the authors observed that the degree of sexual size dimorphism was also greater in the western populations (Appendix A). However, we do not observe sexual size dimorphism in the populations from the Inselbergs. The sexual size differences in our study were very small and statistically not significant, which could be explained by the absence of significant differences between sexes both growth rates and age at sexual maturity (see following sections). Additionally, larger and older *O. asper* individuals than those found in the Inselbergs were observed in other populations analyzed by Otero *et al.* (2001). (Appendix A). These results lead us to consider the remaining question: is there complete isolation among the inselberg amphibian populations such that ecological and genetic factors would generate these observed differences in body size in the absence of gene flow and if so, was there enough time for it to happen? Individually-marks allow us confirms the absence of flow between Inselbergs populations, since we did not find individuals with a mark from one population to another (Piñeiro, 2022). This also was confirmed in our previous mark-recapture studies made in *Homonota taragui*, a critically endangered gecko endemic to the Inselbergs (Courtis *et al.*, 2022). Thus, we might suggest that specific processes, such as selection or genetic drift, might be acting in the hills in the absence of gene flow. However are needed studies primarily aimed at confirming whether there is isolation of the hill populations in relation to the surrounding matrix populations and those throughout the species' distribution range, as well as deeper studies to

determine the role of the evolutionary processes that are taking place in the Inselbergs amphibians of northern Argentina.

Age-related parameters and growth patterns

The study of life history traits, including age structure, size at sexual maturation, and growth rate, is crucial for understanding the evolutionary ecology of isolated populations. Island dwarfism and gigantism syndromes go beyond size differences and encompass significant changes in these traits. Our study showed no significant differences in age-related parameters between the Inselberg spe-
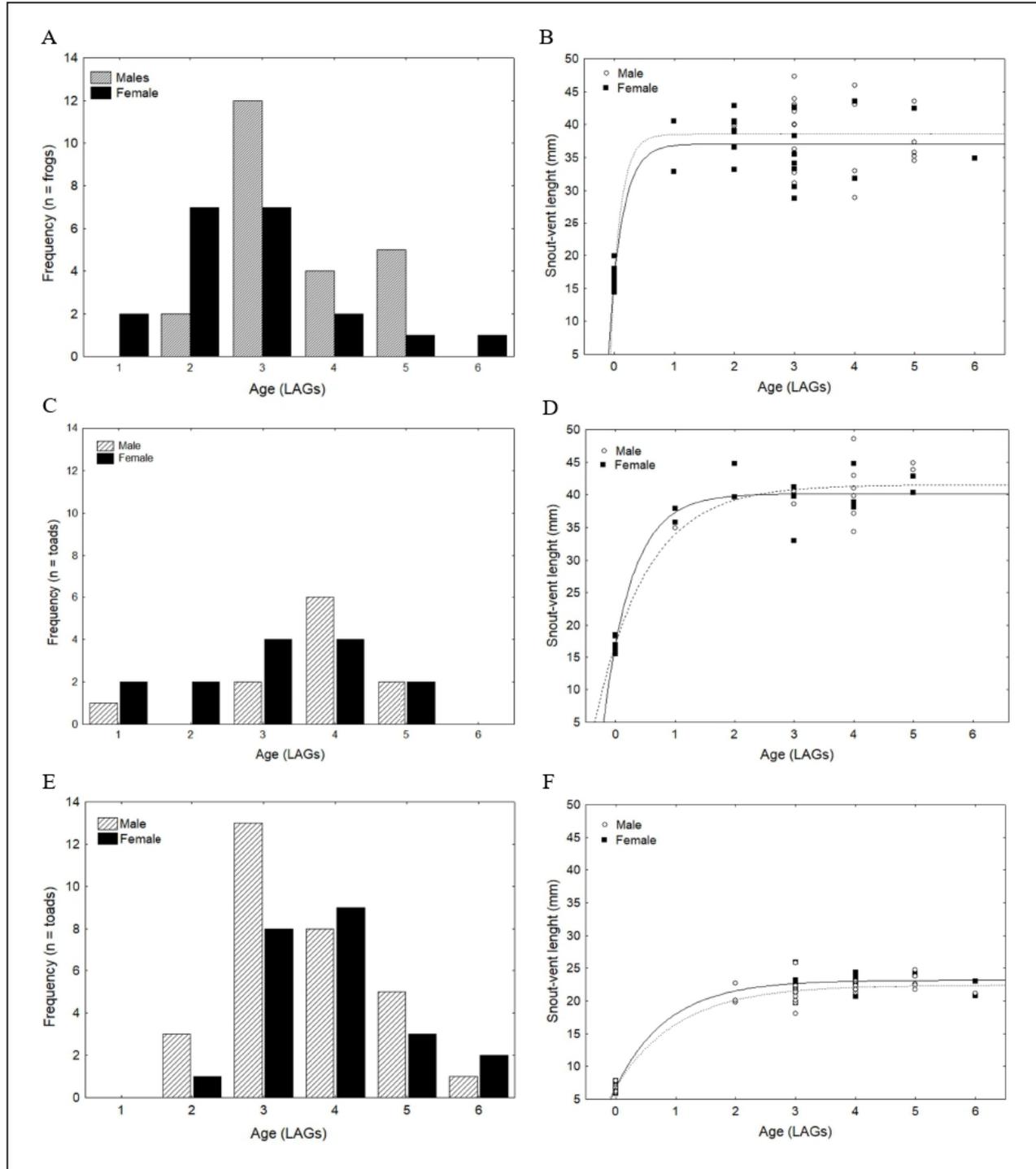
Figure 3. Age structure and growth curves fitted to the von Bertalanffy model, for body growth (SVL) of *Scinax fuscovarius* (A-B), *Odontophrynus asper* (C-D), and *Melanophryniscus atroluteus* (E-F).

cies. Similar ages at sexual maturity and longevity were found in males and females of *O. asper* and *M. atroluteus*, which align with the findings in others species within the same genera or family previously studied (see Appendix A). Nevertheless, females of *S. fuscovarius* matured one year earlier than males, and they are longer-lived than males. There are no other studies on age parameters conducted on *S. fuscovarius* to be discussed, however, differences in longevity were also observed in *Melanophryniscus rubriventris* (males/females = 10/4 years, Quinzio, 2003) and *Rhinella achalensis* (males/females = 8/11 years, Sinsch *et al.*, 2001) and *M. atroluteus* from de Atlantic Forest of Argentina (Marangoni and Baldo, 2023). Differences in ages of sexual maturity and longevity have been explained in terms of differences in growth rates and energy allocation in *R. achalensis* and other bufonids (Sinsch *et al.*, 200; Marangoni *et al.*, 2021; Marangoni and Baldo, 2023). In addition, differential mortality due to the significant energy investment by females in gamete production, which could shorten their life expectancy, was suggested in *M. rubriventris* (Quinzio, 2003). Our results suggest that there is no effect on the variables related to age due to the relative isolation and the potential absence of gene flow among the populations in the Inselbergs. Likewise, we consider our findings to be very preliminary, and more exhaustive studies will be necessary. This includes increasing the number of Inselbergs species and distant populations of the same species, allowing for better comparisons and analysis of the parameters studied. In addition, when analyzing differences in any life history traits between populations, we must analyze other causal sources that may be involved even in lack of genetic isolation (Hyeun-Ji, 2020).

Inselbergs (ISB) vs. Atlantic Forest of Argentina (AFA) populations

Variation in body size and age-related parameters in animals is influenced by many contingent factors, which may lead to intra and inter-population variations at small or large-spatial scales (Bergmann, 1847; James, 1970; Endler, 1977). This phenomenon has been well-documented in amphibians, with the major factors studied being latitude, altitude, and longitude (Bidau *et al.*, 2011; Goldberg *et al.*, 2018; Yu *et al.*, 2019; Hou *et al.*, 2023), terrestrial environment (Gomez-Mestre and Tejedo, 2005; Marangoni *et al.*, 2008), temperature and precipitation (Bidau
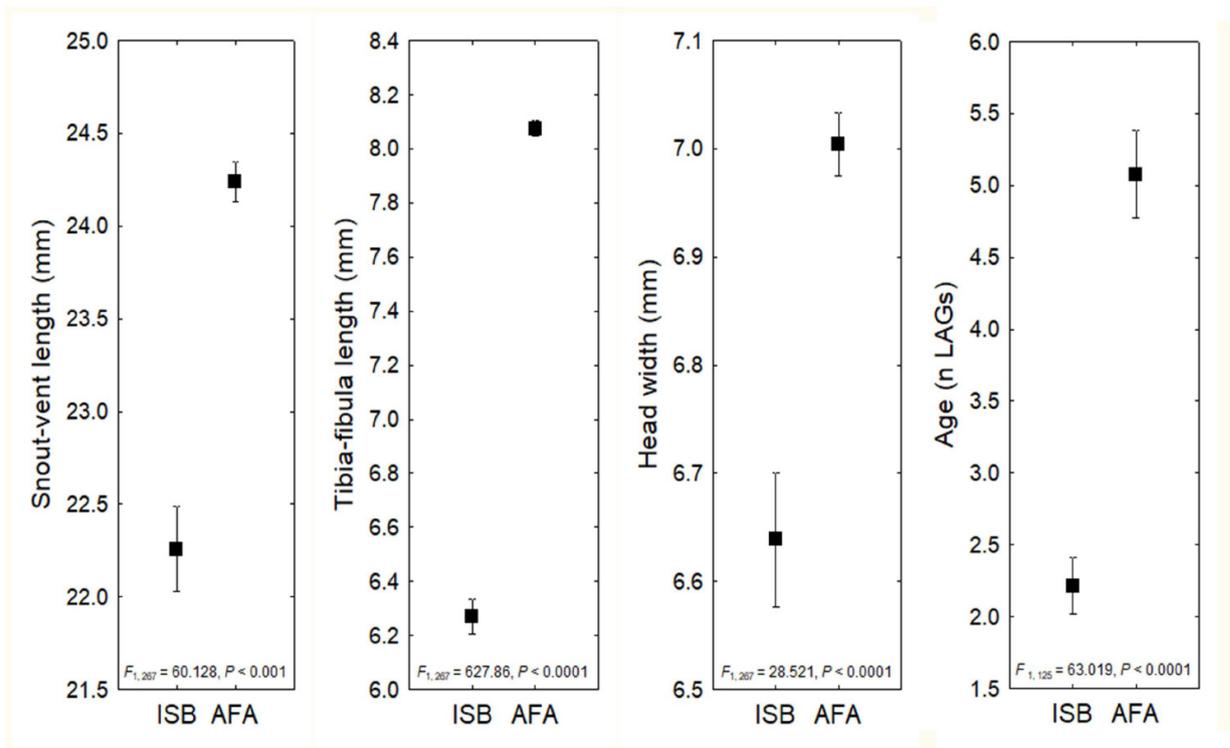
Figure 4. Body size and age differences between Inselberg (ISB, present study) and Atlantic Forest of Argentina (AFA, Marangoni and Baldo, 2023) populations of *Melanophryniscus atroluteus*. Vertical bars denote ± standard errors.
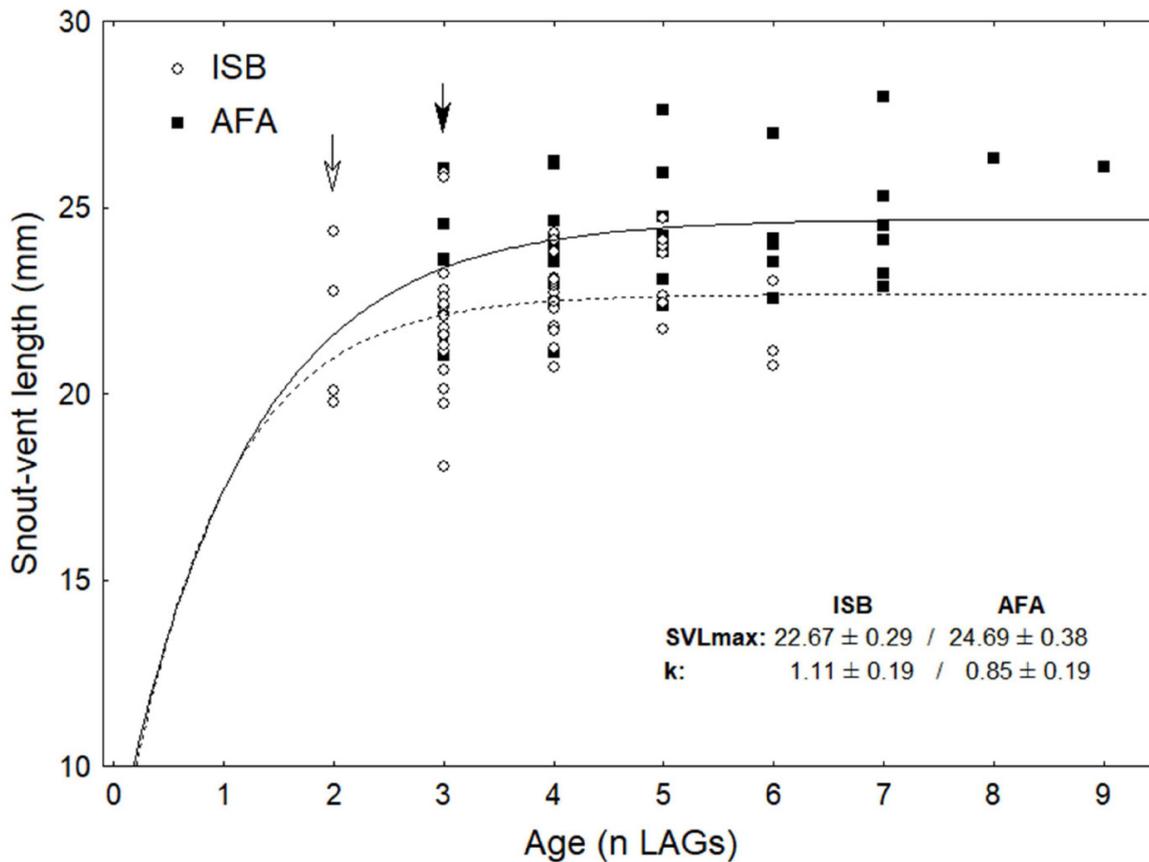
Figure 5. Growth pattern differences between Inselberg (ISB, present study) and Atlantic Forest of Argentina (AFA, Marangoni and Baldo, 2023) populations of *Melanophryniscus atroluteus*. Growth curves fitted to the von Bertalanffy model, for body growth (SVL). Arrows (white = ISB and black = AFA) denote the age at maturity. The parameter estimated by the model (SVLmax and k) are mean ± standard errors.

et al., 2011; Goldberg, 2018; Jiang *et al.*, 2022), food availability (Leips and Travis, 1994; Tracy, 1999), predators risk (Gómez, 2019), abundance (Green and Middleton, 2013), among others. *M. atroluteus* from the ISB showed significant differences in body size, age, and growth compared to the AFA populations analyzed by Marangoni and Baldo (2023). On average, they were smaller, younger, have a shorter lifespan, experience faster growth rates, and reach maturity earlier than the AFA populations (Fig. 4). We suggest that these differences in adult body size could be explained by analyzing the growth pattern and age of sexual maturity. ISB populations demonstrate accelerated growth, reaching sexual maturity earlier, which is associated with a commitment to attaining a smaller adult body size (Fig. 5). This growth pattern, linked to a specific life strategy (Shine, 1989, 1990), has been documented in numerous amphibian species (Halliday and Verrell, 1988;

Marangoni *et al.*, 2021). However, we also propose two alternative mechanisms that could account for the divergence of the ISB and AFA populations of *M. atroluteus*. It is since the size and growth performance of terrestrial juveniles may be better explained by the selective force driving phenotypic differentiation during the larval stage, rather than by terrestrial conditions (e.g., Altwegg, 2003). These two mechanisms are not mutually exclusive and could complement each other in shaping the observed differences. One of them is related to the specific environment where reproductive activity and larval development occur. Reproductive activity was recorded in both populations during autumn-winter, but it took place in a lentic environment (temporary ponds) in the ISB population, whereas it occurred in a lotic environment (streams) in the AFA population. On the other hand, the second's aspect is associated with inter-specific relationships within their habitat.

While we did not observe other amphibian species reproducing synchronously at the study site with *M. atroluteus* in the ISB population, the AFA population reproduces in synchrony with two other species of Melanophryniscus (*M. devincenzii* and *M. krauczuki*). As a result, it is common to observe larvae of the three species living in sympatry. Moreover, we frequently observed fighting or calling interactions among males of *M. atroluteus* in active defense of their calling sites. In addition, we also observed several other amphibian species, such as *Psedupaludicola falcipes*, *Physalaemus riograndensis*, *Leptodactylus gracilis*, *Limnomedusa macroglossa*, *Physalaemus cuvieri*, *Physalaemus* aff. *albonotatus* and *Rhinella azarai*, reproducing synchronously at the same sites throughout most of the breeding season or once the spring rains began (Marangoni and Baldo, 2023). The variation in body shapes due to the interaction between environment and phenotype has recently been studied in 17 species of *Melanophryniscus*, which inhabit different habitats (Severgnini *et al*., 2021). The researchers found that pond-dwelling tadpoles exhibited a higher rate of body shape evolution compared to stream-dwelling tadpoles, although the difference was not statistically significant. Additionally, the authors suggest that differences in the abundance of predators and competitors between environments, could lead to phenotypic variation and the selection of specialized body shapes (see also Huang *et al*., 2020). Thus, we propose that the differences in adult morphometric traits between ISB and AFA populations could also be explained as carryover effects of the environments and inter- and intra-specific competition experienced during the larval stages (Werner, 1986; Reques and Tejedo, 1997).

Literature cited
Altwegg, R. & Reyer, H.U. 2003. Patterns of natural selection on size at metamorphosis in water frogs. *Evolution* 57: 872-882.

Anderson, R.P. & Handley, C.O. Jr. 2002. Dwarfism in Insular Sloths: Biogeography, Selection, and Evolutionary Rate. *Evolution* 56: 1045-1058.

Arana, M.D.; Natale, E.; Ferretti, N.; Romano, G.; Oggero, A.; Martínez, G.; Posadas, P. & Morrone, J.J. 2021. Esquema biogeográfico de la República Argentina. *Opera lilloana* 56.

Argoitia, M.A.; Cajade, R.; Hernando, B.A. & Teta, P. 2021. Bat (Mammalia: Chiroptera) biodiversity in a subtropical inselberg ecosystem of Northeastern Argentina. *Revista de Biología Tropical* 69: 379-390.

Atkinson, D. 1994. Temperature and organism size-A biological law for ectotherms? *Advances in Ecological Research* 25: 1-58.

Baeckens, S. & Van Damme, R. 2020. The island syndrome. *Current Biology* 30: 329 -339.

Barahona, F.; Evans, S.E.; Mateo, J.A.; Garcia-Marquez, M.; Lopez-Jurado, L.F. 2000. Endemism, Gigantism and Extinction in Island Lizards: The Genus Gallotia on the Canary Islands. *Journal of Zoology* 250: 373-388.

Barthlott, W. & Porembski, S. 2000. Why Study Inselbergs? En: Porembski S. & Barthlott W. (eds). Inselbergs: Biotic Diversity of Isolated Rock Outcrops in Tropical and Temperate Regions. 1-6 pp. Ecological Studies, Springer-Verlag, Berlin.

Bergmann C. (1847) Uber die verhältnisse der wärmeökonomie der thiere zu ihrer grösse. *Göttinger Stud* 3: 595-708.

Beverton, R.J.H. & Holt S.J. 1957. On the dynamics of exploited fish populations. London: Fishery Invest Ser II, Vol. XIX, Ministry of Agriculture, Fisheries, and Food.

Bidau C.J.; Marti, D.A. & Baldo D. 2011. Inter- and intraspecific geographic variation of body size in South American redbelly toads of the genus *Melanophryniscus* Gallardo, 1961 (Anura: Bufonidae). *Journal of Herpetology* 45: 66-74.

Bornhardt, W. 1900. Zur OberfHichengestaltung und Geologie Deutsch-Ostafrikas. Reimer, Berlin.

Burke, A. 2003. Inselbergs in a changing world-global trends. D*iversity and Distributions*. 9: 375-383.

Cajade, R.; Etchepare, E.G.; Falcione, C.; Barrasso, D.A. & Álvarez, B.B. 2013a. A new species of *Homonota* (Reptilia: Squamata: Gekkota: Phyllodactylidae) endemic to the

hills of Paraje Tres Cerros Corrientes Province Argentina. *Zootaxa* 3709: 162-176.

Cajade, R.; Medina, W.; Salas, R.; Fandiño, B.; Paracampo, A.; García, I.; Pautasso, A.; Piñeiro, J.M.; Acosta, J.L.; Zaracho, V.H.; Avalos, A.; Gómez, F.; Odriozola, M.P.; Ingaramo, M.D.R.; Contreras, F.I.; Rivolta, M.D.; Hernando, A.B. & Álvarez. B.B. 2013b. Las islas rocosas del Paraje Tres Cerros: un refugio de biodiversidad en el litoral mesopotámico argentino. *Biológica* 16: 147-159.

Calder, W.A. 1984. Size, function and life history. Boston: Harvard University Press.

Castanet, J. & Smirina, E. 1990. Introduction to the skeletochronological method in amphibian and reptiles. *Annales des Sciences Naturelles, Zoologie et Biologie Animale* 11: 191-196.

Coor, R.; Warren, R. & Goudie, A. 1993. Desert Morphology. UCL Press London.

Courtis, A.; Cajade, R., Piñeiro, J. M.; Hernando, A.; Santoro, S. & Marangoni, F. 2022. Population ecology of a critically endangered gecko, endemic to north-eastern of Argentina. *Anais da Academia Brasileira de Ciências* 94: e20200388.

Cracraft, J. 1989. Species as Entities of Biological Theory. In: Ruse, M. (ed.) What the Philosophy of Biology Is. Nijhoff International Philosophy Series, vol 32. Springer, Dordrecht.

Green, D.M. & Middleton, J. 2013. Body size varies with abundance, not climate, in an amphibian population. *Ecography* 36: 947-955.

Donnelly, M.A. & Guyer, C. 1994. Patterns of reproduction and habitat use in an assemblage of Neotropical hylid frogs. *Oecologia* 98: 291-302.

Duellman, W.E. 1970. The hylid frogs of Middle America. *Monographs, Museum of Natural History, University of Kansas* 1: 1-753.

Ellis, L.T.; Afonina, O.M.; Czernyadjeva; I.V.; Konoreva, L.A.; Potemkin, A.D.; et al. 2020. New national and regional bryophyte records. *Journal of Bryology* 42: 1-17.

Endler, J.A. 1977. Geographic variation, speciation, and clines. Princeton University Pess, Princeton, New Jersey.

Esteban, M. & Sanchiz, B. 2000. Differential growth and longevity in low and high altitude *Rana iberica* (Anura, Ranidae). *Herpetological Journal* 10: 19-26.

Fandiño, B.; Fernández, J.M.; Thomann, M.L.; Cajade, R. & Hernando, A.B. 2017. Comunidades de aves de bosques y pastizales en los afloramientos rocosos aislados del Paraje Tres Cerros Corrientes Argentina. *Revista de Biología Tropical* 65: 535-550.

Fassetta, E. 2023. Estudio de rasgos de historia de vida de tres especies sintópicas del género Boana (Anura, Hylidae) mediante esqueletocronología. Undergraduate Thesis Project, Universidad Nacional del Litoral, Santa Fe, Argentina.

Fitzsimons, J.A. & Michael, D.R. 2017. Rocky outcrops: a hard road in the conservation of critical habitats. *Biological Conservation* 211: 36-44.

Foster, S.A. & Endler, J.A. 1999. Thoughts on geographic variation in behavior. Pp. 287-307 In: Foster, S.A. & Endler, J.A. (eds.). Geographic variation in behavior. Oxford University Press, New York, Oxford.

Fredericksen, N.J.; Fredericksen, T.S.; Flores, B.; McDonald, E. & Rumiz, D. 2003. Importance of granitic rock outcrops to vertebrate species in a Bolivian tropical forest. *Tropical Ecology* 44: 185-196.

Gervazoni, P.B. 2017. Diversidad de lepidópteros diurnos (Papilionoidea) del Cerro Nazareno (Reserva Natural Privada Paraje Tres Cerros) Corrientes Argentina. Unpublished Bachelor thesis. Universidad Nacional del Nordeste. Corrientes, Argentina.

Goldberg, J.; Cardozo, D.; Brusquetti, F.; Bueno Villafañe, D.; Caballero Gini, A. & Bianchi, C. 2018. Body size variation and sexual size dimorphism across climatic gradients in the widespread treefrog *Scinax fuscovarius* (Anura, Hylidae). *Austral Ecology* 2018: 35-45.

Gómez, V.I. 2019. The influence of tadpole density and predation on the behavioral responses of two Neotropical anurans. *Phyllomedusa* 18: 293-298.

Gomez-Mestre, I. & Tejedo, M. 2005. Adaptation or exaptation? An experimental test of hypotheses on the origin of salinity tolerance in *Bufo calamita*. *Journal of Evolutionary Biology* 18: 847-855.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16: 183-190.

Halliday, T.R. & Tejedo, M. 1995. Intrasexual selection and alternative mating behaviour. In: Heatwole, H. & Sullivan, B.K. (eds), Amphibian Biology, Social Behaviour, pp. 419-468.

Halliday, T.R. & Verrell, P. 1988. Body size and age in amphibians and reptiles. *Journal of Herpetology* 22: 253-265.

Hemelaar, A. 1985. An improved method to estimate the number of year rings resorbed in phalanges of *Bufo bufo* (l.) and its implications to populations from different latitudes. *Amphibia-Reptilia* 6: 323-341.

Hemelaar, A. 1988. Age, growth and other population characteristics of *Bufo bufo* from different latitudes and altitudes. *Journal of Herpetology* 22: 369-388.

Herczeg, G.B.; Gonda, A.L. & Merilä, J. 2009. Evolution of gigantism in Nine-Spined Sticklebacks. *Evolution* 63: 3190-3200.

Herridge, V.L. & Lister, A.M. 2012. Extreme insular dwarfism evolved in a mammoth. *Proceedings of the Royal Society B* 279: 3193-3200.

Hou, D.; Jia, T.; Ren, Y.; Zhu, W. & Liu, P. 2003. Phenotypic trait variations in the frog *Nanorana parkeri*: differing adaptive strategies to altitude between sexes. *Journal of Vertebrate Biology* 72: 1-11.

Huang, Y.; Wang, X.; Yang, X.; Jiang, J, & Hu, J. 2020. Unveiling the roles of interspecific competition and local adaptation in phenotypic differentiation of parapatric frogs. *Current Zoology* 66: 383-392.

Hyeun-Ji, L.; Broggi, J.; Sánchez-Montes, G.; Díaz-Paniagua, C. & Gomez-Mestre, I. 2020. Dwarfism in close continental amphibian populations despite lack of genetic isolation. *Oikos* 129: 1243-1256.

Isler, R.D. 2016. Tras la huella del ganado en las misiones jesuítas-guaraníes. Identificación de la ruta y catalogación de los caminos y estancias de la Cruz. Tesis Doctoral. Universidad de Granada.

James, F.C. 1970. Geographic size variation in birds and its relationship to climate. *Ecology* 51: 365-90.

Jiang, Y.; Zhao, L.; Luan, X. & Liao, W. 2022. Geographical variation in body size and the Bergmann's rule in Andrew's Toad (*Bufo andrewsi*). *Biology* 11: 1766.

Keogh, J.S.; Scott, I.A. & Hayes, C. 2005. Rapid and repeated

origin of insular gigantism and dwarfism in australian tiger snakes. *Evolution* 59: 226-233.

Leclair, M.H.; Leclair, R. & Gallant, J. 2005. Application of skeletochronology to a population of *Pelobates cultripes* (Anura: Pelobatidae) from Portugal. *Journal of Herpetology*, 39: 199-207.

Leips, J. & Travis, J. 1994. Metamorphic responses to changing food levels in two species of hylid frogs. *Ecology* 75: 1345-1356.

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. *Amphibia-Reptilia* 27: 365-375.

Lomolino, M.V. 1985. Body size of mammals on islands: the island rule reexamined. *American Naturalist* 125: 310-316.

Lomolino, M.V. 2005. Body size evolution in insular vertebrates: generality of the island rule. *Journal of Biogeophy* 32: 1683-1699.

Lovich, J.E. & Gibbons, J.W. 1992. A review of techniques for quantifying sexual size dimorphism. *Growth Develop Aging* 56: 269-281.

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, Spain, Universidad de Sevilla.

Marangoni, F. & Baldo D. 2023. Life-history traits of three syntopic species of the South American Redbelly toads (Anura: Bufonidae: *Melanophryniscus*) from the Atlantic Forest of Argentina. *Herpetological Conservation and Biology* 18: 213-228.

Marangoni, F., Tejedo, M. & Cogălniceanu, D. 2021. Can age and growth patterns explain the geographical variation in the body size of two toad species? *Anais da Academia Brasileira de Ciências* 93: e20190470.

Marangoni, F.; Tejedo, M. & Gomez-Mestre, I. 2008. Extreme reduction in body size and reproductive output associated with sandy substrates in two anuran species. *Amphibia Reptilia* 29: 541-553.

Meregalli, M. 1998. *Gymnocalycium angelae* spec. nov. eine neue Art aus Argentinien. *Kakteen und andere Sukkulenten* 49: 283-290.

Michael, D.R. & Lindenmayer, D.B. 2018. Rocky Outcrops in Australia: Ecology Conservation and Management. Csiro Publishing.

Montesinos, R.; da Silva, H.R. & de Carvalho, A.L.G. 2012. The "Island Rule" Acting on Anuran Populations (Bufonidae: *Rhinella ornata*) of the Southern Hemisphere. *Biotropica*, 44: 506-511.

Mora, C., Tittensor, D.P.; Adl, S.; Simpson, A.G.B. & Worm, B. 2011. How many species are there on Earth and in the ocean? *PLoS Biology* 9: e1001127.

Nadal, M.F.; Achitte-Schmutzler, H.C.; Zanone, I.; Gonzalez, P.Y. & Avalos, G. 2018. Diversidad estacional de arañas en una reserva natural del Espinal en Corrientes Argentina. *Caldasia* 40:129-143.

Odriozola, M.P. 2014. Complementariedad y solapamiento entre los nichos espacial y temporal de tres especies sintópicas de lagartijas del Paraje Tres Cerros, Corrientes, Argentina. Unpublished Bachelor thesis Universidad Nacional del Nordeste, Corrientes, Argentina.

Ojanguren-Affilastro, A.A.; Adilardi, R.S.; Cajade, R.; Ramírez, M.J.; Ceccarelli, F.S. & Mola, L.M. 2017. Multiple approaches to understanding the taxonomic status of an enigmatic new scorpion species of the genus *Tityus* (Buthidae) from the biogeographic island of Paraje Tres Cerros (Argentina). *PLoSONE*.12: e0181337.

Oliva, F. & Panizza, M.C. 2019. Visibilidad y paisaje en el sector centro-oriental de la provincia de Corrientes. *Anuario de Arqueología, Rosario* 11:77-90.

Pardo. L.M.; MacKay, I.; Oostra, B.; van Duijn, C.M. & Aulchenko, Y.S. 2005. The effect of genetic drift in a young genetically isolated population. *Annals of Human Genetics* 69: 288-295.

Peabody, C.E. 1958. A Kansas drought recorded in growth zones of a bullsnake. *Copeia* 1958: 91-94.

Piñeiro, J.M.; Cajade, R.; Hernando, A.B.; Courtis, A.; Ingaramo M.D.R & Marangoni, F. 2021. The isolated rocky outcrops of northeastern Argentina and their role on the herpetofauna conservation. *Anais da Academia Brasileira de Ciências* 93: e20190932.

Piñeiro, J.M 2022. Ampliando la interpretación funcional de los ecosistemas de inselbergs: una perspectiva desarrollada a partir del estudio multifocal de los anfibios y reptiles del Paraje Tres Cerros. PhD thesis, Corrientes, Argentina, Universidad Nacional del Nordeste.

Porembski, S. & Barthlott, W. 2000. Inselbergs: Biotic Diversity of Isolated Rock Outcrops in Tropical and Temperate Regions. Ecological Studies. Springer-Verlag, Berlin.

Porembski, S. 2007. Tropical inselbergs: habitat types, adaptive strategies and diversity patterns. *Revista Brasileira de Botanica* 30: 579-586.

Porembski, S.; Martinelli, G.; Ohlemiiller, R. & Barthlott, W. 1998. Diversity and ecology of saxicolous vegetation mats on inselbergs in the Brazilian Atlantic rainforest. *Diversity and Distributions* 4: 107 -119.

Quinzio, S.I. 2003. Determinación de edad y comportamiento asociado en *Melanophryniscus rubriventris* (Anura: Bufonidae). Undergraduate Thesis Project, Universidad Nacional de Córdoba, Córdoba, Argentina.

Raia, P. & Meiri, S. 2006. The island rule in large mammals: paleontology meets ecology. *Evolution* 60: 1731-1742.

Ravenna, P. 2003. Decisive proof on the validity of *Amaryllis* over *Hippeastrum* as mainly a South American genus including new species and new records of Amaryllidaceae from Argentina Brazil and Paraguay. *Onira* 9: 9- 22.

Ravenna, P. 2009. A survey in the genus *Cypella* and its allies (Iridaceae). *Onira* 12: 1-10.

Rebouças, R.; da Silva, H.R. & Solé, M. 2018. Frog size on continental islands of the coast of Rio de Janeiro and the generality of the Island Rule. *PLoS One* 13: e0190153.

Reques, R. & Tejedo, M. 1997. Reaction norms for metamorphic traits in natterjack toads to larval density and pond duration. *Journal of Evolutionary Biology* 10: 829-851.

Roth, V.L. 1990. Insular dwarf elephants: a case study in body mass estimation and ecological inference. Body size in mammalian paleobiology: estimation and biological implications. *Cambridge University Press:* 151–179.

Roth, V.L. 1990. Insular dwarf elephants: a case study in body mass estimation and ecological inference. In: Damuth, J. & MacFadden, B. J. (eds.), Body size in mammalian paleobiology: estimation and biological implications, pp. 151-179 New York, NY: Cambridge University Press.

Sagor, E.S.; Oullet, M.; Barten, E. & Green, D.M. 1998. Skeletochronology and geographic variation in age structure

in the wood Frog, *Rana sylvatica. Journal of Herpetology* 34: 469-474.

Scheffé, H. 1953. A method for judging all contrasts in the analysis of variance. *Biometrika* 40: 87-110.

Schmidt-Nielsen, K. 1984. Scaling. Why is animal size so important? New York: Cambridge University Press.

Severgnini, M.R.; Baldo, D.; Vera Candioti, M.F. & Provete, D.B. 2021. Environmental influence on the body shape of *Melanophryniscus* tadpoles (Anura: Bufonidae). Poster. https://doi.org/10.6084/m9.figshare.13863566.v1

Shine, R. 1989. Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *Quarterly Review of Biology* 64: 419461.

Shine, R. 1990. Proximate determinants of sexual differences in adult body size. *American Naturalist* 135: 278-283.

Sinsch, U.; Di Tada, I.E. & Martino, A.L. 2001. Longevity, demography and sex-specific growth of the Pampa de Achala toad, *Bufo achalensis* Cei, 1972. *Studies on Neotropical Fauna and Environment* 36: 95-104.

Sinsch, U.; Oromi, N. & Sanuy, D. 2007. Growth marks in Natterjack Toad (*Bufo calamita*) bones: histological correlates of hibernation and aestivation periods. *The Herpetological Journal* 17: 129-137.

Sinsch, U.; Pelster, B. & Ludwig, G. 2015. Large-scale variation of size-and age-related life-history traits in the common frog: a sensitive test case for macroecological rules. *Journal of Zoology* 297: 32-43.

Smirina, E.M. 1972. Annual layers in bones of *Rana temporaria. Zoologichesky Zhurnal* 51: 1529–1534.

Smirina, E.M. 1994. Age determination and longevity in amphibians. *Gerontology* 40: 133-146.

StatSoft. 2007. Statistica (Data analysis software system). Version 8. StatSoft, Tulsa.

Tomašević, N.; Cvetković, D.; Miaud, C.; Aleksić, I. & CrnobrnjaIsailović, J. 2008. Interannual variation in life history traits between neighbouring populations of the widespread amphibian *Bufo bufo. Revue d'Ecologie: La Terre et la Vie* 63: 73- 83.

Tracy, C.R. 1999. Differences in body size among Chuckwalla (*Sauromalus obesus*) populations. *Ecology* 80: 259-271.

Van Valen, L. 1973. Body Size and Numbers of Plants and Animals. *Evolution* 27: 27-35.

von Bertalanffy, L. 1938. A quantitative theory of organic growth. *Human Biology* 10: 181-213.

Wang, S.; Zhu, W.; Gao, X.; Li, X.; Yan, S.; Liu, X.; Yang, J.; Gao, Z. & Li, Y. 2014. Population size and time since island isolation determine genetic diversity loss in insular frog populations. *Molecular Ecology* 23: 637-48.

Werner, E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *The American Naturalist* 128: 319-341.

White, E.O.; Ernest, S.K.M.; Kerkhoff, A.J. & Enquist, B.J. 2007. Relationships between body size and abundance in ecology. *Trends in Ecology and Evolution* 22: 323-330.

Woodward, G.; Ebenman, B.; Emmerson, M.; Montoya, J.M.; Olesen, J.M.; Valido, A. & Warren, P.H. 2005. Body size in ecological networks. *Trends in Ecology and Evolution* 20: 402-409.

Yu, T.L.; Wang, D.L.; Busam, M. & Deng, Y.H. 2019. Altitudinal variation in body size in *Bufo minshanicus* supports Bergmann's rule. *Evolutionary Ecology* 33: 449-460.

Zaracho, V.H.; Céspedez, J.A.; Álvarez, B.B. & Lavilla, E.O. 2012. Guía de campo para la identificación de los anfibios de la provincia Corrientes (Argentina). Fundación Miguel Lillo. Publicación especial.

Species-populations/Sex	Mean AgeLAGs (n)			AM			Longevity			Mean SVLmm (n)			Source
	Male	Female	♂+♀	Male	Female	♂+♀	Male	Female	♂+♀	Male	Female	♂+♀	
Hylidae													
Boana puchella	3.07 ± 0.7 (15)	3.93 ± 1.07* (14)		2	3		4	7		37.06 ± 4.57 (15)	43.6 ± 4.4* (14)		1
Boana puchella (AR)			2.60 ± 0.51 (17)			2			3			40.26 ± 2.39 (17)	2
Boana puchella (LA)			3.29 ± 0.85 (19)			2			5			46.34 ± 2.97 (19)	2
Boana puchella (RC)			2.96 ± 0.76 (27)			2			4			43.27 ± 3.27 (27)	2
Boana cordobae	na	na		2	2		5	5		48.01 ± 4.99 (39)	51.27 ± 5.06* (21)		3
Boana punctata	3.46 ± 0.78 (13)	3.25 ± 0.9 ns (24)		2	2		5	5		32.03 ± 3.27 (13)	29.57 ± 3.4* (24)		1
Boana raniceps	3.77 ± 0.87 (22)	3.66 ± 1.5 ns (6)		3	2		6	6		58.44 ± 4.64 (22)	56.53 ± 6.28 ns (6)		1
Nyctimantis siemersi	3.52 ± 0.13 (40)	3.87 ± 0.17 ns (16)		2	3		5	5		69.17 ± 3.56 (59)	74.19 ± 4.14* (28)		4
Ololygon berthae	na	na		1	2		3	3		18.95 ± 1.14 (15)	21.7 ± 1.71* (12)		5
Scinax fuscovarius	3.52 ± 0.95 (23)	2.80 ± 1.24* (20)		2	1		5	6		38.96 ± 4.85 (31)	37.78 ± 4.3 ns (23)		6
Scinax fuscovarius (East)	na	na		na	na		na	na		43.65 2.58	44.35 1.91	43.92 2.35	7
Scinax fuscovarius (Center)	na	na		na	na		na	na		43.87 2.52	44.48 2.99	44.11 2.73	7
Scinax fuscovarius (West)	na	na		na	na		na	na		46.60 2.93	48.62 3.70	47.77 3.41	7
Cicloramphidae													
Odontophrynus americanus	3.73 ± 0.33 (11)	3.14 ± 0.35 ns (14)		1	1		5	5		40.58 ± 4.31 (11)	39.68 ± 3.04 ns (16)		6
Odontophrynus asper (4n)			4.19 ± 0.9 (37)			2			6			47.81 ± 2.21 (37)	8
Odontophrynus cordobae (2n)			3.94 ± 1.02 (34)			2			7			47.2 ± 2.97 (34)	8
Odontophrynus (Hybrids, 3n)			3.76 ± 0.92 (34)			2			6			46.36 ± 2.58 (34)	8

Species	AM males	AM females					SVL males	SVL females	Source
Odontophrynus cf. *barrioi*	2.05 ± 0.20 (20)	2.20 ± 0.21 ns (18)	1	1	3	4	51.46 ± 4.64 (20)	52.42 ± 4.13 ns (20)	9
Bufonidae									
Melanophryniscus atroluteus (ISB)	3.0 ± 1 (30)	3.87 ± 1.01 ns (23)	2	2	6	6	21.91 ± 1.89 (41)	22.49 ± 1.33* (27)	6
Melanophryniscus atroluteus (AFA)	4.56 ± 1.25 (18)	5.55 ± 1.61* (20)	3	3	7	9	23.63 ± 1.18 (148)	25.76 ± 1.4* (66)	10
Melanophryniscus devincenzii	5.27 ± 1.16 (15)	5.33 ± 0.90 ns (15)	3	4	7	7	23.49 ± 1.16 (67)	27.45 ± 1.54* (30)	10
Melanophryniscus krauczuki	3.06 ± 0.9 (17)	3.5 ± 0.99 ns (18)	2	2	5	5	20.88 ± 1.21 (94)	23.52 ± 1.44* (23)	10
Melanophryniscus rubriventris	4.03 ± 1.53 (32)	3.50 ± 0.52 ns (16)	3	3	10	4	38.75 ± 2.03 (157)	42.76 ± 3.45* (89)	11
Rhinella achalensis	5.27 ± 1.72 (91)	4.89 ± 1.57 ns (114)	1	1	8	11	57.97 ± 6.75 (91)	54.59 ± 6.8* (114)	12
Rhinella arenarum (C1)	1.6 ± 0.7 (15)	1.7 ± 0.6 ns (3)	1	1	3	2	93.8 ± 11.9 (15)	98.3 ± 1.8 ns (3)	13
Rhinella arenarum (C2)	2.2 ± 0.9 (16)	2.7 ± 1.2 ns (3)	2	1	4	4	101.5 ± 7.9 (16)	112.9 ± 3.5* (3)	13
Rhinella arenarum (C3)	1.9 ± 0.8 (15)	2 ± 1.4 ns (3)	1	2	3	3	92.3 ± 12.1 (15)	110.5 ± 4.28* (3)	13
Rhinella arenarum (UL)	2.9 ± 0.9 (14)	3.3 ± 1.2 ns (3)	1	2	4	4	93.5 ± 5.5 (14)	100.8 ± 6.0 ns (3)	13
Rhinella arenarum (SM)	2.7 ± 0.7 (28)	2.9 ± 0.9 ns (14)	1	1	5	4	100.6 ± 7.7 (28)	108.4 ± 9.9* (14)	13
Rhinella arenarum (2000)	2.4 ± 0.9 (105)		1	1	6		99.4 ± 8.8 (105)		14
Rhinella arenarum (2008)	3.0 ± 0.7 (21)	2.6 ± 0.9 ns (12)	2	2	5	4	101.5 ± 7.1 (39)	108.6 ± 9.6* (15)	14
Rhinella arenarum (2000)	2.4 ± 0.9 (105)		1	1	6		99.4 ± 8.8 (105)		14
Rhinella arenarum (2008)	3.0 ± 0.7 (21)	2.6 ± 0.9 ns (12)	2	2	5	4	101.5 ± 7.1 (39)	108.6 ± 9.6* (15)	14

Appendix A : AM = age at maturity. Sources: 1 = Fassetta, 2023; 2 = Baraquet *et al.*, 2021; 3= Otero *et al.*, 2017; 4 = Cajade *et al.*, 2013; 5 = Lezcano 2020; 6 = present study; 7 = Goldberg *et al.* 2018; 8= Otero *et al*, 2021; 9 = Quiroga *et al.*, 2015; 10 = Marangoni and Baldo 2023; 11 = Quinzio 2003; 12 = Sinsch *et al.*, 2001; 13 = Bionda 2018; 14 = Bionda *et al.*, 2015. Descriptive statistics are given as mean ± standard deviation or error (see sources) and sample size (n). Significant differences in SVL and mean age between males and females ($P < 0.05$) are marked with *. ns = not significant. na = not analyzed. ∞ = SVLmax estimated by the von Bertalanffy growth equation.