

# 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.

## 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).

*Inselbergs* (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; Frederickson *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).

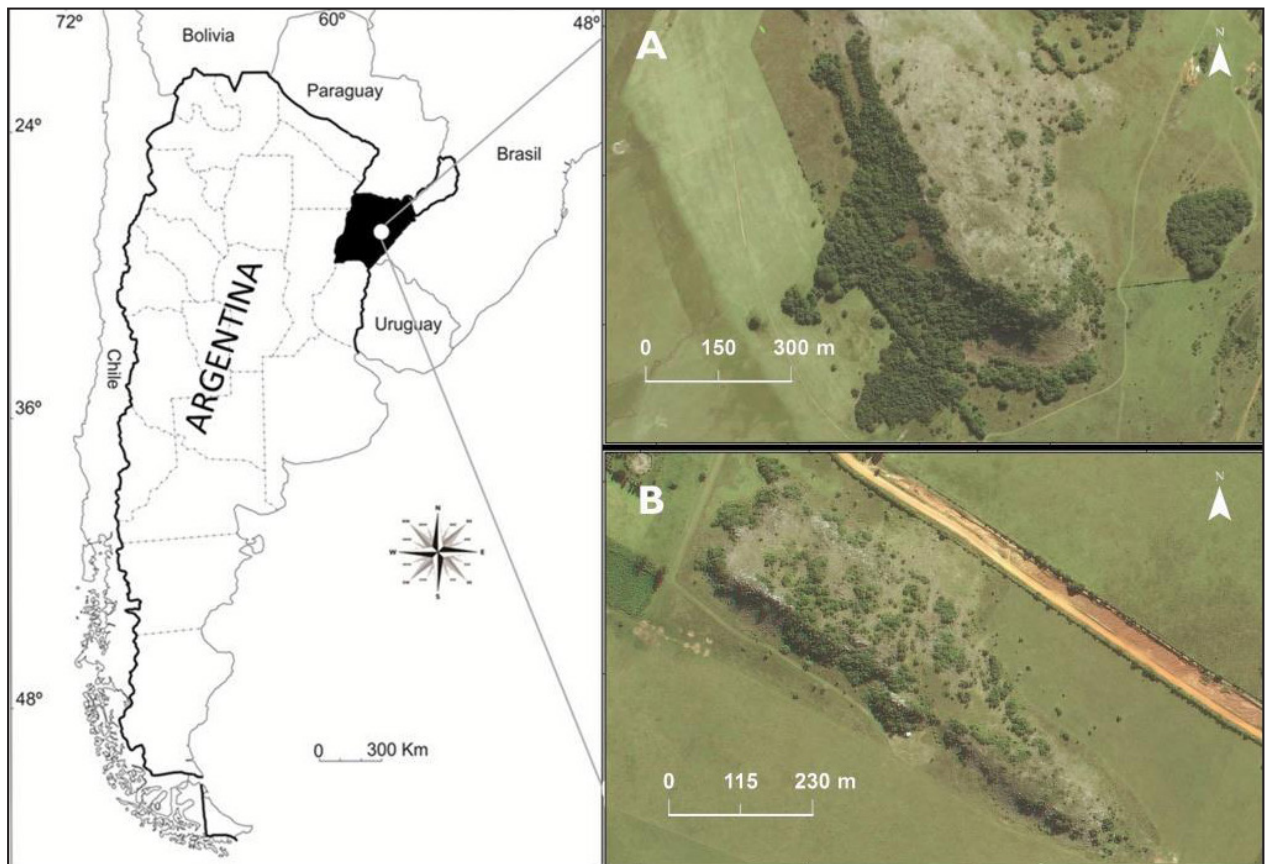
In northern Argentina, three isolated Inselbergs located in the east-central region of Corrientes province (Fig. 1) have been explored through an interdisciplinary program of several projects to study their biodiversity (Meregalli, 1998; Ravena, 2003, 2009; Cajade *et al.*, 2013a,b; Odriozola, 2014; 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 humid Chaco ecoregions in Argentina, Brazil, Paraguay, Uruguay (Zaracho *et al.*, 2012).

#### *Body size and Sexual size dimorphism*

##### *Sampling methods*

We analyzed the 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 ( $29^{\circ} 0.6' 28.8''$  S,  $56^{\circ} 55' 22.8''$  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  $\mu\text{m}$ , 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):  $S_t = S_m - (S_m - S_0) e^{-k(t-t_0)}$

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 ( $S_0$ , see 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 Shapiro-Wilk 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  $\alpha = 0.05$  (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):  $SDI = \text{mean size}_{\text{larger sex}} / \text{mean size}_{\text{smaller sex}}$ , 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  $\lambda = 0.884$ ,  $F_{4,69} = 2.259$ ,  $P = 0.072$ ; *O. asper*: Wilk's  $\lambda = 0.663$ ,  $F_{4,23} = 66.574$ ,  $P < 0.05$  and *M. atroluteus*: Wilk's  $\lambda = 0.585$ ,  $F_{4,8} = 1.419$ ,  $P = 0.312$ ). 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  $\pm$  1 SD..

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

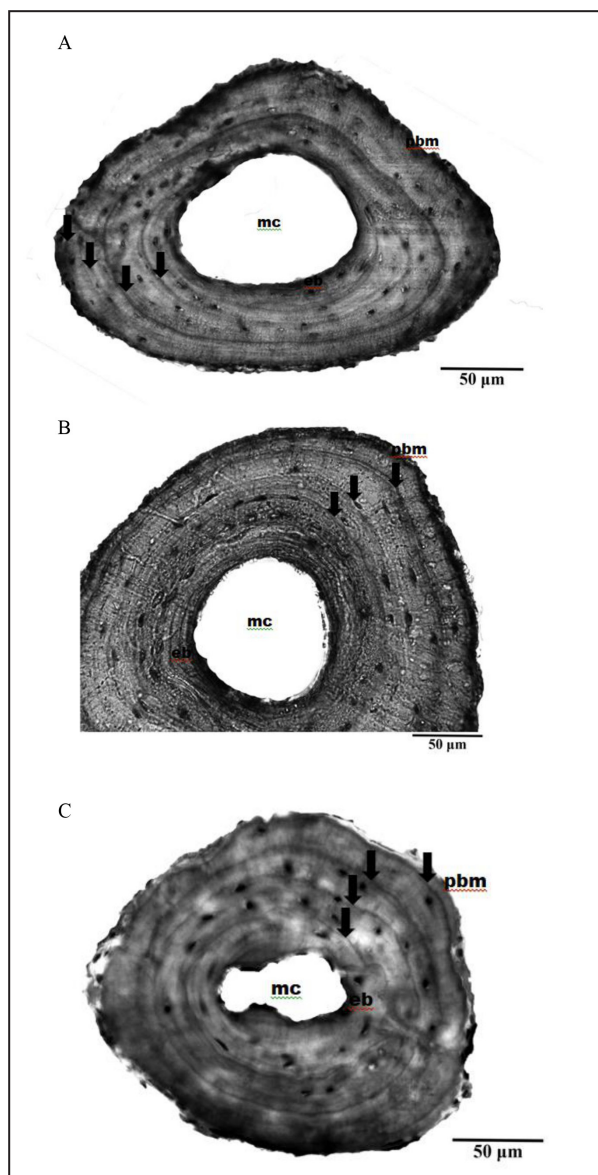
Specie/sex	Regression equation	r2	p	Regression equation	r2	p
<i>Scinax fuscovarius</i>						
		Male (n = 23)			Female (n = 20)	
SVL:BS	y = 7.967 - 0.646*x	0.206	<b>0.029</b>	y = 6.175 - 0.160*x	0.008	0.708
BM:BS	y = 5.861 - 0.172*x	0.159	0.059	y = 5.570 + 0.020*x	0.001	0.887
SVL:Age	y = 2.577 - 0.371*x	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
<i>Odontophrynus asper</i>						
		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
<i>Melanophryniscus atroluteus</i>						
		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	<b>0.002</b>	y = -3.912 + 1.698*x	0.147	<b>0.011</b>
BM:Age	y = 1.3456 - 0.0788*x	0.008	0.555	y = 1.6733 - 0.5504*x	0.178	<b>0.005</b>

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

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).



**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).

#### 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 younger 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;  $\Delta$  = 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 $\pm$ SD [LAGs]	Mode [Frequency]	Median Lifespan [yr]	AM [LAGs]	Longevity [LAGs]	PRLS [yr]	SVL at AM X $\pm$ SD mm [n]	BM at AM X $\pm$ SD mm [n]	Maximum Size SVL-BM mm/g [at age in LAGs]
<i>Scinax fuscovarius</i>									
Male (23)	3.52 $\pm$ 0.95	3 (12)	3	2 $\Delta$	5	3	39.54 $\pm$ 0.23 (n = 2)	4.17 $\pm$ 0.37 (n = 2)	47.31 - 7.59 (3 - 3)
Female (20)	2.80 $\pm$ 1.24	2-3 (7)	2	1 $\Delta$	6	5	36.69 $\pm$ 5.42 (n = 2)	3.88 $\pm$ 2.24 (n = 2)	43.47 - 6.72 (4 - 2)
<i>Odontophrynus asper</i>									
Male (11)	3.73 $\pm$ 0.33	4 (6)	4	1 $\Delta$	5	4	34.88 (n = 1)	8.35 (n = 1)	48.53 - 13.17 (4 - 5)
Female (14)	3.14 $\pm$ 0.35	3-4 (4)	3	1 $\Delta$	5	4	36.84 $\pm$ 1.02 (n = 2)	6.96 $\pm$ 0.61 (n = 2)	44.76 - 12.94 (4 - 2)
<i>Melanophryniscus atroluteus</i>									
Male (30)	3.0 $\pm$ 1	3 (3)	3	2*	6	4	20.87 $\pm$ 1.82 (n = 3)	1.21 $\pm$ 0.08 (n = 3)	25.83 - 2.1 (3-4)
Female (23)	3.87 $\pm$ 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  $\pm 1$  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	$SVL_{max} \pm SE$	$k$	$r^2$
<i>Scinax fuscovarius</i>			
Male (n = 23)	38.54 $\pm$ 0.79 (37 – 40.12)	2.14 $\pm$ 0.52 (0.72 – 2.51)	0.954
Female (n=11)	37.05 $\pm$ 0.76 (35.52 – 38.57)	1.23 $\pm$ 0.35 0.32 – 2.14	0.958
<i>Odontophrynus asper</i>			
Male (n = 11)	41.51 $\pm$ 1.14 (39.11 – 43.91)	1.16 $\pm$ 0.43 0.26 – 2.08	0.974
Female (n=14)	40.16 $\pm$ 0.77 (38.56 – 41.75)	2.08 $\pm$ 0.67 (0.69 – 4.48)	0.979
<i>Melanophryniscus atroluteus</i>			
Male (n = 30)	22.41 $\pm$ 0.38 (21.65 – 23.17)	0.96 $\pm$ 0.15 (0.65 – 1.27)	0.990
Female (n=23)	23.18 $\pm$ 0.37 (22.44 – 23.93)	1.13 $\pm$ 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

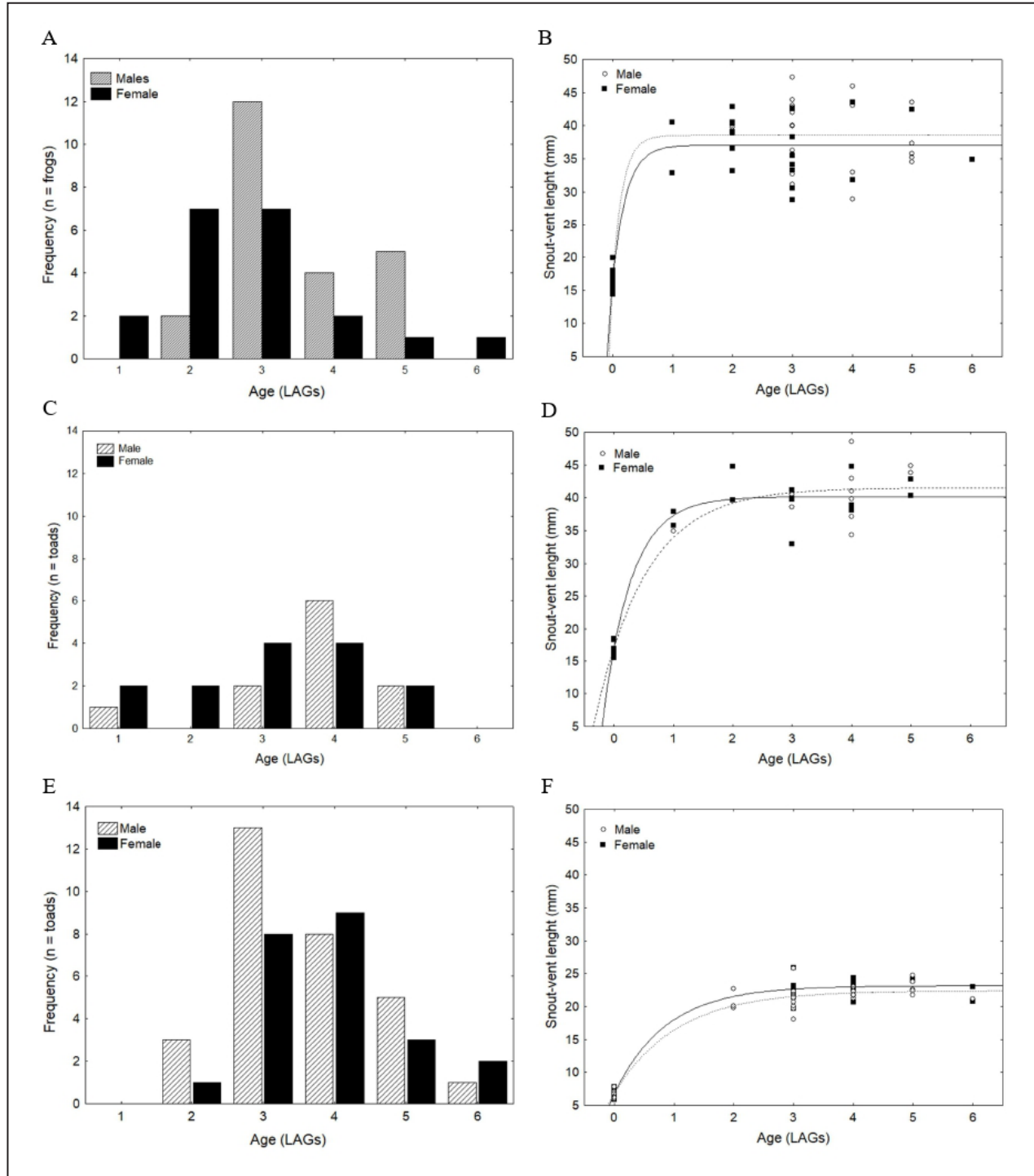
abundant anuran species in these environments. Our results revealed that the hills did not have a significant effect on the analyzed morphometric variables, indicating that differences in body size cannot be attributed to the specific hill where the species inhabit. However, we observed a reduction in 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 taraguí*, 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-



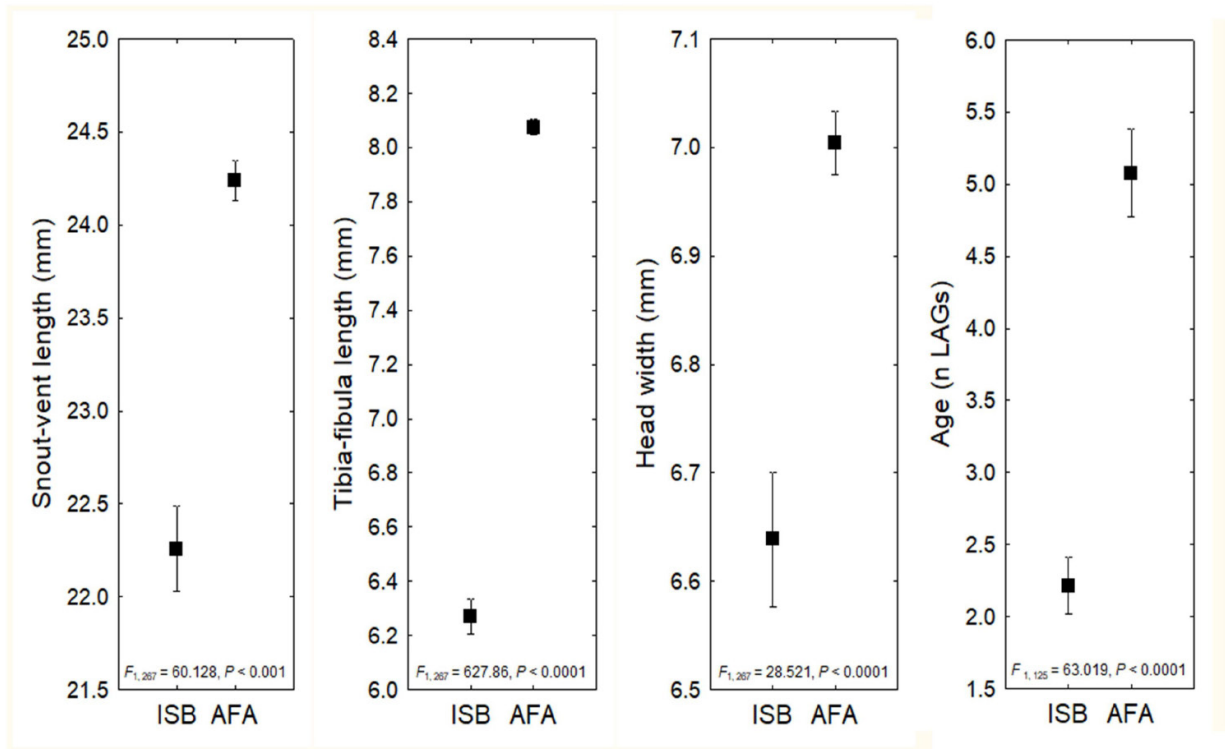
**Figure 3.** Age structure and growth curves fitted to the von Bertalanffy model, for body growth (SVL) of *Scinax fuscovarius* (A-B), *Odontophrynus asper* (C-D), and *Melanophryniscus atroluteus* (E-F).

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

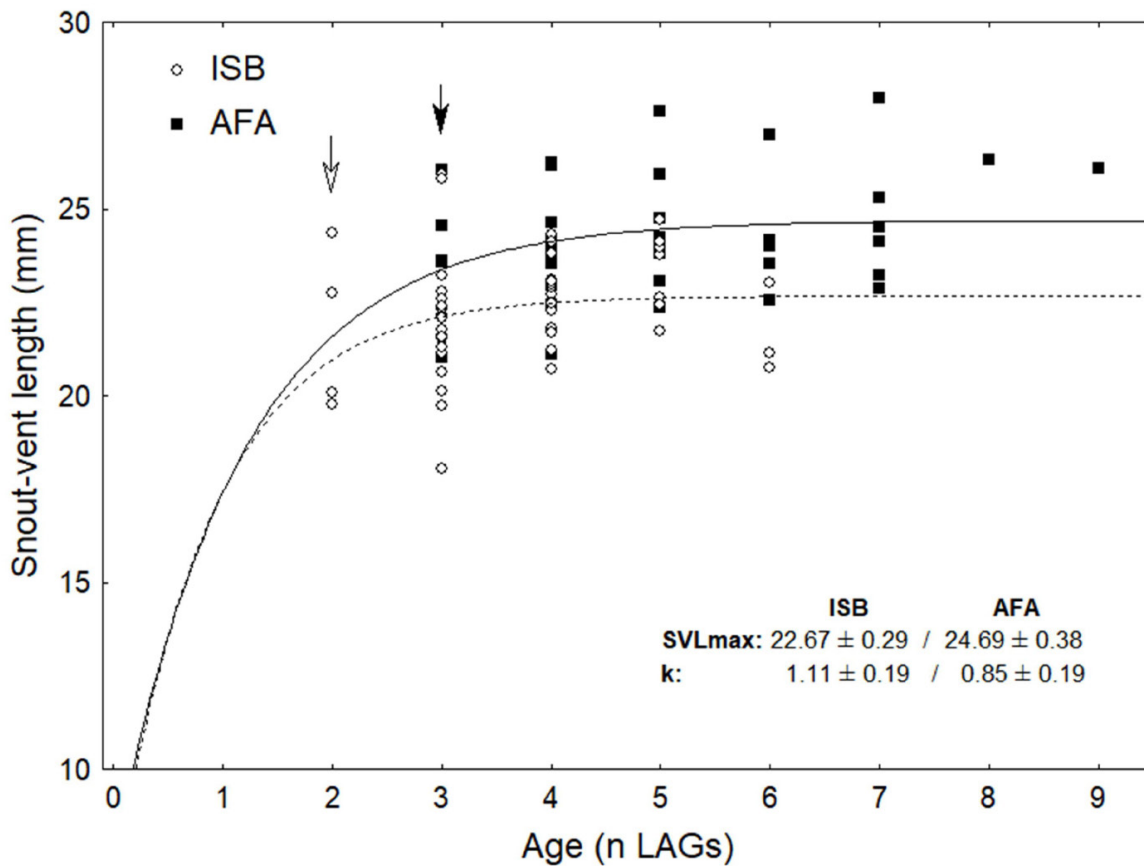
absence of gene flow among the populations in the Inselbergs. Likewise, we consider our findings to be very preliminary, and more exhaustive studies will be necessary. This includes increasing the number of Inselbergs species and distant populations of the same species, allowing for better comparisons 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  $\pm$  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 *Pseudopaludicola 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).

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Species-populations/Sex	Mean AgeLAGs (n)			AM			Longevity			Mean SVLmm (n)			Source
	Male	Female	♂+♀	Male	Female	♂+♀	Male	Female	♂+♀	Male	Female	♂+♀	
<b>Hylidae</b>													
<i>Boana puchella</i>	3.07 ± 0.7 (15)	3.93 ± 1.07* (14)		2	3	2	4	7		37.06 ± 4.57 (15)	43.6 ± 4.4* (14)		1
<i>Boana puchella</i> (AR)		2.60 ± 0.51 (17)				2		3			40.26 ± 2.39 (17)		2
<i>Boana puchella</i> (LA)		3.29 ± 0.85 (19)				2		5			46.34 ± 2.97 (19)		2
<i>Boana puchella</i> (RC)		2.96 ± 0.76 (27)				2		4			43.27 ± 3.27 (27)		2
<i>Boana cordobae</i>	na	na		2	2		5	5		48.01 ± 4.99 (39)	51.27 ± 5.06* (21)		3
<i>Boana punctata</i>	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
<i>Boana rariiceps</i>	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
<i>Nyctimantis siemersi</i>	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
<i>Oloolygon berthae</i>	na	na		1	2		3	3		18.95 ± 1.14 (15)	21.7 ± 1.71* (12)		5
<i>Scinax fuscovarius</i>	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
<i>Scinax fuscovarius</i> (East)	na	na		na	na		na	na		43.65 ± 2.58	44.35 ± 1.91	43.92 ± 2.35	7
<i>Scinax fuscovarius</i> (Center)	na	na		na	na		na	na		43.87 ± 2.52	44.48 ± 2.99	44.11 ± 2.73	7
<i>Scinax fuscovarius</i> (West)	na	na		na	na		na	na		46.60 ± 2.93	48.62 ± 3.70	47.77 ± 3.41	7
<b>Cyclorhynchidae</b>													
<i>Odontophrynus americanus</i>	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
<i>Odontophrynus asper</i> (4n)		4.19 ± 0.9 (37)				2		6			47.81 ± 2.21 (37)		8
<i>Odontophrynus cordobae</i> (2n)		3.94 ± 1.02 (34)				2		7			47.2 ± 2.97 (34)		8
<i>Odontophrynus</i> (Hybrids, 3n)		3.76 ± 0.92 (34)				2		6			46.36 ± 2.58 (34)		8

<i>Odontophrynus cf. barrioi</i>	2.05 ± 0.20 (20)	2.20 ± 0.21 ns (18)	1	1	3	4	51.46 ± 4.64 (20)	52.42 ± 6.413 ns (20)	9
<b>Bufonidae</b>									
<i>Melanophryniscus atroluteus</i> (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
<i>Melanophryniscus atroluteus</i> (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
<i>Melanophryniscus devincenzi</i>	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
<i>Melanophryniscus krauczuki</i>	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
<i>Melanophryniscus rubriventris</i>	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
<i>Rhinella achalensis</i>	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
<i>Rhinella arenarum</i> (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
<i>Rhinella arenarum</i> (C2)	2.2 ± 0.9 (16)	2.7 ± 1.2 ns (3)	1	2	4	4	101.5 ± 7.9 (16)	112.9 ± 3.5* (3)	13
<i>Rhinella arenarum</i> (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
<i>Rhinella arenarum</i> (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
<i>Rhinella arenarum</i> (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
<i>Rhinella arenarum</i> (2000)	2.4 ± 0.9 (105)		1		6		99.4 ± 8.8 (105)		14
<i>Rhinella arenarum</i> (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
<i>Rhinella arenarum</i> (2000)	2.4 ± 0.9 (105)		1		6		99.4 ± 8.8 (105)		14
<i>Rhinella arenarum</i> (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 *et al.*, 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.