



Patterns of growth and natural mortality in *Lysapsus bolivianus* (Anura, Hylidae, Pseudae) in an environmental protection area in the estuary of the Amazon River

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Abstract.—Recent reviews indicate that about one-third of amphibian species are threatened with extinction. Many of these species inhabit tropical areas in developing countries where deforestation and the degradation of natural bodies of water are major threats. *Lysapsus bolivianus* is a poorly known amphibian found throughout much of the central Amazon basin between Bolivia and the Amazon estuary, where it is subject to extensive anthropogenic pressures. The present study was based on samples of this species in an environmental protection area. The data obtained is important for understanding the population structure with respect to size, growth parameters (K , $A_{0.95}$, L_{∞} , \emptyset' , SVL_{max}), and natural mortality of the species. The results showed a sexual dimorphism in size, with females being larger. Both sexes presented fast growth rates ($K_{Male} = 0.71 \text{ year}^{-1}$; $K_{Female} = 0.70 \text{ year}^{-1}$), reaching asymptotic sizes ($SVL_{\infty Male} = 21.20 \text{ mm}$; $SVL_{\infty Female} = 25.60 \text{ mm}$) in less than twelve months, and longevity of <5 years. The species completes its metamorphosis in 20 days, reaching adult age at one month. The estimated natural mortality was 0.64 year^{-1} for males and 0.65 year^{-1} for females. The precocity of this species, as well as the frequency of individuals of various ages and sizes during the whole year, suggests it has developed tactics that allow its survival in this environment with small sizes (average 1.7 cm), which characterizes it as an r-strategist. Anthropogenic pressures in areas where *L. bolivianus* lives in Brazil, as in the area of the present study, make the species vulnerable because they increase its exposure to predators, reduce its breeding sites, and increase its mortality from agricultural pesticides.

Keywords. Amapá, Amphibia, Brazil, Eastern Amazon, population structure

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Introduction

While amphibian research has advanced considerably in recent decades throughout the world, the ecology of most species is still only poorly understood. This is reflected, for example, in the large number of amphibian species classified as Data Deficient by the IUCN (2016). The comprehensive Global Amphibian Assessment (GAA) estimated that more than 30% of the 7,881 recognized amphibian species are currently threatened with extinction, and that several hundred may already be extinct (Barnovsky et al. 2011; IUCN 2016).

The population dynamics of amphibians is still poorly known in comparison with the diversity of this group

(Measey et al. 2016) or other poikilothermic vertebrates, such as fishes and reptiles (Kozłowski and Teriokhin 1999; Shine 2010; Camargo et al. 2010; Loyola et al. 2008). This lack of data is a major obstacle to the development of effective conservation measures (Stuart et al. 2004).

The life history strategies of a species are reflected in a characteristic set of biological and demographic traits, such as age at first sexual maturation, fertility and mortality rates, reproductive patterns, and social organization (Steams 1992; Ricklefs 1977). Growth and mortality rates may be especially important for the understanding of population structure and dynamics, and the capacity of a species to cope with environmental disturbance

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(Radtke and Hourigan 1990; Pauly 1998).

In most tropical organisms, the assessment of growth in rigid structures, such as bones, scales, and woody stems, is impeded by the relative stability of the climate and associated ecological variables (Boujard et al. 1991; Marangoni et al. 2009). In this case, growth parameters may be estimated indirectly through data on parameters such as body size, which are more easily obtained from wild populations. These data can provide insights into the typical body sizes of different age groups, and the definition of cohorts (Basson et al. 1988).

A number of non-linear models have been proposed to describe growth patterns in animals, such as the Brody, von Bertalanffy, and Gompertz functions, and logistic procedures. The Bertalanffy model is the most popular model for analyzing animal population dynamics. It is based on the assumption that growth can be estimated from the difference between the anabolic and catabolic rates of an animal (Bertalanffy 1957; Hota 1994).

The frog *Lysapsus bolivianus* Gallardo, 1961 is a semiaquatic anuran with both nocturnal and diurnal habits, which inhabits the water surface in patches of floating aquatic vegetation (Bosch et al. 1996; Garda et al. 2010; Santana et al. 2013). This species is small in size (mean SVL = 17.6 mm), and is widely distributed in the Amazon basin, ranging from the mouth of the Amazon River in Brazil to northern Bolivia (Frost 2018). It is found in the Rio Curiaú Environmental Protection Area (Rio Curiaú EPA) in Amapá state, northern Brazil, the location of the present study (Melo-Furtado et al. 2014). This protected area has been extensively impacted by human activities, such as deforestation, unregulated fishing, construction of buildings, the accumulation of domestic refuse, landfill of floodplains, and the indiscriminate use of agricultural pesticides in the surrounding areas. All these processes may impact the local biota, especially the anurans, such as *L. bolivianus*.

The present study evaluated the growth and mortality parameters of the *L. bolivianus* population of the Rio Curiaú EPA, together with estimates of longevity and growth performance. These data will hopefully contribute to the development of effective conservation strategies for the study species and other amphibians, as well as the study area in general.

Materials and Methods

Study Area

The present study was conducted in the Rio Curiaú Environmental Protection Area (00°09'00.7"N, 51°02'18.5"W), or Rio Curiaú EPA, which lies to the north of the Amazon River estuary in the state of Amapá, northern Brazil. The Rio Curiaú EPA encompasses 21,700 ha, an area dominated by aquatic systems, such as rivers and seasonal lakes. The local vegetation is mainly Cerrado savanna and floodplain forest. The region's

climate is humid equatorial (*Am*) in the Köppen-Geiger classification system, with a mean monthly temperature of 27.6 °C (range: 25.8–29 °C) and mean annual rainfall of approximately 2,850 mm, with a monsoon period between February and May when the monthly precipitation is around 400 mm (Alvares et al. 2013). The number and sizes of the ponds found within the study area decrease considerably during the dry season.

Sample Collection

Between January and December 2015, frogs were captured randomly by hand during the night, using 9 V flashlights. Frogs were collected by active searches along five 1 km transects in floating vegetation (*Nymphoides indica* [L.] Kuntze and *Salvinia auriculata* Aubl.). The transects were separated by a distance of at least 50 m and were walked by three researchers during each survey (Crump and Scott 1994). The frogs captured were examined to determine their sex and age (adult or juvenile), based on the presence of the nuptial sac in males and the distended or flaccid abdomen (before or after spawning) of the mature females, and their positions in amplexus.

The snout-vent length (SVL, in mm) of each specimen was measured using a tape measure and calipers, and the weight (*Wt*, in g) was recorded using a spring balance (0.01 g precision). Sample collection was authorized by the Brazilian Environment Institute (IBAMA) and the Information and Authorization System (SISBIO) of the Chico Mendes Institute for Biodiversity Conservation (ICMBio) through license number 34238-1. After measurements were taken, all individuals were released at the capture site.

Statistical Analyses

Deviations in the sex ratio were evaluated using a Chi-square test with Yeats' correction. The SVL values were grouped into classes to permit the visualization of the differences between adults and juveniles, and between mature males and females. The difference in the mean body size (SVL) between males and females was analyzed using a *t* test. For this, the assumptions of normality and homoscedasticity were tested *a priori* using the Kolmogorov-Smirnov and Bartlett tests, respectively. A significance level of 5% was considered in all cases.

The total length-weight relationship was determined by the Sparre et al. (1989) allometric equation $Wt = a * L^b$, where *Wt* = body weight (g), *L* = SVL (mm), and 'a' and 'b' = regression constants. Growth parameters were based on the Von Bertalanffy equation, $SVL = SVL_{\infty} * (1 - e^{-k(t-t_0)})$ [Sparre and Venema 1998], where *SVL* = total snout-vent length (mm) at age *t*, *SVL*_∞ = asymptotic snout-vent length (mm), *K* = growth rate (year⁻¹), *t* = the age in years, and *t*₀ = the nominal age at metamorphosis, assumed to be zero. The constants *K* and *SVL*_∞ were estimated by the Ford-Walford model (Ford 1933; Walford 1946).

Growth and mortality of *Lysapsus bolivianus* in Brazil

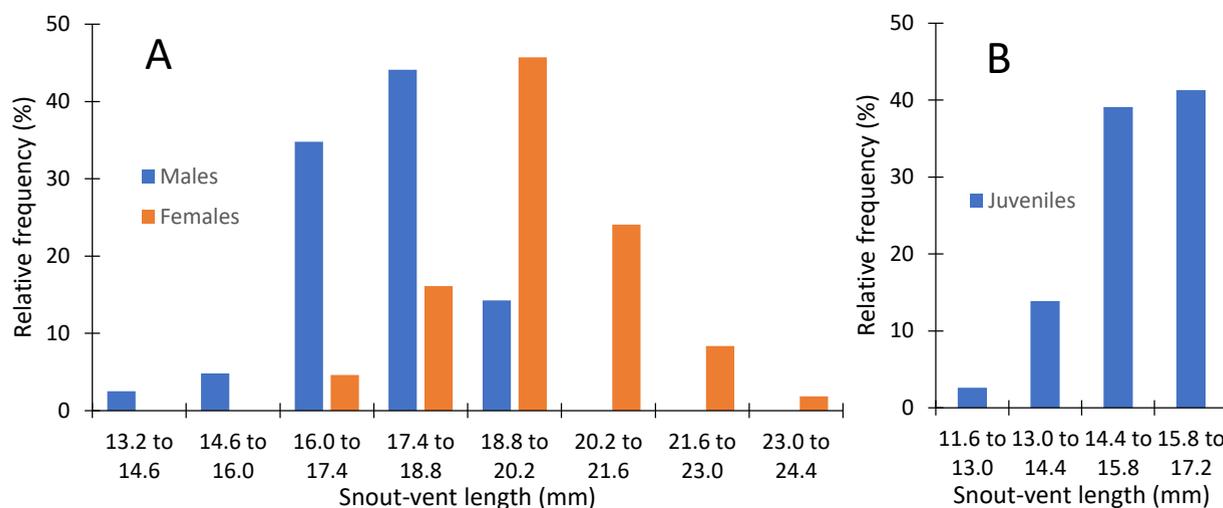


Fig. 1. Relative frequency of the body size classes (SVL, snout-vent length; mm) recorded in the (A) adult males and females and (B) juveniles of the *Lysapsus bolivianus* population from the Rio Curiaú EPA on the estuary of the Amazon River, in northern Brazil.

Longevity ($A_{0.95}$ or t_{max}) was calculated using the Taylor (1958) equation, $A_{0.95} = t_0 + (2.996/K)$, and natural mortality (M) was estimated using the Hoenig (1983) equation which is based on the empirical relationship observed between M and the maximum age described by the equation $\ln M = 1.46 - 1.01[\ln(t_{max})]$, where t_{max} = the maximum age in the population, and M = the natural rate of mortality. The asymptotic weight (W_∞) was estimated by converting L_∞ to the corresponding weight using the Pauly (1998) formula for the length-weight relationship ($W_\infty = a * SVL_\infty^b$). The growth performance (ϕ') was estimated by the Pauly and Munro (1984) formula: $\phi' = \log k + 2 \log SVL_\infty$. Juveniles were analyzed separately from the adults due to their much faster juvenile growth rates (mean = 16.0 ± 3.6 days). As the sex of the juveniles could not be determined, the data were pooled for this age class.

Results

A total of 308 mature *L. bolivianus* individuals were examined, together with 71 individuals classified as juveniles. Overall, mature males ($n = 188$) were significantly more abundant than mature females, with $n = 120$ (χ^2 [with Yates' correction] = 14.57; $df = 1$; $p = 0.001$). However, the females were significantly larger, on average, than males, with a mean SVL in the females of 19.81 ± 1.35 mm (range = 16.40–23.48 mm) versus $17.60 \pm$

1.13 mm (14.11–20.17 mm) in the males ($t = 15.26$; $df = 306.0$; $p < 0.0001$). Clear peaks in body size were observed in both sexes (Fig. 2), with 79.0% of the adult males having an SVL of 16.0–18.8 mm, and 78.2% of the females at 18.8–21.6 mm. The juveniles presented a mean SVL of 15.42 ± 1.07 mm (range: 12.00–16.96 mm), with 81.13% of the specimens lying between 14.4 and 17.2 mm (Fig. 1).

Highly significant coefficients of determination were recorded for the total length-weight relationships in both adult males ($R^2 = 0.63$; $F_{(2,185)} = 67.29$; $p < 0.0001$) and females ($R^2 = 0.74$; $F_{(2,117)} = 521.51$; $p < 0.0001$), indicating different models for the two sexes. The b values of males and females were both lower than 3, which indicate negative allometric growth (Fig. 2).

The relationship between the mean SVL at age t and $t + 1$ (SVL + 1) was described adequately by Walford's equation: $SVL_{t+1} = 12.971 + 0.4192 SVL_t$, $R^2 = 0.989$ for males and $SVL_{t+1} = 12.149 + 0.5198 SVL_t$, $R^2 = 0.959$ for females (Fig. 4). The intersection between the function and the diagonal drawn through the origin provides the value of L_∞ , which was 21.20 mm in males and 25.47 mm in females. Based on the formula $L_\infty \sim L_{max} / 0.95$ (Taylor 1958), the estimated values of SVL_∞ were 21.17 mm for males, and 24.65 mm for females, values which are very close to those derived from the graphs (Fig. 3). Estimated growth parameters are presented in Table 1. In general, the parameters were similar between males and females,

Table 1. Growth parameters for *Lysapsus bolivianus* specimens from the Rio Curiaú EPA in Amapá, Brazil. SVL_{max} = maximum length; SVL_∞ = asymptotic length; k = growth constant; ϕ' = growth performance; M = mortality; $A_{0.95}$ = longevity; n = sample size.

Parameter	Males ($n = 188$)	Females ($n = 120$)	Juveniles ($n = 71$)
K (year⁻¹)	0.71	0.70	0.81
SVL_∞ (mm)	21.20	25.60	59.50
ϕ'	2.50	2.65	3.45
A_{0.95}	4.20	4.28	3.70
SVL_{max} (mm)	20.17	23.48	16.96
M (year⁻¹)	0.64	0.65	12.86

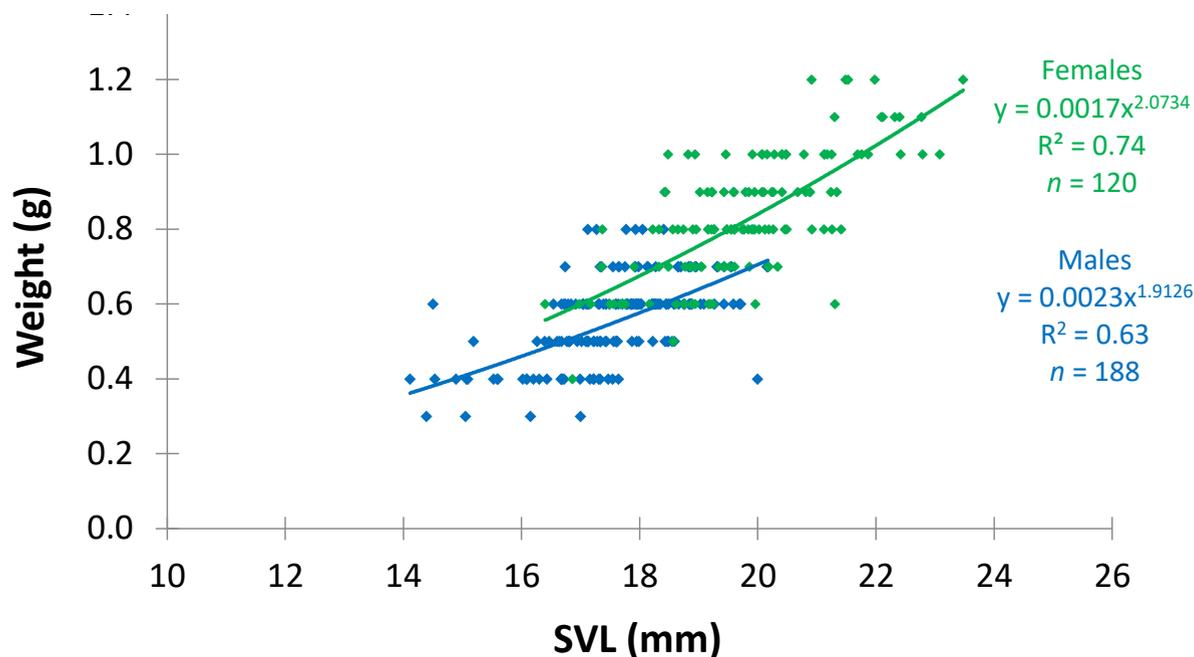


Fig. 2. Weight-length relationships in adult male and female *Lysapsus bolivianus* from the Rio Curiaú EPA on the estuary of the Amazon River, in northern Brazil.

although asymptotic size and growth performance varied between the sexes. Growth rates, longevity, and natural mortality were equal in the sexes.

Males grew 2.22 mm, on average, from an age of 3 to 6 months, 0.26 mm from 6 to 9 months, and only 0.04 mm from 9 to 12 months (Table 2). In females, growth over these same intervals was 2.80 mm, 0.30 mm, and 0.10 mm, respectively. This variation was shown in the Bertalanffy growth curves (Fig. 4), which followed distinct patterns in the males and females.

Discussion

The predominance of males recorded in the present study was consistent with the findings of Melo-Furtado et al. (2014). The male-biased sex ratio in *L. bolivianus* may be advantageous for the fertilization of the largest possible number of eggs. Other studies have related deviations in sex ratio to factors including differential growth and mortality, as well as fluctuations in the availability of nutrients and behavioral variations, all of which may have varying influences on the proportion of the sexes

Table 2. Average snout-vent length at different ages calculated for adult male and female *Lysapsus bolivianus* from the Rio Curiaú EPA in Amapá, Brazil.

	Average SLV (mm) at age indicated			
	3 months	6 months	9 months	12 months
Male	18.68	20.90	21.16	21.20
Female	22.40	25.20	25.50	25.60

at different stages of development (Hamilton and Zuk 1982; Vazzoler 1996; Kraab and Pen 2002; Fawcett et al. 2011; Booksmythe et al. 2013).

The growth rates of both male and female *L. bolivianus* were relatively high (> 0.5), which is typical of species found in highly seasonal habitats, such as that of the study area, as well as those that suffer high rates of predation (Lowe-McConnell 1999). These species grow rapidly, reaching maturity sooner with smaller asymptotic body lengths than larger species with slower growth rates (Pauly 1998).

A reduced asymptotic length, while determined genetically, may also be influenced by variables such as the food supply and population density (Parker 1983; Hubbell and Johnson 1987). Pauly (1998) concluded that low values are typical of tropical species and may be attributed to the combination of a number of factors, particularly temperature, given that higher temperatures accelerate growth and metabolic rates but tend to decrease the asymptotic size (Lomolino and Perault 2007). Faster growth to maturity may also be a strategy to compensate for predation pressure (Reznick et al. 1996).

The natural mortality rates in both sexes were moderate to high, and varied proportionately with growth rates, indicating that the principal causes of mortality in this species are predation and longevity, related to its rapid life cycle (Keiber 1932; Pauly 1998). While the *L. bolivianus* males grow faster than the females, both sexes have similar longevities, which favors reproductive success in a highly seasonal environment that is characterized by long periods of drought.

Growth and mortality of *Lysapsus bolivianus* in Brazil

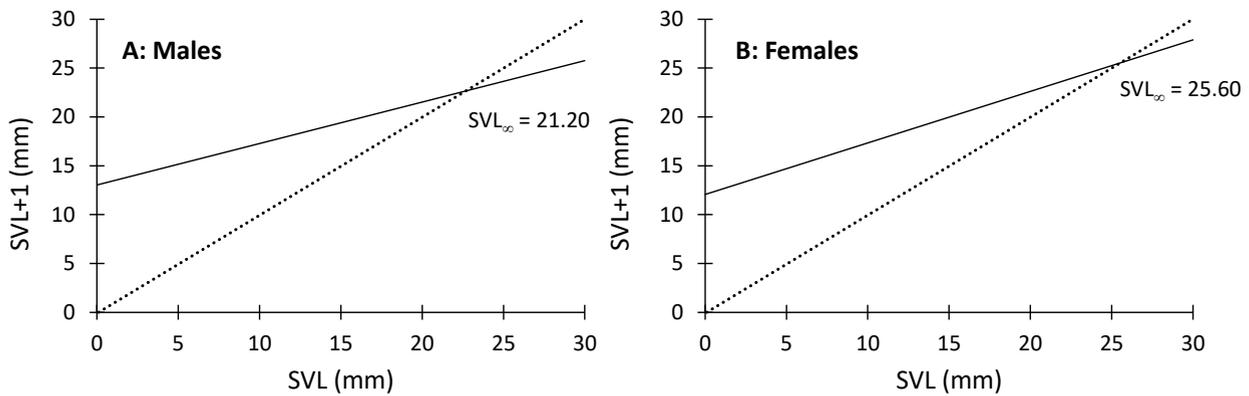


Fig. 3. Plot of the Ford-Walford estimates of growth parameters (SVL_{∞} , k) of adult (A) male and (B) female *Lysapsus bolivianus* from the Rio Curiaú EPA in Amapá, Brazil. The values were estimated by the linear regressions between SVL and SVL+1 for each gender, as $SVL_{\infty} = (a/1-b)$ and $K = -\log_e b$.

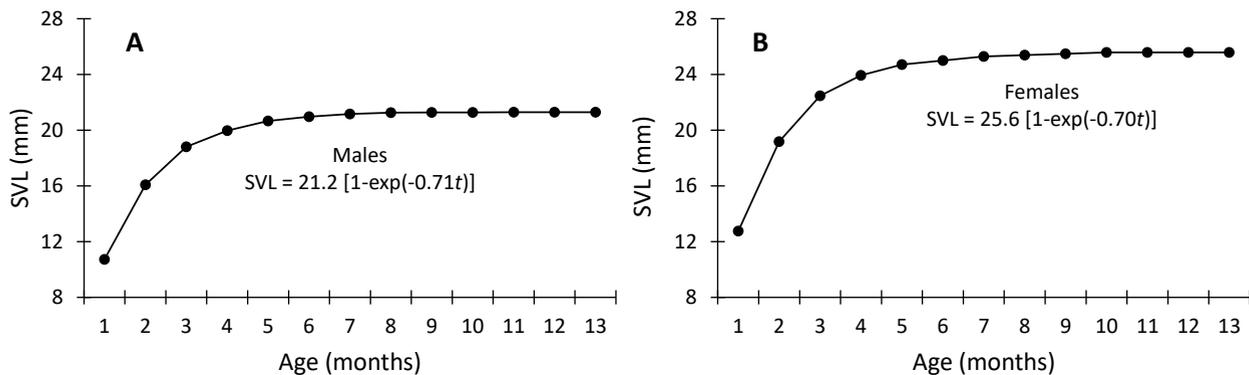


Fig. 4. Von Bertalanffy's growth curves for (A) male and (B) female *Lysapsus bolivianus* from the Rio Curiaú EPA in Amapá, Brazil.

Conclusions

All parameters analyzed indicate an *r* type of life history strategy in *L. bolivianus*. This is typical of species that inhabit highly unstable environments, such as that of the present study area, which is subject to marked seasonal fluctuations in water levels, reinforcing the rapid growth, small size, and reduced longevity of these frogs. Anthropogenic pressures in the areas occupied by *L. bolivianus* in Brazil, such as the present study area, augment the vulnerability of this species due to increasing exposure to predators, the reduction in available breeding sites, and increasing mortality through the use of agricultural pesticides.

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Growth and mortality of *Lysapsus bolivianus* in Brazil

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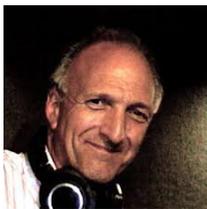
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