Ecology and natural history of *Hypsiboas curupi* (Anura, Hylidae): An endemic amphibian to the southern Atlantic Forest

Ecologia e história natural de *Hypsiboas curupi* (Anura, Hylidae): um anfíbio endêmico do sul da Mata Atlântica

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Abstract

Data on habitat use and life history are essential for evaluation of the conservation status of species, and may direct policy for preservation of natural environments. Over the course of a year, we investigated populations of *Hypsiboas curupi* regarding (i) nighttime activity patterns; (ii) associations with climatic variables; (iii) the spatial distribution patterns of males and females with respect to microhabitat use; and (iv) variation in body size. The study was carried out from August 2010 to July 2011 in the Parque Estadual Fritz Plaumann, a seasonal deciduous forest fragment in the western region of Santa Catarina, southern Brazil. Calling activity was highest from August to November. The number of calling males was highest at 23h, and the frequency of occurrence was influenced by the air humidity. Calling males, non-calling males, and females differed in their distance from the bank and substrate type used. Females were significantly larger and heavier than males. Our results indicate that *H. curupi* is highly dependent on bank vegetation, and that changes in riparian vegetation structure due to human activity can result in the loss of sites for calling and oviposition, and may reduce availability of food and shelter.

Keywords: life history, riparian forest, stream-dwelling amphibians, seasonal deciduous forest, microhabitat use.

Resumo

Informações sobre o habitat e história de vida são essenciais para avaliar o status de conservação das espécies e definir ações para a preservação dos ambientes naturais. Ao longo de um ano, investigamos em populações de *Hypsiboas curupi* (i) o padrão de atividade noturna; (ii) relações com variáveis climáticas; (iii) o padrão de distribuição espcial de machos e fêmeas em termos de uso de microambientes; e (iv) variação no tamanho corporal. Conduzimos o estudo de agosto de 2010 a julho de 2011 no Parque Estadual Fritz Plaumann, um fragmento de Floresta Estacional Decidual na região oeste de Santa Catarina, sul do Brasil. O período de maior atividade foi de agosto a novembro. O maior número de machos vocalizando ocorreu às 23h e a frequência de ocorrência foi influenciada pela umidade do ar. Machos vocalizantes, não-vocalizantes e fêmeas foram discriminados pela distância da margem e tipo de substrato utilizado. Fêmeas foram significativamente maiores e mais pesadas do que machos. Os resultados indicam que *H. curupi* é altamente dependente da vegetação marginal. Assim, mudanças na estrutura da mata ciliar devido a ações humanas podem resultar na perda de sitios de canto e oviposição e reduzir a disponibilidade de alimento e abrigo.

Palavras-chave: história de vida, mata ciliar, anfíbios de riachos, floresta estacional decidual, uso de microambiente.
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**Introduction**

More than 40% of the world’s amphibian species are threatened by extinction (Pimm _et al._, 2014) primarily due to habitat loss and environmental degradation (Young _et al._, 2001; Blaustein and Kiesecker, 2002; Funk _et al._, 2003; Dixo _et al._, 2009; Verdade _et al._, 2010). Species responses to habitat changes vary, making it difficult to identify specific threats; this problem is magnified for species in which information on life history and ecology is lacking (Becker _et al._, 2007, 2010). Species that are sensitive to environmental changes decline faster than more robust species (i.e., those which typically have larger populations and are less affected by habitat disturbance) (Laurance _et al._, 2002; Henle _et al._, 2004; Laurance, 2008).

Stream-dwelling amphibians are continuously impacted by the loss of riparian forest, pollution, and damming, and are particularly vulnerable to environmental change due to strict association with specific niches (Becker _et al._, 2010; Almeida-Gomes _et al._, 2014). Understanding factors that contribute to spatial and temporal variation in species composition is a central question in ecology (Dodd Jr., 2010), and this knowledge is essential for evaluation of the health of populations (Bailey and Nichols, 2010).

*Hypsiboas curupi* Garcia, Faivovich and Haddad, 2007 is a stream-dwelling amphibian endemic to the Atlantic Forest and distributed throughout southwestern Argentina, southeastern Paraguay and southern Brazil (Frost, 2015). *Hypsiboas curupi* belongs to the *H. pulchellus* group, which contains 35 species (Frost, 2015). Some species of this group have been recently described (Garcia _et al._, 2007, 2008; Antunes _et al._, 2008; Kwet, 2008), and difficulty with species identification suggests a complex taxonomy. Ecological aspects of *H. curupi* are not well described, and data are limited to descriptions of larvae, adult (male) vocalization (Garcia _et al._, 2007), geographical distribution (Brusquetti and Lavilla, 2008; Iop _et al._, 2009; Lucas and Garcia, 2011; Zanella _et al._, 2012), behavior (Lipinski _et al._, 2012) and infectious diseases (Preuss _et al._, 2015). *Hypsiboas curupi* populations in Brazil are categorized as “vulnerable” according to the Official List of Brazilian Species Fauna Threatened by Extinction (MMA, 2014), and “endangered” by the List of Species of Threatened Fauna of Santa Catarina State (CONSEMA, 2011). They are restricted to few isolated remnants of mixed ombrophile and seasonal deciduous forest in the Atlantic Forest Biome in southern Brazil (Lucas and Garcia, 2011), where stream habitats have been altered by habitat loss and water pollution (Bonai _et al._, 2009; Ternus _et al._, 2011). We conducted surveys of *H. curupi*, a stream-dwelling amphibian endemic to the southern Atlantic Forest. Observations took place in the evenings over the course of a year, with the goal of increasing knowledge of *H. curupi* ecology and reproductive biology. We (i) analyzed nighttime activity patterns of *H. curupi*; (ii) evaluated *H. curupi* associations with climatic variables; (iii) examined the spatial distribution patterns of males and females with respect to microhabitat use; and (iv) investigated variation in body size of *H. curupi*.

**Materials and methods**

The fieldwork was conducted at the Parque Estadual Fritz Plaumann – PAEFP (27°17’36” S, 52°06’38” W, 400 m a.s.l.), in municipality of Concórdia in western of the state of Santa Catarina, southern Brazil. The PAEFP is near the Itá Hydroelectric Power Plant reservoir in the Uruguay river basin, and covers an area of 717 ha. The area is classified as seasonal deciduous forest, a formation of the Atlantic Forest Biome (Figure 1). Approximately 35% (265 ha) of the area is an island formed during the creation of the reservoir, however we only sampled the continental portion. Seasonal deciduous forest is considered one of the most endangered ecosystems in the Atlantic Forest Biome of southern Brazil (Câmara, 2003; Ribeiro _et al._, 2009), and the PAEFP is the only protected area with this vegetation type in Santa Catarina state.

Climate in the area is humid mesothermal, with an annual temperature of approximately 17 °C (Alvares _et al._, 2013). The mean temperature in the summer ranges between 13 °C and 30 °C, with a mean temperature of 13 °C in the winter (Braga and Ghellere, 1999). Annual rainfall is approximately 2.000 mm and is evenly distributed throughout the year, with indistinct dry seasons (Leite and Leão, 2009).

Frogs were sampled in three streams labeled R1 (Figure 2A), R2 (Figure 2B), and R3 (Figure 2C). These streams had a maximum width of 6 m, and were distanced minimally 50 m and maximally 2 km part. Streams R1 and R2 was composed of predominantly rocky bed with waterfalls and clear backwaters, and depths between 5 and 70 cm. R3 was composed of bedrock and silt, with waterfalls, clear backwaters and depth between 5 and 50 cm. Sampling transects were established spanning approximately 300 m of the stream length, and approximately 1 m beyond the stream edge on each bank. The total sample area was 1.500 m² for R1, and 1.200 m² for R2 and R3. The three streams were sampled in rotation fortnightly. The same 300 m transect was sampled each time for R2 and R3, but due to conditions limiting access and to accommodate the greater length (~1 km), three different 300 m transects were sampled alternately for R1 throughout the survey.

Fieldwork was conducted fortnightly from August 2010 to July 2011 using calling surveys at anuran breeding sites (Scott and Woodward, 1994). The surveys took place between sunset and midnight, for a total sampling effort of approximately 360 hours. Numbers of visually identified males and females were recorded for each stream.
We recorded the number of calling males at three different hours (19h, 21h, and 23h). The maximum number of calling males recorded was used as the monthly frequency of occurrence for each of these times. Mean maximum and minimum air temperatures for all sample days were used in the analysis. Mean rainfall and air humidity data were obtained from the Embrapa’s Weather Station in the municipality of Concórdia, approximately 15 km from the study area. We included mean values from the data set beginning five days prior to the first sampling, and extending throughout the sample period.

Individuals were categorized according to their microhabitats as follows: 1) substrate type (water, leaves, twigs \(> 3 \text{ mm}\), thin branches \(< 3 \text{ mm}\), rocks, soil and leaf litter); 2) perch height (cm); and 3) distance from the nearest bank (cm). Distances from the bank for frogs positioned on the forest side of the bank were designated with a minus sign (-); distances from the bank into the stream were recorded as-is. Frogs were captured to obtain body measurements. Sex was determined, and snout-vent length (SVL) was measured using digital calipers (0.01 mm). Body mass was determined to the nearest 0.1 g using a dynamometer. Calling behavior at the time of capture was also recorded. After morphometric characterization frogs were returned to the location of capture. We analyzed differences in size and body mass between sexes for the month of highest overall abundance. Voucher specimens were deposited in the scientific amphibian collection of the Universidade Comunitária da Região de Chapecó, located in Chapecó, western Santa Catarina, southern Brazil (Research Permit #04/2010/PAEFP/GERUC/DPEC and #13/2011/GERUC/DPEC-FATMA).

We used a Shapiro-Wilk test (Shapiro and Wilk, 1965) to test for homogeneity of variances, a Mann-Whitney U-test (Statsoft, 2007) was used to examine the differences in size and body mass between males and females using individuals captured on the first sample night from each stream. We used Analysis of Variance (ANOVA) to analyze differences in the number of calling males at three observation times (19h, 21h and 23h), using the data from periods with the highest frequency of calling (August to November). We used a Tukey (Zar, 1999) post hoc test to identify significant differences between observation times. We used multiple regression (Zar, 1999) to correlate frog abundance with minimum air temperature, relative humidity, and rainfall. Only the minimum temperature

**Figure 1.** Location of the Parque Estadual Fritz Plaumann, Santa Catarina, southern Brazil and sampled streams.
was used because it explained the greatest amount of variance. Simple linear regression was used to examine relationships between perch height and body mass of female, calling males and non-calling males. ANOVA and linear regression was performed using the BioEstat 5.3 package (Ayres et al., 2007). Mann-Whitney tests and multiple regressions were performed using the Statistica 8.0 package (Statsoft, 2007).

We used General Discriminant Analysis (GDA) to determine which spatial variable (type of substrate, perch height, and distance from nearest bank) best predicted the distribution of females, calling males, and non-calling males. General Discriminant Analysis is a predictive analysis that combines qualitative (categorical) and quantitative (continuous) predictors (Statsoft, 2007). The recent approach of GDA applies the methods of the general linear model to Discriminant Analysis (DA) problem (Statsoft, 2007). Traditional DA is very similar to analysis of variance (ANOVA/MANOVA), but allows identification of explanatory quantitative variables (descriptors) that best describe differences between two or more pre-established groups (Legendre and Legendre, 1998). F-tests can then (Valentin, 1995) be used to determine distance among objects and their significance (the Mahalanobis Distance). DA also generates discriminant functions that represent the linear combination of original predictors, discriminating groups in a multidimensional space (Nelson and Marler, 1990). General Discriminate Analysis was performed using the ‘best selection’ function of Statistica 8.0 (Statsoft, 2007). This method allowed us to value-test the selection criteria for all possible combinations of predictor variables. We also used the Wilk’s lambda value (p<0.05) as the selection criterion for interpreting the ‘best selection’, because the default coefficient range is from 1.0 (no discriminatory power) to 0.0 (perfect discriminatory power) (Statsoft, 2007).

Results

The highest overall abundance of *H. curupi* was found at stream R1 (Figure 3). The maximum number of males recorded in a single nightly observation was 10 (R1-R3). The maximum number of females recorded in a single nightly observation was seven at R1, two at R2, three at R3.
Males vocalized over an eight-month period, with most calling activity from August to November and a decrease in the coldest months (April to June, Figures 4 and 5). During the highest period of activity, the number of calling males differed throughout the night \(F_{(2,9)}=4.40\) and \(p=0.04\), with a significant increase in activity at 23h (Tukey’s \(q=4.0, p<0.05\)). The frequency of vocalization was influenced \(R^2=0.45\), adjusted \(R^2=0.35\), \(F_{(3,17)}=4.69, p<0.01\) by relative humidity \(\beta=-0.74, p<0.01\) but not by the absolute minimum temperature \(\beta=-0.35, p=0.11\) or rainfall \(\beta=0.29, p=0.25\), Figure 4).

*Hypsiboas curupi* used microhabitats close to the stream banks. Most calling males were found using twigs or leaves as a perch (Table 1). Thin branches of vegetation and dry soil were also utilized as substrate, but less commonly. Non-calling males typically used leaves or twigs.

**Figure 4.** The frequency of occurrence of *Hypsiboas curupi* and climatic variables from August 2010 to July 2011 in the Parque Estadual Fritz Plaumann, Santa Catarina, southern Brazil. Males are represented by dark grey bars, and females by light grey bars.

**Figure 5.** The maximum number of *Hypsiboas curupi* calling males at 19h (black bars); 21h (dark grey bars) and 23h (light grey bars) from August 2010 to July 2011 in the Parque Estadual Fritz Plaumann, Santa Catarina, southern Brazil. The dotted line indicates the mean of the absolute minimum air temperature, and the solid line indicates the mean for the absolute maximum air temperature over the sampling period.
thin branches of vegetation, rocks, or wet soil. Females used leaves and thin branches of vegetation. Rarely were females observed on twigs, rock, or leaf litter (Table 1).

Females were significantly larger and heavier than males (Table 2). Calling males, non-calling males and females used perches with a mean height of 47.4±26.3 cm (range: 0-150 cm, N=98), 46.5±24.8 cm (range: 0-100 cm, N=57) and 46.4±39.8 cm (range: 0-120 cm, N=21), respectively. There were no significant relationships between body mass and perch height.

For animals positioned on the stream side of the bank, non-calling males were generally furthest from the bank (mean: 51.9±54 cm, range: 0–200 cm, N=43), while calling males were positioned closer to the bank (mean: 35.8±43 cm, range: 0–115 cm, N=32). Females were intermediate in distance from the bank compared to males (mean: 46.8±55.1 cm, range: 0–150 cm, N=11).

For animals positioned on the forest side, calling males occupied microhabitats closer to bank (mean: 39.2±25.9 cm, range: 0–80 cm, N=10) while non-calling males were further from the bank (near the forest) (mean: 52.5±56.4 cm, range: 0–150 cm, N=12). Females were positioned furthest from the banks (mean: 191±156.3 cm, range: 40–450 cm, N=10).

Axis 1 from GDA analysis accounted for 30.9% of the total data dispersion, whereas the Axis 2 accounted for 18.8%. In this analysis, H. curupi groups were differentiated by the distance from the bank and substrate type. Females were grouped by distance from the banks (F=3.65, p<0.01), calling males were grouped by the type of substrate (F=3.53, p<0.01) and non-calling males were grouped by distance from the bank (F=10.55, p<0.01) and substrate type (F=2.55, p=0.03). Post hoc testing showed that habitat use differed between females and non-calling males (F=4.09, p<0.01), between females and calling males (F=3.43, p<0.01) and between calling and non-calling males (F=2.61, p<0.01). We were not able to discriminate groups based on perch height.

**Discussion**

The H. curupi population in this study had an intermediate reproductive pattern (sensu Wells, 2007), with activity mainly in the warmer months of the year (September to March) and diminishing in the colder months (April to June). This trait is typical of amphibian assemblages in southern Brazil (Both et al., 2008). Although no significant correlations were observed among temperature, rainfall, and reproductive activity, we did detect a decrease in activity during the colder months; this pattern is supported by many studies showing positive influence of climatic variables on the reproductive activity of anurans (Guix, 1996; Pombal, 1997; Camargo et al., 2005; Conte and Rossa-Feres, 2007; Santos et al., 2007; Both et al., 2008; Vasconcelos et al., 2011; Bastiani and Lucas, 2013). The study area is located on the shoreline of a lake that houses a hydroelectric plant, which in conjunction with air humidity, may have influenced the positive correlation of temperature with individual activity. For amphibians which use vegetation as perch for calling, variation in air humidity may result this species susceptible to hydric stress (Pough et al., 1983; Caramaschi and Cruz, 2002).

Our results indicate that H. curupi is highly dependent on bank vegetation. Changes in the structure of riparian
The degree of sexual dimorphism observed in *H. curupi* is similar to other species in the *H. pulchellus* group, such as *H. beckeri* (Acioli and Toledo, 2008), *H. caipora* (Antunes et al., 2008), *H. joaquini* (Garcia et al., 2003) and *H. seminiguttatus* (A. Lutz, 1925) (Garcia et al., 2007). Females larger than males are usually associated with the production of larger and/or more ovules (Crump and Kaplan, 1979; Prado et al., 2000). Dimorphism in *H. curupi* is likely related to different energy investments between the sexes (Freitas et al., 2008). For species with intermediate or prolonged reproductive periods, males may be smaller than females because of energy constraints associated with reproduction, a result of trade-offs involving calling activity, territorial defense, differences in diet, or higher predation pressure (Woolbright, 1983, 1989). As with other species, namely *H. caipora*, *H. ericae* (Caramaschi and Cruz, 2000) and *H. joaquini* (Garcia et al., 2003; Antunes et al., 2008; Garcia and Haddad, 2008), *H. curupi* physically engage in territorial disputes, a trait that is detectable due to scarring on the backs of males. Our study, however, did not observe “satellite male” behavior, which is often reported in Hylidae species (Pombal and Haddad, 2005; Oliveira et al., 2007; Miranda et al., 2008; Muniz et al., 2008). Such behavior might have exacerbated differences in perch height between calling and non-calling males.

The relatively low numbers of individuals observed in this study suggests that the *H. curupi* population is small in this area. Small population sizes are apparently more common in stream-dwelling amphibians in the Atlantic Forest (Almeida-Gomes et al., 2014) than in species in lentic habitat. The population in this study is one of the few currently known in Brazil (see Iop et al., 2009; Lucas and Marocco, 2011; Lucas and Garcia, 2011; Lipinski et al., 2012; Bastiani and Lucas, 2013). Fragmentation and isolation of the interior Atlantic Forest, expansion of hydroelectric projects in the region, and land and soil conversion for agricultural (Câmar, 2003; Bonai et al., 2009; Ternus et al., 2011) and silvicultural projects (Machado et al., 2012) may promote isolation of *H. curupi* populations. Such isolation increases the risk of extinction from inbreeding, genetic and demographic stochasticity, and vulnerability to diseases such as chytridiomycosis (Toledo et al., 2006).

This study contributes to our understanding of *H. curupi* life history, and expands the current knowledge of this species and other amphibians in the Neotropical region. This information is essential for the development of further ecological and evolutionary studies of amphibians (Greene, 1994). Effective conservation strategies aimed to prevent local extinctions can only be formulated with thorough knowledge of the distributions, sizes and vulnerabilities of these populations, and this information must come from field studies (Silvano and Segalla, 2005; Colombo et al., 2008). Future policy and research efforts could focus on predicting the likely geographic distribution of *H. curupi* in southern Brazil. Conservation goals should include a better understanding of the structure and dynamics of populations at a landscape scale, and implementation of plans for riparian zone restoration and reconnection of forest fragments (Becker et al., 2010; Toledo et al., 2010; Almeida-Gomes et al., 2014). Further knowledge of *H. curupi* biology and habitat requirements may aid the design of enhanced conservation strategies for this species in the study region. Further field studies may inform conservation of other groups as well as the lack of effective legislation in riparian habitat is a serious problem for many species that compose the regional biodiversity.

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