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Notes on abundance, size and calling activity of the South American bullfrog, *Leptodactylus pentadactylus* (Anura, Leptodactylidae), in pristine and fragmented forests in Central Amazonia, Brazil

Nuno M. Faria da Costa[1], Diana P. Rojas-Ahumada[2,3], Ronis Da Silveira[1,2], Marcelo Menin[1,2,3,4]

Abstract. *Leptodactylus pentadactylus* is widely distributed in Amazonia and is characterized by a large size, nocturnal habits and terrestrial breeding. The objectives of this study are to describe characteristics of the population structure, abundance, calling activity, and size of adults and juveniles in two primary forests and one urban forest fragment in Central Amazonia. We recorded a total of 158 individuals (males, females and juveniles), most of them on stream shores. Calling males were recorded between September and December predominantly in non-riparian areas of primary forests and only in riparian areas within the urban fragment. Juveniles were found in all sampling periods and recruitment was observed in the middle and at the end of the rainy season. Reproduction and vocalization periods were seasonal, producing discrete generations. The size of adults was different between study areas, with larger individuals in the urban fragment compared to those sampled in primary forests.

Keywords. Calling site, forest fragment, population recruitment, stream shore.

Introduction

Some species of large-sized terrestrial Neotropical frogs of the *Leptodactylus pentadactylus* species group (*sensu* Heyer, 2005) can be recorded in pristine *terra firme* (unflooded upland) forest and in urban forest fragments at Central Amazonia, including *Leptodactylus knudseni* Heyer, 1972 and *L. pentadactylus* (Laurenti, 1768) (Cordeiro and Sanaiotti, 2003; Menin, Waldez and Lima, 2008; Tsuji-Nishikido and Menin, 2011; Lima et al., 2012). However, studies on the population biology of *L. pentadactylus* species group from Amazonia are scarce and dispersed in the literature (but see Galatti, 1992).

The South American Bullfrog, called rã-pimenta in Brazil (*Leptodactylus pentadactylus*), is widely distributed in the Amazonia biome (Frost, 2013). In spite of this wide distribution, there are few studies on reproduction, abundance and size of this species, all of them available primarily from pristine *terra firme* forests (e.g. Galatti, 1992; Heyer, 2005; Menin, Waldez and Lima, 2008; Menin, Lima and Rodrigues, 2010). *Leptodactylus pentadactylus* is found mainly along stream shores in forests of Central Amazonia (Galatti, 1992; Tocher, Gascon and Zimmerman, 1997; Tsuji-Nishikido and Menin, 2011), is active only during the night, and feeds on many types of invertebrates (Duellman, 1978; Galatti, 1992). Sexual dimorphism in the size, with females larger than males, was detected by Heyer (2005). According to Galatti (1992), during the reproductive season, the adults migrate toward the slopes, distant from streams, and then return to the streams after reproduction, with an increase in population density after the reproductive season. However, information on vocal site, calling activity period and population structure in urban fragmented forests is not available.

1 Departamento de Biologia, Instituto de Ciências Biológicas, Universidade Federal do Amazonas, Av. General Rodrigo Otávio Jordão Ramos 3000, 69077-000, Manaus – Amazonas, Brazil.

2 Programa de Pós-Graduação em Diversidade Biológica, Instituto de Ciências Biológicas, Universidade Federal do Amazonas, Av. General Rodrigo Otávio Jordão Ramos 3000, 69077-000, Manaus – Amazonas, Brazil.

3 Instituto Nacional de Ciência e Tecnologia de Estudos Integrados da Biodiversidade Amazônica, Ministério da Ciência, Tecnologia e Inovação, Conselho Nacional de Desenvolvimento Científico e Tecnológico – INCT-CENBAM/MCTI/CNPq, Brazil.

4 Corresponding author: menin_m@yahoo.com.br

In this paper, we describe characteristics of the population structure, abundance, calling activity, call site, and size of adults and juveniles in three areas, two primary forests and one urban forest fragment in Central Amazonia, Brazil. We tested for differences in body size among areas and between sexes.

Material and Methods

Study area. The study took place in three areas: 1) Reserva Florestal Adolpho Ducke (RFAD: between 02º55' and 03º01'S, between 59º53' and 59º59'W), 2) Fazenda Experimental of the Universidade Federal do Amazonas (Fazenda UFAM: between 02º37'17" and 02º39'41" S, between 60º03'29" and 60º07'57" W), both located in the rural or suburban areas of Manaus city, and in 3) campus of the Universidade Federal do Amazonas (campus UFAM: 03º04'34"S, 59º57'30"W), an urban forest fragment in Manaus, Amazonas State, Brazil. RFAD covers 10,000 ha of *terra firme* rainforest that encompass a well-drained forest not subject to seasonal inundation. Fazenda UFAM covers 3,000 ha of *terra firme* forest. A 30-37 m tall closed canopy characterizes the forest in both areas, with emergent trees growing to 40-45 m and an understory that contains abundant sessile palms (Ribeiro et al., 1999). The forest fragment of the campus UFAM encompasses about 600 ha of *terra firme* forest, secondary forest, campinarana sites (a low, relatively light forest with thin-stemmed trees 10–20 m height) and deforested areas (Tsuji-Nishikido and Menin, 2011).

The climate in the region is characterized by a rainy season from November to May, with a reduction of the rainfall in the rest of the year (mensal mean of rainfall varied from 82 to 145 mm among June and October) (Marques Filho et al., 1981). Mean annual temperature is approximately 26 ºC (Marques Filho et al., 1981) and mean annual rainfall was 2,489 mm between 1985 and 2004.

Data collection and analyses. We sampled adults and juveniles of *L. pentadactylus* during five surveys at RFAD, three surveys at Fazenda UFAM, and three surveys at campus UFAM. Data were collected in 72 plots systematically distributed over a 64-km^2 grid at RFAD, 41 plots distributed over a 24-km^2 grid at Fazenda UFAM (see Figure 1 in Waldez et al., 2011), and ten riparian plots along shores of first order streams distributed over 6-km^2 at campus UFAM (see Figure 1 in Tsuji-Nishikido and Menin, 2011). Plots were 250 m long and positioned to follow altitudinal contour lines. All plots in the three study areas were in *terra firme* forest.

We conducted nocturnal surveys at the three areas by simultaneous visual encounter and auditory sampling (Heyer et al., 1994) in the following periods: November–December 2002, March–May 2003, November–December 2003, January–March 2004 and April–May 2004 at RFAD, November–December 2008, January–February 2009 and April–May 2009 at Fazenda UFAM, and September–October 2010, January–April 2011 and February–March 2012 at campus UFAM.

Each plot was sampled for one hour between 18:30 and 22:00 h (more details in Menin, Waldez and Lima, 2008). The surveys in each plot were alternated in each sampling period to avoid differences between plots being associated to activity period of the species.

All individuals located within 20 m of the center line of the plot were recorded. Each time a frog was captured in the visual survey we (1) recorded its snout-vent length (SVL) with a vernier calliper (0.05 mm), (2) classified it as adult (male or female) or juvenile, and (3) recorded the presence and distance of shelters (burrows in the soil among tree roots). Frogs with SVL > 115.0 mm and sexual secondary characters (not detectable in females)

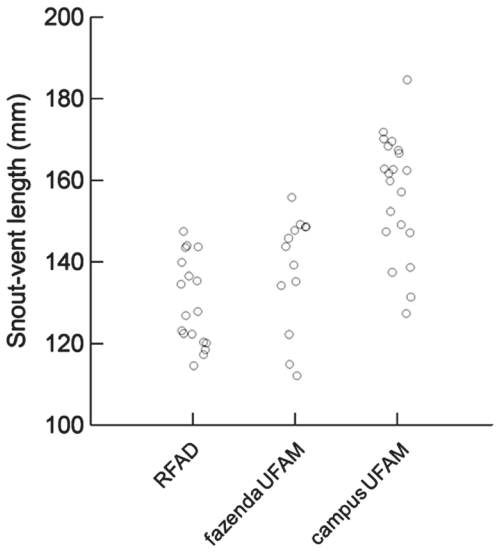

Figure 1. Snout-vent length of *Leptodactylus pentadactylus* adults captured at the Campus of the Universidade Federal do Amazonas (UFAM), at the Fazenda Experimental of the UFAM and at the Reserva Florestal Adolpho Ducke (RFAD), Manaus, Amazonas, Brazil. Each point represents one individual.

Table 1. Number of *Leptodactylus pentadactylus* individuals recorded on visual or auditory nocturnal surveys undertaken in 72 plots at Reserva Florestal Adolpho Ducke (RFAD), in 41 plots at Fazenda Experimental of the Universidade Federal do Amazonas (Fazenda UFAM), and in ten plots at campus UFAM, Manaus, Brazil. Adults: snout-vent length ≥115.0 mm. Values represent the number of individuals in riparian and non-riparian (between parenthesis) plots for RFAD and Fazenda UFAM.

Site/Method/Age class	Nov-Dec/2002	Mar-May/2003	Nov-Dec/2003	Jan-Mar/2004	Apr-May/2004	Total
RFAD						
Visual survey						
Adults	1(0)	4(4)	4(1)	8(0)	5(3)	30
Juveniles	6(0)	1(0)	9(1)	1(1)	1(1)	21
Auditory survey	1(3)	3(3)	0(2)	0(0)	0(0)	12
Total	8(3)	8(7)	13(4)	9(1)	6(4)	63
	Nov-Dec/2008	Jan-Feb/2009	Apr-May/2009			
Fazenda UFAM						
Visual survey						
Adults	5(1)	3(2)	2(1)			14
Juveniles	3(0)	10(0)	14(0)			27
Undetermined	3(0)	0(0)	2(0)			5
Auditory survey	0(0)	0(4)	0(4)			8
Total	11(1)	13(6)	18(5)			54
	Sep-Oct/2010	Jan-Apr/2011	Feb-Mar/2012			
Campus UFAM (only riparian plots)						
Visual survey						
Adults	4	15	12			31
Juveniles	5	2	3			10
Total	9	17	15			41

were considered adults (Galatti, 1992). Individuals that were only heard were classified as male and included in the abundance data. Individuals that were unidentifiable (escaped) were included in the abundance data and classified only as juvenile or adult.

Daily pattern of calling activity and calling site were determined by three auditory surveys at campus UFAM over a 24-hour period during October and November 2010.

We compared the SVL of adults among the three areas by using the Analyses of Variance (ANOVA). When the null hypothesis was rejected, a multiple comparison test (Tukey) was applied to determine which of the samples showed significant differences (Zar, 1999). To verify the presence/absence of sexual dimorphism in the size, we tested the differences in size between males and females at campus UFAM by the t-test (Zar, 1999).

Results

Abundance, sex and size. At RFAD, we detected 63 individuals: 39 individuals were captured, measured, and released and 24 individuals were visualized but not captured. Among the captured individuals, 17 were adults (SVL = 115.0–146.0 mm; mean = 130.2 mm, standard deviation (sd) = 10.5). Of the adults, 10 of them were accurately identified as females (SVL = 122.4–146.0 mm, mean = 134.6 mm, sd = 9.6) and seven were not identified. The other 22 individuals were juveniles (SVL = 23.1–114.8 mm, mean = 76.1 mm, sd = 34.7). Among the individuals not captured, 22 were adults (12 were accurately identified as males by visual inspection and presence of hypertrophied forelimbs or

vocalization activity), ten were not identified, and two were juveniles. The number of juveniles was greater than adults in samplings at the beginning of the rainy season (November and December; Table 1). The smallest juveniles (SVL = 23.1–30.5 mm, n = 5) were found in December 2003 inside a burrow in the soil in a riparian plot (20 m from stream shore).

At Fazenda UFAM, 54 individuals were detected: 37 were captured, measured and released and 17 were not captured. Among the captured individuals, 12 were adults (SVL = 115.1–158.0 mm, mean = 139.6 mm, sd = 12.2). Six of them were accurately identified as females (SVL = 140.3–146.5 mm, mean = 145.0 mm, sd = 2.3) and one was accurately identified as a male (SVL = 158.0 mm). Five were not identified and 25 were identified as juveniles (SVL = 50.9–114.8 mm, mean = 85.7 mm, sd = 19.9). Among the individuals not captured, 14 were adults (8 were accurately identified as males by vocalization activity and six were not identified) and three were juveniles. Juveniles were more abundant than adults in the middle and at the end of the rainy season (January to May; Table 1).

At campus UFAM, we detected 41 individuals: 26 were captured, measured, and released and 15 were not captured (Table 1). Among the captured individuals, 21 were adults (SVL = 127.8–185.4 mm, mean = 156.6 mm, sd = 15.3). Of the adults, nine were accurately identified as females (SVL = 146.3–170.0 mm, mean = 160.1 mm, sd = 7.6), twelve were accurately identified as males (SVL = 127.8–185.4 mm, mean = 154.0 mm, sd = 19.1) and five were juveniles (SVL = 63.7–112.0 mm, mean = 81.3 mm, sd = 18.9). Among the individuals not captured, 14 were adults (5 were accurately identified as males by visual inspection and presence of hypertrophied forelimbs and by vocalization activity and nine were not identified) and one was a juvenile.

We found significant differences in the size of adults among the three study sites ($F_{2,51} = 24.00$, $p < 0.0001$). Those captured at campus UFAM were larger ($p < 0.001$) than those captured at Fazenda UFAM and RFAD, and those captured at Fazenda UFAM were of similar size to those captured at RFAD ($p = 0.103$; Figure 1). Body size of males was not significantly different from that of females ($t = 0.907$, $df = 19$, $p = 0.376$).

Calling activity, call site and shelter. Call activity at RFAD and Fazenda UFAM was recorded predominantly from October to December, but sporadic calls were heard from January to May (see auditory survey at Table 1). Calling males were only recorded in non-riparian plots at Fazenda UFAM while at RFAD calling males were recorded predominantly at non-riparian plots. At riparian plots of both areas we found adults and juveniles in all samples. At campus UFAM, individuals in calling activity were observed from the end of September to December 2010 and 2011 on stream margins. The call site consists of males positioned in the litter, always close to shelters, such as burrows or roots. Seven males of *L. pentadactylus* were recorded calling in the leaf-litter either close to (between 1 m and 10 m) or distant from (> 10 m) stream shores. Males called throughout the night, from 18:00 h to 06:30 h.

We detected shelters close to 12 individuals (five at RFAD and seven at campus UFAM) and they consisted of burrows. One male was observed calling in a shelter formed by tree roots. The distance of each individual from the shelter varied from zero (at entrance of the shelter) to 87 cm (mean = 13.4 cm, sd = 28.2).

Discussion

The adult size of *Leptodactylus pentadactylus* described in the present study is congruent to that described for populations in different regions of Amazonia (Rodriguez and Duellman, 1994; Duellman, 2005; Heyer, 2005). The size of adults found by Galatti (1992) at RFAD was similar to that found in our study also at RFAD and Fazenda UFAM, but smaller than those found at campus UFAM. Sexual dimorphism was observed by the presence of hypertrophied forelimbs and a thumb spine in the males, and no difference in size was detected between the sexes. This pattern disagrees with that reported by Heyer (2005) for *L. pentadactylus* with females larger than males. The absence of difference in size between sexes was already described for other species of the *L. pentadactylus* species group, such as *L. knudseni* Heyer, 1972, *L. labyrinthicus* (Spix, 1824), *L. myersi* Heyer, 1995, *L. paraensis* Heyer, 2005, *L. peritoaktites* Heyer, 2005, *L. rhodomerus* Heyer, 2005, *L. syphax* Bokermann, 1969, and *L. vastus* A. Lutz, 1930 (Heyer, 2005; Silva, Giaretta and Facure, 2005; Silva and Giaretta, 2009).

We found the smaller juveniles in December and January, after the species reproductive period (Galatti, 1992). The greater number of juveniles at the beginning and in the middle of the rainy season at RFAD and Fazenda UFAM indicates recruitment of young born in the previous year for the reproductive population, as observed by Galatti (1992). The presence of juveniles of small size on the margins of streams indicates the recruitment of individuals born during the breeding

season, reflecting variation in the population density throughout the rainy season and the presence of discrete annual generations, corroborating a pattern observed for other anurans (Moreira and Lima, 1991; Ovaska, 1991; Waldez et al., 2011).

At RFAD and Fazenda UFAM, calling males were observed only (Fazenda UFAM) or predominantly (RFAD) in non-riparian areas. In these areas three nests were found by previous studies (Galatti, 1992; Menin, Lima and Rodrigues, 2010). At campus UFAM, males called only from riparian areas, indicating that they did not migrate to slopes in order to reproduce in completely terrestrial sites far from streams (Galatti, 1992). However, their nests were not found. The calling period recorded in the present study (September to December) is similar to the period described by Galatti (1992) and Hero and Galatti (1990). Thus, calls heard at RFAD and Fazenda UFAM throughout the rainy season (January to May) were considered sporadic, as observed in Ecuador (Duellman, 1978) for this species. Sporadic calls were hot heard in the campus UFAM.

The size of adults of *L. pentadactylus* in the urban fragment was larger than those in the pristine forest. This result is contradictory to those found to other vertebrates, such as passerine birds (Wauters et al., 1996) and squirrel (Zanette, Doyle and Trémont, 2000). Despite the absence of a sampling design supporting precise comparisons between fragmented (only one urban fragment) and primary forests, we suggest some speculative hypotheses as a stimulus for future studies in the field: the large body size of the adults of campus UFAM would be explained by (1) reduction of mortality caused by predators, or (2) greater availability of resources (some studies indicate an increase of insects in fragmented areas; Debinski and Holt, 2000).

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