Trabajo

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Natural history notes of *Rhinella proboscidea* (Anura, Bufonidae) from a non-flooded forest in central Amazon

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ABSTRACT

Rhinella proboscidea, a toad of the *Rhinella margaritifera* species group, is found in non-flooded forests in central Amazon. This species is an explosive breeder that reproduces in streamside ponds and uses small seedling plants to rest during the night. In this study, we present data on the variation in abundance, body size and body mass of adults and juveniles, on the relationship between body mass and size, operational sex ratio (OSR), and we characterize the resting sites and some aspects of *R. proboscidea* reproductive events throughout two rainy seasons. We captured 366 individuals (59 males, 84 females, 204 juveniles and19 undetermined). Highest abundance of females and juveniles was found in the fifth sampling period; males were more abundant than females in three sampling periods. We did not detect sexual dimorphism in body size and mass between males and females. The OSR varied from 0.12:1 to 1.50:1 (males:females). There was a positive relationship between body size and mass considering all individuals. Reproductive events were observed from the middle to the end of the rainy season on few occasions. We found ontogenetic variation in the height of resting sites in which juveniles were found in lowest height when compared to adults. In conclusion, juvenile recruitment events occurred in the end of the rainy seasons, and the abundance of individuals are probably related to variation in water body availability for reproduction along the studied periods. Moreover, the absence of sexual dimorphism in body size and mass for this species disagrees with what is known for other species in the *R. margaritifera* group.

Key words: Abundance; Recruitment; Sexual dimorphism; Snout-vent length; Body mass.

RESUMO

Rhinella proboscidea, pertencente ao grupo de espécies de *Rhinella margaritifera*, é encontrada em florestas de terra firme na Amazônia central. Esta espécie possui reprodução explosiva em pequenas poças nas margens de riachos, e utiliza plântulas como sítio de repouso durante a noite. No presente estudo, apresentamos dados sobre a variação na abundância, tamanho e massa de adultos e juvenis, a relação entre massa corporal e comprimento rostro-cloacal, a razão sexual operacional (OSR) e caracterizamos os sítios de repouso e alguns aspectos reprodutivos de *R. proboscidea* ao longo de duas estações chuvosas. Capturamos 366 indivíduos (59 machos, 84 fêmeas, 204 juvenis e 19 indivíduos indeterminados). A maior abundância de fêmeas e juvenis ocorreu no quinto período de amostragem; machos were more abundantes do que as fêmeas em três períodos de amostragem. Não detectamos dimorfismo sexual no tamanho ou massa corporal entre machos e fêmeas. A OSR variou de 0.12:1 a 1.50:1 (machos:fêmeas). Detectamos uma relação positiva entre o tamanho corporal e massa considerando todos os indivíduos. Observamos eventos reprodutivos entre o meio e o fim das estações chuvosas, e em poucas ocasiões. Nós encontramos variação ontogenética na altura dos sítios de repouso onde os juvenis foram encontrados utilizando sítios mais baixos quando comparados aos adultos. Concluindo, os eventos de recrutamento de juvenis ocorreram no final das estações chuvosas, e a abundância de indivíduos está provavelmente relacionada com a variação na disponibilidade de corpos d'água para reprodução ao longo dos períodos estudados. A ausência de dimorfismo sexual no tamanho corporal e massa desta espécie é contrário ao registrado para outras espécies do grupo de *R. margaritifera*.

Palavras-chave: Abundância; Recrutamento; Dimorfismo sexual; Comprimento rostro-cloacal; Massa.

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Introduction

Despite the great Neotropical Anura diversity (Vasconcelos *et al.*, 2019) species population studies are not common and information on the variation in abundance and size of adults and juveniles and on seasonal recruitment events is available only for a few species of Aromobatidae, Bufonidae, Craugastoridae, Leptodactylidae and Microhylidae (e.g. Moreira and Lima, 1991; Galatti, 1992; Donnelly, 1999; Watling and Donnelly, 2002; Waldez *et al.*, 2011). Still, such basic information on population structure and associated variation factors are essential to the understanding of population dynamics and life history aspects of anurans (Schmidt *et al.*, 2002). In this context, information on the population structure are also important to future evaluation on anuran populations due the increasing of "amphibian declines crisis" around the world (Stuart *et al.*, 2004).

Bufonids are widely distributed in temperate and tropical regions, and the Neotropical genus *Rhinella* has the highest species richness, with 92 described species (Frost, 2020). This genus comprises seven species groups, including the *Rhinella margaritifera* group, currently composed of 19 species (Vaz-Silva *et al.*, 2015). Species within this group are found mainly in the leaf litter of primary forests, and reproduce in lentic water bodies (e.g. pools, swamps, Brazil nut empty fruit capsules, artificial ponds) during the rainy season (e.g. Wells, 1979; Aichinger, 1987; Caldwell, 1991; Fouquet *et al.*, 2007; Lima *et al.*, 2007; Roberto *et al.*, 2011), or during the dry season (Bernarde, 2007). These frogs can have explosive or prolonged reproduction patterns (Wells, 1979; Caramaschi and Niemeyer, 2003; Duellman, 2005; Menin *et al.*, 2006).

The leaf toad *Rhinella proboscidea* (Spix, 1824) is distributed along the Amazon River from Peru to Manaus in Brazil (Frost, 2020). It has diurnal habits and can be found during the night, resting perched on the vegetation above the ground (Zimmerman and Bogart, 1988; Lima *et al.*, 2012). However, nocturnal activity was found in males of this species which call during day and night for two or three days after intense rainfall, forming choruses of 50-100 males (Menin *et al.*, 2006). Clutches and tadpoles can be found in temporary ponds near streams or in stream headwaters (Menin *et al.*, 2006).

In this study, developed throughout two rainy seasons in a non-flooded forest in the Brazilian Amazon, we present data on the variation in abundance, size and mass of adults and juveniles, the relationship between body mass and size, and we characterize the resting sites and some reproductive aspects of *R. proboscidea*, a species of the *R. margaritifera* group.

Materials and methods

Study area

Our study was carried out at Reserva Florestal Adolpho Ducke (02°55' and 03°01'S, 59°53' and 59°59'W), in the city of Manaus, State of Amazonas, Brazil. Reserva Ducke covers about 10 000 ha of non-flooded forest, with a closed canopy of 30-37 m height, emergent trees growing up to 40-45 m height, and an understory of abundant sessile palm trees (*Astrocaryum* spp. and *Attalea* spp.; Ribeiro *et al.*, 1999). The climate is Am (Köppen-Geiger system; tropical monsoon with no dry season) (Peel *et al.*, 2007). Rainy season is usually from November to May and average annual rainfall was 2 362 mm between 1985 and 2006 (Rodrigues *et al.*, 2010). The rainiest months are March and April, generally with rainfall > 300 mm per month (Marques-Filho *et al.*, 1981). Air temperature varies between 24.6°C and 26.9°C (Araújo *et al.*, 2002). During the period of our study, annual rainfall was 2 702 mm in 2002, 2 044 mm in 2003 and 2 618 mm in 2004. The months of January (50.4 mm), February (178.1 mm) and March (213.9 mm) 2003 were below the 21-year average for these months (268, 285 and 318 mm, respectively; Rodrigues *et al.*, 2010) (Fig. 1).

Data collection

Individuals of *R. proboscidea* were sampled in five nocturnal surveys (November–December 2002, March–May 2003, November–December 2003, January–March 2004 and April–May 2004) during two rainy seasons. At each sampling period, we collected data for 72 plots (each plot 250 m long and positioned along altitudinal contour lines) systematically distributed over a 64-km^2 grid, formed by 8-km long trails inside Reserva Ducke (Menin *et al.*, 2008). We conducted nocturnal visual encounter surveys (Crump and Scott, 1994) between 18:30 h and 22:00 h. Despite diurnal habits of this species, individuals were easily found during nocturnal samplings than diurnal samplings at the same study area (Menin *et al.*, 2008). At every 5 min, in each

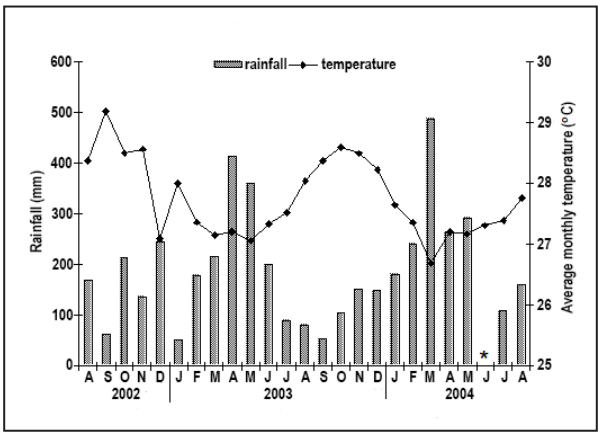

Figure 1. Monthly precipitation levels and average monthly temperatures from August 2002 to August 2004 at the Reserva Florestal Adolpho Ducke, Manaus, Amazonas, Brazil. Data obtained from the meteorological station at the Reserva Ducke. *= missing data.

plot, two observers stopped and searched the litter and the vegetation for specimens. All individuals located within 20 m of the center line of the plot were recorded. Occasional observations were made in areas outside of the plots. We (1) measured the size of each individual as the length from the tip of the snout to the vent (SVL) with a vernier calliper (0.05 mm precision), (2) measured body mass using a Pesola® spring balance (0.1 g precision), (3) classified the individual as male, female or juvenile, (4) recorded its activity (resting, moving, calling, breeding), and (5) characterized the resting site by registering perch height above the ground and type of substrate (leaves, stem, fallen trunks, leaf litter). Males and females were identified by dorsal surface skin or calling activity (dorsal surface is smooth in males and granular in females; Lima *et al.*, 2012). We considered juveniles the individuals with SVL < 39.9 mm (the size of the smallest male found in the samplings). Juveniles that were smaller than 21 mm in SVL and adult individuals that escaped did not have their body mass determined. We measured abundance as the number of individual detections in each sampling period. Vouchers are housed in the Amphibians and Reptiles Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA-H 11815 to 11831, 11855, 11922, 11923).

Statistical analysis

Since our data on body size and body mass showed non-normal distribution, we used non-parametric Mann-Whitney U-Test to compared male and female body size and body mass (Zar, 1999). The differences in perching height between males, females and juveniles were compared using a Kruskal-Wallis test (Zar, 1999). Operational sex ratio was estimated by dividing the number of males by the number of females present in each sampling period and considering total number of individuals in all sampling periods. We also checked the adult sex ratio by using the Chi-square test with Yates' Correction (Fowler *et al.*, 1998). Relationship between body size and mass was determined through Linear Regression model (Zar, 1999). Data was log10 transformed prior to the regression analysis in order to meet the assumptions of the model. The results are expressed as mean ± standard deviation. All statistical analyses were conducted with a significance level of 0.05.

Results

We captured 366 individuals of *R. proboscidea* in the five sampling periods (59 males, 84 females and 204 juveniles; Fig. 2). We could not determine the sex of 19 individuals between 39.1 mm and 42.4 mm of SVL. Among sampled individuals, juveniles were more abundant in four of the five sampling periods (Fig. 2). Males were not detected in the first sampling period. Considering all sampling periods, the operational sex ratio was 0.51:1 males/females (Table 1). However, considering each sampling period individually, the OSR varied from 0.12:1 (males/females) in the fifth sampling period, to 1.50:1 in the fourth sampling period (Table 1). Females were

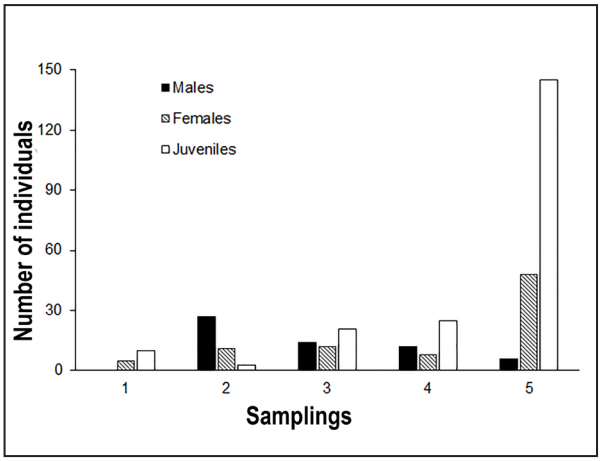

Figure 2. Number of males, females and juveniles of *Rhinella proboscidea* recorded in five nocturnal visual samplings at Reserva Florestal Adolpho Ducke, Manaus, Amazonas, Brazil. The numbers represent sampling periods: 1) November-December 2002, 2) March-May 2003, 3) November-December 2003, 4) February-March 2004, and 5) April-May 2004.

Table 1. Body size (snout-vent length; in mm) and body mass (in grams) of *Rhinella proboscidea*, measured in five nocturnal visual samplings at the Reserva Florestal Adolpho Ducke, Manaus, Amazonas, Brazil. Values are presented as mean ± standard deviation, range (in parenthesis) and sampling size (N), with the exception of periods with only one or two individuals, for which values were given. (*) Juveniles smaller than 21 mm in SVL did not have their body mass determined. A Mann-Whitney U-test was applied for comparisons between body size and body mass of males and females. OSR: operational sex ratio (number of males divided by the number of females). A Chi-squared test with Yates' Correction was used to evaluate adult sex ratio.

Sampling/Sex	Nov-Dec/2002	Mar-May/2003	Nov-Dec/2003	Feb-Mar/2004	Apr-May/2004	Total
Body Size						
Males	-	49.41 ± 3.87 (41.1-54.5) N= 11	45.89 ± 3.42 (38.3-50.5) N= 14	46.64 ± 4.29 (40.0-52.5) N= 12	50.35 ± 3.28 (45.1-54.9) N= 6	47.62 ± 4.06 (38.3-54.9) N= 43
Females	47.24 ± 4.79 (41.7-51.5) N= 5	48.14 ± 4.52 (43.3-55.5) N= 11	48.21 ± 4.21 (42.5-56.6) N= 12	48.39 ± 2.18 (45.0-51.6) N= 8	46.10 ± 3.32 (40.7-56.7) N= 48	46.90 ± 3.70 (40.7-56.7) N= 84
Juveniles	23.07 ± 7.23 (10.4-35.5) N= 10	36.87 ± 1.70 (34.9-37.9) N= 3	25.32 ± 4.02 (17.9-33.9) N= 21	34.55 ± 3.30 (29.6-39.9) N= 24	11.54 ± 8.50 (5.4-38.7) N= 145	16.60 ± 11.40 (5.4-39.3) N= 203
Mann-Whitney test (males *versus* females)	-	U= 50.00 P= 0.490	U= 107.50 P= 0.227	U= 58.00 P= 0.440	U= 49.50 P= 0.009	U= 1,567.00 P= 0.223
OSR (males:females)	-	1:1	1.17:1	1.50:1	0.12:1	0.51:1
Chi-squared test	-	χ^2= 5.92, gl=1, p> 0.05	χ^2= 0.16, gl=1, p> 0.80	χ^2= 0.45, gl=1, p> 0.70	χ^2= 31.13, gl=1, p< 0.01	χ^2= 12.60, gl=1, p< 0.01
Body Mass						
Males	-	9.0-11.0 N= 2	10.36 ± 1.98 (6.0-14.0) N= 14	9.67 ± 2.61 (5.5-14.5) N= 12	11.00 ± 2.02 (9.0-14.0) N= 6	10.20± 2.20 (5.5-14.5) N= 34
Females	9.80 ± 2.59 (6.0-13.0) N= 5	10.50 ± 3.64 (6.0-16.5) N= 8	11.63 ± 3.22 (7.0-18.0) N= 12	10.94 ± 3.91 (7.0-17.0) N= 8	9.33 ± 1.81 (6.0-16.0) N= 48	10.00 ± 2.60 (6.0-18.0) N = 81
Juveniles*	3.50 N= 1	5.17 ± 1.44 (3.5-6.0) N= 3	2.79 ± 0.77 (2.0-4.5) N= 17	3.88 ± 1.11 (2.0-6.0) N= 24	4.8 ± 0.90 (3.0-6.5) N= 15	3.80 ± 1.20 (2.0-6.5) N= 60
Mann-Whitney test (males *versus* females)	-	U= 6.50 P= 0.691	U= 94.50 P= 0.586	U= 53.50 P= 0.668	U= 68.00 P= 0.035	U= 1,181.00 P= 0.227

more abundant than males only in the fifth sampling period (Fig. 2). Highest abundance of juveniles was also found in the fifth sampling period (Fig. 2).

We did not detect significant differences in body size and mass between males and females in most of the sampling periods, and when considering all sampling periods together (Table 1). However, significant differences were found in both body size and body mass in the fifth sampling period, in which the males were greater and heavier than the females (Table 1). Additionally, adult males found at the end of the two rainy seasons (second and fifth sampling periods) were larger than those found in other sampling periods, while the size of females was similar along the sampling periods (Table 1, Fig. 3A and 3B). Juveniles found at the end of the second rainy season were smaller than those found in the other sampling periods (Table 1, Fig. 3C). SVL of smallest juvenile was 5.4 mm. We also detected a positive relationship (Fig. 4) between body size (SVL) and mass (weight) when considering all individuals together (males, females, juveniles and undetermined) ($F_{1,192}$ = 933.690, P < 0.001, n = 194).

Reproductive activity was observed in six occasions but the number of males was estimated in four of these occasions between April and May 2003 (5 males calling in a pool of about 1 m^2, 10 in a pool of about 1 m^2, and 20 in a pool of about 0.5 m^2) and

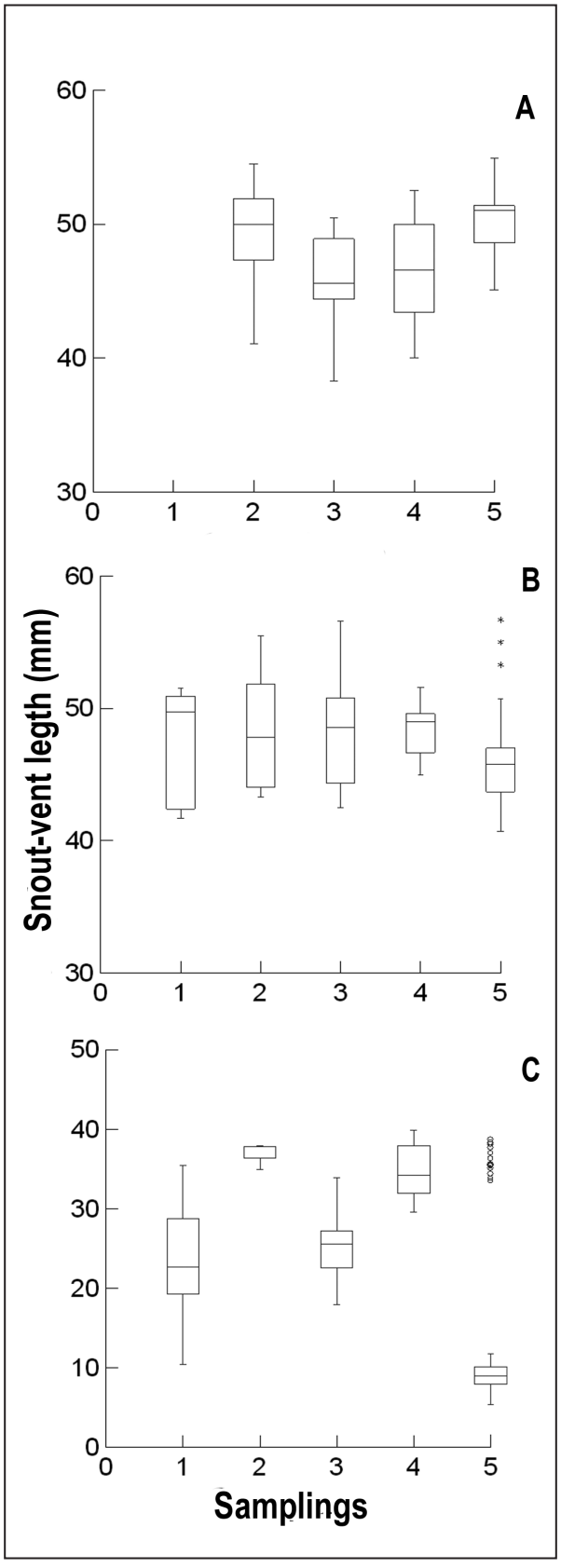

Figure 3. Variation in snout-vent length of 43 males (A), 84 females (B) and 203 juveniles (C) of *Rhinella proboscidea* measured in five nocturnal visual samplings at Reserva Florestal Adolpho Ducke, Manaus, Amazonas, Brazil. Sampling periods as Figure 2.

March and April 2004 (40 males calling in a pool of about 2 m^2). All ponds were shallow, temporary and in edges of streams. SVL of three amplectant pairs varied from 45.8 to 54.5 mm for males and from 50.3 to 55.5 for females.

Most adults and all juveniles were found resting on leaves of small shrubs (319 individuals), on stems or fallen tree trunks (13 individuals) or leaf litter (three individuals); 25 males were found in calling activity and only three individuals were found moving on the leaf litter during the night, far from water bodies. The height of resting sites was significantly highest for males (mean perching height= 25.69 cm ± 18.80, range= 5–100 cm, n= 55) and for females (mean perching height= 23.54 cm ± 17.12, range= 3–100 cm, n= 81) than for juveniles (mean perching height= 15.79 cm ± 13.10, range= 0–70 cm, n= 201) (Kruskall-Wallis= 32.450, df= 2, P< 0.001).

Discussion

Adults and juveniles of *R. proboscidea* were detected throughout the studied rainy seasons, with an abundance peak at the end of the rainy season. The highest abundance of juveniles at the end of the rainy season indicate events of recruitment. This pattern was also observed for *R. hoogmoedi*, another member of the *R. margaritifera* species group (Brito *et al.*, 2013), and for other Neotropical anuran species as *Pristimantis* aff. *fenestratus*, *Adenomera andreae* and *Anomaloglosus stepheni* (Moreira and Lima, 1991; Waldez *et al.*, 2011). Likewise, other anurans have recruitment events that occur mainly in the dry season (Moreira and Lima, 1991; Watling and Donnelly, 2002) or continuously (Chinchilla-Lemus *et al.*, 2020). The high juvenile body size values detected at the beginning of the two studied rainy seasons probably represent individuals that were born in the previous rainy season, a pattern also detected for *Physalameus marmoratus* from the Brazilian Cerrado (Giaretta and Menin, 2004). The same reasoning could be applied to the variation in body size of males: males with smaller sizes were detected in the beginning of the rainy season, indicating the entrance of these individuals in the reproductive stage; whereas those with greater sizes were found in the end of the rainy season - a fact also observed for *R. hoogmoedi* (Brito *et al.*, 2013).

The small number of reproductive events observed in *R. proboscidea* throughout the studied periods indicates seasonal reproduction, with a peak

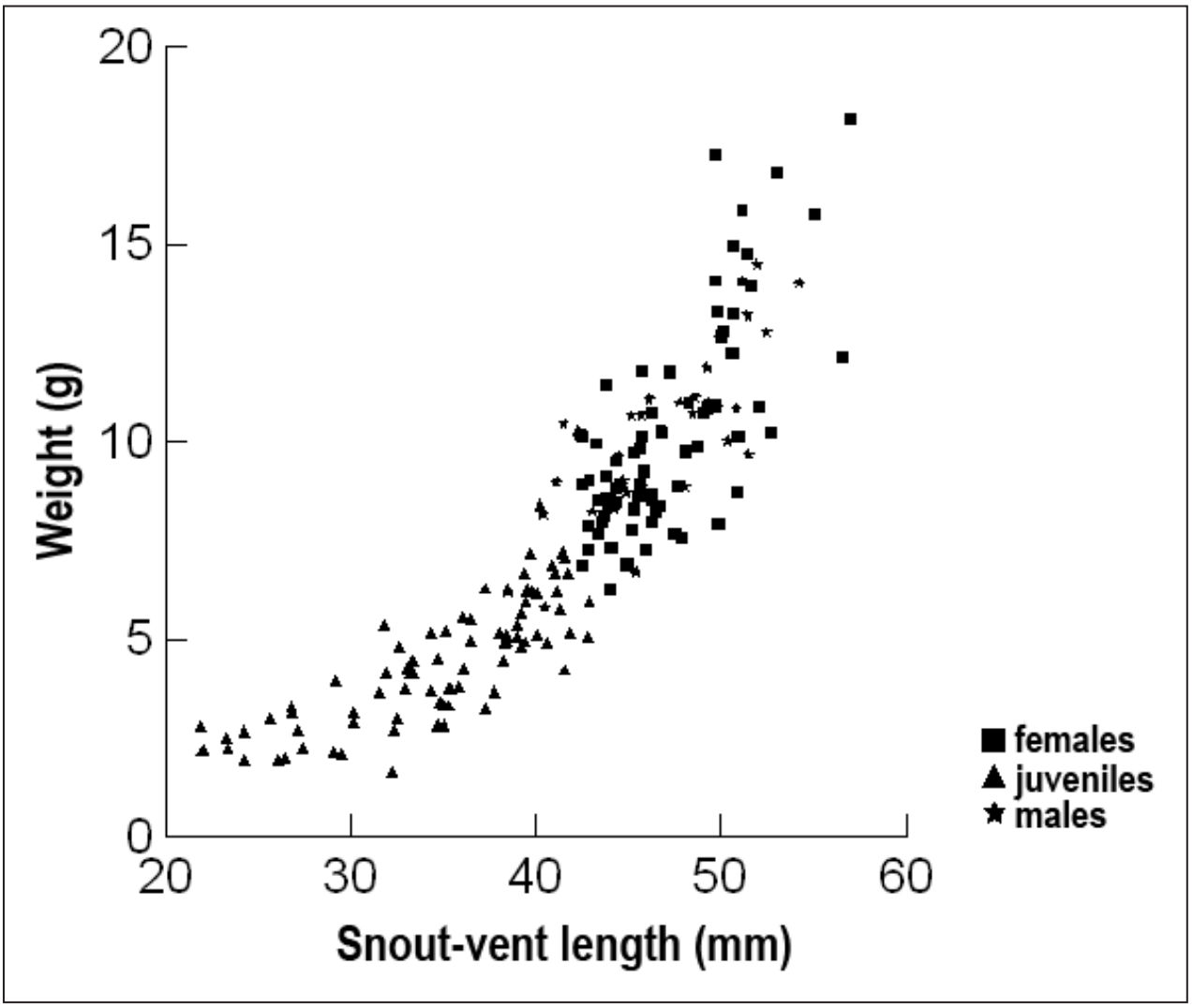

Figure 4. Relationship between body size (snout-vent length, in mm) and mass (in grams) for 191 individuals of *Rhinella proboscidea* measured in five nocturnal visual samplings at Reserva Florestal Adolpho Ducke, Manaus, Amazonas, Brazil. Data not transformed.

from the middle to the end of the rainy season. This fact is probably related to the necessity of temporary ponds for the reproduction of this species, since the availability of temporary water bodies vary in time (Rodrigues *et al.*, 2010). Moreover, the variation in abundance of adults and juveniles throughout the studied rainy seasons should be related to the variation in number of water bodies at the Reserva Ducke. Rodrigues *et al.* (2010) detected precipitation levels below the annual averages for the months of January to March 2003, but observed above average rainfall in March 2004. The authors also stressed that the number of ponds strongly varied, and estimated that there were two times more ponds in 2004 than when compared to 2003 at the Reserva Ducke. We suggest that this variation in water body availability throughout the years should be related to the small number of individuals, mainly juveniles and females of *R. proboscidea,* found in our study at the end of the first rainy season. Anurans with aquatic reproduction in Central Amazonia depend on streamside pond availability (Menin *et al.*, 2011), and should be especially vulnerable to changes in weather patterns. Thus, changes in pond availability due alterations in the precipitation regime should affect juvenile recruitment events and consequently affect the species reproductive success (Greenberg *et al.*, 2017).

The operational sex ratio varied among sampling periods, and it was near to 1:1 (males:females) in three of the sampling periods. However, considering only the fifth sampling period (where we detected the highest number of small-sized juveniles, indicating greater reproductive activity) and all sampling periods together, we found a female-biased sex

ratio. This fact can be a result of the higher motility of females, which makes them conspicuous and easily detected. We suggest that males probably stand more near to the reproduction areas, while females may be more mobile, searching for reproductive male aggregations in explosive reproduction. On the other hand, Izzo *et al.* (2012) detected a male-biased sex ratio for *R. proboscidea* in the same studied area, but the authors collected these data only in water bodies with male aggregations in calling activity. In contrast, we collected our data systematically along standardized sampling units in the two rainy seasons.

We did not detect significant sexual dimorphism in body size and mass between males and females of *R. proboscidea* when considering the whole dataset, although we found significative differences in both measurements in the fifth sampling period. Our results contrast to what was found for all other species in the *R. margaritifera* group, where females are larger (Caramaschi and Niemeyer, 2003; Lima *et al.*, 2007; Ávila *et al.*, 2010) and heavier than males (Hoogmoed and Avila-Pires, 1991; Caldwell and Araújo, 2005; Duellman, 2005). This type of sexual size dimorphism is a common characteristic of most anuran species (Shine, 1979), including many bufonid species (e.g., Monnet and Cherry, 2002; Arantes *et al.*, 2015). The absence of sexual dimorphism in size detected by us may be related to territorial defense and fighting (Shine, 1979). As previously mentioned, *R. proboscidea* is an explosive breeder (Menin *et al.*, 2006), and males use a lot of energy in the search for females in large mating aggregations in small water bodies, and also when fighting for females (Wells, 1977, 2007). The fighting is relatively common, and results in mating balls that are often fatal to some females (Wells, 2007; Izzo *et al.*, 2012).

In our study, adults and juveniles of *R. proboscidea* were found resting during the night, perched on leaves of small shrubs and on stems or fallen tree trunks above the ground, as observed by Zimmerman and Bogart (1988) for both this species and other species of the *R. margaritifera* species group, such as *R. castaneotica*, *R. magnussoni* and *R. hoogmoedi* (Caldwell and Araújo, 2005; Lima *et al.*, 2007; Brito *et al.*, 2013). The use of perching sites during the night should be related to the avoidance of ground dwelling predators, as described for snakes, by Martins (1993). We also detected ontogenetic variations in the use of resting sites: juveniles were found in lowest sites when compared to adults (males and females), which probably relates to the ability of adults in accessing higher resting places.

In conclusion, *R. proboscidea* varies in adult and juvenile body size and body mass throughout the rainy seasons. Juvenile recruitment events occurred mainly at the end of the second rainy season, and the abundance of individuals are probably related to variation in water body availability for reproduction along the studied periods. Moreover, the absence of sexual dimorphism in body size and mass for this species disagrees with what is known for other anuran species and species of the *R. margaritifera* group.

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