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Diet and prey composition of *Leptodactylus mystaceus* from an open area in the Guiana Shield region

Dieta y composición de presas de *Leptodactylus mystaceus* de un área abierta en la región del Escudo Guayanés

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ABSTRACT

In this study we describe the diet of the leptodactylid *Leptodactylus mystaceus* during the rainy season from an open area in the Cancão Natural Municipal Park, municipality of Serra do Navio, a lowland area of the Guiana Shield. Frogs were captured by hand monthly from February to July 2019. We examined 40 individuals of *L. mystaceus*, that were measured and treated with a stomach-flushing method. Three categories of arthropods dominated the diet: Araneae (spiders), Coleoptera (beetles), and Orthoptera (grasshoppers). The most important item in the diet was coleopterans and the niche breadth was narrow. The correlation between the volume of consumed prey and snout-vent length, jaw width, and body mass was not significant. Of the prey items according to hardness and mobility, we found that hard and intermediate prey were more common, respectively. Our results show that *L. mystaceus* shared characteristics with the generalist behavior of typical "sit-and-wait" foragers and "non-ant specialist predators".

Keywords: Eastern Amazon; Food Items; Generalist.

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RESUMEN

En este estudio describimos la dieta del leptodáctilo *Leptodactylus mystaceus* durante la estación lluviosa de un área abierta en el Parque Natural Municipal de Cancão, municipio de Serra do Navio, una zona de tierras bajas del Escudo Guayanés. Las ranas fueron capturadas a mano mensualmente de febrero a julio de 2019. Examinamos 40 individuos de *L. mystaceus*, que fueron medidos y tratados con un método de lavado de estómago. Tres categorías de artrópodos dominaban la dieta: Araneae (arañas), Coleoptera (escarabajos) y Orthoptera (saltamontes). El elemento más importante en la dieta eran los coleópteros y la amplitud del nicho era estrecha. La correlación entre el volumen de presa consumida y la longitud hocico-cloaca, el ancho de la mandíbula y la masa corporal no fue significativa. De las presas según dureza y movilidad, encontramos que las presas duras e intermedias fueron más comunes, respectivamente. Nuestros resultados muestran que *L. mystaceus* comparte características con el comportamiento generalista de los recolectores típicos de "sentarse y esperar" y " depredador especialista no hormiga".

Palabras clave: Alimentos; Amazonía Oriental; Generalista.

INTRODUCTION

Frogs of the family Leptodactylidae are classified as non-ant specialists and exhibit a "sit and wait" foraging behavior (Toft 1980, 1981), and are considered generalist predators that feed mostly on invertebrates, especially arthropods (Baía *et al.* 2020, Oliveira-Souza *et al.* 2021), but several species eat small vertebrates sporadically (Sousa *et al.* 2016, Ceron *et al.* 2018, do Couto *et al.* 2018, Cuestas-Carrillo *et al.* 2019).

Leptodactylus mystaceus (Spix, 1824) is a large-sized species of the *Leptodactylus fuscus* group, that inhabits tropical forests, enclaves of savannahs flooded and open areas and can be found on the ground or around temporary ponds (Affonso *et al.* 2011, Andreani *et al.* 2017, Lima *et al.* 2017). Although *L. mystaceus* has a broad distribution in the Amazon basin and extends as far as the interior portions of the Brazilian states of Minas Gerais and São Paulo, as well as the northern Atlantic Forests of Brazil (Toledo *et al.* 2005, de Sá *et al.* 2014), the natural history of this species, including its diet, restricted to studies of Camera *et al.* (2014) and Oliveira-Souza *et al.* (2021), and anecdotal observations by Figueiredo *et al.* (2018) and Moreira-Brito *et al.* (2020).

In the present study, we aimed to describe the diet of the leptodactylid *L. mystaceus* during the rainy season and correlate morphometric variables of frogs with the ingested prey volume as well as assess the trophic niche amplitude of the species. We also describe the prey preference in the diet in terms of hardness and mobility and constructed the prey category accumulation curve about the number of stomachs studied to determine the sampling efficiency.

MATERIAL AND METHODS

We carried out the study in the Cancão Natural Municipal Park, municipality of Serra do Navio (0°54' North, 52°0' West), a lowland area of the Guiana Shield, the northwestern center portion of the state of Amapá, Brazil for six months, from February to July 2019, as part of a long-term study of natural history of anurans in the eastern Amazon. Our sampling was restricted to an open area parallel to the right bank of the Amapari River to maximize the detection of the species (Silva e Silva and Costa-Campos 2018). The climate of the region is classified as Equatorial (Am) following Koppen's classification, with two well defined seasons, a dry season (August – December) and a rainy season (January – July) with high rainfall (Alvares *et al.* 2013). The average annual temperature and precipitation are 27.6 °C and 2850 mm, respectively (NHMET c2022).

We captured frogs by hand, measured them on site, and conducted stomach flushing (see Solé *et al.* 2005) using a syringe (three ml to 10 ml for anurans of 20 mm–50 mm SVL), soft infusion tube, and water from the site of

capture. We measured snout-vent length (SVL), and jaw width (JW) with an electronic caliper (0.1 mm precision), and body mass with digital balance (precision 0.1 g). After sampling all stomach contents by flushing and taking biometric variables, the frogs were released at the end of the daily sampling, avoiding the recapture of sampled individuals. In the laboratory, the prey contents were analyzed under a stereoscopic microscope ZEISS model Stemi 2000-C, and items were determined to order level according to the identification key of Rafael *et al.* (2012).

We estimated the prey volume using the ellipsoid formula, which uses values of length and width of whole preys (Colli and Zamboni 1999), where $\boldsymbol{V}$ represents prey volume, $\boldsymbol{l}$ = item length e $\boldsymbol{w}$ = item width:

$$\boldsymbol{V = \frac{4}{3}\ \pi\ \left(\frac{l}{2}\right) x \left(\frac{w}{2}\right)^2}$$

The Index of relative importance, which indicates the importance of each item consumed by the animal, was determined from the relative importance (N%), relative occurrence (F%), and relative volume (V%), where IRI = Index of relative importance; F% = frequency of occurrence; N% = numerical frequency; V% = volumetric frequency in each category of prey in the diet.

$$\boldsymbol{IRI = \left(\frac{F\% + N\% + V\%}{3}\right)}$$

For the analysis of food niche breadth, was calculated using the Levins index (B) described by Pianka (1986). In this case, when the value of *B* is between 0 - 0.50 the species will be considered specialist, and values between 0.51 - 1.0 represent generalist individuals.

$$\boldsymbol{B = 1 \Big/ \sum_{i=1}^{n} p_{i^2}}$$

Where $\boldsymbol{B}$ = Levins index (trophic niche breadth); $\boldsymbol{i}$= prey category; $\boldsymbol{n}$ = number of categories; $\boldsymbol{pi}$ = numerical or volumetric proportion of the category of prey $\boldsymbol{i}$ in the diet.

To evaluate the relationship between a frog's snout-vent length, jaw width, and body mass with the volume of the largest prey for each individual we performed a Spearman correlation test using BioEstat 5.0 software (Ayres *et al.* 2007) using morphometric variables as independent variables and prey-volumes as dependent variables (Zar 2010). We classified each prey item into two categories according to hardness (hard, soft, intermediate) and evasiveness (evasive, sedentary, intermediate) following Vanhooydonck *et al.* (2007) and Mohanty and Measey (2018). These qualitative traits of prey are relevant concerning the foraging and feeding strategies of anurans according to Toft (1980).

RESULTS

We examined 40 individuals of *L. mystaceus*, from which 26 stomach contents were obtained by flushing. Stomach flushing revealed 19 prey items belonging to eight taxonomic categories (Table 1), whereby a mean of 1.4 prey items was found per individual (min = 1; max = 6). Based on the Index of Relative Importance (IRI), coleopterans (91.3 %) and orthopterans (44.8 %), constituted the most important prey categories.

The correlation values between SVL (rs = 0.3322; p = 0.2458), jaw width (rs = 0.3011; p = 0.2955), and body mass (rs = 0.0440; p = 0.8813) with the volume of the prey were not significant (Fig. 1). The morphological variables varied approximately two-fold for each variable (Table 2). The Levins index for niche breadth was 0.40. Of the prey items according to hardness, we found that hard prey (63.2 %) was more common than soft (21.1 %) and intermediate prey (15.8 %); according to evasiveness, intermediate prey (57.9 %) was more common than sedentary and evasive prey (Fig. 2).

DISCUSSION

Although the diet of this species has been studied in other locations (see Camera *et al.* 2014) and in the same locality with different methods (see Oliveira-Souza *et al.* 2021), our study adds data on the natural history of the *L. mystaceus*. The use of stomach-flushing in the present study revealed a greater number of prey (n = 8) of which Diplura, Formicidae, anurans, and lizards were not recorded in a previous study (e.g., Oliveira-Souza *et al.* 2021). For this reason, our study showed once again that stomach-flushing is an efficient method to study the diet of anurans (Solé *et al.* 2005, Solé and Rödder 2010, Solé *et al.* 2018).

The diet of *L. mystaceus* was dominated by three prey categories (Coleoptera, Araneae, and Orthoptera), which represent more than 82 % of the prey consumed. The orders Coleoptera, Araneae, and Orthoptera are a group of highly diverse organisms and very abundant in the environment (Rafael *et al.* 2012). The dominance of Coleoptera and Araneae in the diet of *L. mystaceus* has been reported before by Oliveira-Souza *et al.* (2021) for a population in Cancão Natural Municipal Park in Amapá state (78 % prey consumed), and Dermaptera and Coleoptera by Camera *et al.* (2014) in Florentino farm, in Novo Progresso, Pará state (72 % prey consumed). However, we cannot say a preference for these items because available prey in the environment was not assessed. Associated with it, the high number of individuals with empty stomachs suggests that diet could be potentially more diverse than we recorded.

Leptodactylus mystaceus shared some characteristics that agree with the generalist behavior of typical "sit-and-wait" foragers such as cryptic coloration, few food items per stomach (mean = 1.4 items per stomach), high percentage of mobile food items (e.g. Coleoptera, Orthoptera, Formicidae, Annelida, Anura and Lizard) and low trophic niche breadth. Although low trophic niche breadth, *L. mystaceus* is not specialized in any prey category. According to Toft (1980) and based on their trophic niche breadth, *L. mystaceus* is a "non-ant specialist predator", with stomach content with high mobile arthropods.

We found no significant correlation between ingested prey volumes and frog SVL, jaw width, and weight, indicating that a large size did not imply an increase in the size of prey ingested. The lack of correlations between prey volume and SVL and mouth width was also observed for other leptodactylids (e.g., Solé *et al.* 2009, Sanches *et al.* 2019;

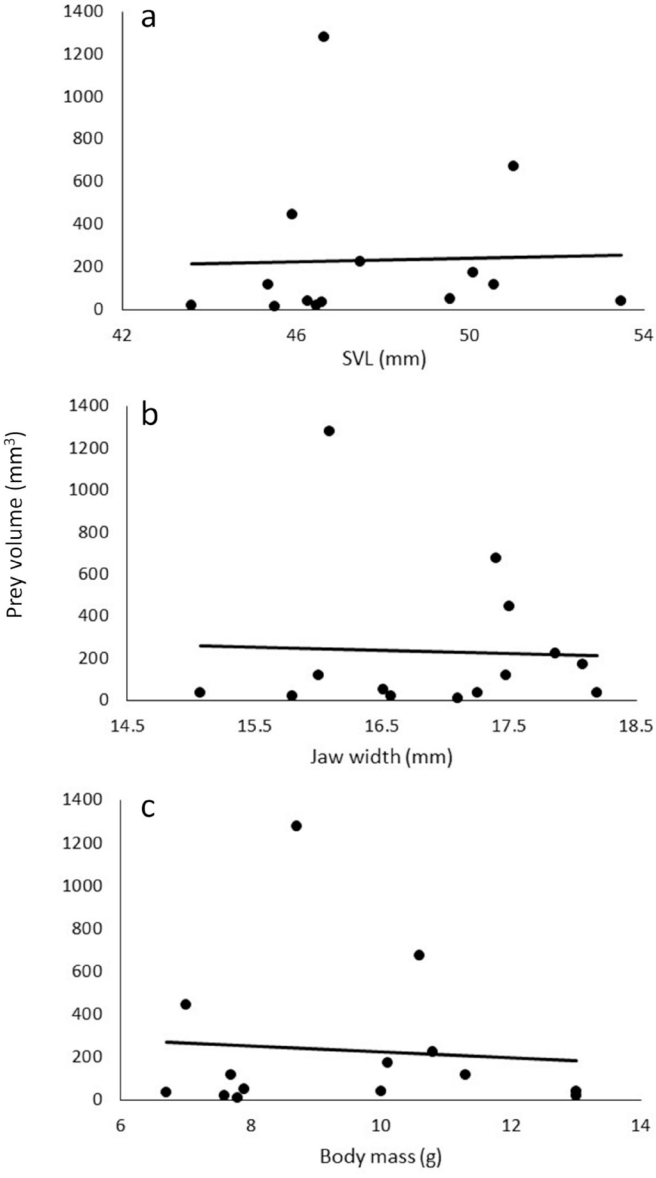

Figure 1. Spearman correlation between (A) snout-vent length (SVL), (B) Jaw width and (C) Body mass with volume of prey consumed of *L. mystaceus*.

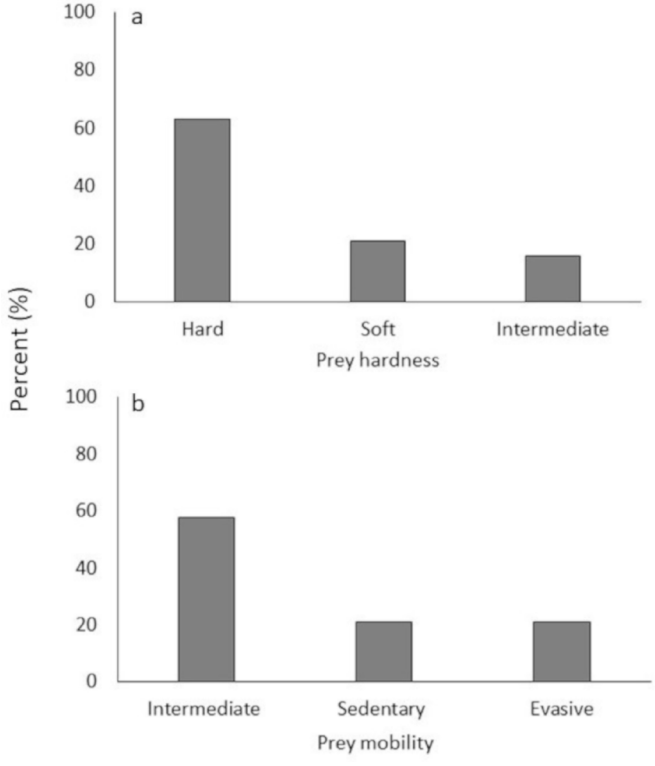

Figure 2. Classification of prey item (%) according to hardness (hard, soft, intermediate) and mobility (evasive, sedentary, intermediate) in the diet of *L. mystaceus*.

Table 1. Composition, Number (N%), Frequency (F%), Volume (V%), Index of relative importance (IRI), Hardness and Mobility of each category of prey recorded in the stomach contents of ***L. mystaceus*** in the municipality of Serra do Navio, Amapá.

Prey item	N	N (%)	F	F (%)	V	V (%)	IRI	Hardness	Mobility
Araneae	3	15.8	3	18.8	57.3	1.9	35.2	Soft	Sedentary
Diplura	1	5.3	1	6.3	61.6	2.1	12.2	Soft	Sedentary
Coleoptera	9	47.4	6	37.5	572.1	19.2	91.3	Hard	Intermediate
Orthoptera	2	10.5	2	12.5	1954.3	65.5	44.8	Intermediate	Evasive
Formicidae	1	5.3	1	6.3	19.6	0.7	11.7	Hard	Intermediate
Annelida	1	5.3	1	6.3	222.9	7.5	14.0	Intermediate	Intermediate
Anura	1	5.3	1	6.3	44.9	1.5	12.0	Hard	Evasive
Lizard	1	5.3	1	6.3	52.3	1.8	12.1	Hard	Evasive

Pedroso-Santos *et al.* 2022), that prefer to ingest smaller prey items than those who feed on fewer but larger prey items. A significant correlation between predator size and jaw width with prey volume may be more common for frogs that prey exclusively on larger prey or possess low metabolism, than generalist species that feed opportunistically and can be positively associated with the number of consumed taxa (Toft 1980, Moser *et al.* 2022). In the same way that body size seems to define the capacity to ingest a large number of prey items, mouth width could be a limiting factor in prey selection (Oliveira *et al.* 2019).

Although the trophic ecology of *L. mystaceus* is well understood, we highlight that stomach-flushing is an efficient method to study the diet of anurans, since exclusive food items in our study (i.e., Diplura, Formicidae, Anura, and lizard) were detected in the trophic guild of this species. We hope that our data will improve the understanding of the trophic ecology of this species, as well as provide support for further studies in other leptodactylids from the Guiana Shield region.

Table 2. Range, mean, and standard deviation of morphological variables for the 40 individuals of *L. mystaceus* analyzed for stomach contents.

Morphometric variables	Range	Mean	SD
Snout-vent length (mm)	43.6–53.5	47.7	2.7
Jaw width (mm)	15.1–18.2	16.9	0.9
Body mass (g)	6.7–13.0	9.4	2.1

AUTHORS CONTRIBUTIONS

CECC field assistance and biological analysis; MMSS field assistance, helped with specimen observations and analysis; AEOS helped with specimen observations and analysis; PRS field assistance, writing, statistical and biological analysis; CECC reviewing and writing the manuscript.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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