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Food habits of *Rhinella proboscidea* (Anura: Bufonidae) in terra firme forests of central Amazonia

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Abstract

Food habits of *Rhinella proboscidea* (Anura: Bufonidae) in terra firme forests of central Amazonia. Anuran species are considered generalist and opportunist feeders. However, some species have dietary specializations. Here we describe the diet composition of *Rhinella proboscidea* based on the stomach content of 29 individuals captured in terra firme forests in Manaus and São Sebastião do Uatumã, state of Amazonas, Brazil. Each prey item was measured and identified to Order, Suborder or Family; ants were identified to Genus. We determined and tested for differences in the trophic niche breadth and the relationships between the frog size and the volume of the largest prey item. We recorded 1614 prey items of 44 taxa. Hymenoptera was the most abundant Order followed by Isoptera, Acari, Coleoptera, Orthoptera, Araneae, Hemiptera, Dermaptera, Diptera, and Collembola. Formicidae comprised 46.56% of the all stomach items and was represented by six subfamilies and 22 genera. Additionally, we found 80 nematodes in a total of 18 frogs. There was a significant difference in the trophic niche breadths of the study areas, and a positive relationship between the toad size and volume of the largest prey item consumed. *Rhinella proboscidea* feeds on a variety of invertebrates, with ants, termites, and mites being the most abundant; this suggests that the toad is an active forager. The most common subfamily was Myrmicinae primarily represented by *Crematogaster* and *Atta.* Differences in trophic niche breadths of the study areas may be related to seasonal differences in the diet composition or local factors.

Keywords: amphibians, diet composition, Formicidae, northern Brazil.

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Resumo

Hábito alimentar de *Rhinella proboscidea* (Anura: Bufonidae) em florestas de terra firme da Amazônia Central. Os anuros são considerados predadores generalistas e oportunistas, mas algumas espécies apresentam especialização na dieta. Neste estudo descrevemos a composição da dieta de *Rhinella proboscidea* baseada no conteúdo estomacal de 29 indivíduos coletados em florestas de terra firme de Manaus e São Sebastião do Uatumã, estado do Amazonas, Brasil. Cada item encontrado foi medido e identificado até o nível de Ordem, Subordem ou Família; as formigas foram identificadas até gênero. Determinamos e testamos a diferença na amplitude do nicho trófico e a relação entre o tamanho dos anuros e o volume da maior presa consumida. Encontramos 1.614 presas pertencentes a 44 categorias taxonômicas. Hymenoptera foi a Ordem mais abundante seguida por Isoptera, Acari, Coleoptera, Orthoptera, Araneae, Hemiptera, Dermaptera, Diptera e Collembola. Formicidae abrangeu 46,56% do total de itens encontrados nos estômagos e foi representada por seis subfamílias e 22 gêneros. Adicionalmente, encontramos 80 nematoides nos estômagos de 18 anuros. Houve diferença significativa na amplitude do nicho trófico entre as áreas de estudo e uma relação positiva entre o tamanho dos anuros e o volume das maiores presas consumidas. *Rhinella proboscidea* alimenta-se de alguns grupos de invertebrados, e formigas, cupins e ácaros são os itens mais abundantes, sugerindo que essa espécie é uma forrageadora ativa. A subfamília mais comum foi Myrmicinae, representada pelos gêneros *Crematogaster* e *Atta.* As diferenças no nicho trófico da espécie entre as áreas estudadas podem estar relacionadas com diferenças sazonais na composição da dieta ou a fatores locais.

Palavras-chave: anfíbios, composição da dieta, Formicidae, norte do Brasil.

Introduction

Bufonids, like other anurans, are considered to be feeding generalists that consume a great variety of prey (Duellman and Trueb 1994)—mainly insects and arachnids (Parmelee 1999, Batista *et al.* 2011). However, studies have reported some dietary specialization in bufonids (Toft 1980, 1981) that feed preferentially on ants (Isacch and Barg 2002, Solé *et al.* 2017). *Rhinella proboscidea* (Spix, 1824) is a medium-sized anuran found in terra firme (unflooded) forests (Lima *et al.* 2012) along the Amazon River ranging from Peru to the city of Manaus, Brazil (Frost 2019). It is a member of the *Rhinella margaritifera* species group (Frost 2019). Individuals are terrestrial, diurnal, and live in the leaf litter of forests (Lima *et al.* 2012). Lima (1998; as *Bufo* cf. *typhonius*) and Lima and Magnuson (1998) showed that in central Amazonia, small individuals of *R. proboscidea* fed largely on mites, whereas adults fed largely on ants and the prey types choice is independent of the prey size. Herein, we provide a detailed description of the composition of the diet of *R. proboscidea* from terra firme forests of central Amazonia, Brazil.

Materials and Methods

We examined 29 *Rhinella proboscidea* that are deposited in the Amphibia Section of the Paulo Bührnheim Zoological Collection of the Universidade Federal do Amazonas, Manaus, Amazonas, Brazil (CZPB-AA 006–020, CZPB-AA 438, and CZPB-AA 476–488). The toads were euthanized 2–5 hr after collection with an overdose of 2% lidocaine hydrochloride, fixed in 10% formalin, and preserved in 70% alcohol. The specimens were collected in the following areas: (1) municipality of Manaus, state of Amazonas, Brazil, in the Adolpho Ducke Forest Reserve (13 females captured in June 2001, between 02°55' and 03°01' S and between 59°53' and 59°59' W) and the Experimental Farm of the Universidade Federal do Amazonas (1 female captured in June 2011, between 02°37'17" and 02°39'41" S and between 60°03'29" and

60°07'57" W); and (2) two forest sites located on opposite margins of the Jatapú River (15 specimens—5 males, 4 females and 6 juveniles—captured in September 2011, left margin at 01°55'53" S and 58°15'21" W; right margin at 02°01'31" S and 58°11'24" W) at the municipality of São Sebastião do Uatumã, state of Amazonas, Brazil. The Adolpho Ducke Forest Reserve has an area of 10,000 ha, whereas the Experimental Farm of the Universidade Federal do Amazonas is 3000 ha. Both locations include the terra firme habitat with a well-drained forest that does not flood seasonally and that has a closed canopy, emergent trees, and abundant sessile palms. The sites along the Jatapú River contain terra firme forests with tall canopy and an abundance of palms, seasonally flooded meadows and patches of dense *campina* forest (Oliveira *et al.* 2014). All sampling sites have a tropical monsoon climate without a dry season (Peel *et al.* 2007), a rainy season lasting from November–May and a mean annual temperature of approximately 26°C (Marques-Filho *et al.* 1981).

The stomach contents were identified to order, suborder or family, following the identification keys of Triplehorn and Johnson (2011) and Rafael *et al.* (2012). The ant genera were determined based on Baccaro *et al.* (2015). We measured the length and width of each prey item with an ocular micrometer connected to a Zeiss Stemi SV 11 stereomicroscope. The volume of each item was estimated using the ellipsoid formula defined by Dunham (1983): $V = (4\pi/3)(\text{length}/2)(\text{width}/2)^2$. We also calculated the index of relative importance (IRI; Pinkas *et al.* 1971) to each prey category and each study area with the formula IRI = (%*N* + %V)%*F*, where %*N* is the numerical percentage, %V is the volumetric percentage, and %*F* is the frequency of occurrence percentage (percentage of occurrence of each prey category in relation to the total samples). The trophic niche breadths in both sites (Manaus and Jatapú River) were determined using the Shannon-Wiener diversity index (H') (Krebs 1999). The values of this index were compared by means of a Student's t-test (Zar 2010). We also measured the snout–vent lengths (SVL) of the toads with digital callipers and used Spearman's correlation coefficient to evaluate correlations between the volume of the largest prey in the stomach content and the SVL. This analysis was performed for the two areas combined because of the small sample sizes. The software Systat 12.0 was utilized for statistical and graphical analysis.

Results

A total of 1614 prey items were found in the stomach of 27 toads from both study areas; two individuals (14.3%) from Manaus had empty stomachs. The prey items represent 44 taxa, with Manaus having 899 prey of 23 taxa and the Jatapú River having 715 prey of 39 taxa (Table 1). The number of prey items per stomach varied from 2–336 items (mean = 59.78 ± 99.89) and the number of prey taxa from 1–12. Of the orders, Hymenoptera was the most abundant (750 individuals), followed by Isoptera (511 individuals), Acari (290), Coleoptera (25), Orthoptera (17), Araneae (12), Hemiptera (5), Dermaptera (2), Diptera (1), and Collembola (1). Formicidae (745 individuals of 6 subfamilies—Dorylinae, Dolichoderinae, Ectatomminae, Formicinae, Myrmicinae, and Ponerinae—and 22 genera) represented 46.56% of the stomach items. Myrmicinae was the most abundant subfamily and *Crematogaster* the most abundant genus, both in Manaus and Jatapú. Additionally, 80 nematodes (Phylum Nematoda) were found in 18 (58.06%) toads, which had from 1–14 nematodes per stomach. Plant material was found in 25.8% of the stomachs, whereas sand grains and silt were present in 6.45% of the stomachs.

The frequency and volume of prey items in each sampling site are listed in Table 1 and are discussed in descending order of importance below. In Manaus toads, the most frequently encountered prey were Araneae, Coleoptera of the family Curculionidae, and unidentified and Formicidae/Ectatomminae of the genus

Table 1. Prey items of *Rhinella proboscidea* in two terra firme forests in central Amazonia, Brazil. *N* = number of prey items; %*N* = relative abundance; %*F* = relative frequency; *V* = volume; %*V* = relative volume; IRI = index of relative importance.

Prey Category	Manaus (*N* = 12)						Jatapú (*N* = 15)					
	N	**%*N***	**%*F***	***V* (mm³)**	**%*V***	**IRI**	***N***	**%*N***	**%*F***	***V* (mm³)**	**%*V***	**IRI**
ARACHNIDA												
Acari												
Oribatida	289	32.15	25.00	8.57	0.12	806.75	1	0.14	6.67	0.95	0.03	1.13
Araneae	5	0.56	33.33	1252.28	16.99	584.94	7	0.98	33.33	268.72	8.51	316.30
ENTOGNATHA												
Collembola												
Neanuridae	1	0.11	8.33	0.61	0.01	1.00	-	-	-	-	-	-
INSECTA												
Coleoptera												
Carabidae	-	-	-	-	-	-	1	0.14	6.67	35.95	1.14	8.54
Ceratocanthidae	-	-	-	-	-	-	1	0.14	6.67	109.36	3.46	24.01
Chrysomelidae	-	-	-	-	-	-	1	0.14	6.67	17.65	0.56	4.67
Curculionidae	5	0.56	33.33	185.21	2.51	102.32	2	0.28	13.33	113.72	3.60	51.72
Scolytidae	-	-	-	-	-	-	1	0.14	6.67	0.57	0.02	1.07
Staphylinidae	-	-	-	-	-	-	5	0.70	6.67	11.53	0.37	7.14
Unidentified	4	0.44	33.33	176.98	2.40	94.66	5	0.70	26.67	111.76	3.54	113.08
Dermaptera												
Anisolabididae	-	-	-	-	-	-	2	0.28	6.67	58.72	1.86	14.27
Diptera												
Phoridae	-	-	-	-	-	-	1	0.14	6.67	1.55	0.05	1.27
Hemiptera	3	0.33	25.00	42.77	0.58	22.75	2	0.28	13.33	17.48	0.55	11.06
Hymenoptera												
Formicidae												
Dolichoderinae												

Table 1. *Continued.*

Prey Category	Manaus (*N* = 12)						Jatapú (*N* = 15)					
	N	**%*N***	**%*F***	***V* (mm³)**	**%*V***	**IRI**	***N***	**%*N***	**%*F***	***V* (mm³)**	**%*V***	**IRI**
Dolichoderus	4	0.44	25.00	13.16	0.18	15.50	37	5.17	60.00	42.90	1.36	391.80
Azteca	-	-	-	-	-	-	18	2.52	13.33	21.32	0.68	42.56
Tapinoma	-	-	-	-	-	-	1	0.14	6.67	6.88	0.22	2.40
Dorylinae												
Unidentified	1	0.11	8.33	2.63	0.04	1.25	5	0.70	13.33	1.95	0.06	10.13
Eciton	2	0.22	8.33	26.31	0.36	4.83	18	2.52	26.67	18.00	0.57	82.41
Labidus	-	-	-	-	-	-	2	0.28	6.67	71.37	2.26	16.94
Ectatomminae												
Gnamptogenys	3	0.33	25.00	14.49	0.20	13.25	33	4.62	26.67	186.73	5.91	280.83
Ectatomma	15	1.67	33.33	413.60	5.61	242.64	1	0.14	6.67	15.88	0.50	4.27
Formicinae												
Camponotus	2	0.22	16.67	39.42	0.53	12.50	62	8.67	53.33	324.84	10.29	1011.14
Nylanderia	-	-	-	-	-	-	3	0.42	13.33	6.95	0.22	8.53
Myrmicinae												
Atta	8	0.89	25.00	35.95	0.49	34.50	139	19.44	33.33	624.93	19.79	1307.54
Crematogaster	26	2.89	16.67	18.31	0.25	52.34	244	34.13	20.00	263.87	8.36	849.80
Blepharidatta	-	-	-	-	-	-	1	0.14	6.67	3.82	0.12	1.73
Cephalotes	1	0.11	8.33	6.50	0.09	1.67	-	-	-	-	-	-
Cyphomyrmex	-	-	-	-	-	-	3	0.42	13.33	10.26	0.32	9.86
Pheidole	2	0.22	8.33	0.42	0.01	1.92	30	4.20	26.67	19.30	0.61	128.28
Sericomyrmex	7	0.78	16.67	15.23	0.21	16.50	-	-	-	-	-	-
Trachymyrmex	1	0.11	8.33	4.69	0.06	1.41	42	5.87	60.00	129.96	4.12	599.40
Unidentified	-	-	-	-	-	-	3	0.42	13.33	5.73	0.18	8.00
Ponerinae												
Anochetus	-	-	-	-	-	-	3	0.42	6.67	8.69	0.28	4.67
Mayaponera	5	0.56	8.33	19.18	0.26	6.83	2	0.28	13.33	4.44	0.14	5.60

Table 1. *Continued.*

Prey Category	Manaus (N = 12)						Jatapú (N = 15)					
	N	%N	%F	V (mm³)	%V	IRI	N	%N	%F	V (mm³)	%V	IRI
Neoponera	-	-	-	-	-	-	7	0.98	26.67	83.55	2.65	96.81
Odontomachus	1	0.11	8.33	24.82	0.34	3.75	7	0.98	26.67	99.21	3.14	109.88
Pachycondyla	-	-	-	-	-	-	2	0.28	13.33	37.20	1.18	19.46
Unidentified	2	0.22	8.33	10.16	0.14	3.00	2	0.28	13.33	6.65	0.21	6.53
Vespoidea	-	-	-	-	-	-	1	0.14	6.67	4.10	0.13	1.80
Platygastridae	-	-	-	-	-	-	1	0.14	6.67	0.72	0.02	1.07
Unidentified	-	-	-	-	-	-	3	0.42	6.67	20.11	0.64	7.07
Isoptera												
Termitidae	511	56.84	33.33	4820.97	65.39	4073.93	-	-	-	-	-	-
Orthoptera	1	0.11	8.33	240.56	3.26	28.07	16	2.24	40.00	389.94	12.35	583.60
TOTAL	**899**			**7372.80**			**715**			**3157.25**		

Ectatomma; volumetrically, the most important items were Isoptera, Termitidae, Araneae, Formicidae/Ectatomminae of the genus *Ectatomma,* and Orthoptera. At the Jatapú River sites, Formicidae/Dolichoderinae of the genus *Dolichoderus,* Myrmicinae of the genus *Trachymyrmex,* Formicinae of the genus *Camponotus* and Orthoptera were the frequent prey items; Formicidae/Myrmicinae of the genus *Atta,* Orthoptera, Formicinae of the genus *Camponotus,* and Araneae were the most important volumetrically. Based on the index of relative importance, the most important prey items for the specimens collected in Manaus were Isoptera Termitidae, Acari/Oribatida, Araneae, Formicidae/Ectatominae of the genus *Ectatomma,* and Coleoptera of the family Curculionidae (Table 1). For the specimens collected in the Jatapú River, the most important prey categories were Formicidae/Myrmicinae of the genus *Atta,* Formicinae of the genus *Camponotus,* Myrmicinae of the genera *Crematogaster* and *Trachymyrmex,* and Orthoptera (Table 1). The trophic niche breadth was significantly (t = -16.62, df = 1862, p < 0.001) greater in the Jatapú River (H'= 1.01) than Manaus (H' = 0.52). We found a positive relationship between the SVL of the toads and the respective size of the largest prey item consumed (r = 0.656, p < 0.001, Figure 1). The SVLs of the individuals of *Rhinella proboscidea* from Jatapú varied from 20.06–48.27 mm (mean = 36.11 ± 9.93), those of the specimens from Manaus varied from 56.32–59.10 mm (mean = 51.31 ± 4.31). The volume of the largest prey item consumed by each specimen from Jatapú and Manaus varied from 4.60–207.77 mm³ (mean = 47.86 ± 54.23) and from 4.62–358.97 mm³ (mean = 128.38 ± 115.09), respectively.

Discussion

Rhinella proboscidea fed on a variety of invertebrates, but the abundance of ants, termites, and mites as prey in the study areas as suggests that the feeding strategy was that of active

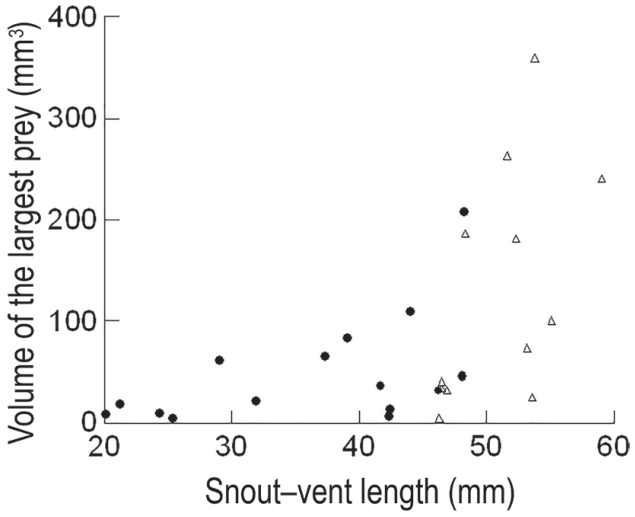

Figure 1. Relationship between the snout–vent lengths of *Rhinella proboscidea* and volume of the largest ingested prey in the two terra firme forests—the municipality of Manaus (open triangles) and Jatapú River (black dots) in central Amazonia, Brazil.

foragers (Toft 1980). This pattern also was observed for other species of *Rhinella,* as well as in other bufonids at localities across the globe (Clarke 1974, Hirai and Matsui 2002, Isacch and Barg 2002, Quiroga *et al.* 2009, Sabagh *et al.* 2012, Solé *et al.* 2017) and members of the neotropical *R. margaritifera* species group (Duellman 1978, Toft 1980, 1981, Parmelee 1999, Maragno and Souza 2011, Fajardo-Martínez *et al.* 2013, Astwood-Romero *et al.* 2016). According to Isacch and Barg (2002), bufonid species probably specialized in ant consumption because they lack teeth, which would constrain the toads to a diet of small prey.

For many species of *Rhinella,* beetles are the second most frequently consumed item (Duellman 1978, Toft 1980, 1981, Parmelee 1999, Isacch and Barg 2002, Quiroga *et al.* 2009, Maragno and Souza 2011, Sabagh *et al.* 2012). However, in our study areas, Isoptera (Termitidae), Acari, and Araneae were also important prey items in the diet of *R. proboscidea.* Additionally, the positive relationship that we observed between the size of toad and the size of the largest prey item consumed indicates that the size of the frog is a limiting factor in the selection of prey (Toft 1980). Eighty-three percent of the diet of smallest frogs (4 juveniles with SVLs from 20.06–25.37 mm) was composed of small Formicidae of the genera *Azteca, Dolychoderus, Pheidole,* and *Trachymyrmex.* Ontogenetic changes in the prey type corresponding to variation in body size of the toads were detected in *R. proboscidea* (Lima and Magnusson 1998) and *Rhinella scitula* (Caramaschi and Niemeyer, 2003) (Maragno and Souza 2011); both are members of the *R. margaritifera* species group. In our study, small prey items such as mites also were consumed by large individuals; 288 of these arachnids were found in the stomachs of only two individuals with SVLs of 52.34 and 46.51 mm. These data agree with those of Lima (1998) in which individuals of *R. proboscidea* of different sizes consumed mites, although large individuals were able to eat other types of prey.

We found a significant difference in the trophic niche breadth of *Rhinella proboscidea* in the study areas; this disparity may be related to seasonal differences in the prey abundance (Burton 1976, Galatti 1992). In Manaus, all the toads were collected in June, which corresponds to the end of the rainy season and the beginning of the dry season. The toads from the Jatapú River were captured in September during the dry season. Seasonal differences in the diet composition were found in anurans of different regions (Santos *et al.* 2004, Maragno and Souza 2011). Alternately, the differences in the diet composition between the study could be related to local factors that affect the prey composition and abundance. The study areas (Manaus and Jatapú) are separated by about 200–215 km; thus, the detected variation should be interpreted with caution.

Ants represent 46.56% of the prey items found in the stomachs of toads, with Myrmicinae being the most typical subfamily primarily represented by the genera *Crematogaster* and *Atta.* Because of its social complexity, Myrmicinae is considered to be the most diverse group of ants. This

subfamily also includes half of the formicid species (Bolton 1995). *Crematogaster* has a wide distribution in terra firme forest. These ants are generalists that can build polidomic nests with massive colonies in the leaf litter (Longino 2003). Members of the genus *Atta*, known as leaf-cutter ants, build complex nests with trails opening up at the soil surface (Cherrett 1968), thereby exposing them as potential prey. Some genera as *Dolichoderus*, *Azteca* (subfamily Dolichoderinae), *Gnamptogenys* (Ectatomminae), and *Camponotus* (Formicinae) are considered shrub or arboreal ants and probably were captured by *Rhinella proboscidea* because these ants typically do their forage on the soil (Baccaro *et al.* 2015).

Nematodes are common parasites found in the lungs, stomachs, and intestines of anurans, such as *Rhinella marina* (Linnaeus, 1758), *R. margaritifera* (Laurenti, 1768), and *R. icterica* (Spix, 1824) (Bursey *et al.* 2001, Goldberg *et al.* 2009, Pinhão *et al.* 2009). We considered the nematodes found in the stomachs of *R. proboscidea* as potential parasites. Other studies have found mainly encysted nematode larva in the stomach of anurans (Goldberg *et al.* 2009, Pinhão *et al.* 2009); thus, the presence of unencysted forms in the stomachs of *R. proboscidea* deserves additional attention.

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