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Diet of the Smoky Jungle Frog, *Leptodactylus pentadactylus*, (Anura, Leptodactylidae) in an urban forest fragment and in a preserved forest in Central Amazonia, Brazil

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Abstract. Understanding the natural history of amphibians in human altered habitats is essential to develop conservation and management actions. In this study, we determined the composition of the diet of *Leptodactylus pentadactylus* in an urban forest fragment and in a preserved forest, both placed in Central Amazonia, Brazil. Diet samples were obtained by stomach flushing of 33 individuals. Each prey item was measured and identified according to its taxonomic group. For each taxon found in the stomachs we determined the number of items, the percentages of volume, frequency and occurrence, and the index of relative importance. We tested for differences in trophic niche breadth, and the relationship between individual size and prey volume. We identified a total of 127 prey items belonging to 18 different taxonomic groups. Arthropods were the main source of food on both areas. Based on the index of relative importance Araneae, Scorpiones, Diplopoda, and Coleoptera were the most important prey items in the forest fragment while Araneae, Diplopoda, Coleoptera, and Diptera were the most important prey items in the preserved forest. Additionally, one small lizard species, *Alopoglossus angulatus*, was consumed by one of the frogs at the forest fragment. There was no significant difference in the trophic niche breath values obtained between the areas, and no correlation between the largest prey items consumed and body sizes of the frog individuals. Overall, the diet of the *L. pentadactylus* was similar in both sites and follows a generalist and opportunistic pattern resembling other species of medium or large-sized *Leptodactylus*.

Keywords. Amphibia, feeding ecology, stomach flushing, trophic niche breadth

Introduction

Knowledge about the natural history of amphibians is essential to understand how different species interact with their environments, and, consequently, to provide a basis for conservation and management actions (Young et al., 2001). Diet is one of the aspects of the biology of amphibians which can be affected by environmental change both in terms of ingested mass and food diversity (e.g. Smith et al., 2004; Cecala et al., 2007; Mahan and Johnson, 2007; Bower et al., 2014). Although some amphibians have shown strong dietary specialization or preferences (Toft, 1981; Das, 1996), most species are regarded as generalist predators (as adults), feeding mainly on invertebrates (Duellman and Trueb, 1994). The composition of invertebrates taxa usually depends on their relative availability (Hirai and Matsui, 2001; Menin et al., 2005). Thus, changes in the environment, affecting the diversity and abundance of a given prey, are expected to be reflected in the diet of generalist species that feeds on it.

In Amazonia, large areas of preserved forest have been degraded in favour of urban development, resulting in habitat loss and fragmentation (Achard et al., 2002). Among many other effects on biodiversity, habitat fragmentation may influence trophic interactions and prey availability due to changes in microclimatic variables (Debinski and Holt, 2000; Carey et al., 2001). Amphibian communities are among the most

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Figure 1. Adult male of *Leptodactylus pentadactylus* from campus of the Universidade Federal do Amazonas, municipality of Manaus, state of Amazonas, Brazil. (Photo: M. Menin).

affected by fragmentation and urbanization processes, with standing changes in diversity, abundance, and population structure caused by disruptions of the life cycle of occurring species (Hamer and McDonnell, 2008). Nevertheless, large urban forest fragments in Amazonia may contain considerable amphibian diversity (e.g. 30 species; Cordeiro and Sanaiotti, 2003) and population sizes (Faria da Costa et al., 2013; Telles et al., 2013).

The Smoky Jungle Frog, *Leptodactylus pentadactylus* (Laurenti, 1768) (Figure 1), is a large neotropical anuran occurring along the Amazon basin in Bolivia, Brazil, Colombia, Ecuador, French Guiana, Peru, and Suriname (Heyer, 2005). In Central Amazonia, populations of *L. pentadactylus* occur both in preserved forest and in urban forest fragments (Galatti, 1992; Tsuji-Nishikido and Menin, 2011). Despite of the wide distribution of this species, the abundance both in preserved and fragmented habitats is relatively low (Faria da Costa et al., 2013) when compared to other anuran species (e.g. Telles et al., 2013). Adults of *L. pentadactylus* exhibit nocturnal and terrestrial behaviour, and are usually found along forest stream banks (Galatti, 1992; Faria da Costa et al., 2013). Previous studies in preserved forest near Manaus (Galatti, 1992) and in Ecuador (Duellman, 1978) showed that *L. pentadactylus* feeds on a variety of invertebrates and occasional vertebrates prey items.

In this study we aimed to determine the composition of the diet of *L. pentadactylus* in an urban forest fragment and in a preserved forest, both in the Brazilian Central Amazonia. We tested for differences in trophic niche breadth to each studied area, and the relationship between individual size and prey volume.

Materials and Methods

Study area.—This study took place in two sites in the state of Amazonas, Brazil: (1) an urban forest fragment, the campus of the Universidade Federal do Amazonas (UFAM: 3.0761° S, 59.9583° W; DATUM = WGS84) in the Manaus municipality, and (2) a preserved forest in Purupuru, Careiro da Várzea municipality, near the BR-319 highway (3.3400° S, 59.8200° W; DATUM = WGS84). Both regions are characterized by tropical monsoon climate, without a dry season (Peel et al., 2007). The mean annual temperature is approximately 26°C and the mean annual rainfall is 2362 mm, with the rainy season lasting from November–May and the dry season lasting from June–October (Marques-Filho et al., 1981). The forest fragment area (1) has 776 ha, including areas of *terra firme* forest (a nonseasonally flooded rainforest), secondary forest, campinarana sites, and deforested areas, with ten first order streams and two second order streams (Tsuji-Nishikido and Menin, 2011; Marcon et al., 2012). The fragment isolation began in 1971, becoming totally surrounded by urban areas about 27 years ago (Tsuji-Nishikido and Menin, 2011). The site preserved forest (2) is characterized by the presence of continuous *terra firme* forest and transitional forests in *paleovárzea* (ancient floodplains of the Amazon River and its tributaries; Rossetti et al., 2005).

Data collection.—We carried out fieldwork during the rainy season in the forest fragment (site 1) between November 2012 and March 2013 and in the preserved forest (site 2) during February 2013. The species *Leptodactylus pentadactylus* was identified according to Hero and Galatti (1990), i.e., specimens with darkly lined dorsolateral folds that continue from the eye to the groin, ventral surface and undersides of limbs black mottling, and usually darkly outlined fold from the eye over the tympanum down the side of body. We surveyed 33 frogs of this species in both sites between 18 h and 01 h (21 frogs in the forest fragment and 12 frogs in the preserved forest). After being visually located with headlamp, we captured each frog with a hand net and we measured the snout-vent length (SVL, in mm) with a digital calliper. We obtained the diet samples by stomach-flushing, following the method described by Solé et al. (2005), and we preserved those

Table 1. Prey items of *Leptodactylus pentadactylus* in an urban forest fragment and a preserved forest in Central Amazonia, Brazil. N: number of items; V: volumetric percentage; N: numerical percentage; F: occurrence-frequency percentage; IRI: index of relative importance.

	Forest fragment (N = 21)					**Preserved forest (N = 12)**				
Prey Category	**N**	**V(%)**	**N(%)**	**F(%)**	**IRI**	**N**	**V(%)**	**N(%)**	**F(%)**	**IRI**
Mollusca										
Gastropoda	1	1.26	1.59	4.76	13.57	1	2.76	1.56	8.33	35.99
Artrophoda										
Chilopoda	1	0.08	1.59	4.76	7.95	-	-	-	-	-
Diplopoda	6	14.70	9.52	28.57	691.97	9	35.13	14.06	50.00	2459.50
Arachnida										
Acari	2	<0.01	3.17	9.52	30.27	2	<0.01	3.13	16.67	52.34
Araneae	6	42.05	9.52	23.81	1227.88	6	33.02	9.38	41.67	1766.81
Scorpiones	6	21.99	9.52	23.81	750.25	-	-	-	-	-
Entognatha										
Collembola	2	<0.01	3.17	9.52	30.27	4	<0.01	6.25	25.00	156.25
Insecta										
Blattaria	3	6.79	4.76	14.29	165.05	4	14.71	6.25	25.00	524.00
Coleoptera	7	3.37	11.11	33.33	482.62	6	8.71	9.38	41.67	753.81
Diptera	4	0.02	6.35	19.05	121.35	12	0.05	18.75	58.33	1096.60
Hemiptera	5	0.04	7.94	19.05	152.02	2	0.01	3.13	16.67	52.34
Hymenoptera (non Formicidae)	3	<0.01	4.76	9.52	45.41	4	<0.01	6.25	33.33	208.65
Formicidae	3	0.24	4.76	9.52	47.60	4	1.67	6.25	33.33	263.97
Isoptera	4	0.14	6.35	4.76	30.89	-	-	-	-	-
Lepidoptera-larva	-	-	-	-	-	2	2.46	3.13	16.67	93.19
Orthoptera	4	2.61	6.35	14.29	128.04	2	1.46	3.13	16.67	76.52
Siphonaptera	1	<0.01	1.59	4.76	7.62	-	-	-	-	-
Chordata										
Squamata	1	6.69	1.59	4.76	39.41	-	-	-	-	-
Unknown	4	-	6.35	14.90		6	-	9.38	41.67	
Plant matter	-	-	-	57.14	-	-	-	-	75.00	-
Total	**63**					**64**				

in 70% ethanol. We conducted the procedure *in situ* and we released the animals immediately after finishing the flushing. We identified most of the prey items at order or family level (only ants were identified at family level: Formicidae), following the identification keys by Adis (2002) and Rafael et al. (2012). We identified Gastropoda, Chilopoda, Diplopoda, and Acari at class or subclass level. We measured each prey item (length and width) using an ocular micrometer (nearest 0.01 mm) connected to a Zeiss Stemi SV 11 stereomicroscope. We estimated the volume of each item using the spheroid volume formula $V = (\pi.\ \text{lenght.width}^2)/6$ (Colli et al., 1992). When only fragments of prey items were found in the diet samples (e.g. chelicerae of arachnids or legs of myriapods), we used invertebrate specimens deposited in the Paulo Bührnheim Zoological Collection of UFAM for identification along with the taxonomic expertise of the collection's curators. If identification was possible, we compared and inferred the original volume of the fragmented prey items by measuring intact specimens of the same taxonomic group and similar size.

Data analysis.—We determined the index of relative importance (IRI), as proposed by Pinkas et al. (1971), for each category using the following formula: $IRI = (N + V).F$, where N is the numerical percentage (percentage of prey items belonging to a certain category); V is the volumetric percentage (percentage of volume occupied by prey items of that category); and F is the frequency of occurrence percentage (percentage of occurrence of

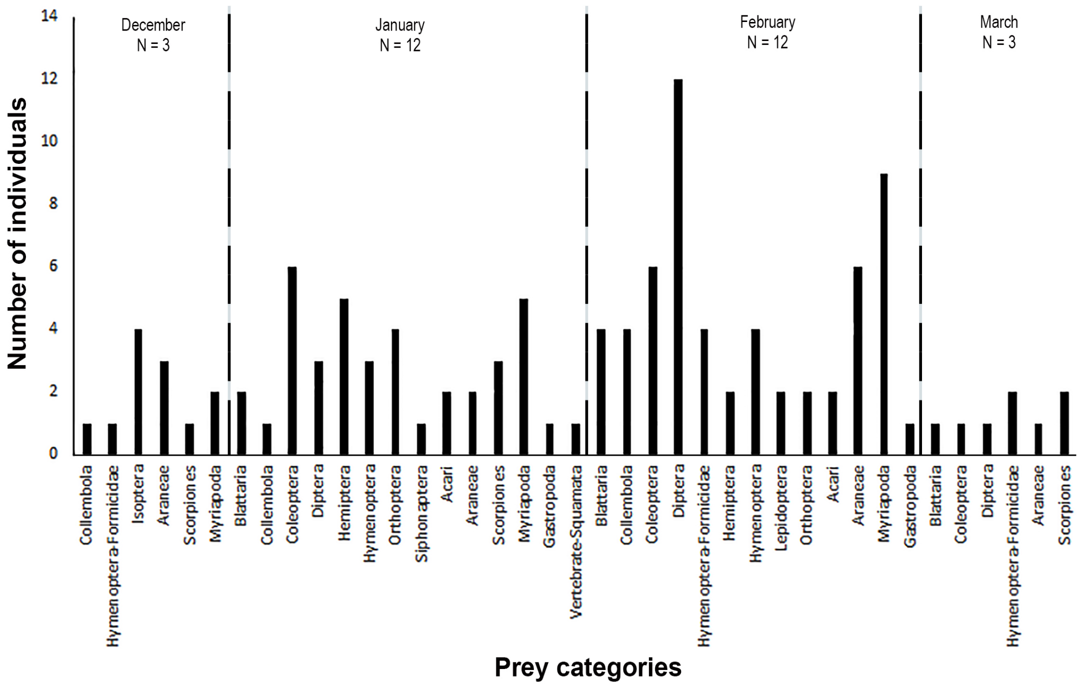

Figure 2. Number of each prey item consumed by *Leptodactylus pentadactylus* individuals per month in an urban forest fragment (December, January and March) and in a preserved forest (February), Central Amazonia, Brazil. N: number of frog individuals.

each item in relation to the total samples). We measured the trophic niche breadth of *L. pentadactylus* in both sites using the Shannon-Wiener diversity index, and we compared the values through a T-test (Zar, 2010). We used Pearson's correlation to test if the volume of the largest prey was related to the SVL of the frog. The significance level for all statistical tests was $\alpha < 0.05$. We used Systat 12.0 software to make the correlation and produce the graph.

Results

In total, we collected 127 prey items belonging to 18 categories (Table 1). From the 21 frogs in the forest fragment, three of which had empty stomachs (one individual per month in November, December, and January). We collected 63 prey items belonging to 17 categories in the fragment. In the preserved forest, all 12 frogs had stomach contents, and we collected 64 prey items belonging to 13 categories. The mean number of prey items per stomach considering all individuals was 3.84 (standard deviation = 2.71; range = 0 – 10).

In both sites, the diet of *L. pentadactylus* was mainly composed of arthropods, especially arachnids, myriapods, and insects (Table 1; Figure 2). The numbers of prey items and categories were higher in the months with a greater number of analysed stomach contents (Figure 2). There was no significant difference between the trophic niche breadth values obtained (forest fragment = 2.67; preserved forest = 2.29; t = 0.02; df = 113; p = 0.99).

Based on the IRI, the most important prey items at the forest fragment were Araneae, Scorpiones, Diplopoda, and Coleoptera, in descending order (Table 1). Together, these categories also represented over 85% of the volume of all the diet samples in this site. One small lizard species, *Alopoglossus angulatus* (Linnaeus, 1758), was consumed by one of the frogs at the forest fragment, accounting for 6.7% of the overall volume in the diet of the frogs from the forest fragment. It was the only vertebrate prey item founded. Similarly, in the preserved forest site the most important categories were Diplopoda, Araneae, Diptera, and Coleoptera, in descending order, representing more than 90% of the overall volume (Table 1).

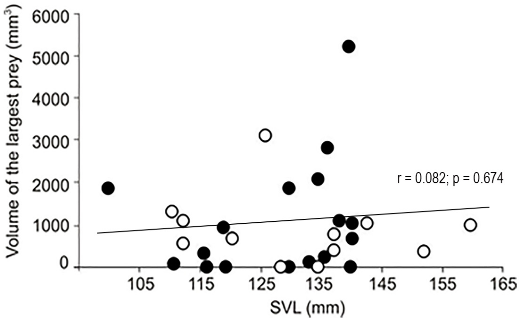

Figure 3. Relationship between the sizes (snout-vent length, SVL) of *Leptodactylus pentadactylus* individuals and volumes of the largest ingested prey in an urban forest fragment (black dots) and in preserved forest (white dots), Central Amazonia, Brazil. Note that there is no significant correlation between the variables.

Spiders were the largest invertebrate prey consumed (forest fragment: V = 5214 mm^3; preserved forest: V = 3120 mm^3). There was no relationship between the sizes of the largest prey item consumed and the respective frogs' body sizes ($r = 0.082$; $p = 0.674$; Figure 3).

Discussion

Overall, the populations of *L. pentadactylus* in the urban fragment and in the preserved forest were similar in terms of diet. Moreover, we observed only small differences in the prey items among the studied months, indicating that prey items do not substantially differ along the rainy season, at least at the taxonomic levels used in our study. So far two other studies have analysed the diet composition of *L. pentadactylus*: Galatti (1992) in Reserve Ducke, a periurban area of Manaus/Brazil; and Duellman (1978) in Santa Cecilia, Ecuador. Similarly to our results, these authors reported *L. pentadactylus* consuming a variety of invertebrates, mainly arthropods, and occasional vertebrates (Duellman, 1978). However, while orthopterans were the most consumed prey both in Reserva Ducke and Santa Cecilia, they were not particularly frequent in either of our study sites. Instead, Aranaeae, Diplopoda, and Coleoptera were important in the diet of *L. pentadactylus* both in the forest fragment and in preserved forest. These taxa were not only consumed frequently, but they also provided important contributions in terms of volume, including many of the largest food items. As Duellman (1978), we also observed a trend, even in smaller individuals, for eating large and potentially dangerous animals, such as tarantulas and scorpions. This latter represents a very substantial part of the diet in the forest fragment, while it is absent in the diet samples collected in the preserved forest, probably due to the high abundance of tarantulas and scorpions in fragments nearby urban areas (M. Menin, pers. obs.). For instance, scorpions are known to reproduce and proliferate at high rates in urban areas (Szilagyi-Zecchin et al., 2012). Likewise, dipterans were a frequent (but not voluminous) prey category in preserved forest only, likely due to their high abundance at the studied site (A.P. Couto, pers. obs.).

The occurrence of very small invertebrates in the diet of *L. pentadactylus* such as Acari, Collembola, and Siphonaptera, could be due to accidental ingestion during capture of larger preys in the leaf litter, which also explains the vegetal matter found in the analysed stomachs of many individuals. For such items, the contribution in terms of volume was considered low.

At the forest fragment, we detected the consumption of a small lizard, *Alopoglossus angulatus* by one of the frogs. This record was already published in detail by Do Couto and Menin (2014) (same specimen given here in Table 1). This and two small anurans in Ecuador (Duellman, 1978) are the only known instances of *L. pentadactylus* eating vertebrates, which suggests that they are not a common part of its diet and apparently they result from opportunistic predation. Occasional predations of vertebrates were also reported to other medium or large-sized *Leptodactylus* (Gouveia et al., 2009; Solé et al., 2009; Fonseca et al., 2012).

Overall, the dietary pattern of *L. pentadactylus* appears to match those of generalist and opportunistic predators, as seen in other large-sized *Leptodactylus* species, such as *L. knudseni*, *L. labyrinthicus*, *L. ocellatus*, *L. rhodomystax*, and *L. rhodonotus* (Duellman, 1978; Parmelee, 1999; França et al., 2004; Solé et al., 2009). Some of these amphibians are able to maintain similar diets even in human altered habitats (Solé et al., 2009). Despite the little diet differences found between the population of the urban forest fragment and that of preserved forest, it is possible that *L. pentadactylus* also exhibits this diet stability. However, future studies are necessary to evaluate this hypothesis even when considering more degraded forest fragments. The campus UFAM is unusually large for an urban forest fragment, still its amphibian diversity is already impacted by edge effects associated with habitat fragmentation (Tsuji-Nishikido and Menin, 2011). With increased disturbance, other factors besides prey availability such as reduced humidity levels, and increased temperatures (Laurance et al., 2011) might act as additional threats to amphibians that lives in fragmented forests, such as *L. pentadactylus*.

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