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Diet of *Leptodactylus spixi* (Anura: Leptodactylidae) from a cacao plantation in southern Bahia, Brazil

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Abstract. We studied the diet of *Leptodactylus spixi* from a cacao plantation in southern Bahia, Brazil. A total of 109 frogs were manually collected between December 2006 and October 2007 and analysed using a stomach flushing method. Of these, 69 stomachs revealed 168 prey items. Acarina, Orthoptera and Formicidae were the most abundant items, while Orthoptera, Diplopoda and Hemiptera were the most important in terms of frequency of occurrence. With more than 50% of total prey volume and the highest Index of Relative Importance, Orthoptera was considered the dominating prey category for this species in the studied area. The comparison between available potential prey in the environment and diet revealed that *L. spixi* fed on most present invertebrates. Electivity values were highest for orthopterans, gastropods and dermapterans. We conclude that *L. spixi* can be considered a "sit-and-wait" predator and also a generalist consuming what is available in the environment.

Keywords: Amphibia, Leptodactylidae, diet, trophic niche, predation, Brazil.

Introduction

The habitat loss in the Neotropics is still the primary threat to amphibian populations (Young et al. 2001, Stuart et al. 2004). Other indirect effects associated to landscape fragmentation, and that can contribute to the decline of amphibians, are those that may cause alteration of trophic interactions due to changes in microclimate causing abundance variations in available prey items (Carey et al. 2001, Young et al. 2001).

The family Leptodactylidae is distributed from the extreme southern USA throughout tropical Mexico, Central America and South America (Frost et al. 2006), wherein the genus *Leptodactylus* currently comprises 74 recognized species mainly distributed in South America (Frost 2016). Compared to other members of the genus, *Leptodactylus spixi* (Fig. 1) is a medium-sized nocturnal frog, which is widely distributed throughout eastern Brazil, from the State of Ceará to eastern Minas Gerais and Rio de Janeiro (Heyer 1978, as *L. mystaceus* and part of *L. amazonicus*; Caramaschi et al. 2008). The altitudinal distribution of this species ranges from sea level up to 900 m a.s.l. The species is closely associated with the Atlantic Rainforest vegetation and inhabits primary and secondary forest including dry forest, but is seldom found in open or strongly anthropogenized areas (authors' unpublished data). Although it is listed as Least Concern by IUCN, its population trend is decreasing (Heyer et al. 2010).

Large species of *Leptodactylus*, such as *Leptodactylus latrans*, have been found to feed mainly on Coleoptera, Orthoptera and Araneae (Maneyro et al. 2004), but also on small vertebrates as other frogs (Mendes et al. 2012). Medium sized species of *Leptodactylus*, such as *Leptodactylus fuscus*, have been reported to feed mainly on Orthoptera and Coleoptera, but not on small vertebrates (Sugai et al. 2012), while *L. natalensis*, also a medium sized species, includes a large number of ants in its diet and is also able to capture other amphibians (Ferreira et al. 2007). Regarding *L. spixi*, previous ecological studies have mainly dealt with bioacoustics (Bilate et al. 2006). Our aim was to study the diet of *L. spixi* from a population from southern Bahia, evaluate potential prey availability in the environment and calculate niche breadth and an Index of Relative Importance for each prey category.

Figure 1. *Leptodactylus spixi* from a cacao plantation in Ilhéus (Bahia, Brazil).

Material and methods

The study was conducted in a small cocoa plantation (25 ha), located on the campus of the State University of Santa Cruz (14°47'45''S, 39°10'20''W), city of Ilhéus, southern Bahia, Brazil. Traditionally, cocoa shrubs are planted in the shade of the Atlantic Rainforest native canopy after removal of the sub-forest. The plantations are locally called *cabrucas* and their understory is periodically removed. Cacao fruits are harvested twice a year. Some studies have suggested that *cabrucas* may serve as alternative forest habitats for many species (Argolo 2004, Pardini 2004, da Rocha et al. 2016). Faria et al. (2007) found these plantations to harbour over 81 % of the amphibian diversity found in primary forests.

Frogs were collected manually at night (18:30 to 21:00) during weekly fieldtrips from December 2006 to October 2007, inside the cocoa plantation sitting on the leaf litter and in small grassland areas on the margins of the cocoa plantation and near paths. They were captured and transferred to the nearby laboratory. Snout-vent length (*SVL*; to nearest 0.1 mm) and mouth width (*MW*) were measured us-

Figure 2. Study area inside the cacao plantation showing leaf litter (A) and an area with grass patches (B).

ing a digital caliper. Body mass (*BM*; to nearest 0.1 g) was recorded using a digital balance. Each frog's stomach was flushed following the methodology proposed by Solé et al. (2005) and specimens were released at the capture site during the same night, about three hours after having been captured. Stomach contents were transferred to vials, fixed in 70% ethanol and later analysed under a stereomicroscope. Prey items were classified to order level with exception of Hymenoptera, which were classified as Formicidae and Non-Formicidae. Completely preserved items were measured and had their volume calculated using the formula for ellipsoid bodies (Griffith & Mylotte 1987): $V = 4/3\pi\ (L/2)\ (W/2)^2$, with L = prey length and W = prey width. If partially digested body parts were retrieved, the regression formulae proposed by Hirai & Matsui (2001) were used to estimate the original prey size, followed by a volumetric calculation using the above-mentioned formula. For regression analyses XLSTAT 2011 (www.addinsoft.de) was used. To meet statistical assumptions, prey volume was log+1 transformed (Zar 1999). The index of relative importance (*IRI*) was applied as a measure that reduces bias in description of animal dietary data (Pinkas et al. 1971): $IRI_t = (PO_t)(PI_t + PV_t)$, where PO_t is the percentage of occurrence (100 x number of stomachs contained t item / total number of stomachs), PI_t is the percentage of individuals (100 x total number of individuals of t in all stomachs/total number of individuals of all taxa in all stomachs), and PV_t is the percentage of volume (100 x total volume of individuals of t in all stomachs/total volume of all taxa in all stomachs). In order to compare the trophic niche breadth, the standardized Shannon-Weaver entropy index J was used (Weaver & Shannon 1949): $J = = H/log(n)$, wherein, $H = -\Sigma p_i\ log(p_i)$, where p_i is the relative abundance of each prey category, calculated as the proportion of prey items of a given category to the total number of prey items (n) in all compared studies. To calculate the trophic niche breadth, the Levins index (B) was used (Krebs 1989): $B = 1/(\Sigma p_i^2)$, wherein p_i = fraction of items in the food category i; range = 1 to N. The index of relative importance (IRI) was computed to compare the importance of each prey category: $IRI_i = (PO_i)\ (PI_i + PV_i)$, where PO_i is the percentage of occurrence of prey item i in the stomachs, PI_i is the percentage of individuals and PV_i is the percentage of the total volume of prey category i (Pinkas et al. 1971).

To estimate the availability of prey items in the microhabitat, we removed 1 m² leaf litter and extracted all potential prey items using a Winkler/Moczarski eclector (Besuchet et al. 1987) for 48 hours. Invertebrates were preserved in alcohol 70% and subsequently identified and quantified in the laboratory. The index of Jacobs (1974) was used to calculate the electivity of food categories consumed by the frogs: $D = (R_k - P_k) / [(R_k + P_k) - (2R_kP_k)]$, where R_k is the proportion of prey in stomach contents and P_k is the proportion of prey in the environment. The index has a symmetrical scale ranging from -1 to +1, where negative values indicate a degree of rejection with respect to the prey and positive values indicate a degree of preference with respect to the prey. This index has been widely used in dietary studies (e.g. Toft 1981, Santana & Juncá 2007, Sousa & Cruz 2008, Lopez et al. 2009).

Results

A total of 109 frogs were captured including predominately adult and subadult specimens measuring from 23.27 to 44.06 mm SVL (mean = 37.24, SD = 4.47). The mouth width ranged from 8.35 to 16.10 mm (mean = 13.15, SD = 1.62) and weight from 1.3 to 9.9 g (mean = 5.8, SD = 4.59).

Stomach flushing revealed that a total of 168 prey items were present in the stomachs of 69 specimens, whereby a mean of 2.44 prey items were found per specimen (min = 1; max = 8). The diet of *L. spixi* was mainly composed of arthropods, including 11 orders of insects, three of arachnids, and a single order of crustaceans (Isopoda). Representatives from the Class Gastropoda, Phylum Annelida and plant material were also recovered (Table 1).

Table 1. Small animals isolated by Winkler sieving from a litter sample collected at site where most of the *Leptodactylus spixi* frogs were captured. Lv = larvae

Taxa	N	%N
Arachnida		
Acarina	1942	60.6
Araneae	59	1.8
Opiliones	6	0.2
Pseudoscorpiones	22	0.7
Crustacea		
Isopoda	16	0.5
Myriapoda		
Diplopoda	43	1.3
Chilopoda	6	0.2
Insecta		
Blattaria	12	0.4
Coleoptera	171	5.3
Coleoptera Lv	20	0.6
Collembola	94	2.9
Dermaptera	3	0.1
Diplura	3	0.1
Diptera	12	0.4
Diptera Lv	39	1.2
Hemiptera	122	3.8
Hymenoptera	15	0.5
Formicidae	536	16.7
Formicidae Lv	24	0.7
Lepidoptera	2	0.1
Lepidoptera Lv	5	0.2
Orthoptera	12	0.4
Thysanoptera	38	1.2
Mollusca		
Gastropoda	3	0.1
Total	3205	100

The most abundant prey items were Acarina (26.8%, n = 45), Orthoptera (18.5%, n = 31), Formicidae (6.5%, n = 11), Diplopoda and Isopoda (6.0%, n = 10). Orthoptera (42%, n = 29), Diplopoda (13%, n = 9), Hemiptera, Hymenoptera and Acarina (10.1% n = 7) were the most important in terms of frequency of occurrence. Orthoptera (3800.32 mm ³ or 52.4% of total), Hemiptera (6.3%), Isopoda (5.5%) and Diptera larvae (5.1%) were the prey categories representing highest volumes. According to the Index of Relative Importance (IRI), the most important item in the diet were orthopterans (IRI = 2979.3). Other important prey items were Acari (IRI = 281.4), Diplopoda (IRI = 114.6), Hemiptera (IRI = 112.6) and Opiliones (IRI = 70.5) (Table 2). Prey diversity was 2.44 (Shannon-Weaver index) and the niche range 7.7 (Levins index).

A positive and significant correlation was found between the SVL and mouth width (Pearson's correlation: $r = 0.86$, $p < 0.0001$, $n = 69$). There was no significant correlation between the SVL and length of the largest prey found in the stomach of frogs (Pearson's correlation: $r = 0.11$, $p < 0.39$, $n = 69$). There was also no significant correlation between the SVL and the total volume of each stomach (Pearson's correlation: $r = 0.13$, $p = 0.29$, $n = 69$).

The analysis of the leaf-litter sample resulted in the identification of 3205 invertebrates. About 60% were mites, 17.6% were ants and 5.9% were coleopterans (Table 1). Electivity indices revealed that the sampled population of *L. spixi* preferred most present prey categories, whereby electivity values were highest in orthopterans, gastropods and dermapterans. Although the electivity index for Isoptera and Oligochaeta was also high, this pattern may be an artefact caused by the absence of specimens trapped in the litter sample (Fig. 3).

Table 2. Dietary items retrieved from the stomachs of *Leptodactylus spixi* (n=69) from a cacao plantation in Ilhéus, Bahia, Brazil, represented as the number of dietary items (N), frequency of occurrence (F), volume (V) in mm³ and Index of Relative Importance (IRI).

Categories	N	N%	F	F %	V	V %	IRI
Arachnida							
Acarina	45	26.8	7	10.1	69.07	1.0	281.4
Araneae	8	4.8	6	8.7	104.76	1.4	54.0
Opiliones	6	3.6	6	8.7	328.55	4.5	70.5
Crustacea							
Isopoda	10	6.0	4	5.8	395.02	5.5	66.1
Myriapoda							
Diplopoda	10	6.0	9	13.0	205.47	2.8	114.6
Chilopoda	2	1.2	2	2.9	74.75	1.0	6.4
Insecta							
Blattaria	4	2.4	3	4.3	201.81	2.8	22.5
Coleoptera	4	2.4	4	5.8	98.08	1.4	21.6
Dermaptera	6	3.6	4	5.8	174.45	2.4	34.7
Diptera Lv	4	2.4	4	5.8	372.91	5.1	43.6
Hemiptera	8	4.8	7	10.1	459.03	6.3	112.6
Hymenoptera	7	4.2	7	10.1	102.6	1.4	56.6
Formicidae	11	6.5	5	7.2	56.01	0.8	53.0
Isoptera	1	0.6	1	1.4	17.34	0.2	1.2
Lepidoptera	1	0.6	1	1.4	364	0.5	1.5
Lepidoptera Lv	3	1.8	3	4.3	264.49	3.6	23.6
Orthoptera	31	18.5	29	42.0	3800.32	52.4	2979.3
Mollusca							
Gastropoda	6	3.6	6	8.7	143.01	2.0	48.2
Annelida							
Oligochaeta	1	0.6	1	1.4	346.04	4.8	7.8
Total	168	100.0	109	157.9	7577.7	100.0	3999.3
Vegetal	15			21.7			

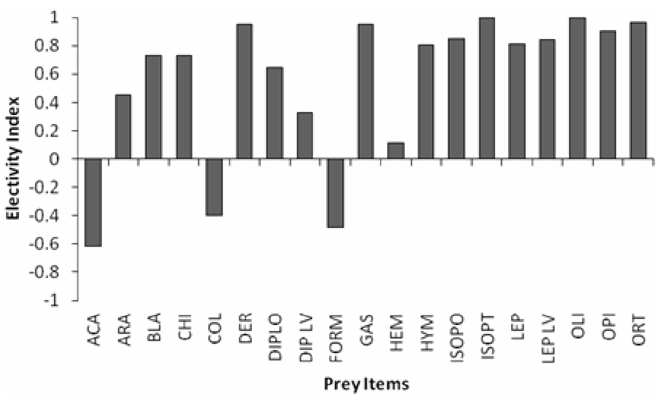

Figure 3. Electivity index of the principal prey categories consumed by *Leptodactylus spixi* in the study area. ACA = Acarina; ARA = Araneae; BLA = Blattaria; CHI = Chilopoda; Col = Coleoptera; DER = Dermaptera; DIPLO = Diplopoda; DIP LV = Diptera larvae; FOR = Formicidae; GAS = Gastropoda; HEM = Hemiptera; HYM = Hymenoptera; ISOPO = Isopoda; ISOPT = Isoptera; LEP = Lepidoptera; LEP LV = Lepidoptera larva; OLI = Oligochaeta, OPI = Opiliones, ORT = Orthoptera.

Discussion

Most frogs of the genus *Leptodactylus* are known as 'sit-and-wait' predators (*sensu* Toft 1981). Frogs using this strategy are often characterized by capturing larger, mobile prey items in small quantities, low metabolic rates, cryptic coloration and wide trophic niche breadth (Perry & Pianka 1997, Pough et al. 1998, Caldas et al. 2016, Ganci et al. 2018, Mageski et al. 2018). The population of *L. spixi* studied herein shared some characteristics that agree with the characteristics of typical sit-and-wait foragers such as cryptic coloration, few food items per stomach (mean = 2.4 items per stomach), high percentage of highly mobile food items (e.g. Orthoptera, Araneae, Opiliones, Hemiptera and Hymenoptera) and broad trophic niche breadth (B = 7.7). Ants are present in the diet of *L. spixi* but do not represent a category of importance (n = 11; V% = 0.8%; F% = 7.2) unlike found for other leptodactylids as *Adenomera thomei* (Rebouças & Solé 2015) or *Physalaemus biligonigerus* (Oliveira et al. 2015). Within the genus *Leptodactylus* several species have been found to prey on large numbers of ants: In a study undertaken in southwestern Colombia 48.12% of the identified stomach contents of *Leptodactylus fragilis* corresponded to ants (Méndez-Narváez et al. 2014). However, in another study on the diet of this species from the municipality Norcasia in Central Colombia ants only represented 6% of all preyed items (González-Duran et al. 2011). In both studies, beetles represented important diet sources, representing 17% and 36%, respectively, of the consumed items. Beetles also were frequent in the diet of *Leptodactylus mystaceus* from Novo Progresso, Pará, Brazil, where Dermaptera and Coleoptera made up 72% of prey items while Formicidae only made up 11% (Camera et al. 2014). In several species of *Physalaemus* and *Engystomops* ants have been found to be important diet components, both numerically and volumetrically (Rodrigues & Santos-Costa 2014, González-Duran et al. 2012, Narváez & Ron 2013).

Toft (1981) and Strüssmann et al. (1984) suggested that leptodactylids are generalists with a broad trophic niche, what is well supported by our data. The electivity test indicated that *L. spixi* has a preference for broad categories of prey found in the environment, rejecting only mites, coleopterans and ants (Fig. 3). These data indicate that the species consumes the prey available at the time of foraging not showing electivity determined by prey. We observed a great plasticity in the diet of the studied frogs, which consumed 11 prey categories with a wide niche breadth (B = 7.7). The categories found in the diet of *L. spixi*, in general, reflected the invertebrates found in the environment.

At the studied *cabruca*, more than half of the prey items consumed were orthopterans in terms of both frequencies of occurrence and in volume. The availability of orthopterans in the environment was estimated at 0.4% of the litter invertebrates. Despite the low percentage estimate for the item, this prey type represents an optimal cost-benefit ratio due to their usually large size. Most *L. spixi* were observed foraging on the ground. In the study area, the leaf litter is sometimes interrupted by small grass patches. Orthopterans are found more frequently in this patches and *L. spixi* may be able to prey on orthopterans resting close to the ground on these grasses.

With 45 items, Acari were the category with the largest number of recovered items, but represented only 1% of total volume, whereby most Acari were found in the stomachs containing orthopterans. Of the seven stomachs containing mites, two did not contain any orthopterans. As several mite species have already been reported as orthopteran ectoparasites (Antonatos & Emmanouel 2014) it is possible that the mites were accidentally ingested with orthopterans.

The presence of six gastropod shells is worth mentioning, representing 2% of total recovered stomach items. Gastropoda are rarely found in the diet of frogs (Dietl et al. 2009, Solé et al. 2009, Bogdan et al. 2013, Gutiérrez-Cárdenas et al. 2016). Because of their slow locomotion they are probably not identified as potential prey by many visually orientating predators. However, for some African amphibians as *Paracassina kounhiensis* and *P. obscura* morphological adaptations have been found that allow them to grasp snails with their jaws and swallow them whole (Drewes & Roth, 1981).

There was no significant correlation between the SVL and the volume of individuals, or between the mouth width and length of the largest prey, indicating that - although there is a tendency for larger animals to predate on larger prey items - they also consume small prey. However, the change in size of the predator not always implies changes in the category of preferred food items, consumed by it and, consequently, in trophic niche of the animal (Whitfield & Donnelly 2006).

We conclude that *L. spixi* is a frog with a "sit-and-wait" feeding strategy, consuming what is available in the environment. Our results represent the first data on the feeding habits of this species, thus contributing to the knowledge of the basic ecology of the frog.

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