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Diet of the Rufous Frog *Leptodactylus fuscus* (Anura, Leptodactylidae) from two contrasting environments

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Abstract

The impact of urbanization on amphibians has received some attention in the conservation literature. Despite the various impacts on animal life, some species can persist along the cities structures by adjusting their natural histories. *Leptodactylus fuscus* is a common anuran species occurring in South America, which can commonly be found in urban environments. Herein, we compare the diet of *L. fuscus* between an urban and a wild environment. We collected 57 individuals of *L. fuscus* and analysed their diet, which differed significantly between the two sites. In the urban environment, Coleoptera were the prevalent prey items, whereas specimens from the wild site had a more diverse diet.

Key Words

anthropic changes, anthropization, Cerrado, resources, trophic ecology

Introduction

Urbanization processes lead to several changes in biological communities (Cushman 2006; Hunter 2007; McDonald et al. 2011). The impact of urbanization on amphibians has received some attention in conservation literature, particularly at broad spatial scales (Riley et al. 2005; MacGregor-Fors et al. 2013; Nicholls et al. 2017; Montezol et al. 2018). However, the few studies that have evaluated amphibians' natural history in urban environments are local or were conducted in temperate regions (e.g. da Rosa et al. 2002; Vallan 2002; Mitchell et al. 2008). In urbanized areas, alterations to the timing and volume of water inputs (Riley et al. 2005; McDonald et al. 2011) can significantly impact amphibian populations (Barret et al. 2010). In addition, some species are capable of persisting in urban environments by adjusting certain natural history behaviors (Mitchell et al. 2008).

The majority of anurans are considered generalist predators and feed mostly on invertebrates (Rodrigues et al. 2004; López et al. 2009; Solé et al. 2009). Anuran diets are primarily influenced by predation risk, body size and condition, and prey availability (Duellman and Trueb 1986). Further, diet composition is directly influenced by habitat and seasonality (da Rosa et al. 2002). In spite of anuran colonization success along edifications (Simon et al. 2009; Threlfall et al. 2012; Scheffers and Paszkowski 2013), the urban environments have lower prey availability (Hunter 2007) as a result of lower overall biomass, abundance, or diversity (Coleman and Barclay 2013; Jaganmohan et al. 2013).

The Rufous Frog *Leptodactylus fuscus* (Schneider, 1799) is a common species occurring in savannas from Panama throughout South America, east of the Andes, south to southern Brazil, Bolivia, Paraguay, and northern Argentina (de Sá et al. 2014). It has nocturnal habits and lives on marshy areas all year round, and the reproduction occurs in the rainy season (Heyer et al. 1990). Previous studies have examined the diet of adults *L. fuscus* in Cerrado areas and found them to be generalist predators (Carvalho et al. 2008; Sugai et al. 2012; Junqueira et al. 2016). In the present work, we study the diet (frequency, volume and importance of preys) of *L. fuscus* between a wild and an urban site.

Material and Methods

Study area

We collected data from contrasting sites in the municipality of Campo Grande, state of Mato Grosso do Sul, Brazil (Fig. 1). The first site is a permanent pond, inserted in a savanna landscape, categorized here as a wild site (WS), located in the surroundings of the Particular Reserve of the Natural Patrimony (RPPN) Brejo Bonito (20°32'13"S, 54°45'04"W; 506 m a.s.l.). The second site (16 km west) is a temporary pond in an abandoned ground among paved streets (20°29'49"S, 54°36'24"W; 550 m a.s.l.) in the southeast area of the city of Campo Grande, categorized here as an urban site (US). Some citizens usually throw garbage and other polluted material at this site. The original vegetation that covered all the municipality territory is characterized by Cerrado phytophysiognomies. The climate in the region is classified as equatorial with two well defined seasons, a dry winter (April to August) and a wet summer (September to March). Köppen-Geiger climate classification is Aw (Kottek et al. 2006). The average annual temperature and precipitation are 22.8 °C and 1,533 mm, respectively (INMET 2005).

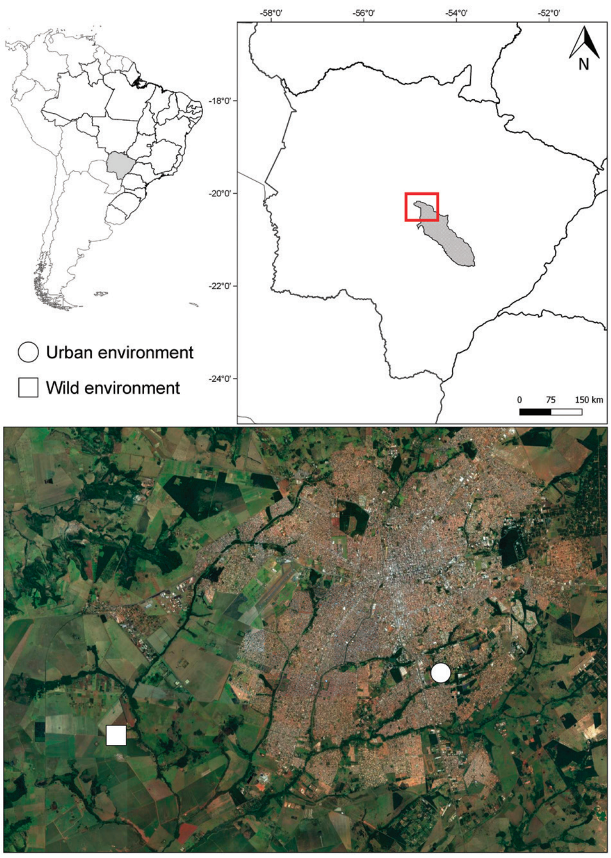

Figure 1. Map showing the sampled areas along the municipality of Campo Grande, state of Mato Grosso do Sul, Brazil.

Data collection

We collected adult *L. fuscus* through nocturnal active searches by "Visual Encounter Surveys" (Crump and Scott 1994), between 19:00h and 22:00h from November 2016 to February 2017, totalizing nine hours for each site. The animals were killed with a 2% lidocaine overdose, fixed in 10% formalin, and conserved in a 70% alcohol solution. The specimens are housed in the Coleção Zoológica da Universidade Federal de Mato Grosso do Sul. We determined the sex of each individual by the presence of vocal sacs and vocal slits in males and their absence in females.

To evaluate the diet, we removed the stomachs of each specimen through a small abdominal incision and extracted their contents. We identified each prey item with a stereoscope microscope to the order level and measured the length and width of the prey with a Mitutoyo digital caliper (0.01 mm precision). Prey items in advanced stages of digestion were considered as unidentifiable.

Statistical analysis

For the diet analysis, we first identified prey to the lowest possible taxonomic level (usually order). The volume of each prey item was then calculated using an ellipsoid formula: $V = \frac{4\pi}{3}\frac{L}{2}\left(\frac{W}{2}\right)^2$ (Griffiths and Mylotte 1987), where L = prey length and W = prey width. To determine the importance of each prey category, we calculated the relative importance index *IRI = F%(N% + V%)*, by using the mean of the percentage of occurrence (F%), the numerical percentage (N%), and the volumetric percentage (V%), according to Pinkas (1971). For the analysis, we removed all unidentifiable items and scorpiones due to partial recovery and the inability to accurately estimate volume.

We performed a PERMANOVA analysis to test if diet composition varies between the two site types, with euclidian distance, using the prey volume, and then we executed a Principal Component Analysis (PCA) to check which prey category most contributed for the differentiation. All statistical analyses were conducted in the R software v.3.4.2 (R Core Team 2017) using the vegan package (Oksanen et al. 2015).

Results

We collected 57 individuals of *Leptodactylus fuscus*, 31 in the urban site (27 males and four females), and 26 in the wild site (12 males and 14 females). Among the analyzed stomachs (n = 57), only two were empty. We found 236 preys belonging to 16 categories as follow by alphabetical order: Araneae, Blattaria, Chilopoda, Coleoptera, Dermaptera, Diptera, Hymenoptera, Hemiptera, Isopoda,

Table 1. Comparison of the diet of *Leptodactylus fuscus* between urban and wild sites in the municipality of Campo Grande, state of Mato Grosso do Sul, Brazil. V = volume, N = number, F = frequency, IRI = important relative index.

	Urban environment				Wild environment			
Prey category	V%	N%	F%	IRI	V%	N%	F%	IRI
Araneae	0.87	4.65	5.43	30	-	-	-	-
Blattaria	0.17	0.58	1.09	0.81	2.93	1.56	2.44	10.96
Chilopoda	-	0.58	1.09	0.63	-	-	-	-
Coleoptera	26.36	34.88	25.00	1531.11	1.72	4.69	7.32	46.91
Dermaptera	4.67	4.65	6.52	60.78	2.32	3.13	4.88	26.54
Diptera	0.27	1.16	1.09	1.56	0.64	9.38	4.88	48.87
Hemiptera	1.18	6.98	9.78	79.76	0.89	3.13	2.44	9.79
Hymenoptera	11.31	21.51	10.87	356.73	0.43	25.00	12.2	310.14
Isopoda	1.36	4.07	2.17	11.8	-	-	-	-
Isoptera	-	-	-	-	0.47	3.13	2.44	8.78
Lepidoptera	4.66	2.33	3.26	22.79	27.05	15.63	9.76	416.34
Not Identified	40.21	11.05	20.65	1058.5	46.3	20.31	34.15	2274.62
Orthoptera	1.18	2.33	4.35	15.23	0.28	1.56	2.44	4.5
Pulmonata	4.16	0.58	1.09	5.16	-	-	-	-
Scorpiones	-	-	-	-	-	4.69	4.88	22.87
Vegetal	3.62	4.65	7.61	62.9	16.95	7.81	12.2	301.94

Isoptera, Lepidoptera, Orthoptera, Plant Material, Pulmonata, Scorpiones and unidentifiable (Table 1).

Diet composition differs between the wild and urban site types (F=7.77; df=53; P<0.001; Fig. 2). Individuals from WS consumed a larger diversity of prey types (Table 1; Fig. 3) than those from the US. Individuals from US primarily consumed coleopterans (IRI=1531.1; 70.26%) in contrast to a more diversified diet from WS individuals, composed of Lepidoptera (IRI=416.34; 34.48%), Hymenoptera (IRI=310.14; 25.68%), Diptera (IRI=48.87; 4.05%) and Coleoptera (IRI=46.91; 3.88%). The orders Isopoda, Araneae, Chilopoda and Pulmonata were record-

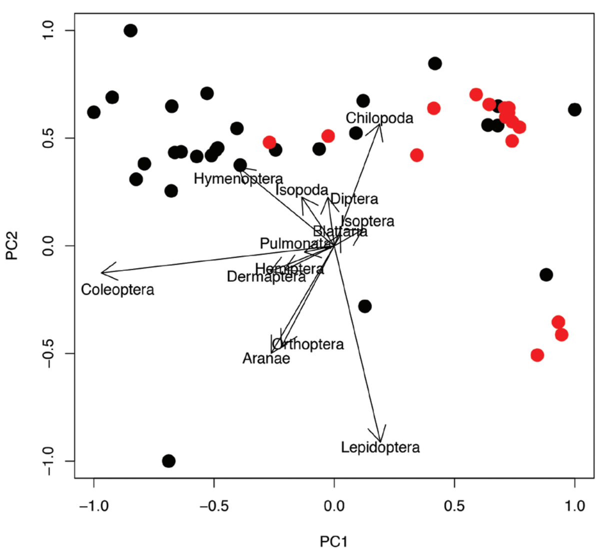

Figure 2. Principal components analysis to evaluate the differences in the diet of *Leptodactylus fuscus* sample in the urban and wild sites in the municipality of Campo Grande, state of Mato Grosso do Sul, Brazil. Black dots indicate individuals from the urban site, and red dots indicate individuals from the wild site. Arrows indicate the contribution in the ordination of each prey item.

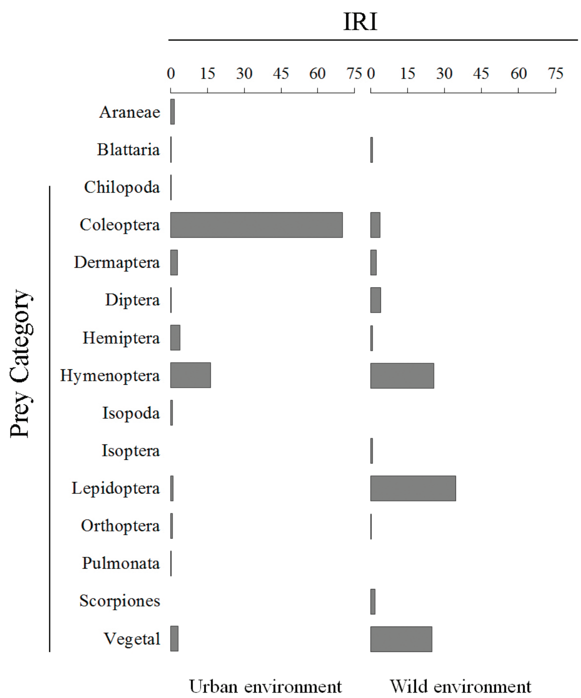

Figure 3. Diet of *Leptodactylus fuscus* in the urban and wild sites in the municipality of Campo Grande, state of Mato Grosso do Sul, Brazil. Bars represent Index of Relative Importance (IRI) (Pinkas 1971). Note the distinctness of the items consumed and their importance to the diet of studied species.

ed only in the urban population, whereas Scorpiones and Isoptera were found only in the wild population.

Discussion

In this study, we report differences in the diet composition of *L. fuscus* between urban and wild sites. Differences between urban and wild environments have been reported for other vertebrates such as birds (Zalewski 1994; Grzedzicka et al. 2013; Chenchouni 2016), snakes (Capizzi et al. 2008), lizards (Balakrishna et al. 2016), and salamanders (Barrett et al. 2012). For anurans, a recent study that evaluated the trophic ecology of anuran assemblages in northeast Argentina found out significant differences in the diet composition of all species along increasingly human-altered environments (López et al. 2015). Balakrishna et al. (2016) also found differences from natural and urban environments for the lizard *Psammophilus dorsalis* (Gray, 1831). The authors reported that individuals from a natural environment had a greater diversity of prey in their stomach contents (Balakrishna et al. 2016).

All previous diet studies of *L. fuscus* reported different prey as the most important in the species diet. We also highlight that each study was conducted in different ecoregions. Firstly, a population from a Cerrado area was studied, and the most important prey item was Coleoptera (Carvalho et al. 2008). Then, a population from the Pantanal floodplain presented Orthoptera as the most important prey item (Sugai et al. 2012). We also highlighted that both areas mentioned are characterized as wild environments. The third known study on *L. fuscus* diet (Junqueira et al. 2016) was performed in the Atlantic Forest ecoregion. However, the area where the individuals were collected is inserted in a fragmented landscape with pastures and crops. The study did not analyze the importance of prey items. However, they show that coleopterans and ants were the most consumed items (Junqueira et al. 2016). Besides, each of our studies found a different most important prey; they therefore support the idea that *L. fuscus*, as most anurans, is a generalist species (Carvalho et al. 2008; Sugai et al. 2012; Junqueira et al. 2016). Our diet results from the wild environment also support the belief that *L. fuscus* is a generalist predator, with a wild range of invertebrate orders (Fig. 3).

The changes from urban to wild environments provide a gradient in the composition of insect communities (McIntyre et al. 2001; McKinney 2008; Raupp et al. 2010). Some insects are positively phototropic (Sivinski 1998), and the urban illumination can attract specific insect orders, contributing to the difference in the insect's community composition. In the same way, the different availability of arthropods between environments, as shown in previous studies (e.g. McIntyre et al. 2001; McKinney 2008; Raupp et al. 2010), allied to the generalist diet of *L. fuscus*, which is influenced by arthropods availability in environment, can lead to the significant differences found in the diet of the animals from both sites.

Leptodactylus fuscus is a common and abundant species, inhabiting many environments (Uetanabaro et al. 2008). Although it is a species of generalist habits (Carvalho et al. 2008; Sugai et al. 2012; Pimenta et al. 2014; Junqueira et al. 2016), the urbanization probably affects its diet composition. However, a study with a more appropriated design would response more precisely how the urbanization process alters the biological traits of the frog. We recommend in a further experiment, at least, sample three ponds of each environment type, as well as repeat the experiment by two years minimum.

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