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Dietary composition and feeding strategy of *Leptodactylus fuscus* (Anura: Leptodactylidae) from a suburban area of the Caribbean Region of Colombia

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ABSTRACT
The use of trophic resources by anurans may be influenced by sexual dimorphism, ontogenetic variation and resources available in the environment. However, most studies on anuran feeding behavior lack of environmental prey availability data. In this study, the dietary composition and the feeding strategy of *Leptodactylus fuscus* were evaluated considering the availability of potential prey in a sub-urban area of the Colombian Caribbean Region. Additionally, differences in diet composition between adult and juvenile' frogs were assessed. Prey items were obtained through forced regurgitation technique and prey availability was assessed using pitfall traps. The importance of each prey category and prey selectivity were evaluated through a relative importance index and a food selection index, respectively. Twenty-four stomachs were analyzed, being Hymenoptera the most important prey category and the most abundant resource in the environment. The population of *L. fuscus* showed a low prey selectivity and prey size was associated with frog's body size. However, there was no variation in numeric and volumetric dietary composition related to ontogeny. Considering the relationship between the diet and prey availability, our results evidenced *L. fuscus* exhibits a generalist and opportunistic feeding behavior, which highlight the importance of including information on prey availability to better understand the anurans dietary behavior.

Key Words: Anurans; Diet; Prey Availability; Prey Selectivity.

The use of trophic resource by anurans is an important aspect related to their natural history and represents basic information associated with their role in community dynamics and ecosystem functioning (Cortés-Gomez *et al*., 2015). Traditionally, it has been recognized that anurans feed on a continuum of two strategies that range from active to passive search for prey (Simon and Toft, 1991). Anuran feeding strategies can be influenced by several factors, such as specific nutrient requirements, energy costs associated with foraging, risk of predation and prey availability (Berazategui *et al*., 2007). Thus, prey availability is a key aspect in order to understand anurans feeding behavior, but this topic is usually neglected in amphibian dietary studies (de Oliveira *et al*., 2019).

Anurans diet can also show sexual and ontogenetic variations regarding composition, size and quantity of prey (Rodrigues *et al*., 2004; Maragno and Souza, 2011). In this sense, females can eat more prey than males, adults can eat larger prey than juveniles and larger individuals can eat prey with larger sizes than small individuals (Sugai *et al*., 2012; Junqueira *et al*., 2016). In general, animals that cannot chew like frogs are limited to the consumption of prey that fits in the mouth and, consequently, ontogenetic changes allow larger individuals to eat larger prey (Lima and Moreira, 1993).

Leptodactylus fuscus is a terrestrial and nocturnal frog, with wide distribution in the Neotropical

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region, generally associated to grasslands, savannahs, swampy areas, degraded forest and urban areas (Reynolds *et al.*, 2004; Frost, 2020). There are several studies on diet composition of *L. fuscus*, which indicate a generalist and opportunist feeding behavior, but none of them considers prey availability (Sugai *et al.*, 2012; Junqueira *et al.*, 2016; Santana *et al.*, 2019). Thus, in the present study, the diet composition and the feeding strategy of *Leptodactylus fuscus* in a suburban area of the Colombian Caribbean region were evaluated, taking into account the availability of prey. In addition, differences in diet composition between adults and juvenile frogs, as well as the association of maximum prey size and body size of frogs were evaluated.

This study was carried out in the Sincelejo municipality, department of Sucre, Colombia (9° 18' 58.1"N, 75° 23' 17.3"W; 213 m a.s.l). Three field trips were conducted between August and October 2016, corresponding to the rainy season. The frogs were collected during night, between 19:00 and 23:00 hs using the technique of systematic survey by visual encounters (Crump and Scott, 2001). The snout-vent length (SVL) of each frog was measured using a Vernier caliper (0.01 mm accuracy) and the stomach contents were removed through forced regurgitation technique; stomach contents were preserved in 70% ethanol (Rivas *et al.*, 1996). Frogs were classified in adults and juveniles taking into account body size characteristics (Heyer, 1978). An analysis of sexual variation of diet was not considered because it was not possible to determine the sex of all individuals in the field. In order to evaluate the availability of potential prey, five pitfall traps were installed separated 10m from each other, in a transect of 50m in the same area where frogs were collected (Campbell and Christman, 1982). These pitfall traps consisted of plastic cups of 250 ml and 10 cm diameter with ethanol and were active between 18:00 and 6:00 hours on the night of sampling. All arthropods collected in the pitfall traps were considered potential prey and were preserved in 70% ethanol.

Stomach contents were identified at the level of order using taxonomic keys (McGavin, 2000). To determine the volume of different prey categories, photographs of each prey item were taken using an assembly with metric reference and then, the images were scanned through tps2DIG program. The length (L) and width (W) of each prey item were measured and the volume was calculated using the ellipsoid formula: $V = 4/3\ \pi\ (\text{length} / 2)\ (\text{width} / 2)^2$, according to Dunham (1983). The importance of each prey category in the population diet was calculated using the Index of Relative Importance (IRI) of Pinkas *et al.* (1971), according to the following equation: $IRI = (N + V) * F$; where N, V and F represents the numerical, volumetric and the frequency of occurrence percentages of each prey category in the diet, respectively. Additionally, a hierarchical classification of prey categories was made using the ranking index (RI) of Montori (1991). Chao 1 and ACE estimators were calculated to know if the number of stomachs analyzed represents a sufficient sample to characterize the diet of *L. fuscus*, using stomachs as sample units and abundance data of prey categories in Estimates version 9.1.0 (Colwell, 2013). To determine the association between prey size and frogs body size a regression analysis was performed, with the volume of the largest prey in each frog as a dependent variable and the SVL as independent variable. In order to evaluate prey selectivity the Jacobs selectivity index was calculated for the two most important prey types (Jacobs, 1974). To evaluate if volumetric and numeric composition of prey differs between adults and juveniles one-way ANOSIM tests were performed (Clarke, 1993). In this sense, Euclidean distance and Bray Curtis index were used for volumetric and numeric composition tests respectively, through the software PAST version 3.24 (Hammer *et al.* 2001).

Twenty-four specimens were captured, of which seven were juveniles and 17 adults (six juveniles and 13 adults had stomach contents). Mean SVL of juveniles was 33 mm (SD=1.63mm, range=31-36mm) and mean SVL of adults was 45mm (SD=4.23mm, range=41-57mm). A total of 92 prey items were retrieved from stomach contents, belonging to 12 categories and 10 orders (Table 1). Chao 1 and ACE estimated 16.45 and 14.67 prey categories respectively, which are values relatively close to the observed number of prey categories for *L. fuscus* in the present study. Hymenoptera and Acariformes showed the highest value of IRI in the diet of *L. fuscus* and were classified, according to the ranking index, as fundamental and secondary prey respectively (Table 1). On the other hand, Scolopendromorpha, Coleoptera and Haplotaxida were classified as accessory prey types, and Gastropoda, Diptera, Hemiptera, Blattodea and Polydesmida were accidental prey in the diet (Table 1).

Adults and juveniles did not differ regarding volumetric composition ($R= -0.054$, $p= 0.63$) neither

Table 1. Diet of *Leptodactylus fuscus*. N: total number of items; % N: numeric percentage; %F: frequency of occurrence percentage; %V: volumetric percentage; IRI: index of relative importance; RI: ranking index.

Class	Prey category	N	%N	%F	%V	IRI	RI
Insecta	Hymenoptera	15	16.3	33.33	0.08	546.14	100
	Coleoptera	10	10.87	12.5	5.92	209.87	38.43
	Diptera	3	3.26	12.5	2.97	77.89	14.26
	Hemiptera	2	2.17	8.33	3.31	45.7	8.37
	Blattodea	1	1.09	4.17	0.78	7.78	1.42
Arachnida	Acariformes	20	21.74	16.67	0.06	363.32	66.52
Chilopoda	Scolopendromorpha	1	1.09	4.17	54.64	232.2	42.52
Diplopoda	Polydesmida	1	1.09	4.17	0.16	5.2	0.95
Mollusca	Gastropoda	7	7.61	16.67	0.51	135.31	24.78
Clitellata	Haplotaxida	7	7.61	4.17	30.97	160.74	29.43
Other categories	Eggs	22	23.91	4.17	0.12	100.14	18.34
	Larvae	3	3.26	4.17	0.49	15.63	2.86

numeric composition of prey (R= 0.044, p= 0.22). In addition, the volume of the largest prey was positively and significantly related with frogs SVL (R^2 = 0.54, p <0.001). Likewise, Scolopendromorpha and Haplotaxida were found only in the stomachs of the largest individuals.

A total of 2643 arthropods were collected through pitfall traps and were identified 10 potential prey categories, being Hymenoptera the most abundant, followed by Collembola and Acariformes (Fig. 1). Among the 10 potential prey categories, six were retrieved from the stomachs of *L. fuscus* (Fig. 1) and according to Jacobs' Index, the two most important prey in the diet were consumed opportunistically

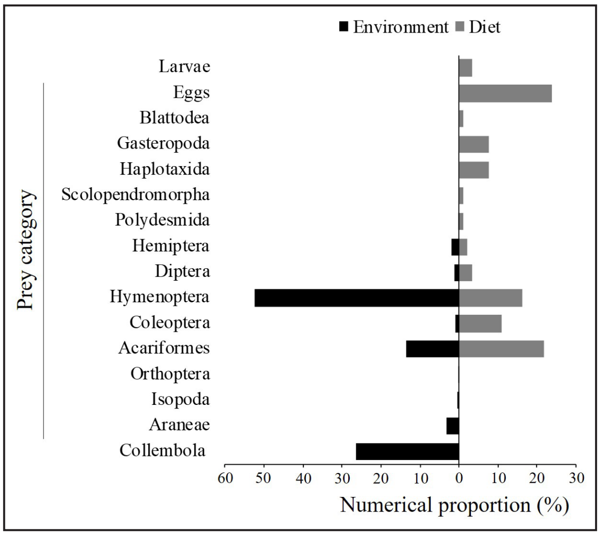

Figure 1. Numerical proportion of different prey categories in the environment and diet of *Leptodactylus fuscus*.

(Hymenoptera, J=0.034; Acariformes, J=0.024).

The relationship between the diet of *L. fuscus* and the availability of potential prey was registered here. The low values in the selectivity index and the variety of consumed prey categories suggest a generalist feeding behavior by *L. fuscus*, similarly to that observed by de Oliveira *et al.* (2019) in *Leptodactylus latrans,* where the individuals consumed prey with higher availability in the environment. The generalist feeding behavior of *L. fuscus* was documented in others studies, but none of that considered the availability of prey (De-Carvalho *et al.*, 2008; Sugai *et al.*, 2012; Santana *et al.*, 2019). On the other hand, the high consumption of small prey in *L. fuscus* suggests an opportunistic feeding strategy, as had previously been registered in this and other species of the genus *Leptodactylus* (Rodrigues *et al.*, 2004; López *et al.*, 2005a-b; De-Carvalho *et al.*, 2008; Solé *et al.*, 2009; Sugai *et al.*, 2012), likewise, the consumption of large prey by some individuals may suggest a possible sit and wait feeding strategy (Solé and Rodder, 2009).

The high consumption of Hymenoptera and Acari may be associated with the high availability of these prey in the study area. Likewise, the high intake of these prey categories differs from those found in other studies (De-Carvalho *et al.*, 2008; Sugai *et al.*, 2012; Junqueira *et al.*, 2016; Santana *et al.*, 2019), which Coleoptera and Orthoptera were the most important prey. These differences may be associated with the type of environment where frogs live, that determines the diversity of potential prey (Santana *et al.*, 2019). On the other hand, the observed association of prey size with body size has been registered in other species of genus *Leptodactylus,* and can be attributed to the fact that larger individuals can eat large prey (e.g. Lajmanovich, 1994; Maneyro *et al.*, 2004; Rodrigues *et al.*, 2004; Lopez *et al.*, 2005a). Furthermore, the intake of some prey categories by few individuals, may suggest the existence of individual specialization in the population or body size constraints on prey types (Amundsen *et al.*, 1996; Cloyed and Eason, 2017). Likewise, an increase in body size can produce an increase in the variety of prey that could be consumed (Solé and Rodder (2009), which can cause variation in the diet according to ontogeny, being associated with the energy requirements, foraging modes, microhabitat use variation between juveniles and adults and prey availability (Rodrigues *et al.*, 2004; Lima and Magnusson 2000; Sugai *et al.*, 2012). However, in this study, adults and juveniles had similar volumetric and numerical composition of consumed prey, which may be associated with the generalist feeding behavior and the availability of prey in the environment.

In conclusion, the diet of the studied population of *L. fuscus* is composed mainly of arthropods, exhibiting low prey selectivity and a food intake based mainly on the most abundant prey in the environment, showing an opportunist and generalist foraging behavior. These results demonstrate the importance of including resource availability data in feeding studies to achieve a better understanding of the trophic ecology of anurans.

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